Title:

Short-term heat acclimation is effective and may be enhanced rather than impaired by dehydration

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Abstract:

Most heat acclimation data are from regimes of longer than one-week duration, and acclimation advice is to prevent dehydration. **Objectives:** We hypothesised that (i) short-term (5-day) heat acclimation would substantially improve physiological strain and exercise tolerance under heat stress, and, of particular interest, (ii) dehydration would provide a thermally-independent stimulus for adaptation. **Methods:** Nine aerobically fit males were heat acclimated using controlled hyperthermia (rectal temperature 38.5°C) for 90 min on five days, on two occasions separated by a five-week washout, in a cross-over design; once euhydrated (EUH) and once dehydrated (DEH) during acclimation bouts. Exercising heat stress tests were completed 1-wk pre and 2-d post acclimation (90-min cycling in T_a 35°C and 60% RH). **Results:** During acclimation bouts, $[aldosterone]_{plasma}$ rose more across DEH than EUH (95%CI for difference: 40 to 411 $pg.mL^{-1}$; *P*=0.03; n=5) and was positively related to plasma volume expansion (r=0.65; *P*=0.05), which tended to be larger in DEH (CI for difference: -1 to 10%; *P*=0.06). In heat stress tests, resting forearm perfusion increased more in DEH (by 5.9 ml 100 Tissue ml^{-1} min^{-1}: -11.5 to -1.0; *P*=0.04), and end-exercise cardiac frequency fell to greater extent (by 11 $b \cdot min^{-1}$: -1 to 22; *P*=0.05). Hydration-related effects on other measured endocrine, cardiovascular and psychophysical responses were unclear. **Conclusions:** Short-term (5-day) heat acclimation induced effective adaptations, some of which were more pronounced after fluid-regulatory strain from permissive dehydration, and unattributable to dehydration effects on body temperature.

Key words: short-term, hypohydration, dehydration, fluid regulation, plasma volume

Introduction

Heat acclimation and acclimatisation confer several thermoregulatory, cardiovascular and neuroendocrine adaptations. These collectively reduce physiological and perceived strain during exertion, and improve functional capacities in both benign (Lorenzo and others 2010; Scoon and others 2007) and heat stressful (Gisolfi and Wenger 1984; Sawka and others 1996) environments. The adaptive effects of medium to long-term heat acclimation (>8-12 d) have received much research attention (Garrett and others 2011). However, many of the important adaptations to heat stress are cardiovascular, and occur relatively rapidly (i.e. nearly complete within 7 d (Patterson and others 2004), and the period available for heat acclimation may often be less than 7 d for sporting, occupational or domestic exposures to heat stress. Therefore, the extent of adaptations from short-term heat acclimation requires more investigation, and was one focus of this study.

Guidelines for heat acclimation typically advocate good hydration during heat exposure (Bergeron and others 2012). However, the reality for many undergoing acclimation is that some dehydration occurs (Greenleaf 1992; Noakes and others 1988). It is also conceivable that dehydration during conditioning bouts may even facilitate some adaptations (Merry and others 2010), hence, the focus of this study. Dehydration increases the fluid-regulatory, cardiovascular and thermal strain, thus generating higher tissue temperatures, more hypovolaemia, and larger responses of the fluid- and stress-regulatory hormones aldosterone, AVP and cortisol (Kenefick and others 2007), as well as thirst (Maresh and others 2004; Sawka and others 1987). One main feature of adaptation to heat stress and exercise is an expansion of plasma volume (PV), which is

mediated through sodium retention and increased intra-vascular albumin concentration (Patterson and others, 2004). This expansion in plasma volume appears to underlie other beneficial physiological and functional outcomes (Lorenzo and others 2010; Racinais and others 2012; Scoon and others 2007). Therefore, we sought to examine if permissive dehydration during exercise and heat acclimation might enhance adaptation independently of effects on body temperature, by PV expansion.

Another important feature of the adaptive response to heat is improved cellular protection from heat stress. The heat shock protein response (HSR) is mediated primarily by heat shock proteins (HSP), with those of moderate molecular weight (HSP70) playing a major role in cytoprotection (Gabai and Sherman 2002; Horowitz 2002; Moseley 1997). The HSR is also important in conferring cross adaptation to other forms of stress (Kee-Bum and others 2004), some of which are present in exercise, but the adaptive HSR in humans has not been well characterised (McClung and others 2008). Intracellular concentrations of HSP70 in leukocytes are increased at rest following 10-d heat acclimation, while the acute response to heat stress is blunted (McClung and others 2008). In contrast, extracellular concentrations of HSP70 may be lower at rest following 2-d heat conditioning, while the acute response to exercising heat stress may be unchanged (Marshall and others 2006). Therefore, we also sought to provide further information on the HSR in humans.

The major aims of this study were, therefore, to examine the hypotheses that (i) short-term (5-day) heat acclimation would confer substantial reduction in physiological strain and increased exercise tolerance during exercise in the heat; and, of particular

interest, (ii) fluid regulatory strain during acclimation would provide a thermally-independent stimulus to further enhance adaptations.

Materials and Methods

Experimental design and overview

A randomly assigned, cross-over design was used (Fig. 1). Nine participants undertook two, 5-d heat acclimation regimes, separated by a 5-week washout; one acclimation with full rehydration (EUH) and one with minimal fluid replenishment (DEH) during each daily acclimation session. Heat acclimation consisted of 90-min exposure on each of 5 consecutive days (40ºC, 60% RH), using controlled hyperthermia (rectal temperature (T_{re}) 38.5ºC), thereby preventing any additional conditioning stimulus from dehydration *per se* on T_{core} (i.e., via hyperosmotic hypovolaemia). Plasma concentrations of fluid-regulatory hormones (aldosterone $[aldosterone]_p$, and AVP $[AVP]_p$) and proteins (Albumin $[albumin]_p$ and total protein $[TP]_p$) were measured at rest and end of acclimation bouts on the first and last days of acclimation. An exercising heat stress test (HST) was completed by participants one week before and on the 2nd day after each acclimation regime. Blood volume (BV) was measured one week before the pre-acclimation HST and the 1st day after each acclimation regime.

Experimental subjects and screening measures.

Participants were 9 healthy, male, well-trained volunteers (Mean ±SD; Age 27 ±7 y; Body mass 74.6 ±4.4 kg; $\dot{V}O_{2\,peak}$ 60 ±7 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ and peak power output 340 ±30 W). Acclimations were carried out in the winter-spring, to minimise seasonal acclimatisation effects. This study was conducted within the bounds of approval granted by the University of Otago Human Ethics Committee.

Procedures and Measurements

The HSTs were in the mornings, all beginning at the same time of the day (9.00 am) for consistency of physiological status. Participants were asked to refrain from strenuous exercise for 24 h prior to each HST. Acclimation bouts took place in the late afternoon/early evening to fit into participants' schedules of availability.

Acclimation: Heat acclimation sessions consisted of cycling (Monark Ergomedic 824E, Sweden) for 90 min in hot and humid conditions (T_{db} 40ºC, 60% RH, with air velocity <0.5 $m \cdot s^{-1}$). Because dehydration increases core temperature, which might provide an additional thermally-mediated improvement in heat adaptations, core temperature was clamped independently of hydration status to nullify this influence. Furthermore, by elevating core temperature to the same level each day, more exertional stress was anticipated as acclimation proceeded (Fig. 2), with matched thermal strain but potentially a compromised volume of exercise being performed in the DEH regime. Modest hyperthermia (rectal temperature (T_{re}) 38.5ºC) was attained as rapidly as practicable, and was maintained by regular adjustment of workload. Participants consumed 250 ml of a 4% carbohydrate fluid before the EUH acclimation bouts. They then consumed a minimum of 250 ml CHO fluid every 15 min during the bouts, for a total consumption of 1500 ml. Participants were allowed to drink *ad libitum* above this dosage. Nominal fluid ingestion (100 mL) was given immediately before the DEH acclimation bouts to limit perception of fluid deprivation.

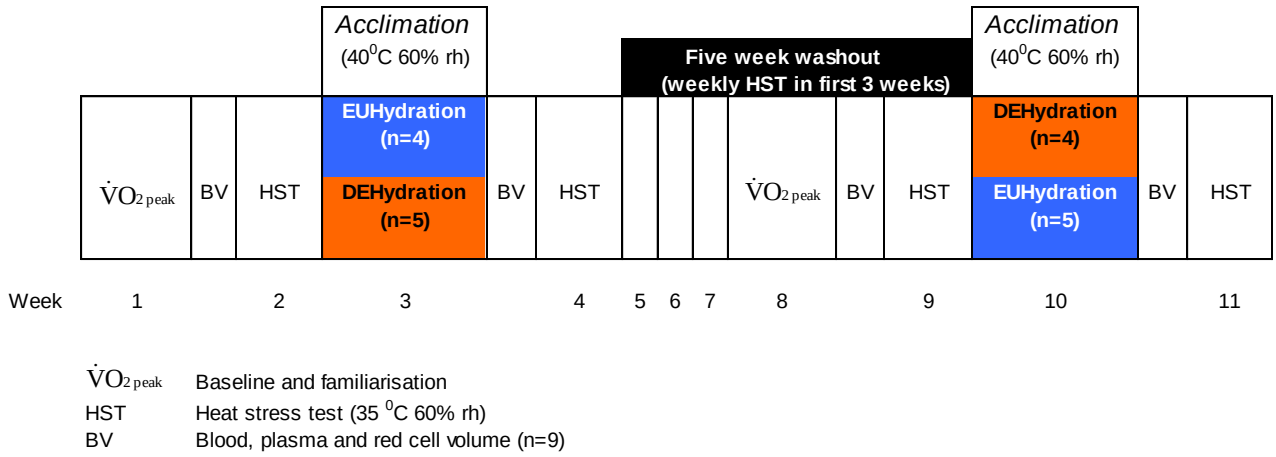

Fig. 1: Experimental design for examining the adaptive effects of short-term heat acclimation with and without dehydration.

Heat stress tests: On arrival at the laboratory (9.00 am) before the HST, participants' thermoregulatory, cardiovascular and fluid-regulatory statuses were measured during 60 min resting and then cycling at 40% of pre-acclimation peak power output (PPO; ~50% $\dot{V}O_{2\,peak}$) for 90 min in hot, humid and calm conditions (T_{db}, 35ºC, 60% RH, with air speed <0.5 $m{\cdot}s^{-1}$). Participants then rested for 10 min before commencing a ramp protocol (2% PPO each 30 s) to volitional fatigue, or the ethical end point of T_{re} ≥39.5ºC. This HST protocol was adapted from a previously-used method (Patterson 1999). Participants consumed 250 mL of the 4% carbohydrate drink before exercise in each HST, and a further 150 mL every 15 min during exercise, for a total consumption of 900 mL in 90 min (Casa and others 2000; Convertino and others 1996).

Exercise was undertaken on an electro-magnetically braked cycle ergometer (Rodby Elektronik AB, Model RE 820/830, Sodertalje, Sweden). Cardiac frequency (f_c) was measured from the R-R interval of ventricular depolarisation (Polar Sport tester

Advantage, Kemplele, Finland). Body core temperature was measured using a general purpose, flexible thermistor (Thermistor 400, Mallinckrodt Medical Inc., St Louis, USA) inserted into the rectum 10 cm beyond the anal sphincter. Skin temperature was measured (Type EUS-U-V5-V2; Grant Instruments, Cambridge, England) at four, right-side sites: calf, upper thigh, chest and bicep. Mean skin temperature ($\overline{T}_{sk}$) was calculated as: $\overline{T}_{sk}$ = 0.3 T_{chest} + 0.3 T_{bicep} + 0.2 T_{thigh} + 0.2 T_{calf} (Ramanathan 1964). Temperatures were logged at 60-s intervals (1200 series, Squirrel Grant Instruments, Cambridge, England).

Thermoeffector function was measured during HSTs from the sudomotor and vasomotor responses to these standardised bouts of stress. Whole-body sweat rate ($mL \cdot h^{-1}$) was calculated from pre- and post-exercise nude body mass corrected for drinking and urinary exchanges (accuracy of scales 0.1 kg; resolution ±20 g; Wedderburn Scales, Teraka Seiko, Tokyo, Japan). Continuous measurement of sweat rate from discrete skin sites was undertaken throughout HSTs using ventilated sweat capsules glued onto the forehead and dorsal forearm (Brengelmann and others 1975; Graichen and others 1982). Forearm blood flow ($\dot{Q}_F$) was measured using venous occlusion plethysmography (Joyner and others 2001; Witney 1953) during HSTs, with a custom-built controller and commercial software for data capture and filtering (Maclab 4e and Chart 4.1 for Windows event Manager software by Power Lab, ADInstruments, USA). Blood pressure was measured by auscultatory aneroid sphygmomanometry, as the average of three individual measurements taken following each $\dot{Q}_F$ measurement series. Forearm vascular conductance (FVC) was calculated as $\dot{Q}_F$ divided by mean arterial pressure (MAP),

where MAP was calculated as diastolic blood pressure plus one third of Pulse Pressure. The $\dot{Q}_F$, MAP and FVC were measured at rest, and at 15, 45 and 75 min during exercise.

Subjects rated their perceptions of exertion (16-point scale ranging 6-20: (Borg 1982), body temperature (1-13) and thermal comfort (1-5) (adapted from Gagge and others 1967), at 20-30 min intervals during HSTs.

Blood measures within acclimation and HSTs: A flexible 20-gauge catheter was placed in a suitable forearm vein before each HST and on days one and five of each acclimation regime. Venous blood samples (15 mL) were taken without stasis, following a 1 mL discard at rest, 30 (HST only), 60 and 90 min. Following extraction of whole blood for measurement of haematocrit (Hct) and haemoglobin (Hb), samples were centrifuged and plasma was stored at -80^{o}C for later measurement of solute concentrations. Aldosterone, AVP and cortisol concentrations were analysed using the radioimmunoassay ^{125}I labelling technique. Using duplicate measures, the intra-assay coefficient of variation for the fluid-regulatory and stress hormones were as follows; $[\text{aldosterone}]_p$ (9.9%), $[\text{AVP}]_p$ (5.6%) and $[\text{cortisol}]_p$ (12.1%). All samples for a given individual were analysed within the same assay. Concentrations of albumin, total protein, Na^+, K^+ and Cl^- were analysed using duplicate colorometric analysis (Cobas Mira Plus, New Jersey, USA). Osmolality was measured using Vapour Pressure Osmometry (Vapro 5520, Wescor Inc., Utah, USA).

Pre- and post-exercise measurements of plasma concentrations of HSP70 were made on the first day (one) and last day (five) of each acclimation regime. enzyme-linked immunosorbent assay (ELISA; DETAILS??) analysis was used to measure HSP70, as described previously (Njemini and others 2003). Briefly, plasma samples were diluted

1/5 and 250 μL added to duplicate wells of a 96 well plate pre-coated with anti-HSP70 monoclonal antibody. After a 2 h incubation at room temperature, HSP70 binding was detected using 100 μL per well of Biotin anti-HSP70 for 1 h followed by 100 μL Avidin-HPP conjugate. Avidin binding was indicated using tetra methyl benzidine (TMB) substrate and measured at 450 nm on a Spectromax Plus Spectrophotometer (Molecular devices, USA). Values were calculated from a standard curve generated using a recombinant HSP70 standard (0.78 to 50 $ng \cdot ml^{-1}$ as a two-fold dilution series). The HSP70 concentrations were corrected for plasma total protein (HSP70/TP) to account for any hydration mediated change in concentration.

Blood volumes: Haemoglobin mass and blood volume were measured one week before the baseline HSTs and on the 1st day after each acclimation, using the carbon monoxide (CO) dilution/rebreathing technique (Ashenden and others 1999; Burge and Skinner 1995). This measurement was taken with the participant seated, following a 20-min, postural equilibration. Artifactual changes due to acclimation-induced changes in iron-containing molecules in muscle were assumed to be negligible due to the habitual training status of these participants. Venous blood samples were obtained after a priming dose (20 mL) and again 10 min after introducing the main CO dose (1.5 mL CO/kg body mass). Measurement of haematocrit, HbCO and total Hb concentrations were determined in triplicate both before and after CO rebreathing. Determination of RCV was corrected for the volume of red cells removed within that acclimation regime. Pilot testing (n=6) indicated that test-retest reliability for accuracy of repeated measurement had a coefficient of variation (CoV) of ~2.9% for blood volume. Plasma volume (PV) was derived by subtraction (CoV; 2.5%). The CO technique was used because of concerns

with repeated dosing of other dilution markers, including of Evans Blue dye, although measurement of PV change is inherently noisy (CV 5-6%) with this and other techniques (Gore et al., 2005). Therefore, in view of the short-duration acclimation regime, change in PV was additionally measured using the Dill and Costill (1974) method, as per several other heat acclimation studies (Lorenzo et al., 2010; Nielsen et al. 1993).

Data analysis

Inferential analyses were performed on data collected at rest before (e.g., blood volumes) within (e.g., heart rate) and at end-exercise in HSTs and acclimation bouts. Data were exported to statistical software packages (Excel Microsoft Windows, SPSS v17, SPSS Inc, Chicago, USA) for analysis. Most data were analysed using two-way analysis of variance (ANOVA) with repeated measures. Factor one (acclimation status) and factor two (acclimation type) each had two levels. Three-way ANOVA was not used because differences at discrete times during acclimation bouts or HSTs were generally of less interest than were other response parameters such as mean or final values or rate of change. Paired t-tests were used to isolate differences between means for significant two-way interactions.

Estimates of population effects for acclimation status (pre vs post) and acclimation type (EUH vs DEH) are reported as means and SD with 95% confidence intervals (95% CI) (Hopkins 2003; Hopkins and others 2009). The relationship between variables was calculated using the Pearson Product Moment Correlation.

Results

Nine participants completed both acclimation regimes and the heat stress tests (HSTs) before and after each acclimation, except that one participant became exhausted in one heat stress test at 67 min, so his end-exercise data were taken as the 60-min measurement in all HSTs. All blood measures were obtained from all participants on HST- and BV-measurement days, but a full cross-over of measures on acclimation days was obtainable from only five participants due to difficulty with cannulation or maintaining cannula patency on one of these additional days in the remaining four participants.

Thermal stress and strain of acclimation bouts

Thermal stress and strain data from the first and last days of EUH and DEH acclimation regimes are shown in Table. 1. Environmental heat stress indices were matched between regimes, as was T_{re} (clamped after reaching 38.5ºC). Time to T_{re} 38.5ºC was ~10% longer in EUH than DEH but by day 5 the time to T_{re} 38.5ºC was similar for EUH and DEH regimes. Less work was performed on the first acclimation day in DEH than EUH ($P<0.05$), but generally not thereafter.

Table. 1 Thermal stress and strain on the first and last days of heat acclimation undertaken with (EUH) and without (DEH) rehydration**.**

	DAY 1		DAY 5		
	EUH	DEH	EUH	DEH	
T_{db} (°C)	39.5±0.1	39.6±0.1	39.6±0.1	39.5±0.1	
RH (%)	62 ±2	62 ±2	62 ±2	61 ±1	
Mean f_c ($b \cdot min^{-1}$)	123 ±13	122±16	123 ±14	121 ±10	
Mean T_{re} (°C)	38.2 ±0.1	38.3±0.1	38.2±0.1	38.2±0.2	
Time to T_{re} 38.5°C (min)	34 ±5	31 ±4	34 ±5	33±4	*†
Fluid consumed (mL)	1930±452	100±0	1861±333	100 ±0	†
Body mass change (%)	0.4 ±0.5	-1.7±0.5	0.1 ±0.7	-2.0±0.7	†

Data are means ±SD for nine males for whom data were available across all sessions. Dry Bulb Temperature (T_{db}), relative humidity (RH), rectal temperature (T_{re}), cardiac frequency (f_c). * P<0.05 for day 1 versus day 5; † P<0.05 for euhydration versus dehydration.

Blood, plasma and red cell volumes

Plasma and blood volumes increased across acclimation (P=0.02 and P=0.04), irrespective of whether dehydration was permitted or not (PV: P=0.52; BV: P=0.40). When calculating ΔPV alternatively with the Dill & Costill method (1974), a tendency (P=0.06) was evident for the expansion to be larger in DEH (8 ±3%) than in EUH (4 ±3%). The 4% mean difference between regimes had a 95%CI of -1 to 10%.

Red cell volume (RCV) also tended (P=0.06) to increase across acclimation, with the increase of 1.7 ±1.6% following EUH (0.6 $mL \cdot kg^{-1}$; CI: -0.7 to 1.9) and 4.1 ±0.9% following DEH (1.5 $mL \cdot kg^{-1}$; CI: 0.7 to 2.3) being unclear (P=0.18; -0.9: 2.5 to 1.0%). An increase in RCV was measured in 5 of 9 individuals after EUH and 9 of 9 after DEH. Thus, PV expansion may have been underestimated for these individuals using the more reliable but less valid method of Dill & Costill (1974).

Fluid regulatory and heat shock protein responses during acclimation bouts

Resting $[aldosterone]_{plasma}$ was not reliably changed across acclimation (Table 2; n=5 as described above). However, the acute response to one bout of stress increased across the 5-day acclimation more in DEH than in EUH (P=0.03; by 225 $pg.mL^{-1}$; CI: 40 to 411), and was positively related to plasma volume expansion (r=0.65; P=0.05). The $[AVP]_{plasma}$ did not change across acclimation at rest or in its magnitude of elevation during the acclimation bout. Similarly, $[TP]_p$ was unchanged across the five-day regimes at rest and in the magnitude of elevation during the bout, but this elevation became larger in DEH than in EUH (P=0.03; Table 2). Total protein-corrected plasma concentration of HSP70 at rest was increased across acclimation, but not differentially between regimes (Fig. 2 and Table 2). At the end of each acclimation bout, the total protein-corrected HSP70 was unchanged across acclimation.

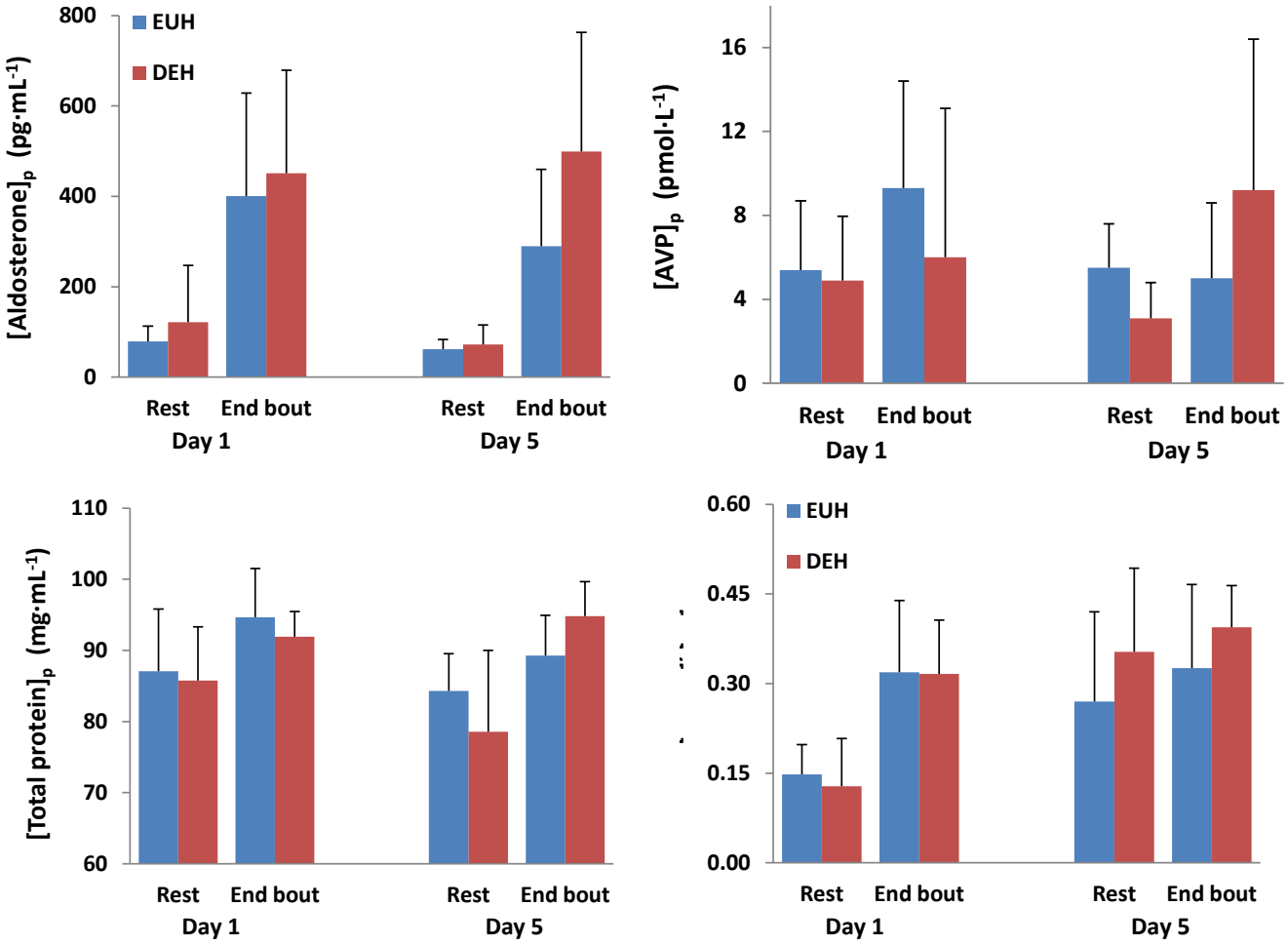

Fig. 2. Plasma concentrations of fluid regulatory hormones, total protein and total protein-corrected heat shock protein (HSP70/TP) responses before and after acclimation bouts on the first and last day of acclimating with euhydration (EUH) or permissive dehydration (DEH). Data means ±SD for five males. See Table 2 for treatment effect magnitudes and inferential statistics.

Table. 2 Change in plasma aldosterone, AVP and total protein concentrations across the five-day acclimation regimes undertaken with (EUH) and without (DEH) rehydration in each bout.

	EUH	DEH	Main effect P value	Interaction P value
Aldosterone ($pg.mL^{-1}$)				
Rest	-2: -30 to 33	-10: -51 to 71	0.65	0.74
90-min exercise	-47: -209 to 115	178: 33 to 324	0.05	0.02
AVP ($pmol.L^{-1}$)				
Rest	0: -4 to 5	1: -1 to 2	0.76	0.91
90-min exercise	-1: -7 to 4	3: -2 to 8	0.78	0.10
Total protein ($mg.mL^{-1}$)				
Rest	-3: -13 to 8	-7: -20 to 6	0.14	0.23
90-min exercise	-6: -11 to 1	3: -4 to 10	0.46	0.03
[HSP70]/[TP]				
Rest	0.12: -0.05 to 0.30	0.22: 0.07 to 0.37	0.003	0.26
90-min exercise	-0.01: -0.17 to 0.18	0.08: -0.02 to 0.17	0.28	0.36

Data are mean and 95% confidence interval of the change across acclimation (Day 5 minus Day 1), for five males for whom samples were obtained before and after bouts on both days in both acclimation regimes. Main effect refers to acclimation status (Day 5-1).

Heat stress tests

Cardiovascular function and Thermoregulation

Resting f_c was not reduced reliably across acclimation. End-exercise f_c was reduced (P=0.01; Fig. 3, upper panel), but to a greater extent for DEH (-19: -29 to -3 $b \cdot min^{-1}$) than for EUH (-10: -17 to 2 $b \cdot min^{-1}$) (interaction P=0.05; 11: -1 to 22 $b \cdot min^{-1}$). The reduction in f_c was moderately related with the extent of PV expansion across DEH (r=0.72; P=0.05), but not across EUH (r=0.25; P=0.72).

Rectal temperature at rest (Fig. 3, lower panel) was not lowered consistently across acclimation. Core temperature at the end of steady-state exercise (90-min) was reduced (P=0.004) to a similar extent (P=0.52) for EUH (-0.2: -0.6 to 0.05^{o}C) and DEH (-0.4: -0.75 to -0.1^{o}C). The true difference between regimes was unclear (0.1: -0.4 to 0.7^{o}C). Mean skin temperature ($\overline{T}_{sk}$) at rest was unaffected by acclimation type (P=0.65) but end exercise temperatures were lower (P=0.003). The mean $\overline{T}_{sk}$ after 90-min exercise was reduced across EUH (-0.3: -1.1 to 0.3^{o}C) and DEH (-0.4: -0.7 to 0.1^{o}C), with the difference between regimes (P=0.15) again being unclear (-0.4: -0.8 to 0.1^{o}C).

The forearm perfusion ($\dot{Q}_F$) at rest was higher following acclimation (Fig. 4, upper panel) but depended on the acclimation type. The increase was evident for DEH (4: 1 to 8 mL·100 mL tissue^{-1}·min^{-1}·) but not EUH (-2: -4 to 1 mL·100 mL^{-1}·min^{-1}); (interaction P=0.04; 6 mL·100 mL^{-1} min^{-1}: -12 to -1). No main or interaction effect on $\dot{Q}_F$ was evident late in exercise (at 75 min; P=0.58). The MAP was not reliably altered across acclimation at rest (P=0.56) or during exercise (75 min; P=0.14). Thus, FVC was higher across acclimation (Fig. 4, lower panel) at rest (P=0.05), but more so (P=0.006) for DEH (0.06: 0.02 to 0.10 mL·100mL Tissue^{-1}·min·mm Hg^{-1}), than for EUH (-0.02: -0.05 to 0.01 mL·100mL Tissue^{-1}·min^{-1}·mmHg^{-1}). FVC was not consistently altered near the end of exercise (75 min; P=0.34).

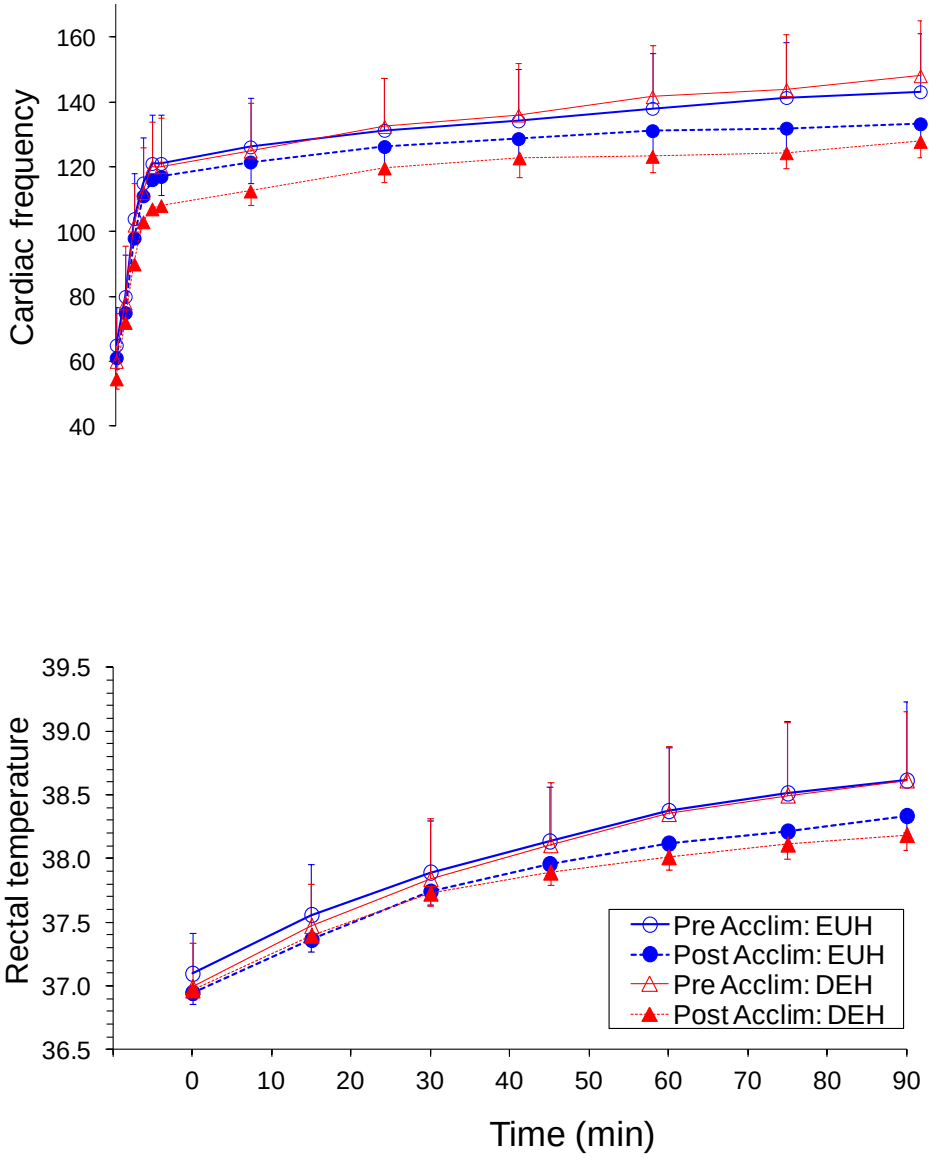

Fig. 3: Cardiac frequency (upper panel) and rectal temperature (lower panel) during 90-min cycling at 40% peak power output in the heat before (pre) and after (post) acclimation, undertaken with (EUH) or without (DEH) rehydration during daily heat acclimation sessions. Data are means ± SD for nine males expressed in $b \cdot min^{-1}$ for cardiac frequency and ^{o}C for rectal temperature.

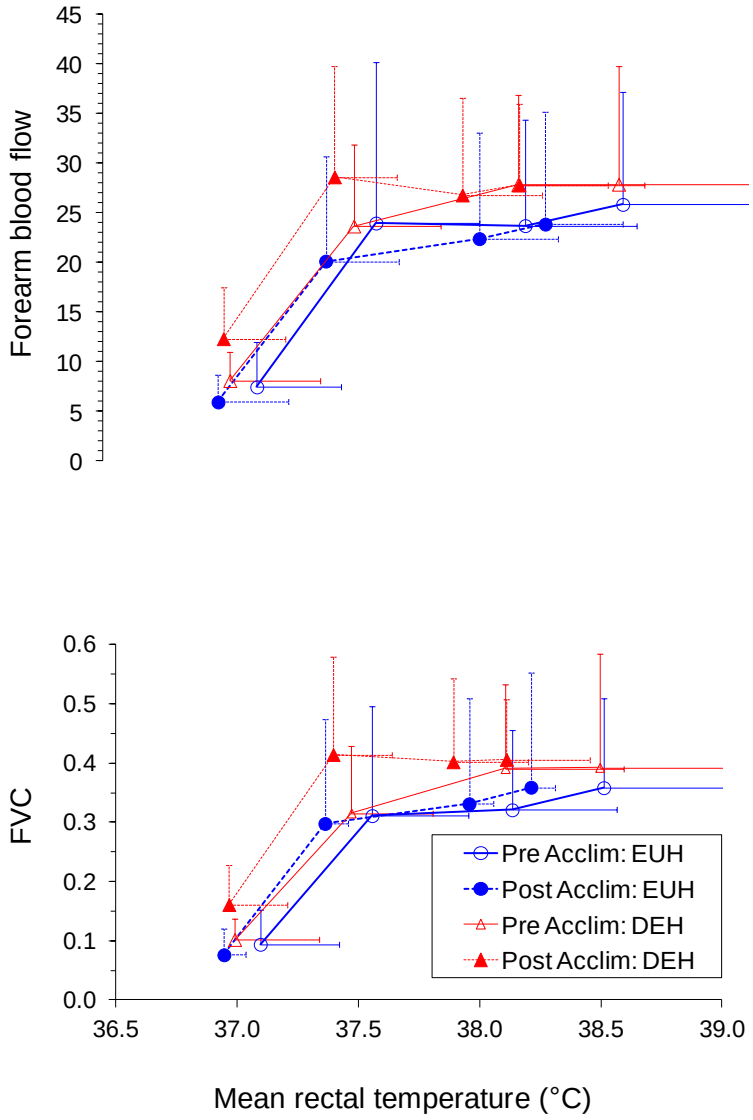

Fig. 4: Forearm blood flow (upper panel) and mean forearm vascular conductance (lower panel) versus mean rectal temperature responses to 90-min cycling at 40% peak power output in the heat (35ºC, 60% RH) before (pre) and after (post) acclimation, undertaken with (EUH) or without (DEH) rehydration during daily heat sessions. Data are means ±SD for eight males. Expressed in $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ for forearm blood flow and $mL \cdot 100mL\ Tissue^{-1} \cdot min^{-1} \cdot mmHg^{-1}$ for FVC.

Perceptions

Perceived body temperature at rest was not reliably changed across acclimation (P=0.09), but by end of exercise the participants felt cooler (P=0.02) and more thermally comfortable (P=0.05), to a similar extent in EUH (P=0.18) and DEH (P=0.38). Perceived exertion at end exercise was reduced (P=0.04), again by a similar extent between acclimation regimes (P=0.65).

Fluid regulation

Participants began HSTs euhydrated before and following each acclimation regime. Neither of the acclimation regimes reliably affected resting concentrations of sodium, albumin or total protein in plasma, nor end-exercise concentrations of albumin or sodium. The fluid regulatory hormones aldosterone and AVP showed substantial blunting in their responses within HSTs following the 5-d acclimation regimes. No differences were evident between acclimation regimes for these improved neuroendocrine sensitivities. Similar findings occurred for both cortisol and total protein responses to the HST (Table.3).

Table 3 Plasma concentrations of fluid regulatory and stress hormones, electrolytes and colloids at rest and 90-min exercise in the heat stress test after EUH and DEH acclimation

	EUH	DEH	Main effect	Interaction
Aldosterone (pg.mL^{-1})				
Rest	-10: -79 to 60	9: -61 to 79	*P*=0.92	*P*=0.59
90-min exercise	-144: -235 to -52	-55: -167 to -43	*P*=0.001	*P*=0.50
AVP (p.mol.L^{-1})				
Rest	-0.1: -0.5 to 0.3	0.9: -2.5 to 4.2	*P*=0.52	*P*=0.61
90-min exercise	-2.9: -6.1 to -0.2	-2.1: -4.1 to 0.1	*P*=0.01	*P*=0.31
Cortisol (ug·dL^{-1})				
Rest	3: -1 to 8	-1: -4 to 2	*P*=0.42	*P*=0.10
90-min exercise	-3: -40 to 13	-2: -15 to 31	*P*=0.02	*P*=0.86
Total protein (mg.mL^{-1})				
Rest	-0.2: -4.3 to 4.6	-3: -6.1 to 0	*P*=0.24	*P*=0.24
90-min exercise	-1.8: -4.6 to 1.0	-1.2: -3.0 to -0.5	*P*=0.02	*P*=0.99
Albumin (mg.mL^{-1})				
Rest	1.2: -3.0 to 5.4	-2.6: -5.8 to 0.7	*P*=0.43	*P*=0.10
90-min exercise	0.3: -2.9 to 3.4	-1.5: -2.8 to -0.2	*P*=0.36	*P*=0.27
Plasma sodium (mmol·L^{-1})				
Rest	-1: -3 to 5.4	0.5: -1 to 2	*P*=0.16	*P*=0.10
90-min exercise	-0.5: -1.5 to 1	1.0: 0.5 to 2	*P*=0.38	*P*=0.11
Plasma osmolality (mmol·kg^{-1})				
Rest	6: -7 to 20	2: -8 to 12	*P*=0.90	*P*=0.28
90-min exercise	-4: -22 to 13	4: -12 to 20	*P*=0.85	*P*=0.66

Data are shown as mean and 95% confidence interval (95% CI) for nine males.

Exercise performance capacity

Work capacity in the incremental test to exhaustion with eight participants increased across acclimation (*P*=0.001), to a similar extent (*P*=0.14) in EUH (time by 14%; 104: 57 to 150 s) and DEH (19%; 146: 101 to 191 s).

Discussion

Hypohydration of 1-2% body mass can increases physiological stress and decrease performance (Casa et al., 2000), depending on fitness (Merry et al., 2010) and ambient heat stress (Cheuvront et al., 2010). However, it is often difficult and sometimes impossible to prevent at least some dehydration during heat stress (Convertino and others 1996) and this has been considered of little consequence by some authors (Greenleaf 1991; Noakes and others 1988). Irrespective, these are issues of acute functional affects, and the purpose of acclimation bouts is to stimulate adaptation. Therefore, the most important issue is whether (and why) the heat-related adaptations are impaired, enhanced, or affected only negligibly by altered hydration status.

The current project was designed to determine whether adaptations to short-term heat acclimation would be more pronounced when dehydration was permitted. The short-term (90 $min \cdot d^{-1}$ for 5-d) heat acclimation substantially attenuated thermal and cardiovascular strain and enhanced exercise capacity to a similar level of long-term exposure to the heat. Compared with the euhydration regime, permissive dehydration during acclimation bouts generally conferred similar adaptive increases in physiological and functional effects, but some cardiovascular-related advantages (resting plasma volume, forearm perfusion, forearm vascular conductance and decreased cardiac frequency at end of exercise heat stress test) were observed with permissive dehydration.

We emphasise that the present findings were obtained using permissive dehydration to a modest level of hypohydration (~1.8%), yet fluid-regulatory stimuli are already induced (Brandenberger and others 1989; Brandenberger and others 1986; McConell and others 1997). Whether even greater dehydration would be more beneficial

or detrimental and how this would compare with a longer acclimation regime is unknown and requires further investigation. However, with increased dehydration the training impulse may become reduced (especially if using the controlled hyperthermic regime) decreasing acclimation (Merry and others 2010), as well as increasing the potential for adverse health effects (Casa and others 2005).

Effectiveness of short-term acclimation to the heat

The adaptations from short-term (5-d) heat acclimation with EUH and DEH acclimation, using the controlled hyperthermia technique, reduced exercising cardiovascular strain and enhanced exercise capacity. The cardiovascular stability was due to increased heat loss rather than lower heat content (~resting core temperature), at the time of day of testing HSTs and this concurs with previous work (Garrett and others 2012; Garrett and others 2009).

In this study there was greater attenuation of end-exercise cardiac frequency after DEH (17%) compared with EUH (7%) acclimation and this difference was significance. Similar findings have previously been reported using short-term heat acclimation regimes, with the controlled hyperthermia technique (Cotter and others 1997). Further, in the present study skin temperature at end-exercise decreased across EUH and DEH acclimation with no difference between regimes and is in line with skin blood flow reduction. These adaptations are characteristic features of heat acclimation (Armstrong and Maresh 1991) and contribute to the greater cardiovascular stability observed (Sawka and others 1985).

Fluid regulation and blood volume response to repeated heat stress

In the present study, there was no difference in environmental parameters between acclimation regimes and this was illustrated by similar dry bulb temperature and relative humidity measures (Table. 1). Similarly, thermal strain was identical between regimes as evidenced by cardiac frequency and rectal temperature. Therefore, participants experienced the same thermal load which is the premise for using the controlled hyperthermia technique for heat acclimation (Taylor 2000). Hydration status was reflected on day five of acclimation by total body water being maintained in EUH (~0.1% body mass loss) and not after DEH (~2% body mass loss) (Table. 1). This is similar to the imposed hypohydration administered by Judelson and colleagues (2008), who reported a modification in the hormonal and metabolic response to resistance exercise, which influenced the post-exercise circulatory milieu. In a review on fluid replacement in athletes, Casa and colleagues (2000) reported that dehydration of 1-2% body mass increases physiological stress and decreases performance. However, in reality, it is often difficult and sometimes impossible to prevent at least some dehydration during repeated heat stress (Convertino and others 1996) and this has been considered of little consequence by some authors (Greenleaf 1991; Noakes and others 1988), on the basis that this readily occurs during repeated heat exposure. It is also unknown if in trained individuals, who are glycogen loaded, if 1-2% mass loss necessarily reflects dehydration. Furthermore, it has been suggested that athletes often over-hydrate due to misconceptions on the risks of hydration (Winger and others 2011). Furthermore, the transient osmolality and volume effects of hypohydration induce fluid-regulatory responses, that could partially mediate the hypervolaemia. Thus, improving the fluid-regulatory efficiency that

is observed with training (Merry and others 2010; Merry and others 2008), provided that the nutritive substrates are ingested following the conditioning bouts (Okazaki and others 2009b; Okazaki and others 2009a). Therefore, we postulate that the increased physiological strain of restricted fluid replenishment during DEH acclimation may have resulted in enhanced adaptation of the fluid-regulatory system.

Fluid regulatory hormones and electrolytes

In the present study, after 90-min of exercise plasma aldosterone increased across DEH bouts, in contrast to a limited response after EUH acclimation which is in line with work of Judelson and others (2008). However, there was no change in plasma AVP after rest and 90-min exercise across acclimation (Table. 2). Therefore, it appears that the exercise-induced response of plasma aldosterone became more pronounced in the DEH compared with the EUH acclimation bouts and this has been previously reported (Judelson and others 2008).

Increased Na^+ (Buono and others 2007) and water retention at the distal tubules (Morris 1981) are important mediators of the rapid PV expansion during the initial hours to days after exercise (Nagashima and others 2001). However, in the present study, although plasma aldosterone was increased an increase in plasma Na^+ was not evident after acclimation for either the EUH or DEH regime, in the standardised exercising heat stress test. This is in contrast with previous findings (Allsopp and others 1998; Brandenberger and others 1989; Francesconi and others 1993) who reported a strong relationship between increased plasma Na^+ with plasma aldosterone response.

Blood and PV responses showed similar findings with plasma aldosterone across DEH acclimation. There was a moderate relationship (r=0.65) between PV expansion

and the increased plasma aldosterone after exercise in the DEH regime (Table. 2). Blood volume expansion at rest increased after EUH (2.2%) and was elevated after the DEH regime (5.3%). This was consistent with the expansion of resting PV after EUH (5.3%) and DEH (9.0%). Patterson and colleagues (2004) used the Evans blue dye dilution technique for establishing resting PV with 12 participants. They reported a 9.8% PV expansion following 7-d heat acclimation, using the controlled hyperthermia technique. However, in this study the CO was the preferred technique because of concerns with repeated dosing of other dilution markers (e.g. Evans Blue dye), although measurement of PV change is inherently noisy (CV 5-6%) with this and other techniques (Gore and others 2005). Therefore, in the present study, the acclimation-induced resting PV was further supported using the ΔPV (Dill and Costill 1974) technique, across HSTs and acclimations. Resting PV increased for EUH (4.2 ±3.4%) but more so following DEH (8.3 ±3.2%).

In summary, we observed that the adaptive response to fluid deficit during five days of heat acclimation, in trained individuals, results in greater cardiovascular stability. It is suggested that the more pronounced biological action of plasma aldosterone observed during exercise with dehydration has a major role in the more robust increase in PV response in comparison with euhydration. This is further supported by the moderate relationship across DEH between PV expansion and plasma aldosterone response after exercise. This further supports the idea that, during exercise, the fluid-regulatory hormone plasma aldosterone may be more consistently altered after a DEH acclimation regime and have a major role in acclimation-induced hypervolaemia. Therefore, it is surprising that the contribution of plasma aldosterone with PV expansion has received limited attention

in the literature. Of the limited information available it has been demonstrated that hormonal and renal adaptations after intense exercise participate in the initial (first 24-48 h) process of PV expansion (Nagashima and others 2001).

Blood, plasma and red cell volume

In addition to renal electrolyte and water retention (Armstrong and others 1987; Convertino 1991) elevated plasma protein content has been instigated in the increased PV observed with exercise (Harrison 1985; Harrison and others 1981). Therefore, it has been suggested that both these mechanisms occur during heat acclimation and there interaction may contribute to PV expansion (Patterson and others 2004). In the present study, resting total protein remained constant from the first to the last day of each of the acclimation regimes. However, the blood volume (BV) expansion observed across EUH and DEH acclimation indicated that resting total protein has increased and may well be a mechanism for the PV expansion that was evident. This is supported by previous research on the notion that PV expansion is mediated by elevated plasma protein content (Harrison and others 1981; Mack and Nadel 1996). There was an increased exercise-induced response of total protein in the present study that became more pronounced within the DEH compared to the EUH acclimation bouts It is suggested that this differential response between regimes was a result of the fluid loss from dehydration. In summary, our data suggest that elevated total protein has a significant role in the mediation of the acclimation-induced PV expansion observed across acclimation.

Heat shock proteins

In the present study, heat shock protein response at rest (Fig 2: lower panel) was impacted by short-term heat acclimation *per se*, indicating a thermo-protective

adaptation. However, there was limited change at end-exercise (Fig.2: lower panel) and no difference between euhydration and dehydration acclimation. The data in the present study is in contrast to the limited information available on hypohydration and HSP70 expression. Kee-Bum and colleagues (2004) demonstrated using the rat model that HSP70 expression in skeletal and cardiac tissue, may not be entirely dependent on T_{re} and hypohydration maybe partly responsible. Further, the influence of cell hydration on HSP70 response has been investigated in the rat model and it has been demonstrated that the heat-induced accumulation of inducible HSP70 increased under hypo-osmotic conditions (Kurz and others 1998; Schliess and Haussinger 1999). Similarly, of the limited information available on the human model, increased HSP70 expression has been demonstrated in the human cell in response to osmotic challenge (Oehler and others 1998). Therefore, it is suggested that the potential limitation in the present study was that the mechanisms of hydration in the human cell were not directly investigated and we had a relatively low participant number (n=5), with a full cross-over of plasma HSP70 measures for the euhydration and dehydration acclimation regimes.

In summary, the present data indicate that during exercise the fluid-regulatory hormone, plasma aldosterone, may be more consistently altered following a DEH acclimation regime. In addition, we suggest that an increase in plasma protein the aldosterone response indicates that at least some portion of the increase in PV is attributable to electrolyte mediated fluid retention. Further, the increased end-exercise responses of plasma aldosterone across DEH acclimation are consistent with the increased PV expansion and larger drops in cardiac frequency (Fig. 3, upper panel) in the standardised exercising heat stress test after the DEH regime.

Conclusions

There are significant physiological adaptations and increased work capacity after short-term (5-day) heat acclimation in trained individuals. The nature of these adaptations was generally to increase heat loss rather than to lower resting body temperature. Compared with euhydration, permissive dehydration during acclimation appears to confer similar adaptive increases in physiological effects, but can further reduce cardiovascular strain via larger increases in resting plasma volume, forearm perfusion, forearm vascular conductance and decreased cardiac frequency at end exercise. This indicates that using permissive dehydration to a modest level of hypohydration (~1.8%) during acclimation does not impair and may even enhance adaptations to exercise in the heat. Secondly, short-term (5-d) heat acclimation increased plasma HSP70 response at rest indicating a thermo protective adaptation but there was no difference at end exercise or between acclimation regimes. In summary, it is recommended that further investigation be conducted to determine to what extent dehydration during acclimation should be encouraged and the extent of any negative consequences.

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Conflict of interests

There are no conflicts of interest for the author’s and the respective institutions in this study.

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