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The sweat glands maximum ion reabsorption rates following heat acclimation in healthy older adults

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New Findings

What is the central question to this study?

Do the sweat glands maximum ion reabsorption rates increase following heat acclimation in healthy older individuals and is this associated with elevated aldosterone concentrations?

What is the main finding and its importance?

Sweat glands maximum ion reabsorption rates improved heterogeneously across body sites, which occurred without any changes in aldosterone concentration following a controlled hyperthermic heat acclimation protocol in healthy older individuals.

Abstract

We examined whether the eccrine sweat glands ion reabsorption rates improved following heat acclimation (HA) in older individuals. Ten healthy older adults (>65 yrs) completed a controlled hyperthermic (+0.9˚C rectal temperature, T_{re}) HA protocol for 9 non-consecutive days. Participants completed a passive heat stress test (lower leg 42˚C water submersion) pre-HA and post-HA to assess physiological regulation of sweat glands ions reabsorption at the chest, forearm and thigh. The maximum ion reabsorption rate was defined as the inflection point in the slope of the relation between galvanic skin conductance and sweat rate (SR). We explored the responses again after a 7-day decay. During passive heating, the T_b thresholds for sweat onset on the chest and forearm were lowered after HA ($P < 0.05$). However, neither sweat sensitivity (i.e. the slope), the SR at a given T_{re}, nor gross sweat loss improved after HA ($P > 0.05$). Any changes observed were lost during the decay. Pilocarpine-induced sudomotor responses to iontophoresis did not change after HA ($P \geq 0.801$). Maximum ion reabsorption rate was only enhanced at the chest ($P = 0.001$) despite unaltered aldosterone concentration after HA. The data suggests that this adaptation is lost after 7-days decay. The HA protocol employed in the present study induced partial adaptive sudomotor responses. Eccrine sweat glands ion reabsorption rates improved heterogeneously across the skin sites. It is likely that aldosterone secretion did not alter the chest sweat ion reabsorption rates observed in the older adults.

Keywords

Aldosterone, controlled hyperthermia, passive heating, sweat gland adaptation

Introduction

The age related decline in sweat gland function typically occurs around 60 yrs, which exacerbates the risk of heat related illness in older adults (Kenney & Fowler, 1988; Abdel-Rahman *et al.*, 1992). Following repeated heat exposure (i.e. heat acclimation (HA)) sudomotor adaptations have been reported amongst healthy older adults (Wagner *et al.*, 1972; Pandolf *et al.*, 1988; Ogawa & Ohnishi, 1989; Armstrong & Kenney, 1993). An important sudomotor adaptive response that is well known to occur in young healthy adults is a lower sweat sodium and chloride concentrations for a given sweat rate; associated with an enhanced reabsorptive capacity of the sweat glands (Allan & Wilson, 1971; Buono *et al.*, 2007*b*). This has been shown by the relation between sweat sodium concentration and sweat rate (Buono *et al.*, 2007*a*) or identifying the maximum reabsorption rates with continuous measurement of galvanic skin conductance (GSC) and sweat rate (SR) (Amano *et al.*, 2016; Gerrett *et al.*, 2019). For the latter method, fluids rich in ions, such as sweat, are well known to be highly conductive and sweat sodium and chloride content is strongly correlated with skin conductance (Shamsuddin & Togawa, 1998; Amano *et al.*, 2016). As such, the relation between GSC and SR has been used to detect when the rate of sweat ion secretion exceeds its reabsorption limit by identifying the inflection point in the slope of the relation between GSC and SR (Amano *et al.*, 2016).

A lower sweat ion concentration on the skin surface increases the water vapour pressure of the skin, which increases the water vapour gradient between the skin and ambient air, enhancing evaporative heat loss for a given skin temperature. It may also play a role in preventing dehydration by conserving sodium and chloride loss through sweating. Such an adaptation would be beneficial in older adults. Conflicting findings exist regarding the sweat sodium loss with ageing. Kirk & Westwood (1989) and Hall (1990) both reported elevated sodium loss with age. Inoue et al. (1991, 1995, 1999) has consistently reported attenuated sweat rates in older adults yet similar, if not higher, sweat [Na+] concentrations compared to younger counterparts. Although not directly measured, these data suggests that sweat [Na+] reabsorption in eccrine sweat glands decreases with ageing. Whilst there is some evidence indicating attenuated sweat sodium concentration after HA in older individuals (Inoue *et al.*, 1995, 1999), the underlying mechanisms of the induction of these adaptive sudomotor responses in this population have not been investigated.

The changes in sweat ions regulation following HA might also be associated with the sweat glands response to the changes in aldosterone secretion. Aldosterone targets basolateral K^+ pump and Na^+/H^+ exchanger in distal human sweat glands via the activation

of protein kinase C (PKC) and calcium signaling (Hegarty & Harvey, 1999; Harvey & Higgins, 2000). The result of which is the promotion of sodium transport from apical to basolateral spaces (i.e. sodium reabsorption). Kirby and Convertino (1986) reported reduced aldosterone and sweat sodium concentrations following a 10-day traditional heat acclimation protocol (fixed endogenous thermal load) in young healthy adults. The elevated ion concentration in older individuals, reported by Kirk & Westwood (1989) and Hall (1990), were associated with the decreasing aldosterone concentration that also accompanies age (Yiallouris *et al.*, 2019), indicating the potential importance of this fluid regulatory hormone on sweat ions regulation in an aged population. No data are currently available regarding the maximum ion reabsorption of the eccrine sweat glands and aldosterone response in older individuals to an induction of HA.

The aim of this study was to determine the induction of sudomotor responses after HA in healthy older adults. We specifically aimed to investigate the adaptations of the eccrine sweat glands maximum reabsorption rates across different locations on the body (forearm, chest and thigh). To investigate this, we applied a controlled hyperthermia technique and protein supplementation to facilitate HA adaptation in older people as some, but not all, of the physiological adaptations have been reported in this cohort using fixed endogenous thermal loads (Robinson *et al.*, 1965; Wagner *et al.*, 1972; Armstrong & Kenney, 1993; Okazaki *et al.*, 2009). The protein supplement was provided as it has been demonstrated to counter the attenuated plasma volume expansion observed in older adults after exercise training/heat exposure and facilitate other thermoregulatory adaptations (Okazaki *et al.*, 2009). We hypothesised that healthy older adults' eccrine sweat glands can adapt by successfully demonstrating a greater maximum ion reabsorption rate after HA. We hypothesise that these adaptations are related to elevated aldosterone concentrations following HA which serve to enhances ion reabsorption. As a secondary aim, we explored whether these adaptations, alongside other phenotypic HA markers such as sweating, skin blood flow and plasma volume, are maintained after short-term (7-day) decay.

Methods

Ethical Approval

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 latest Declaration of Helsinki (except for

registration in a database). Participants were informed about the study purpose and procedures prior to providing verbal and written consent.

Participants

Ten un-acclimated participants (8 males, 2 females, age; 67 ± 1.5 yrs, weight; 60.8 ± 9.0 kg, height; 165.9 ± 7.3 cm, estimated maximal oxygen uptake (VO_{2max}); 32.6 ± 4.5 ml/kg/min) were recruited for this study. Both males and females were recruited, as our previous research indicated no sex related differences in ion reabsorption rates in young adults (Amano *et al.*, 2017). There are no previous studies evaluating the effect of heat acclimation on the ion reabsorption rates in older adults; as such, means, standard deviations (SD) and effect sizes were difficult to estimate for power calculations. Our sample size was based on other keys variables that have been demonstrated to improve after heat acclimation in older adults (Armstrong & Kenney, 1993; Inoue *et al.*, 1999). An α of 0.05 and ß of 0.2 were set for all the following estimates. A 0.3 ± 0.2 ˚C decrement in resting rectal temperature, with an effect size of 1.6, indicated that 5 participants would give enough power to detect significant differences. For sudomotor adaptations such as the reduction of body temperature threshold for sweat onset, a change is 0.4 ± 0.22˚C and an effect size of 1.8 indicated a sample size of 5 would be sufficient. A mean difference in gross sweat loss (GSL) of 38 ± 33 g/m^2/hr and effect size of 1.15 would require a sample size of 9. A 20 ± 10 mEq/L reduction in sweat sodium concentration after heat acclimation, with an effect size of 2, indicated a sample size of 5 would be sufficient. Based on a number of key variables indicating heat acclimation adaptation, we aimed to recruit a minimum of 9 participants. Accounting for potential dropouts in participation of this type of experiment, we finally recruited 10 participants. The decay responses were a secondary aim, and not included in our sample size calculations.

Participants were asked to refrain from consuming caffeine or alcohol and to avoid any strenuous exercise 24 hours preceding the experimental trials. In addition, they were instructed to record their food and beverage intake during the preceding 24 hours and asked to replicate this for all experimental trials (excluding HA sessions). Their diet logs were checked to confirm compliance. They were required to consume 10ml/kg of water ~3 hrs prior to all experiments. Euhydration status, as indicated by a USG value ≤1.025 (Kenefick & Cheuvront, 2012) using handheld refractometer (Atago Co.Ltd, Tokyo, Japan) was confirmed in all participants. All participants were non-smokers and were not taking any

medications. Testing took place between the months of October and May (autumn through spring) in Japan.

Experimental design

Participants visited the laboratory a minimum of 15 times each. The preliminary visit was to familiarise the participants with the experimental procedures and the laboratory. Participants then took part in 2 tests, separated by 24 hours. The first test involved assessments of fitness level, anthropometric characteristics and cholinergic sweat gland responsiveness to transdermal administration of pilocarpine. The second test was a passive heat stress test to monitor sudomotor and other physiological responses to a thermal stimulus. Within 5 days of completing these tests, participants began a 9-day controlled hyperthermia HA protocol, with participants completing the session at any time of day. We employed a 9-day non-consecutive heat acclimation protocol to be completed within 14 days, with no more than 24hrs separating each HA session. After 36-48 hours of completing the final HA session, participants repeated the passive heat test (post-HA) and 24 hours later (i.e. 72 hours after the final HA session) repeated the assessment of fitness level, anthropometric characteristics and cholinergic sweat gland responsiveness. Seven days after the completion of HA an additional passive heat test was conducted to assess the level of decay in thermophysiological measures. The passive heating test measured pre-HA, post-HA and Decay, took place at the same time of day (± 1 hr) for within-experimental trials and within the period of 0800-1000 hrs for all between-experimental trials.

Experimental trials

Fitness, anthropometric and cholinergic sweat gland responsiveness

All participants underwent an anthropometric assessment whereby height, body mass and skin folds were assessed. Body surface area was determined by standard height and body mass calculation (Du Bois & Du Bois, 1916). Percentage body fat was calculated from the assessment of skin fold thickness from 4 sites (biceps, triceps, subscapular suprailiac) (Durnin & Womersley, 1974) using callipers (Eiyoken-Type, Meikosha Co., LTd., Tokyo, Japan). All participants completed the YMCA submaximal fitness test (Golding, 2000) on a cycle ergometer (Aerobike 75XLIII, Combi Wellness Corp, Tokyo, Japan) to estimate VO_{2peak} in a climatic chamber (SR-3000; Nagano Science, Osaka, Japan) maintained at 25˚C, 45% RH, and minimal air movement.

To determine cholinergic sweat gland responsiveness, the number of activated sweat glands (ASG) and the sweat output per a gland (SGO), all participants completed a pilocarpine iontophoresis sweat stimulation test. A small area of the volar forearm was cleaned with an alcohol wipe. Before the iontophoresis, a plastic capsule (6.15cm^2) was filled with 1% pilocarpine dissolved in saline via gauze (F1515; Osaki Medical, Aichi, Japan) and attached to the mid ventral forearm using a spandex rubber band. A 1.5mA iontophoresis current was applied for 5 min between the pilocarpine filled electrode capsule (anode) and a flexible plate electrode (cathode, HV-LLPD, Omro Healthcare, Kyoto, Japan) attached proximal to the wrist joint. Immediately after iontophoresis, pilocarpine filled capsule was removed and the skin surface wiped with a clean gauze. Another capsule (5.31cm^2) was then positioned over the stimulated site to measure SR for 12 minutes. The initial 2 minutes were discarded and then a 10 min average was obtained. Following the sweat rate-sampling period, the sweat capsule was removed, the area wiped dry and then wiped with an iodine-soaked cotton gauze. Residual iodine was removed by blotting the area with tissue paper. Starch paper attached to a small wooden block was then placed over the measurement area for approximately 3 sec. The iodine was transferred from the ASG to the paper as indicated by small dots. The same investigator counted the dots within a defined area (1cm^2). The SGO at the respective site was calculated by dividing the SR by the number of ASG. The fitness anthropometric and cholinergic sweat responsiveness tests were measured pre and post heat acclimation.

Passive heat stress test

All participants completed three heat stress tests (pre-HA, post-HA and Decay) in an environmental chamber maintained at 25˚C, 50% RH with minimal air movement. Prior to entering the chamber participants provide a urine sample for the assessment of hydration status and aldosterone concentration. Nude body weight was also measured using platform scales (ID1 Mettler-Toledo, Germany; resolution of 10g). A rectal thermometer (RET-1, Type T, Copper Constantan thermocouple, Physitemp Instruments, Inc., Clifton, NJ, USA) was then self-inserted 10 cm past the anal sphincter. Rectal temperature was used as a measure of core body temperature (T_{re}). Participants wore shorts (and sports bra for females) and donned a water-perfused suit covering the entire body except the head, neck, hands and below the knee. After entering the chamber, participants rested in a semi-supine position whilst instruments for the measurement of skin temperature, SR, GSC, skin blood flow (SkBF) and mean arterial pressure (MAP) were prepared. SR, GSC and SkBF were

measured from the mid ventral forearm, mid-chest and thigh (one third the length of the thigh from the knee cap).

During preparation and the baseline measurement period, water at 34˚C was passed through the suit to maintain a uniform and stable resting skin temperature. After all instruments were attached and prior to starting a 5-minute baseline sample, a salivary aldosterone sample and a finger prick blood sample were collected. The latter was to measure haemoglobin (Hb) concentration (201+ Hemocue, Sweden) and haematocrit (Hct) to assess the changes in plasma volume. Following baseline measurements, participants submerged their lower legs into a water bath set at 42˚C and the water inside the suit was increased to 38˚C. Once sweating began, the right volar forearm was cleaned and a customised sweat patch for the measurement of sweat NaCl concentration was affixed to the skin. We aimed for all participants to complete 60 minutes of passive heating but some found it too uncomfortable and the test was terminated earlier. A minimum duration of 45 minutes and a maximum of 60 minutes was implemented and was standardised within participants for all three tests. The number of heat-activated sweat glands (HASG) using the starch iodine technique was measured at the chest, forearm and thigh within the final 10 minutes of passive heating. An additional salivary aldosterone sample and finger prick blood sample were collected before the passive heating test was terminated. Participants towel dried themselves and provided a nude body weight measurement for the assessment of GSL.

Heat acclimation

Upon arrival to the laboratory, for each HA session, urine samples were collected for the assessment of hydration status and aldosterone concentration. Participants self-inserted a rectal thermometer (as described earlier) and wore a heart rate (HR) monitor (Polar RS400; Polar Electro Oy, Kempele, Finland). Participants dressed in standardised footwear, shorts and T-shirt. To facilitate the heat load, participants wore knee compression stockings (Mizuno, BioGear Calf Support, Japan) covered with impermeable gaiters and a short-sleeved impermeable jacket. Both the T-shirt and jacket had openings across the left chest to allow free evaporation as the chest was designated as a measurement area of sudomotor function, alongside the forearm and thigh, which were also uncovered. Prior to dressing, a nude body weight measurement was taken.

Participants entered the chamber, set at 35˚C, 45% RH, with minimal air movement and initially rested for 5 minutes for baseline measurements. This was then followed by the

collection of a resting salivary aldosterone sample, thermal sensation, thirst sensation and wetness sensation. They then began the controlled hyperthermic cycling protocol, which usually requires either the elevation of T_c >38.5˚C or an increase in 1.0˚C is used (Taylor *et al.*, 1997). However, from pilot testing this proved too difficult for a number of our healthy recreationally active older individuals. Due to their lower resting T_{re}, this required an ΔT_{re} >2.0˚C or required >2 hours to reach the required T_{re} resulting in approximately 3 hours cycling in the heat which was quite strenuous even for our healthy older active participants. Therefore, we adapted the controlled hyperthermia technique for something more manageable; we aimed for a ΔT_{re} increase of 0.9˚C above baseline and held thereafter for 60 mins. The aim was to increase T_{re} within 45-60 mins, which was typically achieved by cycling for 2-to-3 20-minute blocks interspersed with 5 min rest periods to allow for any delays in T_{re}. The 20-minute exercise blocks initially began at ~75W and the watts increased by 10-20W every 5 minutes. Ratings of perceived exertion (RPE) were recorded at 5-minute intervals, with the aim for RPE to range between 11-16, but not exceed a score of 17. If RPE exceeded 17 the exercise intensity was lowered or a rest period provided. Once T_{re} reached the desired increase of +0.9˚C, participants alternated between cycling (typically around 75W) and resting whilst ensuring a 0.9˚C elevation in T_{re}. Participants were free to drink water *ad libitium* during the heat acclimation sessions. Water bottles were weighed pre and post to record the volume consumed.

During each HA session, thermal sensation, thermal comfort, wetness sensation, thirst sensation and RPE were measured at 5 mins intervals. A sweat sample was collected from the forearm for the assessment of sweat NaCl concentration once T_{re} reached 0.9˚C. At the end of each HA session RPE was scored and a final salivary aldosterone sample collected. Upon the completion of the heat acclimation protocol, participants towel dried and were weighed nude. After each HA session participants ingested 3.2 ml/kg of a protein and CHO mixture, containing 3.2 kcal/kg and 0.18g protein/kg to facilitate thermoregulatory and cardiovascular adaptations (Okazaki *et al.*, 2009). Body fluid loss during each HA was calculated and participants were given water to match this loss within 2 hours of completing the HA session.

Measurement and calculations

Skin temperature was measured using copper-constantan thermocouples (Inui Engineering, Higashi Osaka, Japan) from 8 locations (forehead, chest, back, upper arm, forearm, hand, thigh and calf). A weighted mean skin temperature (T_{sk}) was calculated from

8 sites (Stolwijk & Hardy, 1966) and mean body temperature (T_b) using 0.9 (T_{re}) and 0.1 (mean T_{sk}) weightings (Gisolfi & Wenger, 1984). During the passive heat stress tests and HA sessions the pre and post nude weight, the volume of fluid consumed and the durations were used to calculate GSL (g/m^2/hr). Plasma and blood volume shifts between HA1 and HA9, and between pre-HA, post-HA and Decay were determined as described by Dill & Costill (1974). The core temperature onset thresholds for SR and cutaneous vasodilation at each site were determined using segmental linear regression (Cheuvront *et al.*, 2009). The slopes were defined as the linear portion of the changes in SR and CVC after the onset thresholds.

SR was measured using the ventilated capsule technique. Dry nitrogen gas was flushed (500ml/min) through the apparatus approximately 1 hour prior to each experiment to ensure stable readings. Each capsule (3.46 cm^2) was affixed to the skin using collodion 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. SkBF was measured on each forearm using laser-Doppler velocimetry (ALF21; Advanced, Tokyo, Japan). Cutaneous vascular conductance (CVC) was calculated from the ratio of SkBF to mean arterial blood pressure (MAP). CVC was subsequently calculated as a percentage of the baseline value recorded during the resting phase prior to heating. T_{re}, local T_{sk}, SkBF, SR and GSC were recorded every second by a data logger (MX100; Yokogawa, Tokyo, Japan). Heart rate and arterial blood pressure were continuously measured on the left middle finger using a Finometer (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands); MAP was subsequently calculated.

Sweat NaCl from the right ventral forearm was measured during all the passive heat tests and HA sessions. The area was cleaned with alcohol, rinsed with distilled water and dried with a sterile towel. The sweat patch consisted on a 4 x 4cm cotton gauze (100601; Askul, Tokyo, Japan) placed within a custom designed Parafilm dressing that attached to the skin. Sweat samples were collected from the right ventral forearm. Sterile gloves were worn during application and removal to prevent any contamination and sterile tweezers used to remove patches from the skin. Patches were placed inside an airtight plastic tube (Sarstedt Salivettes) and centrifuged at 4,000 RPM for 10 minutes and re-weighed. Sweat

was extracted and analysed using the Wescor (3120 Sweat-Chek™, Wescor, Logan, UT, USA) which provide a unit of mmol/L (equivalent sodium chloride: NaCl) based on the calculation from the sweat conductivity. The sweat rate of the patch sample was determined gravimetrically (AB54 Mettler-Toledo, Germany; resolution of 0.1mg) as described by Smith & Havenith (2011).

Salivary 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. Urine and salivary samples were frozen at -30˚C until analysis. After thawing, aldosterone (pg/ml) levels were quantified by competitive ELISA (LDN, GmbH & Co.KG, Germany).

As described in our previous experiments (Amano *et al.*, 2016, 2017; Gerrett *et al.*, 2018*a*), 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 (Machado-Moreira *et al.*, 2009; Gerrett *et al.*, 2018*b*). 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 phases 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.

Thermal sensation was rated using a scale with intermediary values ranging from +10 (extremely hot) to −10 (extremely cold) with 0 indicating thermal neutrality (ASHRAE, 2005). Thermal comfort was rated on a 6-point Likert scale with intermediary values; 0=comfortable, 2=slightly uncomfortable, 4=uncomfortable, 6=very uncomfortable (Gagge *et al.*, 1967). RPE was recorded using the 6- to 20-point Borg Scale (Borg, 1954). Wetness sensation and thirst sensation were reported on visual analogue scales ranging from 0 (Not thirsty/ Completely dry) to 10 (Very thirsty/Completely wet) (Ackerley *et al.*,

2012; Millard-Stafford *et al.*, 2012). Scales were presented and reported in Japanese and translated back to English for this manuscript.

Data analysis

Statistical analyses were completed using GraphPad Prism (version 7). Significance was set at $P < 0.05$ and data are presented as mean and SD. The decay responses were collected for exploratory purposes and thus not included in any statistical analysis.

A paired sample *t*-test was used to assess differences in baseline thermoregulatory variables (T_{re}, HR, MAP and saliva aldosterone concentration) between pre-HA and post-HA. As the time for passive heating differed per person, the physiological variables during the last 5 minutes were used to calculate the response to passive heating as a change from baseline (ΔT_{re}, ΔHR, ΔMAP and Δ salivary aldosterone concentration). This data, alongside the remaining physiological data collected once per passive heat test (baseline urine aldosterone, USG, plasma volume, GSL and sweat equivalent Na^+ concentration) were also analysed using a paired sample *t*-test. The sweat response from pilocarpine stimulation test was also analysed with a paired sample t-test. All data were checked for normality.

During passive heating, sudomotor data were collected from three locations and these data (maximum ion reabsorption, onset and slope for both SR and CVC, HASG, SGO and SR at a given T_{re}) were analysed using a two-way ANOVA. Main effects of location (chest, forearm and thigh) and intervention (pre-HA and post-HA) and interactions (i.e. location x intervention) were analysed. Bonferroni adjusted Students *t*-tests were used post-hoc for analysis of main effects but if main effects and interactions were observed, follow up post-hoc testing, were conducted on interaction effects only.

Although not pertinent to the hypothesis, the data collected during the HA sessions were also analysed. For HA data, a one-way ANOVA was used to determine daily changes (HA days 1-9) for the following variables: resting T_{re}, GSL, USG, local SR and equivalent NaCl, urine aldosterone concentration, time for T_{re} to increase 0.9˚C above baseline and the volume consumed during each HA sessions. When significant main effects were observed, post hoc comparisons using Bonferroni test was carried out. To assess the acute effect of training on aldosterone, a salivary aldosterone sample was collected at baseline and at the end of each individual HA training session. This data was analysed using two-way ANOVA with time (baseline vs. exercise) and day (HA days 1-9) as main effects and

interactions. When significant main effects were observed, post hoc comparisons using Bonferroni test was carried out, but if main effects and interactions were observed, follow up post-hoc testing, were conducted on interaction effects only. For HA data, post hoc comparisons were made to HA day 1 only.

When the sphericity assumption was violated, data were adjusted using Greenhouse-Geisser method. As ANOVAs are fairly robust to violations of normality, if the data were approximately normal, then the data were assessed with parametric data analysis. If the data violated this assumption substantially, then a Friedman's test was performed. The relation between forearm SR and forearm sweat equivalent NaCl concentration collected from all HA sessions were analysed using Pearson's correlation coefficient. Baseline and/or the final perceptual data (thermal sensation, thermal comfort, wetness sensation, thirst sensation and RPE) from the HA sessions were analysed using the non-parametric Friedman's test. Whilst for the passive heat stress test they were analysed using Wilcoxon- signed ranked test.

Results

Heat acclimation sessions

All participants successfully completed all 9 HA sessions. Table 1 summarises the exercise responses to each HA session. Despite a decline in resting T_{re} from day 1 of HA (37.45 ± 0.38˚C) to day-9 (37.17 ± 0.39˚C), this was not significant (P = 0.30). The average time taken for participants to increase their T_{re} by 0.9˚C was 57 ± 11 mins and this did not change over the 9 HA sessions (P = 0.850). There was however, considerable variation within and between subjects with the longest time to increase T_{re} by 0.9˚C taking 90 mins and the fastest at 38 mins.

Participants were able to maintain an adequate hydration status throughout the 9-day HA protocol (average USG for all 9 days, 1.015 ± 0.007) and was not different over time (P = 0.917). The volume of water consumed during each HA training sessions did not change over the 9 days (see Table 1, P = 0.992). The average GSL was 0.70 ± 0.26 L/hr and also did not change during the 9-day HA protocol (P = 0.997). Local SR (P = 0.563) and equivalent NaCl concentrations (P = 0.945) from the forearm both remained unchanged during HA. No relation (r^2=0.05, P = 0.961) was observed between daily HA sweat equivalent NaCl and SR from the forearm.

Baseline urine aldosterone concentrations (see Table 1) remained stable during the 9-days of HA and thus there was no main effect of HA session (P = 0.396). For salivary

aldosterone samples collected at baseline and after each HA session, a main effect of time was observed; samples collected after each HA training session were always higher than the baseline samples (P = 0.005). There was no main effect of day or an interaction effect for salivary aldosterone ($P \geq 0.696$).

By day 8, baseline thermal sensation was significantly lower than day 1 (3.7 ± 1.9 to 1.7 ± 1.6, respectively P = 0.002), whilst the end thermal sensation score remained unchanged (daily average; 4.4 ± 2.2, P = 0.873) during the 9 HA training sessions. Baseline (daily average; 4.8 ± 2.1) and end of exercise wetness sensations (daily average; 7.0 ± 1.4) and thirst sensation (daily average baseline 3.8 ± 2.0 and end of exercise: 4.9 ± 1.9) did not change over the course of the 9-day HA protocol ($P < 0.05$). RPE was rated at the end of each HA session and participants scored a similar daily RPE (daily average; 13 ± 1, P = 0.747)

Passive heat stress test – pre, post and decay

Thermophysiological responses

Figure 1A-B illustrates baseline T_{re} and ΔT_{re} from the passive heat stress tests. The intervention had a significant effect on baseline T_{re} with post-HA (36.69 ± 0.33˚C) being significantly lower that pre-HA (36.92 ± 0.27˚C, P = 0.005). Baseline T_{re} after the decay period was 36.77 ± 0.33˚C. For ΔT_{re}, there was no significant difference between pre-HA and post-HA (P = 0.706).

T_{sk} at baseline was not statistically different between pre-HA and post HA (P = 0.298; 33.86 ± 0.75˚C, 33.60 ± 0.26˚C, respectively). Decay was comparable (33.60 ± 0.17˚C). T_{sk} at the end of passive heating was not different between tests (pre-HA; 37.10 ± 0.85˚C, post-HA; 36.72 ± 1.17˚C, P = 0.392). Decay was comparable (36.82 ± 0.92˚C).

Thermal sensation reported at the end of passive heating was not significantly different from pre-HA (7 ± 1) to post-HA (6 ± 2, P = 0.063). Thermal sensation after the Decay period was 6 ± 2. Thirst sensation was not different between trials (pre-HA: 6 ± 1, post-HA 5 ± 2, P = 0.672, Decay: 5 ± 2).

Sudomotor responses

The ΔSR threshold for an increasing ΔGSC for the three locations and each passive heat test are illustrated in Figure 2. Main effects of the intervention (P = 0.003), location and (P = 0.006) an interaction (P = 0.018) were observed for the sweat glands maximum ion reabsorption rate. Post-hoc analysis indicated that the sweat glands maximum ion

reabsorption rate increased only at the chest from pre-HA to post-HA (P = 0.001). The maximum ion reabsorption rates at the forearm during the decay was 0.32 ± 0.21 mg/cm^2/min, which is slightly lower than the post-HA response. The maximum ion reabsorption rates at the forearm followed a similar trend but like the thigh it was not different from pre-to-post-HA (P > 0.05). Forearm sweat equivalent NaCl samples, accounting for local sweat rate did not change from pre-to-post-HA, (3.3 ± 2.6, 4.3 ± 3.0 mmol/L/g/hr, respectively P = 0.558). Forearm sweat equivalent NaCl after the decay was 2.8 ± 1.4 mmol/L/g/hr. There was insufficient sweat collected for NaCl analysis, from some trials for 3 participants, therefore the aforementioned data were based on n=6.

There was no main effect of the intervention (P = 0.052) or an interaction effect (P = 0.251) on HASG (Table 2). There was a main effect of location (P = 0.0001) and post hoc tests indicated that the number of HASG were significantly higher on the forearm than both the chest (P = 0.001) and the thigh (P = 0.001). SR at a given ΔT_{re} (+0.8˚C) also did not differ between pre-HA and post-HA (P = 0.267 and P = 0.987 for main effect of intervention and interaction, respectively) but there was an effect of location with the chest being significantly higher than the thigh (P = 0.007). As a result of the no changes in HASG or SR at a given ΔT_{re} (0.8˚C) throughout the intervention, there were also no differences in SGO between pre-HA and post-HA, on any of the 3 locations (P = 0.339 and P = 0.914 for main effect of intervention and interaction, respectively). A main effect of location (P = 0.022) indicated that the SGO at the chest was significantly higher than thigh (P = 0.026). Decay data are displayed in Table 2 for exploration and preliminary comparison. Table 3 displays the sweat onset and slope for T_b and ΔT_b. There was a main effect of the intervention for the SR onset in relation to T_b, with post-HA being significantly lower than pre-HA (P = 0.004). There was no effect of location, nor an interaction effect ($P \geq$ 0.390 0.375). For the SR onset for ΔT_b, there was no main effect of location, intervention or an interaction effect ($P \geq$ 0.307). The T_b and ΔT_b -SR slope, there was a main effect of location for both parameters, with the thigh being significantly less steep than both the forearm ($P \leq$ 0.011) and chest ($P \leq$ 0.001). GSL was not significantly different pre-HA and post-HA (0.49 ± 0.21, post-HA: 0.55 ± 0.22, respectively, see Figure 1C). Decay values were comparable (0.54 ± 0.25 g/m^2/hr).

Cardiovascular responses

Plasma volume expanded following acclimation (Δ 10.2 ± 10.4%, P = 0.006). After decay, plasma volume appears to have remained slightly elevated following decay (Δ 2.5

± 8.4%). Baseline HR (pre-HA: 60 ± 9, post-HA: 58 ±5, P = 0.354 and-Decay: 61 ± 6 bpm) and the ΔHR from passive heating (pre-HA: 19 ± 6, post-HA: 23 ±4, P = 0.053 and Decay: 21 ± 5 bpm) remained unchanged throughout. Resting MAP also remained unchanged (pre-HA: 99 ± 11, post-HA: 92 ± 6, P = 0.055 and Decay: 98 ± 7 mmHg). There was also no effect of the intervention on ΔMAP at the end of the passive heat (pre-HA: -8 ± 6, post-HA: -3 ± 11, P = 0.179 and Decay: -3 ± 10mmHg).

Table 3 displays the CVC onset and slope for T_b and ΔT_b. For the CVC-T_b onset, there was a main effect of the intervention (P = 0.003) but no effect of location (P = 0.107), nor an interaction effect (P = 0.659). Post hoc testing indicted that the CVC-T_b onset post-HA was significantly lower than pre-HA (P = 0.002). For CVC-ΔT_b onset there was only a main effect of the intervention and post hoc test revealed that pre-HA was significantly higher than post-HA (P = 0.001). The slope for both T_b and ΔT_b was affected by location ($P \leq 0.029$) but not by the intervention ($P \geq 0.117$), nor was there an interaction effect ($P \geq 0.126$). For both parameters, post hoc analysis indicated that the slope for forearm ($P \leq 0.08$) and chest ($P \leq 0.021$) were significantly less steep than the thigh.

Urine and Salivary Aldosterone responses

USG was consistent between the three passive heat stress tests (pre-HA; 1.014 ± 0.006, post-HA; 1.014 ± 0.006, P = 0.964 and Decay; 1.010 ± 0.007). Urine aldosterone concentration was not affected by the intervention (pre-HA: 9.86 ± 5.13; post-HA: 9.21 ± 4.39, P = 0.600 and Decay: 7.49 ± 4.9 µg/l). Baseline salivary aldosterone samples were not different between pre-HA (80.3 ± 35.8 pg/m) and post-HA (69.4 ± 23.0 pg/m, P = 0.364). The values after the decay were 78.2 ± 34.2 pg/ml. Δsalivary aldosterone concentration were not different between pre-HA (18.1 ± 39.9 pg/ml) and post-HA (31.1 ± 42.8, pg/ml, P = 0.596). The values after the decay were 21.8 ± 19.9 pg/ml.

Fitness, anthropometry & pilocarpine sweat test

There were no changes in the sum of skin fold thickness pre-HA vs. post-HA (58.4 ± 13.2 vs. 57.6 ± 14.9 mm, P = 0.743). Estimated VO_{2max} improved post-HA from 30.3 ± 4.3 to 33.3 ± 5.4 ml/kg/min, with a 10% increase (P = 0.007).

Pilocarpine-induced SR (0.61 ± 0.24 vs. 0.62 ± 0.22 mg/cm^2/min, P = 0.801), ASG (114.9 ± 19.7 vs 115.7 ± 26.2 gland/cm^2, P = 0.848), and SGO (5.31 ± 1.9 vs. 5.38 ± 1.8 µg/gland/min, P = 0.853) were not different between pre- and post-HA.

538

539 **Discussion**

540 The primary aim of the present study was to determine whether eccrine sweat glands
541 of healthy older adults (>65yrs) adapt by releasing a more dilute sweat as a result of an
542 enhanced reabsorptive capacity following heat acclimation. Although we hypothesised that
543 this would occur for all three locations investigated (chest, forearm and thigh), we found it
544 only to be true at the chest. We further hypothesised that this adaptation would be a result
545 of elevated aldosterone concentrations following HA but we reported unaltered aldosterone
546 concentrations in healthy older adults (>65yrs) following HA. The mechanism responsible
547 for the elevated maximum sweat ions reabsorptive capacity does not appear to be associated
548 with secreted aldosterone in this population. The data gathered adds important information
549 regarding sweat gland adaptability to HA in an aged population and highlights some
550 important considerations for ensuring an effective HA protocol.

551

552 *Eccrine gland ion reabsorption adaptations*

553 Enhanced ion reabsorption is well reported in young adults (Buono *et al.*, 2007*a*;
554 Amano *et al.*, 2016) but there have been no studies directly assessing this adaptations in
555 older individuals. We provide further evidence that the maximum ion reabsorption rates
556 were enhanced for the sweat glands located only on the chest. Our exploratory analysis of
557 the decay response suggests that this adaptation may be quickly lost after a decay period in
558 older individuals. The forearm followed a non-significant trend and the thigh showed no
559 noticeable changes whatsoever. The adaptation observed at the chest only, may be
560 indicative of the sweat gland activity during HA. Torso SR is well known to be higher than
561 the extremities in young and older adults (Inoue *et al.*, 2004; Smith & Havenith, 2011;
562 Coull *et al.*, 2017). Furthermore, although SR declines with age (Inoue *et al.*, 1991, 2004),
563 it has been reported that the decline is attenuated across the torso and head compared to a
564 greater decline observed at the extremities (Inoue *et al.*, 2004; Coull *et al.*, 2017). During
565 HA, although local SR was not measured it is plausible that chest SR exceeded that of the
566 forearm and thigh. Indeed, the SR of these locations during each passive heat stress test did
567 follow the typical differences in regional SR (i.e. chest>forearm>thigh). Thus, we expect
568 that SR was higher on the chest compared to the forearm and thigh during all heat
569 acclimation sessions. Buono et al. (2009) found that when local forearm sweating was
570 suppressed (via BOTOX injections) during HA, sweat gland adaptations were abolished,
571 whilst the contralateral non-treated arm exhibited changes. This suggests that local sweat

gland activity during HA is required for improved local sweat gland function; the minimal sweat rate required for sudomotor adaptations would be an interesting avenue to explore. Taylor (1997) suggested that GSL should exceed 0.4-0.8 L/h for adequate sudomotor adaptation but only limited information of local SR's during HA sessions exists in literature. Buono et al. (2007) reported enhanced reabsorption rates at the chest in young healthy participants following 10-day HA when local SR increased up to 1.03 ± 0.34 mg/cm^2/min. We measured local SR at the forearm during each HA session using the absorbent patch technique, with reported SR values (averaged over the 9 days HA) of 0.31 ± 0.22 mg/cm^2/min; much lower than values reported previously (Buono *et al.*, 2007*a*). The lower SRs may be indicative of an older population and may suggest a given SR is required to promote sweat gland adaptations. Two participants had visibly greater improvements in the ion reabsorption rate at the chest than other participants (See Figure 2). Exploration of the data indicated that one of these participants had the highest average GSL during HA training (1.25 ± 0.21 L/hr) but the other participant was similar to the mean. In comparison to other HA phenotypes acquired, these participants showed larger improvements than the mean in resting rectal temperature and the T_b threshold and slope for sweating at the chest. There were no other clear distinct patterns in the data but research into inter-individual variance in adaptive responses to HA in older adults would be beneficial.

Whilst sweat gland activity during HA may be one important requirement for enhanced ion reabsorption there is also the reported role of water regulatory hormones such as aldosterone. Aldosterone targets basolateral K^+ pump and Na^+/H^+ exchangers located on the straight reabsorptive duct to enhance sodium reabsorption via the co-activated CFTR and ENaC channels (Harvey & Higgins, 2000). Conn (1949) first reported increased sweat sodium reabsorption in heat acclimated individuals owing to an increased release in aldosterone, which has been supported recently (Neal at al. 2016). However, Kirby & Convertino (1986) reported reductions in both aldosterone and sweated Na^+ concentrations following HA and hypothesised that eccrine gland responsiveness to aldosterone is augmented after heat acclimation. We found no changes in aldosterone concentration following HA but we did observe improved ion reabsorption for the chest sweat glands which supports Kirby & Convertino's hypothesis. However, the same cannot be applied for the sweat glands located on the arm or thigh as ion reabsorption rates were unaltered. The discrepancy between locations suggest that a pre-requisite for enhanced sweat gland ion reabsorption in older adults may be sufficient sweat gland activity (i.e. a given sweat

606 rate) during HA. Without this, improvements in eccrine sweat glands ion reabsorption
607 cannot occur, regardless of any hormonal controlling mechanism. The contribution of
608 sweat gland activity and aldosterone concentration during HA on sweat gland ion
609 reabsorption warrants further investigations.
610
611 *Hormonal adaptations*
612 Elevated aldosterone concentrations occur in response to secretion of ACTH,
613 cortisol, plasma levels of Na^+ and K^+ and activation of the renin-angiotensin-aldosterone
614 system to conserve body fluids and electrolytes. A recent meta-analysis indicated a small
615 positive effect of HA on resting aldosterone concentrations (+25 ± 35%) (Tyler *et al.*,
616 2016), which was mostly based on young healthy adults. We measured aldosterone
617 concentrations during each HA session (baseline vs. exercise in the heat) and in response
618 to the intervention (pre-HA vs. post-HA and Decay) and found no changes in baseline
619 urine, nor saliva concentrations to HA in healthy older individuals. Although we used saliva
620 and urine, rather than plasma, to measure aldosterone concentration, strong correlations
621 have been reported with plasma samples (Mcvie *et al.*, 1979; Few *et al.*, 1986), so we do
622 not expect our sampling method to influence our findings. Furthermore, we reported
623 elevated salivary aldosterone concentrations after an acute bout of heat stress during each
624 HA session (baseline vs. exercise in the heat) which is consistent with other studies
625 measuring plasma and urine aldosterone concentrations to exercise and/or thermal stress
626 (Bonner *et al.*, 1976; Follenius *et al.*, 1979). In young adults, aldosterone has been reported
627 to increase in responses to heat and/or exercise stress (Bonner *et al.*, 1976; Follenius *et al.*,
628 1979) and whilst resting aldosterone concentrations declines with age (Takamata *et al.*,
629 1999; Yiallouris *et al.*, 2019), post exercise and post passive heating values in our study
630 were higher than baseline.
631 Our data indicates that aldosterone mechanisms are still activated in healthy older
632 adults in response to exercise-heat stress but the stimulus required to elevate resting
633 aldosterone concentration after HA was not sufficient within our study. It is unclear
634 whether this is associated with the adapted HA protocol used, an ageing effect which
635 renders one unresponsive, or both. In a dehydrated state aldosterone concentrations in older
636 men are reportedly unaffected by HA compared to younger men (Takamata *et al.*, 1999).
637 Whilst the acclimation status of their participants was not fully confirmed with the usual
638 HA phenotypes, they did report that plasma volume did not expand in the older males whilst
639 it did for the younger participants. In our study, plasma volume expansion was evident (Δ

10.2 ± 10.4%), which may be associated with the protein supplement provided to each participant after each HA session to facilitate recovery and thermoregulatory and cardiovascular adaptations (Okazaki *et al.*, 2009). It may also be associated with the aldosterone secretion observed after each HA session relative to resting values (Francesconi *et al.*, 1983). Given the expanded plasma volume observed in our healthy older adults, it seems unlikely that ageing accounted for the lack of change in resting aldosterone concentration following HA, but this warrants further investigations.

Sudomotor adaptations

Overall, the markers for sweat gland adaptations were incomplete; GSL did not change, HASG, SGO and SR at a given T_{re} during passive heating did not change following HA. Pilocarpine induced sweat rate did not change. The onset for sweating to changes in T_b were observed for some locations but not all and there were no changes in the sensitivity (i.e. the slope). The decay data suggests that the adaptations at these locations could be quickly lost after a short decay period, whilst the reduction in resting and end of passive heating T_{re} and T_b are retained during the decay period. We found a sustained core and body temperature adaptation following a decay period in older individuals. This is in line with previous studies investigating decay in younger adults (Weller *et al.*, 2007; Neal *et al.*, 2016); our exploratory data suggests that this is true for healthy older adults. Whilst this was the case for core body temperatures, it seems that sudomotor adaptations were difficult to acquire and where they did occur they were quickly lost in this cohort.

Taylor (1997) suggested that GSL should exceed 0.4-0.8 L/h for adequate sudomotor adaptation. The average GSL for all HA sessions was 0.69 ± 0.25 L/hr, which is within this so called 'adaptable' range. Despite our cohort being active for their age group, we adapted the controlled hyperthermic protocol to be more manageable for this population. Baseline T_{re} prior to each HA session declined from 37.45 ± 0.38˚C on day 1 to 37.17 ± 0.39˚C by day 9, although this was not significant, it is a meaningful change and it may be that raising T_{re} 0.9˚C above baseline resulted in a progressive reduction in the thermal forcing function (Taylor, 2014). Had we chosen to progressively increase the ΔT_{re} required during HA may have provided the necessary stimulus to promote all the adaptations. However, during our experiment, it was evident that this would not have been feasible for all participants; with one participant requiring (on average) 90 mins to raise T_{re} by 1.0˚C, which was subsequently followed by a further 60 mins of exercise. For this participant, the time to ΔT_{re} 0.9˚C did not change over the course of the HA training and

thus requesting a higher rise in T_{re} would have resulted in >3 hours of training in the heat, quite a considerable load for a healthy older adult (>65 yrs).

Inoue et al. (1999) investigated the response of the sweat glands to cholinergic stimulation in healthy older adults following heat acclimation. Whilst they observed an age-related decline in cholinergic responsiveness they also observed an enhanced response 24 hours after completing an 8-day heat acclimation protocol. Despite recruiting a similar age and fitness status, we did not observe such changes. The pilocarpine test was administered approximately 72 hours after the final HA session and 24 hours after the passive heat stress test. This is much longer than Inoue et al. (1999) and may explain the discrepancy. The longer duration may have accounted for the lack of improvement observed in the pilocarpine induced sweat sensitivity test but a passive heat stress test was conducted within that 72-hour period which would have re-stimulated the sweat glands and reduced decay. Inoue et al. (1999) also implemented 90 minutes per day for 8 days (spread over 10 days) at 43˚C and 30% RH, whilst we used an adapted controlled hyperthermic protocol for 9 days (spread over 14 days) in 35˚C, 45% RH, with all participants exposed >90 minutes to the heat. The more frequent rest periods between HA training sessions in our study may have impaired the sweat gland adaptation but this is difficult to confirm. What is evident, is that the thermal strain experienced (e.g. T_{re} and local SR) during heat acclimation training sessions need to be described fully in order to determine the prerequisite for successful HA protocols for vulnerable populations. Unfortunately, this appears to be a consistent issue in previous studies with this particular age group.

Whilst limited in number, there are a few studies investigating heat acclimation in older adults that have reported sweat gland adaptations (Wagner *et al.*, 1972; Ogawa & Ohnishi, 1989; Armstrong & Kenney, 1993; Inoue *et al.*, 1999) indicating adaptability. The sweat glands appear to be one of the last physiological adaptations to occur, with studies suggesting 8-14 days are required (Daanen *et al.*, 2018). Interestingly, Patterson et al. (2004) suggested that a sustained thermal strain, via controlled hyperthermia heat acclimation protocols, is an important determinant for the attainment of sudomotor adaptations with a short time frame (within just 6 days). This, alongside the reported sudomotor adaptations in other studies with similar populations and our partial heat acclimation status (core body temperature, and cardiovascular but not sudomotor adaptation) would suggest that the protocol used provided an insufficient stimulus to bring about the sudomotor adaptations in older adults. We also employed a 9-day non-consecutive heat acclimation protocol to be completed within 14 days, whereas most

studies with a controlled hyperthermic protocol use consecutive days training. This extended recovery time between HA sessions may have been too long to ensure a constant forcing function exists to exhibit sudomotor adaptations. Given that other adaptations were present (lowered resting and end exercise T_{re} and HR, expanded plasma volume, reduced thermal sensation and discomfort) may suggest that sweat gland adaptations require a short recovery time (<24 hrs) between HA sessions to bring about the adaptations. The data suggests that more sessions and/or less breaks between HA sessions are needed to acquire the adaptations.

Cardiovascular adaptations

In line with previous studies in older males, we observed a significant shift in the T_b threshold for vasodilation but no change in the slope (Armstrong & Kenney, 1993). Unlike the T_b-SR onset, this was observed for all three locations. Our data suggests that these adaptations are retained following a 7-day decay period at the extremities but not the torso. Ikegawa et al. (2011) reported that the adaptations in the control of skin blood flow are heavily dependent on plasma volume expansion, which may explain the adaptations observed post HA and, albeit to a lesser extent, those observed following the decay period. Plasma volume expansion appears to be an important prerequisite for other thermoregulatory adaptations after HA in young and older adults (Okazaki et al 2009; Ikegawa et al. 2011). Plasma volume expansion likely occurred due to the elevated secretion of aldosterone after each heat training session (Δ 10.2 ± 10.4%) (Francesconi *et al.*, 1983) but we also provided a protein supplement after each training session to aid recovery which may have also contributed to the adaptations observed in this age group (Okazaki *et al.*, 2009).

Despite observing a lower resting and end of passive heating T_{re}, we did not observe any changes in HR. Typically, these responses occur simultaneously and indicate a lower physiological strain. HR is generally attenuated in young and older subjects following HA and it is unclear why we did not observe any reductions, especially in the presence of other physiological markers of heat adaptation. There may have been no pronounced reductions in HR due to the relatively low HR of our group prior to HA (60 ± 9 bpm). During passive heating, skin temperature was essentially clamped (with a water perfused suit) during all tests. This elevated yet similar skin temperature resulted in a similar demand for cutaneous blood flow at the end of passive heating (data not shown), resulting in a similar cardiovascular strain during the passive heat stress test. Whether cardiovascular adaptations

742 would be observed if skin temperature was not clamped in our passive heat stress test or
743 during an exercise task is probable given cardiovascular reductions observed in similar
744 studies (Armstrong & Kenney, 1993).

745 **Limitations**

746 Both males and females were recruited for the present study, based on the assumption of
747 no sex related differences supported from previous findings (Baker *et al.*, 2009, 2015;
748 Amano *et al.*, 2017). However, some of these studies are based on younger participants.
749 The small number of females in the present study limits the generalizability of the findings
750 to both males and females. A logical step following this study, would be the confirmation
751 of no sex related differences in maximum ion reabsorption rates amongst older individuals.

752 Our sample size calculation was not based on our primary variable of interest (ion
753 reabsorption rates) but a variety of other HA phenotypes that have been reported to adapt
754 with heat acclimation. A larger sample size, would provide more certainty to confirm the
755 non-significant differences reported, especially due to the high variability within and
756 between participants for sweat ion reabsorption rates. The risk of a Type M (magnitude)
757 error from our data analysis (exaggerated effect size where statistical significance was
758 observed) cannot be ruled out. Furthermore, we included decay as an additional step in the
759 research design but our sample size calculations were not based on the decay response. As
760 such, we only explored the decay data and did not statistically analyse the data. Additional
761 research, addressing these limitations is certainly warranted to advance our knowledge on
762 this topic.

763

764 **Conclusion**

765 Overall, our healthy older adults successfully completed the adapted heat acclimation
766 protocol and showed some, but not all of the adaptations typically reported following heat
767 acclimation. Resting and end-of-passive-heating rectal temperature were successfully
768 lowered, plasma volume expansion was also evident, but the sudomotor adaptations
769 showed modest improvements after heat acclimation. The sweat glands at the chest
770 exhibited an enhanced reabsorptive rate, indicating adaptability but this was not observed
771 at the thigh or forearm. The improvements observed at the chest occurred without any
772 changes in aldosterone concentration. Furthermore, an adapted (i.e. easier) heat acclimation
773 protocol brings about only partial adaptations, but alternative protocols should be
774 investigated to ensure full sudomotor adaptations are obtained in older individuals.

775

Author Contribution

NG and NK conceptualised the research question and all authors contributed to the research design. NG and NK performed experiments; NG analysed data and all authors interpreted the results of the experiments. NG prepared the figures and drafted the manuscript; all authors edited, revised and approved the final version on the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Competing Interest

The authors declare no conflict of interests.

Data availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables and Figures

Figure 1: Resting T_{re} (rectal temperature), B) change in rectal temperature (ΔT_{re}) after passive heating, and C) GSL (gross sweat loss) following passive heating, pre-HA (heat acclimation) (circle), post-HA (square), and Decay (triangle). Grey plots and lines represent individual responses, whilst black solid symbols and lines represent mean responses (n=10) *$P < 0.05$. Decay data are not included in the statistical analysis and are only presented for exploratory purposes.

Figure 2: The ΔSR (sweat rate) threshold for an increasing ΔGSC (galvanic skin conductance), as an index of the eccrine sweat glands maximum ion reabsorption rates pre heat acclimation (pre-HA), post-HA and following a 7-day Decay period from the forearm, chest and thigh. *$P < 0.05$. Data are mean (SD), n=10. Decay data are not included in the statistical analysis and are only presented for exploratory purposes.

971 **Table 1:** Mean ± SD daily responses to each heat acclimation (HA) session (n=10).

	HA1	HA2	HA3	HA4	HA5	HA6	HA7	HA8	HA9
Resting T_{re} (˚C)	37.45 ± 0.38	37.32 ± 0.44	37.19 ± 0.33	37.28 ± 0.28	37.15 ± 0.19	37.08 ± 0.37	37.11 ± 0.33	37.04 ± 0.33	37.17 ± 0.39
GSL (L/hr)	0.67 ± 0.18	0.70 ± 0.23	0.73 ± 0.30	0.67 ± 0.28	0.70 ± 0.30	0.67 ± 0.27	0.70 ± 0.28	0.68 ± 0.22	0.73 ± 0.28
Time to +0.9˚C T_{re} by (mins)	56 ± 14	58 ± 15	51 ±6	57 ± 9	55 ± 6	56 ± 10	60 ± 13	58 ±12	59 ±12
Average T_{re} (˚C) (final 60 mins)	38.38 ± 0.33	38.21 ± 0.38	38.11 ± 0.31	38.22 ± 0.27	38.16 ± 0.17	38.03 ± 0.35	38.05 ± 0.30	37.98 ± 0.29	38.10 ± 0.35
Baseline urine Aldosterone (µg/L)	99.4 ± 24.2	130.1 ± 38.5	105.1 ±16.9	113.4 ± 22.6	121.4 ± 17.6	105.6 ± 24.6	100.8 ±17.81	99.4 ±14.2	91.6 ± 16.8
Baseline Saliva Aldosterone (pg/ml)	66.2 ± 25.9	74.0 ± 20.8	88.7 ± 62.0	88.6 ± 58.9	57.5 ± 22.9	66.3 ± 19.8	70.4 ± 32.8	70.9 ± 28.9	74.6 ± 30.4
Exercise Saliva Aldosterone (pg/ml)	99.4 ± 24.5#	87.7 ±23.8 #	87.7 ± 24.0#	90.4 ± 27.1#	96.8 ± 45.2 #	90.5 ± 66.4 #	102.7 ± 47.1#	85.3 ± 27.0#	96.2 ± 36.0#

Equivalent NaCl	53.7	63.2	55.7	56.4	60.7	54.9	57.4	56.6	63.1
(mmol/L)	(10.9)	(20.6)	(13.2)	(14.8)	(16.1)	(17.5)	(20.3)	(19.8)	(29.8)
Water volume	0.74	0.71	0.61	0.62	0.58	0.57	0.67	0.60	0.58
consumed (L)	(0.46)	(0.43)	(0.40)	(0.48)	(0.37)	(0.36)	(0.53)	(0.49)	(0.46)

[#] indicates a significant difference from pre-HA session ($p<0.05$). HA1-9, heat acclimation day 1-9, T_{re}, rectal temperature; GSL, gross sweat loss, SR, sweat rate.

Table 2: The mean ±SD number of heat-activated sweat glands (HASG), the local sweat rate (SR) when ΔT_{re} increased by 0.8˚C and the sweat gland output (SGO) at the forearm, chest and thigh in responses to a passive heat stress test pre-HA, post-HA and Decay. Decay data are not included in the statistical analysis and are only presented for exploratory purposes.

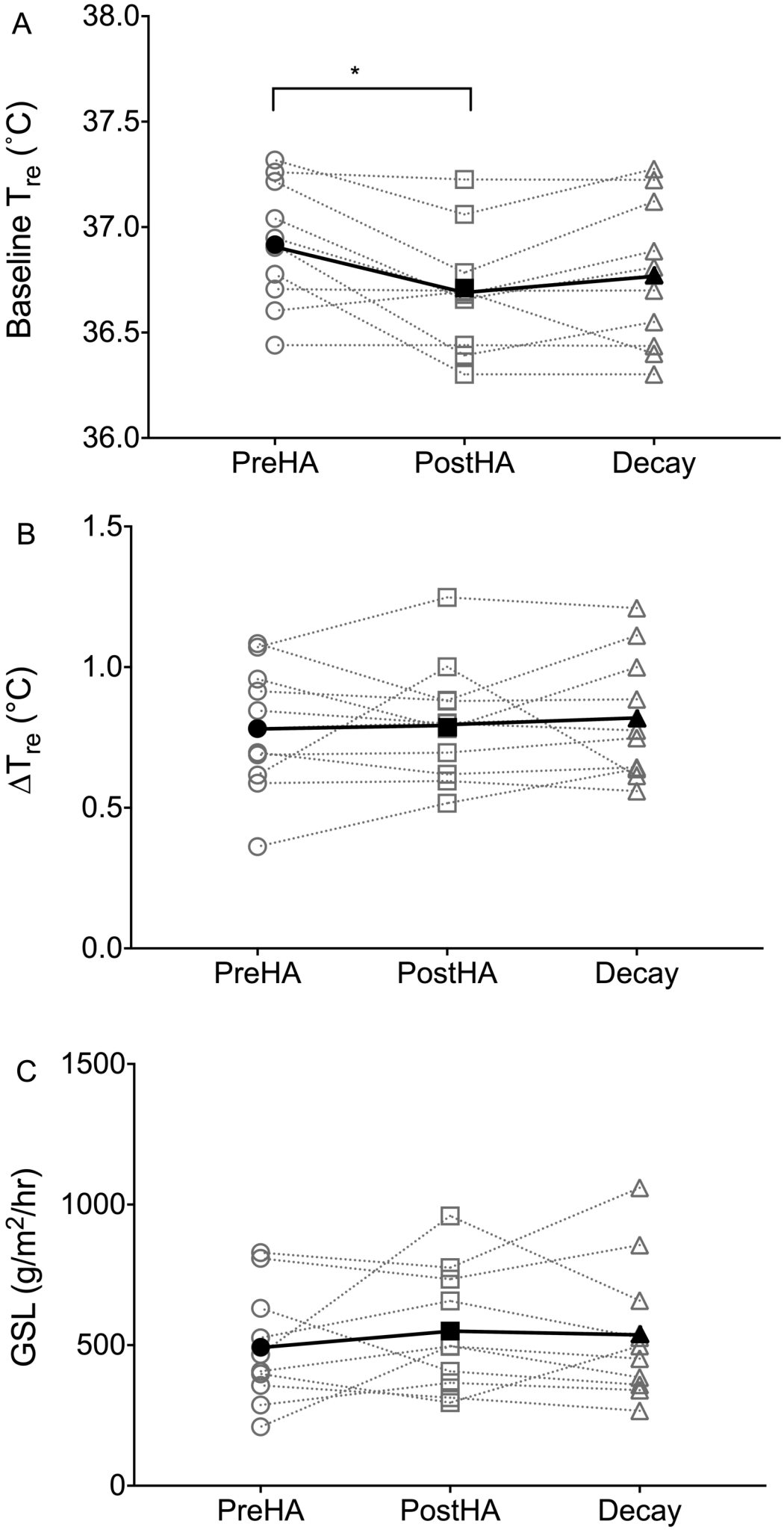
		Forearm	Chest	Thigh
HASG gland/cm^2	Pre-HA	106 ± 27	83 ± 15	65 ± 21
	Post-HA	112 ± 28	83 ± 17	72 ± 23
	Decay	114 ± 41	80 ± 18	66 ± 19
SR (mg/cm^2/min) (at ΔT_{re} of 0.8˚C)	Pre-HA	0.41 ± 0.22	0.50 ± 0.34	0.21 ± 0.09
	Post-HA	0.48 ± 0.32	0.58 ± 0.43	0.27 ± 0.16
	Decay	0.47 ± 0.25	0.56 ± 0.35	0.29 ± 0.22
SGO µg/gland/min	Pre-HA	3.9 ± 2.0	5.9 ± 3.8	3.2 ± 1.0
	Post-HA	4.4 ± 2.8	6.6 ± 4.8	4.0 ±1.7
	Decay	4.4 ± 2.2	6.7 ± 4.2	4.1 ± 2.5

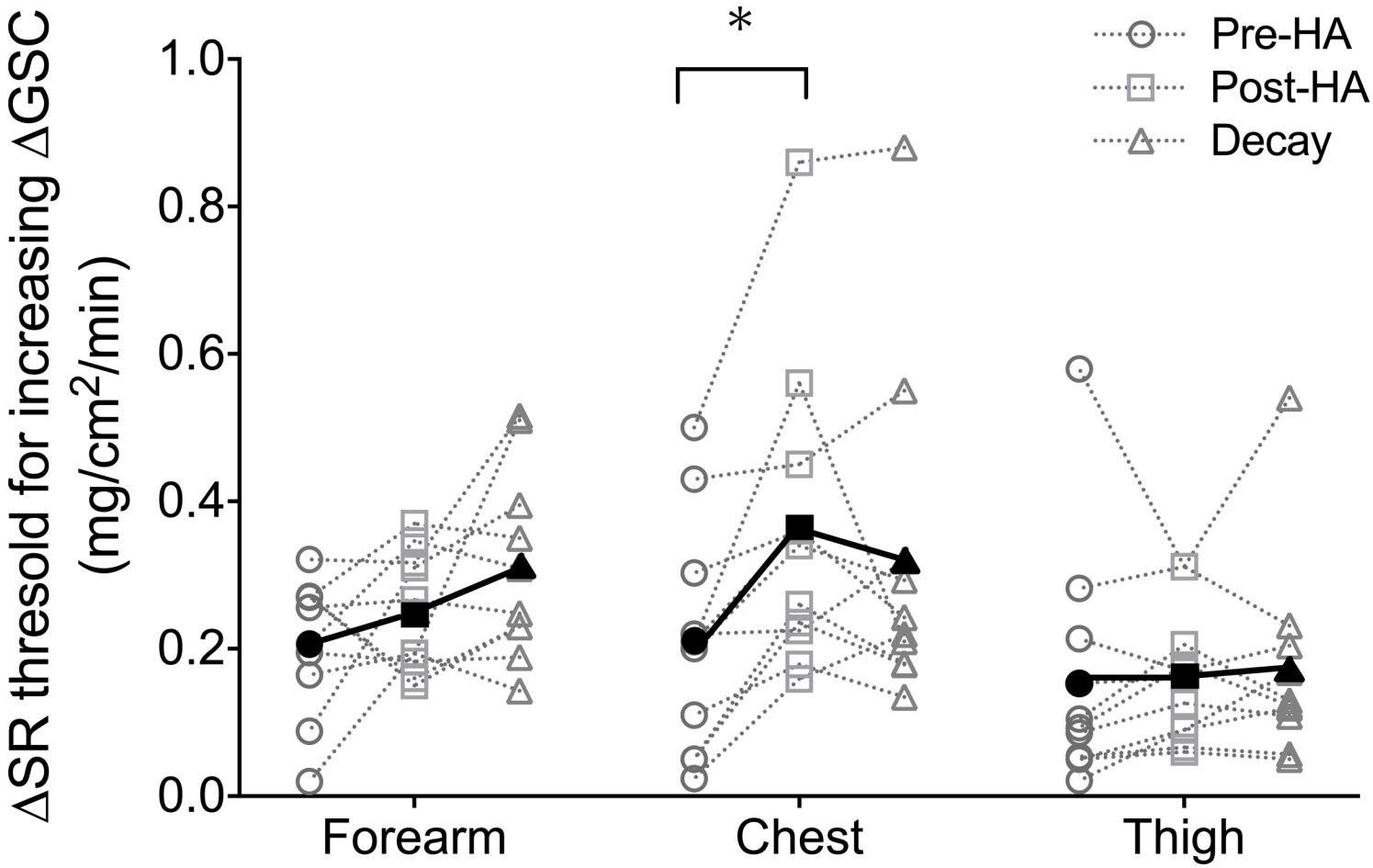
979 **Table 3:** Mean ± SD body temperature (T_b) thresholds and slopes for the sweating and cutaneous vasodilation during passive heat stress tests, pre-
980 HA, post-HA and Decay.

		SR				CVC			
		Pre-HA	Post-HA	Decay	ANOVA	Pre-HA	Post-HA	Decay	ANOVA
Threshold (˚C)	Forearm	37.03 ± 0.22	36.76 ± 0.34	36.87 ± 0.27	$	37.04 ± 0.24	36.67 ± 0.35	36.83 ± 0.27	$
	Chest	37.04 ± 0.22	36.74 ± 0.33	36.87 ± 0.25		37.02 ± 0.34	36.63 ± 0.40	36.87 ± 0.29	
	Thigh	36.99 ± 0.29	36.72 ± 0.38	36.84 ± 0.27		37.15 ± 0.27	36.72 ± 0.40	36.84 ± 0.37	
ΔThreshold (˚C)	Forearm	0.48 ± 0.29	0.43 ± 0.28	0.43 ± 0.27		0.32 ± 0.32	0.43 ± 0.32	0.32 ± 0.32	$
	Chest	0.45 ± 0.3	0.38 ± 0.31	0.44 ± 0.27		0.50 ± 0.29	0.34 ± 0.33	0.45 ± 0.35	
	Thigh	0.50 ± 0.24	0.39 ± 0.29	0.38 ± 0.33		0.56 ± 0.23	0.38 ± 0.33	0.44 ± 0.32	
Slope SR (mg/cm^2/min/˚C) CVC (%/˚C)	Forearm	0.87 ± 0.45	0.48 ± 0.57	0.86 ± 0.38	#[a, b]	703 ± 518	626 ± 350	703 ± 518	#[a, b]
	Chest	1.19 ± 0.61	1.13 ± 0.59	1.05 ± 0.48		533 ± 187	566 ± 271	533 ± 187	

	Thigh	0.53 ± 0.39	0.49 ± 0.30	0.44 ± 0.26	1322 ± 1094	2356 ± 2617	1950 ± 1429

# indicates main effects of location, #[a] indicates differences between thigh and the forearm and # [b] indicates differences between thigh and chest($p<0.05$). $ indicates differences between pre-HA and post-HA ($P > 0.05$). There were no interaction effects (location x condition, $P > 0.05$). Decay data are not included in the statistical analysis and are only presented for exploratory purposes. T_b, mean body temperature; SR, sweat rate; CVC, cutaneous vascular conductance; pre-HA, pre heat acclimation; post-HA, post heat acclimation.


A
38.0
37.5
37.0
36.5
36.0
Baseline T_{re} (˚C)
*
PreHA
PostHA
Decay
B
1.5
1.0
0.5
0.0
ΔT_{re} (°C)
PreHA
PostHA
Decay
C
1500
1000
500
0
GSL (g/m^2/hr)
PreHA
PostHA
Decay


ΔSR thresold for increasing ΔGSC
(mg/cm²/min)
1.0
0.8
0.6
0.4
0.2
0.0
*
Pre-HA
Post-HA
Decay
Forearm
Chest
Thigh