

**Title:**

Effectiveness of dehydration on adaptation to heat

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**Abstract:**

Most advice for heat adaptation is to use long-term (>10 d) regimes, in which hydration status is maintained. We tested the hypothesis that short-term (5-day) heat acclimation would confer substantial improvements in physiological strain and exercise tolerance for exercise in the heat, and fluid regulatory strain provides a thermally-independent stimulus for such adaptations. Ten moderately-fit males were heat acclimated using controlled hyperthermia (rectal temperature 38.5°C) for 90 min on five consecutive days ( $T_a = 40^\circ\text{C}$ , 60% RH), on two occasions separated by a five-week washout, in a randomly assigned, cross-over design; one with euhydration (EUH) and one with dehydration (DEH) during acclimation bouts. One week before, then on the 2<sup>nd</sup> day after each acclimation regime, a heat stress test (HST) was completed, comprising cycling at 40% peak power output for 90 min ( $T_a = 35^\circ\text{C}$ , 60% RH), before incrementing to exhaustion. Plasma volume (PV) at rest was measured using CO rebreathing. Acclimation exercise-induced response of [aldo]<sub>p</sub> became more pronounced across DEH ( $\Delta 178 \text{ pg}\cdot\text{mL}^{-1}$ ; 95%CI: 33 to 324) but not EUH ( $\Delta -47 \text{ pg}\cdot\text{mL}^{-1}$ : -209 to 115) and this difference was significant ( $P=0.02$ ). Compared to EUH, permissive DEH during acclimation bouts conferred larger acclimation-induced increases in resting PV (4.1%: -1.5 to 9.8%;  $P=0.06$ ),  $\dot{Q}_F$  (4.2: 0.7 to 7.8  $\text{ml}\cdot\text{min}^{-1}\cdot 100 \text{ ml}^{-1}$ ;  $P=0.009$ ), FVC (0.06: 0.02 to 0.10  $\text{ml}\cdot 100\text{ml Tissue}^{-1}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ ;  $P=0.006$ ) and decreased end-exercise  $f_c$  by 17% (19: -29 to 9  $\text{b}\cdot\text{min}^{-1}$ ;  $P=0.05$ ). In conclusion, short-term (5-day) heat acclimation was effective with several adaptations more pronounced after fluid-regulatory strain from a dehydration acclimation regime.

**Key words:** hypohydration, fluid regulation, plasma volume

## **Introduction**

The adaptive effects of medium to long-term heat acclimation (>8-12 d) have received much research attention (Nielsen et al. 1993; Regan et al. 1996; Nielsen et al. 1997; Cheung and McLellan 1998; Weller and Harrison 2001; Patterson et al. 2004).. However, many of the important adaptations to heat stress are cardiovascular, and occur relatively rapidly (<7 d). Therefore, the use of short-term heat acclimation regime (Weller and Harrison 2001; Patterson et al. 2004) has been adopted in this study. The use of permissive dehydration during acclimation contradicts the existing fluid replenishment guidelines for heat acclimation, which recommend the maintenance of good hydration status during exposure to heat stress conditions (Armstrong and Maresh 1991; Convertino et al. 1996; Cheung and McLellan 1998; Casa et al. 2000). However, the reality for many people undergoing acclimation bouts is that some level of dehydration is normal, if not frequently inevitable (Noakes et al. 1988; Greenleaf 1992).

It is well recognised that humans normally do not voluntarily replace all water lost during prolonged exercise in the heat (Szlyk et al. 1989; Greenleaf 1992; Armstrong et al. 1997), and in some individuals with high sweating rate, total body water loss may reach 8% of body mass (Armstrong et al. 1986). Heat-and-exercise induced hypohydration manifests itself as hyperosmotic hypovolaemia (Nadel et al. 1980), which impairs cutaneous blood flow and sweat rate, and raises heart rate, core temperature, glycogenolysis, perceived exertion and permeability of tight-membranes (Mack and Nadel 1996; Gonzalez-Alonso et al. 1998; Gonzalez-Alonso et al. 1999; Blatteis 2000; Gonzalez-Alonso and

Calbert 2003; Maughan 2003; Watson et al. 2005). The consequence is impaired exercise tolerance and performance in temperate conditions which has previously been debated (Sawka and Noakes 2007). Dehydration during prolonged exercise in the heat increases the response of the fluid- and stress-regulatory hormones aldosterone, AVP and cortisol, as well as thirst (Sawka et al. 1987), such that hydration is regulated at a lower level until after exercise and/or heat stress (Brandenberger et al. 1986; Brandenberger et al. 1989). The osmolality and volume effects of hypohydration incur fluid-regulatory responses that could partially mediate the hypervolaemia and improved fluid-regulatory efficiency that is observed with training and heat acclimation adaptations and which helps attenuate cardiovascular strain in exercise (Hopper et al. 1988). Further, there are two potential mechanisms explaining PV expansion during heat acclimation. First, greater renal and sweat related retention of electrolytes and fluid would expand the vascular compartment. Secondly, it has been reported that PV expansion after the movement of total plasma protein to the interstitial fluid in the vascular space. However, it is possible that both these mechanisms occur during heat acclimation and that interaction may contribute to PV expansion (Patterson et al. 2004). Therefore, we believe that in contrast to current recommendations of avoiding dehydration during exercise and heat acclimation, permissive dehydration independently facilitates acclimation by increased fluid-electrolyte retention, PV expansion and cardiovascular response to heat stress.

## Methods

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

### Experimental design and overview

A randomly assigned, cross-over design was utilised (Figure 1). Nine participants undertook two, 5-d heat acclimation regimes, separated by a 5-week washout (Barnett and Maughan 1993; Pandolf 1998; Hayden et al. 2004); One acclimation with full rehydration (EUHydrated) and one with minimal fluid replenishment (DEHhydrated) during each daily acclimation session. Heat acclimation consisted of 90-min exposure on 5 consecutive days (40°C, 60% RH), using controlled hyperthermia (rectal temperature ( $T_{re}$ ) 38.5°C). Fluid regulatory measures of plasma aldosterone [aldo]<sub>p</sub>, plasma AVP [AVP]<sub>p</sub> and plasma total protein [TP]<sub>p</sub> were recorded at rest and end exercise on day 1 and day 5 of acclimation. The nine participants completed an exercising heat stress test (HST) administered one week before and the 2<sup>nd</sup> day after EUH and DEH acclimation. On arrival at the laboratory before the HST participants' thermoregulatory, cardiovascular and fluid-regulatory status were measured during 60 min period of rest before cycling at 40% of pre-acclimation peak power output (PPO;  $\sim 50\% \dot{V}O_{2\text{peak}}$ ) for 90 min in the heat ( $T_{db}$ , 35°C 60% RH, with a wind 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 a  $T_{re} \geq 39.5^\circ\text{C}$ .

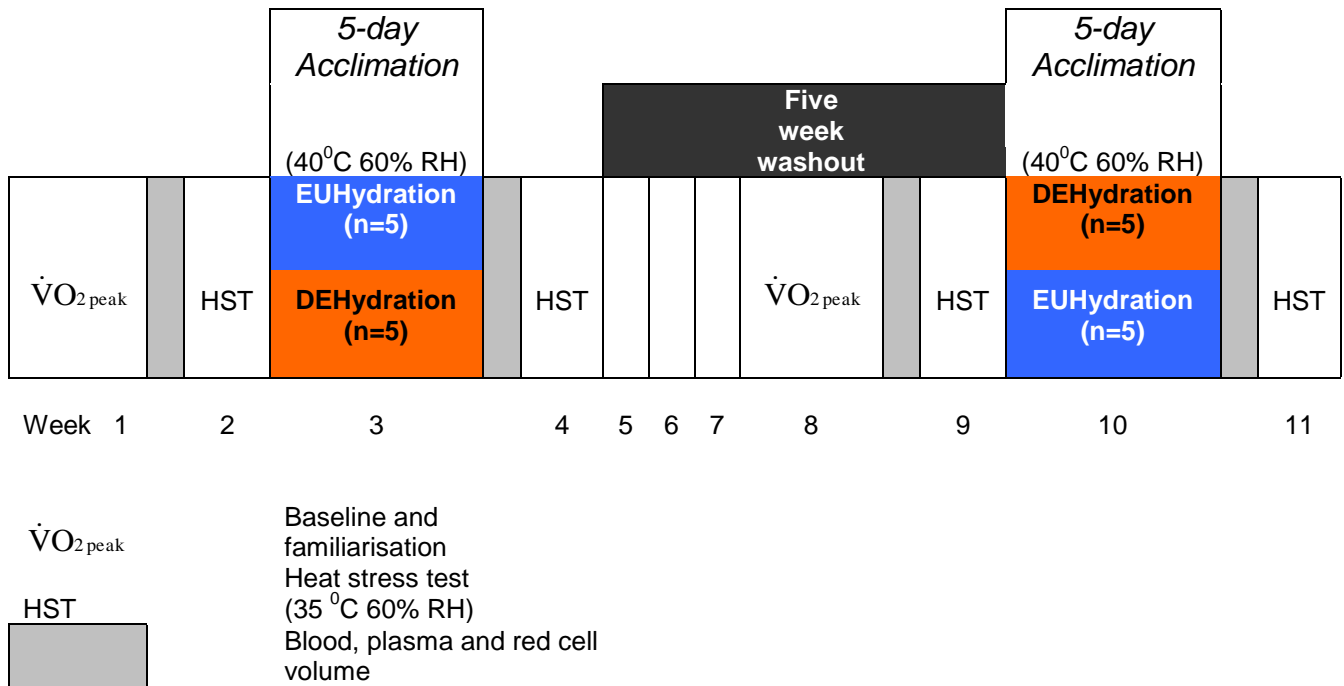
Before the HSTs participants consumed 250 ml of the 4% CHO fluid. At approximately 15 min intervals during HSTs, 150 ml of 4% CHO solution was consumed, for a total consumption of 900 ml in 90 min (Convertino et al. 1996; Casa et al. 2000). Blood volume (BV) was measured one week before the pre-acclimation HST and the 1<sup>st</sup> day after EUH and DEH acclimation using the carbon monoxide (CO) rebreathing technique (Burge and Skinner 1995). Plasma volume (PV) was derived by subtraction.

For logistical reasons regarding usage of the heat chamber facility and participant availability the HSTs were in the mornings, all beginning at the same time of the day (9.00 am). Participants were asked to refrain from strenuous exercise immediately before and 24 h prior to each HST, as it has been demonstrated that lower resting core temperature contributes to reduced physiological strain during acclimation (Kampmann et al. 2008). Acclimation bouts took place in the late afternoon/early evening. This consistency was important to control for changes in core temperature and sweating response that may occur if repeated heat exposures were to take place at different times of the day (Shido et al. 1999). Specifically, Shido and colleagues (1999) reported that the effect of acclimation on resting  $T_{re}$  was specific to the time of day of acclimation.

### **Procedures and Measurements**

*Acclimation:* Heat acclimation sessions consisted of cycling (Monarch Ergomedic 824E, Sweden) for 90 min in hot and humid conditions ( $T_{db}$  40°C 60% RH, with air velocity  $<0.5 \text{ m}\cdot\text{s}^{-1}$ ). Since dehydration increases  $T_c$  under active heat stress, it was necessary to clamp  $T_c$  independently of hydration status to nullify its influence. Elevating  $T_{re}$  to the same level during exposure was intended to permit more work as acclimation

proceeded (Figure 2), and balance thermal strain between regimes. Modest hyperthermia ( $T_{re}$  of 38.5°C) was attained as rapidly as practicable, and was maintained by regular adjustment of workload to alter the physical stress imposed for the participant. Nominal fluid replacement (100 mL) was given immediately before the DEH acclimation bouts to limit perception of fluid deprivation. Fluid-regulatory measures and total protein were recorded on day 1 and day 5 of both (EUH and DEH) acclimation regimes. Blood volume was measured one week before the HST and on the 1<sup>st</sup> day after both (EUH and DEH) acclimations.



**Figure 1:** Experimental design for examining the adaptive effects of short-term heat acclimation with and without dehydration. Acclimation was staggered between participants for logistical reasons.

*Fluid regulatory and stress hormones*

A flexible 20-gauge catheter was placed in a suitable forearm vein before each HST and on days one and five of the EUH and DEH acclimation trials. Venous blood samples (15 mL) were taken without stasis, following a 1 mL discard. The catheter was flushed with saline after sampling. The samples were taken at rest, 30, 60 and 90 min in the HST, and at the same times on days one and five of the acclimation regimes, but with the omission of the 30 min sample (Morel and Doucet 1986; Creasy 2002). Plasma for aldosterone [aldo]<sub>p</sub>, AVP and cortisol were analysed using the radioimmunoassay <sup>125</sup>I labeling technique. The intra-assay coefficient of variation for duplicate measures were as follows; [aldo]<sub>p</sub> (9.9%), [AVP]<sub>p</sub> (5.6%) and [cortisol]<sub>p</sub> (12.1%). All samples for a given individual were analysed within the same assay.

*Heat stress tests:* The 90-min exercise bouts at 40% peak power output ( $T_{db}$ , 35°C 60% RH, with a wind speed <0.5 m·s<sup>-1</sup>), were undertaken on an electro-magnetically braked cycle ergometer (Rodby Elektronik AB, Model RE 820/830, Sodertalje, Sweden). This protocol was adapted from that used by Patterson (1999). Body core temperature was measured using a rectal thermistor (Thermistor 400, Mallinckrodt Medical Inc., St Louis, USA) placed in 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 ( $\bar{T}_{sk}$ ) and mean body temperature ( $\bar{T}_b$ ) were calculated as:  $\bar{T}_{sk} = 0.3 T_{chest} + 0.3 T_{bicep} + 0.2 T_{thigh} + 0.2 T_{calf}$  (Ramanathan 1964) and  $\bar{T}_b = 0.9 T_{re} + 0.1 T_{sk}$  (Sawka et al. 1996). Temperatures were logged at 60-s intervals (1200 series, Squirrel Grant Instruments, Cambridge, England).



### *Sweat rate*

Whole body sweat rate ( $\text{ml}\cdot\text{h}^{-1}$ ) was calculated using measurements of pre- and post-exercise nude body mass using scales (accuracy 0.1 kg; resolution  $\pm 20\text{g}$ ; Wedderburn Scales, Teraka Seiko, Tokyo, Japan). Continuous measurement of sweat rate from discrete skin sites was undertaken throughout HSTs using ventilated sweat capsules glued to the forehead and dorsal forearm (Brenzelmann et al. 1975; Graichen et al. 1982).

### *Forearm blood flow, blood pressure and vascular conductance*

Forearm blood flow ( $\dot{Q}_F$ ) was measured using venous occlusion plethysmography (Witney 1953; Joyner et al. 2001) 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 auscultation, as the average of three individual measurements taken following each  $\dot{Q}_F$  measurement series, using a sphygmomanometer. 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. Forearm blood flow ( $\dot{Q}_F$ ), mean arterial pressure (MAP) and forearm vascular conductance (FVC) were measured at rest, 15, 45 and 75 min during the 90-min exercise stress test.

### *Blood measures*

Haemoglobin (Hb) mass and blood volume were measured one week before the baseline HSTs and on the 1<sup>st</sup> day after each acclimation, using the carbon monoxide (CO) dilution/rebreathing technique (Burge and Skinner 1995; Ashenden et al. 1999). This

measurement was taken in the upright position with the participant seated for a 20 minute equilibrium period. This technique is based on the principle that CO has a strong affinity with Hb, yielding carboxyhaemoglobin (HbCO), which allows Hb mass to be determined from the extent of increase in [HbCO] due to rebreathing a known mass of CO. Venous blood samples were obtained after a priming dose and again after CO rebreathing for ten minutes to obtain [HbCO]. The dosage of CO for aerobically fit males is 20 mL for the priming dose and 1.5 mL CO/kg body mass for the main dose. 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. Blood, plasma and red cell volumes derived from Hb mass were measured before the baseline standardised heat stress tests and on the first day following each acclimation. Changes in plasma volume across HSTs and acclimations were calculated using a previously described method (Dill and Costill 1974).

Duplicate colorimetric analysis (Cobas Mira Plus, New Jersey, USA) was used to analyse albumin, total protein,  $Na^+$ ,  $K^+$  and  $Cl^-$ . Osmolality was analysed using a Wescor Vapro Vapour Pressure Osmometer (5520), Wescor Inc., 459 South main Street, Logan, Utah 84321, USA.

#### *Subjects' perceptions*

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

### **Data analysis**

This research addressed the role of permissive dehydration in facilitating adaptations to the heat. Inferential analyses was performed on data collected at rest before (e.g., blood volumes) or within (e.g., heart rate) HSTs and at end-exercise in HSTs before and after each acclimation, as well as on the first and last days of each acclimation. Data was analysed using two-way analysis of variance (ANOVA) with repeated measures with nine participants. Factor one (time) had two levels for each variable, with factor two (acclimation type) having two levels. Paired t-test analysis has been 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 given and reported as means with 95% confidence intervals (95% CI) (Hopkins 2003). The relationship ( $r$ ) between variables has been calculated using the Pearson Product Moment Correlation and expressed as  $r$ .

### **Results**

All nine participants completed both acclimation regimes, the four heat stress tests (HSTs) and all procedures and measurements associated with them, 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.

## **Acclimation**

### *Thermal stress and strain*

Thermal stress and strain from day 1 and day 5 of EUH and DEH acclimation regimes are shown in Table 1. Measures of dry bulb temperature ( $T_{db}$ ) and relative humidity (RH) indicated that the thermal stress was similar within and between EUH and DEH acclimations, on day 1 and day 5. Similarly, the thermal strain was consistent between regimes illustrated by mean cardiac frequency ( $f_c$ ) and rectal temperature ( $T_{re}$ ). Time to  $T_{re}$  38.5°C was longer on day 1 EUH than DEH but by day 5 the time to  $T_{re}$  38.5°C was similar for EUH and DEH regimes. Therefore, less work was performed during the first acclimation day when comparing DEH and EUH in contrast with day 5, when work output performed in the 90 min was equivalent for DEH versus EUH (Figure 2).

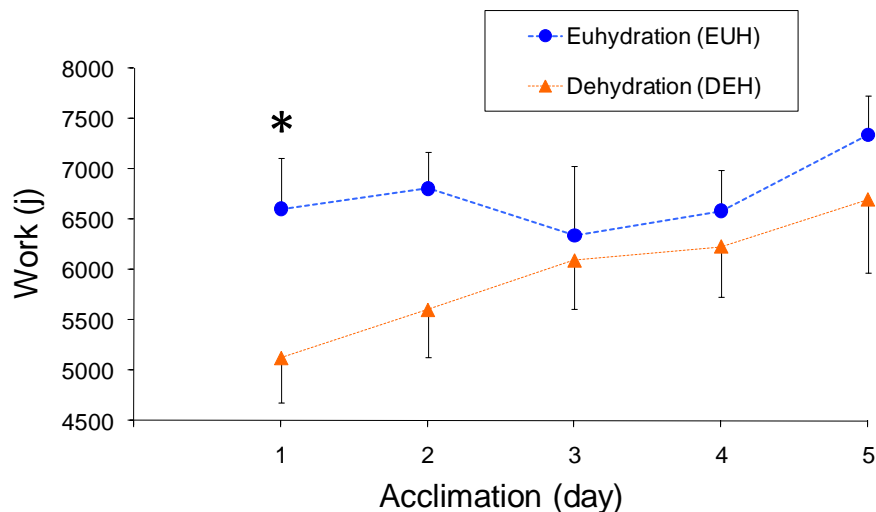
### *Fluid regulatory hormones and total protein*

Individual response data for blood measures are presented from DEH and EUH acclimation bouts for n=5, since a full cross-over of blood samples (i.e., rest and post exercise for day 1 and day 5, for both acclimations) was obtained from only five participants during the acclimation bouts (Table 2). The main effect for [aldo]<sub>p</sub> did not change at rest but at 90-min exercise there was a significant main and interaction effect between EUH and DEH. However, [AVP]<sub>p</sub> did not change across acclimation at rest or 90-min exercise. Similarly, [TP]<sub>p</sub> did not change at rest or 90-min exercise but the interaction effect between EUH and DEH was significant.

**Table 1 Thermal stress and strain.**

|                                   | 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 ±1.6   | 62 ±1.7  | 62 ±1.8   | 61 ±1.4  |    |
| Mean $f_c$ (b·min <sup>-1</sup> ) | 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 ±4.7   | 30.5±4.4 | 34±4.6    | 33±4.2   | *† |
| 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 | †  |

Dry Bulb Temperature ( $T_{db}$ ), relative humidity (RH), rectal temperature ( $T_{re}$ ), time to  $T_{re}$  38.5°C, cardiac frequency ( $f_c$ ), body mass change and fluid consumed on day one and five of acclimation undertaken with (EUH) or without (DEH) fluid rehydration. Data are mean ±SD for nine males for whom data were available across all sessions. Significant difference; day 1 versus day 5 \* ( $P<0.05$ ) and hydration regime † ( $P<0.05$ ) analysed by two-way analysis of variance (ANOVA) with repeated measures.



**Figure 2:** Work output on the first day (1) to the last day (5) of euhydration and dehydration acclimation after 90 min heat exposure. Data are shown as mean  $\pm$ SE for nine males for whom data was available across all sessions. Significant difference \* ( $p < 0.05$ ) EUH cf. DEH acclimation analysed by two-way analysis of variance (ANOVA) with repeated measures. One-way analysis of variance (ANOVA) and Tukey's post-hoc test was used to isolate differences between days of EUH and DEH acclimation.

#### *Blood, plasma and red cell volume*

Plasma volume and thus also blood volume for nine males increased across acclimation ( $P=0.02$  and  $P=0.04$ ), irrespective of whether dehydration was permitted or not (PV:  $P=0.52$ ; BV:  $P=0.40$ ). Expansion of PV across the DEH acclimation was correlated ( $r=0.65$ ) with the increase in  $[\text{aldo}]_p$  measured across the final exercise and heat bout. Applying the Dill and Costill (1974) calculation to estimate the  $\Delta$ PV across acclimations (i.e., assuming unchanged Hb mass), there was further tendency for PV to increase more in DEH ( $8.3 \pm 3.2\%$ ) than in EUH ( $4.2 \pm 3.4\%$ ) ( $P=0.06$ ; difference of  $4.1\%$ :  $-1.5$  to  $9.8$ ).

**Table 2** Change in aldosterone, AVP and total protein response across day one and five EUHydration and DEHydration acclimation

|                                      | EUHydration        | DEHydration      | Main effect          | Interaction          |
|--------------------------------------|--------------------|------------------|----------------------|----------------------|
| Aldosterone (pg.mL <sup>-1</sup> )   |                    |                  |                      |                      |
| Rest                                 | -1.6: -30 to 33    | -10: -51 to 71   | <i>P</i> =0.65       | <i>P</i> =0.74       |
| 90-min exercise                      | -47: -209 to 115   | 178: 33 to 324   | <b><i>P</i>=0.05</b> | <b><i>P</i>=0.02</b> |
| AVP (p.mol.L <sup>-1</sup> )         |                    |                  |                      |                      |
| Rest                                 | 0.1: -3.5 to 5     | 0.9: -0.5 to 2   | <i>P</i> =0.76       | <i>P</i> =0.91       |
| 90-min exercise                      | -1.3: -7 to 4      | 3: -2 to 8       | <i>P</i> =0.78       | <i>P</i> =0.10       |
| Total protein (mg.mL <sup>-1</sup> ) |                    |                  |                      |                      |
| Rest                                 | -3: -13 to 8       | -7: -20 to 6     | <i>P</i> =0.14       | <i>P</i> =0.23       |
| 90-min exercise                      | -5.9: -11.1 to 0.4 | 2.9: -4.2 to 9.9 | <i>P</i> =0.46       | <b><i>P</i>=0.03</b> |

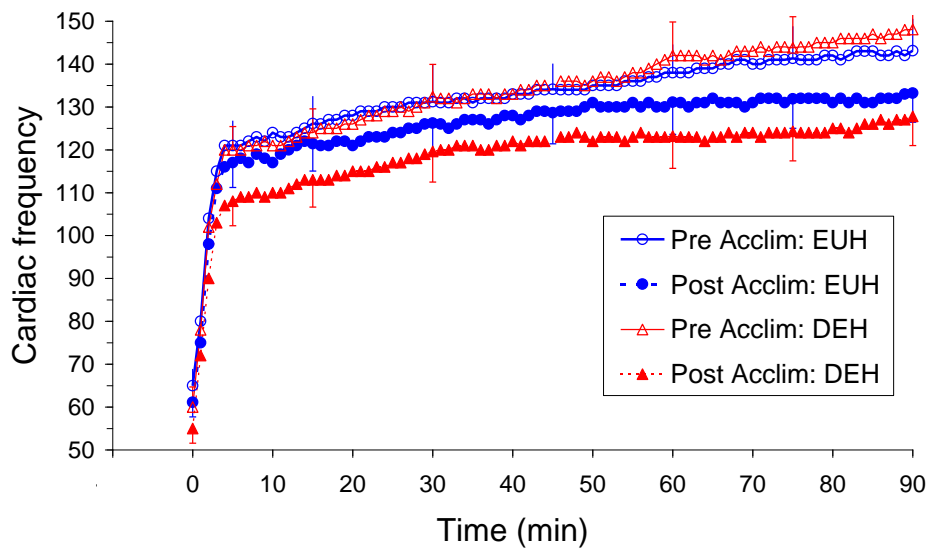
Data are shown as mean and 95% confidence interval (95% CI) for five males for whom data was available across all sessions. Significant difference \* (*P*<0.05) shown in **bold**.

Red cell volume (RCV), corrected for the volume of red cells removed during acclimation bouts did not have a significant main effect across acclimation (*P*=0.06) and the interaction effect between EUH and DEH was not significant (*P*=0.18). However, the mean change was  $1.7 \pm 1.6\%$  following EUH ( $\Delta$  0.6: -0.7 to 1.9 mL·kg<sup>-1</sup>) but  $4.1 \pm 0.9\%$  after DEH ( $\Delta$  1.5: 0.7 to 2.3 mL·kg<sup>-1</sup>). Therefore, this indicates that the difference in PV change between regimes may have been underestimated. Interestingly, 5 of 9 individuals RCV increased after EUH and all 9 of 9 participants following DEH acclimation.

## Heat stress test

### *Cardiac frequency*

End-exercise  $f_c$  (Figure 3) reduced across acclimation ( $P=0.01$ ), and this was more pronounced for DEH (-19  $\text{b}\cdot\text{min}^{-1}$ : -29 to -3) than EUH (-10  $\text{b}\cdot\text{min}^{-1}$ : -17 to 2) ( $P=0.05$ ). This reduction in  $f_c$  across DEH was moderately correlated with the extent of PV expansion ( $r=0.72$ ).



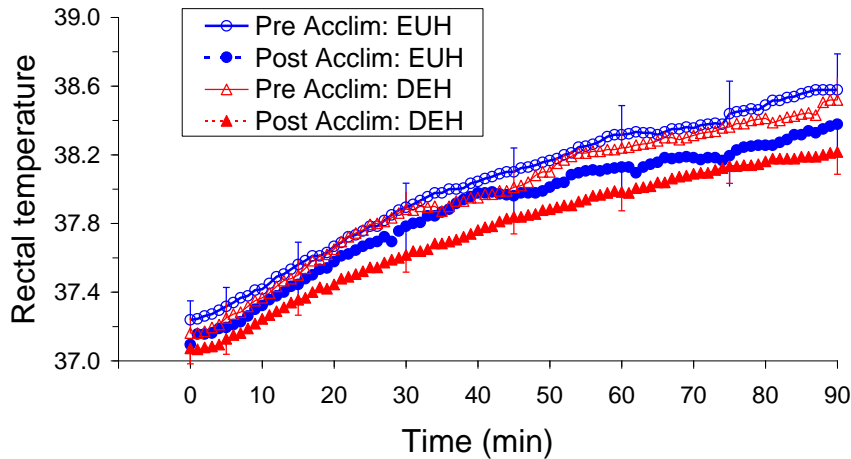
**Figure 3:** Cardiac frequency during 90-min cycling at 40% peak power output in the heat (35°C, 60% RH) before (pre) and after (post) acclimation, undertaken with (EUH) or without (DEH) rehydration during daily heat sessions. Data are means  $\pm$ SE for nine males and expressed in  $\text{b}\cdot\text{min}^{-1}$ .

### *Body temperatures*

The mean change for  $T_{re}$  at rest (Figure 4) was not lowered across EUH ( $\Delta 0.15$ : -0.40 to 0.10°C) or DEH ( $\Delta 0.00$ : -0.2 to 0.2°C). However, the main effect for  $T_{re}$  at 90-min exercise was significant ( $P=0.004$ ) with no interaction effect between EUH and DEH



( $P=0.52$ ). The mean change for  $T_{re}$  at 90-min exercise was reduced by EUH ( $\Delta -0.2$ : -0.6 to 0.05°C) and DEH ( $\Delta -0.4$ : -0.75 to -0.1°C).



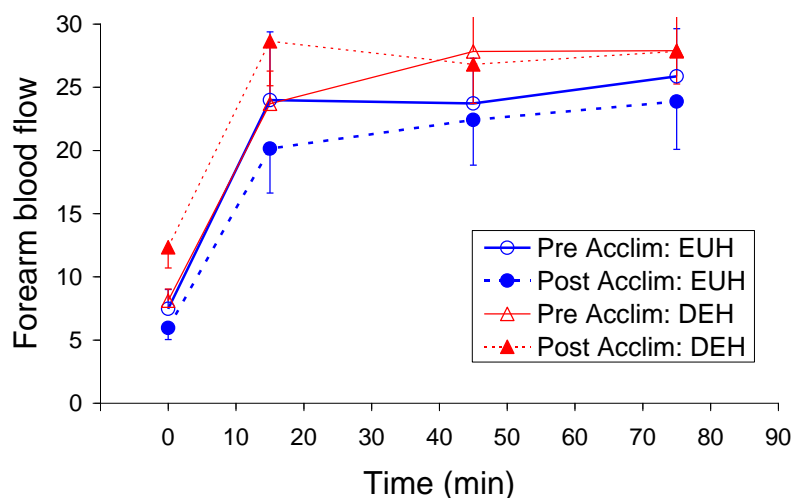
**Figure 4:** Rectal temperature during 90-min cycling at 40% peak power output in the heat (35°C, 60% RH) before (pre) and after (post) acclimation, undertaken with (EUH) or without (DEH) rehydration during daily heat sessions. Data are means  $\pm$ SE for nine males and expressed in °C.

Mean resting  $\bar{T}_{sk}$  did not change across EUH ( $\Delta 0.1$ : -0.1 to 0.5°C) or DEH ( $\Delta 0.2$ : -0.1 to 0.5°C). However, the main effect in  $\bar{T}_{sk}$  at 90-min exercise was significant ( $P=0.003$ ) but with no interaction effect between EUH and DEH ( $P=0.15$ ). The mean change in  $\bar{T}_{sk}$  after 90-min exercise decreased across EUH ( $\Delta -0.3$ : -1.1 to 0.3°C) and DEH ( $\Delta -0.4$ : -0.7 to 0.1°C). Mean change in resting  $\bar{T}_b$  response was unaltered by EUH ( $\Delta -0.2$ : -0.4 to 0.1°C) or DEH ( $\Delta 0.1$ : -0.2 to 0.1°C). The main effect for  $\bar{T}_b$  at 90-min exercise was significant ( $P=0.002$ ) but there was no interaction effect between EUH and

DEH ( $p=0.17$ ). Therefore, at 90-min exercise mean change in  $\bar{T}_b$  reduced across EUH ( $\Delta -0.2$ ;  $-0.4$  to  $0.1^\circ\text{C}$ ) and DEH ( $\Delta -0.4$ ;  $-0.6$  to  $0.1^\circ\text{C}$ ).

*Forearm blood flow, blood pressure and vascular conductance*

The main effect for resting  $\dot{Q}_F$  (Figure 5) was significant across acclimation ( $P=0.05$ ). There was a significant interaction effect for EUH and DEH ( $P=0.009$ ). The mean change for resting  $\dot{Q}_F$  increased across DEH ( $\Delta 4.2$ :  $0.7$  to  $7.8 \text{ ml}\cdot\text{min}^{-1}\cdot 100 \text{ ml}^{-1}$ ) in contrast to limited change for EUH ( $\Delta -1.5$ :  $-3.7$  to  $0.7 \text{ ml}\cdot\text{min}^{-1}\cdot 100 \text{ ml}^{-1}$ ). However, there was no main effect for exercise at 75 min  $\dot{Q}_F$  ( $P=0.58$ ).



**Figure 5:** Forearm blood flow during 90-min cycling at 40% peak power output in the heat ( $35^\circ\text{C}$ , 60% RH) before (pre) and after (post) acclimation, undertaken with (EUH) or without (DEH) rehydration during daily heat sessions. Data are means  $\pm$ SE for nine males and expressed in  $\text{ml}\cdot\text{min}^{-1}\cdot 100 \text{ ml}^{-1}$ .

Resting MAP did not change across acclimation ( $P=0.56$ ) or at 75-min exercise ( $P=0.14$ ). The main effect for resting FVC across acclimation was significant ( $P=0.05$ )

with an interaction effect between EUH and DEH ( $P=0.006$ ). Therefore, the mean change in resting FVC increased across DEH ( $\Delta$  0.06: 0.02 to 0.10 ml $\cdot$ 100ml Tissue $^{-1}\cdot$ min $^{-1}\cdot$ mmHg $^{-1}$ ), in contrast to no change across EUH ( $\Delta$  -0.02: -0.05 to 0.01 ml $\cdot$ 100ml Tissue $^{-1}\cdot$ min $^{-1}\cdot$ mmHg $^{-1}$ ). There was no main effect for FVC at 75-min exercise ( $P=0.34$ ). In summary, the resting  $\dot{Q}_F$  and FVC response were elevated across DEH versus EUH but no such effect was seen at 75-min exercise  $\dot{Q}_F$  and FVC. Therefore, it appears that the resting response of  $\dot{Q}_F$  and FVC became more pronounced after the DEH versus EUH acclimation.

#### *Sudomotor responses*

Forehead and forearm sweat rate were measured at rest and 1-min intervals during the 90-min exercise stress test. Relative increases calculated from the mean data indicate that sweating on the forehead decreased after EUH (11%) and DEH (7%). However, the main effect was not significant for sweat rate at the forehead at 90 min ( $P=0.72$ ) and there was no interaction between EUH and DEH ( $P=0.822$ ). In contrast, sweat rate at the forearm was elevated after 90 min for EUH (19%) and DEH (28%). The main effect was significant ( $P=0.01$ ) but there was no interaction effect between EUH and DEH [ $F$  ( $P=0.77$ )]. Mean changes in sweating at the forearm after 90-min exercise increased for EUH ( $\Delta$  0.3: -0.1 to 0.7 mg $\cdot$ cm $^{-2}\cdot$ min $^{-1}$ ) and DEH ( $\Delta$  0.4: 0 to 0.8 mg $\cdot$ cm $^{-2}\cdot$ min $^{-1}$ ).

#### *Subjects' perceptions*

Perceived body temperature did not change at rest across acclimation ( $P=0.09$ ). However, the main effect at 90 min was significant ( $P=0.02$ ) but there was no interaction effect between EUH and DEH ( $P=0.18$ ). Thermal comfort improved at rest and had a significant main effect ( $P=0.05$ ). There was no interaction effect between EUH and DEH

( $P=0.43$ ). Similarly, after 90-min exercise thermal comfort improved across acclimation ( $P=0.05$ ) but no interaction effect between EUH and DEH ( $P=0.38$ ). Perceived exertion was reduced after 90-min exercise across acclimation and this was significant ( $P=0.04$ ) but there was no interaction effect between EUH and DEH ( $P=0.65$ ).

#### *Fluid regulatory and stress hormones*

Mean change at 90-min exercise  $[\text{aldo}]_p$  substantially decreased ( $P=0.001$ ) across EUH ( $\Delta$  -144: -235 to -52  $\text{pg}\cdot\text{mL}^{-1}$ ) and DEH ( $\Delta$  -55: -167 to -43  $\text{pg}\cdot\text{mL}^{-1}$ ) with no difference between regimes ( $P=0.50$ ). Similarly,  $[\text{AVP}]_p$  decreased across 90-min exercise for EUH ( $\Delta$  -2.9: -6.1 to -0.2  $\text{p}\cdot\text{mol}\cdot\text{L}^{-1}$ ) and DEH ( $\Delta$  -2.1: -4.1 to 0.1  $\text{p}\cdot\text{mol}\cdot\text{L}^{-1}$ ) and this was significant ( $P=0.01$ ) but no differences between EUH and DEH ( $P=0.31$ ). There was a decrease in  $[\text{cortisol}]_p$  across 90-min exercise in EUH ( $\Delta$  -3: -40 to 13  $\text{ug}\cdot\text{dL}^{-1}$ ) and DEH ( $\Delta$  -2: -15 to 31  $\text{ug}\cdot\text{dL}^{-1}$ ) and this was significant ( $P=0.02$ ) with no difference between regimes ( $P=0.86$ ).

#### *Blood measures*

Mean change in  $[\text{TP}]_p$  after 90-min exercise was reduced across EUH ( $\Delta$  -1.8: -4.6 to 1.0  $\text{mg}\cdot\text{ml}^{-1}$ ) and DEH ( $\Delta$  -1.2: -3.0 to -0.5  $\text{mg}\cdot\text{ml}^{-1}$ ) and this was significant ( $P=0.02$ ) but with no difference between EUH and DEH ( $P=0.99$ ). There was no change in plasma albumin  $[\text{alb}]_p$  at rest ( $P=0.43$ ) and there was no interaction effect between EUH and DEH ( $P=0.10$ ). Similarly, after 90-min exercise there was no mean change ( $P=0.36$ ) across EUH ( $\Delta$  0.3: -2.9 to 3.4  $\text{mg}\cdot\text{ml}^{-1}$ ), or DEH ( $\Delta$  -1.5: -2.8 to -0.2  $\text{mg}\cdot\text{ml}^{-1}$ ) with no difference between regimes ( $P=0.27$ ). There was no change in plasma sodium concentration  $[\text{Na}^+]_p$  at rest ( $P=0.38$ ) and no interaction effect between EUH and DEH ( $P=0.11$ ). Similarly, after 90-min exercise there was no mean change ( $P=0.16$ ) across

EUH ( $\Delta$  -0.5: -1.5 to 1 mmol·L<sup>-1</sup>) or DEH ( $\Delta$  1.0: 0.5 to 2 mmol·L<sup>-1</sup>) with no interaction effect between EUH and DEH ( $P=0.10$ ). Plasma osmolality at rest did not change ( $P=0.90$ ) and there was no interaction between EUH and DEH ( $P=0.28$ ). Similarly, after 90-min exercise there was no change ( $P=0.85$ ) across EUH ( $\Delta$  -4: -22 to 13 mmol·kg<sup>-1</sup>) or DEH ( $\Delta$  4: -12 to 20 mmol·kg<sup>-1</sup>) and there was no interaction effect between EUH and DEH ( $P=0.66$ ).

#### *Exercise performance capacity*

Endurance exercise capacity was measured 10 min following the 90-min steady state exercise stress test, using an incremental protocol with 30-s step work increments to voluntary exhaustion. There was a significant main effect [F (1,14)=2.411,  $p=0.001$ ] and a large effect size (eta squared=0.857) but no interaction effect between EUH and DEH [F (1,14)=2.411,  $P=0.14$ ]. Exercise time significantly increased across acclimation by 14% in EUH ( $\Delta$  104: 57 to 150 s) and 19% for DEH ( $\Delta$  146: 101 to 191 s).

## **Discussion**

The current project was designed to determine whether adaptations to short-term heat acclimation would be more pronounced when dehydration was permitted. This study demonstrated the effectiveness of short-term (90 min·d<sup>-1</sup> for 5-d) heat acclimation, using controlled hyperthermia (38.5°C rectal temperature), attenuating thermal strain and enhancing exercise capacity in the heat. Compared with the euhydration regime, permissive dehydration during acclimation bouts conferred larger acclimation-induced increases in resting plasma volume,  $\dot{Q}_F$  and decreased  $f_c$  at the end of the standardised exercise heat stress test.

We emphasise that the present findings were obtained using permissive dehydration to a modest level of hypohydration (~1.8%); a level at which additional fluid-regulatory stimuli are already induced (Table 2) (Brandenberger et al. 1986; Brandenberger et al. 1989; McConell et al. 1997) and thirst becomes stimulated to limit excessive dehydration (Engell et al. 1987; Sawka et al. 1987; Nadel et al. 1993). Whether greater dehydration and/or a longer acclimation regime would be more beneficial or detrimental is unknown and requires further investigation, but if exercise training is an important component of the acclimation then the training impulse would become reduced (especially if using the controlled hyperthermic regime).

#### **Effectiveness of short-term acclimation to the heat**

The adaptations from short-term (5-d) heat acclimation with euhydration, 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 (Turk and Worsley 1974; Weller and Harrison 2001; Creasy 2002). It is suggested the increased capacity for work observed in the present study and the physiological adaptation to heat stress may have been enhanced by using the controlled hyperthermia technique during acclimation. This technique increases work stress progressively as the individual adapts to the heat stress conditions (Taylor 2000; Creasy 2002).

### **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  $T_{db}$  and RH measures (Table 1). Similarly, thermal strain was identical between regimes as evidenced by  $f_c$  and  $T_{re}$ . Therefore, participants experienced the same thermal load which is the basis of using the controlled hyperthermia technique for heat acclimation (Taylor 2000). Individual's hydration status was reflected on day five acclimation by total body water being maintained in EUH (~0.1% body mass) but after DEH the participants experienced a mild hypohydration of ~2% body mass (Table 1). In a review, on the fluid replacement of athletes Casa and colleagues (2000) reported that dehydration of 1-2% body weight 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 et al. 1996) and this has been considered of little consequence by some authors (Noakes et al. 1988; Greenleaf 1991), on the basis that this readily occurs during repeated heat exposure. In summary, we postulate that the increased physiological strain of restricted fluid replenishment during DEH acclimation may have resulted in adaptation of the fluid-regulatory system.

#### *Fluid regulatory hormones and electrolytes*

The neuroendocrine system regulates the volume and composition of plasma volume and intracellular fluid of red blood cells, whose essential components for the retention of  $Na^+$  and water are the renin-angiotensin-aldosterone system and AVP (Brandenberger et al. 1986). In the present study, after 90-min exercise  $[aldo]_p$  increased across DEH bouts in contrast, to a limited response after EUH acclimation and this

difference was significant. However, there was no change in  $[AVP]_p$  after rest and 90-min exercise across acclimation (Table 2). Therefore, it appears that the exercise-induced response of  $[aldo]_p$  became more pronounced in the DEH compared with the EUH acclimation bouts.

The principal effects of aldosterone are the retention of  $Na^+$  and therefore water from the urine output to maintain extracellular fluid volume and thus also blood volume. Increased  $Na^+$  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 et al. 2001). However, in the present study, an exercise-induced response of increased  $[Na^+]_p$  was not evident after acclimation for either the EUH or DEH regime, in the standardised exercising heat stress test. Therefore, this is in contrast with previous findings (Brandenberger et al. 1989; Francesconi et al. 1993; Allsopp et al. 1998) who reported a strong relationship between increased  $Na^+$  with  $[aldo]_p$  response.

Blood and PV responses showed similar findings with  $[aldo]_p$  across DEH acclimation. There was a moderate relationship ( $r=0.65$ ) between PV expansion and the increased  $[aldo]_p$  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. In the present study, the acclimation-induced resting PV was further supported by the  $\Delta PV$  (Dill and Costill 1974), across HSTs and acclimations. Resting PV increased for EUH



( $4.2 \pm 3.4\%$ ) but more so following DEH ( $8.3 \pm 3.2\%$ ) and this was significantly different between regimes.

In summary, in the present study it is proposed that the adaptive response to fluid deficit during acclimation has resulted in greater cardiovascular stability. It is suggested that in comparison with EUH, the more pronounced biological action of  $[\text{aldo}]_p$  observed during exercise in DEH had a major role in the more robust increase in PV response that was evident after that regime. This is further supported by the moderate relationship across DEH between PV expansion and  $[\text{aldo}]_p$  response after exercise. Indicating that the increased  $[\text{aldo}]_p$  response observed after DEH acclimation, may have contributed to the greater PV expansion in that regime. This further elucidates the notion that during exercise the fluid-regulatory hormone,  $[\text{aldo}]_p$ , maybe more systematically altered after a DEH acclimation regime and have a major role in acclimation-induced hypervolaemia. Therefore, it is surprising that the contribution of  $[\text{aldo}]_p$  with PV expansion has received limited attention in the literature. Of the limited information available it has been demonstrated that renal and hormonal adaptations after intense exercise participate in the initial (first 24-48 h) process of PV expansion (Nagashima et al. 2001).

#### *Blood, plasma and red cell volume*

It is accepted that exercise-induced hypervolaemia mediated by PV expansion (Senay et al. 1976; Harrison 1985) has the beneficial effect of enhancing cardiovascular and thermoregulatory responses to exercise, resulting in greater cardiovascular stability (Fellman 1992). It has been established that there are two mechanisms of PV expansion to account for such a change; increased renal electrolyte and water retention (Wyndham 1973; Costill et al. 1975; Armstrong et al. 1987; Convertino et al. 1991) and elevated

plasma protein content (Harrison et al. 1981; Harrison 1985). Therefore, it has been suggested that both these mechanisms occur during heat acclimation and their interaction may contribute to PV expansion (Patterson et al. 2004). In the present study, resting  $[TP]_p$  remained constant from the first to the last day of each of the acclimation regimes (Table 2). However, the blood volume (BV) expansion observed across EUH and DEH acclimation, indicated that resting  $[TP]_p$  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 et al. 1981; Mack and Nadel 1996). There was an increased exercise-induced response of  $[TP]_p$  in the present study that became more pronounced within the DEH compared to the EUH acclimation bouts (Table 2). It is suggested that this differential response between regimes was indicative of the fluid loss from dehydration. In summary, our data suggests that elevated  $[TP]_p$  rather than increased  $[Na^+]_p$  has a major role in the mediation of the greater acclimation-induced PV expansion observed across acclimation.

In summary, the present data indicates that during exercise the fluid-regulatory hormone,  $[aldo]_p$ , may be more systematically altered following a DEH acclimation regime. Therefore, we suggest that the increased biological action of  $[aldo]_p$  may be responsible for the greater increase in PV that was evident across the DEH regime. It is further suggested that the PV expansion observed after EUH and DEH acclimation may be mediated by elevated plasma protein content. The increased end-exercise responses of  $[aldo]_p$  across DEH acclimation are consistent with the increased PV expansion and larger drops in  $f_c$  (Figure 3) in the standardised exercising heat stress after the DEH regime.

### **Adaptation to repeated heat stress**

There was greater attenuation of end-exercise  $f_c$  after DEH (17%) compared with EUH (7%) acclimation (figure 3) and this difference was significant. The end-exercise  $T_{re}$  was lower for EUH (0.7%) and reduced after the DEH (0.8%) regime (Figure 4) with no difference between regimes. Similar findings have previously been reported using short-term heat acclimation regimes, with the controlled hyperthermia technique (Turk and Worsley 1974; Cotter et al. 1997; Patterson et al. 2004). Further, in the present study  $\bar{T}_{sk}$  and  $\bar{T}_b$  at end-exercise decreased across EUH and DEH acclimation with no difference between regimes. These physiological adaptations are characteristic features of heat acclimation (Armstrong and Maresh 1991) and reflected by greater cardiovascular stability (Wyndham et al. 1968; Strydom and Williams 1969; Senay et al. 1976; Sawka et al. 1985). In summary, these findings indicate that after acclimation per se, the cardiovascular system is not under as much physiological strain and more so after the DEH regime.

It is established that the control of  $V_s$  during exercise possibly reflects extramyocardial factors of increased blood volume (Hopper et al. 1988). It has previously been reported by Gonzalez-Alonso and colleagues (1999) that  $\dot{Q}$  reduces with dehydration and hyperthermia because of large decreases in  $V_s$  (19-27%), compared with parallel increases in  $f_c$  (5-10%). There is also a significant reduction in blood flow to the exercising muscle with dehydration during intense exercise in the heat, due to a lowering in perfusion pressure and systemic blood flow rather than increased vasoconstriction (Gonzalez-Alonso et al. 1998). More recently, it has been demonstrated

that in trained individuals, severe heat stress reduces  $\dot{V}O_{2\max}$  by accelerating the decline in  $\dot{Q}$  and mean arterial pressure, leading to reduced exercising muscle blood flow,  $O_2$  delivery and  $O_2$  uptake (Gonzalez-Alonso and Calbert 2003). Therefore, we suggest that the greater PV expansion (Dill and Costill 1974) that was evident after the DEH acclimation (8.3%), across HSTs and acclimations. May have contributed to elevations in end-exercise  $V_s$ , although this measure was not taken in the present work. In a review by Sawka and colleagues (1996),  $V_s$  augmentation has been attributed to heat-acclimation-induced PV expansion (Hales et al. 1996).

Dehydration and hyperthermia are thought to reduce central blood volume during exercise in the upright position, affecting cardiopulmonary and arterial baroreceptors, which can inhibit forearm vascular conductance (FVC) to the skin and other tissues (Gonzalez-Alonso et al. 1999a). Gonzalez-Alonso and colleagues (1999) investigated whether the deleterious effects of dehydration and hyperthermia on cardiovascular function, during upright exercise, were attenuated by elevating central blood volume with supine exercise. It has been established that there is greater  $V_s$ , increased volume of venous return and central blood volume during supine exercise (Poliner et al. 1980). Gonzalez-Alonso and colleagues (1999) observed that the characteristic features of upright exercise during dehydration and hyperthermia of decreased mean arterial pressure, FVC and increased norepinephrine were absent during supine exercise, despite equal dehydration and hyperthermia. They concluded that the reduction in FVC and the increased plasma norepinephrine concentration, independent of hyperthermia, were associated with a reduction in central blood volume during dehydration. Therefore, the mechanisms mediating the reduction in central blood volume are not fully understood but

it has been hypothesised that decreased FVC may play a greater role than skin blood flow in reducing  $V_s$  and central blood volume during exercise in the heat (Gonzalez-Alonso et al. 1999a). In the present study, cutaneous blood flow [estimated from forearm blood flow ( $\dot{Q}_F$ )] (Figure 5) and FVC at rest were elevated across DEH but not EUH acclimation, with a significant differential response between regimes. This indicates elevated forearm perfusion across the DEH acclimation and this may be due to decreased arteriolar vascular resistance rather than increased perfusion pressure.

### **Conclusions**

There were significant physiological adaptations and increased work capacity after short-term (5-day) heat acclimation. The nature of these adaptations were generally to increase heat loss rather than to lower resting body temperature, at least at the time of day at which testing took place. Several adaptations were generally more pronounced when acclimation bouts were undertaken with permissive dehydration versus a euhydration regime. This indicates that using permissive dehydration to a modest level of hypohydration (~1.8%) during acclimation, for enhanced adaptation requires further investigation.

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