Journal of Thermal Biology 93 (2020) 102697

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: http://www.elsevier.com/locate/jtherbio

Sweat rate and sweat composition during heat acclimation

Lisa Klous, Cornelis De Ruiter, Puck Alkemade, Hein Daanen *, Nicola Gerrett

Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, the Netherlands

ARTICLE INFO

Keywords:
Controlled hyperthermia
Heat acclimation
Lactate
Sodium
Sweat composition
Sweat rate

ABSTRACT

The purpose of this study was to determine local sweat rate (LSR) and sweat composition during heat acclimation (HA). For ten consecutive days of HA, eight participants cycled in 33 °C and 65% relative humidity at an intensity such that a rectal temperature of 38.5 °C was reached within ~40 min, followed by a 60-min clamp of this rectal temperature (i.e., controlled hyperthermia). Four participants extended HA by a 28-day decay period and five consecutive days of heat re-acclimation (HRA) using controlled hyperthermia. Sweat from the upper arm and upper back was collected three times during each heat exposure session. LSR and sweat sodium, chloride, lactate, and potassium concentrations were determined. Relative to HA day 1, LSR was increased at the final day of HA (day 10) (arm: +58%, $P < 0.001$; back: +36%, $P < 0.05$). Concentrations of sodium, chloride, and lactate significantly ($P < 0.05$) decreased to ~60% at HA day 10 compared to day 1 on the arm and back. Potassium concentration did not significantly differ on HA day 10 compared to day 1 (arm: +11%, $P > 0.05$; back: +8%, $P > 0.05$). The induction patterns of the sudomotor adaptations were different. Whilst LSR increased from HA day 8 on the arm and from HA day 7 on the back, sodium and chloride conservation already occurred from HA day 3 on both skin sites. Lastly, the sweat lactate reduction occurred from HA day 6 on the arm and back. Initial evidence is provided that adaptations were partly conserved after decay (28 days) and that a 5-day HRA may be sufficient to restore HA adaptations. In conclusion, ten days of exercise-induced HA using controlled hyperthermia led to increases in LSR and concomitant reductions of sweat sodium, chloride, and lactate concentrations, whilst potassium concentrations remained relatively constant.

1. Introduction

Heat acclimation (HA) enhances thermoregulation as indicated by an increased whole-body sweat loss, plasma volume expansion, and a decreased heart rate and core temperature during rest and exercise in the heat (Periard et al., 2015, 2016). Sweating is an important avenue for heat loss and HA results in numerous sudomotor adaptations; a lower core temperature for the onset of sweating and enhanced sweating sensitivity as indicated by a larger sweat output for a given core temperature (Periard et al., 2015). Most of the thermoregulatory and cardiovascular adaptations occur within 4–7 days of HA and are optimised within 10–14 days (Periard et al., 2015, 2016), but sudomotor adaptations reportedly take the longest to acquire (≥ 8 HA days) (Armstrong and Maresh, 1991). An increased sweat production, elicited by HA, enhances the evaporative power and cooling capacity of the human body. On the other hand, the risk of an elevated sweat production is a concomitant elevation in ion losses. Sodium, chloride, and potassium are the ions that are secreted in the largest quantities in sweat (Robinson and Robinson, 1954). These ions are important for maintaining body fluid balance through their major contribution to the osmotic pressure gradient between intracellular and extracellular fluid (Baker and Wolfe, 2020). The benefit of HA is that eccrine sweat glands adapt by conserving sodium loss when sweat production is increased (Buono et al., 2018; Dill et al., 1938; Sargent et al., 1965). This is likely the result of an improved ability to reabsorb sodium through epithelial sodium channels (ENaC) in the straight reabsorptive duct (Gerrett et al., 2018a, 2018b; Reddy and Quinton, 2003).

Whilst changes in whole-body sweat loss may take up to eight days of HA, reductions in local forearm sweat sodium concentration have been reported to occur after just two days of HA (Buono et al., 2018). These reductions in sodium concentration for a given local sweat rate (LSR) can exceed 50%. Improved ion reabsorption to HA is well documented in the literature but tends to be focused on sodium (Buono et al., 2018) or ions in general (Amano et al., 2017), with limited information about

* Corresponding author. Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Van der Boechorststraat 7-9, 1081 BT, Amsterdam, the Netherlands.
E-mail addresses: l.klous@vu.nl (L. Klous), h.a.m.daanen@vu.nl (H. Daanen).

https://doi.org/10.1016/j.jtherbio.2020.102697
Received 11 May 2020; Received in revised form 6 August 2020; Accepted 10 August 2020
Available online 26 August 2020

other important ions in sweat such as chloride and potassium. Research has shown functional interactions between ENaC and cystic fibrosis transmembrane conductance regulator (CFTR) channels (Reddy and Quinton, 2003), that passively reabsorb chloride. Hence, it is plausible to assume that chloride would be reduced in the presence of an elevated LSR following HA, along with sodium. Sweat potassium concentration is thought to be LSR-independent and potassium is most likely not reabsorbed in the straight duct of the eccrine sweat gland (Robinson and Robinson, 1954; Schwartz and Thaysen, 1956). Therefore, sweat potassium concentration is thought to remain mostly unchanged following HA.

In recent years, sweat lactate has been of interest to engineers and developers of wearable sensors as a potential biomarker of the human body's glycolytic metabolism (Buono et al., 2010; Derbyshire et al., 2012). However, sweat lactate bares no relation with blood lactate derived from the exercising muscles, but rather indicates glycolytic activity of the sweat glands (Derbyshire et al., 2012). Wolfe et al. (1970) suggested that 99% of the sweat glands' metabolism is glycolytic. This was disputed by Kealey (1983) and Sato and Dobson (1973) who suggested that only ~50% of the sweat glands' energy requirements come from the anaerobic glycolysis, with the remaining 50% coming from glucose oxidation. Although the metabolic contribution to sweat gland activity remains unclear, it is plausible to observe an increased sweat lactate production resulting from higher rates of sweat production following HA (Poirier et al., 2016; Ravanelli et al., 2018). However, like most physiological responses to a repeated stimulus, adaptations occur, allowing the organism to function more efficiently. Whether the sweat glands become more efficient during HA remains unknown. Since previous research established an inverse relation between LSR and sweat lactate concentration due to dilution (Buono et al., 2010), a proportionally larger reduction of sweat lactate than the increase in LSR could be indicative of increased efficiency, a larger dependency on the aerobic metabolic pathways or potentially a combination. This adaptation may occur at the same time as changes in sweat rate, reportedly at about eight days of HA, or with improved ion reabsorption at reportedly just two days of HA (Buono et al., 2018).

The main purpose of this study was to determine the daily changes in LSR and sweat sodium, chloride, lactate, and potassium concentrations during ten days of HA. It was hypothesised that HA would increase LSR, thereby conserving sodium and chloride to mitigate disturbances in the fluid balance. Thus, leading to lower sodium and chloride concentrations. Potassium concentration was expected to remain unchanged. Secondly, we hypothesised that lactate would be lower after HA due to improved efficiency of the sweat glands or larger dependency on aerobic metabolic pathways. Additionally, we had the opportunity to re-measure four of our participants during a 5-day controlled hyperthermia heat re-acclimation (HRA) protocol following 28 days of decay. Descriptive data on this sub-group may provide initial insights into the sudomotor adaptations following a decay period and during a short HRA period.

2. Material and methods

2.1. Ethical approval

Procedures were approved by the Ethics Committee of the Faculty of Behavioural and Movement Sciences of the Vrije Universiteit Amsterdam (VCWE 2018-160-R1). The study was conducted in accordance with the guidelines of the revised *Declaration of Helsinki* (2013), except for registration in a database. Written informed consent was obtained from all participants before participation in the study.

2.2. Participants

Six un-acclimatised males (age: 36 ± 9 years, height: 180.0 ± 2.3 cm, weight: 76.9 ± 1.7 kg,VO_{2peak}: 50.4 ± 7.1 mL.kg^{-1}.min^{-1}) and two un-acclimatised females (age: 37 ± 10 years, height: 183.1 ± 7.1 cm, weight: 72.7 ± 5.6 kg, VO_{2peak}: 38.1 ± 1.2 mL.kg^{-1}.min^{-1}) participated in this study. Participants had not been in a warm (> 25 °C air temperature) country in the 3 months prior to the study and were instructed to refrain from alcohol, strenuous exercise, and excessive caffeine consumption and to consume plenty of water during the entire protocol. No restrictions were placed on participants' diets. All participants were non-smokers, did not take any prescription medication (except for one female that completed HA only, taking 70 mg alendronic acid weekly, 500 mg calci-chew daily, 7.5 mg mirtazapine daily), had no history of heat-related illnesses, cardiovascular complications, and did not have any known issues with thermoregulation. Menstrual cycle phase was not controlled for in either of the female participants as this was not feasible considering scheduling each part of the protocol (10-day HA, 28-day decay, 5-day HRA).

2.3. Design

All experiments took place during European winter time (Jan–Apr 2019) to limit acclimatisation through natural exposure to high environmental temperatures. Participants visited the laboratory for ten consecutive HA days. Four of the participants extended HA by a 28-day decay period, and another five consecutive days of HRA. Standardised heat stress tests were performed before and after HA and HRA (Fig. 1). Sweat composition data from these tests are not presented here. During the decay period, participants performed their regular exercise activities, but were not allowed to have heat exposure. Experiments took place in a climate chamber (b-Cat, Tiel, The Netherlands) set to 33 °C and 65% relative humidity, and at the same time of day (± 3 h). Upon arrival to the laboratory, participants provided a urine sample. Urine specific gravity (USG) was measured with a handheld refractometer (PAL-S, Atago, Bellevue, USA) to make sure participants were hydrated (USG ≤ 1.025) (Kenefick and Cheuvront, 2012) at the start of every HA and HRA day. Despite following our hydration guidelines, three participants did not meet the criteria for hydration on one occasion, whilst one participant did not meet the criteria on three occasions. If USG >1.025, participants had to drink 5 mL.kg^{-1} body weight before starting. Prior to the HA and HRA sessions, a rectal temperature (T_{re}) probe (MSR, Seuzach, Switzerland or Yellow Springs Instruments, Ohio, USA) was self-inserted 10 cm past the anal sphincter.

2.4. Experimental trials

During HA and HRA, participants cycled (Lode Excalibur Sport, Groningen, The Netherlands) at an intensity chosen such that a T_{re} of 38.5 °C was reached within approximately 40 min (referred to as 'thermal drive'). As participants adapted during the HA and HRA days, exercise load was increased so that T_{re} still reached 38.5 °C within ~40 min. After that, T_{re} was kept slightly above 38.5 °C for 60 min (referred to as 'thermal maintenance'; i.e., controlled hyperthermia). This was accomplished by the researcher adjusting the exercise load and resting where necessary. Water consumption was allowed *ad libitum* at any time. We chose for controlled hyperthermia rather than a fixed workload protocol, because typically exercise duration and mean intensity are reduced using a controlled hyperthermia protocol with similar final adaptations (Gibson et al., 2015). This reduced work load is of practical benefit since athletes in preparation for an important event in the heat have to acclimatise during their taper period, when a substantial reduction in exercise volume is warranted (Mujika, 1998).

2.5. Measurements

Upper arm and upper back sweat were collected during the first and second 15 min in 'thermal drive' and immediately when T_{re} was 38.5 °C (also for ~15 min), using the absorbent patch technique (Morris et al., 2013; Smith and Havenith, 2011). Absorbent patches were covering

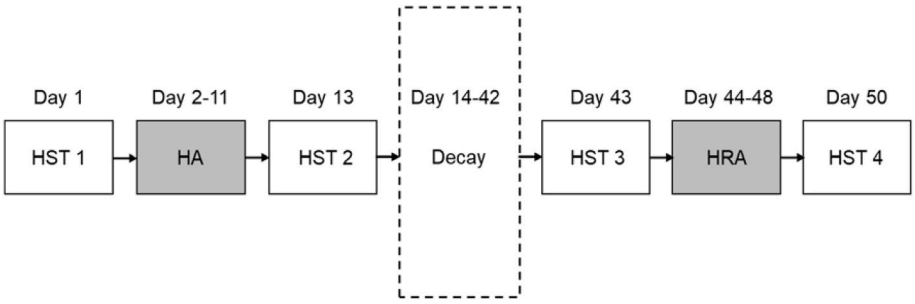

Fig. 1. Schematic overview of the study. The protocol started with a heat stress test (HST), followed by ten consecutive days of heat acclimation (HA), the second HST, a 28-day decay period, the third HST, five consecutive days of heat re-acclimation (HRA), and a final fourth HST. Sweat samples were taken every day during HA ($n = 8$) and HRA ($n = 4$) (grey rectangles).

zones 19 (upper arm) and 13 (upper back) as described by Gerrett et al. (2014). Patches were carefully replaced at the same location by using marks on the skin. The sweat collection period of 15 min was chosen to collect enough sweat for chemical analysis but to prevent saturation of the patch. The sample order was chosen because we expected LSR to increase gradually and in this manner we aimed for three different LSR. Before application, the skin was cleaned with alcohol, deionised water and dried with gauze pads. After collection, the absorbent material was placed in a sealed tube, centrifuged at 1800*g* for 5 min and frozen at −20 °C until analysis. Concentrations of sodium, chloride, and potassium were determined by ion-selective electrodes (Cobas ® 8000 modular analyser, Roche, Almere, The Netherlands). Lactate concentration was analysed enzymatically (Cobas ® LACT2, Roche, Almere, The Netherlands). Limits of detection, coefficients of variation, and sample volumes of the analysers are shown in Table 1. Six patches were analysed for background sodium, chloride, lactate, and potassium concentration and corrections were made accordingly (sodium −7.9 mmol.L^{-1} and chloride −6.6 mmol.L^{-1}). No correction was needed for lactate and potassium. LSR was calculated gravimetrically according to:

$$\mathrm{LSR} = (m_{wet} - m_{dry}) \,/\, \mathrm{t}) \,/\, \mathrm{SA}$$

where m_{wet} refers to mass of the wet patch (mg) after the experiment (Sartorius 1419MP8-1, Sartorius, Göttingen, Germany), m_{dry} to its dry mass (mg), SA to surface area of the covered skin (cm^2), and t to application time (min). LSR is presented in mg.cm^{-2}.min^{-1}. For the participants that completed HRA ($n = 4$), decay was quantified according to:

$$\%\ \mathrm{decay} = \frac{\mathrm{HRA1} - \mathrm{HA10}}{\mathrm{HA1} - \mathrm{HA10}} x\ 100$$

(Pandolf et al., 1977) where HRA1 represents the value of one parameter (LSR or sweat composition) on the first day of HRA, HA10 on the tenth day of HA and HA1 on the first day of HA.

2.6. Statistics

Statistics were reported for HA ($n = 8$), but not for HRA ($n = 4$) considering the small sample size. For the latter, only descriptive data are reported. Generalised Estimating Equations (GEE) modelling (Liang and Zeger, 1993) was used to evaluate LSR and sweat sodium, chloride, lactate, and potassium concentrations over time for the arm and back separately because of large regional differences in sweat rate and composition on different skin sites (Smith and Havenith, 2011, 2012) (IBM SPSS Statistics 26.0). This approach to regression analysis considers measurements within participants (sample order, HA days) as repeated measures and accounts for this dependency. GEE does not assume normally distributed data. An exchangeable correlation structure was used. The independent variables were time (HA day 1−HA day 10) and sample order (first−third). The dependent variables were LSR and sweat sodium, chloride, lactate, and potassium concentration. Interactions of time *x* sample order and LSR, sweat sodium, chloride, lactate, and potassium concentration were explored for significance ($P < 0.05$) and only included in the model when significant.

Table 1
Characteristics of the analysers for sodium, chloride, lactate and potassium concentration in sweat.

	Limit of detection (mmol.L^{-1})	Coefficient of variation (%)	Sample volume (μL)
Sodium	0.4	< 1.0	15
Chloride	0.4	< 2.2	15
Lactate	0.2	< 3.6	2
Potassium	0.2	1.0–1.3	15

3. Results

Eight participants (6 males, 2 females, age: 36 ± 9 years, height: 180.7 ± 4.3 cm, weight: 75.9 ± 3.7 kg, VO_{2peak}: 47.3 ± 8.2 mL.kg^{-1}.min^{-1}) completed ten consecutive days of HA and, as an addition, we had the opportunity to remeasure four of the males (age: 38 ± 6 years, height: 180.9 ± 1.8 cm, weight: 77.0 ± 1.3 kg, VO_{2peak}: 51.8 ± 8.3 mL.kg^{-1}.min^{-1}) during five consecutive days of HRA, after a 28-day decay period. Tables 2 and 3 summarise the daily average (baseline) T_{re} and power output data during each day of the HA protocol ($n = 8$), as well as HA followed by HRA ($n = 4$), respectively.

3.1. LSR

Fig. 2 shows the average LSR on the arm and back during HA for the first, second, and third sample, that respectively were taken during the first and second 15 min of 'thermal drive' and the first 15 min of 'thermal maintenance' (collection period ~15 min). A significant main effect of sample order was found ($P < 0.001$). The parameter estimates revealed that LSR was significantly higher in the second compared to the first sample on the arm ($P < 0.001$) and back ($P = 0.013$). Interactions of time (HA day 1−HA day 10) *x* sample order (first−third) were non-significant ($P \geq 0.279$), indicating that LSR and sweat sodium, chloride, lactate, and potassium concentrations over time were similar for the three samples. Therefore, data of three samples were pooled for further analysis.

3.2. Heat acclimation

There were significant main effects of time on LSR, sweat sodium, chloride, lactate, and potassium concentration ($P < 0.001$, Fig. 3). LSR increased significantly from HA day 8 ($P = 0.004$) on the arm and from HA day 7 ($P = 0.012$) on the back, whilst sweat sodium and chloride concentration were already significantly lower from HA day 3 on the arm and back ($P \leq 0.033$). Sweat lactate concentration decreased from

Table 2
Mean (SD) baseline rectal temperature (T_{re}) (i.e., T_{re} at t = 0), daily time to reach a T_{re} of 38.5 °C (referred to as 'thermal drive'), T_{re} during 'thermal drive', T_{re} during the 60-min T_{re} clamp (at 38.5 °C; referred to as 'thermal maintenance'), power output (PO) during 'thermal drive', PO during 'thermal maintenance', and the amount of rest during 'thermal maintenance' for heat acclimation (HA) (n = 8).

HA (Day)	Baseline T_{re} (°C)	Thermal drive (min)	T_{re} - thermal drive (°C)	T_{re} - thermal maintenance (°C)	PO - thermal drive (W)	PO - thermal maintenance (W)	Rest - thermal maintenance (min)
1	37.4 (0.4)	39 (5)	37.88 (0.43)	38.65 (0.19)	132 (29)	51 (44)	23 (15)
2	37.3 (0.4)	37 (10)	37.75 (0.46)	38.60 (0.25)	125 (34)	55 (48)	21 (12)
3	37.2 (0.4)	37 (5)	37.77 (0.46)	38.59 (0.04)	131 (31)	46 (41)	24 (12)
4	37.3 (0.4)	36 (5)	37.70 (0.49)	38.57 (0.26)	140 (37)	49 (45)	22 (17)
5	37.3 (0.3)	38 (6)	37.79 (0.42)	38.60 (0.13)	136 (38)	41 (45)	21 (17)
6	37.3 (0.4)	41 (8)	37.83 (0.44)	38.57 (0.12)	126 (52)	57 (45)	19 (12)
7	37.2 (0.3)	41 (6)	37.77 (0.47)	38.58 (0.08)	131 (39)	62 (37)	11 (8)
8	37.2 (0.4)	39 (6)	37.74 (0.50)	38.60 (0.11)	134 (37)	59 (40)	13 (7)
9	37.3 (0.3)	37 (5)	37.81 (0.42)	38.61 (0.14)	137 (33)	55 (37)	13 (14)
10	37.2 (0.4)	43 (9)	37.79 (0.49)	38.52 (0.14)	128 (37)	65 (36)	11 (9)

Table 3
Mean (SD) baseline rectal temperature (T_{re}) (i.e., T_{re} at t = 0), daily time to reach a T_{re} of 38.5 °C (referred to as 'thermal drive'), T_{re} during 'thermal drive', T_{re} during the 60-min T_{re} clamp (at 38.5 °C; referred to as 'thermal maintenance'), power output (PO) during 'thermal drive', PO during 'thermal maintenance', and the amount of rest during 'thermal maintenance' for heat acclimation (HA) and heat re-acclimation (HRA) (n = 4).

HA (Day)	Baseline T_{re} (°C)	Thermal drive (min)	T_{re} - thermal drive (°C)	T_{re} - thermal maintenance (°C)	PO - thermal drive (W)	PO - thermal maintenance (W)	Rest - thermal maintenance (min)
1	37.3 (0.5)	41 (6)	37.84 (0.48)	38.78 (0.17)	138 (32)	39 (49)	36 (10)
2	37.2 (0.4)	42 (9)	37.70 (0.51)	38.66 (0.21)	127 (44)	54 (55)	29 (12)
3	37.1 (0.3)	40 (5)	37.69 (0.47)	38.61 (0.12)	139 (30)	42 (45)	30 (8)
4	37.0 (0.3)	37 (6)	37.53 (0.51)	38.62 (0.34)	145 (45)	45 (51)	34 (15)
5	37.1 (0.3)	41 (6)	37.71 (0.45)	38.63 (0.16)	136 (45)	43 (48)	33 (15)
6	37.1 (0.1)	43 (6)	37.74 (0.49)	38.60 (0.12)	119 (66)	65 (50)	25 (11)
7	36.9 (0.1)	43 (6)	37.66 (0.51)	38.60 (0.07)	132 (43)	54 (44)	17 (2)
8	36.9 (0.1)	41 (8)	37.57 (0.52)	38.58 (0.07)	137 (39)	61 (39)	16 (6)
9	37.2 (0.2)	40 (5)	37.72 (0.43)	38.68 (0.16)	140 (32)	46 (36)	22 (15)
10	37.1 (0.3)	43 (4)	37.76 (0.47)	38.59 (0.10)	125 (43)	59 (37)	16 (9)
HRA (Day)							
1	37.0 (0.4)	44 (6)	37.61 (0.50)	38.67 (0.08)	139 (38)	41 (42)	26 (11)
2	36.9 (0.4)	43 (6)	37.56 (0.55)	38.58 (0.11)	141 (32)	60 (44)	18 (5)
3	36.9 (0.2)	42 (6)	37.64 (0.53)	38.68 (0.22)	143 (35)	48 (43)	22 (17)
4	37.2 (0.5)	45 (9)	37.69 (0.50)	38.66 (0.14)	138 (35)	45 (41)	24 (18)
5	36.9 (0.2)	47 (6)	37.51 (0.54)	38.66 (0.15)	135 (44)	48 (46)	24 (13)

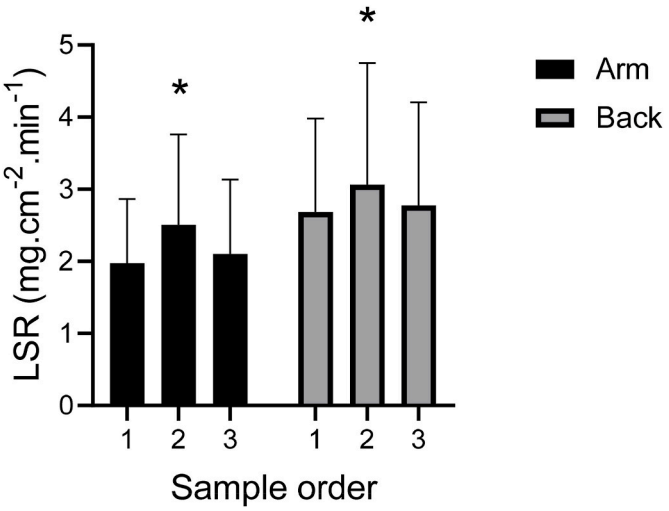

Fig. 2. Mean (SD) upper arm (black bars) and upper back (grey bars) local sweat rate (LSR) during the first, second and third sample for all heat acclimation sessions (n = 8). * denote significant differences from the first sample. LSR on the arm was overall lower compared to the back (P = 0.007).

HA day 6 on the arm and back (P ≤ 0.046). Lastly, sweat potassium concentration significantly increased on HA day 3–6 on the arm (P ≤ 0.028) and HA day 3–7 on the back (P < 0.001).

3.3. Decay and heat re-acclimation

Decay was 39% and 19% for LSR on the arm and back, respectively (Fig. 4). Sweat sodium, chloride, lactate and potassium decay was 69%, 72%, 73%, and 53% on the arm and 75%, 78%, 62%, and 100% on the back. During HRA, LSR was elevated compared to HA day 1 (Fig. 4). As with HA, sweat sodium, chloride and lactate gradually decreased during HRA and changes in sweat potassium concentrations were limited (Fig. 4).

4. Discussion

This is, to our knowledge, the first study to assess the daily changes in LSR and sweat sodium, chloride, lactate, and potassium concentrations on two skin sites during HA using controlled hyperthermia. Exercise-induced HA limited excretion of sweat components in the presence of an elevated LSR from the eccrine sweat glands on the arm and back as shown by the considerable decreases in sweat sodium, chloride, and lactate concentrations. The induction patterns of these sudomotor adaptations were not similar for each of the sweat components. Whilst LSR was increased from HA day 8 on the arm and HA day 7 on the back, sweat sodium and chloride conservation already occurred from HA day 3 on both skin sites. Lastly, sweat lactate reduction occurred from HA day 6 on the arm and back. The first hypothesis that HA conserves components in sweat is confirmed for sodium and chloride, and as expected this was not the case not for potassium. Following HA, sweat lactate concentration is lowered, confirming hypothesis two. We have also provided some initial evidence that considerable parts of the

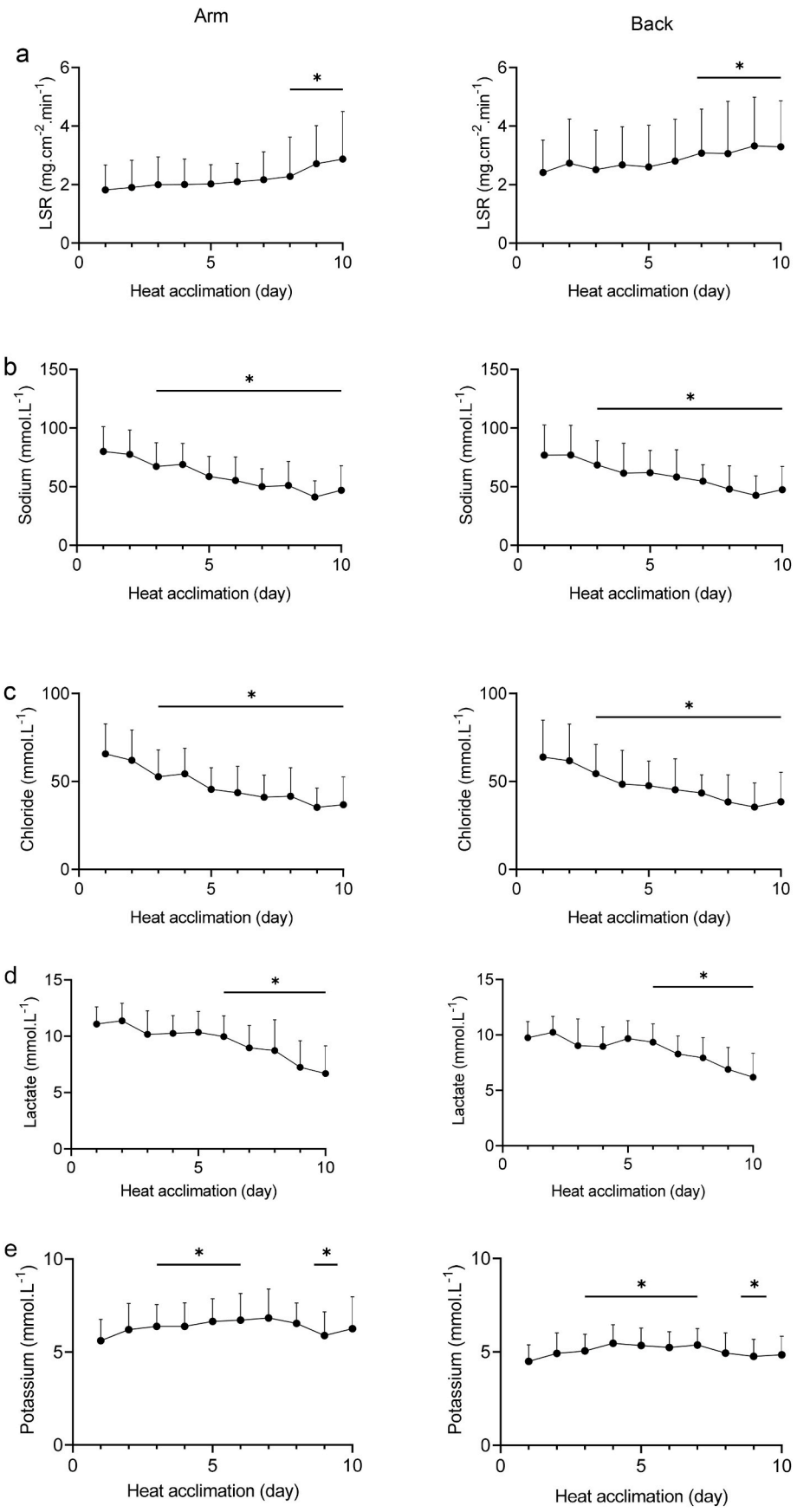

Fig. 3. Mean (SD) of (**a**) local sweat rate (LSR), (**b**) sweat sodium, (**c**) chloride, (**d**) lactate, and (**e**) potassium concentrations for the arm (left panels) and back (right panels) during ten consecutive days of heat acclimation ($n = 8$). Data includes three samples (first to third sample during each heat exposure). * denote significant differences ($P < 0.05$) relative to heat acclimation day 1.

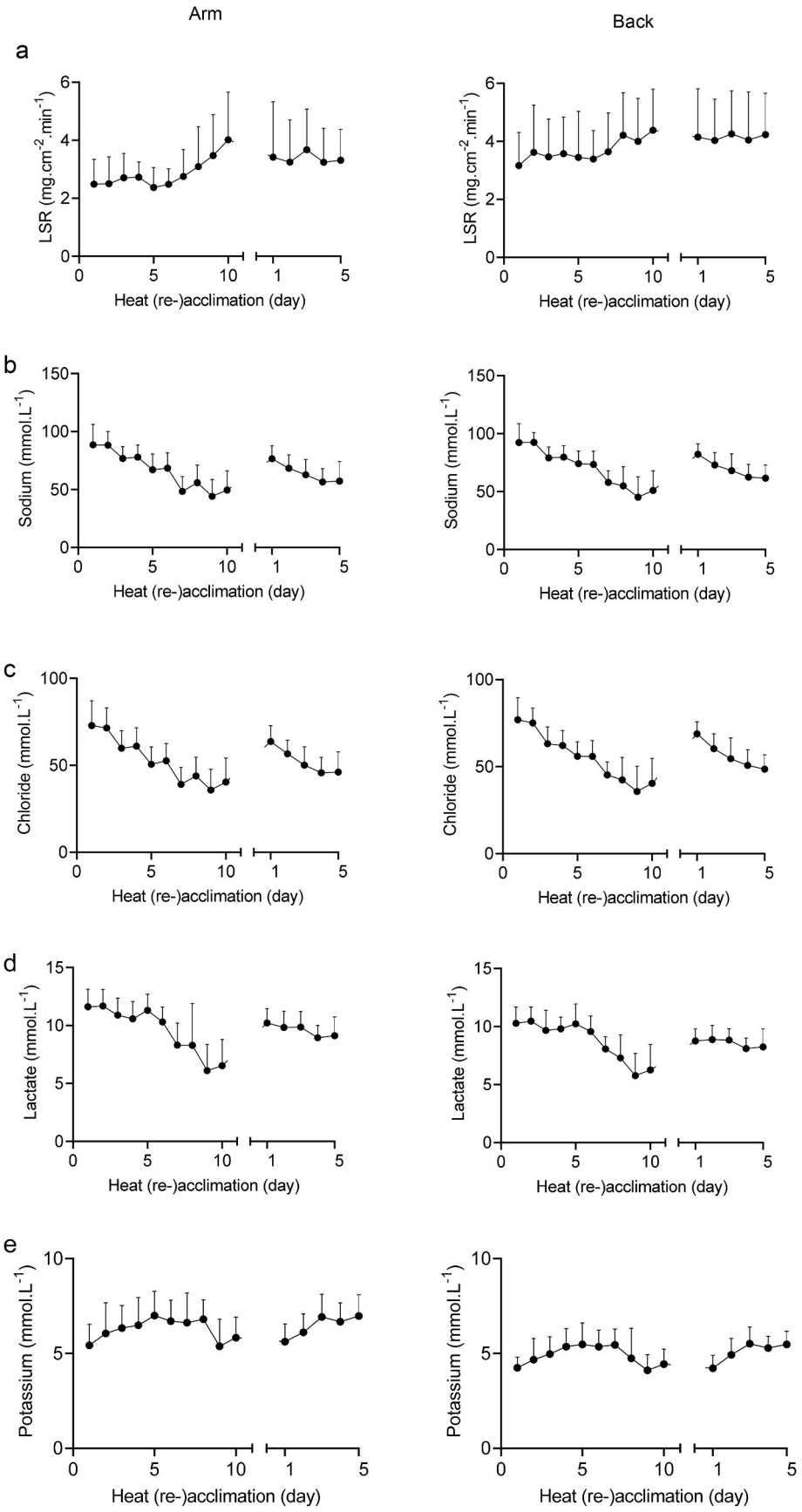

Fig. 4. Mean (SD) of (**a**) local sweat rate (LSR), (**b**) sweat sodium, (**c**) chloride, (**d**) lactate, and (**e**) potassium concentrations for the arm (left panels) and back (right panels) during ten consecutive days of heat acclimation and five consecutive days of heat re-acclimation ($n = 4$). Data includes three samples (first to third sample during each heat exposure). Statistics were not reported due to the small *n*.

sudomotor adaptations were conserved after 28 days of decay (~70%) and that HRA of five days was sufficient to restore HA adaptations.

4.1. Heat acclimation

The three sweat samples during each heat exposure (first and second 15 min of 'thermal drive' and first 15 min of 'thermal maintenance') were taken with the expectation that LSR would gradually increase due to the increase in T_{re} (Nadel et al., 1971). In fact, LSR was lower in the third compared to the second sample on both the arm and back in the presence of a higher T_{re} (Fig. 2, Table 2). Due to the ~two-fold lower power output in 'thermal maintenance' compared to 'thermal drive', metabolic rate was lower in 'thermal maintenance'. As per the heat balance theory, the lower metabolic rate causes a lower required evaporation rate in 'thermal maintenance' (Cramer and Jay, 2016), which occurred in the presence of a higher absolute T_{re}. Even though sweat rate *per se* is not equal to required evaporation, both are closely related, explaining our findings.

In the present study, LSR was rather high compared to previous reported LSR (Buono et al., 2018; Neal et al., 2016; Poirier et al., 2016), potentially because of the high relative humidity (65%). When examining this from a biophysical control perspective, in a humid environment the water vapour pressure gradient is low, which limits the ability to evaporate sweat. The limited ability to evaporate sweat causes core temperature to increase. To still meet the cooling demands, there is a greater need for a higher sweat excretion rate than what is calculated from required evaporation (Cramer and Jay, 2016; Gagnon et al., 2013; Ravanelli et al., 2020). In addition, sweating in a humid environment is not 100% efficient as some sweat drips from the body, which further limits evaporation and increases sweat excretion rate (Baker, 2017).

LSR was found to increase from HA day 8 on the arm and from HA day 7 on the back (Fig. 3). This is in accordance with research on fixed workload HA protocols, showing that whole-body sudomotor adaptations take a relatively long time (~8 HA days) to acquire (Periard et al., 2015). Research into controlled hyperthermia showed earlier sudomotor adaptations, with whole-body sweat rate increasing from HA day 4 (Neal et al., 2016). In the study by Neal et al. (2016), LSR on the upper back did not significantly increase following eight days of HA. Controlled hyperthermia may therefore elicit a more rapid whole-body sweat rate response compared to a fixed workload protocol, but LSR elevations on the upper arm and upper back do not adhere to this pattern. The discrepancy between whole-body and local sudomotor adaptations is a well-known phenomenon and is probably due to regional differences in sweating (Poirier et al., 2016).

Initial sweat sodium and chloride concentrations were rather high in the present study (Fig. 3), presumably caused by the high LSR (Buono et al., 2008), but are within the range of previous reported values (Baker, 2019). Following the HA protocol, sweat sodium concentration decreased from HA day 3 in the presence of an elevated LSR (Fig. 3), indicating improved ability to reabsorb sodium by the eccrine sweat glands. This result is in agreement with a recent study, in which a linear decrease in sweat sodium concentration during a 7-day HA protocol was observed (Buono et al., 2018).

For chloride, we observed a comparable pattern during the 10-day HA protocol as for sodium (Fig. 3). Indeed, Reddy et al. (2003) observed a functional interaction between ENaC and CFTR, which play an important role in sodium and chloride reabsorption in the straight duct, respectively. This functional interaction may account for the similar pattern of sweat sodium and chloride during HA found in the present study, mitigating disturbances in the body's fluid balance. From all adaptations studied here, sodium and chloride show the most rapid response, occurring from HA day 3. Exercise-induced elevations in plasma aldosterone levels were previously associated with improved reabsorption rates of sodium in the eccrine sweat glands (Collins, 1966; Grand et al., 1967). The reduced sweat sodium concentrations to high plasma aldosterone levels were already observed after 24 h with reductions still present after five days. The conservation response to aldosterone most likely explains the rapid and continuous decrease of sodium in final sweat observed here. Since sodium transport by ENaC requires CFTR activation (Reddy and Quinton, 2003), the conservation response to aldosterone may explain the concomitant and rapid chloride decrease as well.

In the present study, a decrease in sweat lactate concentration was found after HA day 6 on both measurement locations (Fig. 3). Metabolically, an elevated lactate concentration could be expected because LSR was higher and lactate in sweat is a metabolic waste product of the eccrine sweat gland (Derbyshire et al., 2012). However, the inverse relation between LSR and sweat lactate concentration due to dilution is well-established (Buono et al., 2010). Therefore, Buono et al. (2018) investigated the lactate excretion rate (LSR *x* sweat lactate concentration) and reported that the lactate excretion rate increased at higher LSR. Such a finding suggests that an increased sweat production is associated with a higher glycolytic rate in the sweat gland. In the present study, a decrease in lactate concentration in the presence of an increased LSR was found. The increase from HA day 1 to HA day 10 in LSR (arm: +58%, back: +36%) was equal or larger compared to the lactate reduction (arm: −40%, back: −37%), indicating that the lactate excretion rate is not reduced. Therefore, our findings cannot exclude that the lactate reduction with the progression of HA is attributed to dilution, confirm a lower glycolytic energy production for a certain LSR (i.e., improved efficiency), or confirm more dependency on aerobic energy production. The reduction in sweat lactate concentration occurred from HA day 6, coinciding most closely with adaptations in LSR (Fig. 3). This suggests that the adaptations in sweat excretion rate and lactate concentration may relate and potentially implies that lactate enters sweat in the secretory coil of the gland, where precursor sweat is produced, rather than in the reabsorptive duct where sodium and chloride are reabsorbed. Future research should investigate the source of lactate production.

Potassium concentrations increased during several consecutive HA days (Fig. 3). The short increase in sweat potassium concentration midway the HA period may relate to the simultaneous decrease in sweat sodium concentration (Robinson and Robinson, 1954), which were both associated with increased adrenal cortical activity during thermal stress. Another explanation could be that the increased LSR during HA potentially caused accumulation of potassium in the absorbent patches, which may account for the (spurious) increases in sweat potassium concentrations.

4.2. Heat re-acclimation

The 28-day decay period in the present study was selected to allow for complete, or at least a substantial, loss of the adaptations that were gained during HA (Weller et al., 2007). Quantitively, LSR decay was 39% and 19% on the arm and back, respectively. Decay on both the arm and back was ~70% for concentrations of sodium, chloride, and lactate. Relative to HA day 1, LSR was elevated and concentrations of components gradually decreased during HRA (Fig. 4). There is limited research on sudomotor adaptations during decay and HRA, but a consistent finding with whole-body sudomotor research that does exist (Ashley et al., 2015; Henschel, 1943; Pandolf et al., 1977; Saat et al., 2005; Stephens and Hoag, 1981; Weller et al., 2007; Wyndham and Jacobs, 1957) points to a shorter HRA program compared to HA with similar final adaptations in phenotype. Indeed, final HRA adaptations appear to approach final HA adaptations here (Fig. 4), but future adequately-powered research on HRA should confirm this.

4.3. Limitations

In the present study, a small sample size was included during HRA ($n = 4$). Please note that similar induction patterns of the adaptations over time were observed for all individuals so we feel confident that the

findings from these four participants are representative for the others. Nevertheless, future research should include more participants.

Another potential limitation is that we did not control for menstrual cycle phase of our two female participants. It is known that core temperature is regulated ~0.4 °C higher in the luteal phase of the menstrual cycle and that simultaneously the core temperature threshold for sweating and vasodilation is increased (Lei et al., 2017). Whilst sweat sodium, chloride and potassium were reported to be unaffected by menstrual cycle phase, it potentially affected our LSR results (Wells and Horvath, 1974). However, logistically it is not feasible to have females in the same phase of their menstrual cycle when utilising a consecutive 10-day HA, 28-day decay and 5-day HRA protocol.

It should be considered that we included middle-aged individuals (≤ 45 years) since aging is thought to affect sweating. However, previous research found that reduced whole-body and LSR were mostly associated with a decline in fitness level and HA status rather than aging *per se* (Inbar et al., 2004; Inoue, 1996; Kenney and Fowler, 1988). The aforementioned studies also include old (> 60 years) rather than middle-aged participants. Since our participants had a relatively high fitness level, were all classified into the same performance level (i.e., recreationally trained) (De Pauw et al., 2013; Decroix et al., 2016), and we only recruited un-acclimatised individuals, aging is not expected to significantly affect our LSR results. Limited data on aging and its effects on sweat composition is available, but one study found no differences in sweat sodium concentration as a function of age (Inoue et al., 1999). The extent to which inclusion of middle-aged individuals affected our sweat composition outcomes remains unknown.

Another factor that potentially affected our results is that we allowed *ad libitum* drinking. If participants were not allowed to drink during the heat exposures, this might have caused more dehydration, which probably would have had unwanted effects on LSR and sweat composition (Montain et al., 1995; Morgan et al., 2004).

5. Conclusions

Exercise-induced heat acclimation by controlled hyperthermia conserved excretion of components from the eccrine sweat glands. The reduction of sodium and chloride concentration occurred from the third day of heat acclimation, whilst lactate concentration was lower from heat acclimation day 6. During heat acclimation, potassium concentrations remained relatively constant. The ability to reabsorb sodium and chloride was presumably improved, whereas sweat lactate may be lower because of dilution.

Author statement

Lisa Klous: Conceptualisation, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing-Original Draft, Writing-Review and Editing, Visualisation. **Cornelis de Ruiter**: Formal analysis, Supervision, Writing-Review. **Puck Alkemade**: Conceptualisation, Methodology, Investigation, Resources, Data Curation, Writing-Review. **Hein Daanen**: Supervision, Writing-Review. **Nicola Gerrett**: Conceptualisation, Methodology, Investigation, Resources, Supervision, Writing-Review.

Funding

This work was supported by Eurostars [grant number: ESTAR 17213]; NWO [grant number: P16-28]; and ZonMw [Thermo Tokyo Project #546001003].

Declaration of competing interest

None.

Acknowledgements

The authors would like to thank all students and participants who dedicated their time to take part in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102697.

References

Amano, T., Hirose, M., Konishi, K., Gerrett, N., Ueda, H., Kondo, N., Inoue, Y., 2017. Maximum rate of sweat ions reabsorption during exercise with regional differences, sex, and exercise training. Eur. J. Appl. Physiol. 117, 1317–1327. https://doi.org/10.1007/s00421-017-3619-8.

Armstrong, L.E., Maresh, C.M., 1991. The induction and decay of heat acclimatisation in trained athletes. Sports Med. 12, 302–312. https://doi.org/10.2165/00007256-199112050-00003.

Ashley, C.D., Ferron, J., Bernard, T.E., 2015. Loss of heat acclimation and time to re-establish acclimation. J. Occup. Environ. Hyg. 12, 302–308. https://doi.org/10.1080/15459624.2014.987387.

Baker, L.B., 2017. Sweating rate and sweat sodium concentration in athletes: a review of methodology and intra/interindividual variability. Sports Med. 47, 111–128. https://doi.org/10.1007/s40279-017-0691-5.

Baker, L.B., 2019. Physiology of sweat gland function: the roles of sweating and sweat composition in human health. Temperature 6, 211–259. https://doi.org/10.1080/23328940.2019.1632145.

Baker, L.B., Wolfe, A.S., 2020. Physiological mechanisms determining eccrine sweat composition. Eur. J. Appl. Physiol. 120, 719–752. https://doi.org/10.1007/s00421-020-04323-7.

Buono, M.J., Claros, R., Deboer, T., Wong, J., 2008. Na+ secretion rate increases proportionally more than the Na+ reabsorption rate with increases in sweat rate. J. Appl. Physiol. 105, 1044–1048. https://doi.org/10.1152/japplphysiol.90503.2008.

Buono, M.J., Kolding, M., Leslie, E., Moreno, D., Norwood, S., Ordille, A., Weller, R., 2018. Heat acclimation causes a linear decrease in sweat sodium ion concentration. J. Therm. Biol. 71, 237–240. https://doi.org/10.1016/j.jtherbio.2017.12.001.

Buono, M.J., Lee, N.V., Miller, P.W., 2010. The relationship between exercise intensity and the sweat lactate excretion rate. J. Physiol. Sci. 60, 103–107. https://doi.org/10.1007/s12576-009-0073-3.

Collins, K.J., 1966. The action of exogenous aldosterone on the secretion and composition of drug-induced sweat. Clin. Sci. 30, 207–221.

Cramer, M.N., Jay, O., 2016. Biophysical aspects of human thermoregulation during heat stress. Auton. Neurosci. 196, 3–13. https://doi.org/10.1016/j.autneu.2016.03.001.

De Pauw, K., Roelands, B., Cheung, S.S., de Geus, B., Rietjens, G., Meeusen, R., 2013. Guidelines to classify subject groups in sport-science research. Int. J. Sports Physiol. Perform. 8, 111–122. https://doi.org/10.1123/ijspp.8.2.111.

Decroix, L., De Pauw, K., Foster, C., Meeusen, R., 2016. Guidelines to classify female subject groups in sport-science research. Int. J. Sports Physiol. Perform. 11, 204–213. https://doi.org/10.1123/ijspp.2015-0153.

Derbyshire, P.J., Barr, H., Davis, F., Higson, S.P., 2012. Lactate in human sweat: a critical review of research to the present day. J. Physiol. Sci. 62, 429–440. https://doi.org/10.1007/s12576-012-0213-z.

Dill, D., Hall, F., Edwards, H., 1938. Changes in composition of sweat during acclimatization to heat. Am. J. Physiol. Legacy Content 123, 412–419. https://doi.org/10.1152/ajplegacy.1938.123.2.412.

Gagnon, D., Jay, O., Kenny, G.P., 2013. The evaporative requirement for heat balance determines whole-body sweat rate during exercise under conditions permitting full evaporation. J. Physiol. 591, 2925–2935. https://doi.org/10.1113/jphysiol.2012.248823.

Gerrett, N., Amano, T., Inoue, Y., Havenith, G., Kondo, N., 2018a. The effects of exercise and passive heating on the sweat glands ion reabsorption rates. Phys. Rep. 6 https://doi.org/10.14814/phy2.13619.

Gerrett, N., Griggs, K., Redortier, B., Voelcker, T., Kondo, N., Havenith, G., 2018b. Sweat from gland to skin surface: production, transport, and skin absorption. J. Appl. Physiol. 125, 459–469. https://doi.org/10.1152/japplphysiol.00872.2017.

Gerrett, N., Ouzzahra, Y., Coleby, S., Hobbs, S., Redortier, B., Voelcker, T., Havenith, G., 2014. Thermal sensitivity to warmth during rest and exercise: a sex comparison. Eur. J. Appl. Physiol. 114, 1451–1462. https://doi.org/10.1007/s00421-014-2875-0.

Gibson, O.R., Mee, J.A., Taylor, L., Tuttle, J.A., Watt, P.W., Maxwell, N.S., 2015. Isothermic and fixed-intensity heat acclimation methods elicit equal increases in Hsp72 mRNA. Scand. J. Med. Sci. Sports 25 (Suppl. 1), 259–268. https://doi.org/10.1111/sms.12430.

Grand, R.J., Di Sant'Agnese, P.A., Talamo, R.C., Pallavicini, J.C., 1967. The effects of exogenous aldosterone on sweat electrolytes. II. Patients with cystic fibrosis of the pancreas. J. Pediatr. 70, 357–368. https://doi.org/10.1016/s0022-3476(67)80132-x.

Henschel, A., Longstreet Taylor, H., Keys, A., 1943. The persistence of heat acclimatization in man. Am. J. Physiol. 140 https://doi.org/10.1152/ajplegacy.1943.140.3.321.

Inbar, O., Morris, N., Epstein, Y., Gass, G., 2004. Comparison of thermoregulatory responses to exercise in dry heat among prepubertal boys, young adults and older males. Exp. Physiol. 89, 691–700. https://doi.org/10.1113/expphysiol.2004.027979.
Inoue, Y., 1996. Longitudinal effects of age on heat-activated sweat gland density and output in healthy active older men. Eur. J. Appl. Physiol. Occup. Physiol. 74, 72–77. https://doi.org/10.1007/BF00376497.
Inoue, Y., Havenith, G., Kenney, W.L., Loomis, J.L., Buskirk, E.R., 1999. Exercise- and methylcholine-induced sweating responses in older and younger men: effect of heat acclimation and aerobic fitness. Int. J. Biometeorol. 42, 210–216. https://doi.org/10.1007/s004840050107.
Kealey, T., 1983. The metabolism and hormonal responses of human eccrine sweat glands isolated by collagenase digestion. Biochem. J. 212, 143–148. https://doi.org/10.1042/bj2120143.
Kenefick, R.W., Cheuvront, S.N., 2012. Hydration for recreational sport and physical activity. Nutr. Rev. 70 (Suppl. 2), S137–S142. https://doi.org/10.1111/j.1753-4887.2012.00523.x.
Kenney, W.L., Fowler, S.R., 1988. Methylcholine-activated eccrine sweat gland density and output as a function of age. J. Appl. Physiol. 65, 1082–1086. https://doi.org/10.1152/jappl.1988.65.3.1082, 1985.
Lei, T.H., Stannard, S.R., Perry, B.G., Schlader, Z.J., Cotter, J.D., Mundel, T., 2017. Influence of menstrual phase and arid vs. humid heat stress on autonomic and behavioural thermoregulation during exercise in trained but unacclimated women. J. Physiol. 595, 2823–2837. https://doi.org/10.1113/JP273176.
Liang, K.Y., Zeger, S.L., 1993. Regression analysis for correlated data. Annu. Rev. Publ. Health 14, 43–68. https://doi.org/10.1146/annurev.pu.14.050193.000355.
Montain, S.J., Latzka, W.A., Sawka, M.N., 1995. Control of thermoregulatory sweating is altered by hydration level and exercise intensity. J. Appl. Physiol. 79, 1434–1439. https://doi.org/10.1152/jappl.1995.79.5.1434.
Morgan, R.M., Patterson, M.J., Nimmo, M.A., 2004. Acute effects of dehydration on sweat composition in men during prolonged exercise in the heat. Acta Physiol. Scand. 182, 37–43. https://doi.org/10.1111/j.1365-201X.2004.01305.x.
Morris, N.B., Cramer, M.N., Hodder, S.G., Havenith, G., Jay, O., 2013. A comparison between the technical absorbent and ventilated capsule methods for measuring local sweat rate. J. Appl. Physiol. 114, 816–823. https://doi.org/10.1152/japplphysiol.01088.2012.
Mujika, I., 1998. The influence of training characteristics and tapering on the adaptation in highly trained individuals: a review. Int. J. Sports Med. 19, 439–446. https://doi.org/10.1055/s-2007-971942.
Nadel, E.R., Mitchell, J.W., Saltin, B., Stolwijk, J.A., 1971. Peripheral modifications to the central drive for sweating. J. Appl. Physiol. 31, 828–833. https://doi.org/10.1152/jappl.1971.31.6.828.
Neal, R.A., Massey, H.C., Tipton, M.J., Young, J.S., Corbett, J., 2016. Effect of permissive dehydration on induction and decay of heat acclimation, and temperate exercise performance. Front. Physiol. 7, 564. https://doi.org/10.3389/fphys.2016.00564.
Pandolf, K.B., Burse, R.L., Goldman, R.F., 1977. Role of physical fitness in heat acclimatisation, decay and reinduction. Ergonomics 20, 399–408. https://doi.org/10.1080/00140137708931642.
Periard, J.D., Racinais, S., Sawka, M.N., 2015. Adaptations and mechanisms of human heat acclimation: applications for competitive athletes and sports. Scand. J. Med. Sci. Sports 25 (Suppl. 1), 20–38. https://doi.org/10.1111/sms.12408.
Periard, J.D., Travers, G.J.S., Racinais, S., Sawka, M.N., 2016. Cardiovascular adaptations supporting human exercise-heat acclimation. Auton. Neurosci. 196, 52–62. https://doi.org/10.1016/j.autneu.2016.02.002.
Poirier, M.P., Gagnon, D., Kenny, G.P., 2016. Local versus whole-body sweating adaptations following 14 days of traditional heat acclimation. Appl. Physiol. Nutr. Metabol. 41, 816–824. https://doi.org/10.1139/apnm-2015-0698.
Ravanelli, N., Coombs, G.B., Imbeault, P., Jay, O., 2018. Maximum skin wettedness after aerobic training with and without heat acclimation. Med. Sci. Sports Exerc. 50, 299–307. https://doi.org/10.1249/MSS.0000000000001439.
Ravanelli, N., Imbeault, P., Jay, O., 2020. Steady-state sweating during exercise is determined by the evaporative requirement for heat balance independently of absolute core and skin temperatures. J. Physiol. https://doi.org/10.1113/JP279447.
Reddy, M.M., Quinton, P.M., 2003. Functional interaction of CFTR and ENaC in sweat glands. Pflügers Archiv 445, 499–503. https://doi.org/10.1007/s00424-002-0959-x.
Robinson, S., Robinson, A.H., 1954. Chemical composition of sweat. Physiol. Rev. 34, 202–220. https://doi.org/10.1152/physrev.1954.34.2.202.
Saat, M., Sirisinghe, R.G., Singh, R., Tochihara, Y., 2005. Decay of heat acclimation during exercise in cold and exposure to cold environment. Eur. J. Appl. Physiol. 95, 313–320. https://doi.org/10.1007/s00421-005-0012-9.
Sargent 2nd, F., Smith, C.R., Batterton, D.L., 1965. Eccrine sweat gland activity in heat acclimation. Int. J. Biometeorol. 9, 229–231. https://doi.org/10.1007/bf02219954.
Sato, K., Dobson, R.L., 1973. Glucose metabolism of the isolated eccrine sweat gland. II. The relation between glucose metabolism and sodium transport. J. Clin. Invest. 52, 2166–2174. https://doi.org/10.1172/JCI107401.
Schwartz, I.L., Thaysen, J.H., 1956. Excretion of sodium and potassium in human sweat. J. Clin. Invest. 35, 114–120. https://doi.org/10.1172/JCI103245.
Smith, C.J., Havenith, G., 2011. Body mapping of sweating patterns in male athletes in mild exercise-induced hyperthermia. Eur. J. Appl. Physiol. 111, 1391–1404. https://doi.org/10.1007/s00421-010-1744-8.
Smith, C.J., Havenith, G., 2012. Body mapping of sweating patterns in athletes: a sex comparison. Med. Sci. Sports Exerc. 44, 2350–2361. https://doi.org/10.1249/MSS.0b013e318267b0c4.
Stephens, R.L., Hoag, L.L., 1981. Heat acclimatization, its decay and reinduction in young Caucasian females. Am. Ind. Hyg. Assoc. J. 42, 12–17. https://doi.org/10.1080/15298668191419280.
Weller, A.S., Linnane, D.M., Jonkman, A.G., Daanen, H.A., 2007. Quantification of the decay and re-induction of heat acclimation in dry-heat following 12 and 26 days without exposure to heat stress. Eur. J. Appl. Physiol. 102, 57–66. https://doi.org/10.1007/s00421-007-0563-z.
Wells, C.L., Horvath, S.M., 1974. Responses to exercise in a hot environment as related to the menstrual cycle. J. Appl. Physiol. 36, 299–302. https://doi.org/10.1152/jappl.1974.36.3.299.
Wolfe, S., Cage, G., Epstein, M., Tice, L., Miller, H., Gordon Jr., R.S., 1970. Metabolic studies of isolated human eccrine sweat glands. J. Clin. Invest. 49, 1880–1884. https://doi.org/10.1172/JCI106407.
Wyndham, C.H., Jacobs, G.E., 1957. Loss of acclimatization after six days of work in cool conditions on the surface of a mine. J. Appl. Physiol. 11, 197–198. https://doi.org/10.1152/jappl.1957.11.2.197.

Lisa Klous. Lisa is a PhD candidate in the field of thermal physiology with emphasis on the process of sweating. Within the XPATCH consortium the main goal is to develop and validate a wearable sweat sensor. She completed her master's degree at the Vrije Universiteit Amsterdam.

Cornelis J. de Ruiter. Cornelis has lots of research experience in the field of muscle physiology. He is a graduate of the Vrije Universiteit Amsterdam. Cornelis is a former Decathlon contestant and has coached national athletes for many years. He now works as a researcher and lecturer at the Vrije Universiteit Amsterdam.

Puck Alkemade. Puck is a PhD candidate in the field of human thermal physiology, investigating thermal strain reduction in (adapted) sports at the Vrije Universiteit Amsterdam. Puck completed her master's degree at the Vrije Universiteit Amsterdam and received part of her training at the Sports Performance Research Institute New Zealand (SPRINZ).

Hein A.M. Daanen. Hein has over thirty years of research experience in human thermal physiology. He is a graduate of the Vrije Universiteit Amsterdam. Hein was a principal scientist at TNO and professor at the Amsterdam Fashion Institute. He now is a full professor at the Vrije Universiteit Amsterdam.

Nicola M. Gerrett. Nicola is a post-doctoral researcher at the Vrije Universiteit Amsterdam. She has previously completed a JSPS fellowship at Kobe University (Japan) investigating ion reabsorption rates of eccrine sweat glands. Her research is predominately focused on physiological and perceptual adaptations to heat stress.