Physiological Reports

Open Access

Physiological Reports ISSN 2051-817X

ORIGINAL RESEARCH

Heat acclimatization blunts copeptin responses to hypertonicity from dehydrating exercise in humans

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Keywords
Aldosterone, arginine vasopressin, iontophoresis, nanoduct.

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Funding Information
This work was supported by the National Institute for Health Research (NIHR) Comprehensive Biomedical Research Centre at Imperial College Healthcare NHS Trust and Imperial College London.

Received: 12 April 2018; Revised: 4 August 2018; Accepted: 10 August 2018

doi: 10.14814/phy2.13851

Physiol Rep, 6 (18), 2018, e13851, https://doi.org/10.14814/phy2.13851

Abstract

Acclimatization favors greater extracellular tonicity from lower sweat sodium, yet hyperosmolality may impair thermoregulation during heat stress. Enhanced secretion or action of vasopressin could mitigate this through increased free water retention. Aims were to determine responses of the vasopressin surrogate copeptin to dehydrating exercise and investigate its relationships with tonicity during short and long-term acclimatization. Twenty-three participants completed a structured exercise programme following arrival from a temperate to a hot climate. A Heat Tolerance Test (HTT) was conducted on Day-2, 6, 9 and 23, consisting of 60-min block-stepping at 50% VO_2peak, with no fluid intake. Resting sweat [Na^+] was measured by iontophoresis. Changes in body mass (sweat loss), core temperature, heart rate, osmolality (serum and urine) and copeptin and aldosterone (plasma) were measured with each Test. From Day 2 to Day 23, sweat [Na^+] decreased significantly (adjusted $P < 0.05$) and core temperature and heart rate fell. Over the same interval, HTT-associated excursions were increased for serum osmolality (5 [−1, 9] vs. 9 [5, 12] mosm·kg^{-1}), did not differ for copeptin (9.6 [6.0, 15.0] vs. 7.9 [4.3, 14.7] pmol·L^{-1}) and were reduced for aldosterone (602 [415, 946] vs. 347 [263, 537] pmol·L^{-1}). Urine osmolality was unchanging and related consistently to copeptin at end-exercise, whereas the association between copeptin and serum osmolality was right-shifted ($P = 0.0109$) with acclimatization. Unchanging urine:serum osmolality argued against increased renal action of vasopressin. In conclusion, where exercise in the heat is performed without fluid replacement, heat acclimatization does not appear to enhance AVP-mediated free water retention in humans.

Introduction

Individuals who perform strenuous physical exercise are at risk of adverse effects associated with excessive body heat ("heat illness"), ranging from incapacity to severe injury and death (Armstrong et al. 2007). In response to this threat, behavioral and physiological adjustments to regulate body temperature (thermoregulation) occur both during and after exercise–heat stress. With repeated exposures, a heat-adapted (HA) phenotype develops (Taylor 2014). This manifests reduced physiological strain from equivalent conditions of exercise–heat stress; (Horvath and Shelley 1946) restored ability to perform maximally under loads equivalent to those achieved under lower heat stress; (Taylor 2014) and protection against heat illness (Maron et al. 1977; Bouchama and Knochel 2002). Increasingly, a distinction between short (<6 days)- and long-term acclimatization (>3 weeks) is drawn to partition the benefits of progressive heat adaptation. Transition from excitatory responses, acting to compensate for

impaired cellular performance and advance adaptation pathways, toward improved metabolic efficiencies and "acclimation homeostasis", is considered the hallmark of long-term HA status (Horowitz 2016).

In addition to cardiovascular, metabolic, and other thermoregulatory enhancements (Taylor 2014), core features of heat adaptation include the lowering of sweat sodium concentration and thermal threshold for sweating, such that cooling capacity is enhanced and body sodium content conserved relative to the heat-naïve state. The increasing volume and decreasing tonicity of sweat with heat adaptation is necessarily accompanied by a progressive elevation in blood osmolality during exercise without fluid replacement (Patterson et al. 2014). This may provide a platform for enhanced plasma volume recovery with rest and subsequent drinking (Patterson et al. 2014; Mack and Nadel 2011). There are many sporting, occupational, and recreational settings in which evolving fluid deficits cannot be entirely replaced during heat stress, however, raising concern that the thermoregulatory benefits of adaptation may be undermined by acute dehydration (Sawka et al. 1983). Indeed, elevations in osmolality during higher intensity exercise are associated with delays to active vasodilation (Mitono et al. 2005; Shibasaki and Crandall 2010) and increased thermal threshold for sweating (Takamata et al. 2001), which may impair heat loss and precipitate heat illness.

In light of this apparent paradox, the question arises as to whether heat adaptation may enhance other mechanisms by which body water is conserved, such as endocrine pathways acting through the kidney. Renal reabsorption of water and electrolytes is regulated by a number of hormones, including the neurohypophyseal peptide arginine vasopressin (AVP) and the mineralocorticoid aldosterone. With moderate and higher intensity exercise, circulating concentrations of both hormones are increased (Convertino et al. 1981; Freund et al. 1991). AVP governs free water retention by acting at vasopressin V2 receptors on the distal nephron, resulting in increased insertion of acquaporin-2 water channels (AQP-2) and activation of AQP-2 gene transcription (Ball 2007). Aldosterone causes renal retention of water with sodium and also regulates reabsorption of sodium at the sweat gland, with the latter role assuming greater importance when heat stress is increased and sustained (Funder 1993; Conn 1949; Ladell and Shephard 1961; Allsopp et al. 1998).

During heat acclimatization, excursions in aldosterone from standard exercise bouts tend to diminish, yet reductions in sweat sodium concentration and content advance progressively and have been attributed to increased responsiveness of the eccrine sweat gland in the HA state (Kirby and Convertino 1986). Studies reporting changes in AVP following heat adaptation have also tended to report a decline in levels at the end of exercise (Greenleaf et al. 1983; Greenleaf 1981; Garrett et al. 2014, 2009) but whether humans show similar augmentation of AVP responses at the kidney is not known. Increased peripheral sensitivity to AVP would accord with the seasonal rise in urine and serum osmolality and increased abundance of renal medullary AQP-2 observed in desert dwelling mammals (Bozinovic et al. 2003). It also is possible that changes associated with longer term heat adaptation, such as upregulation of Heat Shock Protein responses, may influence AQP-2 expression in favor of greater AVP-mediated free water reabsorption (Lu et al. 2007). The assay of AVP is labor intensive, however, and the short half-life of AVP (<30 min) and its instability even in isolated plasma pose considerable challenges to accurate quantification. AVP is secreted in an equimolar ratio with the 39-amino acid glycoprotein copeptin (Morgenthaler et al. 2006), which demonstrates significantly greater stability ex vivo and can be measured using a one-step sandwich immunoassay (Morgenthaler et al. 2006; Christ-Crain and Fenske 2016). Copeptin has found favor as a valid and practical surrogate for AVP in health, with exercise and in various disease states (Morgenthaler et al. 2006; Christ-Crain and Fenske 2016; Mellor et al. 2015). Its concentration in peripheral blood has been proposed as a sensitive marker of the individual stress level (Katan and Christ-Crain 2008).

The acclimatization effects of living and working within a hot climate over weeks and months, compared with undertaking limited hours of laboratory acclimation over days, include optimized mechanisms of heat loss and sweating efficiency (Taylor 2014, 2006). Recent investigations have focused on the potential for permissive dehydration during exercise bouts to narrow the adaptation gap between short- and long-term acclimation, yielding mixed results (Garrett et al. 2014; Akermann et al. 2006; Neal et al. 2016). However, the question of how long-term acclimatization may affect the responsiveness and effects of fluid-regulatory hormones, specifically during exercise without fluid replacement, has been inadequately addressed. Thus, the principle aim of the present investigation was to establish whether the long-term HA phenotype is associated with altered secretion and action of vasopressin/copeptin relative to early heat–stress exposure. Secondary aims were to investigate aldosterone responses, renal function and relationships to copeptin during acclimatization.

Materials and Methods

Volunteers

The study was approved by the United Kingdom (UK) Ministry of Defence Research Ethics Committee and

complied with the standards set in the Declaration of Helsinki (Fortaleza; protocol number 531/MODREC/14). Twenty-three male military personnel were recruited from UK-based military units that had not deployed to a hot climate during the preceding 6 months. Volunteers gave written informed consent. Each volunteer completed a health history questionnaire and medical assessment with the Independent Medical Officer. Volunteers had no prior history of heat illness and were not taking vasoactive or psychotropic medications. They abstained from alcohol for 24 h before all study measures.

Baseline and familiarization measures

Volunteers attended a UK laboratory (Institute of Naval Medicine, Hampshire, England) for detailed baseline measurements and familiarization with the Heat Tolerance Test (HTT) to be performed during the main study. Measurements were undertaken during morning hours. First, volunteer height (±0.01 m), body mass (±0.001 kg) and body composition (Tanita MC 180MA Segmental Multi Frequency Body Composition Monitor Class III; Tanita UK Ltd., Yiewsley, Middlesex, UK) were recorded. A urine sample was provided for estimation of specific gravity and frozen for subsequent measurement of osmolality. Assessment of VO_2 peak was then performed in a climatic chamber (target WBGT 27°C). Volunteers were asked to run on a treadmill following a standardized ramped protocol, starting at 8 $km \cdot h^{-1}$, whereupon reaching 13 $km \cdot h^{-1}$ the gradient was increased by 2% every minute. Expired gas and gas volume was measured by on-line metabolic cart (Cosmed, Quark b^2, Rome, Italy) to determine peak oxygen consumption (peak VO_2) at the point of volitional exhaustion, whereupon the assessment was terminated. Volunteers were rested in a cool environment for ≥60 min, before returning to the chamber to undertake familiarization HTT.

Heat tolerance test

Volunteer Core body temperature (Tc) was monitored during HTT by radiotelemetry pills and paired data loggers (VitalSense, Mini Mitter Company Inc, Oregan, USA). Factory calibration (±0.01°C) of individual pills was confirmed by water bath the day prior. Volunteers were provided with a pill to swallow two hours before attending the testing facility. Pill-ingestion and adequate temperature logging were confirmed on arrival. Volunteers entered the climatic chamber on foot and rested in a standard chair for 30 min. They then performed 60 min of stepping exercise, on/off a 0.32 m-high block. In the UK, the relative exercise intensity was adjusted during the first 5 min to 50% VO_2peak. Stable absolute oxygen consumption was confirmed on three successive measurements. Adjusted stepping rate was maintained thereafter and during all subsequent HTTs, with the use of an in-ear metronome (Seiko SQ50, Seiko Instruments, Chiba, Japan) adjusted to the appropriate cadence. Stepping was performed under direct supervision of observers positioned inside the chamber. Core body temperature, heart rate, and Relative Perceived Exertion (Borg 1970) were recorded manually every 5 min during the HTT.

Deployed (main) study measures

One week after the completion of baseline/familiarization measures, volunteers deployed *en bloc* to the Mediterranean island of Cyprus. Upon arrival, standard UK Ministry of Defence (MOD) guidance on heat acclimatization was followed (https://www.gov.uk/government/publications/prevention-of-climatic-injuries-in-the-armed-forces-medical-policy). This consisted of graded exposure to exercise outdoors in the heat, with increasing levels of military dress and load carriage. HTTs were substituted in place of programmed acclimatization exercise on Day 2, 6, 9 and 23. HTT was conducted in a climatic chamber, during morning hours and at the same time of day for each individual assessed. The environment inside the chamber was maintained at WBGT index 26.6 ± 0.3°C. Outside of HTT assessments, volunteers lived and worked in natural ambient conditions. Food and and nonalcoholic beverages were readily available and provided in sufficient quantities and condition (e.g., chilled water) such that intake was ad libitum, up until arrival for and following departure from each HTT assessment period. From Day 10 onwards, volunteers were free to follow their own physical exercise regimens in addition to programmed activities (i.e., outside of working hours and on allocated rest days). Environmental conditions were recorded by WBGT monitors (Grant, Cambridge, UK) stationed inside the climatic chamber and outside the study facility.

PRE and POST measures

In Cyprus, volunteers were prepared in a room adjacent to the chamber, where they underwent study measures before (PRE) and after (POST) each HTT. Volunteers were instructed to refrain from all eating and drinking from point of arrival at the facility, until after completion of POST measurements. Volunteers were rested for 30 min on arrival, during which time sweat [Na^+] was measured from sweat stimulated by iontophoresis (Nanoduct, Wescorp). The inner surface of the forearm was cleaned with distilled water, dried and remoistened. A pilocarpine gel disc was placed on each of two electrodes, which were

strapped in position at least 2 cm apart. Where sweating rate >1 $g \cdot m^{-2} \cdot min^{-1}$ was achieved, sweat [Na+] measured by continuous flow analysis was recorded. Volunteers then voided, were weighed nude, redressed, and then rested in a standard chair for a period of six minutes. Blood was venesected from an antecubital fossa vein into a serum separator tube (serum osmolality) and EDTA tube (plasma copeptin and aldosterone), after which volunteers entered the climatic chamber. Immediately following HTT, excess sweat was towelled from their skin and they exited the chamber. POST sampling was performed in the same manner as PRE, with seated rest from three min and blood sampled from eight min following HTT. Volunteers were again weighed nude and provided a urine sample, before being allowed to eat and drink.

Blood measures

After applying a lancet and discarding the first drop, 100 μL capillary blood was collected into a plain glass capillary tube and centrifuged for 5 min. Measures were then taken with a hematocrit reader. Venous blood samples were collected from the antecubital fossa, stored in ice and centrifuged within 1 h of collection. All serum, plasma, and urine samples were frozen to −20°C and transported in dry ice back to a supraregional endocrine laboratory in the UK (Royal Victoria Infirmary, Newcastle Upon Tyne), where they were frozen to −80°C until analysis. Creatinine was measured using the Jaffe method on the Roche Modular E platform (Roche Diagnostics, Basel, Switzerland). The laboratory interassay coefficient of variation (CoV) was 1.71–4.59%. Osmolality was measured in duplicate by micro-osmometer (Advanced Model 3320, Advanced Instruments, Norwood, MA, USA) using a suppression of freezing point method (CoV 1.1%). Total serum protein was determined by colorimetric assay, using the Roche cobas c system. Copeptin was assayed using an automated sandwich immunofluorescent assay based on TRACE technology (Brahms CT-proAVP Kryptor Compact Plus, Hennigsdorf, Germany). This assay had a CoV of 2.5–3.7% and a lower limit of detection of 0.9 $pmol \cdot L^{-1}$. Aldosterone was measured in duplicate by chemiluminescent assay, using IDS-iSYS technology (IDS Ltd, Boldon, Tyne and Wear, UK). The lower limit of detection was 103 $pmol \cdot L^{-1}$. Over the range encountered, the intra-assay CoV was <8.4%.

Data analysis

Sample size was calculated using G Power 3.1 (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/). Based on the range and distribution of copeptin values reported in a similar population (Stacey et al. 2018a), a sample of 22 volunteers was required to detect a 50% rise in copeptin at end exercise between Day 2 and Day 23 in Cyprus (power 0.85, alpha 0.05, effect size 0.22). Reductions in plasma volume from PRE to POST were estimated from the increase in total protein concentration (PRE minus POST) expressed as a percentage of PRE values. To allow for potential variation in serum creatinine from changes in muscle mass over the course of the protocol, changes with HTT were expressed as a percentage of the resting (PRE) creatinine measured prior to each HTT. Statistical calculations were performed using the software package GraphPad Prism (GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego USA). Results were assessed for normality using the D'Agostino and Pearson test. Data were summarized as mean ± standard deviation (SD), except where specified. Potential relationships with changes in copeptin and aldosterone from HTT were assessed, using Pearson's (parametric data) or Spearman's (nonparametric data) coefficients. The effects of Time (Acclimatization Day) were assessed by one-way ANOVA and Friedman tests for parametric and nonparametric data, respectively. Thus, where data were distributed parametrically, the effects of Time (Acclimatization Day) and Condition (PRE vs. POST) were determined by two-way ANOVA for repeated measures (RM). A P value of <0.05 was considered significant; where a significant effect or interaction existed, Holm-Sidak (one-way ANOVA) or Dunnett's (two-way ANOVA) multiple comparisons test were applied to assess whether values differed from Day 23 (alpha = 0.05).

Results

Baseline anthropometry and resting (pre-exercise) urine osmolality values are provided with VO_2peak results in Table 1.

In-chamber measurements made at the start (t = 0 min) and end (t = 60 min) of each HTT in Cyprus are presented in Table 2, alongside changes in body mass and sweating sensitivity estimates derived from these data.

PRE and POST results are summarized by HTT in Table 3 and the corresponding difference in biochemical measurements (from PRE to POST, Δ) is presented in Table 4.

Correlation analyses of pooled HTT results for Δ copeptin and Δ aldosterone are shown in Table 5.

Relationships between copeptin and tonicity were determined in the subsample of 20 volunteers with complete POST measurements on Day 6, 9, and 23. Figure 1A shows associations between copeptin and serum osmolality, for which linear correlations existed both on Day 6 and Day 9. These showed no difference

Table 1. Baseline anthropometry, urine osmolality, VO_2peak, and heart rate at VO_2peak for n = 23 volunteers undergoing serial HTT.

Age (y)	Height (m)	Weight (kg)	Body fat (%)	Urine osmolality (mosm·kg^{-1})	VO_2peak (mL·kg·min^{-1})	Peak heart rate (b·min^{-1})
24 ± 3	1.80 ± 0.07	80.86 ± 10.62	17.01 ± 4.28	622 ± 232	55.9 ± 7.9	192 ± 9

Table 2. Core temperature (Tc), heart rate and Relative Perceived Exertion (Borg RPE, as median [IQR]) at the start (t = 0) and end (t = 60) of Heat Tolerance Tests in Cyprus (n = 23).

		Day-2	Day 6	Day 9	Day 23	P
Tc[a,b,c] (°C)	t = 0 min	37.1 ± 0.2*	37.1 ± 0.2*	37.1 ± 02**	36.9 ± 0.4	0.0057
	t = 60 min	38.6 ± 0.4***	38.3 ± 0.4***	38.2 ± 0.4*	38.0 ± 0.4	<0.0001
Heart rate[b,c]	t = 0 min	84 ± 15***	77 ± 14	78 ± 12*	70 ± 10	0.0011
(b·min^{-1})	t = 60 min	152 ± 16***	142 ± 17**	138 ± 17	133 ± 17	<0.0001
Borg RPE	t = 0 min	6 [6, 6]	6 [6, 6]	6 [6, 6]	6 [6, 6]	0.5222
	t = 60 min	13 [10, 14]**	11 [8, 15]	11 [8, 12]	10 [7, 11]	<0.0001
Sweat rate (kg·h^{-1})		1.60 [1.40, 1.80]	1.60 [1.35, 1.85]	1.75 [1.25, 2.30]	1.60 [1.35, 1.9]	0.3199
Sweating sensitivity, (kg·°C^{-1})		1.05 [0.82, 1.24]***	1.52 [1.07, 1.87]	1.62 [1.07, 1.97]	1.47 [1.21, 2.00]	0.0002

Sweat rate and sweating sensitivity derived from PRE to POST changes in body mass. Significant (P < 0.0001) [a]interaction, [b]main effect of Day, [c]main effect of HTT on 2 way ANOVA. Significant difference from Day-23, adjusted P ***<0.0001, **<0.0005, *P < 0.05.

Table 3. Body mass and biochemical measurements from PRE and POST Heat Tolerances Tests in Cyprus (n = 23 unless stated).

		Day-2	Day 6	Day 9	Day 23	P
Body mass[a,b] (kg)	PRE	81.02 ± 10.51***	80.96 ± 10.32***	80.22 ± 10.49***	80.49 ± 10.04	0.0029
	POST	79.36 ± 10.36***	79.29 ± 10.23***	78.48 ± 10.40***	78.85 ± 9.92	0.0024
Sweat [Na^+] mmol·L^{-1} (n = 19)	PRE	45 [34, 50]***	41 [31, 46]**	34 [32, 43]**	32 [29,44]	P = 0.0051
Hematocrit (%)	PRE	45 [44, 46]	44 [42, 46]	42 [41, 44]*	44 [42, 46]	0.0012
Creatinine (μmol·L^{-1})	PRE	92 [83,102]	92 [84, 108]	91 [82, 102]	88 [82, 100]	0.0670
	POST	109 [98, 118]***	112 [97, 118]**	104 [92, 115]	97 [87, 110]	0.0002
Osmolality (mosm·kg$^{-1)}$	PRE	292 [289, 296]	294 [289, 297]	292 [288, 297]	290 [288, 292]	0.1496
	POST	296 [294, 300]**	294 [291, 297]***	297 [295, 302]	300 [297, 302]	<0.0001
Copeptin (pmol·L^{-1})	PRE	5.6 [3.5, 9.7]	6.0 [3.1, 10.1]	5.5 [4.0, 10.4]	7.4 [4.0, 10.1]	0.1403
	POST	14.3 [12.5, 21.3]	15.6 [10.7, 20.9]	13.5 [10.3, 20.8]	14.7 [11.5, 21.2]	0.4853
Aldosterone (pmol·L^{-1})	PRE	197 [104, 246]	215 [103, 399]	226 [167, 322]	230 [158, 296]	0.7060
	POST	904 [621, 1124]***	582 [327, 653]**	531 [276, 790]**	347 [263, 537]	<0.0001
Total protein[a,b] (mmol·L^{-1})	PRE	70 ± 3***	69 ± 3	70 ± 3***	68 ± 3	0.0051
	POST	77 ± 4***	74 ± 3	75 ± 4*	74 ± 4	0.0006
Urine osmolality (mosm·kg^{-1})	PRE	U/A	800 ± 365	773 ± 278	819 ± 295	0.7492
(n = 17)	POST	U/A	732 ± 372	650 ± 251	746 ± 300	0.2005
Uosm:Sosm[b] (n = 17)	PRE	U/A	2.7 ± 1.2	2.6 ± 0.9	2.8 ± 1.0	0.5347
	POST	U/A	2.4 ± 1.3	2.1 ± 0.8	2.4 ± 1.0	0.1660

Significant (P < 0.05 by 2-way ANOVA) [a]main effect of Day, [b]main effect of HTT. Significant difference from corresponding value on Day-23, adjusted P ***<0.0005, **<0.01, *<0.05. U/A = results unavailable due to logistic difficulties prohibiting analysis of samples in UK.

by slope (P = 0.9746), but did exhibit a significant (P = 0.0109) rightward shift over time. Figure 1B shows corresponding data for urine osmolality and copeptin, which correlated at all time points and showed no significant differences by slope (P = 0.7077) or intercept (P = 0.6885).

Discussion

In this study, HTTs were staged across a programme of natural heat acclimatization and generated equivalent bouts of dehydrating exercise–heat stress. Novel findings were that: (1) plasma copeptin responses did not rise with

Table 4. Biochemical changes from PRE to POST Heat Tolerances Tests in Cyprus (n = 23).

	Day-2	Day 6	Day 9	Day 23	P
Δ Creatinine‡, %	18 ± 12	16 ± 10	16 ± 9	13 ± 13	0.2065
Δ Osmolality, mosm·kg^{-1}	5 [−1, 7]**	1 [0, 4]***	5 [2, 10]*	9 [5, 12]	<0.0001
Δ Copeptin, pmol·L^{-1}	9.6 [6, 15]	7.8 [3.5, 13.4]	8.4 [3.5, 10.7]	7.9 [4.3, 14.7]	0.0474
Δ Aldosterone, pmol·L^{-1}	602 [415, 946]***	209 [81, 518]	296 [128, 538]	190 [60, 330]	<0.0001
Δ Plasma volume, (%)	8.7 [6.9, 10.5]	7.3 [5.6, 10.3]	8.2 [4.1, 10.5]	8.7 [5.7, 10.8]	0.3916

Significant difference from corresponding value on Day-23, adjusted P ***<0.0005, **<0.01, *<0.05. ‡Significant linear trend, $P < 0.05$.

Table 5. Correlation analysis of pooled results from 4 Heat Tolerance Tests in Cyprus, highlighting changes in copeptin and aldosterone observed from PRE to POST (n = 23 unless otherwise stated).

	Δ Copeptin	Δ Aldosterone (pmol·L^{-1})
ΔBody mass, kg	r = 0.20, P = 0.060	r = 0.01, P = 0.929
Sweating sensitivity, kg·°C^{-1}	r = −0.12, P = 0.2420	r = −0.39, P = 0.0002
Sweat [Na+], mmol·L^{-1} (n = 19)	r = −0.08, P = 0.523	r = 0.36, P = 0.0020
ΔCreatinine, μmol·L^{-1}	r = 0.31, P = 0.002	r = 0.38, $P < 0.0001$
ΔOsmolality, mosm·kg^{-1}	r = 0.21, P = 0.0520	r = 0.10, P = 0.34
ΔPlasma volume, %	r = 0.30, P = 0.010	r = 0.44, $P < 0.0001$
ΔCopeptin, pmol·L^{-1}	–	r = 0.42, $P < 0.0001$

increasing serum osmolality across serial HTTs, (2) the postexercise relationship between AVP/copeptin and urine osmolality was unchanging with long-term acclimatization and (3) the concentrating action of AVP at the kidney, as evidenced by urine:serum osmolality, was not augmented in the HA state. Important secondary findings were of a progressive reduction in resting sweat [Na^+], demonstrated by the novel application of iontophoresis; a similar, progressive diminution of aldosterone responses throughout short- and long-term acclimatization; and significant associations between changes in aldosterone and resting sweat sodium, sweating sensitivity, and copeptin.

Impact of field conditions and development of natural acclimatization

Almost all previous studies of relevance have been conducted with adaptation to controlled laboratory settings – in which the exposure to heat stress has been limited to small numbers of hours per day – and there is a dearth of evidence in the literature reflecting the lived experience of groups such as athletes, agricultural workers, and military personnel. An important observation, therefore, was the relatively high osmolality of serum and urine measured while living, working, and adapting to the hot climate. Despite the ready availability of fluids for consumption outside of HTT assessments, resting blood osmolalities observed in Cyprus were similar to those resulting from loss of ~4% of body mass after dehydrating exercise in laboratory conditions (Maresh et al. 2004; Kavouras et al. 2006) and were comparable to levels known to elevate vasodilatation and sweating thresholds during exercise (Fortney et al. 1984).

Between Day 6 and Day 9, a step-decrement in resting sweat [Na^+] coincided with the development of serum hypertonicity following exercise, as would be expected from hypotonic sweat losses without fluid replacement (Taylor 2014; Patterson et al. 2014; Mack and Nadel 2011). HA-changes in sudomotor drive and sweat composition – acting not only during exercise, but throughout daytime activities and in warm dormitory accommodation overnight – may have resulted in greater unreplaced free water losses between HTTs than would be observed for laboratory acclimation protocols. Less than 1% variation in body mass from the UK baseline and reductions in hematocrit and total protein concentration argue significant water depletion with acclimatization, however, and instead support HA-expansion of resting plasma volume. Taken together, these data would suggest that the higher-range resting osmolalities reported in Cyprus did not signify the hypertonic hypovolemia of dehydration, but instead reflected a relatively hypertonic hypervolemic state. This may have arisen from reabsorption of sodium (under the influence of aldosterone), contributing to expansion of extracellular

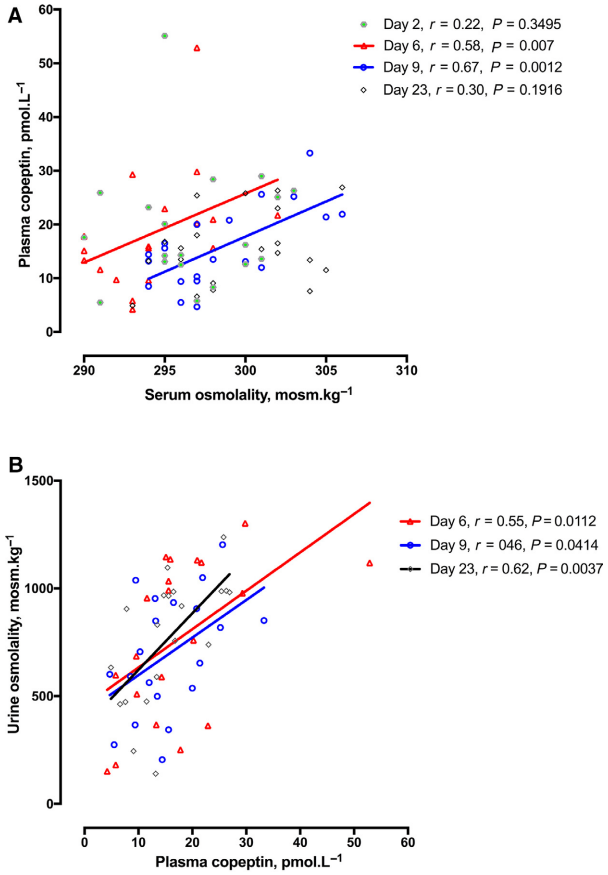

Figure 1. Biochemical associations POST Heat Tolerance Tests in Cyprus (n = 20). (A) plasma copeptin versus serum osmolality on Day 2, 6, 9 and 23 (B) urine osmolality versus plasma copeptin on Day 6, 9 and 23 (Day 2 urine results unavailable).

fluid to a greater extent than free water retention (mediated by AVP and drinking outside of HTT periods).

Influence of nonosmotic factors on AVP/copeptin response

The substantial osmotic stimulus to AVP/copeptin release with exercise was likely counterbalanced by reduced nonosmotic influences. Of the principle factors that may have fallen in magnitude – and thus blunted the response of copeptin to increasing osmotic drive – angiotensin II is a potent stimulus to the peripheral secretion of AVP (Prager-Khoutorsky and Bourque 2010). As angiotensin II also stimulates aldosterone release from the adrenal cortex, the correlation between changes in copeptin and aldosterone reported in this study (r = 0.42, P < 0.001) provides evidence for a relative decline in mutual stimulation with acclimatization. This would be expected from previous work showing that exercise-associated increases in plasma renin activity are blunted with laboratory acclimation (Francesconi et al. 1983) and seasonal acclimatization (Finberg and Berlyne 1977).

On the other hand, aldosterone responses during acclimatization were most similar at end-HTT on Day 6 and Day 9, despite the hypertonicity emerging at this transition point. In support of changes in alternative nonosmotic stimuli, there was a rightward shift in the copeptin–serum osmolality relationship (Fig. 1A) similar to that observed with increased plasma volume and reduced baro-afferent signaling to AVP release (Dunn et al. 1973). This would be consistent with the influence of an enlarged intravascular compartment and would explain the failure of copeptin to rise relative to the increased osmolalities observed with acclimatization. Following prolonged exercise bouts with ad libitum drinking, Hew-Butler et al. (2011) found that copeptin-related more strongly to changes in plasma volume than AVP, which remained more tightly coupled with osmolality; this may reflect differing rates of degradation or clearance, as the half-life of circulating copeptin is considered to be twice that of AVP (26 vs. 12 min) (Fenske et al. 2018).

The act of drinking is known to override both osmotic and volume stimuli to AVP/copeptin release (Morgenthaler et al. 2006; Thompson et al. 1987) yet previous investigators reporting a decline in postexercise AVP with acclimatization have allowed fluid intake during exercise (Greenleaf et al. 1983; Greenleaf 1981; Garrett et al. 2014). Indeed, blunted AVP responses to standard exercise bouts have been explained on the basis of diminishing involuntary dehydration and increasing fluid intake with heat adaptation (Greenleaf et al. 1983). The design of this study, in which participants refrained from fluid intake during exercise and until POST measurements were complete, allowed for an assessment of the integrated effects of exercise in the heat, without interference from drinking. However, it was not possible to isolate endogenous stimulatory or inhibitory factors to AVP/copeptin release. For example, the decline in end-exercise Tc with acclimatization may have reduced osmotic stimulus, as the osmoreceptor is known to show greater sensitivity at higher tissue temperatures (Takamata et al. 1995; Sladek and Johnson 2013). We have also reported a threshold effect of exercising Tc on copeptin response, independent of serum osmolality (Stacey et al. 2018a). The addition of a resting osmotic load test to our protocol may have helped to more definitively locate a change in osmotic sensitivity with acclimatization, independent of body temperature, but was not achievable within the constraints of the deployed study.

Renal concentrating ability with long-term acclimatization

Nevertheless, the relationships reported between copeptin, tonicity, and physiological strain are valid for the real-world context that we sought to investigate and are relevant to current clinical challenges, such as the emerging problem of heat–stress nephropathy (Kupferman et al. 2018) and the potential role of excessive AVP in the pathogenesis of chronic kidney disease (Bankir et al. 2013; Garcia Arroyo et al. 2017). They also raise the question of whether end-organ responsiveness to AVP increased concurrent to the emerging hypertonicity of exercise. In light of the dominant influence of osmolality over blood volume in determining core temperature elevation from exercise in the heat (Montain and Coyle 1992), enhanced renal conservation of free water could provide support to limiting excursions in blood osmolality and the associated risk of thermoregulatory embarrassment during exercise without fluid replacement. In this study, absolute values of urine osmolality were substantially higher than generally reported in laboratory acclimation studies – and the upper limit of renal concentrating ability was approached in some volunteers (Fig. 1B) – but the potential for further increases in urinary concentration that existed in the majority was not fulfilled with acclimatization. This indicates that long-term heat acclimatization does not augment the action of AVP at the kidney and that peripheral sensitivity at the V2 receptor is unchanged in the HA state.

While AVP is elevated by and appears to retain an influence on renal concentrating ability during exercise (Takamata et al. 1994; Hew-Butler et al. 2014) a consistent and seemingly paradoxical increase in free water clearance has been demonstrated both with submaximal and maximal intensity work (Wade and Claybaugh 1980; Wade 1980; Melin et al. 2001). Impaired action of AVP at the renal collecting duct with exercise has been considered the most likely explanation and is indicated by a fall in the ratio of urine to serum osmolality, which was observed as a main effect of HTT in this study. This finding has been proposed to result from reduced glomerular filtration rate (Wade 1980; Melin et al. 2001) antagonism by exercise-induced prostaglandins (Wade 1980), or changes in natriuretic peptides (Melin et al. 2001). Its persistence from short to long-term acclimatization implies a positive biological function, which may outweigh any potential benefits from greater free water retention in the HA state. Indeed, the blunting of AVP–copeptin responses to hyperosmolality, without increased renal responsiveness, may reflect HA protection from kidney injury due to recurrent bouts of vasopressin-dependent hyperfiltration (Bankir et al. 2013; Garcia Arroyo et al. 2017). This hypothesis should be investigated in a future study with the capacity to measure complete urinary volumes and quantify free water and creatinine clearance.

Methodological considerations

The assumption that copeptin reflected AVP is supported by preserved relationships with urine osmolality post-HTT and also by previous work demonstrating the applicability and appropriateness of copeptin as a surrogate for AVP following exercise (Mellor et al. 2015). To the best of our knowledge, the use of serial iontophoresis during heat acclimatization has not been reported previously. In addition to simplifying post-HTT procedures during a busy protocol, this approach was considered likely to reduce confounding from regional collection of sweat during exercise, as the use of occlusive dressings may leach electrolytes from the skin and falsely elevate $[Na^+]$ concentrations (Weschler 2008). Perhaps for this reason, the electrolyte values determined from pharmacologically induced sweating may vary from thermal or exercise-associated sweat (Baker 2017). The relationships reported in this work between changes in sweat $[Na^+]$ and serum osmolality are mechanistically plausible, however, and provide support for the use of this technique as an aid to evaluating heat adaptation. A limitation is the requirement to maintain a collection rate >1 $g \cdot m^{-2} \cdot min^{-1}$, in order to avoid confounding from low sweat volume (LeGrys et al. 2007; Goldberg et al. 2010) and time constraints meant that data was lost from volunteers in whom this could not be achieved.

This investigation employed a traditional method of exercise under constant thermal stress, in order to assess changes in hormonal actors that are known to increase in proportion to the relative intensity of exercise (Convertino et al. 1981; Freund et al. 1991). This was intended to control for a number of independent variables and avoid confounding from increasing intensity and duration of exercise, as may result from constant strain assessments. Heat Tolerance Tests were, however, embedded in an acclimatization program that aimed to progressively increase thermal stress in the 8 days following arrival to Cyprus (Online Appendix 1 Table i). Moreover, exposures to field training later in the study (WBGT during training hours 28.6 ± 0.8°C) achieved the requisite 1°C elevation in Tc considered necessary for maintaining adaptation stimulus. It is argued, therefore, that this hybrid approach allowed for a valid assessment of the effects of advancing heat acclimatization on copeptin, aldosterone and associated biochemical responses to dehydrating exercise–heat stress.

An unanticipated finding, which was possibly attributable to this model of diminishing physiological strain, was

unchanging loss of body mass from exercise-induced sweating with acclimatization. Unchanging contraction of plasma volume with HTT was consistent with the similar sweat rates observed, as Patterson et al. (2014) have shown that exercising fluid losses derive primarily from the intravascular space during extended heat adaptation. Important factors that may have had a bearing on these results include the progressive reduction in body temperature at end-HTT, leading to reduced absolute sudomotor drive, and the higher osmolalities associated with the HA-phenotype, which may have elevated the thermal threshold for sweating. In keeping with the changes in AVP/copeptin response to serum osmolality reported above, Takamata et al. (2001) indicated how other effects of hyperosmolality may be blunted in the long-term HA state, demonstrating reduced inhibition of thermal sweating and cutaneous vasodilation with hypertonic saline infusion and passive heating. It is likely, therefore, that the unchanging loss of body mass with long-term acclimatization derived predominantly from lower Tc stimulus to sudomotor drive.

Conclusions

This investigation builds on the body of work supporting a relative reduction in fluid-conserving responses in the HA phenotype (Patterson et al. 2014; Takamata et al. 2001; Ichinose et al. 2005; Merry et al. 2008; Mayer et al. 2015). Our novel finding of blunted copeptin response to hyperosmolality with heat acclimatization echoes other investigations showing reduced elevations for AVP as a function of plasma osmolality in trained versus untrained subjects exposed to exercise–heat stress (Merry et al. 2008) and reduced ratio of copeptin to serum $[Na^+]$ after 4 weeks of endurance training (Mayer et al. 2015). On one hand, this places the adaptive burden of regulating tonicity on drinking and underlines the importance of superior HA behaviors in responding to thirst, during and at the end of exercise in the heat (Taylor 2014; Periard et al. 2015). On the other hand, the HA phenotype appears more tolerant of dehydrating exercise and hyperosmolality, as demonstrated by improved thermoregulation, diminished cardiovascular strain and reduced adrenal and autonomic responses during exercise without fluid replacement (Patterson et al. 2014; Stacey et al. 2018b). Thus physical performance and health appear not to be protected by increased neuroendocrine stimulus to free water retention in the long-term HA state, but may be more reliant on behavioral regulation of fluid intake with exercise in the heat. However, whether long-term biological benefits arise from the relative blunting of AVP–copeptin responses observed in such circumstances merits further investigation.

Acknowledgments

The following organizations and individuals are acknowledged for their support: the study volunteers and enabling elements of 3rd Battalion The Parachute Regiment, 4th Battalion The Rifles, 45 Commando Royal Marines and Defence Equipment & Support; Surgeon General's Research Steering Committee and Joint Medical Command; the staff of the Biochemistry Department at the Royal Victoria Infirmary, Newcastle-upon-Tyne. The views expressed are those of the authors and not necessarily those of the NIHR, the National Health Service or the UK Department of Health.

Conflict of Interest

No conflicts of interest are declared.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix