Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function

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Junglee NA, Di Felice U, Dolci A, Fortes MB, Jibani MM, Lemmey AB, Walsh NP, Macdonald JH. Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function. Am J Physiol Renal Physiol 305: F813–F820, 2013. —Unaccustomed strenuous physical exertion in hot environments can result in heat stroke and acute kidney injury (AKI). Both exercise-induced muscle damage and AKI are associated with the release of interleukin-6, but whether muscle damage causes AKI in the heat is unknown. We hypothesized that muscle-damaging exercise, before exercise in the heat, would increase kidney stress. Ten healthy euhydrated men underwent a randomized, crossover trial involving both a 60-min downhill muscle-damaging run (exercise-induced muscle damage; EIMD), and an exercise intensity-matched non-muscle-damaging flat run (CON), in random order separated by 2 wk. Both treatments were followed by heat stress elicited by a 40-min run at 33°C. Urine and blood were sampled at baseline, after treatment, and after subjects ran in the heat. By design, EIMD induced higher plasma creatine kinase and interleukin-6 than CON. EIMD elevated kidney injury biomarkers (e.g., urinary neutrophil gelatinase-associated lipocalin (NGAL) after a run in the heat: EIMD-CON, mean difference [95% CI]: 12 [5, 19] ng/ml) and reduced kidney function (e.g., plasma creatinine after a run in the heat: EIMD-CON, mean difference [95% CI]: 0.2 [0.1, 0.3] mg/dl), where CI is the confidence interval. Plasma interleukin-6 was positively correlated with plasma NGAL (r = 0.9, P = 0.001). Moreover, following EIMD, 5 of 10 participants met AKIN criteria for AKI. Thus for the first time we demonstrate that muscle-damaging exercise before running in the heat results in a greater inflammatory state and kidney stress compared with non-muscle-damaging exercise. Muscle damage should therefore be considered a risk factor for AKI when performing exercise in hot environments.

heat stress disorder; exercise; inflammation; interleukin-6; NGAL protein

UNACCUSTOMED STRENUOUS EXERCISE in the heat may lead to exertional heat injury (1). This has been reported in athletes, military personnel, and those in other hazardous occupations such as firefighters (3, 15, 50). Exertional heat injury exists in a spectrum which ranges from relatively mild exertional heat illness to life-threatening heat stroke with reduced consciousness and multiorgan failure (3, 23, 47). In 2011, there were ~3,000 cases of exertional heat injury in US military troops with over 10% sustaining heat stroke as defined by the International Classification of Diseases (ICD-9) (31).

An association between heat stroke and kidney dysfunction has long been recognized, and although not universal, this combination appears to exacerbate the risk of severe acute kidney injury (AKI). This itself portends significant mortality and may require supportive treatment in the form of renal replacement therapy (e.g., hemofiltration) (23, 44, 47). Treatment for severe AKI can be protracted, and full recovery is not guaranteed: survivors of this catastrophic condition may be left with chronic kidney disease, which itself represents significant morbidity (37).

Observations, mainly from longitudinal studies and case reports, suggest that there are a number of factors that are potentially associated with the development of heat illness, including a hot environment, lack of hydration, use of Non-steroidal anti-inflammatory drugs (NSAIDs) and unaccustomed physical activity (1, 3, 12, 15, 22, 23). Some theories also propose that immune dysregulation is partly responsible for the development of heat stroke. In this context, immune dysregulation involves an elevation of proinflammatory cytokines and vasoconstricting mediators including the pleiotropic cytokine interleukin-6 (12).

There is also evidence that AKI per se represents a pro-inflammatory condition with cytokines released by leukocytes and renal tubular cells in the injured kidney. These molecules are believed to be important components of both the initiation and extension of kidney injury (21). Recent findings suggest that interleukin-6 plays a pivotal role in the development of AKI, by promoting its early development via an injurious inflammatory reaction (21, 21, 32). This may be related to trans-signaling and STAT3 activation in renal tubular cells (21).

Interestingly, strenuous unaccustomed exercise can result in muscle damage and consequently elevated circulating interleukin-6 (12, 30, 41). Thus it is plausible that completing exercise in hot environments with muscle damage could generate a more severe proinflammatory response that induces kidney dysfunction typical of severe heat illness. The recent discovery of novel biomarkers of AKI offers a more sensitive and specific method to investigate the kidney in experimental models of heat stress. One such biomarker is neutrophil gelatinase-associated lipocalin (NGAL), a biomarker that has already been utilized in a variety of AKI settings (26–29, 36). Combined
with traditional markers of kidney function, such as creatinine filtration and the production of urine, a more complete understanding of the kidney’s stress response can be gained. Therefore, the unknown contribution of the proinflammatory state resulting from muscle damage to kidney function during heat exposure can, for the first time, be investigated.

Consequently, the purpose of this study was to investigate the effect of muscle damage and subsequent exercise in the heat on markers of kidney injury and function. We hypothesized that following a bout of muscle-damaging exercise, during subsequent exercise in a hot environment 1) kidney injury biomarkers would be upregulated (e.g., urine and plasma NGAL concentrations would be increased); 2) kidney function would be reduced (e.g., plasma creatinine concentration will be increased); and 3) changes in circulating interleukin-6 and consequently biomarkers of kidney injury would be correlated with changes in kidney function.

METHODS

Participants. The study was undertaken in accordance with the Declaration of Helsinki (2008), and the institutional ethics committee gave ethical approval. Recruitment occurred between January and August 2011. Potential participants who were aged between 18 to 30 yr and recreationally active were approached via the university and local sports clubs. Ten healthy Caucasian male subjects were successfully enrolled [age (SD), 20 (2) years]. All provided written consent and completed a brief health questionnaire, which indicated that none had medical conditions, musculoskeletal injury, history of heat illness, or medication use (e.g., regular use of NSAID) that precluded participation. Moreover, none of the subjects were heat acclimatized or consuming alcohol or caffeine.

Study design. A counterbalanced crossover design was employed, with each participant performing exercise in the heat following a treatment that consisted of either muscle-damaging exercise [exercise-induced muscle damage (EIMD); running downhill on a −10% gradient treadmill] or exercise intensity-matched but non-muscle-damaging exercise (CON; running on +1% gradient treadmill) (Fig. 1). All subjects performed both treatments, in random order, on separate occasions with a 14-day washout period in between treatments. The order of presentation of treatments was randomized by one of the authors (M. B. Fortes) using online software (http://www.randomizer.org; Site Statistics, Social Psychology Network), blinded to the identities and demographic details of all participants.

Familiarization and maximal exercise testing. At least 1 wk before the first experimental trial, maximal oxygen uptake (V̇O₂ max) was assessed by a continuous incremental exercise test on a motor-driven treadmill (Mercury 4.0, HP Cosmos, Nussdorf-Traunstein, Germany). Participants ran initially at 8 km/h at a constant 1% gradient with the running speed increased by 2 km/h every 3 min. At 16 km/h, the gradient was increased by 2.5% every 3 min until volitional exhaustion. During this entire period, oxygen uptake and carbon dioxide production were determined using an online breath-by-breath system (Cortex Metalyser 3B, Biophysik, Leipzig, Germany).

Using linear interpolation of the running speed vs. V̇O₂ relationship, the speed elicits 65% V̇O₂ max was calculated. Following a rest period of 15 min, a 4-min speed verification test was conducted to identify the speed that would evoke 65% V̇O₂ max in a subject while running at +1% and −10% gradients. Running speed was adjusted to elicit exactly 65% V̇O₂ max and verified by concomitant measures of expired gas, thus ensuring exercise intensity was the same on both gradients in the experimental arms.

Dietary control. All participants had a standardized breakfast before the start of each trial (cereal bar; 2 kcal/kg body mass). Furthermore, subjects were requested to replicate the diet consumed 24 h before the start of each treatment arm; this was confirmed through written diet diaries. Euhydration was achieved by subjects drinking 40 ml/kg nude body mass (model 705, Seca, Hamburg, Germany) of water during the 24 h before a treatment arm, and consuming an additional 5 ml/kg nude body mass ~30 min before. To confirm euhydration, urine specific gravity was measured using a handheld refractometer (Atago Uricon-Ne refractometer, NSG Precision Cells) on the initial urine sample on the day of study (2).

Experimental protocol (treatment). For both EIMD and CON conditions, participants wore standardized clothing consisting of running shorts, socks, and shoes and performed a warm-up consisting of a 3-min walk on the treadmill at 5 km/h. Depending on the treatment allocation, subjects then ran at their verified speed to elicit 65% V̇O₂ max on either a −10% gradient treadmill (EIMD) or a +1% gradient treadmill (CON) at an ambient temperature of 20°C and 40%
Kidney injury and function. To assess kidney injury, NGAL concentration in blood and urine was determined. To assess kidney function, plasma creatinine concentration (filtration), urine volume, urine specific gravity (urine concentration), and fractional excretion of sodium (integrity of renal tubular reabsorptive function) were measured. Sampling time points are shown in Fig. 1. For micturition, participants were asked to minimize bladder emptying through double voiding. Urine volumes were collected at each time point using 24-h urine containers to determine urine flow rate and to measure urinary specific gravity (see above). The fractional excretion of sodium was calculated as follows:

$$\left\{\frac{\text{sodium}_{\text{urinary}} \times \text{creatinine}_{\text{plasma}}}{\text{sodium}_{\text{plasma}} \times \text{creatinine}_{\text{urinary}}} \times 100\right\}$$

Whole blood (lithium heparin, K$_2$EDTA, and serum) was taken from the antecubital vein using a 22-gauge needle and Vacutainer (BD, Oxford, UK). Serum tubes were left to stand for 1 h before centrifugation. Blood samples were centrifuged for 10 min at 1,500 g. Serum, plasma, and urine aliquots were stored at $-80^\circ$C for subsequent biochemical analysis.

Muscle damage and inflammation. As an indicator of muscle damage, plasma creatine kinase was measured at baseline and 24 h posttreatment. As markers of inflammation, circulating plasma interleukin-6 and tumor necrosis factor-α were measured at baseline and pre- and post-heat stress (Fig. 1).

Biochemical analyses. ELISAs were performed for the following analytes using commercially based kits as per kit instructions: plasma creatine kinase (EnzyChrom creatine kinase assay kit, BioAssay Systems, Hayward, CA); plasma interleukin-6 (Quantikine high sensitivity interleukin-6, HS60B, R&D Systems Europe, Abingdon, UK); plasma tumor necrosis factor-α (High Sensitivity TNF-α ELISA with Signal Amplification, eBioscience, Vienna, Austria); and plasma/urine NGAL (NGAL Rapid ELISA kit, BioPorto, Gentofte, Denmark). Absorbances were read at a wavelength of 450 nm by a microplate reader (FLUOstar Omega, BMG Labtech, Ortenburg, Germany). Intra-assay coefficients of variation (CV) for creatine kinase, interleukin-6, tumor necrosis factor-α, and NGAL (plasma/urine) were 3.8, 5.3, 9.5, and 4.7/8.4%, respectively.

Plasma and urinary creatinine was measured by the Jaffé method using an Olympus AU2700 automated analyzer (assy: OR6178; Olympus UK; Beckmann-Coulter). Plasma and urine sodium was measured by an indirect ion-selective electron probe on the same analyzer. For each biochemical analysis, all participant samples were assayed on the same plate.

Urinary NGAL concentrations are presented as raw values and also when corrected for urinary flow rates (NGAL ng/min). This correction accounts for changes in urinary concentration and/or dilution. Although normalization of urinary NGAL to urinary creatinine is commonly used in clinical and research settings to account for urinary concentration changes (cf. albumin/creatinine ratio), its application in exercise models is likely to be flawed. Marked reductions in glomerular filtration rate and tubular dysfunction occur during high-intensity exercise, and subsequently urinary creatinine excretion becomes a nonconstant (16, 41). Thus, we did not use this method and normalized to urinary flow rate instead (49). All blood analytes were corrected for plasma volume shifts as described by the method of Dill and Costill (11).

Exercise intensity. To confirm energy expenditure was the same in both treatment arms, metabolic energy expenditure (M; W/m²) was determined by the following: oxygen consumption (VO₂) during treadmill running was calculated from 60-s expired air samples collected into a Douglas bag which were analyzed for oxygen and carbon dioxide concentrations (SERVOPRO 1440 gas analyzer, Servomex, Crowborough, UK) and volume (Harvard Apparatus, Edenbridge, UK). This, together with the respiratory exchange ratio (RER; VO₂/VO₂) and body surface area (BSA) by the DuBois and DuBois method, was used in the following equation:

$$\text{metabolic energy expenditure (in W)} = \frac{\text{VO}_2 \times [21.66 \times (0.23 \times \text{RER} + 0.77)]}{60 \times \text{BSA}}$$

Body temperature and hydration status. Core temperature was measured at each time point by a rectal thermistor inserted 12 cm beyond the anal sphincter (Henleys Medical Supplies, Herts, UK) and connected securely to a portable data logger (YSI model 4000A, YSI, Dayton, OH).

Hydration status was assessed through nude body mass change and plasma volume change. The latter was determined by measurement of hemoglobin (β-Hemoglobin Hemocue AB photometer, Hemocue, Dronfield, UK) and hematocrit (Hawksley and Sons, Sussex, UK) measured in whole blood in triplicate and then averaged for subsequent plasma volume shift calculations as described above.

Statistical analysis. Data for all dependent variables were examined for normality using histogram plots and Shapiro-Wilk tests. All data were found to be normally distributed except for urinary NGAL and flow rate corrections. Thus means (SD) are stated for all measures except for urinary NGAL where median (interquartile range) are presented. For the purposes of further statistical analysis, urinary data were successfully transformed to normality using a log₁₀ function.

For all dependent variables, EIMD and CON groups at pre-heat stress and post-heat stress were compared using parametric two-way repeated measures ANOVA (group vs. time) with adjustments made to the degrees of freedom when assumptions of sphericity were violated. Post hoc Bonferroni-adjusted t-tests or Tukey’s tests were used as appropriate to follow up significant interactions.

Mean differences and 95% confidence interval (CI) between EIMD and CON groups at pre-heat stress and post-heat stress were calculated for all measures. In the case of urinary NGAL and urinary NGAL flow rates, the Hodges and Lehman method was used to derive median differences and 95% CI between EIMD and CON.

Plasma creatine kinase concentrations in EIMD and CON arms were compared at baseline and 24 h posttreatment by two-tailed paired Student’s t-tests with Bonferroni correction. Mean energy expenditure in the EIMD and CON arms were compared during the respective treatment bout and during exercise in the heat by two-tailed paired Student’s t-tests with Bonferroni correction.

To determine the possible relationships between plasma interleukin-6, measures of renal function and biomarkers of kidney injury, bivariate Pearson’s correlations were performed on percent change scores from baseline to post-heat stress. For all tests, statistical significance was accepted when $P \leq 0.05$. SPSS version 18.0 (SPSS, IBM, Chicago, IL) was used for all statistical analysis.

For the primary outcome measure of NGAL, a power calculation revealed that six participants were needed per group to detect a smallest important change in means of 4.6 ng/ml. This value is the increase required to elevate urinary NGAL from the normal healthy population mean (5.3 ng/ml) to above the normal range (0.7–9.8 ng/ml) (34). This power calculation assumes a within-subject error of 1.7 ng/ml, and allows for a 5 and 20% chance of making a type I or type II error, respectively (14).
RESULTS

All 10 male participants [age 20 (SD) (2) yr; height 176 (6) cm; \(\text{VO}_2\)\(_\text{max}\) 59 (4) ml·kg\(^{-1}\)·min\(^{-1}\)] successfully completed both trials, and there were no participant dropouts or complications due to heat illness.

**Urinary NGAL and corrections for urinary flow rate.** The heat stress exercise bout elicited a rise in median urinary NGAL concentration following EIMD but not CON exercise (Fig. 2). At post-heat stress, 8 of 10 individuals and 3 of 10 individuals demonstrated rises in urinary NGAL concentrations above the normal range, in EIMD and CON trials, respectively.

Absolute urinary NGAL concentrations were also corrected for urinary flow rates, thus taking into account any alterations in urine production that may confound interpretation of raw values. Between pre- and post-heat stress, CON demonstrated a fall in urinary NGAL flow rate whereas EIMD exhibited a slight rise (baseline, pre-heat stress, post-heat stress for EIMD: 4.6 (2.5–5.6), 10.3 (4.4, 11.7), 12.4 (8.7, 19.2) ng/min; for CON: 9.4 (7.8–12.4), 19.7 (11.6, 29.0), 8.5 (5.7, 11.7) ng/min). For these data, there was a significant interaction (\(P = 0.002\)) but no main effects of time or group (\(P = 0.2\) and 0.3, respectively). Mean difference (EIMD – CON) and 95% CI for urinary NGAL flow rate between both groups were –0.3 [–0.6, –0.1] ng/min and 0.1 [0.007, 0.3] ng/min at pre-heat stress and post-heat stress, respectively.

**Plasma NGAL.** Between pre- and post-heat stress, there were increases in plasma NGAL concentrations that were more prominent in EIMD (Fig. 3).

**Plasma creatinine.** Plasma creatinine increased between pre-heat stress and post-heat stress in both groups, but more so for EIMD (Fig. 4). By post-heat stress, 5 of 10 participants in EIMD met stage 1 of the Acute Kidney Injury Network criteria (plasma creatinine rise of >0.3 mg/dl from baseline), compared with 0 of 10 participants in CON (45).

**Urinary volume, flow rate, and specific gravity.** Between pre- and post-heat stress, there was a reduction in urine volume that was more pronounced in EIMD, exhibiting a significant main effect of time and group \((P < 0.05\) and \(P = 0.05\), respectively), albeit with no interaction \((P = 0.9\), Table 1\).

Urinary flow rate revealed a similar pattern. Between pre- and post-heat stress, urinary flow rate fell to a lower than baseline value (Table 1). This reduction in flow rate was again more pronounced in EIMD, with significant main effects for time and group \((P = 0.001\) and \(P = 0.05\), respectively) but no interaction \((P = 0.7)\).

In line with the above, urine specific gravity rose between pre- and post-heat stress in both groups but more so in EIMD (Table 1). During pre- and post-heat stress, there were significant main effects of time and group \((P = 0.001\) and \(P = 0.04\), respectively) but again no interaction \((P = 0.2)\).

**Fractional excretion of sodium.** Fractional excretion of sodium fell between pre- and post-heat stress but tended to fall more sharply in EIMD (Table 1), as evidenced by a main effect of time \((P = 0.01)\) and a trend for a time \(\times\) group interaction \((P = 0.08)\).

**Muscle damage and inflammation.** As an indirect marker of muscle damage, plasma creatine kinase concentrations at base-
line were similar in CON and EIMD [CON: 78 (58) IU vs. EIMD: 58 (29) IU; \( P = 0.1 \)], suggesting that a sufficient washout period had occurred. By 24 h posttreatment, plasma creatine kinase concentrations were significantly elevated in EIMD [CON, 119 (69) IU vs. EIMD, 250 (69) IU; \( P < 0.001 \)], indicating that the protocol was successful in inducing muscle damage. Mean difference (EIMD − CON) and 95% CI for plasma creatine kinase between both groups were −20 IU [−47, 7 IU] and 131 IU [89, 173 IU] at baseline and post-24 h, respectively.

Furthermore, at baseline plasma interleukin-6 was not different between groups (\( P = 0.4 \); Table 2). Between pre- and post-heat stress, interleukin-6 increased in both groups, but this rise was greater in EIMD (time × group interaction, \( P = 0.005 \)). In contrast, there were no changes in plasma tumor necrosis factor-α between groups at baseline (\( P = 0.8 \); Table 2) or at the pre- to post-heat stress interval (main effect of time: \( P = 0.6 \); main effect of group: \( P = 0.5 \); interaction: \( P = 0.9 \)).

**Exercise intensity.** During treatment, both CON and EIMD exercise was conducted at the same exercise intensity with no difference in mean metabolic energy expenditure between treatments [CON: 504 (56) W/m² vs. EIMD: 503 (57) W/m²; \( P = 0.9 \)]. However, during exercise in the heat, mean metabolic energy expenditure was slightly higher in EIMD compared with CON, despite identical running speed and incline [CON: 522 (50) W/m² vs. EIMD: 556 (57) W/m²; \( P < 0.001 \)]. The mean difference (EIMD − CON) and 95% CI for mean metabolic energy expenditure between groups were −1 W/m² [−15, 13 W/m²] and 34 W/m² [23, 45 W/m²] at pre-heat stress and post-heat stress, respectively.

**Body temperature and hydration status.** Pre- and post-heat stress, rectal core temperature was greater in EIMD compared with CON (Table 2). There was a significant main effect of time and group (\( P < 0.001 \) and \( P = 0.007 \), respectively), but no interaction (\( P = 0.2 \)).

Nude body mass decreased between pre- and post-heat stress in both treatment arms (Table 2). This decrement in body mass was similar for each treatment; hence there was a significant main effect for time but not for group (\( P < 0.05 \) and \( P = 0.8 \), respectively). Moreover, when body mass change between pre- and post-heat stress was calculated as a proxy for fluid balance, there was no difference between EIMD and CON [body mass change EIMD: −0.79 (0.37) kg; CON: −0.78 (0.16) kg, \( P = 0.9 \)].

At baseline, plasma volume was calculated to be 52.3 (3.1) and 51.8 (3.0) % of total blood volume in CON and EIMD groups, respectively. Plasma volume was not different between groups at pre-heat stress and post-heat stress [pre-heat stress: CON, 51.9 (3.6) % vs. EIMD: 51.0 (3.3) %; post-heat stress: CON, 51.2 (3.1) % vs. EIMD, 51.0 (3.0) % of total blood volume]. No main effects were observed for time (\( P = 0.6 \)) or group (\( P = 0.4 \)).

**Correlational analyses.** Plasma NGAL was negatively correlated with urine volume in EIMD (\( r = −0.65 \); \( P = 0.04 \)) but not in CON (\( r = −0.04 \); \( P = 0.9 \)), and plasma NGAL was positively correlated with urinary specific gravity in EIMD.

### Table 1. *Kidney function markers following exercise-induced muscle damage or control exercise*

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<tr>
<td>EIMD</td>
<td>146 (105)</td>
<td>269 (133)†</td>
<td>88 (77)†</td>
<td>−93 [−253, 66]</td>
<td>−81 [−24, −139]</td>
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<tr>
<td>CON</td>
<td>156 (96)</td>
<td>363 (175)</td>
<td>169 (78)*</td>
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**Urine flow rate, ml/min**

| EIMD             | 3.4 (2.3)  | 3.5 (1.9)†         | 1.1 (1.0)†       | −1.3 [−3.3, 0.7]              | −1.0 [−1.7, −0.3]            |
| CON              | 3.2 (1.8)  | 4.8 (2.2)          | 2.0 (1.0)*       |                               |                               |

**Urinary specific gravity**

| EIMD             | 1.011 (0.008) | 1.006 (0.004)†       | 1.012 (0.004)†   | 0.001 [−0.002, 0.046]         | 0.004 [0.001, 0.008]         |
| CON              | 1.010 (0.008) | 1.005 (0.004)        | 1.008 (0.005)*   |                               |                               |

**Fractional excretion of sodium, %**

| EIMD             | 0.39 (0.15)  | 0.33 (0.16)         | 0.13 (0.12)*     | 0.04 [−0.08, 0.16]            | −0.08 [−0.18, 0.01]          |
| CON              | 0.36 (0.12)  | 0.29 (0.12)         | 0.22 (0.19)*     |                               |                               |

Values are means (SD) and mean difference between groups [95% confidence interval]. EIMD, exercise-induced muscle damage; CON, control. By main effect: *significantly different from before heat stress; †significantly different from CON.
Table 2. Treatment responses following exercise-induced muscle damage or control exercise

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<tr>
<td>Interleukin-6, pg/ml</td>
<td>0.7 (0.5)</td>
<td>2.8 (0.8)‡</td>
<td>6.0 (1.9)‡</td>
<td>1.4 [0.6, 2.2]</td>
<td>3.2 [1.5, 5.0]</td>
</tr>
<tr>
<td>Tumor necrosis factor-α, pg/ml</td>
<td>0.6 (0.3)</td>
<td>1.4 (0.9)</td>
<td>2.8 (1.8)†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EIMD</td>
<td>1.9 (0.2)</td>
<td>2.0 (0.1)</td>
<td>2.1 (0.4)</td>
<td>0.06 [−0.13, 0.24]</td>
<td>0.07 [−0.23, 0.37]</td>
</tr>
<tr>
<td>CON</td>
<td>1.9 (0.1)</td>
<td>2.0 (0.2)</td>
<td>2.0 (0.3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Core temperature, °C</td>
<td>37.0 (0.3)</td>
<td>37.8 (0.2)†</td>
<td>39.5 (0.5)†</td>
<td>0.35 [0.05, 0.65]</td>
<td>0.67 [0.13, 1.22]</td>
</tr>
<tr>
<td>EIMD</td>
<td>37.0 (0.4)</td>
<td>37.5 (0.3)</td>
<td>38.8 (0.7)†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CON</td>
<td>37.0 (0.3)</td>
<td>37.5 (0.3)</td>
<td>38.8 (0.7)†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nude body mass, kg</td>
<td>70.1 (7.7)</td>
<td>69.2 (7.5)</td>
<td>68.4 (7.5)*</td>
<td>−0.03 [−0.25, 0.19]</td>
<td>−0.04 [−0.42, 0.33]</td>
</tr>
<tr>
<td>EIMD</td>
<td>69.8 (7.3)</td>
<td>69.2 (7.3)</td>
<td>68.4 (7.3)*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CON</td>
<td>69.8 (7.3)</td>
<td>69.2 (7.3)</td>
<td>68.4 (7.3)*</td>
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Values are means (SD), and mean difference between groups [95% confidence interval]; By main effect or by post hoc test following significant interaction: *
(significantly different from before heat stress; †significantly different from CON.

(r = 0.64; P = 0.05), but not in CON (r = 0.08; P = 0.8). Similarly, a trend existed for plasma NGAL to be correlated negatively with urinary flow rate in EIMD (r = −0.61; P = 0.06), but not in CON (r = −0.05; P = 0.9). Although no associations were noted between plasma interleukin-6 with markers of kidney function, plasma interleukin-6 was positively correlated with the biomarker of kidney injury, plasma NGAL, in EIMD (r = 0.88; P = 0.001). Consistent with other measures, this association was not evident in CON (r = −0.06; P = 0.9).

DISCUSSION

For the first time, we have demonstrated that prior muscle-damaging exercise and its consequent mild inflammatory response led to upregulation of a biomarker of kidney injury (NGAL) when exercise was performed in the heat. Prior muscle-damaging exercise also resulted in alterations to kidney function as evidenced by increased plasma creatinine concentration (by ~20%), decreased urine volume and urine flow rate (by 50%), reduced fractional excretion of sodium (by 40%), and mildly increased urinary specific gravity compared with control exercise. Once Acute Kidney Injury Network criteria for AKI were applied, half of the individuals in the muscle damage group met stage 1 criteria (plasma creatinine >0.3 mg/dl from baseline) compared with no individuals in the control exercise group (45). Moreover, the reduced fractional excretion of sodium in the muscle-damaged group suggests increased Na-K-ATPase activity within renal tubules and therefore higher oxygen and energy demands (18). These findings reveal that even modest muscle damage as typically observed following unaccustomed exercise, combined with relatively mild exercise heat exposure, induces kidney stress.

Such vulnerability of the kidney is of particular relevance to exercise settings and occupations where arduous physical activity has to be completed in the heat. Of note, such functional changes were not a positive adaptation to dehydration. These changes occurred even though fluid intake was strictly controlled and matched between groups, and hydration status and fluid balance were similar in the muscle-damaged state, as evidenced by nearly identical body mass and plasma volume changes. The counterbalanced crossover study design also controlled for potential AKI aggravators such as preceding infectious illnesses, dehydration, and use of NSAID (22). Taking all of the above into account and noting that the study’s population comprised athletic individuals exhibiting a high degree of fitness (mean VO₂ max: 59 ml·kg⁻¹·min⁻¹), it is plausible that even more profound changes in biomarker and kidney function may result in the field where a “perfect storm” of muscle damage-naïve individuals and multiple environmental stressors are likely to exist (8). Thus muscle damage should be considered a novel risk factor for AKI.

Changes in plasma NGAL were correlated with altered kidney function (urinary volume, flow rate, and specific gravity). Although such data may suggest a maladaptive role for NGAL, it is noteworthy that recent in vitro studies of cell cultures exposed to heat stress demonstrate that ectopic expression of NGAL may actually have a protective effect (46). The role of inflammatory cytokines also requires further investigation. The increase in both plasma interleukin-6 and plasma NGAL following muscle damage and the positive correlation between these biomarkers (r = 0.9, P = 0.001 in the muscle damage group) support the argument that an acute-phase response to inflammation from muscle-damaging exercise may lead to the observed kidney stress. However, a lack of a direct correlation between interleukin-6 and kidney function does not discount a relationship and may suggest mediation by other factors (20, 21, 32). Interestingly, tumor necrosis factor-α is unlikely to be such a mediating factor due to the lack of changes following muscle-damaging or control exercise. Alternatively, it is possible that our muscle-damaging protocol did not produce an interleukin-6 response sufficient to be directly associated with alterations in kidney function. Elevations in plasma interleukin-6 are often seen in patients with severe sepsis and AKI, but these tend to be more profound with concentrations of at least several hundred-fold higher than those observed in the present study (6, 24, 39).

Apart from an inflammatory pathway, a reduction in kidney blood flow is another plausible mechanism that may have contributed to the NGAL and kidney function alterations observed herein. Kidney injury, as indicated by elevations of biomarkers such as NGAL, has been observed in recent clinical studies of “prerenal” (reduced kidney perfusion) AKI (33). Prerenal AKI is also known to be associated with heat stroke (23). Although not directly measured in the present study, the exercise protocol was of sufficient intensity (65% of VO₂ max) to reduce kidney blood flow (4, 15, 42, 47). The observed fall in urine production is also consistent with such a reduction in kidney blood flow (42).
From a practical standpoint, these findings suggest that clinicians should take note of individuals who complain of muscle soreness suggestive of muscle damage either before or after performing strenuous exercise in the heat, as they appear to be at greater risk of developing AKI. In the majority of cases, such aberrations will resolve without sequelae. However, if this scenario was amplified and/or occurred as part of a “perfect storm” (as is often the case during heat illness), serious AKI is more likely (8). Currently, unstandardized protocols exist to check for AKI in the days following exertional rhabdomyolysis-associated muscle damage (38). The present results suggest that kidney injury may be induced much earlier. Hence, precautionary measures including serial AKI biomarker sampling may be helpful, particularly given that measurement of traditional markers such as urine volume can be difficult to ascertain following exercise (8, 42).

We recognize that although our elevations of urine NGAL with muscle-damaging exercise were eightfold (to 15 ng/ml), with a highest observed value of 65 ng/ml (expected normal range of 0.7–9.8 ng/ml), and our elevations of plasma NGAL were two-fold (to 109 ng/ml) with a highest observed value of 158 ng/ml (expected normal range of 37–106 ng/ml), these responses are lower than that used to define clinical AKI (typically 250 ng/ml) (32). It is important to note that it was not a study aim to induce AKI. In fact, such subclinical elevations in NGAL have recently been proposed to indicate kidney stress or mild injury (33, 36). In the context of exercise-induced muscle damage, this interpretation is analogous to that of exercise-induced rises in troponin concentrations that have been suggested to be an indicator of mild cardiac stress and possibly subclinical damage following strenuous endurance activity (48).

A key limitation of our study is that we cannot be certain plasma NGAL elevations arose solely from the kidney, as other sources (e.g., respiratory epithelium, liver, heart) cannot be excluded (5). Moreover, fresh urine was not microscopically examined for evidence of tubular damage, e.g., for granular casts and for leukocyte quantification, which can also influence urinary NGAL concentrations (10). However, our plasma elevations were associated with reductions in measures of kidney function, and urinary flow rates were consistent with increased urinary tubular excretion of NGAL in EIMD. Furthermore, the ELISA kit utilized in this study only detects monomeric urinary NGAL, indicating a predominantly tubular rather than leukocyte origin (25). Finally, the current findings are only applicable to a male population. Although the majority of heat stroke and/or acute kidney injury cases following heavy physical exertion are reported in men (3), it remains to be shown whether women demonstrate similar responses given that their hormonal variability can induce a protective effect against muscle damage (9).

In conclusion, performing muscle-damaging exercise before running in the heat results in a mild inflammatory response (as assessed by plasma interleukin-6), upregulation of a biomarker of kidney injury suggesting kidney stress (as assessed by NGAL), and alterations to kidney function (as assessed by plasma creatinine, urine production, and fractional excretion of sodium). Hence, prior muscle-damaging exercise should be considered as a novel risk factor for developing AKI when exercise is performed in the heat. Further controlled studies are required to dissect other potential interacting risk factors such as prior NSAID use and intravascular volume depletion. These may have an additive effect with muscle damage and result in clinically significant changes in NGAL concentrations and kidney function measures. This study also raises the intriguing and as yet unanswered question as to whether acclimatizing naïve individuals to muscle damage can ameliorate the consequent inflammation, elevated AKI biomarkers, and alterations in kidney function, since preconditioning exercise reduces muscle damage through the repeated bout effect (7).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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