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Reliability of biomarkers of physiological stress at rest and post exertional heat stress.

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The purpose of this study was to assess the reliability of blood biomarkers that can signify exercise-induced heat stress in hot conditions. Fourteen males completed two heat stress tests separated by 5-7 days. Venous blood was drawn pre- and post- heat stress for the concentration of normetanephrine, metanephrine, serum osmolality, copeptin, kidney-injury molecule 1 and neutrophil gelatinase-associated lipocalin. No biomarker, except copeptin, displayed systematic trial order bias ($p \geq 0.05$). Normetanephrine, copeptin and neutrophil gelatinase-associated lipocalin presented acceptable reliability (CV range: 0.9-14.3%), while greater variability was present in metanephrine, osmolality and kidney-injury molecule 1 (CV range: 28.6-43.2%). Normetanephrine exhibited the largest increase ($p < 0.001$) in response to heat stress (trial 1 = 1048 ± 461 pmol. L-1; trial 2 = 1067 ± 408 pmol. L-1), whilst kidney-injury molecule 1 presented trivial changes (trial 1 = -4 ± 20 ng. L-1; trial 2 = 2 ± 16 ng. L-1, $p > 0.05$). Normetanephrine, copeptin and neutrophil gelatinase-associated lipocalin demonstrated good reliability and sensitivity to an acute bout of heat stress. These biomarkers may be suitable for application in laboratory and field research to understand the efficacy of interventions that can attenuate the risk of thermal injury whilst exercising in the heat.

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Abstract

The purpose of this study was to assess the reliability of blood biomarkers that can signify exercise-induced heat stress in hot conditions. Fourteen males completed two heat stress tests separated by 5-7 days. Venous blood was drawn pre- and post- heat stress for the concentration of normetanephrine, metanephrine, serum osmolality, copeptin, kidney-injury molecule 1 and neutrophil gelatinase-associated lipocalin. No biomarker, except copeptin, displayed systematic trial order bias ($p \geq 0.05$). Normetanephrine, copeptin and neutrophil gelatinase-associated lipocalin presented acceptable reliability (CV range: 0.9-14.3%), while greater variability was present in metanephrine, osmolality and kidney-injury molecule 1 (CV range: 28.6-43.2%). Normetanephrine exhibited the largest increase ($p < 0.001$) in response to heat stress (trial 1 = 1048 ± 461 pmol. L^{-1}; trial 2 = 1067 ± 408 pmol. L^{-1}), whilst kidney-injury molecule 1 presented trivial changes (trial 1 = -4 ± 20 ng. L^{-1}; trial 2 = 2 ± 16 ng. L^{-1}, $p > 0.05$). Normetanephrine, copeptin and neutrophil gelatinase-associated lipocalin demonstrated good reliability and sensitivity to an acute bout of heat stress. These biomarkers may be suitable for application in laboratory and field research to understand the efficacy of interventions that can attenuate the risk of thermal injury whilst exercising in the heat.

Introduction

Exercise in a hot-humid environment leads to an increase in metabolic heat production, with an increase in skin blood flow and sweating the primary mechanisms for heat loss in humans. An elevated sweat rate, which unless supported by effective fluid intake, can result in dehydration and a concomitant decrease in extracellular volume [1]. Heat acclimation (HA) is considered the most effective countermeasure [2] for optimal human performance and health during endurance-related physical activity performed under heat stress. A standardised heat tolerance test (HTT) has been endorsed for validating heat acclimation (HA) status in athletes and military personnel [2] [3], although it relies on conventional physiological responses (heart rate, core temperature and sweat rate) which may reflect whole-organism strain and resistance to thermal injury incompletely [4]. In addition, current measures of heat stress (i.e. rectal temperature and skin temperature) are relatively short-lived with the acute responses requiring high frequency tracking, and as such are labour intensive and normally necessitate a laboratory environment for reliable data collection. Blood biomarkers, however, offer the potential for measuring the underlying systemic physiological responses more infrequently, lending themselves to measurements following longer heat exposures (hours) and for longitudinal tracking (days) of chronic adaptation. While previous research has utilised blood biomarkers to quantify the magnitude of heat adaptation to hot environmental conditions, their short-term reliability has not been clearly established. This is unexpected considering the significant variation in the exercise-induced change to markers such as, normetanephrine (NMET), metanephrine (MET), copeptin, kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) following a bout of exercise in hot conditions [4-7]. Determining the reliability of these blood biomarkers of physiological stress related to sympathetic activation, fluid regulation and kidney function would help ensure their appropriate application in a laboratory or field-setting to confidently evaluate the efficacy of interventions such as HA for protecting athlete health and optimising performance in the heat.

During heat stress the autonomic nervous system (ANS) buffers increased competition for blood flow between cutaneous circulation and skeletal muscle [8] and is central to cardiovascular regulation during and following a dynamic bout of exercise [9-11]. Observable measures of ANS disturbance and sympathetic activity include catecholamine's adrenaline and noradrenaline [4, 12]. However, research by [13, 14] has shown that these

circulating catecholamines have a very short half-life of (1-2 min), making them unsuitable for monitoring the stress response to longer duration heat exposures. Recent research [4] has identified the nephrine metabolites NMET and MET to be a more stable surrogate marker of sympathoadrenal activity, with application of these biomarkers showing potential utility in evaluating changes in HA status. The hormone arginine vasopressin (AVP) classically rises in response to dehydration and increasing osmolality [15], and is responsible for controlling renal free water regulation. However, AVP is relatively unstable and its analysis labour intensive, with recent research suggesting that copeptin is a valid and practical surrogate biomarker which is more stable and can be analysed on conventional automated biochemistry platforms [16, 17]. Elevated plasma copeptin concentrations are considered a sensitive marker of individual stress level [17, 18] and can reflect changes in fluid-conserving responses associated with the HA phenotype [19]. Another biomarker of fluid regulation, known as serum osmolality (S_{osmo}) is commonly used to assess hydration status, but is subject to short-term variation in response to posture change, exercise, food and fluid intake [20]. S_{osmo} is widely accepted as the most accurate measure of intracellular dehydration [21], and so understanding the biological and analytical variation of these fluid regulatory biomarkers will further confirm their application as markers of hydration status, especially the type and magnitude of dehydration.

During exercise in the heat an increased perfusion of active muscles leads to a ~25% reduction in basal levels of blood flow to the kidneys [22], which along with dehydration, inflammation and oxidative stress is thought to negatively impact kidney function and induce acute kidney stress [23]. Kidney damage biomarkers such as KIM-1 and NGAL have been found to occur in a variety of occupational and athletic settings conducted in hot environments such as endurance running [24, 25] and cycling [7, 26], with recent research investigating the impact HA may have on protecting kidney function [27]. However, the kidney damage biomarker's short-term reliability is yet to be explored, requiring further investigation.

Therefore, given the relative lack of evidence quantifying the variability of the identified relevant blood biomarkers with the potential to provide insight to the heat adaptation status of an individual, the aims of this study were to (i) examine the acute response of those biomarkers to exercise in the heat, and (ii) establish the reliability (5-7 days) in the concentration of these blood biomarkers of exercise heat-stress.

Methods

Participants

Due to the nature of the dependant variables being assessed and statistical approach involving a battery of reliability statistics, it was determined infeasible to perform an '*a priori'* sample size calculation. A sample size of 12 per group is recommended [28] when there is no prior information on which to base sample size. Fourteen healthy males (Table 1) volunteered to participate in the present study. All participants were endurance trained (>5 hours/week), non-smokers and unacclimatised to hot environments. Dietary supplementation (e.g. glutamine, probiotics) and prolonged thermal exposures (e.g. sauna, steam room) were prohibited from 14-days before, and until the end of data collection [29]. Participants were instructed to arrive in a euhydrated state, as indicated by a urine osmolality (<700 mOsm kg^{-1}) and urine specific gravity (<1.020) [30]. The study gained institutional ethical approval and was conducted in line with the principles expressed in the Declaration of Helsinki.

Study design

Participants visited the laboratory on three occasions. During the first visit, preliminary testing occurred, which included baseline anthropometrics and maximal oxygen uptake ($\dot{V}O_{2max}$) assessed as outlined above. The second and third trials were the main experimental trials. These comprised of two repeat 45-minute cycling EHSTs, separated by 5-7 days. Testing was conducted at the same time of day (07-11am) to control for circadian rhythm [31] and thermoregulatory fluctuations [32].

Preliminary testing

On arrival to the laboratory, height (Seca, 220, Germany) and body mass (Seca, 770, Germany) were measured and used to calculate body surface area [33], while body fat (%) was established from a calibrated air displacement plethysmograph (BOD POD, Life measurement systems, USA). A graded exercise test was used to determine an individual's $\dot{V}O_{2max}$. Participants initially cycled at 150 watts, and increased at a rate of 20 $W.min^{-1}$ until voluntary exhaustion, or the individuals cadence dropped below <70 rpm [34]. Exercise was performed on a cycle ergometer (Wattbike, Pro, UK) and under temperate laboratory conditions (19.3 ± 1.6 °C, 46 ± 6% RH). Heart rate (HR) was recorded continuously during all exercise tests using telemetry data from a strap affixed to the chest of the participant (Polar, V800, Finland). Measurements of respiratory gas for $\dot{V}O_2$ and $\dot{V}CO_2$ were taken using

a calibrated breath-by-breath online gas analysis system (Cortex, Metalyser, 3B, Germany) and $\dot{V}O_{2max}$ was determined according to standard criteria [35].

Exertional heat stress test (EHST)

All EHST's were completed within an environmental chamber (TISS, Alton, UK; Sporting Edge, UK, LTD) that was regulated at 32 °C (Trial 1: 31.9 ± 0.1 °C ; Trial 2: 32.0 ± 0.2 °C) and 70% RH (Trial 1: 70.4 ± 0.7%; Trial 2: 70.4 ± 0.8%). Participants were provided with a telemetric pill (e-Celsius, BodyCap, Caen, France) which they were asked to ingest ≥6 hours prior to arrival [36] for continuous measurement of core body temperature (T_{core}). Generalised sensor calibration was conducted through correction of sensor temperature to a reference thermometer by linear regression as recommended [37]. On arrival at the laboratory, participants voided their bladder and provided a fresh, mid-flow urine sample for the assessment of hydration status. Subsequently, participants measured their own nude body mass (Seca, 770, Germany) and positioned a HR strap around their chest. Four skin temperature (T_{skin}) loggers (iButton, Maxim Integrated Products, USA) were affixed to the participant using Opsite, Flexifix tape (Bunzl, UK) and mean T_{skin} calculated using standard equations [38].

Participants entered the environmental chamber and undertook up to 45-minutes of fixed-intensity exercise on a cycle ergometer (Lode, Excalibur Sport, 925900, Netherlands), at a relative workload of 2.5 $W.kg^{-1}$. During the exercise period physiological data (HR, T_{core}, and T_{skin}) were recorded at 2.5-minute intervals, while perceptual responses (rating of perceived exertion (RPE) [39] and thermal sensation (TSS) [40]) were recorded at 5-minute intervals. Peak physiological and perceptual measures were considered the highest recorded value during the EHST. Participants were removed from the environmental chamber and placed in a recovery room if T_{core} ≥39.7 °C (1 incidence). Participants could also terminate the EHST at any time point if they reached volitional exhaustion (4 incidences). Fluid intake was restricted during the EHST and so only urine output was accounted for to calculate total non-urine fluid loss from pre-post changes in nude body mass. Venous blood samples were drawn at rest (PRE) and immediately post termination of the EHST (POST) into two 8.5 mL serum separator tubes (SST) and one 10 mL tube containing EDTA (Becton Dickinson, UK) from a forearm antecubital vein. Blood was analysed for the concentration of NMET, MET, S_{osmo}, copeptin, KIM-1 and NGAL.

Blood analysis

Samples were spun for 15-minutes at 2600 RPM, with plasma EDTA tubes within 10-minutes of being collected and serum SST tubes following clotting for 15-30 minutes at room temperature. Aliquots of plasma and serum were pipetted into 2.0 mL micro-tubes (Eppendorf, UK) and then stored at -80℃ until further analysis. S_{osmo} was measured in duplicate by a micro-osmometer (Advanced Model 3320, Advanced Instruments, Norwood, MA, USA) using a suppression of freezing point method (CV range: 0.5-0.7%). Plasma free nephrines (NMET and MET) were processed using an in-house liquid chromatography/tandem mass spectrometry method (CV: 4–12%). Copeptin was assayed using an automated sandwich immunofluorescent assay based on Time-Resolved Amplified Cryptate Emission (TRACE) technology (Brahms CT-proAVP Kryptor Compact Plus, Hennigsdorf, Germany) and had a CV of 2–10%. KIM-1 and NGAL were analysed using commercially available ELISA kits (R & D systems Europe, Abingdon, Oxon, UK), the intra/inter assay reliability for KIM-1 was 2.6-6.7%, and NGAL 3.1-7.9%. We present the hormone concentrations that are uncorrected for changes in plasma volume.

Statistical Analysis

Statistical calculations were performed using the software packages GraphPad Prism version 8.1.0 (GraphPad Software, San Diego USA). Results were assessed for normality using the Shapiro-Wilk test ($p \geq .05$). A two-way analysis of variance (ANOVA) with repeated measures (time x trial) was used to identify differences between the two trials for blood biomarkers. Where a significant interaction did exist in two-way ANOVA, post-hoc analysis using Bonferroni correction was applied (alpha = 0.05). When there was only a single comparison for physiological, perceptual and blood biomarkers between EHST, a paired-samples *t-test* was used for analysis. Non-parametric perceptual data were analysed using a Wilcoxon signed-rank test.

A selection of *a priori* statistical tests were used to determine inter-trial reliability as recommended [41]. Each biomarker was compared at rest, post-EHST and the pre-post change score. Absolute reliability was assessed using the typical error of measurement (TEM) expressed as a percentage of its respective mean to form the correlation coefficient (CV%), with the TEM calculated from the standard deviation (SD) of the mean difference for each pair of trials using the formula ($TEM = SD_{(diff)}/\sqrt{2}$). Bland-Altman plots with the mean bias and 95% limits of agreement (LOA) were produced. The CVs were considered *very good* (≤10%) and *acceptable* (≤20%) [41]. Relative reliability was assessed using a Pearson's

product-moment correlation coefficient or non-parametric Spearman's rank correlation coefficient. Correlations were classified as small (≤0.69), moderate (0.70-0.89) and high (≥ 0.90) [42]. The standardised mean difference of an effect [43] between EHSTs were determined using effect sizes (Cohens *d*). Effect sizes were categorised as <0.2 as *trivial*, >0.2 *small*, >0.6 *moderate*, >1.2 *large*, >2.0 *very large*, and >4.0 *extremely large* [44]. All data are reported as mean ± SD and statistical significance was set at $P < 0.05$.

Results

Reliability of thermoregulatory, cardiovascular, and perceptual strain

The reliability of peak mean and pre-post change in T_{core} and T_{skin} was considered *very good* (Table 2). T_{core} (time x trial $p = 0.58$) and T_{skin} (time x trial $p = 0.21$) all increased over time to a similar magnitude between repeat trials of the EHST demonstrating no intra-trial differences in response. The reliability of peak mean and pre-post change in HR, RPE and TSS were also all *very good*. HR (time x trial $p = 0.41$), RPE (time x trial $p = 0.07$) and TSS (time x trial $p = 0.71$) all increased during the EHST to a similar extent between trial 1 and trial 2, (Table 2). TNUFL (trial 1 = 1450 ± 356 mL; trial 2 = 1340 ± 320 mL, $p = 0.19$), pre-trial urine osmolality (trial 1 = 511 ± 124 mOsm kg $^{-1}$; trial 2 = 441 ± 176 mOsm kg $^{-1}$, $p = 0.08$), pre-trial urine specific gravity (trial 1 = 1.013 ± 0.005 g.mL^{-1}; trial 2= 1.012 ± 0.005 g.mL^{-1}, $p = 0.64$) and percentage body mass loss (trial 1 = 1.96 ± 0.45 %; trial 2 = 1.75 ± 0.39 %, $p = 0.06$) were similar between trial 1 and trial 2.

Acute responses of blood biomarkers to exertional heat stress

NMET exhibited the largest acute response (*very large*) of all the biomarkers assessed from pre-post EHST (trial 1 = 1048 ± 461 pmol. L^{-1}, $d = 2.8$; trial 2 = 1067 ± 408 pmol. L^{-1}, $d = 3.8$), see Figure 1. Blood biomarker MET also exhibited a significant ($p \leq 0.0001$) acute response to repeat trials of the EHST (trial 1 = 159 ± 87 pmol. L^{-1}, $d = 1.7$, *large*; trial 2 = 115 ± 95 pmol. L^{-1}, $d = 2.1$, *very large*). In addition, copeptin (trial 1 = 11.7 ± 9.6 pmol. L^{-1}, $d = 1.9$, *large*; trial 2 = 8.1 ± 8.9 pmol. L^{-1}, $d = 1.3$, *large*) and plasma NGAL (trial 1 = 38 ± 23 µg. L^{-1}, $d = 1.8$, *large*; trial 2 = 24 ± 31 µg. L^{-1}, $d = 0.9$, *moderate*) also demonstrated significant increases ($p \leq 0.001$) in concentration during both repeat trials of the EHST. In contrast, there was non-significant ($p > 0.05$) responses during the EHST's for S_{osmo} (trial 1 = 5 ± 4 mosm·kg^{-1}, $d = 1.0$, *moderate*; trial 2 = 1 ± 6 mosm·kg^{-1}, $d = 0.2$, *trivial*) and KIM-1 (trial 1 = -4 ± 20 ng. L^{-1}, $d = 0.1$, *trivial*; trial 2 = 2 ± 16 ng. L^{-1}, $d = 0.1$, *trivial*).

Reliability of blood biomarkers

Bland-Altman plots are presented to illustrate bias for post-EHST concentration in each biomarker (Figure 2). None of the biomarkers, except copeptin at the pre-post change time point, displayed systematic trial order bias ($p \leq 0.05$), figure 2. Of all biomarkers assessed NMET was the only one to have *moderate* relative reliability at pre- and post-EHST, (Table 3), with copeptin, NGAL and KIM-1 demonstrating *moderate* relative reliability at rest. The absolute reliability (CV) was considered *very good* for S_{osmo} and copeptin at pre- and post-EHST, with NMET and copetin observing *acceptable* absolute reliability at rest only, (Table 3). In contrast, MET and KIM-1 had very high relative reliability at all time points, and NGAL at the post-EHST time point (Table 3).

Discussion

The short-term reliability of blood biomarkers of physiological stress was assessed in the present study, along with their acute response to exercise under hot-humid conditions. NMET, copeptin and NGAL presented good correlations, acceptable absolute reliability, and significant increases in concentration following exertional heat stress. In contrast, MET, S_{osmo} and KIM-1 presented poor reliability and/or little response to exercise in the heat. Thus, NMET, copeptin and NGAL may be appropriate for application in field-based or laboratory research settings to reliably quantify individual physiological responses to acute heat stress or adaptations to a hot environment following interventions such as heat acclimation.

Changes in plasma nephrines (NMET and MET) can be assumed to better reflect sympathoadrenal activation than their parent catecholamines during standardised exercise bouts, and increases in MET and NMET have been shown to reflect the level of environmental challenge faced [45]. In this study we show that plasma NMET had good absolute reliability (CV) at rest (13%) and following exercise-heat stress (14.3%). Contrastingly, plasma MET showed unacceptable absolute reliability at rest (28.6%) and post-EHST (29.9%). The current study demonstrated increases in NMET by approximately 260% or Δ 1057 pmol. L^{-1} and MET by 65% or Δ 137 pmol. L^{-1} following repeat trials of the EHST. This response is comparable to research [4] of a similar duration/intensity exercise protocol in which participants completed a 60-minute HTT involving stepping (50% VO_{2peak}), and reported increases in NMET (201% or 459 pmol. L^{-1}) and MET (26% or 50 pmol. L^{-1}). Together these findings advocate NMET, of the two plasma nephrines assessed, as a more suitable biomarker of sympathetic activity to an acute bout of exercise heat stress.

In the present study baseline copeptin concentrations (4.7 pmol. L^{-1}) were consistent with previous evidence (5.5 + 2.4 pmol. L^{-1}) in 153 healthy male subjects [46]. Copeptin demonstrated the best absolute reliability at rest (1.2%) and post-EHST (0.9%) of all biomarkers assessed and large acute responses by 208% or 9.9 pmol. L^{-1} to exercise in the heat. This response is comparable to previous research [5], which also reported an increase in copeptin by 155% or 9.9 pmol. L^{-1} following a 60-minute HTT. However, copeptin did display systematic trial order bias (p <0.05) between repeat trials of the EHST. This could partly be explained by a trend towards a lower percentage body mass loss and reduced TNUFL (8% or 110 mL) during the second EHST. Despite not reaching significance, this may have caused a reduced stress response and thus a reduced concentration in post-EHST copeptin concentration. Further, it is noted that changes in plasma volume associated with exercise and fluid loss can lead to apparent alterations in biomarkers, which we didn't adjust for using estimated changes in plasma volume. However, it is suggested that values unadjusted for changes in plasma volume should be reported since these are the concentrations to which potential target tissues are exposed [47-49]and this applies equally if hormone clearance is reduced. S_{osmo} presented very good absolute reliability, yet poor relative reliability at all time points. As expected minimal changes were demonstrated in response to exercise in the heat (1%, 3 mosm·kg^{-1}) and was comparable to [5] who also demonstrated a small change in S_{osmo} concentration (1.4%, 4 mosm·kg^{-1}) following a 60-minute HTT. Taking

these factors into account, copeptin appears to be a more reliable and sensitive marker of changes in fluid balance during exercise-heat stress.

Kidney injury biomarkers KIM-1 and NGAL have been extensively researched in multiple clinical settings [50], and more recently in situations considered to be clinically benign for the kidneys. These markers have demonstrated considerable predictive value in the early detection of acute kidney injury (AKI), or at least an increased potential to develop AKI [51]. KIM-1 demonstrated poor absolute reliability (CV >20%) and poor relative reliability post-EHST, except at rest displaying a strong correlation (r = 0.88). In contrast, acceptable absolute reliability was shown for NGAL (11%) at rest and unacceptable reliability post-EHST (24.2%). Following both EHST's, KIM-1 demonstrated minimal, non-significant changes (6% or 0.58 ng. L^{-1}). While we demonstrated a mean increase of 2.0 °C in core body temperature during the EHST, elevations in KIM-1 have typically occurred following more prolonged exercise [52] and can help explain why we did not observe significant changes in KIM-1 despite moderate hyperthermia, which is suggested to cause elevations in AKI biomarkers [6]. The combination of physical exertion, thermal strain and heat illness has been associated with rises in serum creatinine [53], although it is unclear if these changes reflect kidney injury. As such, we included KIM-1 as a more specific AKI marker, and clearly the minimal response was not associated with any significant kidney injury. In contrast, another marker of kidney damage (plasma NGAL) demonstrated significant increases in the current study (46% or 32 µg. L^{-1}). These findings agree with similar research duration/intensity exercise protocols [6, 54]. In the study by [6] participants completed 40-minutes treadmill walking (4.8 km·h^{-1}, 5% incline) under hot-humid conditions (38 °C , 50% RH) and demonstrated an increase in plasma NGAL by 12.7 µg.L^{-1}. While in the study by [54] a 2-fold increase in plasma NGAL (109 µg.L-1) was demonstrated after 40-minutes of running at 65% VO_{2max}. As previously reported, exercise in the heat can increase the risk of AKI in humans and this risk is in proportion to the magnitude of heat strain and dehydration [51]. Despite observing significant elevations in NGAL in the current study, with the highest demonstrated value of 197 µg. L^{-1} (expected normal range of 37-106 µg. L^{-1}), these responses are lower than that used to define clinical AKI (cut off value = 250 µg. L^{-1}) and are more suggestive of a degree of renal stress. Therefore, application of this marker in the present EHST could be used to monitor the effectiveness of strategies such as HA on kidney function in athletic populations.

Limitations

Although the implementation of a tightly controlled methodological design, which accounted for most extraneous variables, the presented results are not without some limitations. The

blood biomarker analysis was limited to a single time-point after the EHST (termination), which may not have coincided with the peak response. Further research is required within the biomarkers assessed during more prolonged exercise periods in the heat and following periods of passive heat stress, thus informing their ability to quantify the magnitude of heat adaptation to a hot environment.

Conclusion

In the present study we comprehensively assessed the reliability of biomarkers associated with sympathoadrenal responses, fluid regulation and kidney stress at rest and following exercise in the heat. Quantifying the inherent variation of biological systems affected by exercise in the heat can inform the selection of biomarkers for application in field and laboratory settings. The biomarkers NMET, copeptin and NGAL presented good reliability and sensitive responses to an acute bout of heat stress. These biomarkers could be utilised to evaluate the efficacy of interventions that can attenuate the risk of thermal injury and reduction in exercise capacity when exercising the heat. Compared to traditional physiological markers of heat stress, blood biomarkers offer the potential for measurement of underlying systemic physiological responses less frequently, affording themselves to measurements following chronic exposures such as heat acclimation.

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Table 1. Participant demographic characteristics.

Maximal oxygen uptake (VO_{2max}), peak power output (PPO), standard deviation (SD).

Table 2. Relative and absolute reliability of physiological and perceptual responses.

*Significant correlation ($p \leq 0.05$); **Significant correlation ($p \leq 0.01$); ***Significant correlation ($p \leq 0.001$); ****Significant correlation ($p \leq 0.0001$). CVs were considered *very good* (≤10%) and *acceptable* (≤20%).

Table 3. Relative and absolute reliability of all blood biomarkers.

*Significant correlation ($p \leq 0.05$); **Significant correlation ($p \leq 0.01$); +Significant difference between trials from a paired *t-test* ($p < 0.05$). CVs were considered *very good* (≤10%) and *acceptable* (≤20%).

Figure.1 Blood biomarker responses to EHST1 (white box) and EHST2 (grey box), at rest (PRE) and immediately following exercise in the heat (POST). Significant overall effect of time (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$). Significant overall effect of trial (+$p < 0.05$). Normetanephrine, (NMET, n =12) metanephrine, (MET, n =12), serum osmolality (S_{osmo}, n =12), copeptin (n =13), kidney injury molecule-1 (KIM-1, n = 13) and neutrophil gelatinase-associated lipocalin (NGAL, n =13).

Figure 2. Bland-Altman mean bias and 95% LoA between post-EHST trial 1 and trial 2: (a) = NMET (n =12), (b) = MET (n =12), (c) = S_{osmo} (n =12), (d) = Copeptin (n =13), (e) = KIM-1 (n = 13) and (f) = NGAL (n =13).

Table 1. Participant demographic characteristics.

Measure	Mean ± SD
Age (years)	29 ± 10
Stature (cm)	179 ± 5
Body mass (kg)	74.5 ± 8.0
Body fat (%)	11.1 ± 5.1
Body surface area (m^2)	1.93 ± 0.11
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	59 ± 8
PPO (Watts)	369 ± 47

Maximal oxygen uptake (VO_{2max}), peak power output (PPO), standard deviation (SD).

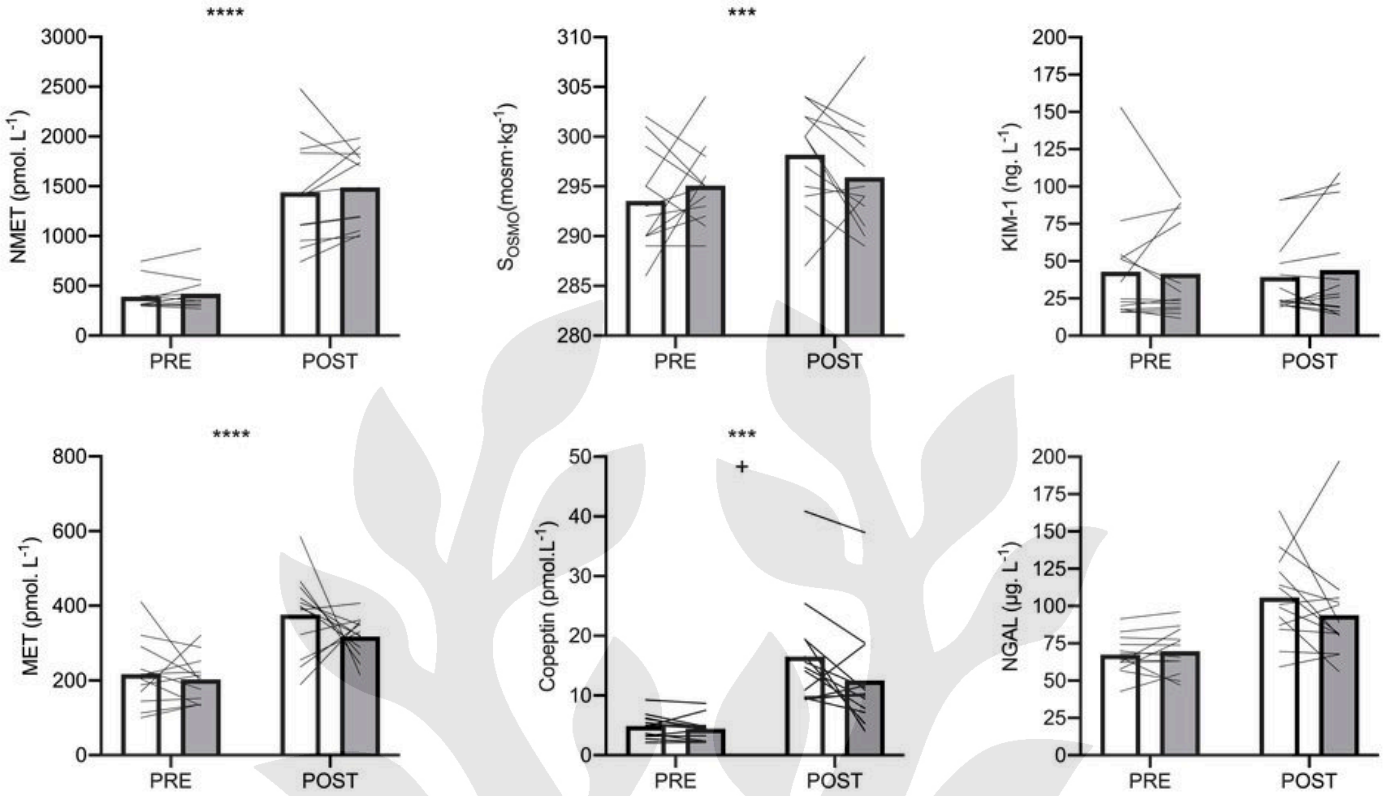
Table 2. Relative and absolute reliability of physiological and perceptual responses.

		Trial 1 (SD)	Trial 2 (SD)	*P*	*d*	*r*	TEM	CV%	Bias (LoA)
T_{core} (°C)	Peak	38.8 ± 0.5	38.8 ± 0.6	0.73	0.06	0.79***	0.3	0.7	0.0 ± 0.4
	Mean	37.8 ± 0.4	37.7 ± 0.3	0.65	0.11	0.69**	0.2	0.5	0.0 ± 0.3
	Pre-post	2.0 ± 0.5	2.0 ± 0.6	0.63	0.08	0.84****	0.2	18.3	0.0 ± 0.3
T_{skin} (°C)	Peak	36.5 ± 0.3	36.5 ± 0.5	0.88	0.03	0.80***	0.2	0.6	0.0 ± 0.3
	Mean	35.6 ± 0.6	35.5 ± 0.5	0.27	0.20	0.65**	0.2	0.7	-0.1± 0.3
	Pre-post	2.8 ± 0.6	2.9 ± 0.8	0.17	0.24	0.85****	0.3	22.2	0.2 ± 0.4
HR ($b \cdot min^{-1}$)	Peak	172 ± 11	170 ± 11	0.16	0.17	0.91****	3	1.9	-2 ± 5
	Mean	155 ± 10	153 ± 12	0.23	0.16	0.90****	4	2.3	-2 ± 5
	Pre-post	109 ± 13	107 ± 13	0.42	0.13	0.83***	6	9.6	-2 ± 8
RPE (AU)	Peak	17 ± 2	16 ± 2	0.13	0.31	0.82***	1	5.3	-1 ± 1
	Mean	14 ± 2	13 ± 2	0.15	0.27	0.82***	1	6.7	-1 ± 1
	Pre-post	11 ± 2	10 ± 2	0.08	0.31	0.82***	1	15	-1 ± 1
TSS (AU)	Peak	7.1 ± 0.7	7.1 ± 0.7	0.67	0.05	0.90****	0.2	3.1	0.0 ± 0.3
	Mean	6.3 ± 0.6	6.2 ± 0.7	0.56	0.17	0.78**	0.5	5.3	0.5 ± 0.5
	Pre-post	2.5 ± 0.6	2.4 ± 0.8	0.81	0.10	0.43	0.5	25.2	-0.1 ± 0.8
TNUFL (mL)	Mean	1450 ± 356	1340 ± 320	0.19	0.32	0.77**	142	6.9	-110 ± 242

*Significant correlation ($p \leq 0.05$); **Significant correlation ($p \leq 0.01$); ***Significant correlation ($p \leq 0.001$); ****Significant correlation ($p \leq 0.0001$). CVs were considered *very good* (≤10%) and *acceptable* (≤20%).

		Trial 1 (SD)	Trial 2 (SD)	*p*	*d*	*r*	TEM	CV%	Bias (LoA)
NMET (pmol. L^{-1})	Pre	391 ± 150	422 ± 167	0.26	0.20	0.75**	53	13	31 ± 78
	Post	1439 ± 503	1489 ± 360	0.59	0.11	0.82**	209	14.3	50 ± 309
	Pre-post	1048 ± 461	1067 ± 408	0.85	0.04	0.70**	301	28.5	19 ± 343
MET (pmol. L^{-1})	Pre	217 ± 89	203 ± 62	0.60	0.19	0.35	60	28.6	-14 ± 89
	Post	376 ± 105	318 ± 52	0.22	0.70	0.70*	104	29.9	-58 ± 153
	Pre-post	159 ± 87	115 ± 95	0.31	0.48	-0.26	65	47.4	-44 ± 145
S_{osmo} (mosm·kg^{-1})	Pre	294 ± 5	295 ± 4	0.35	0.36	0.25	4	1.3	1 ± 6
	Post	299 ± 5	296 ± 5	0.18	0.45	0.46	4	1.2	-2 ± 6
	Pre-post	5 ± 4	1 ± 6	0.12	0.77	-0.23	4	135.1	-4 ± 8
Copeptin (pmol. L^{-1})	Pre	4.9 ± 2.0	4.5 ± 1.9	0.26	0.23	0.78**	0.9	1.2	-0.4 ± 1.3
	Post	16.5 ± 8.8	12.5 ± 8.6	0.04+	0.48	0.15	4.2	0.9	-3.6 ± 6.2
	Pre-post	11.7 ± 9.6	8.1 ± 8.9	0.06	0.39	0.26	7	65.7	-4.0 ± 6.0
KIM-1 (ng. L^{-1})	Pre	43 ± 38	42 ± 31	0.85	0.04	0.88**	18	43.2	-1 ± 1
	Post	39 ± 26	44 ± 35	0.34	0.16	0.44	12	29.6	5 ± 17
	Pre-post	-4 ± 20	2 ± 16	0.31	0.33	0.04	13	2231	6 ± 25
NGAL (µg. L^{-1})	Pre	67 ± 12	70 ± 15	0.43	0.18	0.70**	8	11	3 ± 11
	Post	106 ± 29	94 ± 35	0.24	0.38	0.68*	24	24.2	-12 ± 34
	Pre-post	38 ± 23	24 ± 31	0.16	0.52	0.25	21	66.3	-14 ± 34

Table 3. Relative and absolute reliability of all blood biomarkers.
*Significant correlation ($p \leq 0.05$); **Significant correlation ($p \leq 0.01$); +Significant difference between trials from a paired *t-test* ($p < 0.05$). CVs were considered *very good* (≤10%) and *acceptable* (≤20%).



Kommentare

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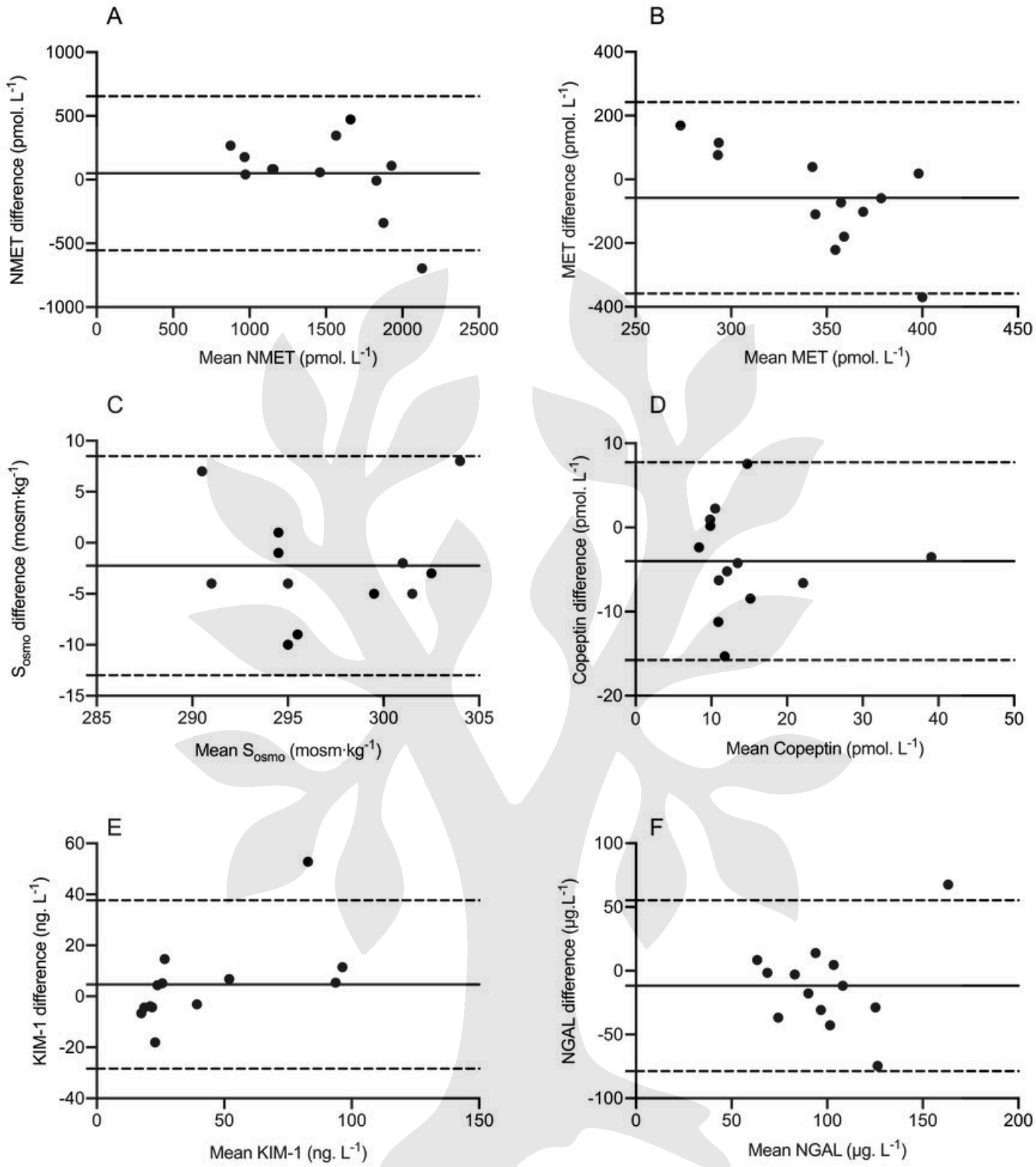

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