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RESEARCH ARTICLE

Both hyperthermia and dehydration during physical work in the heat contribute to the risk of acute kidney injury

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Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, 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. First published February 20, 2020; doi:10.1152/japplphysiol.00787.2019.—Occupational heat stress increases the risk of acute kidney injury (AKI) and kidney disease. This study tested the hypothesis that attenuating the magnitude of hyperthermia (i.e., increase in core temperature) and/or dehydration during prolonged physical work in the heat attenuates increases in AKI biomarkers. Thirteen healthy adults (3 women, 23 ± 2 yr) exercised for 2 h in a 39.7 ± 0.6°C, 32 ± 3% relative-humidity environmental chamber. In four trials, subjects received water to remain euhydrated (*Water*), continuous upper-body cooling (*Cooling*), a combination of both (*Water* + *Cooling*), or no intervention (*Control*). The magnitude of hyperthermia (increased core temperature of 1.9 ± 0.3°C; $P < 0.01$) and dehydration (percent loss of body mass of −2.4 ± 0.5%; $P < 0.01$) were greatest in the *Control* group. There were greater increases in the urinary biomarkers of AKI in the *Control* trial: albumin (increase of 13 ± 11 μg/mL; $P \leq 0.05$ compared with other trials), neutrophil gelatinase-associated lipocalin (NGAL) (increase of 16 ± 14 ng/dL, $P \leq 0.05$ compared with *Cooling* and *Water* + *Cooling* groups), and insulin-like growth factor-binding protein 7 (IGFBP7) (increase of 227 ± 190 ng/mL; $P \leq 0.05$ compared with other trials). Increases in IGFBP7 in the *Control* trial persisted after correcting for urine production/concentration. There were no differences in the AKI biomarker tissue inhibitor of metalloproteinase 2 (TIMP-2) between trials ($P \geq 0.11$). Our findings indicate that the risk of AKI is highest with greater magnitudes of hyperthermia and dehydration during physical work in the heat. Additionally, the differential findings between IGFBP7 (preferentially secreted in proximal tubules) and TIMP-2 (distal tubules) suggest the proximal tubules as the location of potential renal injury.

NEW & NOTEWORTHY We demonstrate that the risk for acute kidney injury (AKI) is higher in humans with greater magnitudes of hyperthermia and dehydration during physical work in the heat and that alleviating the hyperthermia and/or limiting dehydration equally reduce the risk of AKI. The biomarker panel employed in this study suggests the proximal tubules as the location of potential renal injury.

AKI; exercise; heat stress, hydration; kidney function

INTRODUCTION

Data from rodent models demonstrate that recurrent exposures to heat stress induces nephropathy, which is likely caused by repeated bouts of kidney injury (20, 48, 50, 51). The extent of kidney injury from a singular exposure or repeated bouts is likely proportionate to the magnitude of hyperthermia (i.e., an increase in core temperature) and/or dehydration (i.e., a hypovolemic, hyperosmotic state) incurred during the heat stress. For instance, data in rodents indicate that drinking water after heat exposure does not prevent kidney injury, but there is a protective effect of limiting dehydration by drinking water during the heat exposure (51). Moreover, kidney injury may also be independently caused by greater increases in core temperature during the heat exposure (54). The mechanisms by which kidney injury is evoked by hyperthermia and/or dehydration are multifactorial and likely include an interplay of reductions and/or a redistribution of renal blood flow, increases in circulating vasopressin, hyperuricemia, reductions in renal ATP, and the activation of the polyol-fructokinase pathway (16, 50, 51, 54, 58, 62).

Consistent with the data from rodent models, data in humans indicate that prolonged physical work (e.g., exercise) in hot environments can reduce kidney function and elevate biomarkers of acute kidney injury (AKI) (26, 32, 36, 57). Increases in these AKI biomarkers may not always reflect true kidney damage (i.e., clinical AKI) but rather likely indicate an increased risk of developing AKI when these AKI biomarkers are elevated alongside decreases in renal function (58). The mechanisms by which AKI risk is elevated with prolonged physical work in the heat in humans have not been fully elucidated. The largest gap in knowledge is likely the relative importance of hyperthermia versus dehydration on AKI risk, given that this information could transform occupational heat stress and hydration recommendations aiming to safeguard renal health in workers. We have previously demonstrated that increases in AKI biomarkers are influenced by the magnitude of hyperthermia and dehydration as a consequence of longer durations of physical work in the heat (57). For methodological reasons, however, we were unable to isolate hyperthermia from dehydration; although, we found no statistical evidence that dehydration was of more (or less) importance than hyperthermia. With this background, the purpose of the present study

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was to determine the independent and combined effects of hyperthermia and dehydration on increases in AKI biomarkers during physical work in the heat. We hypothesized that preventing dehydration and/or attenuating the rise in core temperature attenuates increases in AKI biomarkers during physical work in the heat compared with a nonintervention control condition.

METHODS

Participants. Thirteen healthy adults (3 women) participated in this study. Subject characteristics were age of 22.6 ± 2.0 yr, a height of 176 ± 8 cm, a weight of 78.1 ± 13.9 kg, and a body mass index of 25 ± 3 kg/m^2. All subjects were nonsmokers and reported to be free from any known cardiovascular, renal, metabolic, neurological, or gastrointestinal diseases. Subjects self-reported to exercise at least 3 days/wk and were recreationally active. The study was conducted during the months of October–April in Buffalo, NY. Thus, subjects were assumed to not be heat acclimatized during this time. Female subjects 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. Additionally, female subjects were not on birth control and were tested during the first 10 days of their self-identified menstruation. Subjects visited the laboratory on five separate occasions. During the first visit, subjects were screened and familiarized with the instrumentation and exercise protocol. The final four visits were the experimental trials. This study was approved by the Institutional Review Board at the University at Buffalo in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects before their participation in this study.

Instrumentation and measurements. Height and nude body weight were measured using a stadiometer and scale (Satorius, Bohemia, NY). Subjects ingested a telemetry pill (HQ Inc., Palmetto, FL) 6–8 h before arriving at the laboratory for measurement of core temperature. Skin temperature was measured using four thermochron iButtons (Maxim Integrated Products, San Jose, CA) attached the right side of the body on the chest, triceps brachii, quadriceps, and posterior calf. Urine specific gravity was measured in duplicate using a refractometer (Atago, Bellevue, WA). Heart rate was measured using a wireless monitor (Polar Electro, Bethpage, NY). Systolic and diastolic blood pressure were measured manually in duplicate by the same member of the research team throughout the study. In addition to wearing shorts and athletic shoes during exercise, subjects wore a tube-lined water-perfused suit top that covered the entire upper body except for the head and hands (Med-Eng, Ottawa, ON, Canada) during all four experimental trials. To ensure maximal contact between the water-perfused suit top and the skin, a long sleeve polyester-blend compression top was worn over the suit. Women wore sports bras, and men were shirtless underneath the water-perfused suit. Measurements of thermal sensation (14), thermal comfort (15), and rating of perceived exertion (4) were taken every 10 min during the exercise protocol using standard analog scales.

Renal artery and segmental artery blood velocities were obtained via Doppler ultrasound (GE Vivid-Q, Chicago, IL) and a phased-array transducer (2.5–3.5 MHz) using the approach previously described in detail (6, 56). In brief, the coronal approach was utilized with subjects in the left lateral recumbent position to measure blood velocity in the distal segment of the right renal artery (renal) and in the middle portion of the segmental arteries in the right kidney (segmental). Indelible ink was used to mark the transducer location during baseline measurements during the first experimental visit, and this location was used for all subsequent measurements. Subjects were provided with a marker and were instructed to retrace the area to keep the transducer location viable for all subsequent visits. Blood velocity measurements were averaged over the course of nine cardiac cycles. During a midexhalation, non-Valsalva breath hold, three cardiac cycles were measured, and this was immediately repeated two more times. This approach yielded average blood velocity measurements over the course of ~30 s. Mean blood velocity in the segmental arteries was averaged across three segmental arteries (n = 9) and across two segmental arteries (n = 3). Because of acoustic shadowing of the kidney during three of the four experimental trials, 1 subject was excluded from the analysis for this measurement, resulting in n = 12 for blood velocity measurements. All blood velocity measurements were obtained by the same sonographer (C. L. Chapman) with a within-subject test-retest coefficient of variation for renal blood velocity measurements of 3.9 ± 0.8% (renal artery) and 3.9 ± 1.2% (segmental artery). Given the depth of the renal vasculature, it is not possible to accurately measure vessel diameter using Doppler ultrasound. Therefore, renal and segmental blood velocity were interpreted to reflect changes in renal blood flow (6, 10, 39, 56, 66) because reductions in renal blood flow during pharmacological intervention have been shown to be due to decreases in blood velocity with a maintenance of the vessel diameter (35).

Hemoglobin was measured in duplicate using the Hemopoint H2 (Alere, Orlando, FL), and hematocrit was measured in triplicate using microcentrifugation. Serum and urine osmolality were measured in duplicate via freezing point depression (model 3250; Advanced Instruments, Norwood, MA). Serum measurements of sodium, potassium, creatinine, blood urea nitrogen and uric acid, and urine measurements of sodium, potassium, creatinine, and albumin were measured via standard clinical techniques by Kaleida Health, Department of Pathology and Laboratory Medicine (Williamsville, NY). Comparative elevations in urine albumin were interpreted as indicative of greater challenges to glomerular (e.g., permeability and/or hyperfiltration) or renal tubular reabsorption of albumin (2). Serum copeptin was measured to indirectly assess vasopressin release using a human copeptin ELISA kit (LifeSpan BioSciences, Seattle, WA) (1). Neutrophil gelatinase-associated lipocalin (NGAL) was measured in the plasma and urine using a commercially available human NGAL ELISA kit (Toronto Bioscience, Toronto, Canada). NGAL was measured in the plasma as an indirect indicator of renal ischemia and/or glomerular dysfunction (30). NGAL was measured in the urine to allow for comparisons of the magnitude of general renal tubular injury across experimental conditions (30, 58). Insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) were measured in the urine using separate human IGFBP7 and TIMP-2 ELISA kits (RayBiotech Life, Peachtree Corners, GA). IGFBP7 and TIMP-2 are proteins that induce G1 cell cycle arrest (12), which occurs in renal epithelial cells during AKI (67). During AKI, IGFBP7 and TIMP-2 originate from damage to the glomeruli and/or renal tubules (23). However, IGFBP7 is preferentially secreted in renal proximal tubules, whereas TIMP-2 is preferentially secreted in the distal tubules (11). Importantly, urine IGFBP7 and TIMP-2 received US Food and Drug Administration approval in 2014 as a screening tool to estimate the risk of AKI development in clinical practice (12). Notably, the aggregate findings from our biomarker panel of urine NGAL, IGFBP7, and TIMP-2 likely allow for the indirect examination of the source of kidney injury (e.g., glomerular versus proximal tubules versus distal tubules) during prolonged physical work in the heat (58).

Experimental protocol. A quasi-randomized crossover design was employed, with each subject participating in four experimental trials in which they walked on a treadmill in a 39.7 ± 0.6°C, 32 ± 3% relative humidity environment for two hours (Fig. 1). These environmental conditions were specifically chosen to reflect the thermal environment for outdoor work during late morning shifts encountered by workers at risk for AKI (18). In the 4 experimental conditions, subjects were either provided with a volume of thermoneutral water (35.1 ± 2.0°C) every 15 min that matched the amount of sweat loss as estimated by changes in body weight (*Water*), provided continuous upper-body cooling via a tube-lined suit perfusing 2°C water throughout exercise (*Cooling*), received both interventions (*Water + Cool-*

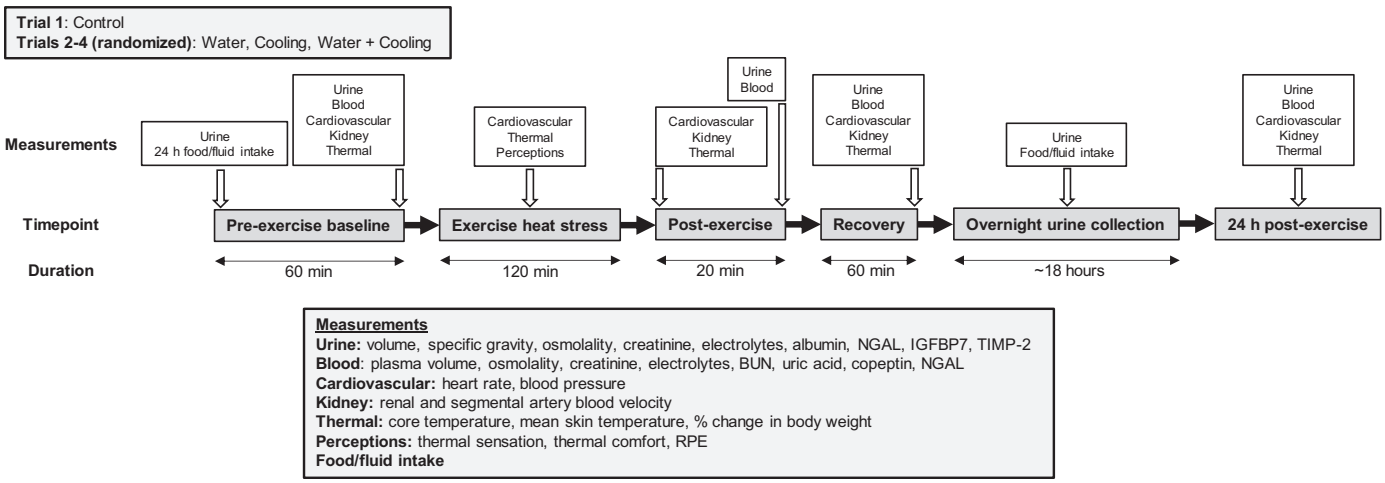

Fig. 1. Schematic of study protocol. In a quasi-randomized crossover design, subjects undertook 2 h of treadmill walking in a 39.7 ± 0.6°C, 32 ± 3% relative humidity environment. During exercise heat stress, subjects received water to remain euhydrated (*Water*; $n = 13$), continuous upper body cooling (*Cooling*; $n = 12$), a combination of both (*Water + Cooling*; $n = 13$), or no intervention (*Control*; $n = 13$). BUN, blood urea nitrogen; IGFBP7, insulin-like growth factor-binding protein 7; NGAL, neutrophil gelatinase-associated lipocalin; RPE, rating of perceived exertion; TIMP-2, tissue inhibitor of metalloproteinase 2.

ing), or received neither intervention (*Control*). The water-perfused suit top was worn in all four experimental conditions to keep exercise attire the same, but the suit was not perfusing water (i.e., there was no cooling) in the *Water* and *Control* trials. Because of the greatest risks of participant volitional fatigue or reaching our ethical limit for core temperature (39.5°C), the Control trial was always performed first so that the three remaining trials could be matched to the same exercise duration if stopping criteria were met. The three remaining experimental trials were randomized. During the *Control* trials, 11 subjects completed 2 h of exercise, 1 subject stopped at 90 min because of volitional fatigue, and1 subject stopped at 86 min after reaching our ethically approved core temperature limit. In these instances, the three remaining trials were matched to the duration of the *Control* trials. Thus, the average exercise duration across subjects was 115 ± 12 min. At least 7 days separated the experimental trials to minimize any potential effects of heat acclimation. Subjects also came into the laboratory 24 h after the start of each trial for additional data collection.

Subjects recorded their dietary and fluid intakes during the 24-h period preceding the start of the trial and during the overnight period between the end of the experimental trial and the 24-h follow-up visit. Subjects were instructed to keep dietary and fluid intakes the same across each preceding 24-h period and across the overnight periods. Importantly, there were no differences between experimental trials in food and fluid intakes for the 24 h preceding the start of the trial, and in the overnight period, which occurred from when the subject departed the laboratory after recovery from exercise until they arrived at the laboratory the following day (~18 h) (Table 1). Subjects arrived at the laboratory having refrained from strenuous exercise, alcohol, and caffeine for 12 h, and from food for 2 hours. Upon arrival, subjects voided their bladders, and euhydration was confirmed via a urine specific gravity of <1.020 (Table 2) (55). Subjects were then weighed nude and instrumented with a heart rate monitor and four thermochron iButtons. Thirty minutes after voiding their bladder, subjects assumed a supine position in a 24.5 ± 2.6°C, 30 ± 15% relative-humidity environment. After 20 min of supine rest, measurements of core temperature, heart rate, blood pressure, and renal and segmental artery blood velocity were taken. Then, a venous blood sample was taken. Following blood collection and precisely 1 h after the previous urine void, subjects voided their bladders into a collection container for measurement of urine volume and calculation of pre-exercise urine flow rate during this 1-h baseline period. Finally, a nude body weight measurement was taken.

Following the pre-exercise baseline period, subjects entered the environmental chamber and immediately measured their body weight while wearing sneakers and athletic shoes (women with sports bra). This body weight was used as the reference point during the exercise period for water volumes provided during the *Water* and *Water + Cooling* trials. Immediately after measuring body weight, subjects donned the water-perfused suit and compression top. Then, subjects

Table 1. *Pre-experimental trial and overnight period fluid and food intake*

	Pre-experimental Trial				Overnight			
	Control	Water	Cooling	Water + Cooling	Control	Water	Cooling	Water + Cooling
Fluid volume, mL	3,847 (1,121)	3,933 (1,179)	3,845 (1,303)	3,628 (937)	2,753 (949)	2,833 (811)	3,086 (1,072)	2,747 (789)
Total energy, kcal	2,403 (671)	2,315 (624)	2,574 (1,011)	2,707 (917)	1,885 (747)	2,010 (691)	2,387 (717)	2,574 (824)
Fat, g	95 (39)	93 (40)	87 (41)	95 (35)	69 (40)	67 (25)	83 (37)	95 (54)
Protein, g	108 (46)	113 (42)	109 (40)	125 (56)	89 (43)	92 (33)	108 (38)	121 (51)
Total carbohydrate, g	281 (106)	260 (99)	323 (134)	321 (122)	233 (114)	265 (134)	299 (119)	311 (117)
Sugar, g	88 (59)	84 (54)	127 (61)	98 (68)	77 (76)	95 (105)	110 (66)	100 (64)
Sodium, g	3,064 (1,378)	2,942 (1,741)	3,200 (2,190)	3,352 (1,281)	2,447 (1,163)	2,759 (1,421)	3,031 (1,868)	2,958 (1,564)

Values are expressed as mean (SD). Dietary intakes for the 24 h preceding the start of the experimental trial (Pre-experimental trial) and during the time from when subjects left the laboratory after the experimental trial until they returned exactly 24 h from the start of pre-exercise for follow-up data collection (an ~18 h period; Overnight). Subjects were instructed to keep all intakes for the pre-experimental trial period the same and to keep all overnight intakes the same across trials. Thus, pre-experimental trial data and overnight data were analyzed using separate one-way ANOVAs to examine statistical differences between *Control* ($n = 13$), *Water* ($n = 13$), *Cooling* ($n = 12$), and *Water + Cooling* ($n = 13$) groups.

Table 2. *Thermoregulation and hydration status*

	Control				*Water*				*Cooling*				*Water + Cooling*			
Parameter	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h
Core temperature, °C	36.9 (0.2)	38.6 (0.4)^{W,C,*}	37.3 (0.3)^{W,*}		36.9 (0.4)	37.9 (0.6)	37.0 (0.3)		36.9 (0.3)	38.0 (0.4)*	37.1 (0.3)*		36.9 (0.3)	37.6 (0.3)	36.9 (0.2)	
Heart rate, beats/min	58 (9)	102 (16)^{W,C,*}	71 (11)*	57 (11)	58 (11)	87 (13)	68 (11)	56 (7)^{C}	59 (12)	84 (13)*	67 (12)	61 (7)	60 (7)	77 (12)	63 (8)	56 (7)
Δ body weight, %	0.0 (0.0)	−2.4 (0.5)^{W,C,*}	−2.1 (0.5)^{W,C,*}	−0.7 (0.9)	0.0 (0.0)	−0.2 (0.4)^{C}	−0.4 (0.4)^{C}	−0.2 (1.5)	0.0 (0.0)	−1.5 (0.3)*	−1.1 (0.3)*	−0.1 (0.9)	0.0 (0.0)	0.1 (0.4)	−0.2 (0.5)	0.3 (0.9)
Blood samples																
Δ plasma volume, %		−6 (4)^{W,*}	−2 (4)*			1 (7)	2 (9)			−3 (4)*	−1 (5)			2 (3)	3 (3)	
Plasma osmolality, mosmol/g H_2O	281 (3)	288 (3)^{W,*}	285 (5)	283 (6)	281 (6)	281 (4)^{C}	282 (3)^{C}	286 (5)	281 (4)	287 (6)*	286 (3)	284 (5)	283 (4)	282 (3)	283 (4)	285 (5)
Serum sodium, mmol/L	139 (2)	143 (2)^{W,*}	142 (2)^{W,*}	139 (1)	139 (3)	139 (2)^{C}	139 (2)	140 (3)	139 (2)	142 (2)*	140 (2)	140 (1)	139 (2)	139 (2)	140 (2)	139 (2)
Serum potassium,mmol/L	4.4 (0.3)	4.4 (0.2)	4.5 (0.4)	4.4 (0.4)	4.3 (0.2)	4.4 (0.2)	4.4 (0.3)	4.5 (0.3)	4.4 (0.5)	4.7 (0.5)	4.4 (0.3)	4.6 (0.4)	4.2 (0.4)	4.5 (0.4)	4.2 (0.3)	4.4 (0.3)
Urine samples																
Specific gravity	1.003 (0.006)	1.008 (0.006)	1.019 (0.005)^{W,*}	1.015 (0.008)	1.004 (0.006)	1.008 (0.007)	1.011 (0.009)	1.016 (0.008)	1.004 (0.006)	1.010 (0.006)	1.018 (0.003)*	1.013 (0.009)	1.004 (0.005)	1.010 (0.007)	1.011 (0.006)	1.013 (0.008)
Osmolality, mosmol/gH_2O	221 (216)	363 (209)	732 (140)^{W,*}	642 (269)	252 (216)	384 (239)	533 (300)^{C}	705 (263)	274 (242)	495 (235)	771 (153)*	568 (300)	242 (187)	440 (209)	493 (253)	559 (309)
Sodium, mmol/L	36 (34)	43 (31)	76 (38)	67 (45)	38 (36)	28 (14)	51 (47)	74 (45)	42 (33)	56 (39)	91 (53)	62 (45)	39 (34)	45 (27)	56 (44)	78 (50)
Potassium, mmol/L	12.8 (8.8)	44.7 (23.9)	97.5 (32.5)^{W,*}	41.5 (19.2)	16.5 (12.2)	37.0 (19.8)^{C}	46.4 (21.0)	59.5 (38.2)	24.0 (22.1)	61.3 (26.8)	94.6 (36.1)*	44.1 (31.1)	16.5 (11.2)	46.2 (20.6)	45.5 (22.9)	38.4 (29.5)

Measures of thermoregulation and hydration status immediately before exercise (Pre), immediately after exercise (Post), after one-h recovery period following Post (Rec), and 24 h from the start of Pre (24 h). Data were analyzed using a mixed-effects two-way ANOVA with post hoc Tukey's test pairwise comparisons between *Control* (n = 13), *Water* (n = 13), *Cooling* (n = 12), and *Water + Cooling* (n = 13). Values expressed as mean (SD). ^{W}Different from *Water* ($P < 0.04$), ^{C}Different from *Cooling* ($P < 0.03$), *Different from *Water + Cooling* (W+C) ($P \leq 0.05$).

commenced the exercise period by walking on a treadmill at 4.8 km/h. The grade of the treadmill was set to elicit 55% of estimated heart rate maximum within 3 min during the *Control* trial. Importantly, this relative heart rate intensity is the average relative heart rate recorded over ~8 h shift in outdoor workers at risk for AKI (33). This grade was kept constant for the remainder of the *Control* trial and for the three additional experimental trials. Subjects were permitted to watch a movie or listen to music of their choice during exercise. Every 10 min throughout the entire exercise period, participants were monitored for core temperature, heart rate, perceptions of thermal comfort and thermal sensation, and ratings of perceived exertion. Subjects took a brief respite from exercise every 15 min to measure body weight. After removing the water-perfused suits and compression tops, subjects completely towel-dried their entire body to remove excess sweat before measuring body weight. During the *Water* and *Water + Cooling* trials, a volume of water equal to the amount of body-weight loss was provided, and subjects were instructed to finish drinking this water before the next 15 min measurement of body weight. The water provided was warm (35.1 ± 2.0°C) so that any additional amount of cooling received because the temperature of the water was negligible during the *Water* trial. Exercise duration was 2 h or until stopping criteria were met. The National Institute for Occupational Safety and Health (NIOSH, Centers for Disease Control and Prevention) guidelines recommend drinking sport drinks with balanced electrolytes when working in the heat for more than 2 h (22). Thus, we specifically chose a duration of 2 h to eliminate the need to provide a sports drink, which contains potentially confounding variables like sugar or electrolytes.

Upon completion of the exercise portion, participants were moved back to the pre-exercise testing room. Subjects immediately assumed a supine position, and measurements of core temperature, heart rate, blood pressure, and renal and segmental artery blood velocity were taken. Then, after 20 min of supine rest, a venous blood sample was taken (post exercise). After this, nude body weight was measured, and subjects completely voided their bladders into a urine collection container. Then, the recovery period commenced. Because the primary end points for this study were biomarkers in the urine, subjects were given 500 mL of water to consume in the *Control* and *Cooling* trials (in which water was not provided during exercise) to ensure adequate urine production after a 1-h recovery period. Subjects assumed a supine position 30 min into the recovery period. After 20 min of supine rest, measurements of core temperature, heart rate, blood pressure, and renal and segmental artery blood velocities were taken. Then, a venous blood sample was taken (recovery). Subjects then measured their nude body weight and completely voided their bladders into a urine collection container (occurring 60 min after the pervious urine void). In the *Control* and *Cooling* trials, subjects were provided with the remaining volume of water to match the amount of estimated sweat loss through changes in body weight (minus the 500 mL provided during the beginning of the recovery period). This volume of water ensured that, at minimum, subjects rehydrated completely after exercise to match their loss of body weight in the *Control* and *Cooling* trials compared with the *Water* and *Water + Cooling* trials (in which hydration matched loss of body weight during exercise). Importantly, analogous to previously mentioned data in rodents, this approach yielded findings that were due to dehydration occurring during the exercise protocol. In all trials, subjects were given a food and beverage log to complete until they returned to the laboratory the following day, exactly 24 h after the start of pre-exercise. Additionally, during this ~18 h period from when they left the laboratory and to when they returned to the laboratory the following morning, subjects collected their voided urine into a urine collection container. Subjects were instructed to perform a final urine void into the same overnight container exactly 1 h before returning to the laboratory the following day so that a measure of urine flow rate over a 1-h period could be compared with that from the pre-exercise period. Upon returning to the laboratory for the 24-h post-exercise

visit, subjects voided their bladders into a urine collection container and measured their nude body weight. Then, subjects assumed a supine position for 20 min. After 20 min of supine rest, measurements of heart rate, blood pressure, and renal and segmental artery blood velocities were taken. Finally, a venous blood sample was taken.

Data and statistical analyses. Data are presented as absolute values. AKI biomarkers are also presented as the absolute change (Δ) from baseline because AKI is clinically defined as a rapid reduction in kidney function (27). Fluid and food intakes from pretrial and overnight diaries were analyzed for fluid volume and amount of total energy, fat, protein, carbohydrate, sugar, and sodium using online software (myfitnesspal, Under Armour, Inc., Baltimore, MD). Mean skin temperature was calculated according to Ramanthan (47). Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Percentage changes in plasma volume were estimated using standard equations (9). Urine flow rates were calculated over 1-h periods for pre-exercise, recovery, and 24 h post exerciseas well as over an approximately 140-min period for post exercise. Creatinine clearance was calculated as the quotient of urine and serum creatinine concentration multiplied by urine flow rate. The fractional excretions of sodium and potassium were calculated from concentrations of sodium and potassium in the serum and urine and from the change in serum and urine creatinine concentrations. Osmolar clearance (C_{osm}) was calculated as the quotient of osmolality of the urine and plasma multiplied by urine flow rate. Free water clearance (C_{H2O}) was calculated as the difference between urine flow rate and C_{osm}. The ratio of albumin to urine creatinine (albumin:creatinine) was calculated because of the recommended advantage in quantifying albuminuria when correcting to urine creatinine across a series of urine samples for a given subject (8). The ratio of blood urea nitrogen (BUN) to serum creatinine (BUN:creatinine) was calculated as a general indicator of the cause of increased biomarkers of AKI, with ratios >20:1 suggesting prerenal origin (41). Vascular resistance in the renal and segmental arteries was calculated as mean arterial pressure divided by blood velocity. Resistive index in the renal and segmental arteries, which has clinical utility as a predictor of AKI (5), was calculated as the difference between peak systolic velocity and end diastolic velocity, divided by peak systolic velocity. Urine NGAL, IGFBP7, and TIMP-2 were normalized to urine flow rate to provide an index of biomarker excretion rate and were normalized to urine concentration (i.e., creatinine and osmolality) (3, 7, 26, 58, 63). According to the Acute Kidney Injury Network (AKIN) criteria, Stage 1 AKI was defined as an increase in serum creatinine ≥0.3 mg/dL from pre-exercise, and Stage 2 AKI was defined as an increase in serum creatinine greater than two-to threefold or urine output <0.5 $mL \cdot kg^{-1} \cdot h$ for >12 h (37). Albuminuria was defined as a spot sample of urine albumin during post-exercise, recovery, and 24 h of ≥ 20 mg/L or timed sample of urine albumin ≥20 μg/min (8).

One subject was lost to follow-up for their final experimental trial, a *Cooling* trial. Thus, *Control*, *Water*, and *Water* + *Cooling* trials are reported as $n = 13$, and *Cooling* is reported as $n = 12$. To account for the loss to follow-up, a mixed-effects model ANOVA using GraphPad Prism (version 8, GraphPad Prism, La Jolla, CA) was used to analyze the data. If the ANOVA revealed a significant F value, post hoc two-tailed Tukey's test pairwise comparisons were made. Preexperimental trial and overnight food and fluid intakes and overnight urine collection data across trials were each analyzed using a one-way ANOVA. For urine albumin, one subject was excluded from the mean data and statistical analyses during the *Control* trial at post-exercise and recovery and at 24 h post exercise because the subject was >3 standard deviations away from the mean. However, because the subject's values were in the physiological ranges, they were plotted as individual outliers in Fig. 3. An a priori power analysis was performed using an effect size calculated based on the urine NGAL data presented in our previous work (7). Using this effect size ($f = 0.27$) and the observed within-subject correlation of 0.9, 12 subjects were required to achieve a power of 0.80 with $\alpha = 0.05$ to identify a significant interaction (G × Power). Unfortunately, to our knowledge, there were no data to a priori determine the sample size required for adequate statistical power for IGFBP7 and TIMP-2. Statistical significance was set a priori at $P \leq 0.05$, and actual P values are reported where possible. Data are reported as means (SD).

RESULTS

Experimental control of core temperature and hydration during exercise. At the end of exercise, the greatest increase in core temperature from pre-exercise occurred in *Control*

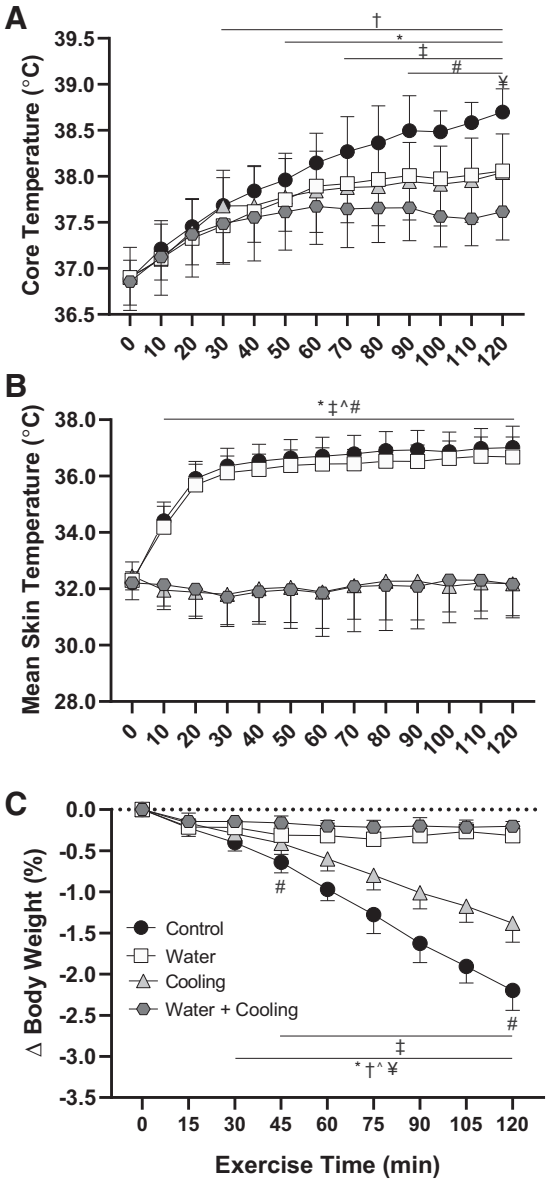

Fig. 2. Core temperature (*A*), mean skin temperature (*B*), and percent loss of body weight (*C*) during 2 h of physical work in the heat. During physical work, subjects received either no intervention (*Control*), water only to maintain hydration throughout (*Water*), continuous upper-body cooling only (*Cooling*), or both interventions (*Water* + *Cooling*). Data are presented as means (SD). Statistical analyses are from post hoc two-tailed Tukey's test pairwise comparisons following a mixed-effects model ANOVA: †*Control* is different from *Water* ($P < 0.05$), ‡*Control* is different from *Cooling* ($P < 0.03$), **Control* is different from *Water* + *Cooling* ($P < 0.04$), ^*Water* is different from *Cooling* ($P < 0.05$), #*Water* is different from *Water* + *Cooling* ($P < 0.03$), and ¥Cooling is different from *Water* + *Cooling* ($P < 0.05$).

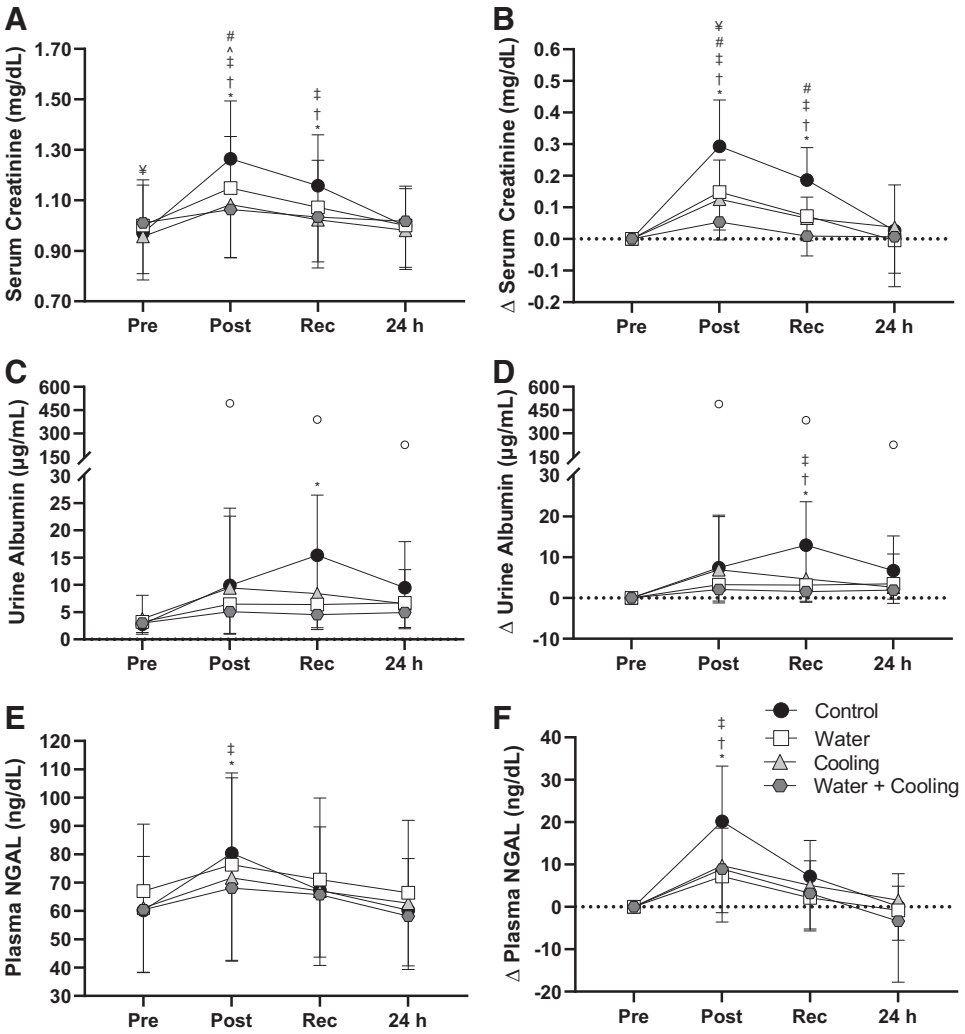

Fig. 3. Absolute values and changes (Δ) in serum creatinine (*A* and *B*), urine albumin (*C* and *D*), and plasma neutrophil gelatinase-associated lipocalin (NGAL) (*E* and *F*) before (Pre), immediately after (Post), at 1 h of recovery after Post (Rec), and at 24 h after Pre-physical work (24 h) in the heat. During, subjects received either no intervention (*Control*), water only to maintain hydration throughout (*Water*), continuous upper-body cooling only (*Cooling*), or both interventions (*Water* + *Cooling*). For urine albumin, data from the *Control* trial (Post and Rec) and *Cooling* trial (24 h) in 1 subject are plotted as singular data points (open circles). These values are in physiological ranges but were excluded from the mean data and statistical analyses given that they are outliers (>3 standard deviations from the mean) to clarify data interpretation. Statistical analyses are from post hoc two-tailed Tukey's test pairwise comparisons following a mixed-effects model ANOVA: †*Control* is different from *Water* ($P < 0.05$), ‡*Control* is different from *Cooling* ($P \leq 0.04$), **Control* is different from *Water* + *Cooling* ($P < 0.05$), ^*Water* is different from *Cooling* ($P = 0.05$), #*Water* is different from *Water* + *Cooling* ($P < 0.03$), and ¥*Cooling* is different from *Water* + *Cooling* ($P \leq 0.05$).

(1.9 ± 0.3°C; $P < 0.01$). These increases were attenuated in *Water* (1.2 ± 0.4°C), *Cooling* (1.1 ± 0.4°C), and *Water* + *Cooling* (0.8 ± 0.3°C) (Fig. 2*A*). Mean skin temperature was higher by ~3.9 ± 0.3°C throughout the exercise period in *Control* and *Water* compared with *Cooling* and *Water* + *Cooling* ($P < 0.01$; Fig. 2*B*). The 15-min rehydration periods in *Water* and *Water* + *Cooling* minimized dehydration during exercise (Fig. 2*C*). The percentage of body weight loss was greatest in *Control* and was comparatively attenuated in *Cooling* (Table 2). Furthermore, there were no differences in the percentage change in body weight post-exercise in *Water* and *Water* + *Cooling* compared with pre-exercise ($P \geq 0.87$; Table 2). Post-exercise decreases in plasma volume and increases in plasma osmolality were greatest in the *Control* trial, and these responses were attenuated in the *Cooling* trial (Table 2). Compared with pre-exercise, plasma volume and plasma osmolality were relatively maintained at post-exercise in the *Water* and *Water* + *Cooling* trials (Table 2). At the end of exercise, ratings of perceived exertion were higher in *Control* [15 ± 2 arbitrary units (au), ~"hard"; $P < 0.01$] compared with *Cooling* (11 ± 1 au, ~"light") and *Water* + *Cooling* (11 ± 2 au, ~light) but were not different compared with *Water* (13 ± 2, ~ "somewhat hard"; $P = 0.11$). Subjects reported to be hotter at the end of exercise in *Control* (6 ± 0 au, ~"hot"; $P < 0.01$) compared with *Cooling* (5 ± 1 au, ~"slightly warm") and *Water* + *Cooling* (4 ± 1 au, ~"neutral") but not *Water* (6 ± 1 au, ~"warm"; $P = 0.56$). Additionally, subjects reported to be most thermally uncomfortable in the *Control* trial (3 ± 1 au, ~"uncomfortable"; $P \leq 0.02$) compared with *Water* (3 ± 1 au, ~uncomfortable), *Cooling* (2 ± 1 au, ~"slightly uncomfortable"), and *Water* + *Cooling* (2 ± 1 au, ~slightly uncomfortable).

Kidney function and biomarkers of AKI. Urine flow rate at recovery decreased most in *Control* (−6.9 ± 4.8 mL/min; $P < 0.01$) compared with *Water* (−4.5 ± 3.5 mL/min), *Cooling* (−3.4 ± 4.2 mL/min), and *Water* + *Cooling* (−3.1 ± 4.8 mL/min) (Table 3). The largest magnitude of increase in serum creatinine occurred in *Control* post exercise (0.29 ± 0.15 mg/dL; $P < 0.04$) and at recovery (0.19 ± 0.10 mg/dL; $P < 0.03$) (Fig. 2, *A* and *B*). Urine creatinine was higher at recovery in *Control* compared with the other conditions ($P \leq 0.04$; Table 3). Creatinine clearance was lower post exercise in *Control* compared with *Cooling* ($P < 0.01$) and *Water* + *Cooling* ($P < 0.01$) but not with *Water* ($P = 0.16$; Table 3). Creatinine clearance only differed between conditions at recovery in *Cooling* and *Water* + *Cooling* ($P < 0.05$; Table 3). The fractional excretion of sodium was reduced at recovery in *Control* compared with *Cooling* ($P = 0.05$) and *Water* +

Table 3. *Indices of kidney function*

	Control				*Water*			*Cooling*			*Water + Cooling*		
Parameter	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h
Urine flow rate, mL/min	8.4 (4.6)	1.3 (0.2)	0.7 (0.2)^{W,C,*}	1.4 (0.7)	5.9 (3.2)	1.3 (0.6)	1.7 (1.1)	1.4 (1.6)	5.9 (3.4)	1.3 (0.6)	1.0 (0.2)	2.5 (2.5)	5.7 (3.5)	1.7 (1.0)	2.0 (1.2)	2.7 (3.7)
Creatinine clearance, mL/min	145 (28)	87 (31)^{C,*}	165 (39)	127 (63)	139 (29)	106 (17)*	161 (47)	135 (35)	149 (28)	122 (31)	160 (19)*	146 (36)	152 (22)	126 (22)	150 (24)	121 (30)
Albumin/creatinine ratio	11.2 (7.3)	7.3 (4.8)	5.6 (2.7)	7.3 (9.5)	10 (5.6)	5.9 (4.9)	4.6 (2.0)	4.3 (2.3)	9.6 (3.8)	6.0 (3.6)	4.3 (1.7)	6.5 (3.6)	9.9 (6.9)	4.6 (1.8)	4.8 (2.1)	5.9 (4.8)
Urine creatinine, mg/dL	44 (48)	142 (107)	280 (120)^{W,C,*}	159 (99)	52 (53)	113 (87)	157 (108)	165 (94)	61 (81)	130 (96)	180 (69)*	130 (100)	49 (43)	106 (57)	105 (61)	111 (69)
BUN, mg/dL	14 (4)*	15 (4)	15 (3)	15 (3)	16 (5)	16 (4)	16 (4)	15 (2)	16 (4)	16 (3)	16 (4)	15 (2)	16 (4)	16 (4)	16 (4)	16 (3)
BUN:creatinine, au	14 (4)^{C,*}	12 (3)^{C,*}	13 (3)^{C,*}	15 (3)	16 (5)	14 (4)	15 (4)	16 (3)	17 (5)	16 (5)	16 (5)	16 (3)	16 (4)	15 (3)	15 (3)	16 (4)
FENa, %	0.9 (0.4)	0.3 (0.2)	0.3 (0.2)^{C,*}	0.4 (0.3)	0.8 (0.3)	0.3 (0.2)	0.4 (0.5)	0.4 (0.3)	0.8 (0.4)	0.4 (0.3)	0.4 (0.3)	0.5 (0.4)	0.8 (0.4)	0.4 (0.2)	0.5 (0.3)	0.7 (0.6)
FEK, %	2.0 (1.9)	1.6 (1.2)	0.8 (0.7)	0.9 (0.9)	1.8 (1.8)	1.9 (1.2)	1.0 (0.7)	1.5 (2.9)	2.2 (2.1)	1.7 (1.3)	1.1 (1.5)	1.6 (3.2)	1.8 (2.2)	1.4 (1.0)	1.3 (1.1)	1.6 (2.7)
Osmolar clearance, mL/min	3.3 (0.8)	1.5 (1.2)	1.9 (0.6)*	3.3 (2.2)	3.4 (1.3)	1.5 (0.9)	2.5 (1.1)	2.5 (1.3)	3.6 (1.0)	2.1 (0.9)	2.4 (1.0)	2.7 (1.0)	3.3 (0.7)	2.4 (1.3)	2.7 (0.5)	2.8 (1.0)
Free water clearance, mL/min	4.6 (4.2)	−0.2 (0.9)	−1.2 (0.5)*	−1.8 (1.7)	2.5 (3.8)	−0.2 (0.8)	−0.7 (1.5)	−1.2 (1.3)	2.3 (3.6)	−0.7 (0.8)	−1.5 (0.7)	−0.1 (2.0)	2.5 (3.4)	−0.7 (0.8)	−0.7 (1.2)	−0.2 (3.1)
Serum uric acid, mg/dL	5.4 (0.7)	6.3 (0.7)	6.2 (0.7)	5.7 (0.8)	5.7 (1.0)	6.0 (1.0)	6.0 (1.0)	5.9 (0.7)	5.7 (1.1)	6.2 (1.2)	6.1 (1.2)	5.8 (1.2)	5.9 (1.6)	6.0 (1.5)	5.9 (1.7)	5.8 (1.3)
Δ serum uric acid, mg/dL		0.9 (0.5)^{W,*}	0.8 (0.5)^{W,*}	0.3 (0.8)		0.3 (0.2)*	0.4 (0.2)	0.2 (0.7)		0.4 (0.3)*	0.4 (0.3)	0.2 (0.4)		0.1 (0.2)	0.1 (0.2)	−0.2 (0.7)
Serum copeptin, pmol/L	5.9 (5.0)	5.8 (5.2)	6.3 (5.6)	5.7 (5.4)	3.8 (3.9)*	5.1 (5.9)	5.5 (4.5)	6.1 (4.7)	4.8 (4.3)	5.0 (4.6)	5.9 (4.9)	6.0 (5.4)	6.3 (4.2)	6.0 (5.1)	5.6 (3.9)	6.2 (3.8)
Δ serum copeptin, pmol/L		−0.0 (2.0)	0.4 (2.2)	−0.2 (2.3)		1.3 (2.3)	1.7 (1.6)	1.8 (2.5)		0.2 (1.1)	1.1 (1.8)	0.7 (2.9)		−0.3 (3.6)	−0.1 (3.3)	−0.1 (2.6)

Values expressed as mean (SD). Indices of kidney function immediately before exercise (Pre), immediately after exercise (Post), after 1-h recovery period following Post (Rec), and 24 h from the start of Pre (24 h). Data were analyzed using a mixed-effects two-way ANOVA with post hoc Tukey's test pairwise comparisons between *Control* (n = 13), *Water* (n = 13), *Cooling* (n = 12), and *Water + Cooling* (n = 13). au, arbitrary units; BUN, blood urea nitrogen; FEK, fractional excretion of potassium; FENa, fractional excretion of sodium. ^{W}Different from *Water* ($P < 0.05$), ^{C}Different from *Cooling* ($P \leq 0.05$), *Different from *Water + Cooling* (W+C) ($P \leq 0.05$).

Cooling ($P = 0.06$; Table 3). Increases in serum uric acid were greater in *Control* post exercise and at recovery compared with *Water* and *Water + Cooling* ($P < 0.02$; Table 3). Changes in serum copeptin were not different between trials ($P \geq 0.08$, Table 3). Blood velocity was higher and vascular resistance in the segmental artery was lower post-exercise in *Cooling* compared with *Water + Cooling* ($P \leq 0.02$), but there were no other differences between trials post exercise in blood velocity, vascular resistance, and resistive index in the renal and segmental arteries ($P \geq 0.13$; Table 4). Increases in urine albumin were greater at recovery in *Control* compared with other experimental conditions ($P \leq 0.05$; Fig. 3*A*). Much larger, but physiologically relevant, increases in urine albumin occurred in 1 subject during *Control* at post-exercise (by 488 μg/mL) and recovery (by 384 μg/mL) compared with the mean data (Fig. 3*D*). Increases in plasma NGAL were greater at post-exercise in *Control* compared with the other conditions ($P < 0.04$; Fig. 3*F*). There no differences between trials in indices of renal function and biomarkers of AKI during the overnight urine collection (Table 5). Positive findings of stage 1 AKI occurred in 9 out of 13 (69%) subjects following *Control*, in 3 out of 13 (23%) subjects following *Water*, 1 out of 12 (8%) subjects following *Cooling*, and 0 out of 13 (0%) subjects following *Water + Cooling*. Positive findings for stage 2 AKI were found in 3 out of 13 (23%) subjects for *Control*, 1 out of 13 (8%) subjects for *Water*, 1 out of 12 (8%) subjects for *Cooling*, and 1 out of 13 (8%) subjects for *Water + Cooling*. Albuminuria was found in 6 out of 13 (46%) subjects in *Control*, 3 out of 13 (23%) subjects in *Water*, 0 out of 12 (0%) subjects in *Cooling*, and 0 out of 13 (0%) subjects in *Water + Cooling*.

Increases in urine NGAL were greater at recovery in *Control* compared with *Cooling* ($P = 0.03$) and *Water + Cooling* ($P = 0.05$; Fig. 4*B*). There were greater increases in urine IGFBP7 at recovery in *Control* compared with *Water* ($P = 0.05$), *Cooling* ($P = 0.05$), and *Water + Cooling* ($P < 0.01$) (Fig. 4*D*). Changes in urine TIMP-2 were not significant between trials ($P \geq 0.11$; Fig. 4*F*). Normalization of urine NGAL to urine osmolality revealed increases in *Control* compared with *Water + Cooling* at recovery ($P = 0.05$; Fig. 5*B*), but there were no differences in urine NGAL between trials after normalizing to flow rate and creatinine (Fig. 5, *A* and *C*). Additionally, IGFBP7 in the urine revealed increases in the *Control* trial at recovery compared with all other conditions when normalizing for urine flow rate ($P \leq 0.05$; Fig. 5*D*) and urine osmolality ($P < 0.02$; Fig. 5*E*). Normalizing urine IGFBP7 to urine creatinine yielded increases at recovery in *Control* compared with *Water* ($P < 0.02$) and *Cooling* ($P < 0.03$) (Fig. 5*F*). TIMP-2 in the urine did not differ between trials when normalizing for urine flow rate, urine osmolality, or urine creatinine ($P \geq 0.08$, Fig. 5, *G–I*).

DISCUSSION

The experimental design of the present study allowed for direct comparisons of the independent and combined effects of hyperthermia and dehydration during prolonged physical work in the heat on AKI biomarker responses. The primary findings are that *1*) increased magnitudes of hyperthermia and dehydration during physical work in the heat increase the risk of AKI, *2*) alleviating hyperthermia and/or preventing dehydration

Table 4. *Doppler ultrasound assessment of renal hemodynamics*

Parameter	*Control*				*Water*				*Cooling*				*Water + Cooling*			
	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h	Pre	Post	Rec	24 h
Mean arterial pressure, mmHg	86 (6)	90 (8)	83 (6)	83 (5)	86 (5)	90 (6)	84 (5)	82 (8)	85 (6)	87 (7)	84 (6)	84 (8)	85 (7)	87 (7)	84 (6)	84 (8)
Renal artery Blood velocity, cm/s	38 (7)	40 (7)	41 (9)	37 (9)	38 (5)	38 (8)	39 (8)	37 (8)	37 (7)	40 (6)	41 (7)	37 (7)	36 (7)	37 (7)	38 (7)	34 (7)
Vascular resistance, $mmHg \cdot cm \cdot s^{-1}$	2.4 (0.5)	2.3 (0.5)	2.1 (0.5)	2.4 (0.8)	2.3 (0.3)	2.5 (0.5)	2.3 (0.5)	2.3 (0.4)	2.3 (0.5)	2.2 (0.3)	2.1 (0.3)	2.3 (0.4)	2.4 (0.5)	2.4 (0.5)	2.3 (0.5)	2.5 (0.6)
Resistive index	0.62 (0.07)	0.64 (0.06)	0.58 (0.07)	0.65 (0.08)	0.63 (0.08)	0.61 (0.09)	0.59 (0.09)	0.63 (0.07)	0.62 (0.06)	0.63 (0.08)	0.60 (0.09)	0.63 (0.08)	0.62 (0.07)	0.64 (0.08)	0.60 (0.06)	0.62 (0.08)
Segmental artery Blood velocity, cm/s	20 (4)	22 (3)	23 (4)	20 (4)	21 (4)	20 (4)	22 (4)	18 (3)	21 (3)	22 (3)*	23 (4)	21 (3)	20 (3)	19 (3)	21 (4)	20 (5)
Vascular resistance, $mmHg \cdot cm \cdot s^{-1}$	4.4 (0.5)	4.3 (0.9)	3.7 (0.5)	4.3 (0.8)	4.4 (0.8)	4.7 (0.9)	3.9 (0.6)	4.6 (0.7)	4.2 (0.4)	4.1 (0.5)*	3.8 (0.7)	4.2 (0.5)	4.6 (0.7)	4.7 (0.6)	4.3 (0.8)	4.4 (0.9)
Resistive index	0.61 (0.06)	0.63 (0.06)	0.56 (0.06)	0.61 (0.06)	0.61 (0.06)	0.60 (0.06)	0.56 (0.06)	0.63 (0.05)*	0.60 (0.05)	0.60 (0.07)	0.60 (0.05)	0.61 (0.05)	0.60 (0.07)	0.59 (0.06)	0.56 (0.06)	0.59 (0.06)

Values expressed as mean (SD). Renal hemodynamics measured via Doppler ultrasound immediately before exercise (Pre), immediately after exercise (Post), after 1-h recovery period following Post (Rec), and 24 h from the start of Pre (24 h). Data were analyzed using a mixed-effects two-way ANOVA with post hoc Tukey's test pairwise comparisons between *Control* ($n = 12$), *Water* ($n = 12$), *Cooling* ($n = 11$), and *Water + Cooling* ($n = 12$). *Different from *Water + Cooling* (W+C) ($P < 0.05$).

Table 5. *Overnight urine collection*

Parameter	Control	Water	Cooling	Water + Cooling
Urine flow rate, $mL \cdot kg \cdot h^{-1}$	1.4 (0.9)	1.4 (0.7)	1.5 (0.8)	1.6 (1.0)
Sodium, mmol/L	54 (37)	58 (36)	56 (22)	66 (36)
Potassium, mmol/L	33 (26)	34 (18)	28 (14)	27 (14)
Creatinine, mg/dL	100 (74)	104 (69)	93 (52)	100 (71)
Albumin, μg/mL	4.2 (2.6)	3.9 (2.3)	3.9 (1.8)	4.5 (3.7)
Albumin/creatinine ratio, mg/g	5.1 (2.2)	4.3 (1.2)	4.8 (1.7)	4.9 (1.8)
NGAL, ng/dL	4.9 (2.9)	5.4 (3.4)	4.7 (2.2)	6.2 (4.5)
IGFBP7, ng/mL	40.8 (27.2)	39.9 (16.6)	38.6 (23.4)	39.4 (29.6)
TIMP-2, ng/mL	0.11 (0.09)	0.10 (0.07)	0.09 (0.09)	0.11 (0.10)

Values expressed as mean (SD). Measures of kidney function and biomarkers of acute kidney injury during an overnight urine collection that occurred approximately over an ~18 h period from the end of recovery from exercise (i.e., 80 min after the end of exercise) until 24 h from the start of pre-exercise. Data were analyzed using a one-way ANOVA to examine statistical differences between *Control* ($n = 13$), *Water* ($n = 13$), *Cooling* ($n = 12$), and *Water + Cooling* ($n = 13$). IGFBP7, insulin-like growth factor-binding protein 7; NGAL, neutrophil gelatinase-associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinase 2.

equally reduces the risk of AKI, and *3*) the proximal tubules are the likely site of renal injury provoked by hyperthermia and dehydration caused by physical work in the heat.

The magnitude of hyperthermia and dehydration influences the risk of AKI. A majority of previous work investigating the link between heat stress and AKI has focused on either dehydration and/or the type of beverage used for rehydration (7, 16, 17, 48, 51) or the duration of physical work (57) as the primary modulators of increased AKI risk during heat stress. Sato et al. (54) were the first to report hyperthermia as a modulator for the extent of kidney damage during heat exposure. Our findings indicate that hyperthermia and dehydration both contribute the risk of AKI following physical work in the heat. In the present study, the risk of AKI was highest in the *Control* trial, which elicited the greatest magnitudes of hyperthermia and dehydration. There were greater increases in AKI biomarkers at post-exercise (plasma NGAL; Fig. 3) and recovery (albumin, NGAL, and IGFBP7 in the urine; Figs. 3 and 4) in the *Control* trial that were accompanied by reductions in urine flow rate and creatinine clearance (Table 3). To our knowledge, we are the first to report the responses of the cell cycle arrest markers IGFBP7 and TIMP-2 in the context of physical work in the heat. Notably, our most consistent findings were the increases in the urine biomarker IGFBP7, which remained consistently prevalent even after correcting for urine production (flow rate) and concentration (osmolality and creatinine) (Fig. 5). These findings indicate that, independent of changes in urine output, IGFBP7 is more highly secreted in response to greater magnitudes of hyperthermia and dehydration during physical work in the heat.

The albuminuria in the *Control* trial coincided with greater increases in urine NGAL and IGFBP7, but differences in TIMP-2 were not observed across trials. Concomitant increases in urine NGAL and albumin supports the renal tubules as the location of injury (59, 64). However, the specific finding that IGFBP7 was increased but that there were no differences in TIMP-2 likely isolates the renal proximal tubules as the site of injury with greater magnitudes of hyperthermia and dehydra-

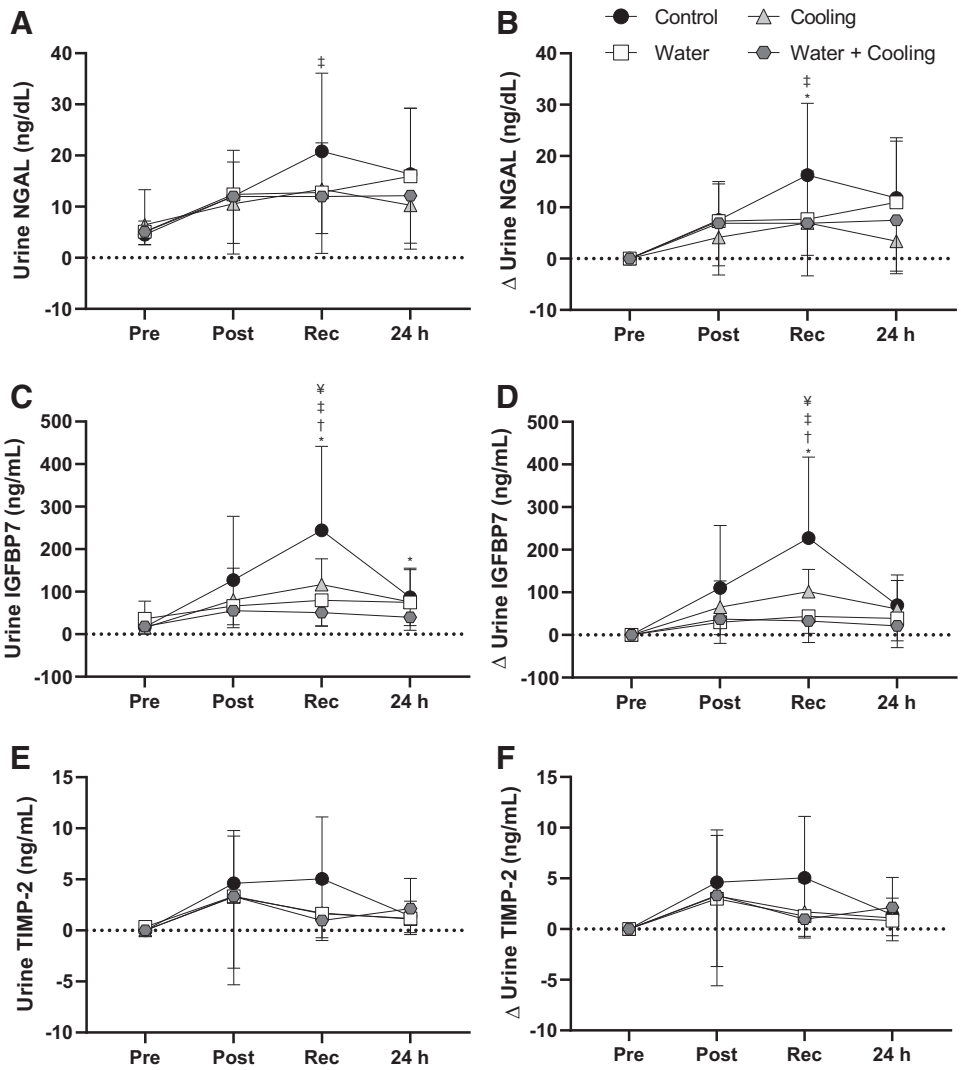

Fig. 4. Absolute values and changes (Δ) in urine neutrophil gelatinase-associated lipocalin (NGAL) (*A* and *B*), urine insulin-like growth factor-binding protein 7 (IGFBP7) (*C* and *D*), and urine tissue inhibitor of metalloproteinase 2 (TIMP-2) (*E* and *F*) before (Pre), immediately after (Post), at 1 h of recovery after Post (Rec), and 24 h after Pre-exercise (24 h) in the heat. During exercise, subjects received either no intervention (*Control*), water only to maintain hydration throughout (*Water*), continuous upper-body cooling only (*Cooling*), or both interventions (*Water* + *Cooling*). Statistical analyses are from post hoc two-tailed Tukey's test pairwise comparisons following a mixed-effects model ANOVA: †*Control* is different from *Water* ($P \leq 0.05$), ‡*Control* is different from *Cooling* ($P \leq 0.05$), **Control* is different from *Water* + *Cooling* ($P \leq 0.05$), and ¥*Cooling* different from *Water* + *Cooling* ($P < 0.01$).

tion during physical work in the heat. Notably, IGFBP7 is expressed in both proximal and distal tubular epithelial cells but is preferentially secreted in proximal tubule cells (11). This is in contrast to TIMP-2, which is expressed and secreted to a larger degree in distal tubule cells (11). The findings from our biomarker panel indicate that the injury likely occurs in the renal proximal tubules, which aligns with many of the proposed mechanisms of kidney injury in rodent models (see *Potential mechanisms of increased risk of renal proximal tubular injury*).

Despite the fact that clinical AKI and proteinuria have been previously associated with physical work in the heat (60), to our knowledge, the present study is the first to report of the effect of hyperthermia and/or dehydration on urine albumin when exercise duration and intensity are controlled. Post-exercise proteinuria is a well-documented phenomenon that is more dependent on the intensity of exercise compared with exercise duration (43, 44). Poortmans and Vanderstraeten (45) have suggested that post-exercise proteinuria occurs because of an increased glomerular permeability and saturation of the renal tubular reabsorption processes resulting in greater albumin excretion in the urine. It is likely that this albuminuria in the *Control* trial is due to tubular causes given that the tubular etiology of the observed increases in urine NGAL and IGFBP7 and that albuminuria is also a function of reductions in tubular albumin reabsorption and/or tubular albumin synthesis (64).

Potential mechanisms of increased risk of renal proximal tubular injury. The mechanisms for the comparatively greater increases in biomarkers of AKI in the *Control* trial are likely multifactorial. Exercise (28), hyperthermia (46), and dehydration (40) independently reduce renal blood flow, with greater reductions occurring when these conditions are combined (61). Additionally, data in animals reveal that hyperthermia (38) and dehydration (21) elicit profound reductions in blood flow in the renal cortex, the predominant location for sodium reabsorption in the proximal tubules. Sodium reabsorption is increased with greater magnitudes of dehydration during prolonged work in the heat (42) and, in addition to the reduced oxygen delivery to the renal cortex, may contribute to greater renal ATP depletion. This conclusion is supported by data from Sato et al. (54), which indicate that hyperthermia independently reduces renal ATP. Renal ATP depletion is believed to be one of the potential mechanisms by which physical work in the heat increases risk for AKI (58). In the present study, we were unable to directly measure renal ATP or renal blood flow during the exercise protocol. However, our data indicate that

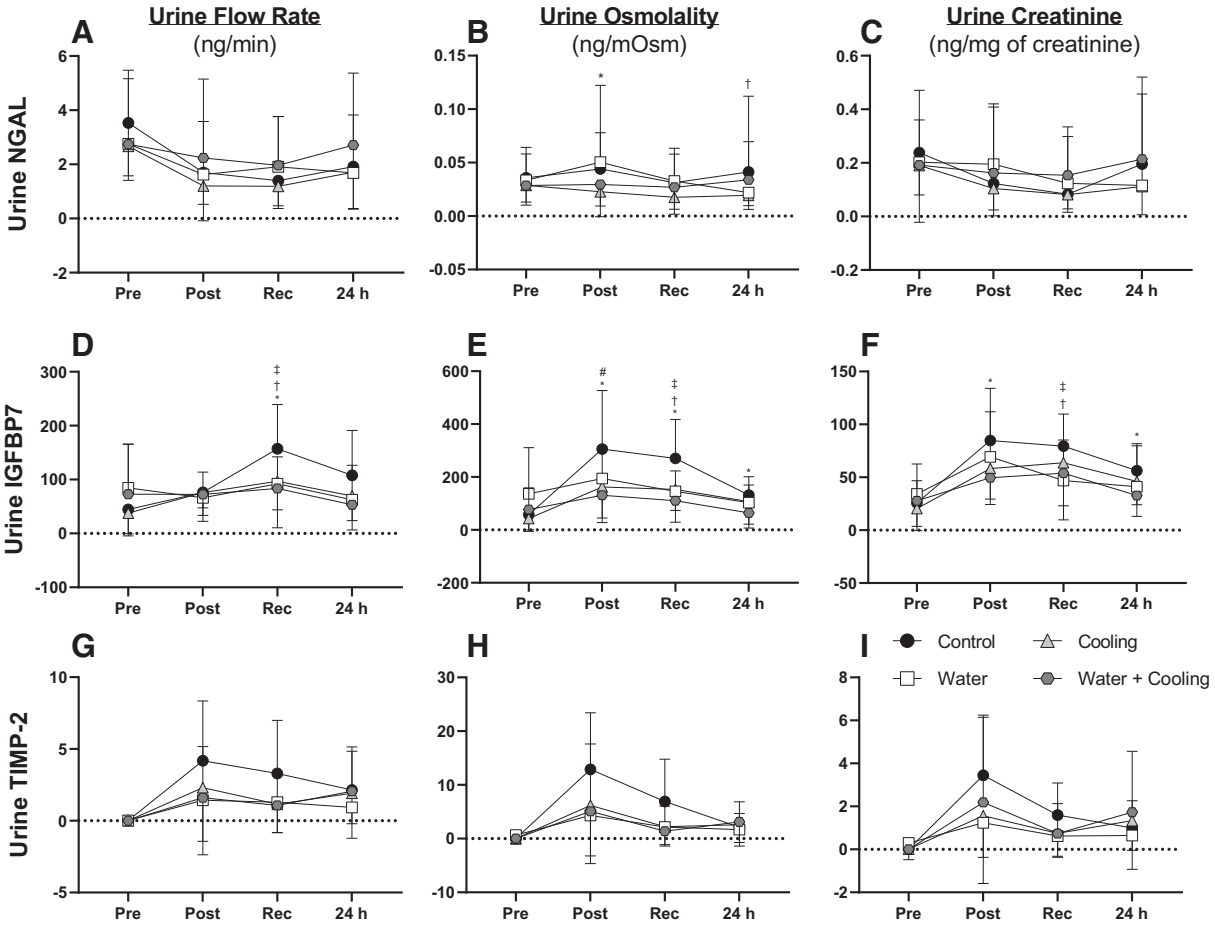

Fig. 5. Normalization of urine biomarkers of acute kidney injury. Neutrophil gelatinase-associated lipocalin (NGAL) (*A–C*), insulin-like growth factor-binding protein 7 (IGFBP7) (*D–F*), and tissue inhibitor of metalloproteinase 2 (TIMP-2) (*G–I*) are normalized to urine flow rate, urine osmolality, and urine creatinine before (Pre), immediately after (Post), at 1 h of recovery after Post (Rec), and at 24 h after Pre-exercise (24 h) in the heat. During exercise, subjects received either no intervention (*Control*), water only to maintain hydration throughout (*Water*), continuous upper-body cooling only (*Cooling*), or both interventions (*Water* + *Cooling*). Statistical analyses are from post hoc two-tailed Tukey's test pairwise comparisons following a mixed-effects model ANOVA: †*Control* is different from *Water* ($P \leq 0.05$), ‡*Control* is different from *Cooling* ($P < 0.03$), **Control* is different from *Water* + *Cooling* ($P \leq 0.05$), and #*Water* is different from *Water* + *Cooling* ($P < 0.04$).

the increased risk of AKI with greater hyperthermia and dehydration were not related to differential changes in renal or segmental artery vascular resistance in the post-exercise and recovery periods (Table 4). Unfortunately, it is not feasible to utilize the Doppler ultrasound technique for renal and segmental artery blood velocity during treadmill walking. Thus, we are unable to determine if differences in renal and/or segmental artery vascular resistance during the exercise protocol may have contributed to the AKI biomarker responses. However, our data support the idea that an increased sodium reabsorption occurred during the Control trial, which may have contributed to greater reductions in renal ATP (Table 3).

Renal cortical vasoconstriction (53) and reductions in intracellular ATP in renal proximal tubular cells (69) are also independently induced by uric acid. In the present study, the greatest increase in serum uric acid occurred with the largest combination of hyperthermia and dehydration, and these findings were sustained into the recovery period (Table 2). The potential for uric acid as a mechanism by which AKI risk is increased with greater magnitudes of hyperthermia and dehydration is supported by data in rodent models that report protective effects against heat stress-induced renal injury with treatment from the uric acid-lowering drug allopurinol (50). Recent findings from Xiao et al. (69) indicate that hyperuricemia induces proximal tubular injury through impairments of sodium potassium ATPase in the proximal tubule. Uric acid is also released via the polyol-fructokinase pathway as a byproduct of fructose metabolism from fructokinase in the renal proximal tubules, which can occur secondary to endogenous fructose production in the kidneys in response to hyperosmolality (34). In the present study, AKI risk was greatest in the *Control* trial, which elicited a hypovolemic, hyperosmotic dehydration (Table 2) and potentially may have increased polyol-fructokinase activation. In a rodent model, attenuating dehydration during heat stress reduces the extent of both polyol-fructokinase pathway activation and kidney injury (51). Additionally, activation of the polyol-fructokinase pathway is energetically costly and depletes renal ATP (31). Interestingly, uric acid activates aldose reductase and stimulates endogenous fructose production in hepatocytes (52), but to our knowledge, there has not been direct investigation of a

hyperuricemia induced activation of the polyol-fructokinase pathway in renal proximal tubular cells.

Notably, the polyol-fructokinase pathway is also partially mediated by vasopressin release (49, 62) and has been implicated in renal injury resulting from greater magnitudes of dehydration during heat stress in rodent models (51). To our knowledge, it is not currently possible to quantify fructokinase activation in human renal tubules. Thus, our understanding of the potential role of the polyol-fructokinase pathway as a mechanism by which AKI risk is increased following physical work in the heat is incomplete and speculative. However, data in rodent models indicate that vasopressin mediates fructokinase activity in the context of heat stress-induced dehydration (49), which is partially supported by previous findings from our laboratory that increases in serum copeptin are greater when consuming a high-fructose soft drink during and following physical work in the heat (7). In contrast, the present study does not support vasopressin as a mechanism for the increased risk of AKI with hyperthermia and dehydration from physical work in the heat because there were no observed differences in serum copeptin across trials (Table 3). Thus, we speculate that vasopressin is more likely to increase the risk of AKI when a large volume of fructose is ingested during physical work in the heat. In support of this, increases in vasopressin have been reported independent of osmolality with fructose exposure in humans (68), and increases in plasma copeptin are not modified with antioxidant supplementation during and/or after fructose consumption in a recurrent heat-stress rodent model (17).

Considerations. There are a few considerations that warrant discussion. First, the extent to which absolute AKI risk is increased with the observed increases in AKI biomarkers in the context of physical work in the heat is not known. Thus, interpretation of these biomarkers must be generalized as relative to the other experimental trials in our study. Increases in these AKI biomarkers (IGFBP7 and NGAL) together with albuminuria in the *Control* trial likely represent a greater risk of pathology with combined hyperthermia and dehydration during physical work in the heat. Notably, a larger AKI biomarker response to a given trial is probably indicative of a comparatively greater pathological response, but this situation may not reflect true (clinical) AKI that is characterized by kidney damage. The magnitude by which these AKI biomarkers must increase to be classified as clinical AKI is not currently known in the context of physical work in the heat. We believe it is unlikely that true AKI occurred, despite the fact that more subjects met the clinically defined criteria for Stage 1 and Stage 2 AKI in this trial, because these AKI biomarkers recovered within ~18 h following exercise in the heat. Second, the findings presented herein are constrained to the conditions employed. For instance, because of the relatively short duration of physical work (2 h), it is possible that our findings are underestimating the response of these biomarkers in occupations with sustained hyperthermia and dehydration caused by longer work shift durations (e.g., agricultural workers). Third, the work intensity employed in the present study was kept constant to reflect the average intensity across a work shift of agricultural workers at risk of AKI (33). Further investigation is warranted in models simulating manual labor over a longer work duration, in which intensity fluctuates between moderate and high, with planned rest periods. Fourth, it is not clear if the reductions in urine flow rate and creatinine clearance in the *Control* trial in the present study are indicative of reduced kidney function occurring secondary to injury or simply represent the normal physiological response to hyperthermia and dehydration. For instance, it may be that renal blood flow was reduced during the exercise portion of the noncooling trials, despite no differences between trials at post-exercise, which resulted in a reduced creatinine clearance in the *Control* and *Water* trials. Fifth, although the data obtained from pre-exercise urine samples were consistent across the four trials with a 1-h timed sample, a better baseline comparison could be made with a 24-h baseline urine collection. We recommend future studies incorporate a 24-h baseline urine collection. Sixth, it is not known if cardiorespiratory fitness is protective against the risk of AKI following physical work in the heat. Subjects in the present study self-reported to be recreationally active, and exercise intensity was performed at a relative intensity rather than absolute intensity to account for potential differences in cardiorespiratory fitness. Future work should consider the impact of cardiorespiratory fitness or physical activity levels on biomarkers of AKI following physical work in the heat. Seventh, in the present study, women were tested within the first 10 days of their menstrual cycle. The extent to which changes in the hormonal profile across the menstrual cycle, which influences thermoregulation and the regulation of volume status (19), impacts biomarkers of AKI following physical work in the heat remains unknown. Lastly, the extent to which the positive findings of serum creatinine-based Stage 1 and Stage 2 AKI and albuminuria are indicative of a true AKI versus an increased risk of AKI (or kidney strain) in the context of physical work in the heat are not known.

Perspectives. There is increasing evidence of chronic kidney disease of unknown etiology (CKDu) and/or AKI among individuals who frequently perform outdoor, physical work in the heat (e.g., agricultural workers) (18, 25, 32). One of the primary hypotheses for the etiology of CKDu is that repetitive insults of transient (i.e., lasting $<$3 days) or subclinical AKI caused by physical work in the heat can progress into nephropathy, a risk that could increase because of climate change (20, 24). Indeed, a meta-analysis by Flouris et al. (13) found an ~15% incidence of kidney disease or AKI in individuals whose occupation requires them to work in the heat for a minimum of 2 mo/yr for 6 h/day and 5 days/wk. Thus, understanding the mechanisms underlying the increased risk of AKI from physical work in the heat may mitigate the risk of CKDu in these populations (58). Notably, the findings in the present study are supported by renal biopsies obtained from patients with CKDu, which reveal mild-to-moderate chronic tubulointerstitial damage with additional signs of tubular damage present in the urine (65). Based on the data from the present study, countermeasures designed to reduce AKI risk should attenuate the magnitude of hyperthermia and/or dehydration. Ice cooling vests have been shown to attenuate increases in core temperature during exercise in the heat (29) and thus, have the potential to reduce the risk of AKI. Additionally, it is not known if ad libitum water intake during physical work in the heat, which attenuates the magnitude of dehydration but does not prevent dehydration (42), is sufficient to attenuate the risk of AKI. The efficacy of cooling devices and rehydration protocols in protecting against AKI warrants further investigation. Additionally, together with our previous work (7), our findings highlight the importance of the timing of when biological samples are

collected. In the present study, blood-based biomarkers were increased closer to the cessation of the exercise protocol compared with the urine-based biomarkers. This may have important ramifications given the continued need to measure AKI biomarkers in both experimental and field settings. Moreover, the kinetic response of each biomarker may be different. For example, the urine biomarkers IGFBP7 and TIMP-2 appear to increase before injury occurs, whereas urine NGAL may only increase in response to an injury (70). Thus, further investigation into the kinetic profile of the biomarkers of AKI in the context of physical work in the heat is warranted, so that detection of the risk of AKI is not missed due to mistiming the collection of biological samples.

Conclusions. The present study provides evidence in humans that greater magnitudes of hyperthermia and dehydration increase the risk for AKI following physical work in the heat. Additionally, this study is the first to report the response of urine biomarkers IGFBP7 (which increased to a greater extent in Control) and TIMP-2 (no differences between trials) following physical work in the heat. The biomarker panel employed in this study indicates that hyperthermia and dehydration codependently contribute to the increased risk of AKI, and that the location of this injury is likely in the renal proximal tubules.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

C.L.C., B.D.J., and Z.J.S. conceived and designed research; C.L.C., N.T.V., and Z.J.S. performed experiments; C.L.C. and Z.J.S. analyzed data; C.L.C., B.D.J., N.T.V., D.H., M.D.P., and Z.J.S. interpreted results of experiments; C.L.C. prepared figures; C.L.C. and Z.J.S. drafted manuscript; C.L.C., B.D.J., N.T.V., D.H., M.D.P., and Z.J.S. edited and revised manuscript; C.L.C., B.D.J., N.T.V., D.H., M.D.P., and Z.J.S. approved final version of manuscript.

REFERENCES

1. **Balanescu S, Kopp P, Gaskill MB, Morgenthaler NG, Schindler C, Rutishauser J.** Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar states. *J Clin Endocrinol Metab* 96: 1046–1052, 2011. doi:10.1210/jc.2010-2499.
2. **Birn H, Christensen EI.** Renal albumin absorption in physiology and pathology. *Kidney Int* 69: 440–449, 2006. doi:10.1038/sj.ki.5000141.
3. **Bongers CCWG, Alsady M, Nijenhuis T, Hartman YAW, Eijsvogels TMH, Deen PMT, Hopman MTE.** Impact of acute versus repetitive moderate intensity endurance exercise on kidney injury markers. *Physiol Rep* 5: e13544, 2017. doi:10.14814/phy2.13544.
4. **Borg G.** Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2: 92–98, 1970.
5. **Bossard G, Bourgoin P, Corbeau JJ, Huntzinger J, Beydon L.** Early detection of postoperative acute kidney injury by Doppler renal resistive index in cardiac surgery with cardiopulmonary bypass. *Br J Anaesth* 107: 891–898, 2011. doi:10.1093/bja/aer289.
6. **Chapman CL, Benati JM, Johnson BD, Vargas NT, Lema PC, Schlader ZJ.** Renal and segmental artery hemodynamics during whole body passive heating and cooling recovery. *J Appl Physiol (1985)* 127: 974–983, 2019. doi:10.1152/japplphysiol.00403.2019.
7. **Chapman CL, Johnson BD, Sackett JR, Parker MD, 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. doi:10.1152/ajpregu.00351.2018.
8. **de Jong PE, Curhan GC.** Screening, monitoring, and treatment of albuminuria: Public health perspectives. *J Am Soc Nephrol* 17: 2120–2126, 2006. doi:10.1681/ASN.2006010097.
9. **Dill DB, Costill DL.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247–248, 1974. doi:10.1152/jappl.1974.37.2.247.
10. **Drew RC, Muller MD, Blaha CA, Mast JL, Heffernan MJ, Estep LE, Cui J, Reed AB, Sinoway LI.** Renal vasoconstriction is augmented during exercise in patients with peripheral arterial disease. *Physiol Rep* 1: e00154, 2013. doi:10.1002/phy2.154.
11. **Emlet DR, Pastor-Soler N, Marciszyn A, Wen X, Gomez H, Humphries WH IV, Morrisroe S, Volpe JK, Kellum JA.** Insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2: differential expression and secretion in human kidney tubule cells. *Am J Physiol Renal Physiol* 312: F284–F296, 2017. doi:10.1152/ajprenal.00271.2016.
12. **Endre ZH, Pickering JW.** Acute kidney injury: cell cycle arrest biomarkers win race for AKI diagnosis. *Nat Rev Nephrol* 10: 683–685, 2014. doi:10.1038/nrneph.2014.198.
13. **Flouris AD, Dinas PC, Ioannou LG, Nybo L, Havenith G, Kenny GP, Kjellstrom T.** Workers' health and productivity under occupational heat strain: a systematic review and meta-analysis. *Lancet Planet Health* 2: e521–e531, 2018. doi:10.1016/S2542-5196(18)30237-7.
14. **Gagge AP, Stolwijk JA, Hardy JD.** Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res* 1: 1–20, 1967. doi:10.1016/0013-9351(67)90002-3.
15. **Gagge AP, Stolwijk JA, Saltin B.** Comfort and thermal sensations and associated physiological responses during exercise at various ambient temperatures. *Environ Res* 2: 209–229, 1969. doi:10.1016/0013-9351(69)90037-1.
16. **García-Arroyo FE, Cristóbal M, Arellano-Buendía AS, Osorio H, Tapia E, Soto V, Madero M, Lanaspa MA, Roncal-Jiménez C, Bankir L, Johnson RJ, Sánchez-Lozada LG.** Rehydration with soft drink-like beverages exacerbates dehydration and worsens dehydration-associated renal injury. *Am J Physiol Regul Integr Comp Physiol* 311: R57–R65, 2016. doi:10.1152/ajpregu.00354.2015.
17. **García-Arroyo FE, Gonzaga G, Muñoz-Jiménez I, Osorio-Alonso H, Iroz A, Vecchio M, Tapia E, Roncal-Jiménez CA, Johnson RJ, Sánchez-Lozada LG.** Antioxidant supplements as a novel mean for blocking recurrent heat stress-induced kidney damage following rehydration with fructose-containing beverages. *Free Radic Biol Med* 141: 182–191, 2019. doi:10.1016/j.freeradbiomed.2019.06.016.
18. **García-Trabanino R, Jarquín E, Wesseling C, Johnson RJ, González-Quiroz M, Weiss I, Glaser J, José Vindell J, Stockfelt L, Roncal C, Harra T, Barregard L.** Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador–A cross-shift study of workers at risk of Mesoamerican nephropathy. *Environ Res* 142: 746–755, 2015. doi:10.1016/j.envres.2015.07.007.
19. **Giersch GEW, Charkoudian N, Stearns RL, Casa DJ.** Fluid balance and hydration considerations for women: review and future directions. *Sports Med* 50: 253–261, 2020. doi:10.1007/s40279-019-01206-6.
20. **Glaser J, Lemery J, Rajagopalan B, Diaz HF, García-Trabanino R, Taduri G, Madero M, Amarasinghe M, Abraham G, Anutrakulchai S, Jha V, Stenvinkel P, Roncal-Jimenez C, Lanaspa MA, Correa-Rotter R, Sheikh-Hamad D, Burdmann EA, Andres-Hernando A, Milagres T, Weiss I, Kanbay M, Wesseling C, Sánchez-Lozada LG, Johnson RJ.** Climate change and the emergent epidemic of CKD from heat stress in rural communities: the case for heat stress nephropathy. *Clin J Am Soc Nephrol* 11: 1472–1483, 2016. doi:10.2215/CJN.13841215.
21. **Hope A, Tyssebotn I.** The effect of water deprivation on local renal blood flow and filtration in the laboratory rat. *Circ Shock* 11: 175–186, 1983.
22. **Jacklitsch B, Williams W, Musolin K, Coca A, Kim J, Turner N.** *NIOSH Criteria for a Recommended Standard: Occupational Exposure to Heat and Hot Environments*. Washington, DC: DHHS (NIOSH). Publication No. 2016-106, 2016.
23. **Johnson ACM, Zager RA.** Mechanisms underlying increased TIMP2 and IGFBP7 urinary excretion in experimental AKI. *J Am Soc Nephrol* 29: 2157–2167, 2018. doi:10.1681/ASN.2018030265.

24. **Johnson RJ, Sánchez-Lozada LG, Newman LS, Lanaspa MA, Diaz HF, Lemery J, Rodriguez-Iturbe B, Tolan DR, Butler-Dawson J, Sato Y, Garcia G, Hernando AA, Roncal-Jimenez CA.** Climate change and the kidney. *Ann Nutr Metab* 74, *Suppl* 3: 38–44, 2019. doi:10.1159/000500344.
25. **Johnson RJ, Wesseling C, Newman LS.** Chronic kidney disease of unknown cause in agricultural communities. *N Engl J Med* 380: 1843–1852, 2019. doi:10.1056/NEJMra1813869.
26. **Junglee NA, Di Felice U, Dolci A, Fortes MB, Jibani MM, Lemmey AB, Walsh NP, Macdonald JH.** Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function. *Am J Physiol Renal Physiol* 305: F813–F820, 2013. doi:10.1152/ajprenal.00091.2013.
27. **Kellum JA, Lameire N, Aspelin P, Barsoum RS, Burdmann EA, Goldstein SL, Herzog CA, Joannidis M, Kribben A, Levey AS.** Kidney disease: improving global outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl* 2: 1–138, 2012.
28. **Kenney WL, Zappe DH.** Effect of age on renal blood flow during exercise. *Aging (Milano)* 6: 293–302, 1994. doi:10.1007/BF03324255.
29. **Kenny GP, Schissler AR, Stapleton J, Piamonte M, Binder K, Lynn A, Lan CQ, Hardcastle SG.** Ice cooling vest on tolerance for exercise under uncompensable heat stress. *J Occup Environ Hyg* 8: 484–491, 2011. doi:10.1080/15459624.2011.596043.
30. **Koyner JL, Parikh CR.** Clinical utility of biomarkers of AKI in cardiac surgery and critical illness. *Clin J Am Soc Nephrol* 8: 1034–1042, 2013. doi:10.2215/CJN.05150512.
31. **Lanaspa MA, Ishimoto T, Cicerchi C, Tamura Y, Roncal-Jimenez CA, Chen W, Tanabe K, Andres-Hernando A, Orlicky DJ, Finol E, Inaba S, Li N, Rivard CJ, Kosugi T, Sanchez-Lozada LG, Petrash JM, Sautin YY, Ejaz AA, Kitagawa W, Garcia GE, Bonthron DT, Asipu A, Diggle CP, Rodriguez-Iturbe B, Nakagawa T, Johnson RJ.** Endogenous fructose production and fructokinase activation mediate renal injury in diabetic nephropathy. *J Am Soc Nephrol* 25: 2526–2538, 2014. doi:10.1681/ASN.2013080901.
32. **Laws RL, Brooks DR, Amador JJ, Weiner DE, Kaufman JS, Ramírez-Rubio O, Riefkohl A, Scammell MK, López-Pilarte D, Sánchez JM, Parikh CR, McClean MD.** Biomarkers of kidney injury among nicaraguan sugarcane workers. *Am J Kidney Dis* 67: 209–217, 2016. doi:10.1053/j.ajkd.2015.08.022.
33. **Lucas RA, Bodin T, García-Trabanino R, Wesseling C, Glaser J, Weiss I, Jarquin E, Jakobsson K, Wegman DH.** Heat stress and workload associated with sugarcane cutting-an excessively strenuous occupation! *Extrem Physiol Med* 4, *S1*: A23, 2015. doi:10.1186/2046-7648-4-S1-A23.
34. **Madero M, García-Arroyo FE, Sánchez-Lozada LG.** Pathophysiologic insight into MesoAmerican nephropathy. *Curr Opin Nephrol Hypertens* 26: 296–302, 2017. doi:10.1097/MNH.0000000000000331.
35. **Marraccini P, Fedele S, Marzilli M, Orsini E, Dukic G, Serasini L, L'Abbate A.** Adenosine-induced renal vasoconstriction in man. *Cardiovasc Res* 32: 949–953, 1996. doi:10.1016/S0008-6363(96)00128-9.
36. **McDermott BP, Smith CR, Butts CL, Caldwell AR, Lee EC, Vingren JL, Munoz CX, Kunces LJ, Williamson K, Ganio MS, Armstrong LE.** Renal stress and kidney injury biomarkers in response to endurance cycling in the heat with and without ibuprofen. *J Sci Med Sport* 21: 1180–1184, 2018. doi:10.1016/j.jsams.2018.05.003.
37. **Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A; Acute Kidney Injury Network.** Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 11: R31, 2007. doi:10.1186/cc5713.
38. **Miyamoto M.** Renal cortical and medullary tissue blood flow during experimental hyperthermia in dogs. *Thermal Medicine* 10: 78–89, 1994. doi:10.3191/thermalmedicine.10.78.
39. **Momen A, Leuenberger UA, Ray CA, Cha S, Handly B, Sinoway LI.** Renal vascular responses to static handgrip: role of muscle mechanoreflex. *Am J Physiol Heart Circ Physiol* 285: H1247–H1253, 2003. doi:10.1152/ajpheart.00214.2003.
40. **Nadal JW, Pedersen S, Maddock WG.** A comparison between dehydration from salt loss and from water deprivation. *J Clin Invest* 20: 691–703, 1941. doi:10.1172/JCI101262.
41. **Needham E.** Management of acute renal failure. *Am Fam Physician* 72: 1739–1746, 2005.
42. **Otani H, Kaya M, Tsujita J.** Effect of the volume of fluid ingested on urine concentrating ability during prolonged heavy exercise in a hot environment. *J Sports Sci Med* 12: 197–204, 2013.
43. **Poortmans JR, Labilloy D.** The influence of work intensity on postexercise proteinuria. *Eur J Appl Physiol Occup Physiol* 57: 260–263, 1988. doi:10.1007/BF00640673.
44. **Poortmans JR, Mathieu N, De Plaen P.** Influence of running different distances on renal glomerular and tubular impairment in humans. *Eur J Appl Physiol Occup Physiol* 72: 522–527, 1996. doi:10.1007/BF00242285.
45. **Poortmans JR, Vanderstraeten J.** Kidney function during exercise in healthy and diseased humans. An update. *Sports Med* 18: 419–437, 1994. doi:10.2165/00007256-199418060-00006.
46. **Radigan LR, Robinson S.** Effects of environmental heat stress and exercise on renal blood flow and filtration rate. *J Appl Physiol* 2: 185–191, 1949. doi:10.1152/jappl.1949.2.4.185.
47. **Ramanathan NL.** A new weighting system for mean surface temperature of the human body. *J Appl Physiol* 19: 531–533, 1964. doi:10.1152/jappl.1964.19.3.531.
48. **Roncal-Jimenez C, Lanaspa MA, Jensen T, Sanchez-Lozada LG, Johnson RJ.** Mechanisms by which dehydration may lead to chronic kidney disease. *Ann Nutr Metab* 66, *Suppl* 3: 10–13, 2015. doi:10.1159/000381239.
49. **Roncal-Jimenez CA, Milagres T, Andres-Hernando A, Kuwabara M, Jensen T, Song Z, Bjornstad P, Garcia GE, Sato Y, Sanchez-Lozada LG, Lanaspa MA, Johnson RJ.** Effects of exogenous desmopressin on a model of heat stress nephropathy in mice. *Am J Physiol Renal Physiol* 312: F418–F426, 2017. doi:10.1152/ajprenal.00495.2016.
50. **Roncal-Jimenez CA, Sato Y, Milagres T, Andres Hernando A, García G, Bjornstad P, Dawson JB, Sorensen C, Newman L, Krisher L, Madero M, Glaser J, Gárcía-Trabanino R, Romero EJ, Song Z, Jensen T, Kuwabara M, Rodriguez-Iturbe B, Sanchez-Lozada LG, Lanaspa MA, Johnson RJ.** Experimental heat stress nephropathy and liver injury are improved by allopurinol. *Am J Physiol Renal Physiol* 315: F726–F733, 2018. doi:10.1152/ajprenal.00543.2017.
51. **Roncal Jimenez CA, Ishimoto T, Lanaspa MA, Rivard CJ, Nakagawa T, Ejaz AA, Cicerchi C, Inaba S, Le M, Miyazaki M, Glaser J, Correa-Rotter R, González MA, Aragón A, Wesseling C, Sánchez-Lozada LG, Johnson RJ.** Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int* 86: 294–302, 2014. doi:10.1038/ki.2013.492.
52. **Sanchez-Lozada LG, Andres-Hernando A, Garcia-Arroyo FE, Cicerchi C, Li N, Kuwabara M, Roncal-Jimenez CA, Johnson RJ, Lanaspa MA.** Uric acid activates aldose reductase and the polyol pathway for endogenous fructose and fat production causing development of fatty liver in rats. *J Biol Chem* 294: 4272–4281, 2019. doi:10.1074/jbc.RA118.006158.
53. **Sánchez-Lozada LG, Soto V, Tapia E, Avila-Casado C, Sautin YY, Nakagawa T, Franco M, Rodríguez-Iturbe B, Johnson RJ.** Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia. *Am J Physiol Renal Physiol* 295: F1134–F1141, 2008. doi:10.1152/ajprenal.00104.2008.
54. **Sato Y, Roncal-Jimenez CA, Andres-Hernando A, Jensen T, Tolan DR, Sanchez-Lozada LG, Newman LS, Butler-Dawson J, Sorensen C, Glaser J, Miyazaki M, Diaz HF, Ishimoto T, Kosugi T, Maruyama S, Garcia GE, Lanaspa MA, Johnson RJ.** Increase of core temperature affected the progression of kidney injury by repeated heat stress exposure. *Am J Physiol Renal Physiol* 317: F1111–F1121, 2019. doi:10.1152/ajprenal.00259.2019.
55. **Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS; American College of Sports Medicine.** Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 377–390, 2007. doi:10.1249/mss.0b013e31802ca597.
56. **Schlader ZJ, Chapman CL, Benati JM, Gideon EA, Vargas NT, Lema PC, Johnson BD.** Renal hemodynamics during sympathetic activation following aerobic and anaerobic exercise. *Front Physiol* 9: 1928, 2019. doi:10.3389/fphys.2018.01928.
57. **Schlader ZJ, Chapman CL, Sarker S, Russo L, Rideout TC, Parker MD, Johnson BD, Hostler D.** Firefighter work duration influences the extent of acute kidney injury. *Med Sci Sports Exerc* 49: 1745–1753, 2017. doi:10.1249/MSS.0000000000001254.
58. **Schlader ZJ, Hostler D, Parker MD, Pryor RR, Lohr JW, Johnson BD, Chapman CL.** The potential for renal injury elicited by physical work in the heat. *Nutrients* 11: 2087, 2019. doi:10.3390/nu11092087.

59. **Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM.** Biomarkers in acute kidney injury - pathophysiological basis and clinical performance. *Acta Physiol (Oxf)* 219: 556–572, 2017. doi:10.1111/apha.12764.
60. **Schrier RW, Henderson HS, Tisher CC, Tannen RL.** Nephropathy associated with heat stress and exercise. *Ann Intern Med* 67: 356–376, 1967. doi:10.7326/0003-4819-67-2-356.
61. **Smith JH, Robinson S, Pearcy M.** Renal responses to exercise, heat and dehydration. *J Appl Physiol* 4: 659–665, 1952. doi:10.1152/jappl.1952.4.8.659.
62. **Song Z, Roncal-Jimenez CA, Lanaspa-Garcia MA, Oppelt SA, Kuwabara M, Jensen T, Milagres T, Andres-Hernando A, Ishimoto T, Garcia GE, Johnson G, MacLean PS, Sanchez-Lozada LG, Tolan DR, Johnson RJ.** Role of fructose and fructokinase in acute dehydration-induced vasopressin gene expression and secretion in mice. *J Neurophysiol* 117: 646–654, 2017. doi:10.1152/jn.00781.2016.
63. **Waikar SS, Sabbisetti VS, Bonventre JV.** Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. *Kidney Int* 78: 486–494, 2010. doi:10.1038/ki.2010.165.
64. **Ware LB, Johnson AC, Zager RA.** Renal cortical albumin gene induction and urinary albumin excretion in response to acute kidney injury. *Am J Physiol Renal Physiol* 300: F628–F638, 2011. doi:10.1152/ajprenal.00654.2010.
65. **Wijkström J, González-Quiroz M, Hernandez M, Trujillo Z, Hultenby K, Ring A, Söderberg M, Aragón A, Elinder CG, Wernerson A.** Renal morphology, clinical findings, and progression rate in mesoamerican nephropathy. *Am J Kidney Dis* 69: 626–636, 2017. doi:10.1053/j.ajkd.2016.10.036.
66. **Wilson TE, Sauder CL, Kearney ML, Kuipers NT, Leuenberger UA, Monahan KD, Ray CA.** Skin-surface cooling elicits peripheral and visceral vasoconstriction in humans. *J Appl Physiol (1985)* 103: 1257–1262, 2007. doi:10.1152/japplphysiol.00401.2007.
67. **Witzgall R, Brown D, Schwarz C, Bonventre JV.** Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest* 93: 2175–2188, 1994. doi:10.1172/JCI117214.
68. **Wolf JP, Nguyen NU, Dumoulin G, Berthelay S.** Influence of hypertonic monosaccharide infusions on the release of plasma arginine vasopressin in normal humans. *Horm Metab Res* 24: 379–383, 1992. doi:10.1055/s-2007-1003340.
69. **Xiao J, Zhang X, Fu C, Yang Q, Xie Y, Zhang Z, Ye Z.** Impaired Na^+-K^+-ATPase signaling in renal proximal tubule contributes to hyperuricemia-induced renal tubular injury. *Exp Mol Med* 50: e452, 2018. doi:10.1038/emm.2017.287.
70. **Zarbock A, Schmidt C, Van Aken H, Wempe C, Martens S, Zahn PK, Wolf B, Goebel U, Schwer CI, Rosenberger P, Haeberle H, Görlich D, Kellum JA, Meersch M; RenalRIPC Investigators.** Effect of remote ischemic preconditioning on kidney injury among high-risk patients undergoing cardiac surgery: a randomized clinical trial. *JAMA* 313: 2133–2141, 2015. doi:10.1001/jama.2015.4189.