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RESEARCH ARTICLE

Ad libitum drinking does not mitigate acute kidney injury risk nor elevations in markers of oxidative stress and inflammation during simulated occupational heat stress

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Abstract

This study tested the hypothesis that ad libitum fluid intake during a 2-h occupational heat stress simulation attenuates increases in renal oxidative stress, inflammation, and acute kidney injury (AKI) risk compared with fluid restriction. Thirteen healthy adults (5 women) completed two 2-h occupational heat stress simulations consisting of eight circuits of treadmill walking and rowing exercise in a wet bulb globe temperature of 33.1 ± 0.2°C. In the drinking trial (Drink), participants were provided 237 mL of a noncaloric sport drink every 15 min and drank ad libitum. In the fluid restriction trial (No Drink), no fluid was provided. Urine and blood samples were analyzed for markers of oxidative stress (thioredoxin-1, TRX-1), inflammation (monocyte chemotactic protein-1, MCP-1), and AKI risk ([IGFBP7·TIMP-2]). During Drink, ad libitum fluid intake was 1,394 ± 316 mL, and reductions in body weight were greater in No Drink (1.3 ± 0.8% vs. 2.8 ± 0.9%, $P < 0.001$). Peak core temperature was not different between Drink (38.5 ± 0.4°C) and No Drink [38.6 ± 0.4°C, mean difference (upper, lower CI): 0.1 (0.4, −0.1)°C; $P = 0.346$]. Urine, but not serum TRX-1 ($P = 0.254$), was elevated at postrecovery and recovery ($P < 0.001$) but not different between trials ($P = 0.743$). Serum and urine MCP-1 were elevated at postrecovery and recovery ($P < 0.001$) but not different between trials ($P \geq 0.407$). Urine [IGFBP7·TIMP-2] was elevated at postrecovery and recovery ($P < 0.001$) but not different between trials ($P \geq 0.096$). Ad libitum fluid intake during a 2-h occupational heat stress simulation does not modify biomarkers of systemic and renal oxidative stress and inflammation, nor AKI risk, compared with when fluid is restricted.

NEW & NOTEWORTHY Ad libitum fluid intake, consistent with occupational hydration recommendations, does not attenuate renal oxidative stress, inflammation, or acute kidney injury risk during simulated heat stress. These findings challenge the assumption that current hydration recommendations are protective, suggesting that additional strategies are needed to mitigate heat-induced kidney injury in occupational settings.

dehydration; hyperthermia; kidney function; work-related heat stress

INTRODUCTION

Approximately 2.4 billion workers (i.e., ~70% of the global workforce) are frequently exposed to occupational heat stress, with over 90% of these exposures occurring outside of a heat wave (1). Occupational heat stress is associated with both acute kidney injury (AKI) (2) and the development of chronic kidney disease of nontraditional origin (CKDnt) (3), which has been described as an epidemic in manual laborers in Central America and other global hotspots (4). It has been hypothesized that CKDnt may develop through single or repeated episodes of AKI triggered by occupational heat stress (2). This potential etiology is supported by findings that interventions reducing heat stress exposure by mandating structured breaks in shaded locations and improved hydration practices attenuate the incidence of clinical AKI (4) and cross harvest falls in estimated glomerular filtration rate (4) in Central American agricultural workers. Notably, despite the seasonal nature of heat, the consistent potential for heat stress during the summer months in many regions has raised concerns about kidney health among workers in the United States who are regularly exposed to occupational heat stress (5, 6).

Reducing the risk of AKI may be an accessible intervention point to help prevent CKDnt. Notably, the mechanism(s) by which physical work in the heat increases AKI risk is

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multifactorial. One key factor is hyperthermia (i.e., increases in core temperature), which has been shown to increase the risk of AKI during physical work in hot environments, even among young, healthy adults (7–10). This risk is further exacerbated by dehydration, an acute loss of body water (7). AKI risk is thought to be initially manifested through hyperthermia-mediated reductions in blood flow to the kidneys and splanchnic organs (11) that are exacerbated when combined with physical work and dehydration (12). In this state, it is speculated that hyperthermia leads to a relative mismatch between oxygen supply, via renal blood flow, and oxygen demand, which increases due to enhanced sodium reabsorption in the renal tubules (13), while this supply-demand mismatch is likely exacerbated with dehydration and physical exertion. This proposed etiology is believed to induce renal oxidative stress and inflammation, particularly within the renal tubules (14), which may be occurring on a background of heat-stress-provoked systemic inflammation that is potentially mediated by increased gastrointestinal permeability (15, 16).

Previous findings from our laboratory broadly support these mechanisms. Specifically, markers of renal oxidative stress and inflammation have been found to be elevated with heat stress exposure and associated with markers of AKI risk during 8 h of passive heat exposure that rendered participants both hyperthermic and dehydrated (8), as well as 4 h of simulated occupational heat stress that rendered participants hyperthermic, but mostly euhydrated (17). However, the role of hydration status as a modifier of renal oxidative stress and inflammation during simulated occupational heat stress remains unknown. We have previously reported that ad libitum fluid intake consistent with occupational heat stress recommendations (18) prevents the development of dehydration during simulated occupational heat stress (19, 20). Therefore, this study tested the hypothesis that, when compared with fluid restriction, free access to fluids during 2 h of simulated occupational heat stress attenuates increases in markers of renal oxidative stress and inflammation and AKI risk.

METHODS

Participants

Before study commencement, an effect size was calculated based on recent data from our laboratory demonstrating that increases in a marker of renal oxidative stress (urinary liver type fatty acid binding protein) was modified by the magnitude of dehydration and elevations in core temperature ($f = 0.29$; 21). Using this effect size and the observed correlation coefficient among repeated measures ($r = 0.75$), a sample size of 12 participants was found to provide at least 80% power to detect a significant trial × time interaction using a two-sided test and an alpha = 0.05 (G*Power 3.1.9.4). With this background, 13 healthy adults (5 women) participated in this study to try to ensure that at least 12 data sets were obtained for most outcome variables. Participant characteristics are reported in Table 1. All participants were nonsmokers and reported to be free from any known cardiovascular, renal, metabolic, neurologic, or gastrointestinal diseases. In addition, participants self-reported to be regularly engaged in regular moderate physical activity 3–5 days/wk (22, 23). Women self-reported to be normally menstruating and were not pregnant at any point during the study, which was confirmed via a urine pregnancy test before each trial. Women were not on any form of birth control and were tested in the first 5 days of self-identified menstruation, a time in which estrogen and progesterone are at their lowest levels, which is similar to other heat stress-focused studies (24). The study was conducted throughout the calendar year in Bloomington, IN, USA.

Table 1. *Participant characteristics (n = 13, 5 women, 8 men)*

	Mean ± SD	(min, Max)
Age, yr	30 ± 6	(20, 40)
Race (White/Black/Native Hawaiian or Pacific Islander/Asian/Native American/Not reported)	12/0/0/0/1	
Ethnicity (Hispanic/non-Hispanic/Not reported)	3/9/1	
Height, cm	175.1 ± 10.4	(160.0, 191.5)
Weight, kg	80.2 ± 17.3	(54.2, 117.1)
Body surface area, m^2	1.95 ± 0.26	(1.55, 2.42)
Body mass index, kg/m^2	25.9 ± 3.1	(21.2, 33.1)
Resting heart rate, beats/min	65 ± 8	(51, 78)
Age-predicted heart rate max, beats/min	187 ± 4	(180, 194)
Mean arterial pressure, mmHg	82 ± 8	(71, 95)

Participants visited the laboratory on three separate occasions. The first visit included screening, familiarization, and a graded submaximal exercise test, the latter of which was used to determine exercise intensity during the subsequent experimental trials. The following two visits were experimental trials (see *Experimental Protocol*) and were separated by at least 3 days. The mean duration between experimental trials was 38 ± 70 days; the variability is due to the greater length of time between trials to control for the menstrual cycle in women (73 ± 102 days) compared with men (17 ± 32 days). This study was approved by the Institutional Review Board at Indiana University (IRB No.: 15402) and conformed to the Declaration of Helsinki, except for registration in a database. Informed written consent was obtained from all participants before participating in this study.

Measurements

Anthropometric, thermal, and cardiovascular measures.

Height and nude body weight were measured using a stadiometer (Seca, Hamburg, Germany) and scale (Sauter, Balingen, Germany). Percent changes in body mass were calculated as the difference between postexposure and preexposure nude body mass divided by preexposure body mass and multiplied by 100. Sweat rate was calculated from the change in nude body weight adjusted for fluid intake and urine output. Core body temperature was measured by participants ingesting a telemetry pill (HQ Inc., Palmetto, FL) 2 h before visit start ($n = 2$) or self-inserting a rectal thermistor (Covidien, Medtronic, Minneapolis, MN; $n = 11$). Skin temperature was measured using four Thermocron iButtons (Maxim Integrated, San Jose, CA) attached to the right side of the body on the chest, triceps brachii, quadriceps, and posterior calf. Weighted mean skin temperature was calculated using a standard four-site

equation (25). Systolic and diastolic blood pressure were measured using electrosphygmomanometry (Tango M2, SunTech Medical, Morrisville, NC). Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Heart rate expressed as a percentage of age-predicted maximal heart rate was calculated as heart rate divided by the maximal age-predicted heart rate (26), multiplied by 100. Ratings of perceived exertion (27), thermal comfort (28–33), thermal sensation (28, 30, 33), thirst sensation (28–32), and sweating sensation (27, 33) were measured using standard visual analog scales.

Hydration and kidney function.

Urine specific gravity was measured in duplicate via refractometry (ATAGO Co., Ltd, Tokyo, Japan). Urine flow rate was calculated over ∼60-min periods for pre-exercise and recovery, and over an ∼165-min period for postexercise. Hemoglobin concentration was measured in duplicate with the Hemopoint H2 (Alere, Orlando, FL). Hematocrit was measured in triplicate by microcentrifugation (34). Plasma and urine osmolality were measured in duplicate using freezing-point depression (model 3250; Advanced Instruments, Norwood, MA). Osmolar clearance was calculated as the quotient of osmolality of the urine and plasma multiplied by urine flow rate. Free water clearance was calculated as urine flow rate multiplied by the difference between 1 and the ratio of urine to plasma osmolality. Serum and urine sodium and potassium were measured in duplicate with a point-of-care electrolyte analyzer (EasyLyte Plus; Medica Corporation, Bedford, MA). The fractional excretions of sodium and potassium were calculated from concentrations of sodium and potassium in the serum and urine and serum and urine creatinine concentrations. Serum and urine creatinine and albumin were measured with a Roche Analyzer (F. Hoffmann-La Roche AG, Indianapolis, IN; intra-assay CV: 3.1%). Creatinine clearance was calculated as the quotient of urine and serum creatinine concentration multiplied by urine flow rate to provide an estimate of changes in glomerular filtration rate. Serum cystatin C was measured using commercially available ELISA kits (U-PLEX Human Cystatin-C Assay; K151M9K-1; intra-assay CV: 3.5%, inter-assay CV: 8.5%).

Acute kidney injury risk.

AKI risk was quantified as the product of urine insulin-like growth factor-binding protein 7 (IGFBP7) and urine tissue inhibitor metalloproteinase 2 ([IGFBP7·TIMP-2]) (35). Urine IGFBP7 is a marker of G1 cell cycle arrest in the renal epithelium that is preferentially secreted in the renal proximal tubules (36, 37). Urine TIMP-2 is a marker of G1 cell cycle arrest in the renal epithelium that is preferentially secreted in the renal distal tubules (36). IGFBP-7 and TIMP-2 were measured with commercially available ELISA kits (human IGFBP-7; ELH-IGFBPRP1-1; intra-assay CV: 10%, inter-assay CV: 12%; and human TIMP-2; ELH-TIMP2-1; intra-assay CV: 10%, inter-assay CV: 12%). Urinary [IGFBP7·TIMP-2] is presented over time and as the peak change (Δ) from preexposure, which is consistent with the FDA-approved indication where the time course is not considered (2).

Oxidative stress and inflammation.

Monocyte chemoattractant protein-1 (MCP-1) is a proinflammatory cytokine (38). Urine MCP-1 is increased with clinical AKI due to increased expression of MCP-1 in the kidneys (39), whereas elevated urine MCP-1 is associated with reductions in kidney function in Mesoamerican agricultural workers (40). Serum MCP-1 is a marker of systemic inflammation (41, 42). Although little is known of the role of serum MCP-1 in heat-related AKI risk, serum MCP-1 is increased with diabetic nephropathy (43) and is associated with increased risk of chronic kidney disease progression (44). Thioredoxin-1 (TRX-1) is a redox protein that plays a role in maintaining redox homeostasis (45). Urinary TRX-1 has been previously reported to increase during prolonged passive heat stress (8) and is reflective of oxidative stress and/or hypoxia from proximal tubular epithelial cells (46). In addition, this oxidative stress-specific biomarker has been shown to be a useful tool for distinguishing AKI from chronic kidney disease and healthy kidneys (46). Serum TRX-1 is an important biomarker in the diagnosis and prognosis of a variety of clinical conditions, such as various cancers (47, 48), including renal carcinoma (49), renal tubular damage in heart failure (50), diabetes (49), and AKI (46). Serum and urine MCP-1 and TRX-1 were measured using commercially available ELISA kits (MCP-1 mesoscale; K151NND-1; intra-assay CV: 3.6%, inter-assay CV: 10.4%; and TRX-1 mesoscale; EH451RB; intra-assay CV: 10%, inter-assay CV: 12%).

Gastrointestinal permeability.

Intestinal fatty acid binding protein (I-FABP) is a protein expressed in the epithelial cells of the intestinal mucosal layer (51). Increased serum concentrations of I-FABP reflect intestinal enterocyte damage (52), which has been previously reported to increase during exercise in the heat (53–60). Serum lipopolysaccharide binding protein (LBP) is a marker of bacterial translocation arising secondary to gut permeability (61). Increases in LBP have been reported during prolonged heat stress (62) and exercise in the heat (63, 64). Serum I-FABP and LBP were measured using commercially available ELISA kits (I-FABP Mesoscale; K151M4R-2; intra-assay CV: 3.5%; and LBP Mesoscale; K151K5R-2; intra-assay CV: 2.1%).

Renal perfusion.

Renal segmental artery blood velocity was measured as a marker of renal perfusion using methods we have described previously (10, 65). To summarize, segmental artery blood velocity was measured via Doppler ultrasound (Toshiba Aplio 300, Canon Medical Systems) using a phased-array transducer (2.5–3.5 MHz) using the coronal approach with the participant in the upright seated position. All measurements were made in the right kidney with the participant in the same position. Indelible ink was used to mark the transducer's location during the first measurement, and this location was used for all subsequent measurements. During the measurement of segmental artery blood velocity, participants were instructed to perform a mid-exhalation non-Valsalva breath hold for no longer than 10 s during the ultrasound measurement. Mean segmental artery blood velocity was measured over at least three cardiac cycles. All segmental artery blood velocity measurements were obtained by the same sonographer (E. Tourula) with a within-subject test-retest coefficient of variation for segmental artery blood velocity of 4.2 ± 0.5%. The renal vasculature is at a depth where it is not possible to accurately measure vessel diameter using

Doppler ultrasound. Therefore, segmental artery blood velocity was interpreted to reflect segmental artery blood flow because reductions in renal blood flow during pharmacological intervention have been shown to be a result of decreases in blood velocity and not changes in vessel diameter (66, 67). Segmental artery vascular resistance was calculated as mean arterial pressure divided by segmental artery blood velocity, which provided an index of segmental artery vasomotor tone.

As in our previous studies (10, 65, 68), participants completed a cold pressor test (CPT) to assess changes in renal vascular control to sympathetic stimulation, conducted before, immediately after, and following recovery from simulated occupational heat stress. At each timepoint, following initial measurement of segmental artery renal blood velocity, blood pressure, and heart rate, the CPT test started when an ice slurry (0.7 ± 0.1°C) was poured into a container in which the participant had placed their foot (69–71). The ice slurry covered the entire foot and malleolus and was continuously stirred. A foot cooling-based CPT was used to minimize movement of the upper body, which was important when measuring renal blood velocity in the upright seated position. Blood pressure, heart rate, and renal segmental blood velocity measurements were measured during each minute throughout the duration of the 2-min CPT. The ultrasound transducer remained in place throughout the entire duration of the CPT.

Plasma volume measurement.

Carbon monoxide rebreathing was used to measure total hemoglobin mass from which absolute plasma volume was calculated (72, 73). Briefly, following 20 min of supine rest, the carbon monoxide rebreathing procedure consisted of a participant inhaling a bolus of 99.5% chemically pure carbon monoxide in the amount of 1.0 mL/kg body weight delivered via a 100 mL syringe. Although remaining in the supine position, the participant rebreathed for 2 min from a closed spirometer system connected to a 5 L anesthesia bag filled with 100% oxygen. Before the start of the rebreathing procedure, venous blood was collected. Capillary blood samples were collected at 6 and 6 min after the start of the rebreathing procedure. Venous and capillary blood was sampled by a blood gas analyzer (OSM3, Radiometer) for the determination of carboxy-hemoglobin concentration in duplicate. Carbon monoxide rebreathing was only completed once during each experimental trial. Thus, at each timepoint, absolute plasma volume was back calculated from total blood volume minus erythrocyte volume, assuming that hemoglobin mass was unaffected by exercise in the heat (74).

Experimental Protocol

This study used a block randomized, crossover design. An overview of the experimental protocol is presented in Fig. 1. Participants arrived at the laboratory for the two experimental visits, having refrained from strenuous exercise, alcohol, and caffeine for 12 h and from food for 2 h. Upon arrival, participants voided their bladder, and euhydration was confirmed via a urine specific gravity of ≤1.020 (75), and a negative pregnancy test was confirmed for women. Participants were weighed nude in a private room and then provided 250 mL of room temperature water to promote urine production over the preceding 60 min. Participants then assumed a supine position, and a venous blood sample was taken after 20 min to account for shifts in plasma volume due to changes in posture (76). A pre-exposure urine sample was collected by participants completely voiding their bladder ~60 min after the previous urine void. A nude body measurement was then obtained. Following presimulated occupational heat stress measurements, participants entered the environmental chamber maintained at 38.6 ± 0.4°C and 54 ± 1% relative humidity. These conditions result in a wet bulb globe temperature of 33.1 ± 0.2°C and were chosen because outdoor workers in the United States (e.g., construction, agriculture, etc.) are often exposed to this level of environmental heat (77).

Upon entering the environmental chamber, participants assumed the seated position for presimulated occupational heat stress renal ultrasound measurements, which was immediately followed by a CPT. Participants then engaged in 2 h of simulated occupational heat stress consisting of 10 min of walking on a treadmill and 5 min of rowing exercise every 15 min throughout the 2 h (8 rounds total). For both treadmill walking and rowing, the external workload was set to an intensity sufficient to elicit an absolute oxygen consumption of 1.2 L/min. The external workload was established during the screening visit, and the external workload was maintained constant throughout both experimental trials. A target of 1.2 L/min was chosen because it represents the absolute

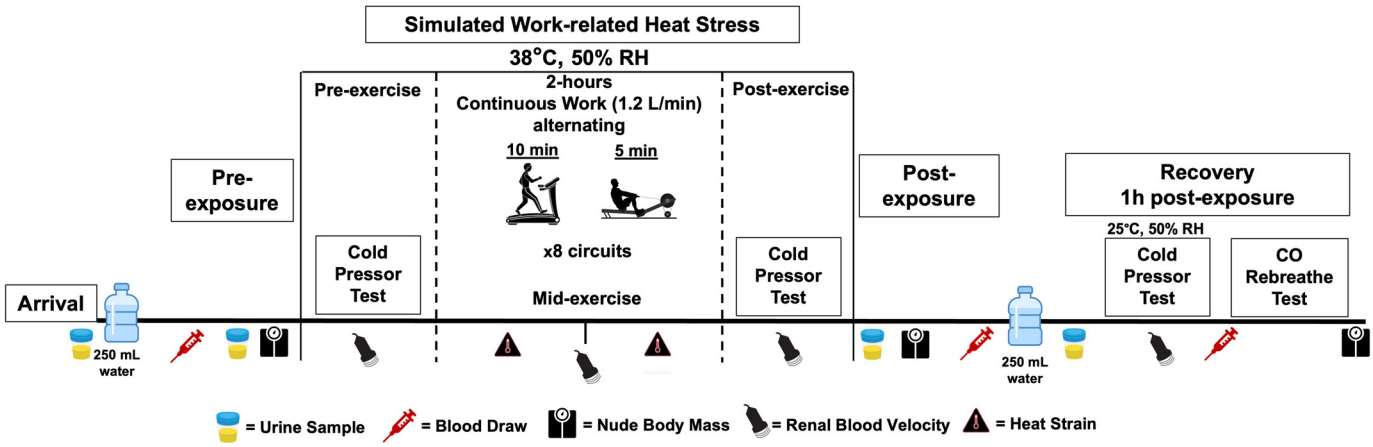

Figure 1. Overview and order of data collection during the experimental trials. CO, carbon monoxide; %; RH, relative humidity.

oxygen consumption agricultural and construction workers experience when performing moderate work (78, 79). A novel aspect of this study is the use of rowing ergometry to simulate the upper-body work that is common in manual labor. Specifically, rowing was used because it is a whole body, continuous exercise utilizing large muscle groups in the legs, back, shoulders, and arms, and better mimics manual tasks like hoeing, shoveling, or manual harvesting compared with treadmill walking alone (80). Thus, the combined use of treadmill walking and rowing better mimicked tasks workers experience. For example, workers often must walk to different areas on a job site to perform the necessary upper-body work.

In one trial, participants were provided 237 mL of a noncaloric sports drink (Gatorade Zero) every 15 min during exercise to encourage ad libitum drinking (Drink trial). This approach is consistent with current occupational heat stress and hydration recommendations (81), and we have shown previously that it results in minimal dehydration (9, 20). In the other trial, the participants were not given fluids to drink (No Drink trial).

Following the first hour of exercise, participants were quickly (~1 min) moved to the seated position for mid-exercise measurement of segmental artery blood velocity. At the end of the second hour of exercise, participants were again seated and underwent segmental artery blood velocity measurements, which was immediately followed by the postsimulated occupational heat stress CPT. Participants exited the environmental chamber after these measurements were conducted.

Immediately upon leaving the environmental chamber, a postsimulated occupational heat stress urine sample was collected. Participants then toweled off before the measurement of nude body weight. Participants assumed the supine position, and a postsimulated occupational heat stress venous blood sample was obtained following 20 min of supine rest. Participants were then given 250 mL of room-temperature water to promote urine production, as we have done previously (7). Approximately 60 min after postexposure urine collection, the participants provided a recovery urine sample. Participants then entered the environmental chamber maintained at 26.9 ± 0.8°C and 49 ± 4% relative humidity for recovery segmental artery blood velocity measurements and a recovery CPT. Immediately following the CPT, participants left the environmental chamber and assumed a supine position to collect a venous blood sample before the start of the carbon monoxide rebreathing test. Finally, participants provided a recovery nude weight before they were permitted to leave the laboratory.

During the Drink trial, 12 participants completed the 2-h simulated occupational heat stress, whereas one subject stopped at 90 min due to lightheadedness. During the No Drink trial, nine participants completed the 2-h simulation. Three participants stopped at 90 min due to core temperature reaching 39.5°C (the ethical cutoff), and one participant stopped at 60 min due to lightheadedness. When the simulated occupational heat stress was terminated early, postrecovery and recovery measurements were obtained in the same manner as if the entire 2-h period were completed. One participant was excluded from the CPT analysis due to the inability to tolerate the CPT, which rendered ultrasound-based images inaccurate. Two participants could not provide a urine sample at the recovery timepoint in the No Drink trial. Therefore, the number of samples for all parameters from urine samples reported at pre-exposure and postexposure in both trials is $n = 13$, whereas recovery samples were $n = 13$ in the Drink trial and $n = 11$ in the No Drink trial.

Data and Statistical Analyses

Before statistical analysis, all urinary markers were normalized to urine specific gravity to account for differences in urine concentration (82), and serum biomarkers were corrected to percent changes in plasma volume to account for differences in plasma volume between conditions. Data collected over time during simulated occupational heat stress were analyzed using two-way linear mixed models with repeated measures of trial and time. After visual inspection of predicted and actual residuals (i.e., QQ plots), it was identified that most urinary markers were not normally distributed. Therefore, statistical inference of these variables was completed after Log_{10} transformation before statistical analyses, which normally distributed the data in all instances. Markers of AKI risk, inflammation, oxidative stress, and gastrointestinal permeability are presented as peak changes from pre-exposure. A two-tailed paired *t*-test or Wilcoxon test for non-normally distributed data was used to compare the peak changes in these variables between trials. Spearman's rank correlations, to account for nonparametric data, were used to examine relations between the peak change in [IGFBP7·TIMP-2] and peak changes in urinary and serum markers of oxidative stress, inflammation, and gastrointestinal permeability in both trials. Responses to the CPTs were assessed as the change from minute zero of the CPT, and these data were analyzed using independent two-way linear mixed models with repeated measures of trial and CPT time at prerecovery, postrecovery, and recovery timepoints. The peak elevations (heart rate, mean arterial pressure, segmental artery vascular resistance) and nadir (segmental artery blood velocity) responses relative to minute zero during each CPT were calculated, and comparisons between trials and over time were made using two-way linear mixed models with repeated measures of trial and time. Two-tailed paired *t*-tests were used to examine changes in the percent change in nude body weight and sweat rate between trials. In all instances, the Geisser–Greenhouse correction was applied if sphericity could not be assumed. As determined a priori, if a significant interaction or main effect of trial was found, post hoc analyses were completed with Tukey's multiple comparisons tests, which adjusts for multiple comparisons. All data were analyzed with GraphPad Prism software (version 8; La Jolla, CA). Data are presented as mean ± SD and individual values where possible. For pairwise comparisons, Cohen's d_z effect size is provided along with the mean difference and 95% confidence intervals (CI). Statistical significance was set a priori at $P \leq 0.05$, and actual P values are reported where possible.

RESULTS

Thermoregulation and Fluid Regulation

Core and mean skin temperature data are presented in Fig. 2. In summary, core temperature increased during

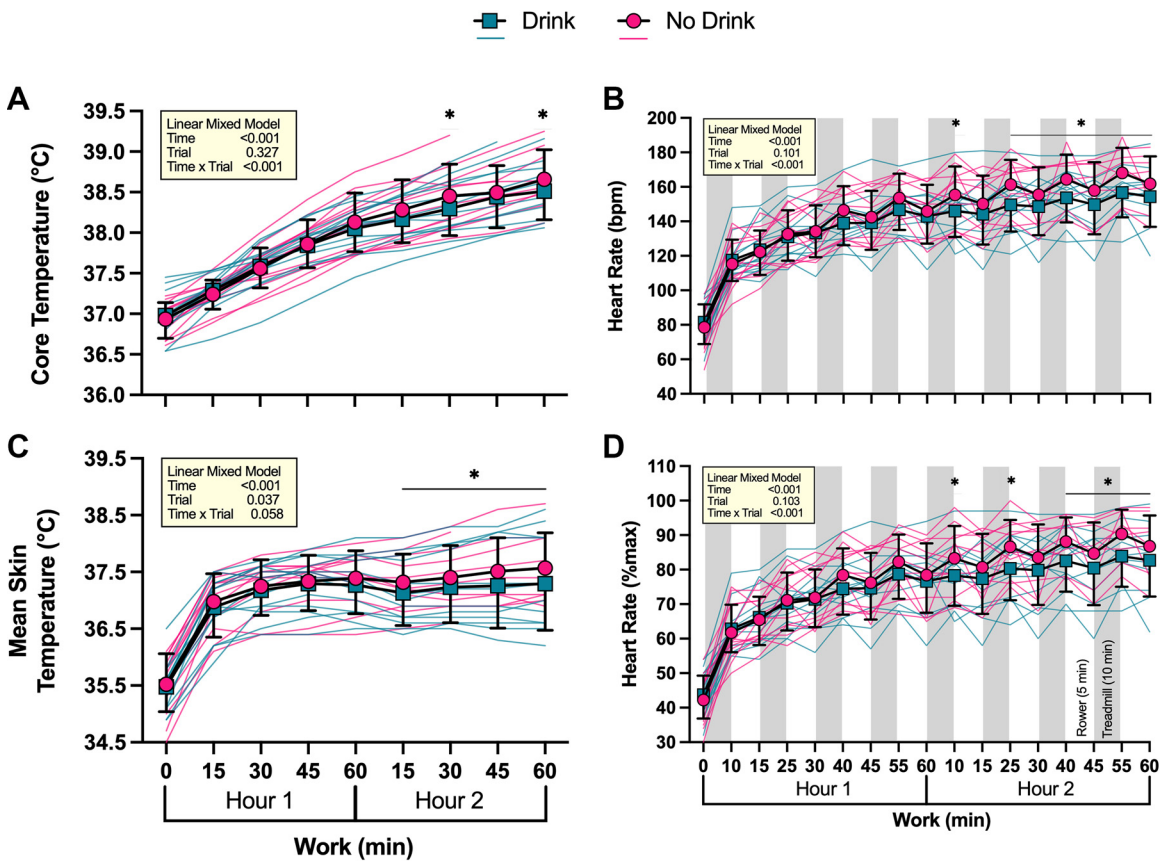

Figure 2. Core temperature (*A*) and mean skin temperature (*C*), and heart rate (*B*) and heart rate presented as a percentage of age-predicted max (*D*) are presented throughout the 2-h occupational heat stress simulation. Data are presented as means ± SD and were analyzed using a two-way linear mixed model with Tukey's post hoc comparisons. *P* values from the two-way linear mixed models are reported. All data are $n = 13$. No Drink, fluid restriction trial.*Significantly different from No Drink trial ($P \leq 0.05$).

simulated occupational heat stress in both trials (Main effect of Time: $P < 0.001$). The increase in core temperature changed differently over time between trials (Time × Trial: $P < 0.001$) and was higher in the No Drink trial at 90 min (38.2 ± 0.3°C vs. 38.5 ± 0.4°C; $P = 0.042$) and 120 min (38.5 ± 0.4°C vs. 38.7 ± 0.4°C; $P = 0.023$). The rate of rise in core temperature in the No Drink trial (1.0 ± 0.3°C/h) was greater than the Drink trial [0.8 ± 0.2°C/h; mean difference

Table 2. *Markers of hydration*

Measurement	Timepoint	Drink	No Drink	*P* Values Paired *t*-test
Sweat rate, L/h		1.2 ± 0.4	1.1 ± 0.3	0.623
Body mass loss, %		1.3 ± 0.8	2.7 ± 1.0	<0.001
				Linear Mixed Model
Nude body mass, kg	Pre	80.8 ± 17.1	80.9 ± 17.6	Time: <0.001
	Post	79.7 ± 16.8*	78.7 ± 17.4	Trial: 0.028
	Recovery	79.8 ± 16.8*	78.4 ± 17.6	Time × Trial: <0.001
Plasma volume, mL	Pre	4,829 ± 1,022	4,983 ± 1,285	Time: 0.016
	Post	4,588 ± 849	4,632 ± 1,203	Trial: 0.168
	Recovery	4,934 ± 1,011*	4,686 ± 1,199	Time × Trial: 0.025
Serum osmolality, mosmol/kgH_2O	Pre	293 ± 8	288 ± 10	Time: 0.008
	Post	294 ± 7*	301 ± 9	Trial: 0.235
	Recovery	241 ± 5	297 ± 8	Time × Trial: 0.015
Urine osmolality, mosmol/kgH_2O	Pre	235 ± 99	225 ± 130	Time: <0.001
	Post	362 ± 166	303 ± 151	Trial: 0.940
	Recovery	650 ± 172	677 ± 87	Time × Trial: 0.658
Urine specific gravity	Pre	1.006 ± 0.003	1.006 ± 0.003	Time: <0.001
	Post	1.010 ± 0.005	1.009 ± 0.004	Trial: 0.860
	Recovery	1.022 ± 0.007	1.023 ± 0.003	Time × Trial: 0.367

Data are presented as means ± SD and analyzed using two-way linear mixed models with Tukey's post hoc comparisons. *P* values are reported. Data at pre- and postexposure in both trials are $n = 13$. Data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$. Drink, drinking trial; No Drink, fluid restriction trial. *Significantly different from No Drink trial ($P \leq 0.05$).

(upper, lower CI): 0.2 (0.4, 0.0)°C/h; Cohen's d_z: 0.739; $P = 0.021$]. Despite the lack of difference between peak core temperature in the Drink (38.5 ± 0.4°C) and No Drink trial [38.6 ± 0.4°C; mean difference (upper, lower CI): 0.1 (0.4, −0.1)°C; Cohen's d_z: 0.272; $P = 0.346$], the change in core temperature matched for the total time between trials was greater in the No Drink trial [1.7 ± 0.4°C vs. 1.5 ± 0.4°C; mean difference (upper, lower CI): 0.3 (0.4, 0.1)°C; Cohen's d_z: 0.795; $P = 0.014$]. Mean skin temperature increased over time (Main effect of Time: $P < 0.001$) in both trials and differed between trials (Main effect of Trial: $P = 0.037$), with pairwise comparisons indicating that mean skin temperature was higher in No Drink throughout the second hour of simulated occupational heat stress ($P \leq 0.020$).

Heart rate data during simulated occupational heat stress are presented in Fig. 2. To summarize, heart rate increased over time in both trials (Main effect of Time: $P < 0.001$), and the rise in heart rate was greater in the No Drink trial during most of the second hour ($P \leq 0.049$). Similarly, heart rate represented as a percentage of age-predicted maximal heart rate increased in both trials over time (Main effect of Time: $P < 0.001$) and was higher in the No Drink trial during most of the second hour ($P \leq 0.032$).

Hydration-related measurements are presented in Table 2. In summary, participants in the No Drink trial lost 1.5 ± 0.5% more body weight compared with the Drink trial [mean difference (upper, lower CI): −1.5 (−1.2, −1.7)%; Cohen's d_z: 3.001; $P < 0.001$] (Table 2). Whole body sweat rate was not different between trials ($P = 0.623$). During the Drink trial, ad libitum fluid consumption was 1,394 ± 316 mL.

Table 3. *Markers of kidney function*

Measurement	Timepoint	Drink	No Drink	Linear Mixed Model *P* Values
Serum creatinine, mg/dL	Pre	0.94 ± 0.18	0.88 ± 0.17	Time: <0.001
	Post	1.19 ± 0.27	1.12 ± 0.17	Trial: 0.108
	Recovery	1.08 ± 0.23	1.06 ± 0.19	Time × Trial: 0.495
Urine creatinine, mg/dL	Pre	49 ± 36	41 ± 21	Time: <0.001
	Post	133 ± 92	108 ± 57	Trial: 0.976
	Recovery	380 ± 215	417 ± 136	Time × Trial: 0.462
Creatinine clearance, mL/min	Pre	145 ± 28	159 ± 27	Time: <0.001
	Post	96 ± 44	97 ± 44	Trial: 0.820
	Recovery	179 ± 64	158 ± 53	Time × Trial: 0.174
SerumCystatin C, mg/L	Pre	1.9 ± 0.4	1.9 ± 0.4	Time: 0.003
	Post	2.3 ± 0.6	2.3 ± 0.4	Trial: 0.724
	Recovery	2.2 ± 0.5	2.2 ± 0.6	Time × Trial: 0.923
Serum albumin, g/dL	Pre	4.4 ± 0.2	4.4 ± 0.3	Time: <0.001
	Post	4.7 ± 0.3	4.8 ± 0.3	Trial: 0.434
	Recovery	4.7 ± 0.3	4.8 ± 0.3	Time × Trial: 0.086
Urine albumin, g/dL	Pre	0.05 ± 0.02	0.05 ± 0.02	Time: <0.001
	Post	0.06 ± 0.02	0.05 ± 0.02	Trial: 0.653
	Recovery	0.08 ± 0.02	0.08 ± 0.03	Time × Trial: 0.701
Urine flow rate, mL/min	Pre	4.2 ± 2.4	4.7 ± 2.6	Time: <0.001
	Post	1.0 ± 0.5	1.1 ± 0.3	Trial: 0.732
	Recovery	0.8 ± 0.6	0.5 ± 0.6	Time × Trial: 0.366
Osmolar clearance, mL/min	Pre	2.8 ± 1.4	3.0 ± 1.34	Time: <0.001
	Post	1.1 ± 0.8	1.1 ± 0.61	Trial: 0.562
	Recovery	1.5 ± 1.1	1.0 ± 1.32	Time × Trial: 0.240
Free water clearance, mL/min	Pre	1.3 ± 1.8	1.7 ± 2.7	Time: 0.002
	Post	−0.1 ± 0.4	0.0 ± 0.6	Trial: 0.466
	Recovery	−0.7 ± 0.6	−0.6 ± 0.7	Time × Trial: 0.744
Serum sodium, mmol/L	Pre	139 ± 2	138 ± 2	Time: 0.8063
	Post	140 ± 2	143 ± 2	Trial: 0.1156
	Recovery	139 ± 3	143 ± 4	Time × Trial: 0.1025
Urine sodium, mmol/L	Pre	133 ± 66	134 ± 65	Time: 0.002
	Post	114 ± 59	133 ± 85	Trial: 0.263
	Recovery	193 ± 96	237 ± 131	Time × Trial: 0.300
Serum potassium, mmol/L	Pre	4.4 ± 0.5	4.5 ± 0.8	Time: 0.326
	Post	4.1 ± 0.3	4.4 ± 0.6	Trial: 0.203
	Recovery	4.3 ± 0.4	4.4 ± 0.4	Time × Trial: 0.597
Urine potassium, mmol/L	Pre	106 ± 54	95 ± 45	Time: <0.001
	Post	177 ± 67	179 ± 90	Trial: 0.563
	Recovery	355 ± 126	394 ± 80	Time × Trial: 0.217
Fractional excretion of sodium, %	Pre	2.7 ± 2.0	2.5 ± 1.4	Time: <0.001
	Post	1.0 ± 0.8	1.1 ± 0.8	Trial: 0.942
	Recovery	0.5 ± 0.4	0.6 ± 0.6	Time × Trial: 0.590
Fractional excretion of potassium, %	Pre	59 ± 36	52 ± 21	Time: <0.001
	Post	45 ± 18	46 ± 16	Trial: 0.343
	Recovery	28 ± 11	25 ± 12	Time × Trial: 0.497

Data are presented as means ± SD and were analyzed using a two-way linear mixed model with Tukey's post hoc comparisons. *P* values are reported. Data at pre- and postexposure in both trials are $n = 13$. Data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$. Drink, drinking trial; No Drink, fluid restriction trial.

Kidney Function and AKI Risk Biomarkers

Markers of kidney function are presented in Table 3, and urinary biomarkers of AKI risk are presented in Fig. 3. To summarize, all urinary kidney injury risk biomarkers increased over time (Main effect of Time: $P < 0.001$), but there were no differences between trials over time (Time × Trial: $P \geq 0.096$). Although there were no differences between trials in peak changes of IGFBP7 [mean difference (upper, lower CI): −28.5 (200.5, −257.4) ng/mL Cohen's d_z: 0.070; $P = 0.946$] or [IGFBP7·TIMP-2] [mean difference (upper, lower CI): 5.2 (12.7, −2.3) ng/mL Cohen's d_z: 0.391; $P = 0.273$], the peak change in TIMP-2 was greater in the No Drink trial [mean difference (upper, lower CI): 6.4 (12.2, 0.5) ng/mL Cohen's d_z: 0.616; $P = 0.021$].

Inflammation Biomarkers

Serum and urinary inflammation biomarkers are presented in Fig. 4. To summarize, serum and urinary MCP-1 increased over time (Main effect of Time: $P \leq 0.012$) but did not change differently between trials (Time × Trial: $P \geq 0.407$). Moreover, the peak change in serum [Drink: 0.04 ± 0.07 vs. No Drink: 0.07 ± 0.10 ng/mL; mean difference (upper, lower CI): 0.02 (0.07, −0.03) ng/mL Cohen's d_z: 0.257; $P = 0.337$] and urine [Drink: 0.34 ± 0.40 vs. No Drink: 0.56 ± 0.91 ng/dL; mean difference (upper, lower CI): 0.22 (0.74, −0.31) ng/mL Cohen's d_z: 0.233; $P = 0.735$] MCP-1 did not differ between the trials. Peak changes in [IGFBP7·TIMP-2] were not significantly correlated with peak changes in serum MCP-1 ($P = 0.092$; $r_s = 0.337$) but significantly correlated with peak changes in urine MCP-1 ($P < 0.001$; $r_s = 0.794$).

Oxidative Stress Biomarkers

Oxidative stress serum and urinary biomarkers are presented in Fig. 5. To summarize, serum TRX-1 did not change over time (Main effect of Time: $P = 0.310$) and did not change differently between trials (Time × Trial: $P = 0.539$). However, urinary TRX-1 increased over time (Main effect of Time: $P < 0.001$) but did not change differently between trials (Time × Trial: $P = 0.743$). Moreover, the peak change in serum [Drink: 21.0 ± 45.5 vs. No Drink: 32.3 ± 77.5 ng/mL; mean difference (upper, lower CI): 10.1 (51.5, −31.2) ng/mL Cohen's d_z: 0.139; $P = 0.585$] and urine [Drink: 72.7 ± 95.0 vs. No Drink: 83.6 ± 130.1 ng/mL; mean difference (upper, lower

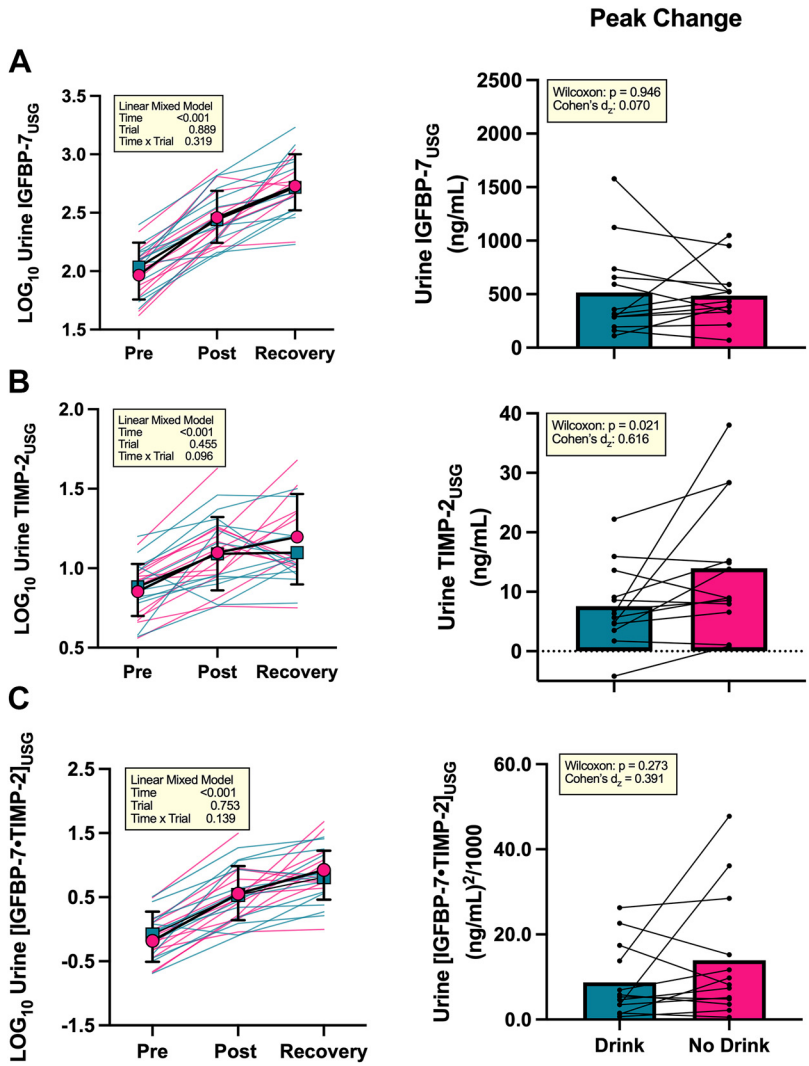

Figure 3. Acute kidney injury biomarker responses, urine insulin-like growth factor-binding protein 7 (IGFBP7) (*A*), urine tissue inhibitor metalloproteinase 2 (TIMP-2) (*B*), and the product of urine IGFBP7 and TIMP-2 [(IGFBP7·TIMP-2)] (*C*), during the 2-h occupational heat stress simulation. *Left*: urinary biomarkers normalized to urine specific gravity (USG) and presented pre- (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Data were log transformed before statistical analyses and were analyzed using two-way linear mixed models with *P* values reported. These data are represented as means ± SD and individual values. *Right*: peak changes in urine kidney injury biomarkers normalized to USG. Data were analyzed using Wilcoxon tests, and Cohen's d_z effect size is reported. Data are represented as means and individual values. Data at pre- and postexposure in both trials are $n = 13$. Data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$.

CI): 10.9 (65.8, −41.0) ng/mL Cohen's d_z: 0.119; $P = 0.685$] TRX-1 did not differ between trials. Peak changes in [IGFBP7·TIMP-2] were not significantly correlated with peak changes in serum TRX-1 ($P = 0.548$; $r_s = 0.123$) but were significantly correlated with peak changes in urinary TRX-1 ($P = 0.008$; $r_s = 0.511$).

Gut Permeability

Gut permeability serum biomarkers are presented in Fig. 6. To summarize, serum LBP did not change over time (Main effect of Time: $P = 0.333$) nor change differently between trials (Time × Trial: $P = 0.435$). Serum I-FABP increased over time (Main effect of Time: $P < 0.001$) but did not change differently between trials (Time × Trial: $P = 0.323$). Neither the peak change in LBP [Drink: 231.6 ± 294.1 vs. No Drink: 173.1 ± 284.5 ng/mL; mean difference (upper, lower CI): 46.3 (219.3, −126.7) ng/mL Cohen's d_z: 0.151; $P = 0.563$] or I-FABP [Drink: 0.4 ± 0.5 vs. No Drink: 0.5 ± 0.6 ng/mL; mean difference (upper, lower CI): 0.1 (0.6, −0.4) ng/mL Cohen's d_z: 0.127; $P = 0.623$] differed between trials. Peak changes in [IGFBP7·TIMP-2] were not significantly correlated with peak changes in LBP ($P = 0.920$; $r_s = 0.021$) or I-FABP ($P = 0.346$; $r_s = 0.193$).

Renal Perfusion

Indices of segmental artery blood velocity and hemodynamic data at pre-, mid-, postexercise, and recovery are presented in Fig. 7. To summarize, resting segmental artery blood velocity and vascular resistance were not different over time (Main effect: $P \geq 0.415$) and did not change differently between trials (Time × Trial: $P \geq 0.491$). Resting heart rate and mean arterial pressure showed an effect of time (Main effect: $P \leq 0.018$) but did not change differently between trials (Time × Trial: $P \geq 0.157$).

Changes in indices of segmental artery perfusion and hemodynamic data during the CPTs are presented in Figs. 8 and 9. To summarize, in all CPT's segmental artery blood velocity was not different relative to baseline (Main effect of Time: $P \geq 0.321$) or between trials (Time × Trial: $P \geq 0.190$). Changes in segmental artery vascular resistance increased relative to baseline at the pre-CPT (Main effect of Time: $P = 0.038$) but not at postrecovery or recovery (Main effect: $P \geq 0.192$). At no time did segmental artery vascular resistance change differently between trials during the CPT (Time × Trial: $P \geq 0.487$). Maximal reductions in segmental artery blood velocity and peak increases in segmental artery vascular resistance were not different between trials (Time × Trial: $P \geq 0.392$). Changes in mean arterial pressure from baseline increased at pre-CPT (Main effect of Time: $P = 0.042$), but there were no differences between trials (Time × Trial: $P = 0.590$). At the post-timepoint, changes in MAP did not differ over time (Main effect of Time: $P = 0.346$) or between trials (Time × Trial: $P = 0.220$). Similarly, changes in mean arterial pressure did not differ over time at the recovery timepoint CPT (Main of effect Time: $P = 0.249$) but indicated a difference between trials (Time × Trial: $P = 0.038$). Post hoc pairwise comparisons indicated that the increase in mean arterial pressure was higher in the Drink trial at *minute 1*

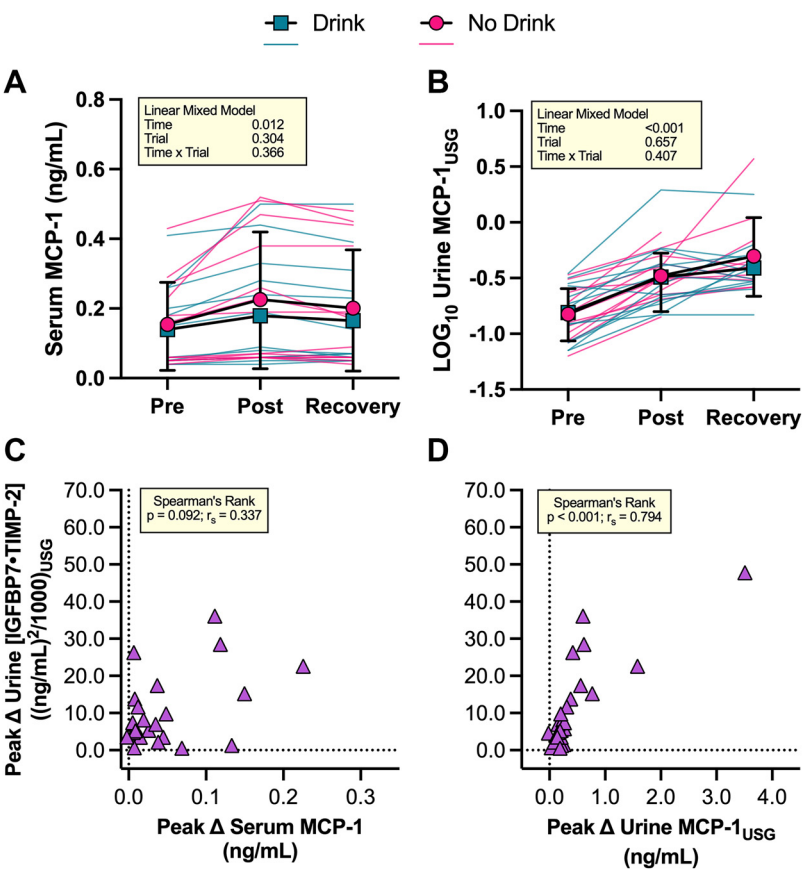

Figure 4. Serum and urine inflammatory biomarkers. Serum (*A*) and urine (*B*) monocyte chemoattractant protein-1 (MCP-1) are presented prerecovery (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Serum MCP-1 data were corrected to percentage changes in plasma volume. Urine MCP-1 data were normalized to urine specific gravity and log transformed before statistical analyses. Data over time were analyzed using a two-way linear mixed model with *P* values reported accordingly. Data at pre- and postexposure in both trials are $n = 13$, data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$. Data are presented as mean ± SD and individual values. Spearman's rank correlations between the peak change (Δ) in serum MCP-1 and urine MCP-1 with change in [IGFBP7·TIMP-2] are presented in *C* and *D*. Exact *P* values and r_s values are shown. Drink, drinking trial; No Drink, fluid restriction trial.

(Drink: 8 ± 17 mmHg; No Drink: −2 ± 11 mmHg; $P = 0.054$) and *minute 2* (Drink: 10 ± 17 mmHg; No Drink: 2 ± 14 mmHg; $P = 0.055$) of the recovery CPT. Peak changes in mean arterial pressure during each CPT did not change over time (Main effect of Time: $P = 0.570$) but indicated a difference between the Drink and No Drink trial (Time × Trial: $P = 0.033$) during recovery, where there was a greater pressor response in the Drink trial (12 ± 18 vs. 1 ± 14 mmHg, $P = 0.022$). In all CPTs, heart rate changed over time (Main effect of Time: $P \leq 0.007$), but changes in heart rate did not differ between trials (Time × Trial: $P \geq 0.129$).

DISCUSSION

The primary purpose of this study was to determine the effect of ad libitum fluid intake on renal oxidative stress and inflammation and AKI risk during 2 h of simulated occupational heat stress. The primary findings are: *1*) ad libitum fluid intake does not mitigate AKI risk during 2 h of simulated occupational heat stress, and *2*) elevations in markers of renal oxidative stress and inflammation are positively associated with AKI risk during simulated occupational heat stress, but these responses are not modified by ad libitum fluid intake. Collectively, these findings do not support our hypothesis that providing free fluid access, consistent with occupational hydration recommendations (81), throughout 2 h of simulated occupational heat stress reduces biomarkers of renal oxidative stress and inflammation or AKI risk compared with restricted fluid intake.

AKI Risk and Hydration during Simulated Occupational Heat Stress

In the present study, attenuating the development of dehydration during simulated occupational heat stress did not mitigate AKI risk, defined as elevations in urinary [IGFBP7·TIMP2] (Fig. 3). Although the change in core temperature was greater in the No Drink trial, participants achieved similar peak core temperatures (Fig. 2) and sweat rates (Table 2), which allowed for a focus on fluid intake (i.e., hydration status) and its role in the risk of developing AKI, alongside renal oxidative stress and inflammation (see *Inflammation, Oxidative Stress, and Gut Permeability during Simulated Occupational Heat Stress*). Fluid restriction elicited greater dehydration in the No Drink trial as evidenced by a greater loss in nude body mass (Table 2), increases in serum osmolality, and reductions in plasma volume (Table 2). However, we would be remiss not to acknowledge that ad libitum drinking did not completely ameliorate body fluid losses, given that participants lost ~1% of their body weight even in the drinking trial (Table 2). Thus, it is possible that had we used a complete fluid replacement trial, we may have observed an attenuated rise in AKI risk during simulated occupational heat stress. It should be noted, however, that providing readily available cool liquids, and not prescribed drinking, is recommended during occupational heat stress (18). Moreover, the observed rate of fluid intake was in a range similar to that observed in agricultural workers during heat stress (0.7 L/h vs. 0.8 L/h) (83, 84). Therefore, the present study indicates that although ad libitum fluid intake attenuates the magnitude of dehydration, this was insufficient

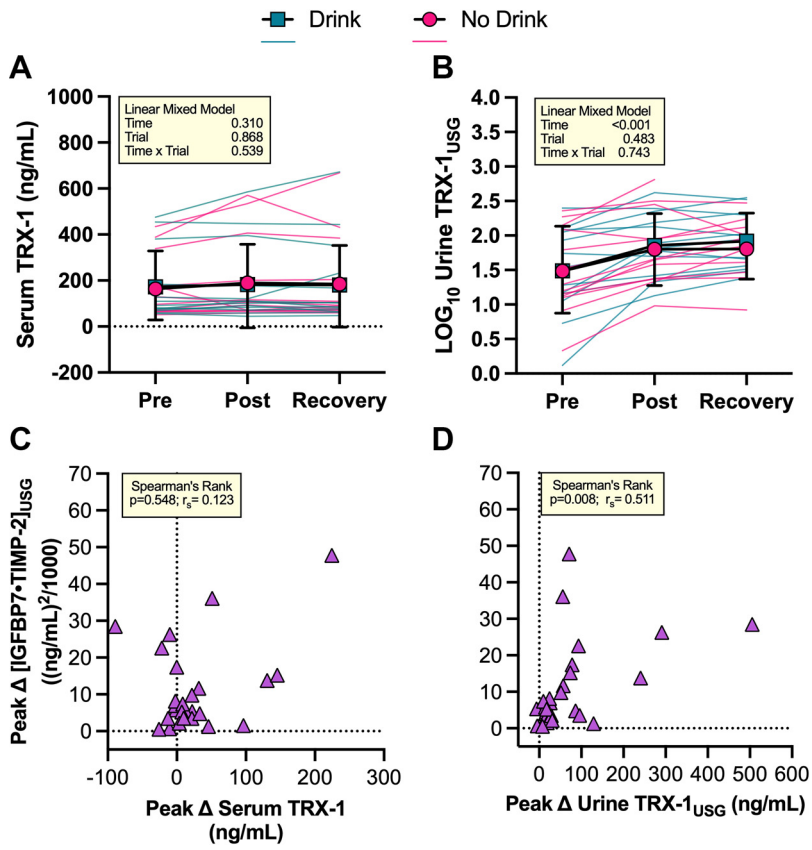

Figure 5. Serum and urine oxidative stress biomarkers. Serum (*A*) and urine (*B*) thioredoxin-1 (TRX-1) are presented prerecovery (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Serum TRX-1 data were corrected to percentage changes in plasma volume. Urine TRX-1 data were normalized to urine specific gravity and log-transformed before statistical analyses. Data were analyzed using a two-way linear mixed model, and *P* values are reported accordingly. Data at pre- and postexposure in both trials are $n = 13$. Data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$. Data are presented as means ± SD and individual values. Spearman's rank correlations between the peak change (Δ) in serum TRX-1 and urine TRX-1 with the change in [IGFBP7·TIMP-2] are presented in *C* and *D*. Exact *P* values and r_s values are shown. Drink, drinking trial; No Drink, fluid restriction trial.

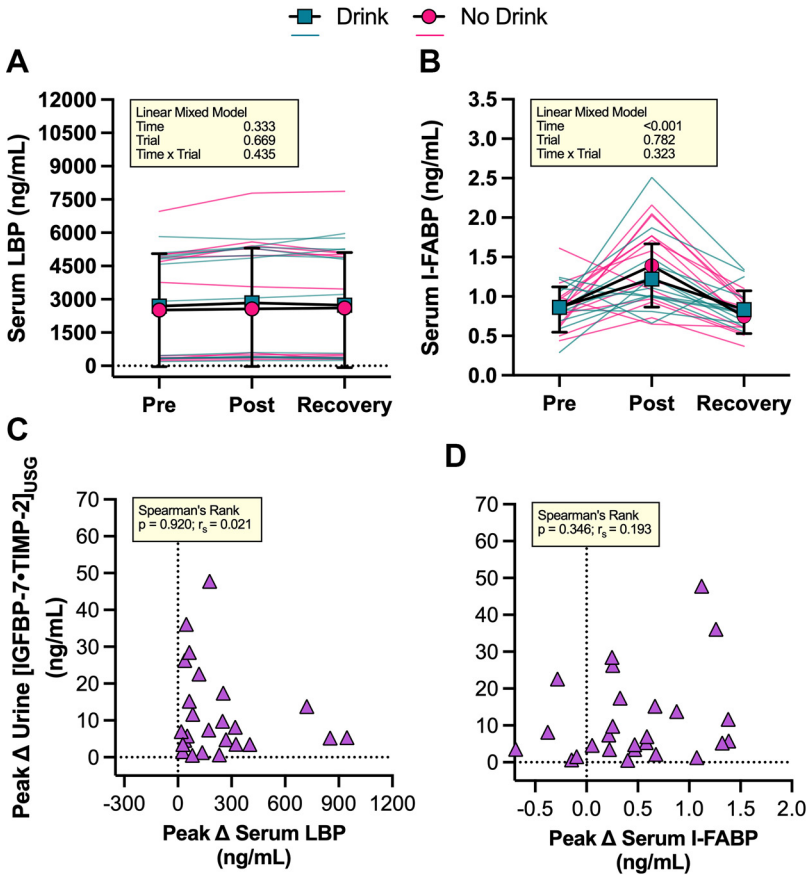

Figure 6. Gut permeability biomarkers. Serum lipopolysaccharide-binding protein (LBP) (*A*) and intestinal fatty acid binding protein (*B*) are presented prerecovery (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Serum LBP and I-FABP data were corrected to percentage changes in plasma volume. Data were analyzed using a two-way linear mixed model, and *P* values are reported accordingly. Data at pre- and postexposure in both trials are $n = 13$. Data at recovery in the Drink trial are $n = 13$, and No Drink trial are $n = 11$. Data is presented as mean ± SD and individual values. Spearman's rank correlations between the peak change (Δ) in serum LBP (*C*) and serum I-FABP (*D*) with change in [IGFBP7·TIMP-2] are presented in *C* and *D*. Exact *P* values and r_s values are shown.

to attenuate elevations in urinary [IGFBP7·TIMP2]. Such findings suggest that at a given level of hyperthermia, there may be a dehydration threshold after which AKI risk will be magnified, and that this threshold likely exceeds ~2.7% body weight loss [i.e., the mean body weight loss observed in the No Drink trial (Table 2)].

Seemingly in contrast to our findings, Juett et al. (85) found that during high intensity intermittent running in a temperate environment, biomarkers of AKI risk were augmented with dehydration, but unfortunately, the authors did not measure core temperature. This is important because Chapman et al. (86) found that independently attenuating the magnitude of hyperthermia (via body cooling) and dehydration (via prescribed drinking) mitigated the rise in AKI biomarkers, but body cooling trial or prescribed drinking resulted in peak core temperatures that were similar between trials (approximately +1.2°C). Thus, based on the findings of the present study, it may be that core temperature, rather than hydration status, plays a comparatively more important role in the development of AKI risk. This may be particularly true when the magnitude of hyperthermia exceeds some threshold value, which is likely greater than 38.0°C (9), but has not yet been defined. Moreover, it is likely that the interactive effects of hyperthermia and dehydration on AKI risk differ depending on the magnitude of changes in each of these factors.

Although urinary [IGFBP7·TIMP2], the a priori established biomarker of AKI risk, did not differ between the trials (Fig. 3), it is interesting to note that the peak increase in urinary TIMP-2, which is preferentially secreted in the distal tubules (87), was greater in the No Drink trial (Fig. 3). This finding is in contrast with previous work that identified no effect of prescribed drinking on the rise in urinary TIMP-2 concentrations during simulated occupational heat stress (7). These seemingly conflicting findings may be explained, at least in part, by the higher average relative work intensity in the present study [75% vs. ~65% (88) of estimated maximum heart rate] and the inclusion of upper body exercise to better simulate the physical demands of occupational tasks. These are notable differences because the cardiovascular response and likely the redistribution of blood flow away from the kidneys is influenced by the relative work intensity (11, 89) and the muscle mass utilized with upper body work known to provoke a greater cardiovascular response (90, 91). We speculate that with this greater cardiovascular response, oxygen delivery to the renal medulla, which anatomically houses portions of the distal tubule, may have been compromised to a greater extent in the No Drink trial due to augmented sympathetic outflow. This is supported by evidence that sympathetic activation induced by lower body negative pressure reduces renal medullary oxygenation in healthy adults (92). Although we do not have regional renal blood flow measurements, in the current study we observed no effect of physical work or drinking on segmental artery vascular resistance (Fig. 7), a marker of renal vasomotor tone (93–95), nor the segmental artery vascular responsiveness to sympathetic stimulation (i.e., CPT) (Fig. 8), a marker of renal vascular control (65, 93, 94). Moreover, although indicators of kidney and tubular function (e.g., creatinine clearance, fractional excretion of sodium, etc.) exhibited the expected changes with simulated occupational heat stress (86, 96, 97), these indicators did not

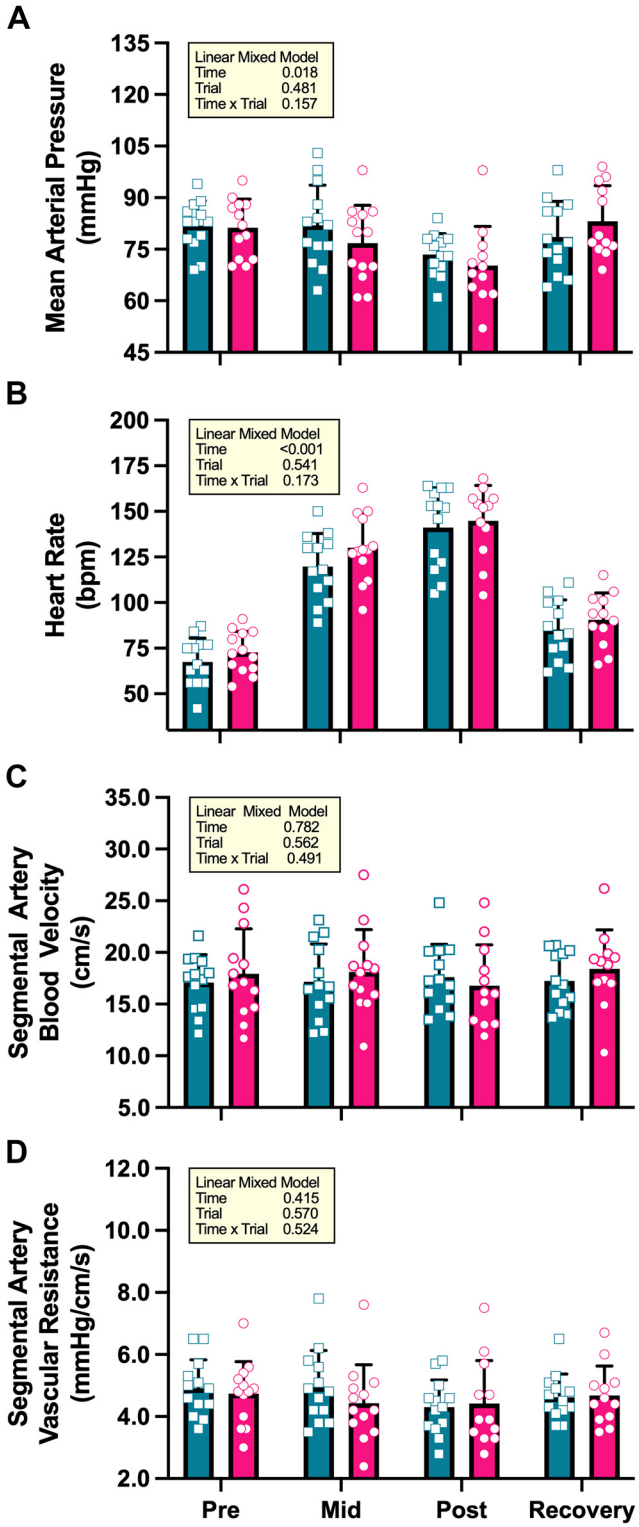

Figure 7. Resting mean arterial pressure (*A*), heart rate (*B*), segmental artery blood velocity (*C*) and segmental artery vascular resistance (*D*) presented pre- (Pre), halfway (Mid), postrecovery (Post), and at recovery following the occupational heat stress simulation. Data were analyzed using a two-way linear mixed model, and *P* values are reported. All data are $n = 12$ and presented as means ± SD and individual values.

differ between trials (Table 3) and are unlikely to explain the greater increase in urinary TIMP-2 in the No Drink trial. Thus, from the current study, the mechanisms underlying the observed greater increase in urinary TIMP-2 in the No Drink trial remain unclear. Collectively, these findings highlight the need for future studies to clarify the role of hydration across a range of core temperatures and over longer work durations, to better understand its impact on AKI risk during occupational heat stress.

Inflammation, Oxidative Stress, and Gut Permeability during Simulated Occupational Heat Stress

Contrary to our hypothesis, ad libitum fluid intake did not reduce markers of systemic (serum) or renal (urinary) inflammation (Fig. 4) or oxidative stress (Fig. 5) during simulated occupational heat stress. Serum MCP-1, our primary biomarker of systemic inflammation, was increased following simulated occupational heat stress but was unaffected by fluid intake. This finding is corroborated by previous work indicating that the systemic inflammatory response to heat stress is not modified by hydration in healthy young men (98). Similarly, urinary MCP-1, our primary biomarker of renal inflammation, was elevated following simulated occupational heat stress irrespective of hydration status. This is a novel finding. For example, previous work has demonstrated that the urinary inflammatory marker IL-18 is increased with prolonged passive heat exposure that rendered participants both hyperthermic and dehydrated (8) and that urinary MCP-1 is increased with simulated occupational heat stress when ad libitum drinking was allowed (17). The current study extends these findings to indicate that this increase in renal inflammation is not worsened when fluid intake is restricted. Similar to our study (Fig. 7), this previous work also indicated that passive heat stress (8) and simulated occupational heat stress (17) resulted in an increase in urinary TRX-1, but not serum TRX-1, suggesting increases in oxidative stress are localized to the kidneys. Notably, independent of hydration status, peak changes in [IGFBP7·TIMP-2] and urinary MCP-1 and TRX-1 were significantly correlated (Figs. 4 and 5), whereas correlations between peak changes in [IGFBP7·TIMP-2] and serum MCP-1 and TRX-1 were not statistically significant. This observation supports previous work during prolonged passive heat stress (8), which suggests a relation between renal inflammation and renal oxidative stress and the development of AKI risk. However, it should be noted that these relationships are only associative and should not be considered causal. As a result, future research is required to identify causal relationships between renal and/or systemic inflammation and oxidative stress and AKI risk in the context of occupational heat stress.

It has been proposed that heat-induced gastrointestinal permeability and the resultant systemic proinflammatory state occurring secondary to immune system activation caused by bacterial translocation is a contributor to AKI risk during occupational heat stress (62, 99). In the current study, biomarkers of gut permeability indicating enterocyte damage (I-FABP), but not bacterial translocation (LBP), were found to increase during simulated occupational heat stress but were not modulated by ad libitum fluid intake (Fig. 6). This observation is in contrast to previous work demonstrating that

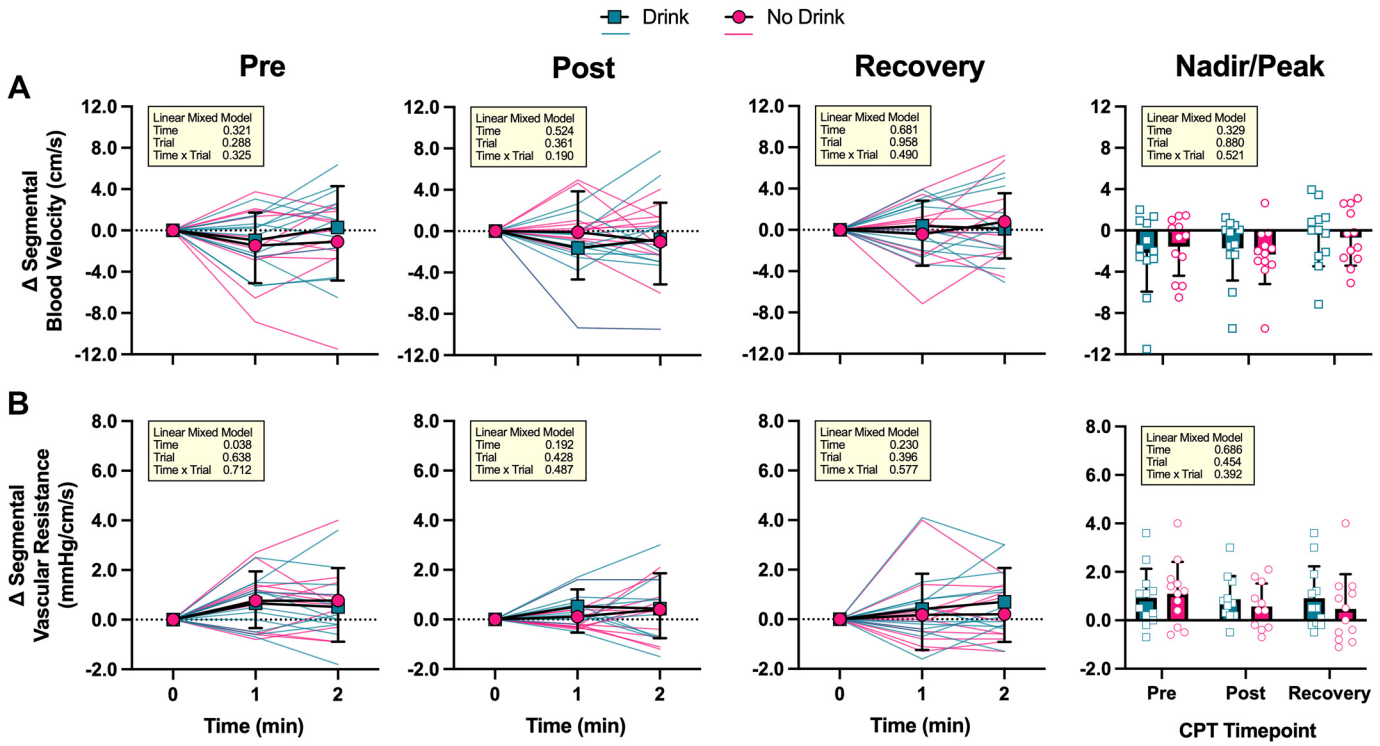

Figure 8. Segmental artery blood velocity (*A*) and segmental artery vascular resistance (*B*) during the cold pressor test prerecovery (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Nadir changes in segmental blood velocity and peak changes in segmental vascular resistance at each of these timepoints are also presented. Data were analyzed using a two-way linear mixed model, and *P* values are reported. All data are $n = 12$ and presented as means ± SD and individual values.

minimizing dehydration mitigates the rise in markers of gastrointestinal permeability during exercise in the heat (100). That said, although in this previous study markers of gastrointestinal permeability were attenuated by fluid replacement, the fluid restricted trial resulted in a body mass loss of ~1.5%, which was similar to the body mass lost in the Drink trial in the present study (~1.3%), highlighting the need for further research on the dose-response effects of fluid replacement on markers of gastrointestinal permeability. Notably, although we observed seemingly parallel rises in I-FABP and serum MCP-1, our marker of systemic inflammation, none of these variables significantly correlated with the urinary [IGFBP7·TIMP-2], our primary marker of AKI risk (Figs. 4 and 6). Thus, although markers indicative of gastrointestinal permeability, systemic inflammation, and AKI risk all increased independent of fluid intake, there is little evidence to link gastrointestinal permeability and the resulting systemic inflammation to AKI risk. This may be because the magnitude of hyperthermia was relatively homogenous and did not regularly exceed 39°C, which has been shown to consistently be associated with increased markers of gastrointestinal permeability (101). That said, further research is required to better discern the potential causal effects of immune system activation during occupational heat stress and its impact on kidney function and injury.

Experimental Considerations

There are a few considerations that warrant attention. First, we would like to note that the actual number of participants with complete data sets ($n = 11$) was less than the a priori power calculation ($n = 12$). This is because two participants were unable to provide a urine sample at the recovery timepoint in the No Drink trial. Thus, it is possible that we were underpowered to observe an effect for the measured urinary markers. As a result, we have provided effect size metrics to aid in data interpretation. Although we acknowledge this limitation, given the small effect size for the effect of trial on our primary outcome measurement of AKI risk (urinary [IGFBP7·TIMP2]), we think it is unlikely that one additional observation would have meaningfully changed the conclusions of this study. Second, a strength of this study is that it was a well-controlled study. Limitations of this approach, however, are that the obtained findings may not accurately reflect real-world exposures in which work shifts are 8–12 h in duration, are repeated for several days, and coexposure to other environmental factors (e.g., dust, noise, etc.). To highlight, this study involved a single 2 h occupational heat stress simulation. Therefore, it is possible that longer durations or repeated days of physical work with no fluid replacement would have unveiled greater elevations in renal and systemic oxidative stress and inflammation and AKI risk. However, it is worth noting that previous work utilizing 2 h of simulated occupational heat stress showed that prescribed fluid intake was able to avert elevations in AKI risk (7), suggesting that work duration is unlikely the primary explanation of our findings. Rather, our data are likely explained by the magnitude of hyperthermia experienced and the statistically significant, but relatively small, differences in hydration status. This conclusion is supported by recently published field work, in

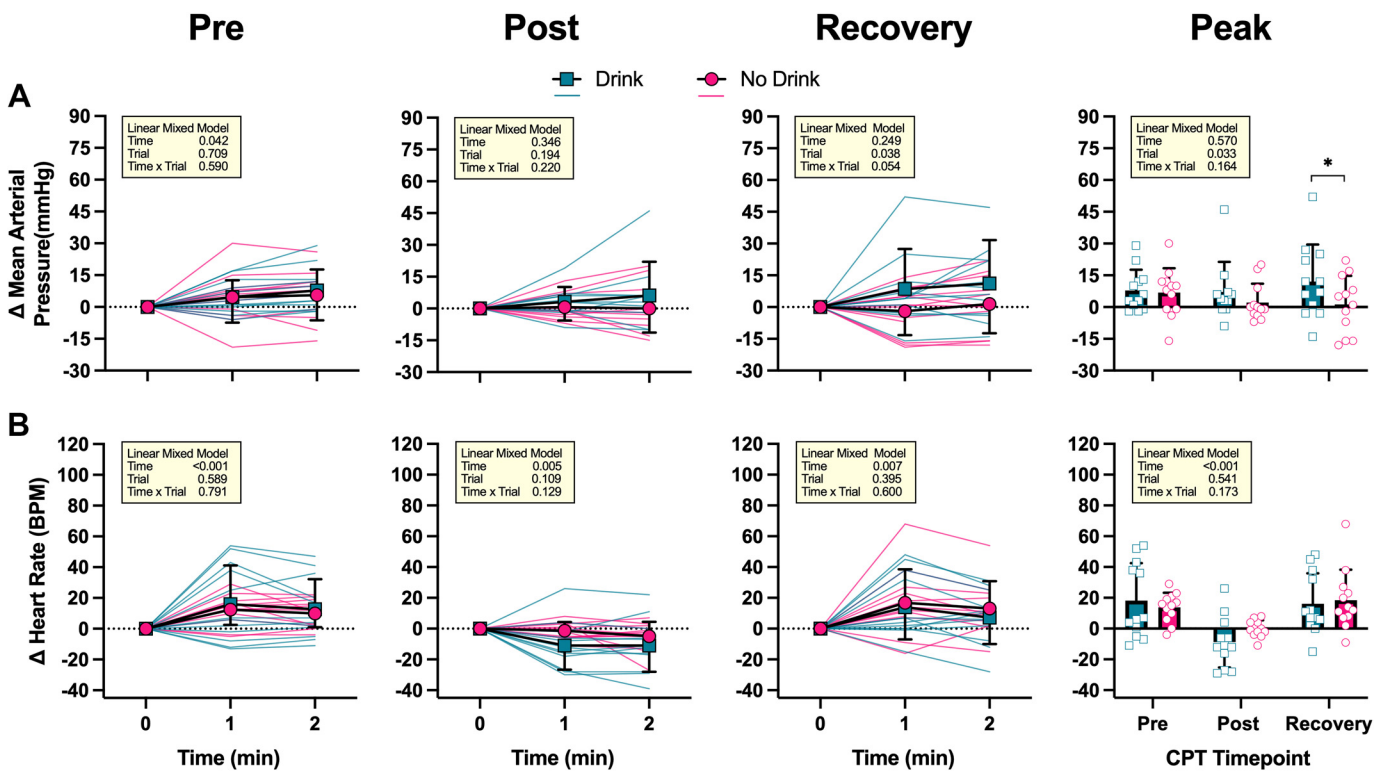

Figure 9. Changes in mean arterial pressure (*A*) and heart rate (*B*) during the cold pressor test prerecovery (Pre), postrecovery (Post), and at recovery following the occupational heat stress simulation. Peak changes in mean arterial pressure and heart rate are also shown. Data were analyzed using a two-way linear mixed model with Tukey's post hoc comparisons when necessary. *P* values are reported. All data are $n = 12$ and presented as means ± SD and individual values. *Significantly different from No Drink trial ($P \leq 0.05$).

which the magnitude of hyperthermia, not hydration status, was identified as an independent predictor of cross-shift increases in AKI risk (urinary [IGFBP7·TIMP2]) in construction workers (102). Second, it is important to note that the biomarkers of AKI risk measured in this study are subclinical in nature and reflect an increased risk of AKI but do not indicate a clinical diagnosis. To this end, there is no established threshold for a meaningful increase in urinary [IGFBP7·TIMP2] in the context of occupational heat stress. Interestingly, however, all observations in the current study, independent of the experimental trial, displayed an increase in urinary [IGFBP7·TIMP2] greater than 0.3 (ng/mL)^2/1,000 (Fig. 3), the threshold used clinically (103). Thus, future research is required to improve the understanding and interpretation of AKI risk biomarkers in the context of occupational heat stress. Moreover, how biomarkers of AKI risk across a single or repeated episodes of occupational heat stress translate to the risk of developing CKDnt remains poorly understood (104). Third, the U.S. National Insititue of Occupational Safety and Health recommends that workers arrive at the worksite hydrated and that the worksite have cool fluids readily available (18). These recommendations guided our study design. However, we acknowledge that this may not always be what is encountered in the workplace, where, for example, workers regularly arrive at the worksite dehydrated (18, 79, 105). Lastly, renal blood flow was not directly measured. Instead, Doppler ultrasound was used to measure segmental artery blood velocity, which was interpreted to reflect changes in renal blood flow, as done previously (96). To increase the reliability of the operator-dependent ultrasound measurements and to ensure transparency, the intraoperator coefficient of variation of our sonographer was measured and reported to aid in the interpretation of measurements obtained from Doppler ultrasound (94). Nevertheless, future studies should consider incorporating more direct measures of renal blood flow and/or renal plasma flow; particular focus should be on methods (e.g., MRI) that can quantify the regional distribution of blood flow within the kidneys.

Perspectives

Workers frequently experiencing occupational heat stress are at risk for heat-related illnesses (1), including heat-related AKI (1, 5) and CKDnt (1), the latter of which has been hypothesized to arise from a single clinically diagnosed AKI event or repeated subclinical AKI episodes (106). Experimental studies demonstrate that hyperthermia and dehydration are potential modulators of the development of heat-related AKI (7), whereas workplace-based interventions that mitigate the development of hyperthermia and dehydration reduce the prevalence of clinical AKI (7, 105, 107, 108). Interestingly, our study found that attenuating dehydration via ad libitum fluid intake during simulated occupational heat stress, which followed occupational hydration recommendations (81, 109, 110), did not reduce AKI risk. We interpret such findings to demonstrate the relative importance of hyperthermia such that relatively small differences in hydration status have minimal effect of AKI risk during occupational heat stress when a

given, not well-defined, level of hyperthermia is present. Notably, we have also identified that simulated occupational heat stress increased markers of renal oxidative stress and inflammation, but this effect was not modified by ad libitum drinking. Moreover, while we observed increases in markers of systemic and renal inflammation and oxidative stress, only markers of renal inflammation and oxidative stress were correlated with increases in AKI risk. Such findings suggest that endogenous processes in the kidneys may contribute to the increased risk of AKI during occupational heat stress. Thus, future work should examine the potential causal role of renal inflammation/oxidative stress in the increased AKI risk during occupational heat stress. This information will be vital toward the development of novel strategies to protect the renal health of outdoor workers.

Conclusions

This study demonstrates that preventing dehydration by adhering to current occupational hydration guidelines did not attenuate increases in renal oxidative stress, renal and systemic inflammation, or AKI risk following 2 h of simulated occupational heat stress. In addition, the data show that elevations in AKI risk are associated with markers of renal inflammation and oxidative stress, regardless of fluid intake. To better protect the kidney health of outdoor workers, future research should focus on understanding the causal mechanisms by which occupational heat stress triggers renal oxidative stress and/or inflammation, thereby increasing AKI risk.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

E.T., M.J.H., H.W.H., F.T.A., T.D.M., B.D.J., D.H., and Z.J.S. conceived and designed research; E.T., M.J.H., M.E.H., H.W.H., T.D.M., B.D.J., and Z.J.S. performed experiments; E.T., M.J.H., M.E.H., T.D.M., B.D.J., and Z.J.S. analyzed data; E.T., M.J.H., M.E.H., H.W.H., F.T.A., T.D.M., B.D.J., D.H., and Z.J.S. interpreted results of experiments; E.T., M.J.H., and Z.J.S. prepared figures; E.T., M.J.H., and Z.J.S. drafted manuscript; E.T., M.J.H., M.E.H., H.W.H., F.T.A., T.D.M., B.D.J., D.H., and Z.J.S. edited and revised manuscript; E.T., M.J.H., M.E.H., H.W.H., F.T.A., T.D.M., B.D.J., D.H., and Z.J.S. approved final version of manuscript.

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