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Effects of acute or chronic heat exposure, exercise and dehydration on plasma cortisol, IL-6 and CRP levels in trained males

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ABSTRACT

This study examined the acute and chronic effects of euhydrated and hypohydrated heat exposure, on biomarkers of stress and inflammation. Eight trained males [mean (SD) age: 21 (3) y; mass: 77.30 (4.88) kg; $\dot{V}O_{2max}$: 56.9 (7.2) mL kg^{-1} min^{-1}] undertook two heat acclimation programmes (balanced cross-over design), once drinking to maintain euhydration and once with restricted fluid-intake (permissive dehydration). Days 1, 6, and 11 were 60 min euhydrated exercise-heat stress tests (40 °C; 50% RH, 35% peak power output), days 2–5 and 7–10 were 90 min, isothermal-strain (target rectal temperature: 38.5 °C) exercise-heat sessions. Plasma was obtained pre- and post- exercise on day 1, 2, and 11 and analysed for cortisol, interleukin-6 (IL-6), and C-reactive protein (CRP). Cortisol and CRP were also assessed on day 6. IL-6 was elevated following the initial (acute) 90 min isothermal heat strain exercise-heat exposure (day 2) with permissive dehydration ((pre exercise: 1.0 pg mL^{-1} [0.9], post-exercise: 1.8 pg mL^{-1} [1.0], P = .032) and when euhydrated (pre-exercise: 1.0 pg mL^{-1} [1.4], post-exercise: 1.6 pg mL^{-1} [2.1], P = .048). Plasma cortisol levels were also elevated but only during permissive dehydration (P = .032). Body mass loss was strongly correlated with Δcortisol (r = −0.688, P = .003). Although there was a trend for post-exercise cortisol to be decreased following both heat acclimation programmes (chronic effects), there were no within or between intervention differences in IL-6 or CRP. In conclusion, acute exercise in the heat increased IL-6 and cortisol only when fluid-intake is restricted. There were no chronic effects of either intervention on biomarkers of inflammation as evidenced by IL-6 and CRP returning to basal level at the end of heat acclimation.

1. Introduction

Heat acclimation improves perceptions of thermal comfort and submaximal, as well as maximal, aerobic exercise performance in warm-hot conditions [34,37,42] and possibly cool environments [12,31,38]. The performance and perceptual benefits associated with heat acclimation are achieved via an earlier onset of sweating, plasma volume expansion, and alterations in fluid-electrolyte balance which ultimately lead to an attenuated rise in body core temperature and lower cardiac frequency during rest and exercise [12,22,37,41]. Consequently, various heat acclimation protocols are employed in both occupational and sporting settings in an attempt to minimise the physiological challenges encountered during prolonged exercise in the heat [30].

Some benefits of heat acclimation, including improved cardiovascular stability, occur within the first few days [36,37]. The logistics, in relation to time and cost, associated with longer heat acclimation protocols may be difficult for many athletes and occupational personnel e.g. prior to competition or preceding military deployment. Therefore, the extent of adaptations from short- (≤7 heat exposures) and medium-term (8–14 heat exposures) heat acclimation programmes have recently received interest [9,42]. In an attempt to enhance the beneficial effect of shorter term heat acclimation Garett and colleagues [21] provided evidence that in contrast to traditional hydration guidance [3], restricting fluid intake (permissive dehydration) during a 5-day heat acclimation may provide a supplemental stimulus with some positive effects. Specifically, compared to a euhydrated heat acclimation programme, daily mild dehydration led to higher resting body mass, a

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tendency for a greater expansion of plasma volume, a larger rise in resting forearm perfusion by way of higher vascular conductance, and a larger reduction in cardiac frequency during exercise [21]. However, the current literature base is contradictory and Neal et al. [33] have recently reported that permissive dehydration during a medium term heat acclimation protocol did not positively affect the acquisition and decay of heat acclimation, or endurance performance parameters, although no negative effects were reported either.

Regardless of these inconsistent findings, short- and medium-term heat acclimation programmes are becoming increasingly popular in team sports and in various occupational settings [9,34,42]. However, few researchers have considered the potential negative side effects [26,42,47]. It is well established that strenuous endurance exercise results in an acute immune and stress response. Typical features of this response are alterations in cortisol [4,48], and the peripheral distribution pattern of leukocytes [35], and increases in cytokine concentration (e.g. interleukin-6 [IL-6]; [2,26]) as well as other inflammatory biomarkers such as C-reactive protein (CRP) [35].

Further, exercise in high ambient temperatures is known to exert a synergistic impact on the stress response to exercise [2,19,26]. The acute stress (e.g. cortisol) and inflammatory responses (e.g. cytokines) following exercise in hot temperatures exceed those seen in cooler conditions [39,45]. Despite reports suggesting that restricting fluid intake during acute exercise augments the plasma cortisol response [4,6,17,29] and cytokine production [25,26], the independent effects of hydration status on the stress and inflammatory responses to heat acclimation has received little attention. The increased physical and psychological strain experienced during heat acclimation, combined with the possibility of inadequate recovery, may increase potential for minor illnesses [26,46] and ultimately impair subsequent exercise performance [42,47].

Accordingly, the primary aim of this study was to investigate the acute and chronic effects of 11-day euhydrated and dehydrated heat acclimation protocols on biomarkers of stress and inflammation. It was hypothesised that circulating levels of cortisol, IL-6, and CRP, after an initial (acute exposure) isothermal-strain exercise-heat session, would be elevated and that the permissive dehydration would exaggerate this response. Similarly, we hypothesised that resting and post-exercise levels of cortisol, IL-6 and CRP would be augmented at the end (chronic exposure) of the permissive dehydration heat acclimation protocol.

2. Methods

2.1. Participants

Eight un-acclimated, trained male athletes (> 150 km cycling weekly) [mean (SD) age: 21 (3) years, body mass: 77.3 (4.9) kg, stature: 181 (5) cm, $\dot{V}O_{2max}$: 56.9 (7.2) mL kg^{-1} min^{-1}; peak power output: 338 (46) W] volunteered and provided written informed consent for the study, which was conducted in accordance with the Institution's ethics and governance committee and Declaration of Helsinki [2013]. Participants were all engaged in recreational endurance exercise (running, cycling, and triathlon) and free from any symptoms of illness, inflammation or soreness at the start of the study. Participants were also instructed not to take any medication, including anti-inflammatories, for the duration of the experiment.

2.2. Experimental design

This study was part of a larger project investigating effects of heat acclimation on performance variables in trained cyclists, and the experimental design has been described in detail elsewhere [33]. Briefly, a within-participant, balanced cross-over design (with three months washout) was employed, with participants undertaking both control [euhydrated heat acclimation (HAEu)] and intervention [permissive dehydration (HADe)] HA programmes (target ambient conditions: 40 °C; 50% RH). Four participants completed the HAEu first. Each HA programme lasted 11-days and consisted of three bouts of exercise at a fixed external work rate [heat stress test (HST)], undertaken on day 1 (HSTpre), day 6, and day 11 (HSTpost). The HST was completed on a CompuTrainer cycle ergometer (RacerMate Inc., Seattle, Washington, USA) for 60 min at 35% of peak power output. During the HSTs 1.25 L of 3.6% carbohydrate-electrolyte solution (drink temperature 20 °C) was ingested to replace fluid losses, divided into five boluses (0.25 L) and consumed immediately prior to commencing exercise and every 15 min thereafter.

HSTs were interspersed with eight (days 2–5 and 7–10) isothermal strain exercise-heat exposures (ISO). The ISO consisted of cycling (RacerMate Inc., Seattle, WA, USA), at a work rate eliciting a rating of perceived exertion (RPE; [5]) of 15 (anchored by the word 'Hard') until rectal temperature reached 38.3 °C, at which point external power output was adjusted as appropriate to maintain the target rectal temperature of 38.5 °C. Each ISO lasted a total of 90 min. Rectal temperature was recorded using a thermistor (Grant Instruments, Cambridge, UK) self-inserted 15 cm beyond the anal sphincter. Nude body mass (dry) was measured pre- and post-each test session (Industrial Electronic Weight Indicator, ModelI 10, Ohaus Corporation, Parsippany, NJ, USA). As previously described [33], during the HAEu intervention all participants consumed 0.25 L of 3.6% carbohydrate-electrolyte fluid (Science in Sport, Nelson, UK) every 15 min, including immediately before and after each ISO (total fluid consumed = 1.75 L); drink temperature was 20 °C. After exercise, participants were encouraged to drink *ad libitum* to ensure similar hydration for the following day. Permissive dehydration was achieved by restricting fluid intake during exercise [21] and no fluid was provided in the HADe during each ISO, or for 10 min after. Thereafter, participants consumed 1.75 L of the same beverage and were encouraged to drink *ad libitum* to ensure adequate hydration for the following day. Blood was sampled at a similar time of day for all participants to limit diurnal variation.

2.3. Blood sampling procedure

Immediately before and after exercise on day 1, 2, 6, and 11 a 10 mL venous blood samples was obtained (K2 EDTA blood collection tubes, Beckton Dickson & Company, Plymouth, UK) from the antecubital vein following 10 min of seated rest. Haemoglobin concentration (201^{+} HemoCue, Sweden) and haematocrit (Hawksley, Lancing, UK) were measured in triplicate. These values were used to estimate plasma volume changes using the method of Dill and Costill [13]. Whole blood samples were centrifuged (1500*g* for 15 min at 4 °C, Heraeus^{TM} Multifuge^{TM} 3 S-R, ThermoElectron Corporation, Germany) and the resultant plasma stored at −80 °C. IL-6 (Promo Kine, PromoCell, Sickingenstrasse, 69,126 Heidelberg, Germany; Sigma Aldrich, St Louis, MO 63,103 USA), CRP (RayBiotech, Inc., Norcross, GA 30,092, USA) and cortisol (Sigma-Aldrich, St Louis, MO 63,103 USA) were measured in plasma using commercially available quantitative human enzyme linked immunosorbent assays (ELISA) kits. Manufacturer instructions were followed for high sensitivity for each of the kits and repeated freeze-thaw cycles of plasma were minimized. Intra-assay coefficient of variations for IL-6 was 6.8% (acute: Promokine) and 19.6% (chronic: Sigma-Aldrich), and 5.8% and 4.0% for CRP and Cortisol respectively. Minimum detectable plasma concentrations were 0.92 pg mL^{-1} for IL-6, 34 pg mL^{-1} for CRP, and 26.3 ng mL^{-1} for Cortisol. All analytes were corrected for changes in plasma volume before statistical analysis.

2.4. Statistical analyses

The distribution of data was assessed using descriptive methods (skewness, outliers, and distribution plots) and inferential statistics (Shapiro–Wilk test). Acute body mass loss on day 2 and chronic body mass losses (average of HADe and HAEu sessions) were analysed using separate paired sample t-tests. The acute and chronic effects of