

Published in final edited form as:
J Appl Physiol (1985). 2025 March 01; 138(3): 706–717. doi:10.1152/jappphysiol.00590.2024.

Influence of work intensity on acute kidney injury risk during simulated occupational heat stress

Hayden W. Hess^{1,3}, Molly E. Heikkinen¹, Erica Tourula¹, M. Jo Hite¹, Kelli Rivers¹, Roger S. Zoh², Blair D. Johnson¹, David Hostler³, Zachary J. Schlader¹

¹Department of Kinesiology, Indiana University School of Public Health-Bloomington, Bloomington, Indiana, USA

²Department of Epidemiology and Biostatistics, Indiana University School of Public Health-Bloomington, Bloomington, Indiana, USA

³Center for Research and Education in Special Environments, Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY; Current Address for H. W. Hess.

Abstract

Violation of the National Institute of Occupational Safety and Health (NIOSH) heat stress recommendations by exceeding the allowable wet bulb globe temperature (WBGT) for a given work intensity and work-rest ratio augments acute kidney injury (AKI) risk. Here we tested the hypothesis that exceeding the allowable work intensity at a given WBGT and work-rest ratio would also worsen AKI risk. Twelve healthy adults completed two NIOSH recommendation compliant trials and one noncompliant trial consisting of a 4 h (half workday) exposure. Work-rest ratio was fixed at 30 min of walking and 30 min of rest each hour. Work intensity (metabolic heat production) was prescribed as a function of WBGT– 412±51 W (27.3±0.3°C; high intensity compliant [C_{high}]), 290±75 W (31.6±0.2°C; low intensity compliant [C_{low}]), and 410±61 W

Correspondence: Zachary J. Schlader, 1025 E. 7th St., Bloomington, IN 47405, zschlade@iu.edu.
 AUTHOR CONTRIBUTIONS

	H.W.H.	M.E.H.	E.T.	M.J.H.	K.R.	R.S.Z.	B.D.J.	D.H.	Z.J.S.
Conceived and designed research	x						x	x	x
Performed experiments	x	x	x	x					
Analyzed data	x				x	x			x
Prepared figures	x								
Drafted manuscript	x								x
Edited and revised manuscript	x	x	x	x	x	x	x	x	x
Approved final version of manuscript	x	x	x	x	x	x	x	x	x

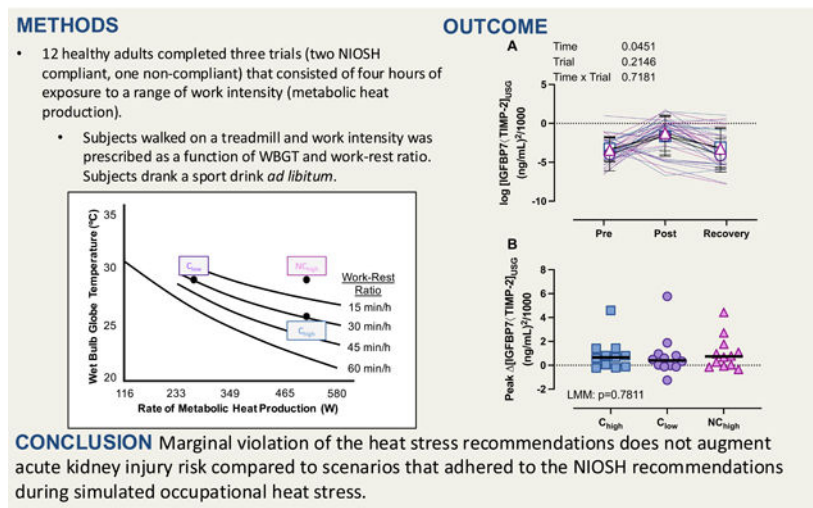
DISCLOSURES
 This article’s contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute for Occupational Safety and Health. Z.J.S. has received consultant fees from Otsuka Holdings Co., Ltd. No other conflicts of interest, financial or otherwise, are declared by the authors.

DISCLAIMERS
 None.

($31.7 \pm 0.2^\circ\text{C}$; high intensity noncompliant [NC_{high}]). AKI risk was quantified by the product of urinary insulin-like growth factor-binding protein 7 and tissue inhibitor of metalloproteinase 2 normalized to urine specific gravity ($[\text{IGFBP7} \cdot \text{TIMP-2}]_{\text{USG}}$). Peak core temperature was higher in NC_{high} trial ($38.3 \pm 0.4^\circ\text{C}$) compared to the compliant trials (C_{high} : $38.0 \pm 0.3^\circ\text{C}$; C_{low} : $37.8 \pm 0.4^\circ\text{C}$; $p \leq 0.0095$). $[\text{IGFBP7} \cdot \text{TIMP-2}]_{\text{USG}}$ increased from pre- to immediately postexposure in all trials (time effect: $p = 0.0454$) but the peak increase was not different between trials (C_{high} : $0.89 \pm 1.7 [\text{ng/mL}]^2/1000$; C_{low} : $0.78 \pm 1.7 [\text{ng/mL}]^2/1000$; NC_{high} : $1.0 \pm 1.4 [\text{ng/mL}]^2/1000$; $p = 0.7811$). Violating the NIOSH recommendations by exceeding either the allowable work intensity (i.e., NC_{high} versus C_{low}) or WBGT (i.e., NC_{high} versus C_{high}) resulted in a modest elevation in peak core temperature but did not modify AKI risk.

Graphical abstract

Influence of work intensity on acute kidney injury risk during simulated occupational heat stress



Keywords

acute kidney injury; kidney function; heat stress; hydration

INTRODUCTION

Outdoor workers frequently exposed to heat stress are at risk of developing chronic kidney disease (CKD). This kidney pathology is not associated with traditional risk factors (e.g., hypertension, diabetes, obesity), but is elicited following a single or recurrent episodes of acute kidney injury (AKI) caused by exertional hyperthermia and/or dehydration stemming from occupational heat exposures typically experienced in the agriculture, construction, and manufacturing sectors (1, 2). As such, this CKD is of non-traditional origin (CKDnt) or is often referred to as heat stress nephropathy (1, 3–5). Several field (6, 7) and laboratory-based studies (8–13) have identified that the risk of developing AKI is largely modified by the magnitude of heat strain and/or dehydration, duration of exposure, and nature of the physical work (1, 2, 14).

Federal and non-federal agencies issue exposure limit recommendations that aim to mitigate hyperthermia and ultimately, heat-related injury/illness. In the US, the National Institute for Occupational Safety and Health (NIOSH) heat stress recommendations (15) are designed to prevent peak core temperature during a workday from exceeding 38.0°C in unacclimatized workers. We have previously reported that adherence to the NIOSH heat stress recommendations were largely effective at accomplishing this objective when work-rest ratios were prescribed on the basis of wet-bulb globe temperature (WBGT) at a fixed work intensity (i.e., metabolic heat production) (11). Importantly, this translated to a maintenance of glomerular filtration rate (i.e., kidney function) (16, 17) and attenuated AKI risk compared to a work-rest matched positive control scenario that violated the recommendations (11). Whether these observations remain consistent across work intensities has rarely been explored. In fact, to our knowledge, only one study has examined the implications of work intensity on AKI risk during physical work in the heat. Houck et al. (18) reported that high-intensity intermittent work in the heat exacerbated AKI risk compared to work-matched, moderate intensity continuous work. That said, whether this conclusion translates to occupational scenarios is unclear. For example, the prescribed work in Houck et al. (18) was likely not reflective of manual labor such that work intensity oscillated between 80% and 30% of each participant's peak oxygen uptake every 5 minutes. Therefore, the purpose of the present study was to examine AKI risk during simulated occupational heat stress that adhered to the NIOSH heat stress recommendations by fixing the work-rest ratio and adjusting the work intensity (metabolic heat production) as a function of WBGT. Importantly, we examined the effects of work intensity under steady-state conditions. To do so, we compared two scenarios that were compliant with the NIOSH recommendations to a positive control scenario that violated the recommendations by exceeding the allowable work intensity for a given WBGT at a given work-rest ratio. We tested the hypothesis that violating the recommendations would augment hyperthermia resulting in elevated AKI risk during simulated occupational heat stress.

METHODS

Participants

To determine sample size, effect size was calculated from the product of urinary insulin like growth factor binding protein 7 (IGFBP7) and tissue inhibitor metalloproteinase 2, (TIMP-2), an biomarker of overall AKI risk (see below) from Chapman et al. (10). Using this effect size ($f = 0.365$), $\alpha = 0.05$, $1 - \beta = 0.80$, and a moderate correlation coefficient between repeated measures ($r = 0.77$), the calculated total sample size needed to detect a significant within participant, repeated measures interaction using a repeated measures ANOVA (the a priori primary analysis) was 9 participants (G*Power 3.1.9.7). However, to account for potential missing samples/data, we recruited an additional 3 subjects to ensure a complete data set. Therefore, twelve healthy adult nonsmokers (7 women; age: 27 y [range: 20–39]; body mass index: 24 kg/m² [range: 21–31]) participated in this study with all trials completed from July 2022 until March 2023. All subjects were free of chronic disease, were not heat acclimatized (19), and regularly engaged in physical activity as reported via the International Physical Activity Questionnaire (20). All had normal kidney function at screening (i.e., eGFR >60 mL/min/1.73 m²) measured via a point-of-care device

(StatSensor Creatinine, Nova Biomedical). Women were not pregnant, which was confirmed via urine pregnancy test prior to each visit, self-reported to be normally menstruating, and had no diagnosis of a menstrual cycle disorder. Women were tested at any point during their menstrual cycle because in real-world settings women work across their entire menstrual cycle. The study was approved by the Institutional Review Board at Indiana University (IRB# 1902420140), conformed to the Declaration of Helsinki, and was registered at [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04767347) (NCT04767347). Before participation, each subject was informed of the procedures and risks before providing informed written consent.

Experimental Protocol

Participants visited the laboratory on four occasions separated by at least three days. The first visit involved screening and visits two through four were the experimental trials. The three experimental trials (Figure 1) consisted of four hours (half a workday) at a fixed work-rest ratio of 30:30 minutes with exposure to WBGTs of $31.6 \pm 0.2^\circ\text{C}$ (C_{low}), $27.3 \pm 0.3^\circ\text{C}$ (C_{high}), and $31.7 \pm 0.2^\circ\text{C}$ (NC_{high}). The NC_{high} either violated the NIOSH heat stress recommendations by exceeding the allowable work intensity (metabolic heat production) at a given WBGT and 30:30 min work-rest ratio (i.e., NC_{high} vs. C_{low}) or by exceeding the allowable WBGT at a given metabolic heat production and 30:30 min work-rest ratio (i.e., NC_{high} vs. C_{high}). Subjects walked on a treadmill evoking an average rate of metabolic heat production (H_{prod}) of 412 ± 51 W (C_{high}), 273 ± 47 W (C_{low}), 410 ± 61 W (NC_{high}). The experimental trials were completed in a block-randomized order and subjects were blinded to the environmental conditions where possible.

The experimental protocol was the same as previously reported (11, 16, 17) except for the conditions of the 4 h exposures outlined above. All subjects were euhydrated upon arrival, defined as a urine specific gravity (USG) ≤ 1.020 (21). Following urine collection subjects drank 367 ± 62 mL (0.5% body mass) of cool tap water. Subjects then rested supine for ~60 minutes in a $\sim 22^\circ\text{C}$ environment before a blood sample was obtained. Subjects then voided their bladder, were weighed nude, and donned long pants and long shirt, a short sleeve cotton t-shirt served as an undershirt, and athletic shoes. Subjects then entered the environmental chamber and the four-hour exposure commenced. Compliant with the NIOSH guidelines (15), subjects were provided 237 mL of a cool ($\sim 10^\circ\text{C}$) flavor preferred sport drink (Gatorade; 248 kcal/L; 62 g CHO/L; 450 mg Na/L) every 15 minutes and were permitted to drink *ad libitum*. Total volume consumed across the four-hour exposure was measured. Additionally, we calculated the percentage of potential volume (i.e., if participants drank all potential provided fluids up to 237 mL every 15 minutes) as $([\text{total volume} \div \text{potential volume}] * 100)$. During rest periods, subjects sat on a mesh chair in the environmental chamber. Subjects were permitted to urinate as needed during the four-hour exposure. Urine volume and atomic time were recorded to determine urine flow rate at the postexposure urine collection. Following the exposure, subjects were weighed nude, voided their bladder, and then rested supine for 20 minutes before a venous blood sample was obtained. Subjects then completed a 60-minute recovery period where an additional fluid replacement equal to the preexposure fluid bolus outlined above (i.e., 367 ± 62 mL [0.5% body mass]) but of their flavor preferred sports drink. Subjects then rested supine for ~60 minutes before measurements were repeated (i.e., Recovery).

Instruments and Measurements

Height and nude body mass were measured using a stadiometer and scale. Percentage changes in body mass were calculated as $[(\text{postexposure} - \text{preexposure nude body mass}) / \text{preexposure body mass}] \times 100$. Core temperature was measured via rectal temperature probe inserted ~10 cm beyond the anal sphincter (n=11) or via telemetry pill (HQ Inc.) swallowed ~6–8 hours before each experimental trial (n=1). The method of core temperature measurement was consistent for all trials for each subject to avoid any differences between the two methods that might exist (22). Heart rate was measured using a wireless monitor (Polar Electro, Bethpage, NY, USA). Systolic and diastolic blood pressure were measured manually. Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Heart rate and mean arterial pressure are reported every 15 minutes during the exposure. Finally, perceptual data (thermal perception, comfort and sweating sensation (23), rating of perceived exertion (24), and thirst perception (25)) were measured every 15 minutes and presented as the mean during the four-hour exposure.

Changes in plasma volume were calculated from hemoglobin and hematocrit using standard equations (26). Serum creatinine (sCr) and urine creatinine (uCr) were measured via a clinical analyzer (COBAS INTEGRA 400+ Analyzer, Roche Diagnostics, Indianapolis, IN, USA). Urine flow rate was calculated as urine volume divided by time (minutes between each bladder void). Glomerular filtration rate (GFR) was estimated from creatinine clearance (i.e., urinary creatinine $\times$ urine flow rate $\div$ serum creatinine). Urine specific gravity was measured using refractometry (Atago, Tokyo, Japan). Urine and plasma osmolality (Advanced Instruments, Norwood, MA, USA), and sodium and potassium concentrations (Medica Corporation, Bedford, MA, USA) were measured in duplicate using commercially available systems. Fractional excretion (FEx) was calculated as $100 \times ([U_x \times sCr] \div [S_x \times uCr])$, where U_x and S_x represent concentrations of 'x' in the urine and serum, and uCr and sCr are serum and urinary creatinine concentrations (2). Free water clearance was calculated as $UFR \times (1 - [U_{osm} \div P_{osm}])$, where UFR is urine flow rate and U_{osm} and P_{osm} are the osmolality of the urine and plasma. Osmolar clearance was calculated as $(U_{osm} \div P_{osm}) \times UFR$ (27).

Insulin-like growth factor-binding protein 7 (IGFBP7; a marker of G1 cell cycle arrest in the renal epithelium preferentially secreted in the renal proximal tubules (2)), tissue inhibitor metalloproteinase 2 (TIMP-2; a marker of G1 cell cycle arrest in the renal epithelium preferentially secreted in the renal distal tubules (2)), monocyte chemoattractant protein-1 (MCP-1; a proinflammatory cytokine expressed in acute kidney injury (28) and observed in cases of CKDnt among heat stressed Mesoamerican agricultural workers (29)), and thioredoxin-1 (TRX-1; a marker of systemic oxidative stress [serum] (30) and reflective of oxidative stress and/or hypoxia from proximal tubular epithelial cells [urine] (31) previously reported to increase during prolonged passive heat stress (12)) were measured in the serum and urine using separate commercially available human assay kits. Consistent with the FDA approved indication in clinical settings, the product of urinary IGFBP7 and TIMP-2 ($[IGFBP7 \cdot TIMP-2]$) provided an index of AKI risk (2). Urinary $[IGFBP7 \cdot TIMP-2]$ is presented over time and as the peak change (Δ) from preexposure, which is consistent with the FDA approved indication where time course is not considered (2). To account

for differences in urine concentration, [IGFBP7•TIMP-2] was normalized to USG (i.e., [IGFBP7•TIMP-2]_{USG}). Additionally, urinary IGFBP7, TIMP-2, MCP-1, and TRX-1 were independently normalized to urine flow rate and urine specific gravity. Because changes in urinary MCP-1 and TRX-1 concentrations could be contributed to by extra-renal sources, we calculated the fractional excretion of each biomarker (e.g., FeMCP-1) as $100 \times ([U_x \times sCr] \div [S_x \times uCr])$, as outlined above. The fractional excretion data provide an estimate of the origins of MCP-1 and TRX-1 urinary markers. As such, increases in the fractional excretion of MCP-1 and TRX-1 were interpreted as increased renal production of these proteins (32).

Data and Statistical Analysis

We examined dependent variables between trials via repeated measures, one-way linear mixed models (LMM) or dependent variables over time (pre- to postexposure and following a 1-hour recovery) and between trials with two-way (Time $\times$ Trial) LMMs. All urinary TRX-1, MCP-1, IGFBP7, TIMP-2, and [IGFBP7•TIMP-2]_{USG} data over time were not normally distributed. In these instances, data were log₁₀ transformed prior to analysis, which corrected the data to a normal distribution. When the assumption of sphericity was violated, the Geisser-Greenhouse correction was applied. In all instances, when a significant effect or interaction was identified, multiple comparisons were carried out using Sidak's test, which corrects for multiple comparisons. Data were analyzed with GraphPad Prism software (Version 10.2.0). All data are presented as individual values, absolute or change (from preexposure) values using mean $\pm$ SD. $n=12$ unless otherwise reported. There are several reasons for missing data: 1) failure to obtain a blood draw or urine sample within a given subject/trial/timepoint; and/or 2) samples were outside of the range of the assay kit or of the osmometer and/or electrolyte analyzer. A priori statistical significance was set at $p \leq 0.05$. Effect sizes (Cohen's d_z) are reported for statistically significant pairwise comparisons in accordance with published recommendations (33). Actual p-values are reported where possible. In instances referring to more than one pairwise comparisons the highest (for groups of comparisons where $p \leq 0.05$) or lowest (for groups of comparisons where $p \geq 0.05$) p-values are reported using $\leq$ or $\geq$ symbols, respectively.

RESULTS

Core temperature and hemodynamic responses to simulated occupational heat stress are presented in Figure 2A-F. Briefly, core temperature increased throughout the exposure in all trials ($p < 0.0001$), but non-compliance with the recommendations (NC_{high}) resulted in a greater increase in core temperature starting at minute 30 ($p \leq 0.0059$). This resulted in a greater mean ($C_{high} : 37.6 \pm 0.2^\circ\text{C}$; $C_{low} : 37.5 \pm 0.3^\circ\text{C}$; NC_{high} : $37.8 \pm 0.2^\circ\text{C}$; $p = 0.0009$; $d_z : 0.99$) and peak ($C_{high} : 38.0 \pm 0.3^\circ\text{C}$; $C_{low} : 37.8 \pm 0.4^\circ\text{C}$; NC_{high} : $38.3 \pm 0.4^\circ\text{C}$; $p = 0.0002$; $d_z : 1.25$) core temperature in the non-compliant vs. both compliant trials. Perceptual data are presented in Figure 3A-E. Indices of hydration and fluid intake are presented in Figure 4A-D. Percentage change in body weight was not different between the non-compliant and compliant trials ($C_{high} : -0.1 \pm 0.8\%$; $C_{low} : 0.1 \pm 1.0\%$; NC_{high} : $0.0 \pm 1.2\%$; $p = 0.6784$) despite differences in whole-body sweat rate ($C_{high} : 0.4 \pm 0.1$ L/hour; $C_{low} : 0.3 \pm 0.1$ L/hour;

NC_{high} : 0.5 ± 0.1 L/hour; $p < 0.0001$ *vs* 0.99). The augmented fluid losses via sweating were offset by increased *ad libitum* fluid intake ($p = 0.0016$).

Fluid and electrolytes are presented in Table 1 and creatinine clearance and indices of fluid conservation are presented in Table 2. Briefly, plasma volume increased from preexposure in the C_{high} trial ($p \leq 0.0036$), but did not change in the C_{low} ($p \geq 0.0513$) and NC_{high} ($p \geq 0.8125$) trials. Serum creatinine increased from pre- to postexposure in all trials (C_{high} : $+0.06 \pm 0.05$ mg/dL; C_{low} : 0.05 ± 0.04 mg/dL; NC_{high} : 0.11 ± 0.06 mg/dL; $p \leq 0.0213$; *vs* 0.90) and remained elevated at recovery in the NC_{high} trial ($+0.09 \pm 0.05$ mg/dL; $p < 0.0001$). Creatinine clearance was reduced from pre- to postexposure in the NC_{high} trial ($p = 0.0053$; *vs* 0.86). Urine flow rate was not changed from pre- to postexposure and following recovery or different between trials (Time $\times$ Trial: $p = 0.5891$), but other indices of fluid conservation (i.e., osmolar clearance, fractional excretion of sodium and potassium) were reduced from preexposure in all trials ($p \leq 0.0104$).

Serum and urinary biomarkers of oxidative stress, inflammation, and acute kidney injury risk are presented in Table 3 and Figure 4A-F. Serum TRX-1, IGFBP7, and TIMP-2 did not change over time or differ between trials ($p \geq 0.1657$). In contrast, serum MCP-1 increased from pre- to postexposure in the NC_{high} trial ($p < 0.0468$; *vs* 0.77), was higher than C_{low} at postexposure ($p = 0.0017$; *vs* 0.71) and C_{high} at recovery ($p = 0.0332$; *vs* 0.67). All urinary biomarkers were elevated from pre- to postexposure in each trial ($p \leq 0.0009$) when normalized to urine flow rate. However, there were only main effects of time for urinary MCP-1, IGFBP7, and TIMP-2 when normalized to urine specific gravity ($p \leq 0.0461$), but *posthoc* analyses did not reveal any within trial changes. There were no changes in the fractional excretion of TRX-1, MCP-1, IGFBP7, or TIMP-2 ($p \geq 0.0673$). Finally, acute kidney injury risk ($[IGFBP7 \cdot TIMP-2]_{USG}$) is presented in Figure 5. There was a main effect of time ($p = 0.0451$), but no differences between trials over time (Figure 5A) or as a peak change (Figure 5B).

DISCUSSION

The purpose of the present study was to examine the influence of work intensity (i.e., H_{prod}) on AKI risk and indices of kidney function/fluid conservation during simulated occupational heat stress that was either compliant (C_{high} and C_{low}) or non-compliant (NC_{high}) with the NIOSH heat stress recommendations. We tested the hypothesis that exceeding the allowable work intensity at a given WBGT and fixed work-rest ratio would augment AKI risk. In the present study, non-compliance with the NIOSH recommendations (NC_{high}) resulted in a marginally higher magnitude of hyperthermia (Figure 2A-D) compared to NIOSH compliant trials. In contrast with our hypothesis, this resulted in no difference in AKI risk between the three experimental trials (Figures 5A-H, 6A-B). In disagreement with the first study (11, 16) as part of our registered clinical trial ([NCT04767347](#)), in the present study we observed that modest non-compliance with the NIOSH recommendations (i.e., exceeding allowable H_{prod} by ~ 120 W or environmental limits by $\sim 4^\circ\text{C}$ WBGT at 30 minutes work-rest per hour) did not augment AKI risk quantified via urinary biomarkers. This highlights subtle differences with regards to the severity of non-compliance and when rest is issued in compensable (present study) versus uncompensable conditions (11) (discussed in more detail below).

The NIOSH heat stress recommendations aim to keep peak core temperature below 38.0°C for non-acclimated workers (15). ~62% (15/24) of compliant trials maintained a peak core temperature below 38.0°C, whereas non-compliance resulted in 100% of trials exceeding a peak core temperature of 38.0°C (Figure 2B). In the current study, this was paralleled by a greater mean core temperature and time above 38.0°C in the non-compliant versus compliant trials (Figures 2C and 2D). These findings are consistent with our previous study (11, 16). Also in agreement with our previous study (17), hydration status was maintained in both compliant and non-compliant trials with *ad libitum* fluid intake despite differences in environmental conditions, work intensity (metabolic heat production), and sweat rates (Figures 4A-D). This is important to note, as AKI risk and kidney function are modulated by hydration status and fluid intake (2, 10). Thus, any anticipated differences in kidney function and/or AKI risk between compliant and non-compliant trials (although not observed in the present study) would have been reflective of differences in hyperthermia, but unlikely total work (11) completed during the simulated occupational heat stress.

Urine specific gravity and serum osmolality did not change nor was different between trials (Table 1). However, plasma volume was expanded in all trials (i.e., a main effect of trial was observed). Serum creatinine was elevated from pre- to postexposure in each trial and remained elevated following recovery during the C_{low} and NC_{high} trials. Furthermore, serum creatinine was higher in the non-compliant trial compared to C_{low}. Our previous study revealed that the change in serum creatinine is related to peak core temperature (11), which likely explains the higher serum creatinine in the non-compliant trial in the present study given the greater hyperthermia. An interesting finding in the present study is that several indices of fluid conservation remained unchanged from pre- to postexposure and following a one-hour recovery (Table 2). Urine flow rate and free water clearance were unchanged in all trials, which is likely due to the magnitude of fluid intake during the four-hour exposure. This contrasts with our previous studies that have reliably shown a reduction in urine flow rate and free water clearance reflective of fluid conservation during heat strain and/or dehydration (9–13, 16, 34). However, the fractional excretion of sodium and osmolar clearance was reduced from pre- to postexposure in all trials and remained reduced from preexposure following a one-hour recovery, but was not different between trials despite differences in heat strain (Figure 2A-D).

Creatinine clearance (our marker of glomerular filtration rate) was unchanged in the compliant trials (Table 2), which is consistent with the compliant trials in our previous study (16). However, creatinine clearance was reduced from preexposure in the NC_{high} trial but was not different from the two compliant trials at any timepoint (Table 2). This is an interesting finding given that non-compliance in the present study only resulted in a modest difference in heat strain and no difference in hydration status compared to the compliant trials. However, this is consistent with previous work from our lab (9, 10) despite showing in our previous NIOSH recommendation study that creatinine clearance is maintained even under non-compliant conditions as long as fluids are freely available (11).

Perhaps the most interesting finding in the present study, AKI risk (quantified via [IGFBP7•TIMP-2]) was not different between the compliant and non-compliant trials (Figures 5 and 6). [IGFBP7•TIMP-2]_{USG} increased from pre- to postexposure and returned

to preexposure levels following a one-hour recovery in all trials. Both IGFBP7 (a marker of proximal tubular injury) and TIMP-2 (a marker of distal tubular injury) increased similarly indicating the both the proximal and distal tubules were involved in the elevation in $[IGFBP7 \cdot TIMP-2]_{USG}$ (2). Interestingly, we observed more muted responses when normalizing to concentration (urine specific gravity), which only revealed a time main effect, compared to when normalizing to urine flow rate in which all trials had a significant increase from pre- to postexposure. Combined with the findings from our previous study (11), there appears to be a threshold in core temperature from which AKI risk is augmented under non-compliant scenarios. We previously reported augmented AKI risk in participants that reached/exceeded 38.0°C. However, it is important to note that this was due to a $\sim 1.0^{\circ}\text{C}$ difference in peak core temperature between the group of participants that did or did not reach/exceed 38.0°C. In the present study, the difference in peak core temperature between compliant and non-compliant trials was $\sim 0.3^{\circ}\text{C}$. Thus, the differences in work intensity (metabolic heat production) had marginal impact on our markers of AKI risk. Notably, while there was a statistical difference in workload between the high and low intensity trials (high: 5.4 ± 0.7 METs vs. low: 3.5 ± 0.9 METs; $p < 0.001$), both were relatively moderate intensity workloads (35). Thus, greater differences in workload may be necessary to elicit differential elevations in AKI risk. This is supported by previous field work by Moyce et al. in which it was reported that AKI (quantified by KDIGO criteria for elevations in serum creatinine) across a single day (average daily maximum WBGT: $\sim 30^{\circ}\text{C}$) in agricultural workers in California was associated with increased workload, but AKI risk was particularly marked when the workload exceeded moderate levels (36). Further, it is important to note that duration of exposure is an integral component to AKI risk when completing physical work in the heat (12–14). The combination of identical exposure times and small differences in core temperature responses among trials resulted in similar total heat loads among trials. Additionally, core temperature across each 30-minute rest period decreased, which was not observed in our previous investigation in which rest occurred in a hotter environment (WBGT: $\sim 36^{\circ}\text{C}$ (11)). These findings highlight the importance of rest to reduce heat strain, particularly when exceeding the heat stress recommendations for work intensity, findings that support interventions put in place in the field that reduce the renal burden of heat stress (37). Together, we speculate that there is a (yet undefined) core temperature threshold above 38.0°C, particularly when sustained, upon which AKI risk disproportionately increases. It is also important to note that these findings are representative of the primary effects of hyperthermia, as hydration status was well maintained via ad libitum drinking.

In the present study, we measured urinary markers of inflammation and oxidative stress. In all trials, regardless of NIOSH recommendation compliance, urinary markers of inflammation (MCP-1) and oxidative stress (TRX-1) were elevated immediately postexposure but returned to preexposure levels at recovery (Figures 5A-D). Additionally, we measured all markers in the serum (Table 3) to capture any changes in systemic inflammation (serum MCP-1) and/or oxidative stress (serum TRX-1). Serum TRX-1 was unchanged in all trials, while serum MCP-1 increased from pre- to postexposure in the NC_{high} trial and was higher than the C_{low} trial at postexposure and C_{high} trial at recovery. This could indicate that the greater heat strain, albeit marginal, resulted in an elevation in

systemic inflammation under non-complaint conditions but this did not seemingly modify markers of AKI risk. These findings suggest that the elevated AKI risk in the present study could be contributed to by the systemic inflammatory response to hyperthermia, as is known to occur with exertional heat injury (38). This speculation is supported by our fractional excretion of MCP-1 and TRX-1 data, which were unchanged, indicating that the increases in urinary MCP-1 and TRX-1 likely reflect increases in systemic (serum) MCP-1 and TRX-1. We would have anticipated an increase in the fractional excretion of these markers if they were endogenous to the kidneys (32). Future work is required to elucidate the role of systemic inflammation in the pathophysiology of exertional heat injury, particularly as it pertains to the development of AKI.

Experimental Considerations

Given that the present study was the second component to the registered clinical trial ([NCT04767347](#)), there are several experimental considerations pertaining to the previous study (11, 16, 17) that also apply here. Most importantly, particularly with regards to the generalizability of the findings, the compliance with NIOSH heat stress recommendations were only permitted under laboratory-controlled environmental conditions where metabolic heat production remained constant during the 4-hour exposure. This contrasts what often occurs on the job where during half of a typical workday fluctuations in environmental conditions and metabolic heat production variation would be anticipated (1, 6). However, the selected work intensities (metabolic heat production) represent the average of several physical labor activities (e.g., walking, lifting, shoveling) as identified from the compendium of physical activities (39). Second, since the examination of kidney-related indices occurred after a simulated half workday, it remains unknown if these responses would be the same if the duration had been extended (e.g., 6–12 hours, overnight). Much like the first study, this is integral with regards to the trials where workers were non-compliant with the NIOSH heat stress recommendations (11). However, as we have demonstrated in the present and previous studies (11, 16, 40), the simulated half-day is indeed sufficient for examining differences between NIOSH-compliant and non-compliant trials. Third, much like all our work in this area, we are relegated to measuring circulating and urinary biomarkers only at limited and discrete timepoints so it is possible that we missed dynamic changes in kidney-related indices during the simulated occupational heat stress, as well as the postexposure and recovery periods (41). Finally, taken in its entirety, [NCT04767347](#) was completed in participants that were self-reported to be healthy and aged 20–40 years. Thus, combined with the findings of the previous study (11, 16, 17, 42), it may not be broadly generalizable to the general population of workers exposed to heat stress that are aged across the entire adult lifespan (3) or carry traditional risk factors that may further challenge the kidneys during occupational heat stress (e.g., hypertension, diabetes, obesity) (42).

Conclusions

In conclusion, AKI risk is elevated immediately following four hours of simulated occupational heat stress that was compliant with the NIOSH heat stress recommendations (via adherence to metabolic heat production [work intensity] at a given WBGT and fixed work-rest ratio) but is not augmented under a scenario of modest non-compliance.

ACKNOWLEDGMENTS

All biochemical analyses were conducted by the Center for Diabetes and Metabolic Diseases Translation Core at the Indiana University School of Medicine. We thank the participants for participating in our study. Additionally, we thank the research staff and other students for assistance during data collection.

GRANTS

This study was supported by an award from the National Institute for Occupational Safety and Health (R01OH011528; to Z.J.S.).

REFERENCES

1. Chapman CL, Hess HW, Lucas RAI, Glaser J, Saran R, Bragg-Gresham J, Wegman DH, Hansson E, Minson CT, **and** Schlader ZJ. Occupational heat exposure and the risk of chronic kidney disease of nontraditional origin in the United States. *Am J Physiol Regul Integr Comp Physiol* 321: R141–r151, 2021. [PubMed: 34161738]
2. Chapman CL, Johnson BD, Parker MD, Hostler D, Pryor RR, **and** Schlader Z. Kidney physiology and pathophysiology during heat stress and the modification by exercise, dehydration, heat acclimation and aging. *Temperature* 1–52, 2020.
3. Johnson RJ, Wesseling C, **and** Newman LS. Chronic Kidney Disease of Unknown Cause in Agricultural Communities. *N Engl J Med* 380: 1843–1852, 2019. [PubMed: 31067373]
4. Roncal-Jimenez C, García-Trabanino R, Barregard L, Lanaspa MA, Wesseling C, Harra T, Aragón A, Grases F, Jarquin ER, González MA, Weiss I, Glaser J, Sánchez-Lozada LG, **and** Johnson RJ. Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy. *Am J Kidney Dis* 67: 20–30, 2016. [PubMed: 26455995]
5. Roncal-Jimenez CA, García-Trabanino R, Wesseling C, **and** Johnson RJ. Mesoamerican Nephropathy or Global Warming Nephropathy? *Blood Purif* 41: 135–138, 2016. [PubMed: 26766409]
6. Glaser J, Hansson E, Weiss I, Wesseling C, Jakobsson K, Ekström U, Apelqvist J, Lucas R, Arias Monge E, Peraza S, Hogstedt C, **and** Wegman DH. Preventing kidney injury among sugarcane workers: promising evidence from enhanced workplace interventions. *Occup Environ Med* 77: 527–534, 2020. [PubMed: 32404530]
7. Hansson E, Glaser J, Weiss I, Ekström U, Apelqvist J, Hogstedt C, Peraza S, Lucas R, Jakobsson K, Wesseling C, **and** Wegman DH. Workload and cross-harvest kidney injury in a Nicaraguan sugarcane worker cohort. *Occup Environ Med* 76: 818–826, 2019. [PubMed: 31611303]
8. Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, **and** Schlader ZJ. Both hyperthermia and dehydration during physical work in the heat contribute to the risk of acute kidney injury. *J Appl Physiol* 128: 715–728, 2020. [PubMed: 32078468]
9. Chapman CL, Johnson BD, Sackett JR, Parker MD, **and** Schlader ZJ. Soft drink consumption during and following exercise in the heat elevates biomarkers of acute kidney injury. *Am J Physiol Regul Integr Comp Physiol* 316: R189–r198, 2019. [PubMed: 30601706]
10. Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, **and** Schlader ZJ. Both hyperthermia and dehydration during physical work in the heat contribute to the risk of acute kidney injury. *J Appl Physiol* (1985) 128: 715–728, 2020. [PubMed: 32078468]
11. Hess HW, Baker TB, Tarr ML, Zoh RS, Johnson BD, Hostler D, **and** Schlader ZJ. Occupational Heat Stress Recommendation Compliance Attenuates Acute Kidney Injury Risk Compared to a Work-Rest Matched, Positive Control Scenario. *Kidney360* 2023.
12. Hess HW, Stooks JJ, Baker TB, Chapman CL, Johnson BD, Pryor RR, Basile DP, Monroe JC, Hostler D, **and** Schlader ZJ. Kidney injury risk during prolonged exposure to current and projected wet bulb temperatures occurring during extreme heat events in healthy young men. *J Appl Physiol* (1985) 133: 27–40, 2022. [PubMed: 35616302]
13. Schlader ZJ, Chapman CL, Sarker S, Russo L, Rideout TC, Parker MD, Johnson BD, **and** Hostler D. Firefighter Work Duration Influences the Extent of Acute Kidney Injury. *Med Sci Sports Exerc* 49: 1745–1753, 2017. [PubMed: 28272268]

14. Schlader ZJ, Hostler D, Parker MD, Pryor RR, Lohr JW, Johnson BD, **and** Chapman CL. The Potential for Renal Injury Elicited by Physical Work in the Heat. *Nutrients* 11: 2019.
15. Jacklitsch B, Williams J, Musolin K, Coca A, Kim JH, **and** Turner N. NIOSH Occupational exposure to heat and hot environments NIOSH, CDC 2016.
16. Hess HW, Baker TB, Tarr ML, Zoh RS, Johnson BD, Hostler D, **and** Schlader ZJ. Creatinine clearance is maintained in a range of wet-bulb globe temperatures and work-rest ratios during simulated occupational heat stress. *Am J Physiol Renal Physiol* In Press: 2024.
17. Hess HW, Tarr ML, Baker TB, Hostler D, **and** Schlader ZJ. Ad libitum drinking prevents dehydration during physical work in the heat when adhering to occupational heat stress recommendations. *Temperature (Austin)* 9: 292–302, 2022. [PubMed: 36211944]
18. Houck J, McKenna Z, Fennel Z, Ducharme J, Wells A, Mermier C, Deyhle M, Laitano O, Specht J, **and** Amorim F. The effect of interval and continuous work on markers of acute kidney injury in a hot environment. *Eur J Appl Physiol* 122: 2437–2450, 2022. [PubMed: 35999474]
19. Bain AR, **and** Jay O. Does summer in a humid continental climate elicit an acclimatization of human thermoregulatory responses? *Eur J Appl Physiol* 111: 1197–1205, 2011. [PubMed: 21127900]
20. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, **and** Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381–1395, 2003. [PubMed: 12900694]
21. Cheuvront SN, Ely BR, Kenefick RW, **and** Sawka MN. Biological variation and diagnostic accuracy of dehydration assessment markers. *Am J Clin Nutr* 92: 565–573, 2010. [PubMed: 20631205]
22. Bogerd CP, Velt KB, Annaheim S, Bongers C, Eijssvogels TMH, **and** Daanen HAM. Comparison of two telemetric intestinal temperature devices with rectal temperature during exercise. *Physiol Meas* 39: 03nt01, 2018.
23. Gagge AP, Stolwijk JA, **and** Hardy JD. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res* 1: 1–20, 1967. [PubMed: 5614624]
24. Borg G Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2: 92–98, 1970. [PubMed: 5523831]
25. Adams JD, Myatich AI, **and** McCullough AS. Thirst as an ingestive behavior: A brief review on physiology and assessment. *Nutr Health* 26: 271–274, 2020. [PubMed: 32297552]
26. Dill DB, **and** Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247–248, 1974. [PubMed: 4850854]
27. Wesson LG Jr., and Anslow WP Jr. Effect of osmotic and mercurial diuresis on simultaneous water diuresis. *Am J Physiol* 170: 255–269, 1952. [PubMed: 12985890]
28. Wang Y, Chen J, Chen L, Tay YC, Rangan GK, **and** Harris DC. Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J Am Soc Nephrol* 8: 1537–1545, 1997. [PubMed: 9335381]
29. Hansson E, Broberg K, Wijkström J, Glaser J, Gonzalez-Quiroz M, Ekström U, Abrahamson M, **and** Jakobsson K. An explorative study of inflammation-related proteins associated with kidney injury in male heat-stressed workers. *J Therm Biol* 112: 103433, 2023. [PubMed: 36796890]
30. Kishimoto C, Shioji K, Nakamura H, Nakayama Y, Yodoi J, **and** Sasayama S. Serum thioredoxin (TRX) levels in patients with heart failure. *Jpn Circ J* 65: 491–494, 2001. [PubMed: 11407728]
31. Kasuno K, Shirakawa K, Yoshida H, Mori K, Kimura H, Takahashi N, Nobukawa Y, Shigemi K, Tanabe S, Yamada N, Koshiji T, Nogaki F, Kusano H, Ono T, Uno K, Nakamura H, Yodoi J, Muso E, **and** Iwano M. Renal redox dysregulation in AKI: application for oxidative stress marker of AKI. *Am J Physiol Renal Physiol* 307: F1342–F1351, 2014. [PubMed: 25350977]
32. Prodjosudjadi W, Daha MR, Gerritsma JS, Florijn KW, Barendregt JN, Bruijn JA, van der Woude FJ, **and** van Es LA. Increased urinary excretion of monocyte chemoattractant protein-1 during acute renal allograft rejection. *Nephrol Dial Transplant* 11: 1096–1103, 1996. [PubMed: 8671975]
33. Sullivan GM, **and** Feinn R. Using Effect Size-or Why the P Value Is Not Enough. *J Grad Med Educ* 4: 279–282, 2012. [PubMed: 23997866]

34. Freemas JA, Worley Gabler MC, Hess HW, McDeavitt J, Baker TB, Johnson BD, Chapman CL, and Schlader ZJ. Glomerular filtration rate reserve is reduced during mild passive heat stress in healthy young adults. *Am J Physiol Regul Integr Comp Physiol* 323: R340–r350, 2022. [PubMed: 35816723]
35. Jetté M, Sidney K, and Blümchen G. Metabolic equivalents (METs) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin Cardiol* 13: 555–565, 1990. [PubMed: 2204507]
36. Moyce S, Armitage T, Mitchell D, and Schenker M. Acute kidney injury and workload in a sample of California agricultural workers. *Am J Ind Med* 63: 258–268, 2020. [PubMed: 31773783]
37. Masoud A, McKenna ZJ, Li Z, Deyhle MR, Mermier CM, Schlader ZJ, and Amorim FT. Strategies to mitigate acute kidney injury risk during physical work in hot environments. *Am J Physiol Renal Physiol* 2024.
38. Oki K, Henderson CG, Ward SM, Ward JA, Plamper ML, Mayer TA, Caldwell AR, and Leon LR. Identification of therapeutic targets in a murine model of severe exertional heat stroke. *Am J Physiol Regul Integr Comp Physiol* 323: R935–r950, 2022. [PubMed: 36283086]
39. Herrmann SD, Willis EA, Ainsworth BE, Barreira TV, Hastert M, Kracht CL, Schuna JM Jr., Cai Z, Quan M, Tudor-Locke C, Whitt-Glover MC, and Jacobs DR Jr. 2024 Adult Compendium of Physical Activities: A third update of the energy costs of human activities. *J Sport Health Sci* 13: 6–12, 2024. [PubMed: 38242596]
40. Hess HW, Tarr ML, Baker TB, Hostler D, and Schlader ZJ. Ad libitum drinking prevents dehydration during physical work in the heat when adhering to occupational heat stress recommendations. *Temperature* 1–11, 2022.
41. Chapman CL, and Schlader ZJ. Assessing the risk of acute kidney injury following exercise in the heat: Timing is important: Comment on: Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, and Schlader ZJ Hyperthermia and dehydration during physical work in the heat both contribute to the risk of acute kidney injury, *J Appl Physiol* (1985), 2020. DOI: 10.1152/jappphysiol.00787.2019. *Temperature (Austin)* 7: 304–306, 2020.
42. Chapman CL, Hess HW, Lucas RAI, Glaser J, Saran R, Bragg-Gresham J, Wegman D, Hansson E, Minson CT, and Schlader Z. Occupational heat exposure and the risk of chronic kidney disease of non-traditional origin in the United States. *Am J Physiol Regul Integr Comp Physiol* 2021.

NEW & NOTEWORTHY

We demonstrate that 1 violation of the National Institute for Occupational Safety and Health heat stress recommendations by exceeding allowable work intensity by ~120 W or environmental limits by ~4°C WBGT at 30 minutes work-rest per hour results in a modest elevation in peak core temperature but does not augment acute kidney injury risk compared to scenarios that adhered to the NIOSH recommendations during simulated occupational heat stress.

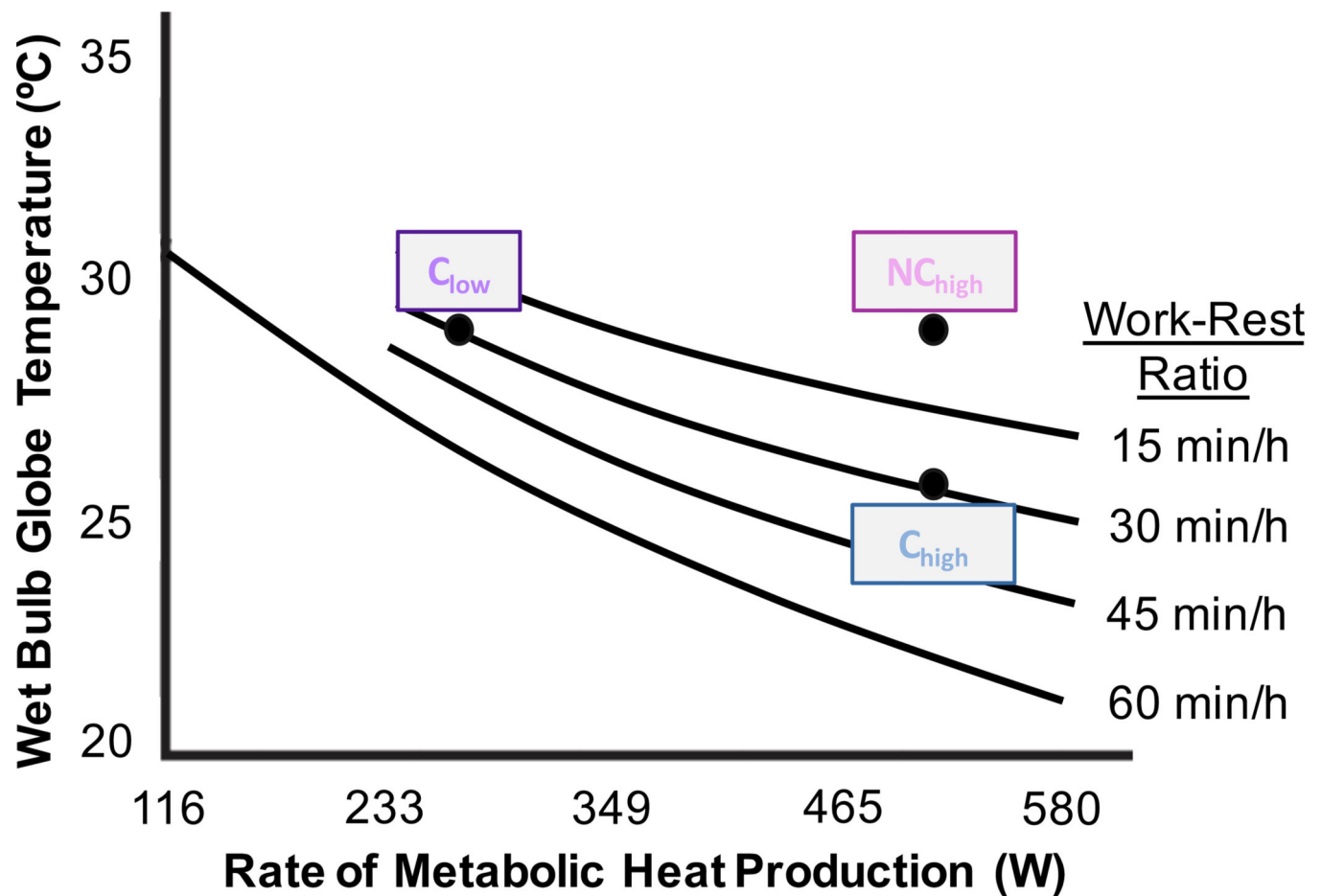
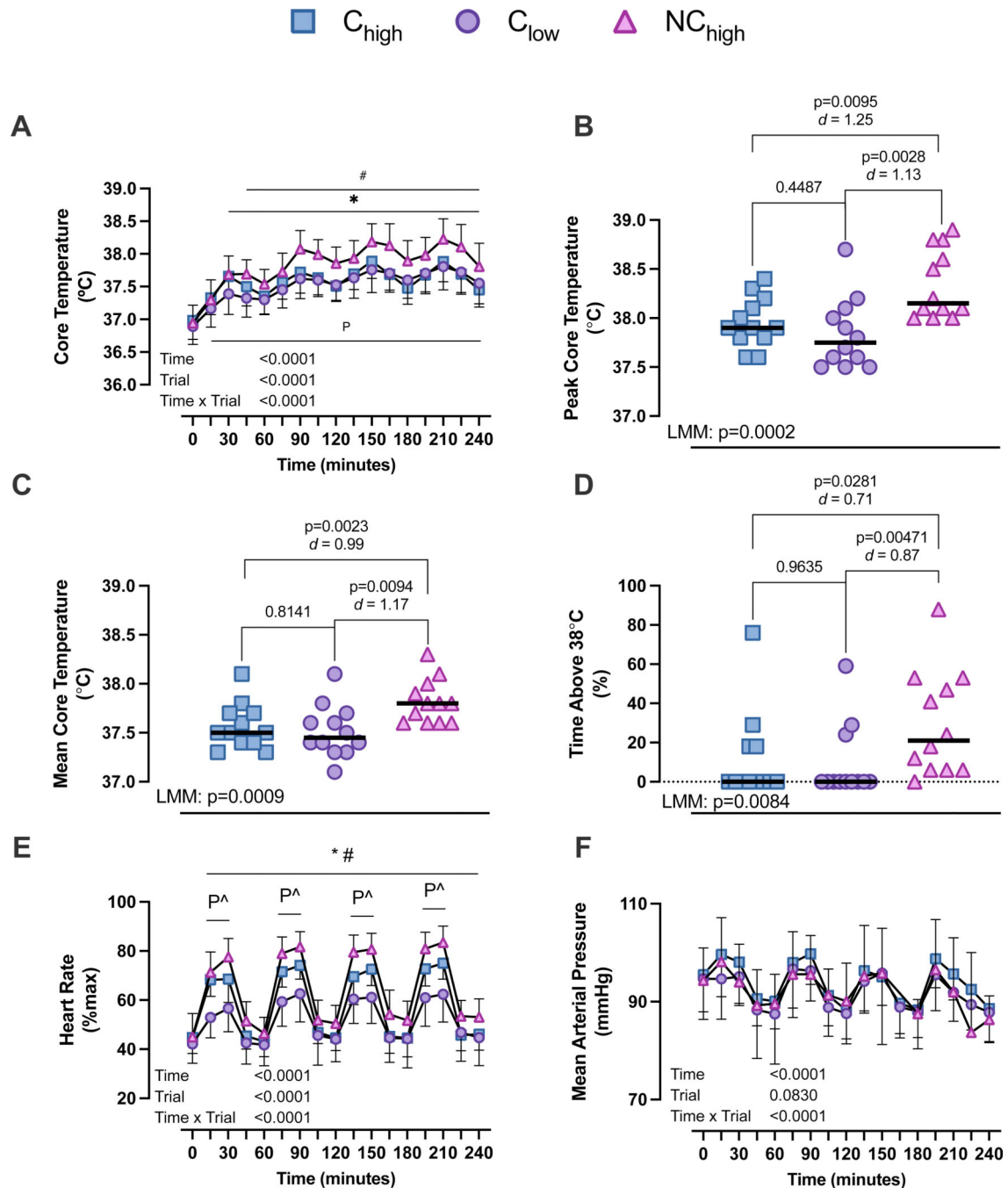
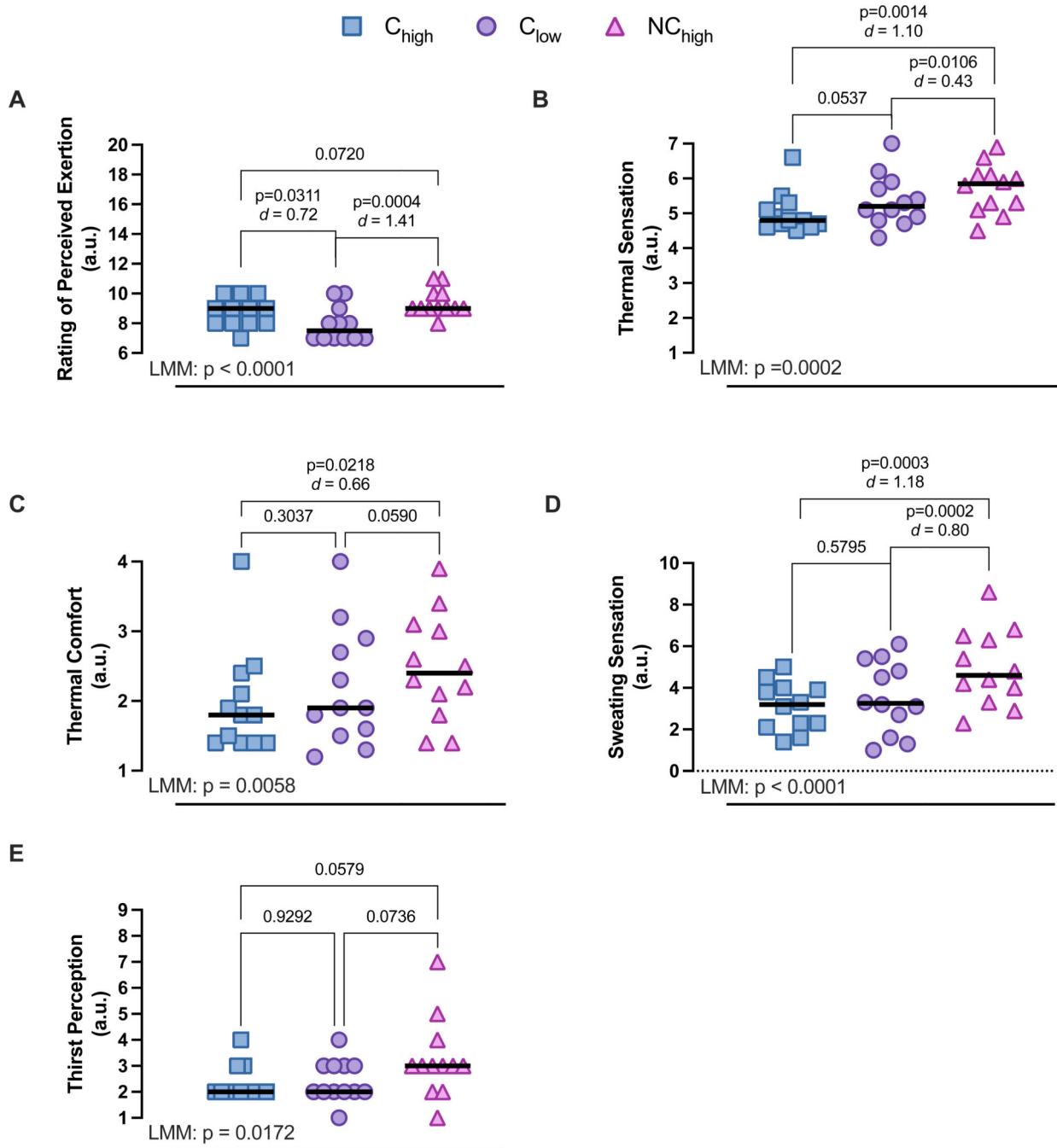


Figure 1.

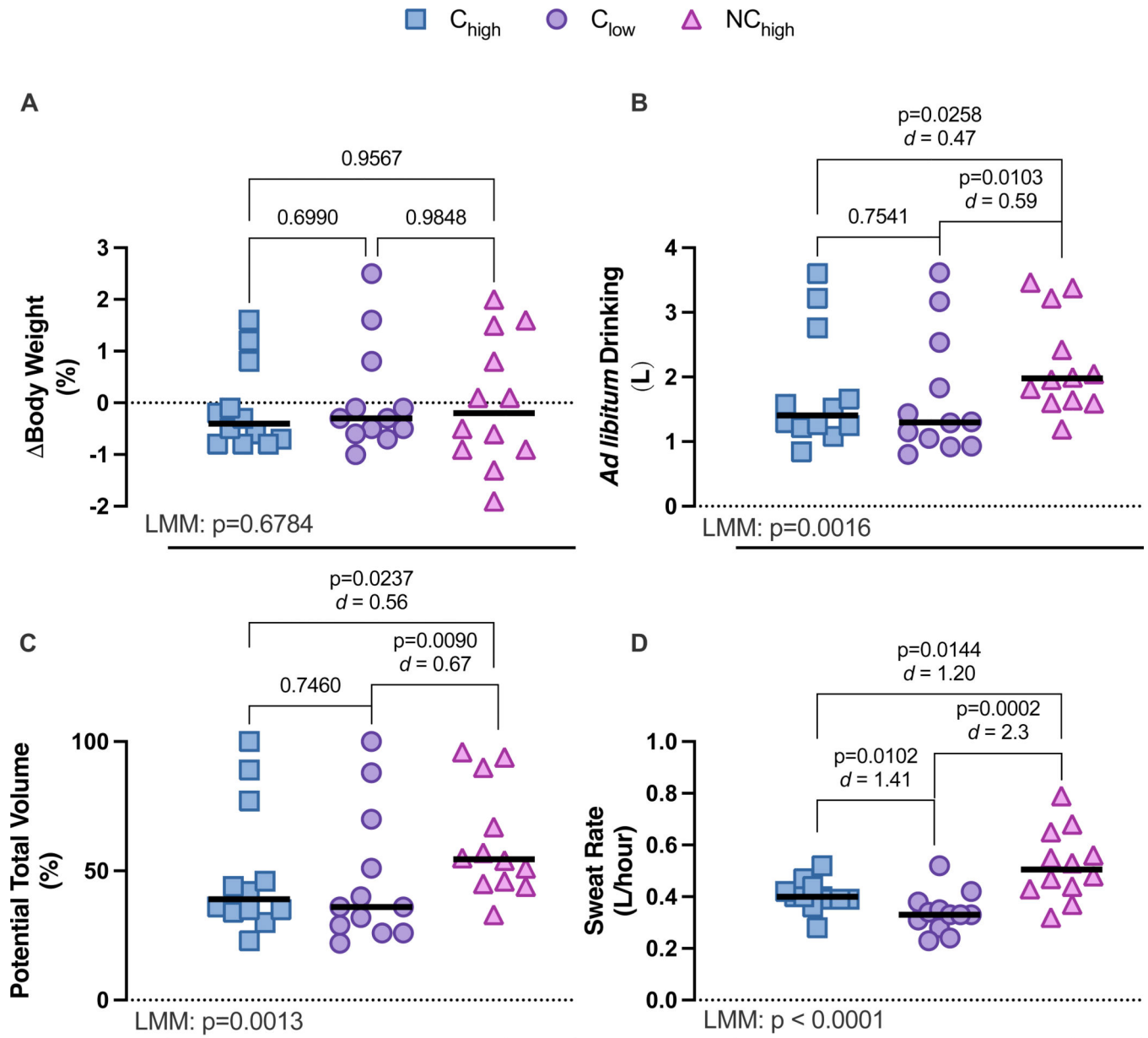
Study design and experimental trials on the basis of the NIOSH heat stress recommendations (15). Participants walked on a treadmill at NIOSH-compliant work intensities (i.e., metabolic heat production) as a function of wet-bulb globe temperature (WBGT) and work-rest ratio (Compliant – Low Intensity [C_{low}] and Compliant – High Intensity [C_{high}]). Participants also completed an experimental trial that was not compliant with the NIOSH heat stress recommendations by violating allowable work intensity at a given WBGT at a work-rest ratio of 30:30 min per hour (Non-compliant – High Intensity [NC_{high}]).

**Figure 2A-F.**

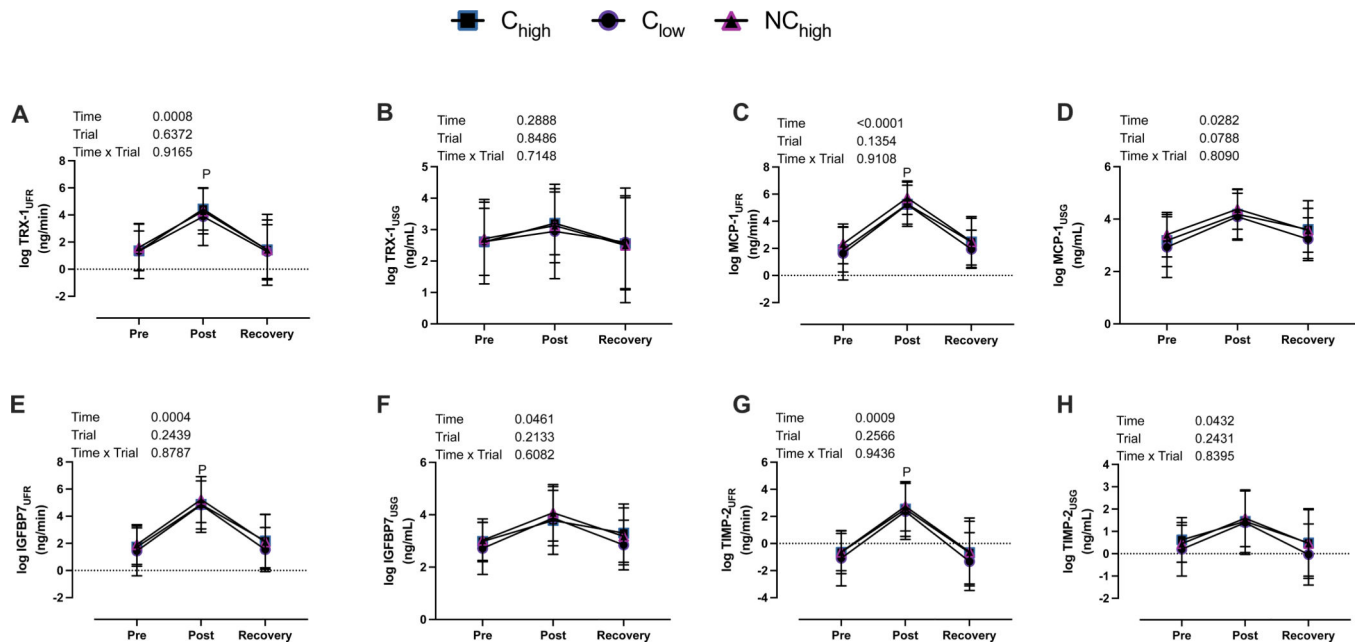
Core temperature and hemodynamic responses to simulated occupational heat stress. Data are presented as mean (SD) or as individual data and mean (black line). $n = 12$ except for metabolic heat production in the C_{low} trial where $n = 11$. Data were analyzed using either a two-way (Time × Trial; A) or one-way (B-E) linear mixed model (LMM) and *posthoc* analyses completed using Sidak's multiple comparisons. Exact p-values reported where possible. P = different from preexposure (minute 0) ($p < 0.05$); * = NC_{high} different from C_{low} ($p < 0.05$); # = NC_{high} different from C_{high} ($p < 0.05$); ^C_{high} different from C_{low} ($p < 0.05$).

**Figure 3.**

A-E. Perceptual responses to simulated occupational heat stress. Data are presented as mean (SD) or as individual data and mean (black line). $n = 12$. Data were analyzed using either one-way linear mixed model (LMM) and *posthoc* analyses completed using Sidak's multiple comparisons. Exact p-values reported.

**Figure 4.****A-D.** Indices of hydration and fluid intake during the simulated occupational heat stress.

Data are presented as mean (SD) or as individual data and mean (black line). $N = 12$. Data were analyzed using a one-way linear mixed model (LMM) and *posthoc* analyses completed using Sidak's multiple comparisons. Exact p-values reported.

**Figure 5.**

Urinary biomarkers of oxidative stress (A-B: thioredoxin-1 [TRX-1]), inflammation (C-D: monocyte chemoattractant protein-1 [MCP-1];) and acute kidney injury (E-F: insulin like growth factor binding protein 7 [IGFBP7]; G-H: tissue inhibitor metalloproteinase 2 [TIMP-2]) normalized to both urine flow rate (UFR) and urine specific gravity (USG). Urinary biomarkers were measured preexposure (Pre), postexposure (Post), and following a one hour recovery. Data are presented as mean (SD) and $N=12$. Data were analyzed using either a one-way linear mixed model and *posthoc* analyses completed using Sidak's multiple comparisons. Exact p-values reported. P = different from preexposure ($p<0.05$).

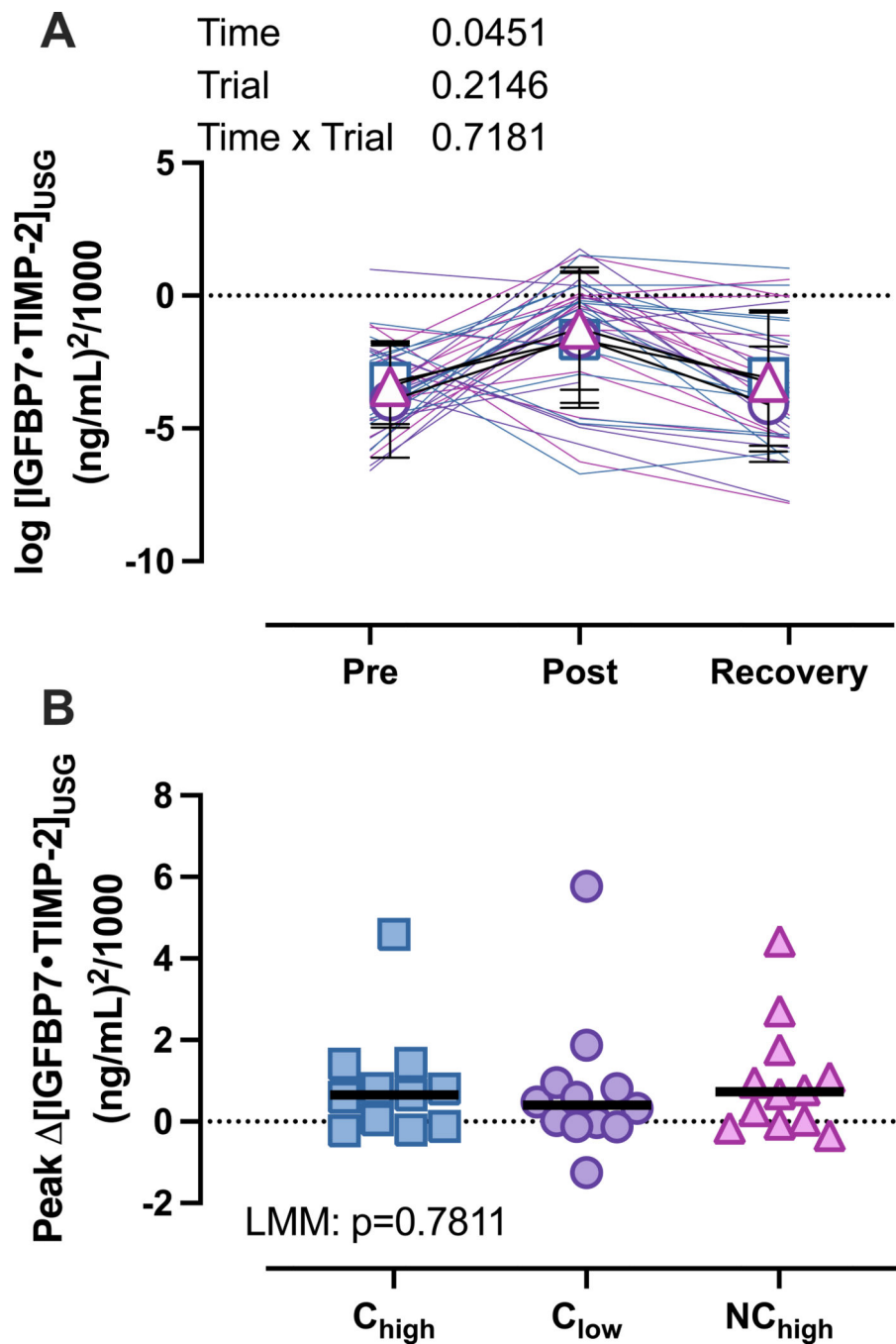


Figure 6.

A) $[IGFBP7 \cdot TIMP-2]_{USG}$ at pre-, post- and at recovery following simulated occupational heat stress and B) peak $\Delta [IGFBP7 \cdot TIMP-2]_{USG}$. Individual and mean (SD) data are presented for $n = 12$ in each trial and all timepoints except in C_{high} where $n = 11$. Data were analyzed using a two-way linear mixed model (A) or one-way linear mixed model (B) and *posthoc* analyses completed using Sidak's multiple comparisons. Exact p-values reported. Δ indicates change from pre-; C_{high} , Compliant – High Intensity; C_{low} , Compliant – Low

Intensity; IGFBP7, Insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor metalloproteinase 2; NC_{high}, Non-compliant – High Intensity; USG, urine specific gravity.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

	H.W.H.	M.E.H.	E.T.	M.J.H.	K.R.	R.S.Z.	B.D.J.	D.H.	Z.J.S.
Conceived and designed research	x						x	x	x
Performed experiments	x	x	x	x					
Analyzed data	x				x	x			x
Prepared figures	x								
Drafted manuscript	x								x
Edited and revised manuscript	x	x	x	x	x	x	x	x	x
Approved final version of manuscript	x	x	x	x	x	x	x	x	x

Table 1.

Fluid and electrolytes.

Experimental Trial	C _{high}			C _{low}			NC _{high}			LMM
Parameter	Pre	Post	Rec	Pre	Post	Rec	Pre	Post	Rec	
ΔPlasma Volume (%)	-	+ 5.0 (4.3) ^P	+ 5.2 (4.2) ^P	-	+1.3 (3.1)	+1.9 (2.7)	-	+1.0 (6.4)	+1.2 (7.7)	Time: p=0.0125 Trial: p=0.1190 Time × Trial: p=0.1561
Urine Specific Gravity	1.005 (0.005) <i>n=11</i>	1.009 (0.007)	1.007 (0.006)	1.005 (0.006)	1.010 (0.007)	1.005 (0.004)	1.009 (0.008)	1.008 (0.006)	1.006 (0.006)	Time: p=0.2720 Trial: p=0.5946 Time × Trial: p=0.3825
Serum Osmolality (mOsm/kgH ₂ O)	300 (7)	299 (5)	300 (4)	298 (5)	296 (6)	297 (4)	297 (7)	298 (4)	297 (3) *	Time: p=0.5495 Trial: p=0.0265 Time × Trial: p=0.4821
Urine Osmolality (mOsm/kgH ₂ O)	222 (151)	311 (222)	243 (216)	230 (194)	359 (232)	183 (147)	288 (225)	308 (190)	253 (231)	Time: p=0.2861 Trial: p=0.7094 Time × Trial: p=0.5168
Serum Na ⁺ (mmol/L)	138 (1)	139 (1)	138 (1)	138 (1)	139 (4)	138 (1)	138 (1)	138 (1)	139 (2) <i>n=11</i>	Time: p=0.1390 Trial: p=0.5740 Time × Trial: p=0.1254
Urine Na ⁺ (mmol/L)	120 (124)	113 (99)	114 (122) <i>n=11</i>	142 (127)	160 (174)	76 (63) <i>n=11</i>	200 (197)	97 (66) <i>n=11</i>	69 (81) <i>n=11</i>	Time: p=0.2390 Trial: p=0.9016 Time × Trial: p=0.1319
Serum K ⁺ (mmol/L)	4.3 (0.1)	4.3 (0.1)	4.3 (0.1)	4.3 (0.1)	4.3 (0.1)	4.3 (0.1)	4.3 (0.1)	4.2 (0.1)	4.3 (0.1) <i>n=11</i>	Time: p=0.1032 Trial: p=0.6549 Time × Trial: p=0.0598
Urine K ⁺ (mmol/L)	93 (61)	142 (71)	71 (52) <i>n=11</i>	87 (62)	140 (88)	55 (44) <i>n=11</i>	91 (65)	143 (69) <i>n=11</i>	76 (80) <i>n=11</i>	Time: p=0.0210 Trial: p=0.7519 Time × Trial: p=0.8076
Serum Creatinine (mg/dL)	0.85 (0.14)	0.91 (0.15) ^P	0.88 (0.14)	0.84 (0.13)	0.89 (0.16) ^P	0.88 (0.15) ^P	0.84 (0.14)	0.96 (0.14) <i>p*</i>	0.93 (0.14) <i>p*</i>	Time: p<0.0001 Trial: p=0.0419 Time × Trial: p=0.0021
Urine Creatinine (mg/dL)	46.9 (39.7)	97.6 (79.2)	61.8 (57.3)	45.0 (47.2)	108.2 (82.6)	46.9 (39.9) <i>n=11</i>	53.6 (43.4)	120.3 (97.2)	69.4 (66.7)	Time: p=0.0290 Trial: p=0.3824

Experimental Trial	C _{high}			C _{low}			NC _{high}			LMM
	Pre	Post	Rec	Pre	Post	Rec	Pre	Post	Rec	
										Time × Trial: p=0.8342
Serum Albumin (g/dL)	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	4.3 (0.2)	4.3 (0.2)	4.3 (0.2)	4.3 (0.2)	4.4 (0.2)	4.4 (0.2)	Time: p=0.0538 Trial: p=0.1994 Time × Trial: p=0.0171
Urine Albumin (g/dL)	0.021 (0.012)	0.028 (0.015)	0.023 (0.009)	0.022 (0.010)	0.023 (0.012)	0.020 (0.013) <i>n=11</i>	0.023 (0.011)	0.025 (0.013)	0.024 (0.013) <i>n=11</i>	Time: p=0.3667 Trial: p=0.6073 Time × Trial: p=0.8253
Serum Cystatin C (mg/L)	6.5 (3.2)	6.9 (4.2)	6.8 (3.4)	6.0 (2.9)	6.0 (3.4)	6.5 (3.5)	5.8 (3.5)	7.0 (4.5)	6.2 (4.6)	Time: p=0.4436 Trial: p=0.7856 Time × Trial: p=0.0610

Data are presented as mean (SD). Data were analyzed using a linear mixed effects model (LMM). Actual p-values are reported. All data are *n*=12 unless otherwise reported.

P = different from preexposure;

* = NC_{high} different from C_{low};

= NC_{high} different from C_{high}

Table 2.

Indices of fluid conservation and creatinine clearance.

Experimental Trial	C _{high}			C _{low}			NC _{high}			LMM
Parameter	Pre	Post	Rec	Pre	Post	Rec	Pre	Post	Rec	
Urine Flow Rate (mL/min)	4.4 (2.5)	2.7 (3.1)	4.3 (3.0)	4.9 (3.4)	2.4 (2.0)	4.7 (3.2)	3.6 (2.1)	1.9 (2.4)	4.3 (3.7)	Time: p=0.1531 Trial: p=0.2022 Time × Trial: p=0.5891
Creatinine Clearance (mL/min)	143.8 (19.4)	127.3 (27.8)	149.5 (30.7)	137.8 (25.3)	141.7 (25.2)	146.7 (31.8)	148.1 (30.8)	119.5 (35.7) ^P	147.0 (39.4)	Time: p=0.0221 Trial: p=0.7075 Time × Trial: p=0.249
Free Water Clearance (mL/min)	2.1 (2.4)	1.3 (2.9)	2.5 (3.1)	2.7 (3.2)	0.9 (2.3)	2.9 (3.3)	1.2 (2.3)	0.8 (2.1)	2.3 (4.5)	Time: p=0.3515 Trial: p=0.2288 Time × Trial: p=0.5322
Osmolar Clearance (mL/min)	2.3 (0.8)	1.4 (0.6) ^P	1.8 (0.5) ^P	2.2 (0.9)	1.6 (0.6) ^P	1.8 (0.4)	2.4 (1.0)	1.1 (0.4) ^P	1.4 (0.4) ^{P*}	Time: p<0.0001 Trial: p=0.2319 Time × Trial: p=0.0587
FeNA (%)	1.9 (1.2)	1.0 (0.8) ^P	1.4 (0.9) ^P	2.4 (1.2)	1.2 (1.0) ^P <i>n=11</i>	1.3 (0.7) ^P	2.5 (1.4)	0.6 (0.4) ^P	0.9 (0.6) ^{P*} <i>n=11</i>	Time: p<0.0001 Trial: p=0.7094 Time × Trial: p=0.0146
FeK (%)	8.0 (4.3)	6.8 (2.9)	5.3 (3.5)	8.2 (4.7)	5.2 (1.0)	4.6 (1.3)	6.1 (2.1)	4.3 (2.0) ^P	4.3 (2.0) ^P	Time: p=0.0104 Trial: p=0.1518 Time × Trial: p=0.3711

Data are presented as mean (SD). Data were analyzed using a linear mixed effects model (LMM). Actual p-values are reported. All data are *n*=12 unless otherwise reported.

P = different from preexposure (p<0.05);

* = NC_{high} different from C_{low} (p<0.05)

Table 3.
Markers of Inflammation, Oxidative Stress, and Acute Kidney Injury

Experimental Trial	C _{high}			C _{low}			NC _{high}			LMM
Parameter	Pre	Post	Rec	Pre	Post	Rec	Pre	Post	Rec	
Serum TRX-1, ng/mL	197.6 (254.8) <i>n</i> =9	210.3 (369.2) <i>n</i> =8	134.6 (131.1) <i>n</i> =9	99.6 (90.9) <i>n</i> =8	2123.3 (5091.1) <i>n</i> =10	81.6 (62.0) <i>n</i> =8	236.5 (300.9) <i>n</i> =10	1279.8 (2863.1) <i>n</i> =10	561.0 (954.3) <i>n</i> =10	Time: p=0.1650 Trial: p=0.3500 Time × Trial: p=0.8335
Serum MCP-1, pg/mL	223.0 (47.4)	241.0 (86.6)	188.2 (75.1)	225.5 (73.6)	220.0 (85.0)	236.1 (77.3)	223.2 (55.9)	286.1 (101.4) <i>p</i> *	249.7 (104.9) [#]	Time: p=0.1606 Trial: p=0.0330 Time × Trial: p=0.0204
Serum IGFBP7, ng/mL	88.8 (17.9)	86.3 (14.2)	87.3 (13.9)	87.2 (18.0)	84.1 (20.2)	85.5 (18.9)	88.8 (15.8)	85.8 (16.6)	85.1 (18.6)	Time: p=0.0502 Trial: p=0.6475 Time × Trial: p=0.8215
Serum TIMP-2, ng/mL	79.5 (20.9)	85.6 (29.5)	84.3 (21.9)	90.7 (26.2)	86.5 (37.6)	88.3 (24.9)	87.1 (30.9)	91.5 (24.7)	85.9 (26.4)	Time: p=0.8185 Trial: p=0.4384 Time × Trial: p=0.6691
FeTRX-1 (%)	0.71 (0.81) <i>n</i> =9	0.51 (0.45) <i>n</i> =8	0.54 (0.54) <i>n</i> =9	0.95 (0.89) <i>n</i> =8	0.50 (0.53) <i>n</i> =10	1.42 (1.52) <i>n</i> =8	0.55 (0.73) <i>n</i> =10	0.48 (0.80) <i>n</i> =10	0.66 (1.03) <i>n</i> =10	Time: p=0.0699 Trial: p=0.1768 Time × Trial: p=0.0673
FeMCP-1 (%)	0.31 (0.18)	0.46 (0.34)	0.57 (0.50)	0.29 (0.24)	0.43 (0.38)	0.43 (0.58)	0.33 (0.20)	0.36 (0.21)	0.40 (0.32)	Time: p=0.0756 Trial: p=0.2030 Time × Trial: p=0.0713

Data are presented as mean (SD). Data were analyzed using a linear mixed effects model (LMM). Actual p-values are reported. All data are *n*=12 unless otherwise reported.

P = different from preexposure (p<0.05);

* = NC_{high} different from C_{low} (p<0.05);

[#] = NC_{high} different from C_{high} (p<0.05)