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## Title

Variation in renal responses to exercise in the heat with progressive acclimatisation

## Short title

Renal function and heat acclimatisation

Authors (no conflicting or competing interests declared)

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## **Abstract**

**Objectives:** To investigate changes in renal status from exercise in the heat with acclimatisation and to evaluate surrogate markers of Acute Kidney Injury.

**Design:** Prospective observational cohort study.

**Methods:** 20 male volunteers performed 60 min standardised exercise in the heat, at baseline and on four subsequent occasions during a 23-day acclimatisation regimen. Ratings of Perceived Exertion were reported every 5 minutes during exercise. Blood was sampled before and after exercise for serum creatinine, copeptin, interleukin-6, normetanephrine and cortisol. Fractional excretion of sodium was calculated for corresponding urine samples. Acute Kidney Injury was defined as serum creatinine rise  $\geq 26.5 \mu\text{mol.L}^{-1}$  or fall in estimated Glomerular Filtration Rate  $>25\%$ . Predictive values of each candidate marker for developing Acute Kidney Injury were determined by ROC analysis.

**Results:** From baseline to Day 23, serum creatinine did not vary at rest, but showed a significant ( $P<0.05$ ) reduction post-exercise ( $120 [102, 139]$  versus  $102 [91, 112] \mu\text{mol.L}^{-1}$ ). Acute Kidney Injury was common (26/100 exposures) and occurred most frequently in the unacclimatised state. Log-normalised fractional excretion of sodium showed a significant interaction (exercise by acclimatization day), with post-exercise values tending to fall with acclimatisation. Ratings of Perceived Exertion predicted AKI (AUC 0.76, 95% confidence interval 0.65-0.88), performing at least as well as biochemical markers.

**Conclusion:** Heat acclimatization is associated with reduced markers of renal stress and AKI incidence, perhaps due to improved regional perfusion. Acclimatisation and monitoring Ratings of Perceived Exertion are practical, non-invasive measures that could help to reduce renal injury from exercise in the heat.

**Keywords:** heat stroke; renal insufficiency, chronic; thermotolerance; cytokines; vasopressins.

## Practical Implications

1. With bouts of exercise in the heat sufficient to elicit moderate levels of dehydration, changes in serum creatinine meet the diagnostic threshold for Acute Kidney Injury at a high frequency in unacclimatised subjects. This effect is substantially and progressively reduced with effective heat acclimatisation.
2. Serum creatinine changes meeting the criteria for AKI diagnosis do not associate with greater haemoconcentration than subthreshold changes, but do associate with elevated markers of cardiovascular strain and systemic inflammation. This indicates the potential role of factors other than plasma volume loss in reducing regional bloodflow and impacting upon kidney function.
3. Ratings of Perceived Exertion perform equally well to these biological markers in discriminating renal status. Effective heat acclimatisation regimens, perhaps coupled with RPE monitoring, could play a role in preventing renal injury.

## Introduction

High-intensity or prolonged exercise can generate significant thermal stress and give rise to a spectrum of heat-related disorders, especially in hot environments.<sup>1,2</sup> Heat stroke is the most severe and life threatening manifestation of heat illness and may be defined as a form of hyperthermia associated with a systemic inflammatory response, leading to a syndrome of encephalopathy and multi-organ dysfunction. This clinical picture commonly includes renal failure,<sup>1,3</sup> the severity of which may be classified by international consensus criteria using the magnitude of increase in serum creatinine, decrease in glomerular filtration rate (GFR) or reduction in urine output.<sup>4,5</sup>

It is increasingly appreciated that, even in the absence of frank incapacitation or debility, the combination of exercise and heat stress can result in changes in serum creatinine (sCr) and other biomarkers that are compatible with Acute Kidney Injury (AKI).<sup>6-8</sup> While severe AKI requiring medical intervention is rare following prolonged endurance exercise, significant increases in sCr are common with an average rise of 29 (+/-12.3)  $\mu\text{mol/L}$  (above the threshold for stage I AKI) following a marathon or ultra-marathon.<sup>7</sup> In marathon running,<sup>9</sup> endurance cycling<sup>8</sup> and submaximal exercise bouts of shorter duration,<sup>6,10</sup> sCr has been reported to rise in association with direct markers of renal tubular injury, such as neutrophil gelatinase-associated lipocalin (NGAL)<sup>8-11</sup> and kidney injury molecule-1 (KIM-1).<sup>9,11</sup> This has raised concern that repeated bouts of prolonged, strenuous exercise could contribute to long-term renal dysfunction.<sup>7</sup> Indeed, in populations exposed occupationally to strenuous physical activity in the heat, recurrent bouts of AKI - identified by elevations in sCr alone - have been associated with later development of chronic kidney disease (CKD).<sup>12,13</sup> This has been recognised as a serious global health problem ([http://www.regionalnephropathy.org/wp-content/uploads/2016/08/MeN-2015-Scientific-Report-high-resolution\\_final.pdf](http://www.regionalnephropathy.org/wp-content/uploads/2016/08/MeN-2015-Scientific-Report-high-resolution_final.pdf)), with the potential to deepen and expand in line with regional and world climate change predictions.

While a proportion of the rise in sCr following exertion may reflect muscle breakdown with increased solute load rather than just the fall in GFR, sCr is considered to under-estimate the true decrease in GFR that accompanies exercise.<sup>14</sup> This reduction occurs in association with decreased renal perfusion, with regional vasoconstriction from moderate intensity exercise reducing renal blood flow by 25% or more compared with resting values.<sup>15</sup> Volume depletion, secondary to sweating and hypohydration, is another mechanism by which renal perfusion is diminished, therefore high ambient temperatures increase the risk of exercise-associated AKI.<sup>1,7,16</sup> Rarely, AKI can occur with skeletal muscle damage (exertional rhabdomyolysis)<sup>17</sup> due to the release and precipitation of myoglobin in the kidney.<sup>8,16</sup> Despite this understanding of the various pathways to reduced kidney function and/or AKI from exercise in the heat, the impact of progressive heat adaptation on renal physiology and pathophysiology has not been well explored. Thus our principle aim was to investigate how renal function might vary with repeated bouts of exercise-heat stress conducted over a programme of structured, progressive heat acclimatisation (HA).

The measures necessary to make a diagnosis of AKI need to be taken over an extended period of time, e.g. blood tests for sCr before and after an exercise bout,<sup>4,5</sup> or collection of urine to show a threshold reduction ( $<0.5 \text{ mL.kg}^{-1}.\text{h}^{-1}$ ) over a period of at least six hours.<sup>4</sup> In field settings, this can also lead to difficulties in establishing the true incidence and magnitude of AKI as it may be difficult to access target populations, for example: at the start of an agricultural shift, during firefighting, in the middle of a military exercise or on a prolonged (recreational or competitive) endurance event. Therefore developing a 'spot marker' that reflected instantaneous renal status, without the requirement for recent antecedent measurements, could facilitate practical surveillance and perhaps help to prevent adverse outcomes following participation. For example, prompt treatment of pre-renal AKI with rehydration, or protection of individuals with nascent AKI from further exercise-heat stress, might serve to mitigate short- and longer-term complications such as acute metabolic insult or unrecoverable loss of renal function.

In keeping with observations of higher heart rate and body core temperature (T<sub>c</sub>) in agricultural workers affected by AKI,<sup>16</sup> an optimal biomarker of renal risk from heat stress might be expected to reflect the contribution of thermoregulatory strain to renal hypoperfusion and early tubular injury.<sup>18</sup> Candidate biomarkers that are activated in the neurohumoral response to exercise and heat stress include the adrenal hormones cortisol and noradrenaline and the posterior pituitary hormone arginine vasopressin (AVP). Additionally, the cytokine interleukin-6 (IL-6) is reported to rise with physical exertion, reflecting the magnitude of elevation in T<sub>c</sub>, and has been implicated in the inflammatory responses known to drive and progress AKI.<sup>19,20</sup> The rise in IL-6 above baseline has also been shown to parallel that of sCr at 24 hours following a marathon event and plasma values correlate highly ( $r=0.9$ ) with NGAL following shorter duration exercise in the heat.<sup>10</sup>

Whereas the collection, processing and assay of cortisol and IL-6 are relatively straightforward, due to their greater stability *ex vivo* and longer half-lives, both noradrenaline and AVP are subject to rapid metabolism in the blood and require complex measurement techniques for quantification. Surrogate markers of the systemic release of noradrenaline (free normetanephrine) and AVP (copeptin) have been shown to provide practical and appropriate alternatives in the investigation of exercise and external environmental stress,<sup>21,22</sup> having more favourable analytical profiles and – in the case of copeptin, cortisol and IL-6 – emerging potential for point-of-care measurement in the field.<sup>23-25</sup> Accepting the limitations of using changes in sCr for diagnosis, we hypothesized that AKI from exercise in the heat would associate with elevated levels of these neurohumoral and inflammatory markers, relative to bouts with no AKI (nAKI). In addition, as self-reported Borg Ratings of Perceived Exertion (RPE) increase with elevated heat stress, this simple index was also included alongside the biochemical markers. Thus, in addition to our primary aim of evaluating the impact of HA and its associated adaptations on AKI incidence, we aimed to secondarily investigate the discriminatory value of post-exercise blood measures (cortisol,

free normetanephrine, copeptin and IL-6) and RPE in the identification of AKI from exercise-heat stress.

## Methods

The ethical approval for this project was obtained by the United Kingdom (UK) Ministry of Defence (MoD) Research Ethics Committee (protocol number 531/ MoDREC/ 14) and conformed with the standards set in the declaration of Helsinki. The conduct of military activities undertaken was further subject to the MoD Joint Service Publication 539 on climatic illness prevention (<https://www.gov.uk/government/publications/prevention-of-climatic-injuries-in-the-armed-forces-medical-policy>). All participants gave written informed consent.

With the exception of IL-6 assays and the determination of AKI status, the methods used in this study and a range of parallel findings compatible with declining strain from HA have been reported previously.<sup>22,26</sup> A cohort of male United Kingdom (UK) military personnel were recruited, of whom 20 provided complete measures relevant to the analysis. Volunteers first attended the UK Institute of Naval Medicine to undergo baseline anthropometric measures, peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ) assessment and Heat Tolerance Test (HTT). This first assessment was defined as HTT1 in relation to later HTTs, which were conducted during HA in Cyprus. Each HTT comprised block-stepping for 60 minutes at relative exercise intensity (50%  $\text{VO}_{2\text{peak}}$ ), with stepping rate calibrated at five minutes after three successive stable measurements of  $\text{VO}_2$  and maintained thereafter with the aid of an in-ear metronome (Seiko SQ50, Seiko Instruments, Chiba, Japan). All exercising measures were undertaken in a climatic chamber (WBGT 27 °C). Volunteers were instructed to refrain from eating and drinking from point of arrival for study measures pre-HTT, until after completion of measurements post-HTT. This meant that HTT was performed without replacement of fluid losses. RPE was recorded manually every 5 minutes during HTT, by investigators stationed inside the chamber.



Volunteers were deployed to Cyprus two weeks later, where they undertook the standard UK military HA program followed by graded exposure to structured occupational training (Table i and ii, Online Appendix). Programmed exercise was substituted for HTTs in a local climatic chamber on Day 2, 6, 9 and 23 (corresponding to HTT2, HTT3, HTT4 and HTT5). These deployed HTTs were conducted in the same fashion as in the UK, including PRE and POST blood sampling and maintenance of the stepping rate determined in the UK, again by metronome and under the direct observation of investigators.

Venous blood and urine were sampled at resting baseline (PRE) and 8 minutes after each HTT (POST). Samples of serum (sodium, creatinine, total protein, cortisol, IL-6) and plasma (free normetanephrine, copeptin) were centrifuged and frozen with urine samples (creatinine, [Na<sup>+</sup>]) to -20 °C until analysis. Sodium (Na) and creatinine were measured by the Jaffe method on the Roche Modular E platform (Roche Diagnostics, Basel, Switzerland). The laboratory interassay coefficients of variation (CoV) were 0.6-1.7%. and 1.71–4.59%, respectively. Total protein was determined by colorimetric assay, using the Roche cobas c system. Changes in plasma volume were then estimated from the relative change in total serum protein concentration. Cortisol was assayed by competitive immunoassay (Roche) using fully automated electrochemiluminescence technology (functional sensitivity < 8.5 nmol L<sup>-1</sup> ). IL-6 concentration was determined by single-molecule counting technology using a quantitative fluorescent sandwich immunoassay (Erenna, Singulex, Inc.) with CoV 2–8%.(30) Plasma free normetanephrine was measured using an in-house liquid chromatography/tandem mass spectrometry method (CoV 4–12%). Copeptin was assayed by TRACE technology using an automated sandwich immunofluorescent assay (Brahms CT-proAVP Kryptor Compact Plus, Hennigsdorf, Germany), with CoV of 2.5–3.7% and a lower limit of detection of 0.9 pmol\_L<sup>-1</sup>.

Urinary fractional excretion of Na (FENa) was determined from measured concentrations of sCr, serum Na (sNa), urine creatinine (uCr) and urine Na (uNa), as:  $(uNa \times sCr)/(sNa \times uCr)$

x 100. Estimated GFR (eGFR) was calculated from sCr using the CKD Epidemiology Collaboration (CKD-EPI) formula, using an iteration appropriate to the study population and observed sCr range:<sup>27</sup>

$$\text{eGFR (mL}^{-1}\cdot\text{min}^{-1}\cdot 1.73 \text{ m}^{-2}) = 141 \times (\text{sCr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$$

Exposures were classified as AKI or no AKI (nAKI), using two definitions applied commonly in clinical practice: the Kidney Disease Improving Global Outcomes (KDIGO) criteria, which define AKI as a sCr rise  $\geq 26.5 \mu\text{L}^{-1}$  and the RIFLE (Risk, Injury, Failure, Loss, Endstage Renal Disease) criteria, where the threshold for AKI is a fall in eGFR  $>25\%$ .<sup>4,5</sup>

Statistical analyses were performed using GraphPad Prism version 7.0d for Mac (GraphPad Software, San Diego USA). Data were assessed for normality using the D'Agostino and Pearson test. One-way ANOVA and Kruskal-Wallis analyses were used to assess changes in biomarkers and eGFR with acclimatisation. Paired t-test (parametric data) or Mann-Whitney (non-parametric data) were used to assess whether POST variables differed by AKI status across the combined HTTs. Two-way ANOVA (HTT by HA status) was performed for repeated measures of log-normalised FENa. Receiver operating characteristic (ROC) curves were plotted to investigate the performance of individual POST variables in reflecting AKI. Statistical significance was specified as  $\alpha=0.05$ .

## Results

The 20 volunteers were age  $25 \pm 3$  years old and had body mass index  $25.0 \pm 2.6 \text{ kg.m}^{-2}$ , percentage body fat  $16.9 \pm 4.9 \%$  and  $\text{VO}_{2\text{peak}}$   $56.7 \pm 8.8 \text{ mL.kg}^{-1}.\text{min}^{-1}$ . As reported previously, this HA regimen was associated with significant reductions in physiological strain and evidence for appropriate adaptation in Cyprus, including significant reductions in heart rate, body core temperature ( $T_c$ ), autonomic excitability, sweat  $[\text{Na}^+]$  and haematocrit.<sup>22,26</sup>

Table 1 displays PRE and POST results for sCr and candidate biomarkers. With HA, the fall in eGFR from HTT varied significantly ( $P < 0.0001$ ), showing a negative linear trend (slope -4.2,  $P < 0.05$ ). Across all 5 HTTs, 18 episodes of AKI were identified according to KDIGO criteria, showing a mean rise in sCr of  $38.7 \pm 10.3 \mu\text{mol/L}$ . Defining AKI by RIFLE criteria added a further 8 instances (mean loss of eGFR  $32.3 \pm 6.0\%$ ), such that AKI affected 26 out of 100 exposures. Other data pertaining to the performance of the Heat Tolerance Test are displayed in Table iii (Online Appendix). Corresponding results from the more extended bouts of field acclimatisation exercise that were undertaken in the later stages of HA are provided for reference in Table iv (Online Appendix).

Logistical challenges prevented urine sampling entirely in relation to HTT2 on Day 2 in Cyprus; on other occasions, a small number of volunteers failed to produce a sample. This reduced both PRE and POST urine samples to 73/100 exposures, with asymmetrical loss reducing the completeness of PRE-POST sampling to 11 out of 20 volunteers across four HTTs (HTT1, HTT3, HTT4 and HTT5). For this subset, log-normalised FENa showed a significant main effect of HTT ( $F=22.9$ ,  $P=0.0007$ ) and a significant interaction (HTT by heat

acclimatisation status,  $F=3.3$ ,  $P=0.0341$ ), with post-exercise FENa tending to increase with progressive HA (adjusted  $P=0.097$  for UK baseline vs Day 23 in Cyprus).

For combined HTT exposures ( $n=100$ ), Figure 1 displays the variables of interest dichotomised by AKI status. In every POST parameter except plasma volume loss, AKI-associated values were significantly greater than nAKI. The AUC [95% confidence interval] from ROC analysis (AKI vs nAKI) was 0.76 [0.65 - 0.88] for RPE; 0.65 [0.52 - 0.78] for copeptin; 0.74 [0.63 - 0.86] for IL-6; 0.77 [0.66 - 0.87] for normetanephrine; and 0.69 [0.57 - 0.82] for cortisol. Cut-off values for the diagnosis of AKI, with corresponding sensitivity and specificity, are displayed in Table v of the Online Appendix.

## Discussion

We believe this is the first study in which the risk of AKI has been systematically assessed in relation to human heat adaptation. Our results show that AKI is prevalent in unacclimatised volunteers performing standardized bouts of exercise-heat stress at baseline, but is almost entirely absent after the first week of HA has been achieved. Given the association of early AKI, as defined by sCr rise, with later CKD in agricultural workers undertaking hot-season harvesting,<sup>12,13</sup> our results provide novel evidence to support the implementation of graded HA protocols in protecting at-risk groups. This could potentially encompass athletes intermittently travelling to and from hot environments, who in the intervening periods may be unable to maintain adequate heat adaptation i.e. acclimatization specific to the thermal stress encountered when training and competing in the heat.<sup>28</sup> Significant differences by AKI status were also observed in RPE, neurohumoral and inflammatory markers, with the performance of RPE being comparable with that of the other candidate biomarkers. This reinforces and develops existing evidence for RPE as a practical, non-invasive means of identifying and protecting against deleterious levels of physiological strain.<sup>29</sup>

In the present work, practical limits to volunteer availability and sample shipping volumes prevented the continuous collection of urine, which could otherwise have been of utility in deriving creatinine clearance and further identifying and characterising AKI. In mitigation, FENa was calculated from PRE and POST samples of blood and urine, as a marker of the renal response to changes in volume status and regional bloodflow. The significant

interaction of exercise-heat stress (from HTT) with day (HA status), indicated a reduction in the impact of exercise on FENa and improving renal perfusion as adaptation progressed. This finding was paralleled in the post-exercise association of lower FENa with AKI, pointing to a pre-renal reduction in kidney function in those with greater excursions in sCr.

This finding appeared to stand in contrast, however, with the lack of differential haemoconcentration by renal status, which would argue against absolute changes in hydration status being the leading factor contributing to AKI in this setting. The same protocol was associated with a fall in resting haematocrit in these volunteers<sup>22,23</sup> however, and it may be that increased plasma volume with HA contributed to greater renal perfusion during exercise. It has been suggested that cardiovascular adaptations to exercise training – which, as with HA,<sup>21,26</sup> may include increased plasma volume, diminished vasopressin/copeptin responses and reduced sympathetic activation/sensitivity – result in relatively less vasoconstriction of the renal vascular beds during exercise,<sup>30</sup> perhaps reducing the risk of tubular injury.

This aspect of our data reflects the results of investigations into renal injury in agricultural labourers in California, in which the level of physiological strain, but not the degree of fluid loss, increased the odds of AKI across a working shift.<sup>16</sup> In this same setting, piece rate work - which incentivises labourers to work harder by paying by the weight of produce collected, rather than by the hour – has been shown to be associated with increased AKI.<sup>16,18</sup> Labourers with more physically demanding jobs, such as sugar cane cutters, are known to be at increased risk of AKI relative to other workers exposed to the same conditions,<sup>10,14</sup> with post- and cross-shift elevations in sCr predicting longitudinal decline in eGFR and development of CKD among this group.<sup>13,14</sup>

These findings are relevant when considering the association of RPE with AKI in the present work. RPE is known to relate linearly to exercise intensity in thermoneutral conditions;<sup>26</sup> shows divergent responses to environmental manipulation, which parallel changes in

cardiovascular strain (increased RPE and heart rate with heating, reductions with cooling);<sup>30</sup> and rises as a function of the elevation in body temperature during exercise in the heat.<sup>31</sup> Moreover, the reproducibility of work output guided by RPE alone is high and maintaining RPE below 15 in thermoneutral conditions is associated with lower cardiovascular stress.<sup>32</sup> It is possible, therefore, that the non-invasive, simple tool represented by RPE has potential for exploitation in preventing renal injury from physical exertion. While exercising to a given RPE in competitive sport is clearly not practicable, it may have utility during the HA process. However, we see it has most potential in possibly protecting those populations exposed occupationally to strenuous physical activity in the heat, where recurrent bouts of AKI are associated with the development of CKD.<sup>12,13</sup> This might take the form of primary prevention through imposing a ceiling of RPE (e.g. 13, 'somewhat hard' intensity) below which individuals are advised to operate; dynamic case finding through monitoring RPE remotely e.g. where physical stress is expected to vary across an exposure, as with changing work output or environmental conditions; or secondary prevention based on end-of-shift reporting. Given the potential for RPE to dissociate from other markers of physical strain with adaptation over time,<sup>33</sup> it is however advisable to evaluate the renal protective effects of 'iso-intense' activity (i.e. exercise in the heat to a range of pre-specified RPE levels) in future work addressing both short and longer-term HA.

Unlike the body of evidence accumulating for occupational heat stress, data are not available to confirm or refute an association between AKI and subsequent CKD in endurance athletes.<sup>7</sup> Furthermore, while the post-exercise rise and fall in sCr has been shown to accompany similar changes in markers of tubular injury (NGAL and KIM-1),<sup>8-11</sup> a solid pathological basis for acute changes in sCr reflecting 'true' contemporaneous injury has not been established in this population. The ultimate significance of variability in sCr with exercise and HA remains to be determined; while the addition of tubular injury markers to future investigations is merited, the most pressing requirement is for the provision of longer

term post-exposure follow-up, in order to establish the extent to which acute changes confer susceptibility to chronic disease.

We acknowledge the limits to quantifying HTT intensity in Cyprus, where volunteers were required to ensure that stepping rate was maintained at the rate specific to 50%  $\text{VO}_{2\text{peak}}$  in the UK, under the direct observation of study investigators. It is considered unlikely that  $\text{VO}_{2\text{peak}}$  changed substantially between the start and end of the study, however, with training regimens replicated after baseline in the UK and on arrival to Cyprus (see Table ii, Online Appendix) and only ~5% variation from unacclimatised baseline reported with similar HA regimens.<sup>34</sup> Like much of the literature, our findings related to male volunteers who were relatively young and fit. This means that more work is necessary to investigate exercise-associated changes in renal status in female, older and less athletic populations. Moreover, the exercise bouts were relatively short in duration, and of higher intensity than would be expected with sporting events such as marathons. Nevertheless, the use of standardised exercise-heat exposures in a relatively homogeneous population represents a good starting point for further enquiry.



## **Conclusion**

Increasing rates of participation in prolonged endurance events, combined with climate change and unavoidable occupational exposure, present significant potential for recurrent renal insults and perhaps long-term reduction in renal function among at-risk groups. The obvious flaws in the current gold standard AKI diagnostic criteria highlight the importance of conducting further research in this area. We have shown the potential benefit of adequate HA in preventing rises in sCr consistent with AKI. We have also shown an association between these excursions and neurohumoral, inflammatory and RPE markers, which could have practical utility in surveillance for and prevention of AKI. Further work is required in larger, mixed gender cohorts with longer duration exercise bouts.

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|                                         |      | UK<br>(HTT 1)     | Day 2<br>(HTT 2)  | Day 6<br>(HTT 3)  | Day 9<br>(HTT 4)  | Day 23<br>(HTT 5) | Kruskal-<br>Wallis |
|-----------------------------------------|------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| Creatinine<br>$\mu\text{mol.L}^{-1}$    | PRE  | 85 [78, 98]       | 92 [84, 103]      | 94 [86, 108]      | 97 [83, 103]      | 93 [86, 103]      | P=0.3006           |
|                                         | POST | 120 [102, 139]    | 110 [105, 119]    | 115 [104, 120]    | 110 [96, 115]     | 102 [91, 112]     | P=0.0154           |
| eGFR loss, %                            |      | 31.4 [24.3, 37.5] | 18.3 [13.6, 24.7] | 14.9 [11.4, 25.2] | 14.1 [9.40, 18.2] | 11.9 [3.95, 20.1] | P<0.0001           |
| AKI cases, n                            |      | 14                | 5                 | 6                 | 0                 | 1                 | -                  |
| Plasma volume<br>loss, %                |      | 8.44 [5.41, 13.3] | 8.45 [6.55, 11.2] | 6.96 [5.24, 10.7] | 8.02 [3.42, 10.4] | 8.83 [5.80, 11.2] | P=0.5448           |
| FENa, %<br>(n=11)                       | PRE  | 0.87 [0.43, 0.93] | -                 | 0.45 [0.28, 0.88] | 0.52 [0.28, 1.11] | 0.71 [0.34, 0.92] | 0.2099             |
|                                         | POST | 0.25 [0.14, 0.42] | -                 | 0.30 [0.24, 0.50] | 0.43 [0.18, 0.65] | 0.48 [0.23, 0.58] | 0.2881             |
| RPE                                     | PRE  | 6 [6,6]           | 6 [6,6]           | 6 [6,7]           | 6 [6,6]           | 6 [6,6]           | 0.8820             |
|                                         | POST | 15 [13, 18]       | 13 [9, 14]        | 12 [7, 15]        | 11 [8, 12]        | 10 [7, 11]        | P<0.0001           |
| Copeptin<br>$\text{pmol.L}^{-1}$        | PRE  | 5.6 [4.0, 7.8]    | 6.5 [3.5, 9.8]    | 8.0 [3.4, 11.0]   | 6.0 [3.9, 10.9]   | 7.6 [4.5, 10.2]   | P=0.5538           |
|                                         | POST | 23.8 [18.7, 41.6] | 14.3 [12.5, 24.6] | 15.8 [11.0, 20.7] | 13.6 [11.0, 21.3] | 15.1 [11.9, 22.6] | P=0.0229           |
| IL-6<br>$\text{pg.mL}^{-1}$             | PRE  | 0.60 [0.49, 0.71] | 0.88 [0.66, 1.35] | 0.79 [0.66, 1.24] | 0.87 [0.70, 1.23] | 0.64 [0.60, 0.93] | P=0.0044           |
|                                         | POST | 2.63 [2.04, 4.73] | 1.85 [1.35, 2.96] | 1.54 [1.11, 2.14] | 1.84 [1.25, 2.57] | 1.39 [1.02, 2.00] | P=0.0016           |
| Normetanephrine<br>$\text{pmol.L}^{-1}$ | PRE  | 288 [240, 371]    | 208 [155, 265]    | 255 [181, 319]    | 302 [229, 346]    | 252 [180, 311]    | P=0.0034           |
|                                         | POST | 931 [635, 1187]   | 626 [509, 794]    | 532 [424, 680]    | 605 [494, 819]    | 461 [339, 545]    | P<0.0001           |
| Cortisol<br>$\text{nmol.L}^{-1}$        | PRE  | 385 [329, 458]    | 433 [378, 498]    | 334 [279, 414]    | 404 [313, 576]    | 377 [310, 449]    | P=0.1094           |
|                                         | POST | 611 [404, 769]    | 453 [347, 626]    | 318 [205, 420]    | 325 [284, 402]    | 287 [208, 447]    | P<0.0001           |

**Tables**



**Table 1.** Biochemical results and Relative Perceived Exertion (RPE) scores for the 20 volunteers before (PRE) and immediately after (POST) Heat Tolerance Test (HTT) assessments, which were conducted at baseline in the United Kingdom (UK) and during progressive heat acclimatisation in Cyprus. AKI=Acute Kidney Injury, IL-6 = Interleukin-6.

### Figure legends

**Figure 1:** Biochemical results and Relative Perceived Exertion (RPE) scores following Heat Tolerance Test (HTT), dichotomised by renal status as Acute Kidney Injury (AKI) or no AKI (nAKI): (a) copeptin, (b) interleukin-6 (IL-6), (c) normetanephrine, (d) cortisol (e) fractional excretion of sodium (FENa) (f) Rating of Perceived Exertion (RPE) and (g) fall in plasma volume (PV) with HTT. Significant difference (AKI vs nAKI), adjusted  $P < 0.05$ , \*\*\*0.001, \*\*\*\*0.0001.

