Hydrating with a higher-fructose sport drink does not worsen acute kidney injury risk during simulated work-related heat stress

Erica Tourula [1], Molly E. Heikkinen [1], Amanda Regester [1,2], Marley G. Owen [1], Hayden W. Hess [1,3], Lisa Spence [2,4], Timothy D. Mickleborough [1,2,5], Blair D. Johnson [1,2], Nana Gletsu-Miller [2,4], Zachary J. Schlader [1,2]

[1] Department of Kinesiology, Indiana University School of Public Health – Bloomington, Bloomington, IN, USA

[2] Nutrition and Exercise Research Center, Indiana University School of Public Health – Bloomington, Bloomington, IN, USA

[3] Center for Research and Education in Special Environments, Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY, USA

[4] Department of Applied Health Sciences, Indiana University School of Public Health – Bloomington, Bloomington, IN, USA

[5] Area of Exercise Physiology, Faculty of Sports Science, Chulalongkorn University, Bangkok, Thailand.

Corresponding Author:
Zachary J. Schlader
Address: 1025 E. Seventh St, Ste 112, Bloomington, IN 47405
Phone: 812-855-6953
Email: zschlade@iu.edu

Running Title: Higher-fructose sport drink and acute kidney injury

Abstract

Fructose containing soft drinks worsen acute kidney injury (AKI) risk during simulated work related heat stress (WrHS), but the fructose content is comparatively lower in sport drinks. This study tested the hypothesis that consuming a higher-fructose sport drink exacerbates AKI risk compared to consuming a sucrose-sweetened sport drink during a four-hour WrHS simulation. In this block randomized, double-blind crossover study, 12 healthy adults (26±7 y; 6 women) completed two trials involving ingesting six 500 mL doses of a higher-fructose sport drink (55% fructose & 45% glucose; Fructose55) or a sucrose-sweetened sport drink (50% fructose & 50% glucose; Sucrose) before, during, and following a 4 hour WrHS simulation that comprised three circuits of treadmill walking (10 minutes) and rowing (5 minutes) at five metabolic equivalents and 15 minutes of rest every hour. Drinks were matched for total carbohydrates, calories, and electrolytes and modestly matched for osmolality. Urine and blood samples were collected pre- and post-exposure, at recovery, and 24 hours post-visit. AKI risk was quantified as the product of urine insulin-like growth factor-binding protein 7 and urine tissue inhibitor of metalloproteinase-2 ([IGFBP7•TIMP-2]) normalized to specific gravity. Peak rectal temperature (Fructose55: 38.3±0.7°C, Sucrose: 38.3±0.7°C, p=0.991) and percent change in body weight (Fructose55: -0.7±1.0%, Sucrose: -0.9±1.0%, p=0.466) were not different between trials. Urinary [IGFBP7•TIMP-2] increased over time ($p<0.001$) but did not differ between trials (peak change – Fructose55: 1.7±1.6 $(ng/mL)^2/1000$, Sucrose: 1.6±1.8 $(ng/mL)^2/1000$, p=0.677). Consuming a higher-fructose containing sport drink does not exacerbate AKI risk during WrHS when compared to a sucrose-sweetened equivalent.

Keywords: Dehydration, occupational heat stress, rehydration, kidney function

New and Noteworthy:

This study tested whether drinking a sport drink with a higher proportion of fructose influences acute kidney injury (AKI) risk during simulated work-related heat stress. In a block randomized, double-blind crossover design, healthy adults consumed either a higher-fructose or sucrose-sweetened sport drink matched for calories, electrolytes, and carbohydrates. Despite increases in AKI biomarkers with heat stress, the type of sugar in the sport drink did not alter AKI risk, indicating that modest differences in fructose content does not meaningfully impact kidney stress.

Introduction

Workers performing physically demanding tasks in hot environments experience work-related heat stress (WrHS) (1). WrHS can lead to dehydration, hyperthermia (i.e., elevations in core temperature), and increase the likelihood of acute kidney injury (AKI) (2, 3). Preventing dehydration attenuates the rise in core temperature during simulated WrHS, which reduces AKI risk (4). However, the type of beverage consumed during WrHS may influence kidney-related outcomes. For example, in rodent models, rehydration with a high-fructose solution activates the intra-renal polyol–fructokinase pathway and is accompanied by increased vasopressin concentrations, and renal inflammation, oxidative stress and mitochondrial dysfunction (5–7). Data from our laboratory have shown that consuming a high-fructose corn syrup (HFCS) sweetened soft drink during simulated WrHS exacerbates seemingly normal, physiologically mediated reductions in kidney function and provokes further increases in circulating biomarkers of AKI compared with water (8). These effects are thought to be related to fructose mediated augmented vasopressin responses (8) and greater renal vasoconstriction (9), although hyperosmolality may also contribute due to plasma volume contraction that occurs by drinking a hyperosmotic beverage (10).

Occupational guidelines recommend that workers consume sport drinks when WrHS exceeds two hours in duration, yet the optimal carbohydrate composition is not defined (11). Most commercially available sport drinks contain sugar, but their formulations vary widely (12). Recent studies have shown that different sport drink types have a limited effect on AKI risk during simulated WrHS (13, 14). However, previous comparisons have not matched beverages across variables such as calories, electrolyte concentrations, and osmolality, leaving the independent influence of sugar composition in sport drinks unknown.

Ingestion of a commercially available sucrose-sweetened sport drink does not worsen kidney function or elevate AKI risk versus a sugar-free sport drink during simulated WrHS (13). Therefore, the primary objective of this study was to compare the AKI risk biomarker responses between two sport drink formulations that differed only by fructose concentration yet are similar in calories, electrolytes, carbohydrates and osmolality to those that are commercially available. We tested the hypothesis that consuming a higher-fructose sport drink exacerbates AKI risk compared with a sucrose-sweetened sport drink during a four-hour WrHS simulation. Previous work demonstrates that drinking a HFCS sweetened, hyperosmotic, soft drink elicits renal vasoconstriction during a sympathoexcitatory test (i.e., cold pressor

test) (9). Whether such findings translate to sport drinks, which have substantially lower energy content and osmolality remains unknown. Therefore, the secondary objective of this study was to test the hypothesis that consuming a higher-fructose sport drink augments renal vasoconstriction during the cold pressor test compared to a sucrose-sweetened sport drink.

METHODS

Participants

Effect size was calculated based on previous data from our laboratory that examined the effect of consuming a HFCS-sweetened soft drink on the increase in urinary neutrophil gelatinase associated lipocalin, an AKI risk biomarker, following four hours of simulated WrHS. This resulted in a Cohen's d_z effect size of 0.72. Using this effect size, sample size was estimated using G*Power 3.1.9.4 software and the following parameters - power=80%, alpha=0.05, and a moderate correlation between repeated measures (r=0.5). The resulting sample size calculation revealed that we needed twelve participants to detect a statistically significant trial x time interaction in a two-way repeated measures ANOVA.

This study was approved by the Institutional Review Board at Indiana University (IRB#: 19683) 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. Twelve healthy adults (6 women) participated in this study. 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. Participants self-reported to be regularly engaged in regular moderate physical activity 3-5 days per week (15, 16). The study was conducted throughout the calendar year in Bloomington, IN, USA. While heat acclimatization status was not controlled, participants were studied within the same season (e.g., summer, winter, etc.) and it is unlikely that the climate resulted in meaningful differences in acclimatization status (17). Women were not pregnant at any point during the study, which was confirmed via a urine pregnancy test before each trial. Women self-reported that they were eumenorrheic and not on birth control. Women were tested throughout the menstrual cycle given that women often undergo WrHS without regards for menstrual cycle phase and testing occurred 14 ± 10 (mean ± SD) days from the onset of the last self-reported menses.

Participants visited the laboratory on three separate occasions. The first visit included screening, familiarization, and graded submaximal exercise tests. The following two visits were experimental trials

(see *Experimental protocol*) and were separated by at least three days. The mean duration between experimental trials was 20 ± 18 days.

Sport drink formulations

To accomplish the objectives of this study, we aimed to compare the effects of a fructose load like that in a commercially available HFCS sweetened sport drink versus an equivalent commercially available beverage sweetened with sucrose. The sucrose-sweetened beverage was designed to mimic the composition of a popular commercially available sport drink (Gatorade G2) sweetened only with sucrose (Sucrose). The higher-fructose beverage was designed to be identical with regards to calories, electrolytes, and total carbohydrate content, with the exception being that the experimental beverage was sweetened with 55% fructose and 45% glucose (Fructose55, Table 2). Food-grade HFCS was not utilized as a sweetener because using HFCS resulted in a hyperosmotic beverage (~664 mOsm/L), which can impair fluid absorption by increasing the net secretion of water into the intestinal lumen (10). Moreover, there is precedent to utilize an HFCS mimic by modifying the fructose-glucose ratio, such that previous work in rats indicates that rehydrating with a 60% fructose, 40% glucose beverage worsens AKI provoked by heat exposure (5). In the present study, a 55% fructose, 45% glucose ratio was used because this mimics food grade HFCS-55, which is common in sugar sweetened beverages (18). Finally, given the chemical differences between sucrose and the combination of 55% fructose, 45% glucose sweetened beverage it was impossible to precisely control for osmolality and keep all other aspects of the beverages the same. Although osmolality differed between beverages, the absolute difference was modest compared to previous soft-drink (8) and sport drink (13, 14) studies and was considered acceptable given the need to match calories, electrolytes, and carbohydrate content. It should also be noted, that based on current understanding, the difference in osmolality between the Sucrose and Fructose55 beverages (see Table 2) was unlikely to result in meaningful differences in fluid absorption (19). All sport drinks were formulated and made by the Indiana University School of Public Health – Bloomington, Nutrition and Exercise Research Center. Notably, a non-caloric control beverage (e.g., water or a sugar-free sport drink) was not included in the study for two reasons – 1) occupational heat stress and hydration recommendations suggest that workers consume a sport drink containing electrolytes when work / exposure to heat stress exceeds two hours (11), and 2) previous evidence indicates that drinking a commercially available sucrose-sweetened sport drink does not worsen kidney function or elevate AKI risk versus a sugar-free sport drink (13). Hence, the Sucrose beverage served as the control beverage.

Experimental protocol

This study employed a block randomized, double-blind crossover design. Participants kept a diet log for 24 hours prior to the start of each experimental visit. Participants were encouraged to replicate their diet and fluid intake from the first visit during the second visit. Prior to arrival, participants were instructed to drink a pre-calculated volume of water equivalent to 0.5% of their body mass two hours prior to each experimental visit to ensure they were equally well hydrated for both experimental visits. Participants arrived at the laboratory for the two experimental visits, having refrained from strenuous exercise, alcohol, and caffeine for 12 hours and from food for 2 hours. A timeline of each experimental trial can be found in Figure 1.

Upon arrival, participants provided a urine sample by completely voiding their bladder into a collection urinal. Euhydration was confirmed via a urine specific gravity of ≤1.025 (20). Participants were then weighed nude in a private room. Participants then assumed a supine position, and a venous blood sample was taken after 20 minutes to account for shifts in plasma volume due to changes in posture (21). The assigned sport drink was then consumed within a 5-minute period. Participants then re-assumed a supine position and rested for 30 minutes, after which a second venous blood sample was collected before commencing the CPT. Following the CPT, a post-drink urine sample was collected (Figure 1). Consistent with the secondary purpose of this study, this before WrHS assessment was specifically designed to examine the acute effects of sport drink ingestion on renal hemodynamics. The measurements and measurement timing were modeled after the study by Chapman et al. (9), which examined the renal vascular response to consumption of a soft drink sweetened with HFCS.

Immediately following the before WrHS assessment, participants measured their nude body mass and then donned a standard work clothing ensemble, which included a white cotton short sleeved undershirt, a long-sleeved button-up over shirt, and worker pants, with women also wearing a sport bra. Following pre-chamber baseline measurements, participants entered a hot and humid environmental chamber (38.5 ± 0.7°C; 41 ± 3% relative humidity). These conditions were chosen because outdoor workers in the U.S. (e.g., construction, agriculture, etc.) are often exposed to this level of environmental heat (22, 23). Following initial measurements taken in a standing position, participants then engaged in four hours of simulated WrHS. Each hour consisted of 3 cycles of 10 minutes of walking on a treadmill and 5 minutes of rowing exercise, for a total of 45 minutes of work, followed by 15 minutes of seated rest, which is the approximate work-to-rest ratio recommended for agriculture workers at known risk of

AKI due to WrHS (24). During each rest period, participants were given 500 mL of the assigned sport drink, totaling four drinks (2000 mL) over the four hours. We have shown previously that this volume of fluid replacement results in modest reductions in body mass during similarly intense WrHS simulations (8). The external workload was set to elicit five metabolic equivalents, which was determined during the initial visit to the laboratory. This workload was chosen because it falls within the range of light to moderate work intensity documented to be experienced by outdoor workers (25). We have previously used rowing ergometry to simulate work tasks that utilize upper and lower body continuous work (26). The use of rowing allows for a whole body, continuous motion utilizing large muscle groups in the legs (drive phase), back, shoulders, and arms (pull phase), better mimicking manual tasks like raking, shoveling, or manual harvesting compared to treadmill walking alone (27).

Immediately after leaving the chamber, post-WrHS urine was collected. Participants then toweled off prior to the measurement of nude body weight. Participants assumed the supine position, and post-exposure venous blood samples were taken 20 minutes later. Participants were then given an additional 500 mL of their assigned sport drink and reassumed a supine position for at least 30 minutes before a venous blood sample was obtained approximately 75 minutes following the end of the WrHS simulation. The after-WrHS CPT then commenced which permitted assessment of the comparative effects of Fructose55 and Sucrose on renal vasomotor responses following simulated WrHS, with CPT testing conducted at the equivalent time point after ingestion of the final 500 mL sport drink dose as during the pre-WrHS CPT. Another urine sample was collected immediately following the CPT, after which the carbon monoxide rebreathing test commenced. Lastly, participants provided a recovery nude weight before leaving the laboratory.

Participants returned to the laboratory approximately 24 hours from the beginning of their experimental trial for a final urine and venous blood sample. During the overnight period, participants were not given specific instructions except to relax and not undertake any exercise until arriving at the laboratory the following morning. Participants were instructed to record all food and beverage items and volumes from the time leaving the experimental visit until the 24-hour follow-up visit the following day to help replicate their overnight diet for their next experimental visit.

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, multiplied by 100. Sweat rate was calculated from the change in nude body weight adjusted for fluid intake and output. Core temperature was measured by participants self-inserting a rectal temperature probe approximately 10 cm past the anal sphincter. Skin temperatures were 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 and weighted mean skin temperature was calculated accordingly (28). Systolic and diastolic blood pressure were measured using electrosphygmomanometry (Tango M2 Stress Test Monitor, Morrisville, NC, USA; or Omron Hoffman Estates, Illinois, USA). Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Percent of age predicated maximal heart rate was calculated as heart rate divided by the maximal age-predicted heart rate (29) multiplied by 100.

Hydration and kidney function: Urine specific gravity was measured in duplicate via refractometry (ATAGO Co., Ltd, Tokyo, Japan). Urine flow rate was calculated as urine volume divided by the duration of time between urine collection periods and was determined over two periods: approximately 360 minutes from baseline to post-WrHS and about 75 minutes from post-WrHS to recovery. Hemoglobin concentrations were measured in duplicate using the Hemopoint H2 device (Alere, Orlando, FL), and hematocrit was assessed in triplicate by microcentrifugation (30). Plasma and urine osmolality were evaluated in duplicate via freezing-point depression (model 3250; Advanced Instruments, Norwood, MA). Osmolar clearance (Cosm) was calculated by multiplying urine flow rate (V) by the ratio of urine (Uosm) to plasma (Posm) osmolality ($Cosm = V \times Uosm/Posm$) (31). Free water clearance (CH_2O) was derived by subtracting Cosm from urine flow rate ($CH_2O = V - Cosm$) (31). Biomarkers in urine and serum related to inflammation, oxidative stress, acute kidney injury (AKI) risk, kidney function, and intestinal permeability were analyzed using standardized commercial methods at the Center for Diabetes and Metabolic Diseases Translation Core in the Indiana University School of Medicine (described below). Serum electrolytes, glucose, creatinine and albumin and urinary electrolytes, creatinine, and albumin were obtained from comprehensive metabolic profile panels run by the Indiana University Health Pathology Laboratory. All urinary markers were normalized to specific gravity (denoted using subscript USG) prior to statistical analysis to account for variations in urine concentration across repeated spot urine samples (32), consistent with prior studies (4, 8, 26, 33). The advantage of normalizing to specific

gravity is that the units are unchanged, aiding data interpretation. Urinary biomarkers have also been normalized to urine flow rate (denoted using subscript UFR) providing an index of biomarker excretion rate. That said, it should be noted that the *a priori* primary outcome variables are the urinary markers normalized to specific gravity. Creatinine clearance, serving as an estimate of glomerular filtration rate, was calculated as the product of urine flow rate and the ratio of urine to serum creatinine concentrations. The fractional excretions of sodium and potassium were calculated using serum and urine concentrations of these electrolytes, along with changes in creatinine concentrations. Serum copeptin, a stable surrogate marker for arginine vasopressin, was measured using a commercially available immunoassay [Vector laboratories, Inc., Newark, CA: Isbio, LS-F26731-1 (intra-assay CV: 7.6%, lower limit of detection: 7.8 pg/mL)].

Acute kidney injury risk: Urine insulin-like growth factor-binding protein 7 (IGFBP7), which is preferentially secreted in the renal proximal tubules, and urine tissue inhibitor metalloproteinase 2 (TIMP-2), which is preferentially secreted in the renal distal tubules, are markers of G1 cell cycle arrest in renal epithelial cells (34, 35). IFGBP-7 and TIMP-2 were measured with commercially available ELISA kits [RayBiotech Life, Inc., Peachtree Corners, GA, USA: RayBio human IGFBP-7; ELH-IGFBPRP1-1 (intra-assay CV: 10%, inter-assay CV: 12%, lower limit of detection: 0.065 ng/mL) and RayBio human TIMP-2; ELH-TIMP2-1 (intra-assay CV: 10%, inter-assay CV: 12%, lower limit of detection: 2 pg/mL)]. Consistent with the FDA-approved indication in clinical settings, the product of urinary IGFBP7 and TIMP-2 ([IGFBP7•TIMP-2]) provided an index of AKI risk (36). Kidney injury molecule-1 (KIM-1), an established biomarker of proximal tubular injury (37), was measured in urine samples using commercially available ELISA kits [Mesoscale Diagnostics LLC, Rockville, MD, USA: KIM-1 mesoscale; K1514UR-2 (intra-assay CV: 2.7%, inter-assay CV: 6.5%, lower limit of detection: 10 pg/mL)]. Urine KIM-1 has demonstrated utility in detecting early tubular damage (37, 38). Neutrophil gelatinase-associated lipocalin (NGAL), another sensitive marker of tubular injury (39), was quantified in urine using commercially available ELISA kits [Meso Scale Diagnostics, Rockville, MD, USA: U-PLEX Human NGAL mesoscale; K151Z1K-1 (intra-assay CV: 4.8%, inter-assay CV: 14%, lower limit of detection: 0.78 pg/mL)]. Urine NGAL is an established biomarker of AKI across a range of conditions (40, 41). Urine (intra-assay CV: 1.7%, lower limit of detection: 2.2 mg/dL) and serum (intra-assay CV: 1.9%, lower limit of detection: 0.2 mg/dL) uric acid concentrations were measured using a Roche Analyzer (F. Hoffmann-La Roche AG, Indianapolis, IN). Elevated uric acid is associated with ingestion of fructose (42), which may lead to an increased risk of AKI during heat stress and dehydration (43, 44).

Inflammation: Monocyte chemoattractant protein-1 (MCP-1), a proinflammatory cytokine (45), was evaluated in both urine and serum. Urinary MCP-1 levels rise in clinical AKI due to increased renal expression (46), and are linked to reduced kidney function in Mesoamerican agricultural workers (47). Serum MCP-1 serves as an indicator of systemic inflammation (48, 49). MCP-1 in serum and urine was measured using commercially available ELISA kits [Meso Scale Diagnostics, Rockville, MD, USA: V-PLEX Human MCP-1 mesoscale; K151NND (intra-assay CV: 11.2%, inter-assay CV: 8.9%, lower limit of detection: 0.09 pg/mL)]. Additional systemic inflammatory markers, interleukin-6 (IL-6; intra-assay CV: 4.5%, inter-assay CV: 7.3%, lower limit of detection: 0.06 pg/mL) and tumor necrosis factor-alpha (TNF-α; intra-assay CV: 3.4%, inter-assay CV: 10.1%, lower limit of detection: 0.04 pg/mL) were analyzed in serum using high-sensitivity multiplex assays (Meso Scale Diagnostics, Rockville, MD, USA: V-PLEX Human Proinflammatory Panel I; K15052D-1). IL-6 is rapidly produced in response to heat stress and exercise, reflecting both muscle-derived myokine activity and systemic inflammatory signaling (50–52). TNF-α, a key pro-inflammatory cytokine, has been implicated in the pathogenesis of AKI (53–55).

Oxidative stress: Thioredoxin-1 (TRX-1), a redox-regulating protein (56), was also assessed. Urinary TRX-1 has been shown to increase during prolonged passive heat stress (33), and reflects oxidative stress and/or hypoxia from proximal tubular epithelial cells (57). Serum TRX-1 is recognized as a diagnostic and prognostic biomarker in many clinical conditions (58–60). TRX-1 in serum and urine was measured using commercially available ELISA kits [Thermo Fisher Scientific, Waltham MA, USA: Invitrogen Human Thioredoxin-1 mesoscale; EH451RB (intra-assay CV: 10%, inter-assay CV: 12%, lower limit of detection: 0.2 ng/mL].

Gastrointestinal permeability: Serum intestinal fatty acid binding protein (I-FABP), which is specifically expressed in intestinal enterocytes, was measured as a marker of gastrointestinal permeability. Elevated serum I-FABP concentrations indicate enterocyte damage (61), and levels have been reported to increase during physical work (exercise) in the heat (62–65). Serum lipopolysaccharide binding protein (LBP), a marker of bacterial translocation resulting from gut permeability (66), rises with prolonged heat stress (67) and exercise in the heat (68, 69). Serum concentrations of I-FABP and LBP were determined using commercial ELISA kits [Meso Scale Diagnostics, Rockville, MD, USA: R-Plex Human I-FABP Mesoscale (intra-assay CV: 4.1%, lower limit of detection: 27 pg/mL); K151M4R-2 and R-Plex Human LBP Mesoscale; K151K5R-2 (intra-assay CV: 3.4%, lower limit of detection: 101 pg/mL)].

Renal perfusion: Renal and segmental artery blood velocity was used as a marker of renal perfusion (70). Renal and 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) in the coronal approach with the participant in the left lateral recumbent position. All measurements were made with the participant in the same position. Indelible ink was used to mark the transducer location during the first baseline measurement. Participants were instructed to perform a mid-exhalation non-Valsalva breath hold for no longer than 10 seconds during the ultrasound measurement. Baseline mean renal and segmental artery velocities were measured over 2-4 cardiac cycles. All blood velocity measurements were obtained by the same sonographer (E. Tourula) with a within-subject test-retest coefficient of variation for renal and segmental artery blood velocity measurements of 4.0 ± 0.3 % and 3.8 ± 0.6 %, respectively. The renal vasculature is at a depth where it is not possible to measure vessel diameter using Doppler ultrasound accurately. Therefore, renal and segmental artery blood velocity was interpreted to reflect renal blood flow because reductions in renal blood flow during pharmacological intervention have been shown to be due to decreases in blood velocity and not changes in vessel diameter (71, 72). Renal and segmental artery vascular resistance was calculated as mean arterial pressure divided by segmental artery blood velocity.

As we have done previously (9, 73, 74), participants completed a cold pressor test (CPT) to assess changes in renal vascular control to sympathetic stimulation before and after simulated WrHS (see details below). Following initial measurement of renal and segmental artery renal blood velocity, blood pressure, and heart rate, the CPT commenced by placing the participant's right hand in agitated ice water (0.9 ± 0.3°C) up to the wrist for two minutes. Blood pressure, heart rate, and renal segmental artery blood velocity were measured during each minute throughout the CPT. Renal segmental artery velocity was used during the CPT because only one artery could be measured accurately without moving the transducer while the renal segmental artery is comparatively more responsive to heat stress during the CPT than the renal artery (73).

Plasma volume measurement: Carbon monoxide (CO) rebreathing was used to measure total hemoglobin mass, from which total blood volume and plasma volume were calculated (75, 76). Briefly, following 20 minutes of supine rest, the CO rebreathing procedures consisted of a bolus of 99.5% chemically pure CO in the amount of 1.0 mL per kg body weight delivered via a 100 mL syringe and

inhaled by the participant. While remaining in the supine position, the participant rebreathed for 2 minutes from a closed spirometer system connected to a 5 L anesthetic bag filled with 100% oxygen. Before the start of the rebreathing, venous blood was collected. At 8 and 10 minutes after the start of rebreathing, finger-prick blood samples were collected in duplicate. The collected venous and capillary blood was sampled by a blood gas analyzer (OSM3, Radiometer) for the determination of carboxy-hemoglobin concentration, which permitted calculation of hemoglobin mass. Assuming that hemoglobin mass is unaffected by four hours of simulated WrHS (77) absolute plasma volume was calculated from total blood volume minus erythrocyte volume at each timepoint.

Data and statistical analyses

Before WrHS, resting (minute-0) renal perfusion and hemodynamic data were collected from twelve participants. In the Sucrose trial, twelve participants contributed CPT data at minute-1 and eleven at minute-2. In the Fructose55 trial, segmental artery blood velocity was obtained from twelve participants at minute-1 and eleven at minute-2, and blood pressure and heart rate were available for eleven participants at both time points. Accordingly, segmental artery vascular resistance was calculated for twelve participants at minute-1 and eleven at minute-2 in the Sucrose trial, and for eleven participants at both time points in the Fructose55 trial. Missing data resulted from equipment malfunction or poor Doppler image quality. During WrHS, one Fructose55 participant stopped exercising at 2 h 30 min due to lightheadedness, remained seated to consume the third drink, exited the chamber at 2 h 45 min, and consumed the fourth drink over the next 30 minutes. Another Fructose55 participant completed exercise but was removed from the chamber during hour four after reaching the IRB-mandated core temperature cutoff (39.5°C) and consumed the final drink within 15 minutes outside the chamber. In the Sucrose trial, one participant stopped at 3 h 40 min due to nausea and completed the rest period and final drink outside heat exposure. All deviations occurred in the first trial and were replicated in the second. After WrHS, resting renal perfusion and hemodynamic measurements were again obtained from twelve participants. In the Sucrose trial, twelve participants had CPT data at minute-1 and eleven at minute-2. In the Fructose55 trial, CPT data were available for eleven participants due to one unusable dataset. Thus, segmental artery vascular resistance was calculated for eleven participants at both time points in the Fructose55 trial, and for eleven at minute-1 and twelve at minute-2 in the Sucrose trial. Missing data were due to equipment malfunction or inadequate Doppler imaging. Seven Fructose55 and nine Sucrose participants completed the carbon monoxide rebreathing test; missing data were due to

equipment malfunction or difficulty obtaining carbon monoxide. Plasma volume data reflect these sample sizes.

For the primary objective (i.e., AKI risk during WrHS), the analysis focused on blood and urine samples collected immediately following arrival (i.e., baseline), post-WrHS, at recovery and 24-hours post-. The resulting data, together with the temporal cardiovascular and thermoregulatory data collected during WrHS, were analyzed with two-way linear mixed models with repeated measures of both trial and time. For the secondary objective (i.e., acute effects of sport drink consumption), the analysis focused on blood and urine samples collected at baseline and following the first drink (Drink #1). The resulting data were analyzed using two-tailed paired t-tests. The before WrHS and after WrHS CPT data were analyzed as a change from minute zero of the CPT to isolate the effect of the cardiovascular and renal vascular response to sympathetic stimulation. These data were analyzed with independent two-way linear mixed models with repeated measures of both trial and time. The cardiovascular and renal vascular data at minute zero of the CPT were compared using two-tailed paired t-tests. The effect of WrHS on the responses to the CPT were explored by comparing peak changes in the cardiovascular and renal vascular responses in the Sucrose and Fructose55 trials using two-way linear mixed models with repeated measures of both trial and time. Two-tailed paired t-tests were used to examine differences in nude body mass loss and sweat rate between trials. In all instances, data were checked for a normal distribution via visual inspection of predicted and actual residuals (i.e., QQ plots). Data determined to be not normally distributed were either Log_{10} transformed before executing the linear mixed models (i.e., urinary IGFBP7, [IGFBP7 • TIMP-2], NGAL and TRX-1 normalized to urine specific gravity, all urinary markers when normalized to urine flow rate, and serum TRX-1, MCP-1, IL-6 and TNF-α). When applicable, the Geisser–Greenhouse correction was applied if sphericity could not be assumed. If a significant interaction or main effect of trial was identified in the linear mixed models, post hoc analyses were completed with Šídák's multiple comparisons tests. All data were analyzed with GraphPad Prism software (version 8; La Jolla, CA). Statistical significance was set a priori at $p \leq 0.05$, and actual p-values are reported where possible. Data are presented as mean ± SD and individual values where possible.

Results

Before WrHS

Resting renal and segmental artery perfusions are presented in Table 3. In summary, resting renal artery and segmental artery blood velocity and vascular resistance did not differ between the Fructose55 and Sucrose trials ($p \geq 0.377$) after consuming a 500 mL sport drink.

Changes in segmental artery perfusion and hemodynamic data during the CPT are presented in Figure 2. To summarize, segmental artery blood velocity decreased over time (Main effect of Time: $p = 0.048$) but did not change differently between trials (Time x Trial: $p = 0.529$). Segmental artery vascular resistance increased over time (Main effect of Time: $p < 0.001$) and changed differently between trials (Main effect of Trial: $p = 0.017$). Post hoc pairwise comparisons indicated the increase in segmental artery vascular resistance was greater in the Sucrose trial at 2 minutes (Sucrose: +2.0 ± 0.9 mmHg/cm/s; Fructose55: +1.3 ± 1.0 mmHg/cm/s; $p = 0.038$). Mean arterial pressure increased during the CPT (Main effect of Time: $p < 0.001$) and changed differently over time (Time x Trial: $p < 0.001$). Post hoc pairwise comparisons indicated the increase in mean arterial pressure was greater in the Sucrose trial at 1 minute (Sucrose: +21 ± 8 mmHg; Fructose55: +14 ± 9 mmHg; $p = 0.001$) and 2 minutes (Sucrose: +25 ± 7 mmHg; Fructose55: +20 ± 10 mmHg; $p < 0.033$). Heart rate increased during the CPT (Main effect of Time: $p < 0.001$), but the increase in heart rate did not differ between trials (Time x Trial: $p = 0.993$).

Serum and urinary markers of fluid and electrolyte control before (Baseline) and after (Post Drink #1) acute consumption of the assigned 500 mL sport drink are reported in Table 4. In summary, serum glucose indicated an effect of time (Main effect of Time: $p = 0.014$) but changed differently over time (Time x Trial: $p = 0.011$). Post hoc comparisons indicated serum glucose was higher at Post Drink #1 in the Sucrose trial (107 ± 20 mg/dL) compared to the Fructose55 trial (90 ± 12 mg/dL; $p = 0.011$). Urine and serum creatinine, sodium, potassium all decreased (Main effect of Time: $p \leq 0.006$) but did not change differently between trials (Time x Trial: $p \geq 0.369$). Urine flow rate and creatinine clearance increased over time (Main effect of Time: $p \leq 0.046$) but did not change differently between trials (Time x Trial: $p \geq 0.688$).

During and after WrHS

Thermoregulation and fluid regulation

Core and mean skin temperature data are presented in Figure 3. In summary, core temperature increased during WrHS in both trials (Main effect of Time: $p < 0.001$) but was not different between trials (Time x Trial: $p=0.180$). Mean skin temperature increased over time (Main effect of Time: $p < 0.001$) in

both trials. Heart rate data during WrHS is also presented in Figure 3. To summarize, heart rate increased over time in both trials (Main effect of Time: $p < 0.001$) but did not change differently between trials (Time x Trial: $p = 0.380$). Similarly, heart rate when represented as a percentage of age predicted maximal heart rate increased in both trials over time (Main effect of Time: $p < 0.001$) but did not change differently between trials (Time x Trial: $p = 0.605$).

Hydration-related measures are presented in Table 5. In summary, percent changes in nude body mass (Wilcoxon: $p = 0.466$) and whole-body sweat rate (Wilcoxon: $p = 0.910$) did not differ between trials. Likewise, plasma volume, serum osmolality, urine osmolality, and urine specific gravity did not differ between trials (Time x Trial: $p \geq 0.341$).

Kidney Function and AKI Risk Biomarkers

Markers of kidney function and electrolyte and fluid balance are presented in Tables 6 and 7. To summarize, serum creatinine, creatinine clearance, and serum albumin increased over time (Main effect of Time: $p \leq 0.013$) but there were not different between trials (Time x Trial: $p \geq 0.409$). Osmolar and free water clearance increased over time (Main effect of Time: $p \leq 0.027$) and were not different between trials (Time x Trial: $p \geq 0.144$). Although free water clearance had an effect of trial (Trial: $p = 0.039$), post hoc comparisons did not indicate a difference between trials at any timepoint. Serum and urine sodium, and fractional excretion of sodium decreased, while urine potassium increased over time (Main effect of Time: $p \leq 0.029$) but were not different between trials (Time x Trial: $p \geq 0.116$). Serum glucose increased over time (Main effect of Time: $p = 0.030$) and changed differently between trials (Time x Trial: $p = 0.005$). Post hoc comparisons indicated a greater increase in serum glucose in the Sucrose trial at Recovery (Sucrose: 1.8 ± 2.9 mg/dL; Fructose55: 0.5 ± 1.6 mg/dL; $p = 0.003$).

Markers of AKI risk are presented in Figure 4 and Table 8. To summarize, urinary IGFBP-7_{USG} and TIMP-2_{USG} individually and as a product [IGFBP-7•TIMP-2] $_{USG}$ increased over time (Main effect of Time: $p \leq 0.004$), but there were no differences between trials over time (Time x Trial: $p \geq 0.375$). Likewise, serum uric acid and urinary KIM-1_{USG} increased over time (Main effect of Time: $p \leq 0.015$) but did not differ between trials (Time x Trial: $p \geq 0.251$). Urinary NGAL $_{USG}$ and uric acid $_{USG}$ did not show an effect of time ($p \geq 0.266$) or time x trial ($p \geq 0.238$). Urinary markers of AKI risk normalized to urine flow rate did not meaningfully differ from when these markers were normalized to specific gravity. Specifically, IGFBP-7_{UFR} and TIMP-2 $_{UFR}$ increased over time (Main effect of Time: $p \leq 0.002$), but there were no differences

between trials over time (Time x Trial: $p \geq 0.238$). Likewise, urinary excretion of KIM-1_{UFR} and NGAL $_{UFR}$ increased over time (Main effect of Time: $p \leq 0.014$) but did not differ between trials (Time x Trial: $p \geq 0.135$).

Oxidative stress biomarkers

Oxidative stress serum and urinary biomarkers are presented in Table 9. To summarize, serum TRX-1 did not change over time (Main effect of Time: $p \geq 0.079$) but indicated a difference between trials (Main effect of Trial: $p \geq 0.040$). Post hoc comparisons did not indicate a difference between the Fructose55 and Sucrose trial at any time point ($p \geq 0.105$). Urinary TRX-1_{USG} did not change over time (Main effect of Time: $p = 0.388$) and did not change differently between trials (Time x Trial: $p = 0.150$). Urinary TRX-1_{UFR} did increase over time (Main effect of Time: $p = 0.013$) and did not change differently between trials (Time x Trial: $p = 0.069$).

Inflammation biomarkers

Serum and urinary inflammation biomarkers are presented in Table 9. To summarize, serum and urinary MCP-1_{USG} increased over time (Main effect of Time: $p \leq 0.026$) but did not change differently between trials (Time x Trial: $p \geq 0.131$). Serum IL-6, but not TNF-α ($p = 0.379$), increased over time (Main effect of Time: $p < 0.001$), but both were not different between trials (Time x Trial: $p \geq 0.290$). Urinary TRX-1_{UFR} did increase over time (Main effect of Time: $p = 0.013$) and did not change differently between trials (Time x Trial: $p = 0.069$).

Gut permeability

Gut permeability biomarkers are presented in Table 9. To summarize, serum LBP increased over time (Main effect of Time: $p < 0.001$) but were not different between trials (Time x Trial: $p = 0.219$). Increases in serum I-FABP were not significant over time (Main effect of Time: $p = 0.055$) and were not different between trials (Time x Trial: $p = 0.546$).

Renal perfusion

Resting renal and segmental artery perfusion and hemodynamics following 4 h WrHS are presented in Table 10. In summary, mean arterial pressure, heart rate, renal artery and segmental artery blood velocity, vascular resistance did not differ between the Fructose55 and Sucrose trials ($p \geq 0.444$) following WrHS.

Indices of segmental artery perfusion and hemodynamic data during the CPT are presented in Figure 5. To summarize, segmental artery blood velocity decreased during the CPT (Main effect of Time: $p = 0.007$) but did not differ between trials (Time x Trial: $p = 0.606$). Segmental artery vascular resistance increased during the CPT (Main effect of Time: $p < 0.001$) but was not different between trials (Time x Trial: $p \geq 0.456$). Mean arterial pressure increased during the CPT (Main effect of Time: $p < 0.001$) but did not differ between trials (Time x Trial: $p = 0.879$). Heart rate increased during the CPT (Main effect of Time: $p = 0.005$) but and did not differ between trials (Time x Trial: $p = 0.928$).

Peak changes in blood pressure, heart rate and segmental vascular resistance, and reductions in segmental blood velocity in response to the CPT before and after WrHS are presented in Figure 6. To summarize, peak changes in mean arterial pressure, were greater in the CPT that occurred before-WrHS (Main effect of Time: $p = 0.021$), but these changes did not differ between trials (Time x Trial: $p = 0.182$). Peak changes in heart rate and segmental artery vascular resistance as well as reductions in segmental blood velocity during the CPT were not different from before-WrHS to after-WrHS (Main effect of Time: $p \geq 0.099$) and did not change differently between trials (Time x Trial: $p \geq 0.204$).

Discussion

The primary objective of this study was to compare the effect of consuming a higher-fructose sport drink with a sucrose-sweetened sport drink matched for energy, total carbohydrates, and electrolyte content and relatively well matched for osmolality on AKI risk during a four-hour WrHS simulation. The secondary objective was to determine the acute effect of a single 500 mL dose of these sport drinks on renal hemodynamics during a sympathoexcitatory stimulus (i.e., CPT). The main findings are: 1) Drinking a higher-fructose sport drink does not exacerbate AKI risk during four hours of simulated WrHS compared to drinking a sucrose-sweetened sport drink (Figure 4). And 2) a single 500 mL dose of a sucrose-sweetened sport drink elicits a greater increase in mean arterial pressure and segmental vascular resistance compared to a higher-fructose sport drink (Figure 2). Collectively, these findings do not support our hypothesis that drinking a higher-fructose sport drink throughout four hours of simulated WrHS exacerbates elevations in biomarkers of AKI risk and renal vascular resistance during a sympathoexcitatory stimulus compared to a sucrose-sweetened sport drink.

AKI risk during- and after- WrHS

Hydrating with both a higher-fructose and sucrose-sweetened sport drink elicited an increase in AKI risk (i.e., $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$) during simulated WrHS (Figure 4) and the magnitude of the observed increase in these biomarkers is largely consistent with that observed in both lab- (26, 78–80) and field- (2) based studies. Increases in $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$ were due to elevations in both IGFBP7 and TIMP-2, a finding supported by previous work examining AKI risk during simulated WrHS (4, 26, 78). As an aside, it is notable that the urinary markers of AKI risk normalized to urine flow rate did not meaningfully differ from when these markers were normalized to specific gravity, the exception being urinary NGAL that demonstrated the expected increases when expressed as excretion rate but not when normalized to urine specific gravity (Table 8). Nevertheless, and contrary to our hypothesis, the consumption of a higher-fructose sport drink did not exacerbate the increase in AKI risk biomarkers following simulated WrHS (Figure 4). This is in contrast with the study by Chapman et al., (8), who reported that HFCS-sweetened soft drink consumption elevated AKI risk. In the present study, sport drink formulations were designed to be matched for total energy, total carbohydrates and electrolytes, and relatively well matched for osmolality (Table 2). Matching sport drink constituents allowed comparisons of sugar type, with a modest, and likely a physiologically inconsequential (19), difference in osmolality (of ~133 mOsm/L). Interestingly, our findings are strongly aligned with the two previous studies that have attempted to answer this question previously, albeit with some limitations. Specifically, Atkins et al. (14) identified that AKI risk following two hours of simulated WrHS did not differ between a commercially available sport drink sweetened with HFCS compared to water with non-caloric sweetener. These findings were seemingly corroborated by Lignier et al. (13) who found that AKI risk following four hours of simulated WrHS did not differ between commercially available sucrose-sweetened and non-caloric sport drinks. Given that these previous studies were unable to comprehensively control for other beverage constitutions (13, 14), the present study provides direct evidence that modest differences in fructose content in sport drinks does not differentially elevate AKI risk.

Atkins et al. (14) proposed that a threshold level of fructose intake may need to be reached before significant differences in AKI risk become apparent during or after WrHS. It is worth noting that previous soft drink studies administered a total fructose load of 276 g (8), whereas in previous commercially available sport drink studies, Atkins et al. (14) administered a more moderate amount of fructose (~145 g total), and Lignier et al., (13) administered a relatively low amount of fructose (50 g). Notably, the Sucrose condition in the current study had a similar the fructose load per milliliter compared with the sport drink trial of Lignier et al. (13) given that the Sucrose trial in our study mimicked Gatorade G2.

However, due to differences in volume consumed, the calculated total fructose load was 84 g in the Sucrose trial. Moreover, the total fructose load of 92.4 g in the Fructose55 trial is ~1.5x less than that in Atkins et al. (14) and ~3x less than the HFCS soft drink study (8). It has been proposed that the relatively small fructose load in previous sport drink studies could explain the lack of differences in AKI risk between different types of sport drinks (13, 14). This contention is supported by observations in the present study, as the fructose load difference between trials was only 8.4 g, and there were no differences in AKI risk between sport drinks. It is worth noting that this small difference in fructose content was intentional, as our goal was to isolate the effect of fructose at a concentration consistent with commercially available sport drinks, while holding constant other beverage characteristics (e.g., electrolytes, total carbohydrate content, and energy), an approach that was designed to directly inform the sugar composition of sport drinks from the perspective of kidney health during WrHS. Nevertheless, it is likely that this modest difference in fructose load was not sufficient to differentially modify the renal and/or systemic inflammation and oxidative stress (Table 9) or differences in renal vascular control (Table 10, Figure 5), which have been linked to AKI risk in the context of WrHS (8, 9, 26, 74). Thus, the present study provides strong evidence that modestly elevated fructose content in sport drinks does not increase AKI risk.

In the present study, the sport drinks were relatively well matched for osmolality. To this point, the HFCS-sweetened soft drink used previously (8) was extremely hyperosmotic (~834 mOsm/kg), which likely promoted a shift of water into the intestinal tract leading to reductions in plasma volume (10). Decreases in plasma volume and increases in plasma osmolality lead to the release of angiotensin II, via activation of the renin-angiotensin-aldosterone system, and the release of vasopressin (81). Angiotensin II causes renal sodium reabsorption (82, 83), vasoconstriction in the renal afferent and efferent arterioles (84), and the release of aldosterone, leading to more sodium reabsorption (85). Vasopressin increases water reabsorption (86) and induces vasoconstriction of the efferent kidney arterioles (87). In the context of WrHS, all of these factors could exacerbate energy utilization in the kidneys, resulting in ATP depletion that may promote renal interstitial inflammation (5, 7) and oxidative stress (43). Thus, a primary role for beverage osmolality in modifying AKI risk may be likely in the context of WrHS. For example, in contrast to the HFCS-sweetened soft drink study (8), in the present study copeptin, a marker of vasopressin (88), was not different from baseline (Table 7), observations that can likely be attributed to differences in beverage osmolality between soft drinks and sport drinks.

Not controlling for sport drink electrolyte content is also an important consideration in previous studies. For example, while Atkins et al. (14) reported that a commercially available sport drink sweetened with HFCS had approximately half the osmotic load of a soft drink (~420 mOsm/kg) and did not modify AKI risk during simulated WrHS compared to water containing a noncaloric sweetener, the water containing a noncaloric sweetener had very low electrolyte content (15 mg vs. 666 mg of sodium per serving). Thus, in the water condition the requirement for sodium reabsorption may have been increased, which could have elevated the work of the kidneys (89). Therefore, it remains possible that the effect of the fructose load from the HFCS sweetened sport drink on AKI risk was masked by the increased demand for electrolyte reabsorption in the water containing noncaloric sweetener. The present study overcame this limitation by matching the sport drinks for electrolyte content to ensure the need for electrolyte reabsorption did not differ between Fructose55 and Sucrose (e.g., 210 mg Na^+ and 65 mg K^+). In the present study, this was confirmed by the lack of observed differences in measures of electrolyte and fluid reabsorption (e.g., free water clearance, fractional excretion of sodium) and kidney function (e.g., creatinine clearance) between the Fructose55 and Sucrose trials (Tables 6 & 7). Collectively, given that AKI risk was elevated in all conditions, independent of sport drink type (Figure 4, (13, 14)), future research should examine the independent effects of beverage osmolality and electrolyte concentrations as a means to optimize hydration beverage composition to best alleviate WrHS provoked elevations in AKI risk.

Before WrHS - Renal hemodynamics following a single 500 mL dose of sport drink

Previous research has identified that consuming a HFCS-sweetened soft drink, compared to water, augments vascular resistance in the segmental renal artery both at rest and during the CPT (9). In this same study, it was identified that the augmented renal vasoconstriction was primarily due to HFCS in the soft drink and not necessarily osmolality, caffeine or energy content (9). The present study aimed to determine the isolated, acute effects of consuming a higher-fructose sport drink versus a sucrose-sweetened sport drink on renal hemodynamic responses to sympathetic stimulation. To this end, it is noteworthy that no differences in resting renal hemodynamics were observed from baseline to Post Drink #1 in either trial (Table 3). Moreover, in contrast to the augmented blood pressure response observed previously with acute HFCS-sweetened soft drink consumption (9), we found that the Sucrose trial elicited a comparatively augmented increase in mean arterial pressure and segmental vascular resistance during the CPT versus the Fructose55 trial (Figure 2). While surprising, this heightened pressor / renal vasoconstrictor response is likely attributable to the higher serum glucose levels in the Sucrose

trial versus the Fructose55 trial (Table 3). This observation is supported by previous research demonstrating that ingestion of a glucose sweetened beverage augments the blood pressure response to the CPT, which was associated with the rise in blood glucose despite that circulating insulin concentrations were reduced (90). Additional observations support that acute glucose ingestion acutely raises blood pressure, a response that is only weakly associated with insulin sensitivity (91). When considered together, these findings suggest that the differential blood pressure response following a single 500 mL dose of a higher-fructose or sucrose-sweetened sport drink during the CPT was likely driven by differential elevations in serum glucose. However, given the differing digestion and absorption kinetics of sucrose and fructose (92–94), it remains possible that the peak hemodynamic effects of fructose ingestion were not fully captured within the timeframe of our measurements. Future research should investigate the time course of renal and hemodynamic responses following ingestion of different sugar types, particularly to determine whether fructose-induced effects occur later than those of sucrose. Additionally, studies utilizing continuous or more frequent monitoring over an extended postprandial period are warranted to better capture the full temporal profile and physiological impact of each sugar type on renal vascular function and blood pressure regulation.

Experimental considerations

There are a few experimental considerations that warrant attention. First, this study involved drinking six 500 mL sport drink doses throughout simulated WrHS. It is possible that ad libitum drinking, rather than prescribed drinking of these sport drinks, and/or rehydrating with a higher-fructose sport drink following fluid restricted simulated WrHS would have unveiled greater elevations of AKI risk. However, this study was designed under the premises that NIOSH recommends that workers arrive at the worksite hydrated and that the worksite have cool fluids readily available (11). Despite this, we acknowledge that this may not always be what is encountered in the workplace, where, for example, workers regularly arrive at the worksite dehydrated (23, 95). In addition, we acknowledge that our study attempted to replicate half a workday and may not capture the entirety of a workday or repeated workdays, and therefore, may not have captured the potential effect of prolonged higher-fructose sport drink consumption on AKI risk. To this end, it is possible that deleterious renal consequences of chronic high fructose beverage ingestion could develop secondary to the independent effects of fructose on the cardiovascular system (96). Second, the sport drinks were the same for every person, despite differences in body size between participants. Thus, the calorie content and fructose load on a per kilogram of body weight basis differed between people. It is possible that a better approach would be to match fructose load / body weight

between participants. However, this study was designed to mimic workers who consume commercially available sport drink, where workers of varying body weights would consume a single bottle (e.g., ~500 mL). Thus, the sport drink volume was fixed between trials. Third, it is important to note that the biomarkers of AKI risk measured in this study are subclinical in nature in that they reflect only an increased risk of AKI but do not indicate a clinical diagnosis. As a result, the clinical implications of these findings remains unclear. Fourth, 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 (97). To increase the reliability of the operator-dependent ultrasound measurements and to ensure transparency the intra-operator coefficient of variation of our sonographer was measured and reported to aid in the interpretation of measurements obtained from Doppler ultrasound (73). Fifth, given the difference in timing of digestion and absorption kinetics between sucrose and fructose (92–94), it remains possible that the peak hemodynamic effects of fructose ingestion were not fully captured within the timeframe of our measurements. Finally, menstrual cycle phase was not controlled for in the women participants. While recent work has identified that AKI risk during simulated WrHS may be higher during the early follicular compared to the mid-luteal phase of the menstrual cycle, this effect is small and AKI risk did not differ from men at any phase of the menstrual cycle (79). Nevertheless, future studies may want to explore interactions between menstrual cycle phase, fructose-sweetened beverage intake and AKI risk in the context of WrHS.

Perspectives

An epidemic of chronic kidney disease has been identified as occurring primarily in agricultural workers regularly exposed to WrHS (98). This disease often occurs in the absence of traditional risk factors (e.g., older age, diabetes, hypertension, obesity), and has therefore been described as non-traditional in nature (98) (CKDnt). It has been proposed that a single heat provoked clinical AKI bout or repeated subclinical AKI bouts can lead to CKDnt (22). Experimental studies demonstrate that hyperthermia and dehydration are potential modulators of the development of heat-related AKI (4), while workplace based interventions that mitigate the development of hyperthermia and dehydration reduce the prevalence of clinical AKI and the risk of CKDnt (3). Occupational hydration recommendations suggest to drink a sport drink when WrHS lasts longer than two hours (11), while previous work in humans and rodents has identified that high-fructose in the beverages could exacerbate AKI risk during WrHS (5, 6, 8). Our study provides strong evidence that fructose in a concentration expected in sport drinks does not elevate AKI

risk, even after controlling for electrolyte concentrations, energy content and large differences in osmolality. Thus, it is unlikely that the modest dose of fructose often found in commercially available sport drinks modify the risk for AKI during WrHS. Therefore, occupational hydration recommendations do not require updates specific to the sugar composition of sport drinks to protect against AKI risk. However, future research is needed to identify the optimal beverage osmolality and electrolyte concentrations to best alleviate WrHS provoked elevations in AKI risk, particularly during longer or repeated WrHS bouts.

Conclusions

This study demonstrates that consuming a higher-fructose sport drink does not independently exacerbate AKI risk following four hours of simulated WrHS compared to drinking a sucrose-sweetened sport drink matched for energy, total carbohydrate, and electrolyte concentrations and relatively well matched for osmolality. These findings suggest that the modest fructose content typical of many sport drinks is unlikely to meaningfully influence AKI risk.

Acknowledgments

We thank the participants for participating in our study. Additionally, we thank our research technicians and other students for their assistance during data collection. Access to raw data can be obtained upon reasonable request via email to the corresponding author.

Grants

This work was supported by an award from the U.S. National Institute of Occupational Safety and Health (R01OH011528).

Conflicts of interest

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

Author contributions

Conceived and designed research: ET, HWH, LS, TDM, BDJ, NGM, ZJS

Performed experiments: ET, MEH, AR, MGO, ZJS

Analyzed the data: ET, MEH, AR, ZJS

Interpreted results of experiments: ET, MEH, AR, MGO, HWH, LS, TDM, BDJ, NGM, ZJS

Prepared figures: ET, ZJS

Drafted manuscript: ET, ZJS

Edited and revised manuscript: ET, MEH, AR, MGO, HWH, LS, TDM, BDJ, NGM, ZJS

Approved final version of manuscript: ET, MEH, AR, MGO, HWH, LS, TDM, BDJ, NGM, ZJS

References

1. **Manal Azzi, Andreas Flouris, Halshka Graczyk, Balint Nafradi, and Natasha Scott, eds.** Heat at work: Implications for safety and health | International Labour Organization [Online]. 2024. https://www.ilo.org/publications/heat-work-implications-safety-and-health [16 Oct. 2024].

2. **Tourula E**, **Specht JW**, **Hite MJ**, **Walker C**, **Garcia S**, **Khandpekar O**, **Yoder HA**, **Zoh RS**, **Johnson BD**, **Wegman DH**, **Glaser J**, **Amorim F**, **Schlader ZJ**. Hyperthermia Predicts Cross-Shift Acute Kidney Injury Risk in Construction Workers. *Kidney International Reports* 10: 2856–2859, 2025. doi: 10.1016/j.ekir.2025.05.044.

3. **Hansson E**, **Jakobsson K**, **Glaser JR**, **Wesseling C**, **Chavarría D**, **Lucas RAI**, **Wegman DH**. Association Between Acute Kidney Injury Hospital Visits and Environmental Heat Stress at a Nicaraguan Sugarcane Plantation. *Workplace Health & Safety* 72: 131, 2024. doi: 10.1177/21650799241235410.

4. **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. *Journal of Applied Physiology* 128: 715–728, 2020. doi: 10.1152/japplphysiol.00787.2019.

5. **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 L-G**. Rehydration with soft drink-like beverages exacerbates dehydration and worsens dehydration-associated renal injury. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 311: R57–R65, 2016. doi: 10.1152/ajpregu.00354.2015.

6. **Milagres T**, **García-Arroyo FE**, **Lanaspa MA**, **Garcia G**, **Ishimoto T**, **Andres-Hernando A**, **Kuwabara M**, **Jensen T**, **Sato Y**, **Glaser J**, **Sánchez-Lozada LG**, **Johnson RJ**, **Roncal-Jimenez C**. Rehydration with fructose worsens dehydration-induced renal damage. *BMC Nephrology* 19: 180, 2018. doi: 10.1186/s12882-018-0963-9.

7. **García-Arroyo FE**, **Tapia E**, **Blas-Marron MG**, **Gonzaga G**, **Silverio O**, **Cristóbal M**, **Osorio H**, **Arellano-Buendía AS**, **Zazueta C**, **Aparicio-Trejo OE**, **Reyes-García JG**, **Pedraza-Chaverri J**, **Soto V**, **Roncal-Jiménez C**, **Johnson RJ**, **Sánchez-Lozada LG**. Vasopressin Mediates the Renal Damage Induced by Limited Fructose Rehydration in Recurrently Dehydrated Rats. *International Journal of Biological Sciences* 13: 961, 2017. doi: 10.7150/ijbs.20074.

8. **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. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 316: R189–R198, 2019.

9. **Chapman CL**, **Grigoryan T**, **Vargas NT**, **Reed EL**, **Kueck PJ**, **Pietrafesa LD**, **Bloomfield AC**, **Johnson BD**, **Schlader ZJ**. High-fructose corn syrup-sweetened soft drink consumption increases vascular resistance in the kidneys at rest and during sympathetic activation. *American Journal of Physiology-Renal Physiology* 318: F1053–F1065, 2020. doi: 10.1152/ajprenal.00374.2019.

10. **Evans GH**, **Shirreffs SM**, **Maughan RJ**. Acute effects of ingesting glucose solutions on blood and plasma volume. *Br J Nutr* 101: 1503–1508, 2009. doi: 10.1017/S0007114508076290.

11. **Jacklitsch BL**, **Williams WJ**, **Musolin K**, **Coca A**, **Kim J-H**, **Turner N**. Occupational exposure to heat and hot environments: revised criteria 2016 [Online]. https://stacks.cdc.gov/view/cdc/37911 [19 Mar. 2025].

12. **Baker LB**, **Jeukendrup AE**. Optimal Composition of Fluid-Replacement Beverages. *Comprehensive Physiology* 4: 575–620, 2014. doi: 10.1002/j.2040-4603.2014.tb00555.x.

13. **Lignier MJ**, **Hess HW**, **Freemas JA**, **Johnson BD**, **Hostler D**, **Schlader ZJ**. The effect of consuming a sucrose-containing sports drink on acute kidney injury risk during a 4 h simulated occupational heat stress. *Appl Physiol Nutr Metab* 50: 1–12, 2025. doi: 10.1139/apnm-2024-0261.

14. **Atkins WC**, **McKenna ZJ**, **McDermott BP**. Sports drinks do not increase acute kidney injury risk in males during industrial work in the heat when euhydration is maintained, a randomized crossover trial. *Appl Physiol Nutr Metab* 49: 844–854, 2024. doi: 10.1139/apnm-2023-0393.

15. **Craig CL**, **Marshall AL**, **Sjöström M**, **Bauman AE**, **Booth ML**, **Ainsworth BE**, **Pratt M**, **Ekelund U**, **Yngve A**, **Sallis JF**, **Oja P**. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381–1395, 2003. doi: 10.1249/01.MSS.0000078924.61453.FB.

16. **Lee PH**, **Macfarlane DJ**, **Lam T**, **Stewart SM**. Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. *Int J Behav Nutr Phys Act* 8: 115, 2011. doi: 10.1186/1479-5868-8-115.

17. **Bain AR**, **Jay O**. Does summer in a humid continental climate elicit an acclimatization of human thermoregulatory responses? *Eur J Appl Physiol* 111: 1197–1205, 2011. doi: 10.1007/s00421-010-1743-9.

18. **White JS**, **Hobbs LJ**, **Fernandez S**. Fructose content and composition of commercial HFCS-sweetened carbonated beverages. *Int J Obes* 39: 176–182, 2015. doi: 10.1038/ijo.2014.73.

19. **Rowlands DS**, **Kopetschny BH**, **Badenhorst CE**. The Hydrating Effects of Hypertonic, Isotonic and Hypotonic Sports Drinks and Waters on Central Hydration During Continuous Exercise: A Systematic Meta-Analysis and Perspective. *Sports Med* 52: 349–375, 2022. doi: 10.1007/s40279-021-01558-y.

20. **American College of Sports Medicine**, **Sawka MN**, **Burke LM**, **Eichner ER**, **Maughan RJ**, **Montain SJ**, **Stachenfeld NS**. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 377–390, 2007. doi: 10.1249/mss.0b013e31802ca597.

21. **Hagan RD**, **Diaz FJ**, **Horvath SM**. Plasma volume changes with movement to supine and standing positions. *J Appl Physiol Respir Environ Exerc Physiol* 45: 414–417, 1978. doi: 10.1152/jappl.1978.45.3.414.

22. **Chapman CL**, **Hess HW**, **Lucas RAI**, **Glaser J**, **Saran R**, **Bragg-Gresham J**, **Wegman DH**, **Hansson E**, **Minson CT**, **Schlader ZJ**. Occupational heat exposure and the risk of chronic kidney disease of nontraditional origin in the United States. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 321: R141–R151, 2021. doi: 10.1152/ajpregu.00103.2021.

23. **Specht JW**, **Garcia S**, **Wegman DH**, **Glaser J**, **Schlader ZJ**, **Amorim FT**. Heat strain in road construction workers during the summer in New Mexico: a preliminary study. *Annals of Work Exposures and Health* 69: 225–229, 2025. doi: 10.1093/annweh/wxae097.

24. **Glaser J**, **Hansson E**, **Weiss I**, **Wesseling C**, **Jakobsson K**, **Ekström U**, **Apelqvist J**, **Lucas R**, **Arias Monge E**, **Peraza S**, **Hogstedt C**, **Wegman DH**. Preventing kidney injury among sugarcane workers: promising evidence from enhanced workplace interventions. *Occup Environ Med* 77: 527–534, 2020. doi: 10.1136/oemed-2020-106406.

25. **Herrmann SD**, **Willis EA**, **Ainsworth BE**. The 2024 Compendium of Physical Activities and its expansion. *Journal of Sport and Health Science* 13: 1–2, 2024. doi: 10.1016/j.jshs.2023.09.008.

26. **Tourula E**, **Hite MJ**, **Heikkinen ME**, **Hess HW**, **Amorim FT**, **Mickleborough TD**, **Johnson BD**, **Hostler D**, **Schlader ZJ**. Ad libitum drinking does not mitigate acute kidney injury risk nor elevations in markers of oxidative stress and inflammation during simulated occupational heat stress. *Am J Physiol Renal Physiol* 330: F71–F88, 2026. doi: 10.1152/ajprenal.00234.2025.

27. **Hase K**, **Kaya M**, **Yamazaki N**, **Andrews BJ**, **Zavatsky AB**, **Halliday SE**. Biomechanics of Rowing. *JSME International Journal Series C Mechanical Systems, Machine Elements and Manufacturing* 45: 1073–1081, 2002. doi: 10.1299/jsmec.45.1073.

28. **Ramanathan NL**. A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology* 19: 531–533, 1964. doi: 10.1152/jappl.1964.19.3.531.

29. **Tanaka H**, **Monahan KD**, **Seals DR**. Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology* 37: 153–156, 2001. doi: 10.1016/S0735-1097(00)01054-8.

30. **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.

31. **Hall JE**, **Hall ME**. *Guyton and Hall textbook of medical physiology*. 14th edition. Philadelphia, PA: Elsevier, 2021.

32. **Cone EJ**, **Caplan YH**, **Moser F**, **Robert T**, **Shelby MK**, **Black DL**. Normalization of urinary drug concentrations with specific gravity and creatinine. *J Anal Toxicol* 33: 1–7, 2009. doi: 10.1093/jat/33.1.1.

33. **Hess HW**, **Stooks JJ**, **Baker TB**, **Chapman CL**, **Johnson BD**, **Pryor RR**, **Basile DP**, **Monroe JC**, **Hostler D**, **Schlader ZJ**. Kidney injury risk during prolonged exposure to current and projected wet bulb temperatures occurring during extreme heat events in healthy young men. *J Appl Physiol (1985)* 133: 27–40, 2022. doi: 10.1152/japplphysiol.00601.2021.

34. **Kashani K**, **Al-Khafaji A**, **Ardiles T**, **Artigas A**, **Bagshaw SM**, **Bell M**, **Bihorac A**, **Birkhahn R**, **Cely CM**, **Chawla LS**, **Davison DL**, **Feldkamp T**, **Forni LG**, **Gong MN**, **Gunnerson KJ**, **Haase M**, **Hackett J**, **Honore PM**, **Hoste EAJ**, **Joannes-Boyau O**, **Joannidis M**, **Kim P**, **Koyner JL**, **Laskowitz DT**, **Lissauer ME**, **Marx G**, **McCullough PA**, **Mullaney S**, **Ostermann M**, **Rimmelé T**, **Shapiro NI**, **Shaw AD**, **Shi J**, **Sprague AM**, **Vincent J-L**, **Vinsonneau C**, **Wagner L**, **Walker MG**, **Wilkerson RG**, **Zacharowski K**, **Kellum JA**. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care* 17: R25, 2013. doi: 10.1186/cc12503.

35. **Kashani K**, **Cheungpasitporn W**, **Ronco C**. Biomarkers of acute kidney injury: the pathway from discovery to clinical adoption. *Clinical Chemistry and Laboratory Medicine (CCLM)* 55: 1074–1089, 2017. doi: 10.1515/cclm-2016-0973.

36. **Nalesso F**, **Cattarin L**, **Gobbi L**, **Fragasso A**, **Garzotto F**, **Calò LA**. Evaluating Nephrocheck® as a Predictive Tool for Acute Kidney Injury. *International Journal of Nephrology and Renovascular Disease* 13: 85, 2020. doi: 10.2147/IJNRD.S198222.

37. **Han WK**, **Bailly V**, **Abichandani R**, **Thadhani R**, **Bonventre JV**. Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int* 62: 237–244, 2002. doi: 10.1046/j.1523-1755.2002.00433.x.

38. **Vaidya VS**, **Ferguson MA**, **Bonventre JV**. Biomarkers of acute kidney injury. *Annu Rev Pharmacol Toxicol* 48: 463–493, 2008. doi: 10.1146/annurev.pharmtox.48.113006.094615.

39. **Buonafine M**, **Martinez-Martinez E**, **Jaisser F**. More than a simple biomarker: the role of NGAL in cardiovascular and renal diseases. *Clin Sci (Lond)* 132: 909–923, 2018. doi: 10.1042/CS20171592.

40. **Mishra J**, **Dent C**, **Tarabishi R**, **Mitsnefes MM**, **Ma Q**, **Kelly C**, **Ruff SM**, **Zahedi K**, **Shao M**, **Bean J**, **Mori K**, **Barasch J**, **Devarajan P**. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 365: 1231–1238, 2005. doi: 10.1016/S0140-6736(05)74811-X.

41. **Haase-Fielitz A**, **Bellomo R**, **Devarajan P**, **Story D**, **Matalanis G**, **Dragun D**, **Haase M**. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery--a prospective cohort study. *Crit Care Med* 37: 553–560, 2009. doi: 10.1097/CCM.0b013e318195846e.

42. **Johnson RJ**, **Perez-Pozo SE**, **Lillo JL**, **Grases F**, **Schold JD**, **Kuwabara M**, **Sato Y**, **Hernando AA**, **Garcia G**, **Jensen T**, **Rivard C**, **Sanchez-Lozada LG**, **Roncal C**, **Lanaspa MA**. Fructose increases risk for kidney stones: potential role in metabolic syndrome and heat stress. *BMC Nephrol* 19: 315, 2018. doi: 10.1186/s12882-018-1105-0.

43. **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.

44. **Roncal-Jimenez C**, **García-Trabanino R**, **Barregard L**, **Lanaspa MA**, **Wesseling C**, **Harra T**, **Aragón A**, **Grases F**, **Jarquin ER**, **González MA**, **Weiss I**, **Glaser J**, **Sánchez-Lozada LG**, **Johnson RJ**. Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy. *Am J Kidney Dis* 67: 20–30, 2016. doi: 10.1053/j.ajkd.2015.08.021.

45. **Deshmane SL**, **Kremlev S**, **Amini S**, **Sawaya BE**. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. *J Interferon Cytokine Res* 29: 313–326, 2009. doi: 10.1089/jir.2008.0027.

46. **Liu Y**, **Xu K**, **Xiang Y**, **Ma B**, **Li H**, **Li Y**, **Shi Y**, **Li S**, **Bai Y**. Role of MCP-1 as an inflammatory biomarker in nephropathy. *Frontiers in Immunology* 14: 1303076, 2024. doi: 10.3389/fimmu.2023.1303076.

47. **Hansson E**, **Broberg K**, **Wijkström J**, **Glaser J**, **Gonzalez-Quiroz M**, **Ekström U**, **Abrahamson M**, **Jakobsson K**. An explorative study of inflammation-related proteins associated with kidney injury in male heat-stressed workers. *Journal of Thermal Biology* 112: 103433, 2023. doi: 10.1016/j.jtherbio.2022.103433.

48. **Lagzdina R**, **Rumaka M**, **Gersone G**, **Tretjakovs P**. Circulating Levels of IL-8 and MCP-1 in Healthy Adults: Changes after an Acute Aerobic Exercise and Association with Body Composition and Energy Metabolism. *Int J Mol Sci* 24: 14725, 2023. doi: 10.3390/ijms241914725.

49. **Singh S**, **Anshita D**, **Ravichandiran V**. MCP-1: Function, regulation, and involvement in disease. *International Immunopharmacology* 101: 107598, 2021. doi: 10.1016/j.intimp.2021.107598.

50. **Chan MHS**, **Carey AL**, **Watt MJ**, **Febbraio MA**. Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 287: R322–R327, 2004. doi: 10.1152/ajpregu.00030.2004.

51. **Febbraio MA**, **Hiscock N**, **Sacchetti M**, **Fischer CP**, **Pedersen BK**. Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. *Diabetes* 53: 1643–1648, 2004. doi: 10.2337/diabetes.53.7.1643.

52. **Pedersen BK**, **Steensberg A**, **Fischer C**, **Keller C**, **Keller P**, **Plomgaard P**, **Wolsk-Petersen E**, **Febbraio M**. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Proceedings of the Nutrition Society* 63: 263–267, 2004. doi: 10.1079/PNS2004338.

53. **Ramesh G**, **Reeves WB**. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest* 110: 835–842, 2002. doi: 10.1172/JCI15606.

54. **Akcay A**, **Nguyen Q**, **Edelstein CL**. Mediators of Inflammation in Acute Kidney Injury. *Mediators Inflamm* 2009: 137072, 2009. doi: 10.1155/2009/137072.

55. **Lee DW**, **Faubel S**, **Edelstein CL**. Cytokines in acute kidney injury (AKI). *Clin Nephrol* 76: 165–173, 2011. doi: 10.5414/cn106921.

56. **Hasan AA**, **Kalinina E**, **Tatarskiy V**, **Shtil A**. The Thioredoxin System of Mammalian Cells and Its Modulators. *Biomedicines* 10: 1757, 2022. doi: 10.3390/biomedicines10071757.

57. **Kasuno K**, **Shirakawa K**, **Yoshida H**, **Mori K**, **Kimura H**, **Takahashi N**, **Nobukawa Y**, **Shigemi K**, **Tanabe S**, **Yamada N**, **Koshiji T**, **Nogaki F**, **Kusano H**, **Ono T**, **Uno K**, **Nakamura H**, **Yodoi J**, **Muso E**, **Iwano M**. Renal redox dysregulation in AKI: application for oxidative stress marker of AKI. *American Journal of Physiology-Renal Physiology* 307: F1342–F1351, 2014. doi: 10.1152/ajprenal.00381.2013.

58. **Hofer S**, **Rosenhagen C**, **Nakamura H**, **Yodoi J**, **Bopp C**, **Zimmermann JB**, **Goebel M**, **Schemmer P**, **Hoffmann K**, **Schulze-Osthoff K**, **Breitkreutz R**, **Weigand MA**. Thioredoxin in human and experimental sepsis. *Crit Care Med* 37: 2155–2159, 2009. doi: 10.1097/CCM.0b013e31819fff67.

59. **Liu X**, **Dong X**, **Hu Y**, **Fang Y**. Identification of thioredoxin-1 as a biomarker of lung cancer and evaluation of its prognostic value based on bioinformatics analysis. *Front Oncol* 13, 2023. doi: 10.3389/fonc.2023.1080237.

60. **Lee YJ**, **Kim Y**, **Choi BB**, **Kim JR**, **Ko HM**, **Suh KH**, **Lee JS**. The blood level of thioredoxin 1 as a supporting biomarker in the detection of breast cancer. *BMC Cancer* 22: 12, 2022. doi: 10.1186/s12885-021-09055-1.

61. **Lau E**, **Marques C**, **Pestana D**, **Santoalha M**, **Carvalho D**, **Freitas P**, **Calhau C**. The role of I-FABP as a biomarker of intestinal barrier dysfunction driven by gut microbiota changes in obesity. *Nutrition & Metabolism* 13: 31, 2016. doi: 10.1186/s12986-016-0089-7.

62. **Foster J**, **Mckenna ZJ**, **Atkins WC**, **Jarrard CP**, **Crandall CG**. Aging Increases Enterocyte Damage during a 3-Hour Exposure to Very Hot and Dry Heat: A Preliminary Study. *Biology* 12: 1088, 2023. doi: 10.3390/biology12081088.

63. **Walter E**, **Gibson OR**, **Stacey M**, **Hill N**, **Parsons IT**, **Woods D**. Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse. *Eur J Appl Physiol* 121: 1179–1187, 2021. doi: 10.1007/s00421-021-04603-w.

64. **Sumi D**, **Nagatsuka H**, **Matsuo K**, **Okazaki K**, **Goto K**. The Impact of Heat Acclimation on Gastrointestinal Function following Endurance Exercise in a Hot Environment. *Nutrients* 15: 216, 2023. doi: 10.3390/nu15010216.

65. **Snipe RMJ**, **Costa RJS**. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile? *Journal of Science and Medicine in Sport* 21: 771–776, 2018. doi: 10.1016/j.jsams.2017.12.014.

66. **Mohr AE**, **Crawford M**, **Jasbi P**, **Fessler S**, **Sweazea KL**. Lipopolysaccharide and the gut microbiota: considering structural variation. *FEBS Letters* 596: 849–875, 2022. doi: 10.1002/1873-3468.14328.

67. **McKenna ZJ**, **Atkins WC**, **Wallace T**, **Jarrard CP**, **Crandall CG**, **Foster J**. Gastrointestinal permeability and kidney injury risk during hyperthermia in young and older adults. *Exp Physiol* 110: 79–92, 2024. doi: 10.1113/EP092204.

68. **Wallett AM**, **Etxebarria N**, **Beard NA**, **Saunders PU**, **Welvaert M**, **Périard JD**, **McKune AJ**, **Pyne DB**. Running at Increasing Intensities in the Heat Induces Transient Gut Perturbations. .

69. **Etxebarria N**, **Beard NA**, **Gleeson M**, **Wallett A**, **McDonald WA**, **Pumpa KL**, **Pyne DB**. Dietary Intake and Gastrointestinal Integrity in Runners Undertaking High-Intensity Exercise in the Heat. .

70. **Chapman CL**, **Johnson BD**, **Hostler D**, **Lema PC**, **Schlader ZJ**. Reliability and agreement of human renal and segmental artery hemodynamics measured using Doppler ultrasound. *J Appl Physiol (1985)* 128: 627–636, 2020. doi: 10.1152/japplphysiol.00813.2019.

71. **Manoharan G**, **Pijls NHJ**, **Lameire N**, **Verhamme K**, **Heyndrickx GR**, **Barbato E**, **Wijns W**, **Madaric J**, **Tielbeele X**, **Bartunek J**, **De Bruyne B**. Assessment of renal flow and flow reserve in humans. *J Am Coll Cardiol* 47: 620–625, 2006. doi: 10.1016/j.jacc.2005.08.071.

72. **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.

73. **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. *Journal of Applied Physiology* 127: 974–983, 2019. doi: 10.1152/japplphysiol.00403.2019.

74. **Freemas JA**, **Worley ML**, **Gabler MC**, **Hess HW**, **Goss CS**, **Baker TB**, **Johnson BD**, **Chapman CL**, **Schlader ZJ**. Renal vascular control during normothermia and passive heat stress does not differ between healthy younger men and women. *American Journal of Physiology-Renal Physiology* 326: F802–F813, 2024. doi: 10.1152/ajprenal.00034.2024.

75. **Foss JL**, **Constantini K**, **Mickleborough TD**, **Chapman RF**. Short-term arrival strategies for endurance exercise performance at moderate altitude. *Journal of Applied Physiology* 123: 1258–1265, 2017. doi: 10.1152/japplphysiol.00314.2017.

76. **Gore CJ**, **Bourdon PC**, **Woolford SM**, **Ostler LM**, **Eastwood A**, **Scroop GC**. Time and Sample Site Dependency of the Optimized CO-Rebreathing Method. *Medicine & Science in Sports & Exercise* 38: 1187–1193, 2006. doi: 10.1249/01.mss.0000222848.35004.41.

77. **Belviranli M**, **Okudan N**, **Kabak B**. The Effects of Acute High-Intensity Interval Training on Hematological Parameters in Sedentary Subjects. *Medical Sciences* 5: 15, 2017. doi: 10.3390/medsci5030015.

78. **Hess HW**, **Baker TB**, **Tarr ML**, **Zoh RS**, **Johnson BD**, **Hostler D**, **Schlader ZJ**. Occupational Heat Stress Recommendation Compliance Attenuates AKI Risk Compared with a Work–Rest Ratio–Matched, Positive Control Scenario. *Kidney360* 4: 1752, 2023. doi: 10.34067/KID.0000000000000288.

79. **Freemas JA**, **Goss CS**, **Ables R**, **Baker TB**, **Bruinvels G**, **Mündel T**, **Martin BJ**, **Carter SJ**, **Chapman RF**, **Khandpekar OM**, **Zoh RS**, **Schlader ZJ**. Fluid balance, kidney function and acute kidney injury risk in healthy men and women during simulated work-related heat stress. .

80. **Hess HW**, **Heikkinen ME**, **Tourula E**, **Hite MJ**, **Rivers K**, **Zoh RS**, **Johnson BD**, **Hostler D**, **Schlader ZJ**. Influence of work intensity on acute kidney injury risk during simulated occupational heat stress. *Journal of Applied Physiology* 138: 706–717, 2025. doi: 10.1152/japplphysiol.00590.2024.

81. **Sparks MA**, **Crowley SD**, **Gurley SB**, **Mirotsou M**, **Coffman TM**. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol* 4: 1201–1228, 2014. doi: 10.1002/cphy.c130040.

82. **Cogan MG**. Angiotensin II: a powerful controller of sodium transport in the early proximal tubule. *Hypertension* 15: 451–458, 1990. doi: 10.1161/01.hyp.15.5.451.

83. **Coppola S**, **Frömter E**. An electrophysiological study of angiotensin II regulation of Na-HCO3 cotransport and K conductance in renal proximal tubules. I. Effect of picomolar concentrations. *Pflugers Arch* 427: 143–150, 1994. doi: 10.1007/BF00585953.

84. **Casellas D**, **Carmines PK**, **Dupont M**, **Redon P**, **Moore LC**. Arteriolar renin and vascular effects of angiotensin II in juxtamedullary nephrons. *Kidney Int Suppl* 30: S60-64, 1990.

85. **Spät A**, **Hunyady L**. Control of aldosterone secretion: a model for convergence in cellular signaling pathways. *Physiol Rev* 84: 489–539, 2004. doi: 10.1152/physrev.00030.2003.

86. **Danziger J**, **Zeidel ML**. Osmotic homeostasis. *Clin J Am Soc Nephrol* 10: 852–862, 2015. doi: 10.2215/CJN.10741013.

87. **Skorecki KL**, **Brown D**, **Ercolani L**, **Ausiello DA**. Molecular Mechanisms of Vasopressin Action in the Kidney. *Comprehensive Physiology* 1991: 1185–1218, 1991. doi: 10.1002/j.2040-4603.1991.tb01583.x.

88. **Morgenthaler NG**, **Struck J**, **Alonso C**, **Bergmann A**. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 52: 112–119, 2006. doi: 10.1373/clinchem.2005.060038.

89. **Chapman CL**, **Johnson BD**, **Parker MD**, **Hostler D**, **Pryor RR**, **Schlader Z**. Kidney physiology and pathophysiology during heat stress and the modification by exercise, dehydration, heat acclimation and aging. *Temperature (Austin)* 8: 108–159, [date unknown]. doi: 10.1080/23328940.2020.1826841.

90. **Stornello M**, **Valvo EV**, **Distefano M**, **Golino M**, **Cocuzza F**, **Vasques E**, **Modicano V**, **Leone S**, **Giustolisi V**. Influence of glucose load on cardiovascular and humoral responses to a cold pressure test. *J Hum Hypertens* 9: 93–99, 1995.

91. **Monnard CR**, **Fellay B**, **Scerri I**, **Grasser EK**. Substantial Inter-Subject Variability in Blood Pressure Responses to Glucose in a Healthy, Non-obese Population. *Front Physiol* 8, 2017. doi: 10.3389/fphys.2017.00507.

92. **Dong Y**, **Li W**, **Yin J**. The intestinal-hepatic axis: a comprehensive review on fructose metabolism and its association with mortality and chronic metabolic diseases. *Critical Reviews in Food Science and Nutrition* 64: 12473–12486, 2024. doi: 10.1080/10408398.2023.2253468.

93. **Gonzalez JT**, **Fuchs CJ**, **Betts JA**, **Van Loon LJC**. Glucose Plus Fructose Ingestion for Post-Exercise Recovery—Greater than the Sum of Its Parts? *Nutrients* 9: 344, 2017. doi: 10.3390/nu9040344.

94. **Fuchs CJ**, **Gonzalez JT**, **van Loon LJC**. Fructose co-ingestion to increase carbohydrate availability in athletes. *J Physiol* 597: 3549–3560, 2019. doi: 10.1113/JP277116.

95. **Mix J**, **Elon L**, **Mac VVT**, **Flocks J**, **Economos E**, **Tovar-Aguilar AJ**, **Hertzberg VS**, **McCauley LA**. Hydration Status, Kidney Function, and Kidney Injury in Florida Agricultural Workers. *Journal of Occupational and Environmental Medicine* 60: e253–e260, 2018.

96. **Brown CM**, **Dulloo AG**, **Yepuri G**, **Montani J-P**. Fructose ingestion acutely elevates blood pressure in healthy young humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 294: R730–R737, 2008. doi: 10.1152/ajpregu.00680.2007.

97. **Chapman CL**, **Holt SM**, **O'Connell CT**, **Brazelton SC**, **Medved HN**, **Howells WAB**, **Reed EL**, **Needham KW**, **Halliwill JR**, **Minson CT**. Hypohydration attenuates increases in creatinine clearance to oral protein loading and the renal hemodynamic response to exercise pressor reflex. *J Appl Physiol (1985)* 136: 492–508, 2024. doi: 10.1152/japplphysiol.00728.2023.

98. **Wesseling C**, **Glaser J**, **Rodríguez-Guzmán J**, **Weiss I**, **Lucas R**, **Peraza S**, **da Silva AS**, **Hansson E**, **Johnson RJ**, **Hogstedt C**, **Wegman DH**, **Jakobsson K**. Chronic kidney disease of non-traditional origin in Mesoamerica: a disease primarily driven by occupational heat stress. *Rev Panam Salud Publica* 44: e15, 2020. doi: 10.26633/RPSP.2020.15.

Figure Legends

Figure 1: Overview and order of data collection during the experimental trials. Relative Humidity (RH), Carbon Monoxide (CO).

Figure 2: Mean arterial pressure (A), heart rate (B), segmental artery blood velocity (C) and segmental artery vascular resistance (D) during the cold pressor test (CPT) following consumption of 500 mL of a sport drink sweetened with sucrose (Sucrose) or 55% fructose / 45% glucose (Fructose55). Data are presented as a change from minute 0. Data was analyzed using a two-way linear mixed model with Šidák's post hoc comparisons. Data are n=12 at baseline (0-minutes) and n=11 at minute-2 in both trials, with data at minute-1 n=11 in the Fructose55 trial and n=12 in the Sucrose trial. Data are presented as mean ± SD and individual values. * Indicates significantly different from Sucrose trial ($P \leq 0.05$).

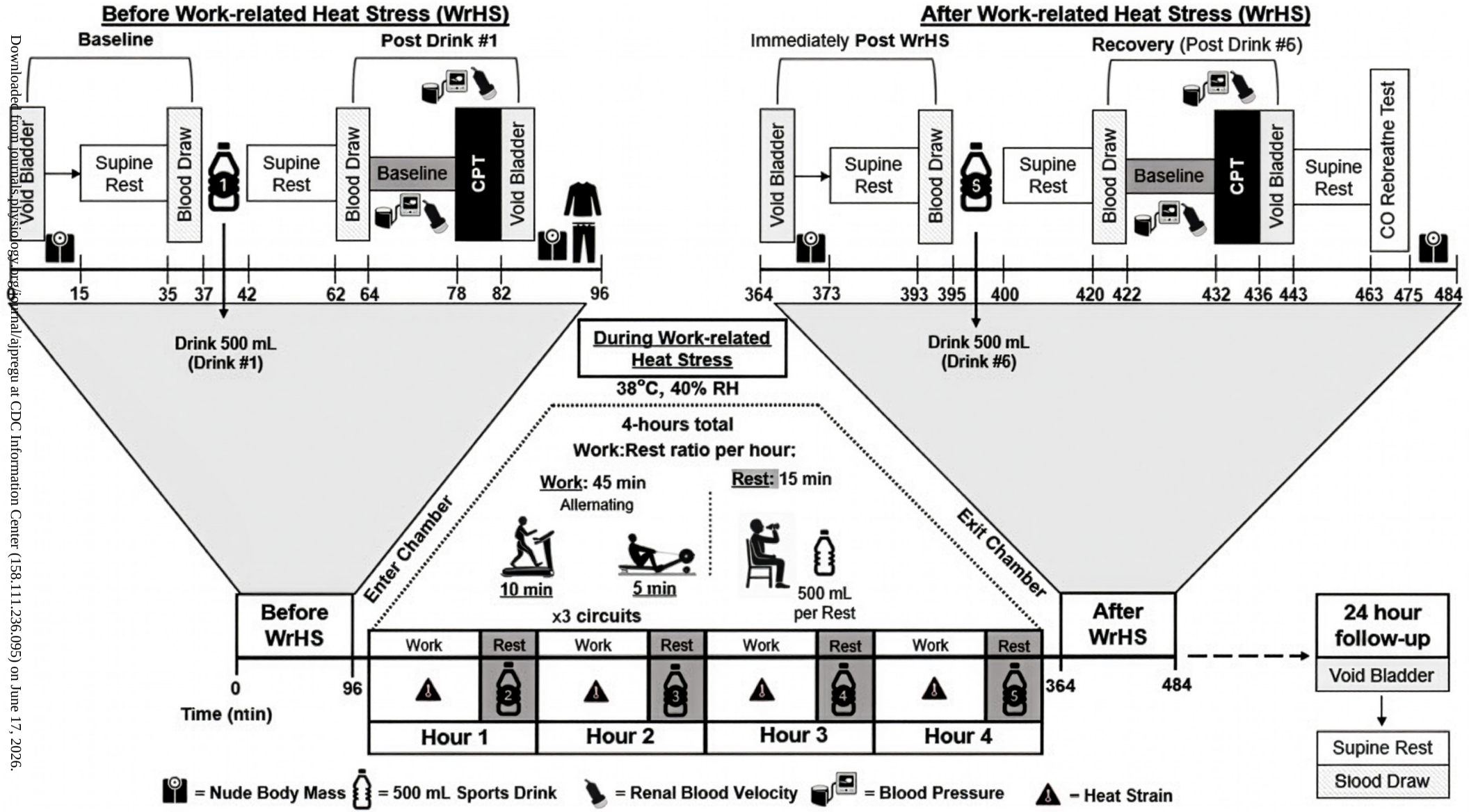
Figure 3: Core temperature (A), mean skin temperature (B), heart rate (C), and heart rate presented as a percentage of age-predicted maximum (D) throughout the 4-hour work-related heat stress (WrHS) simulation. Data are presented as mean ± SD and were analyzed using a two-way linear mixed model with Šidák's post hoc comparisons where necessary. P-values from the two-way linear mixed models are reported. All data are n=12. * Indicates significantly different from Sucrose trial ($p \leq 0.05$).

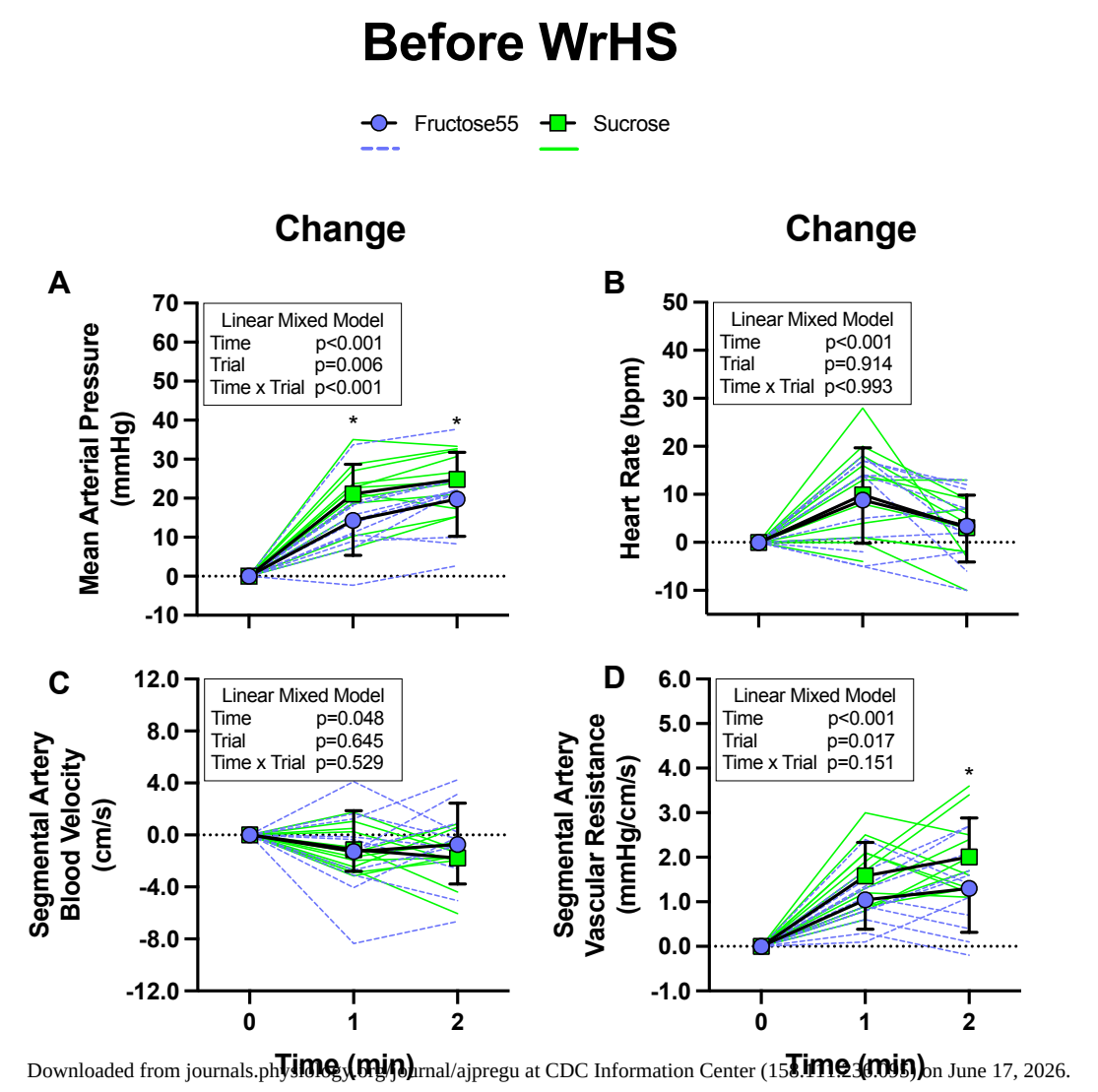
Figure 4: Acute kidney injury risk biomarker responses before, during and following work-related heat stress. Urine insulin-like growth factor-binding protein 7 ($IGFBP7_{USG}$) (A), urine tissue inhibitor metalloproteinase 2 ($TIMP\text{-}2_{USG}$) (B) and the product of urine IGFBP7 and TIMP-2 ($[IGFBP7 \bullet TIMP\text{-}2]_{USG}$) (C). Urinary biomarkers normalized to urine specific gravity and presented before (Baseline), immediately after WrHS (Post-WrHS), ~75 minutes after-WrHS (Recovery), and at the 24-hour follow-up (24 h). Data were log transformed prior to statistical analyses if required and were analyzed using a two-way linear mixed models with p-values reported. All data are represented as mean ± SD and individual values. Urine data at all timepoints in the Fructose55 trial are n=12 and n=12 in the sucrose trial, except at the 24-hour follow-up (24 h), which are n=11.

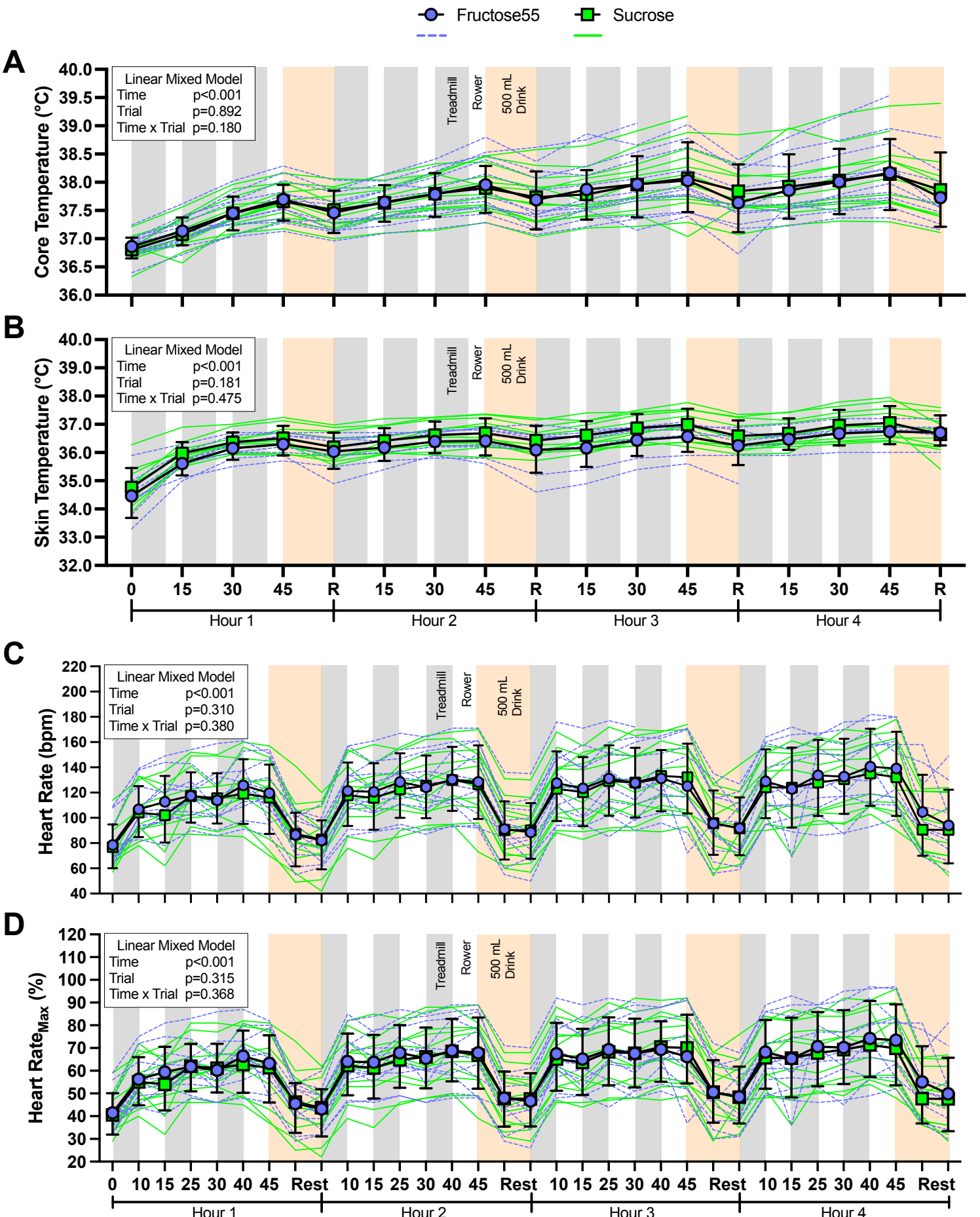
Figure 5: Mean arterial pressure (A), heart rate (B), segmental artery blood velocity (C) and segmental artery vascular resistance (D) during the cold pressor test (CPT) after work-related heat stress (WrHS).

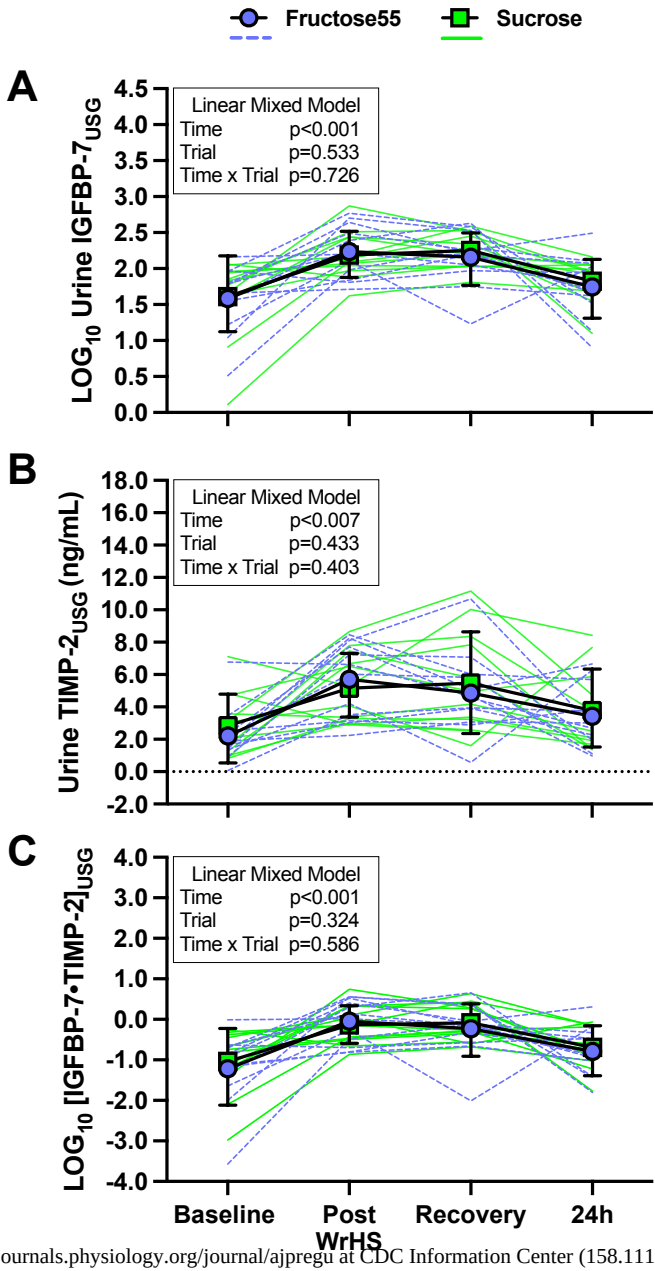
Data are presented as a change (D) from minute 0. Data were analyzed using a two-way linear mixed model. All data are n=11 in the Fructose trial. Data are n=12 at rest (0-minutes), n=11 at minute-1, and n=12 at minute-2 in the Sucrose trial. Data are presented as mean ± SD and individual values. * Indicates significantly different from Sucrose trial ($P \leq 0.05$).

Figure 6: Peak change (Δ) in mean arterial pressure (A), heart rate (B), segmental artery blood velocity (C) and segmental artery vascular resistance (D) during the cold pressor test before- work-related heat stress (WrHS) and after-WrHS. Data were analyzed using a two-way linear mixed model. All data before-WrHS in both trials are n=12. After-WrHS, all data was n=11 in the Fructose55 trial and n=12 in the Sucrose trial. Data are presented as mean ± SD and individual values. * Indicates significantly different from Sucrose trial ($P \leq 0.05$).

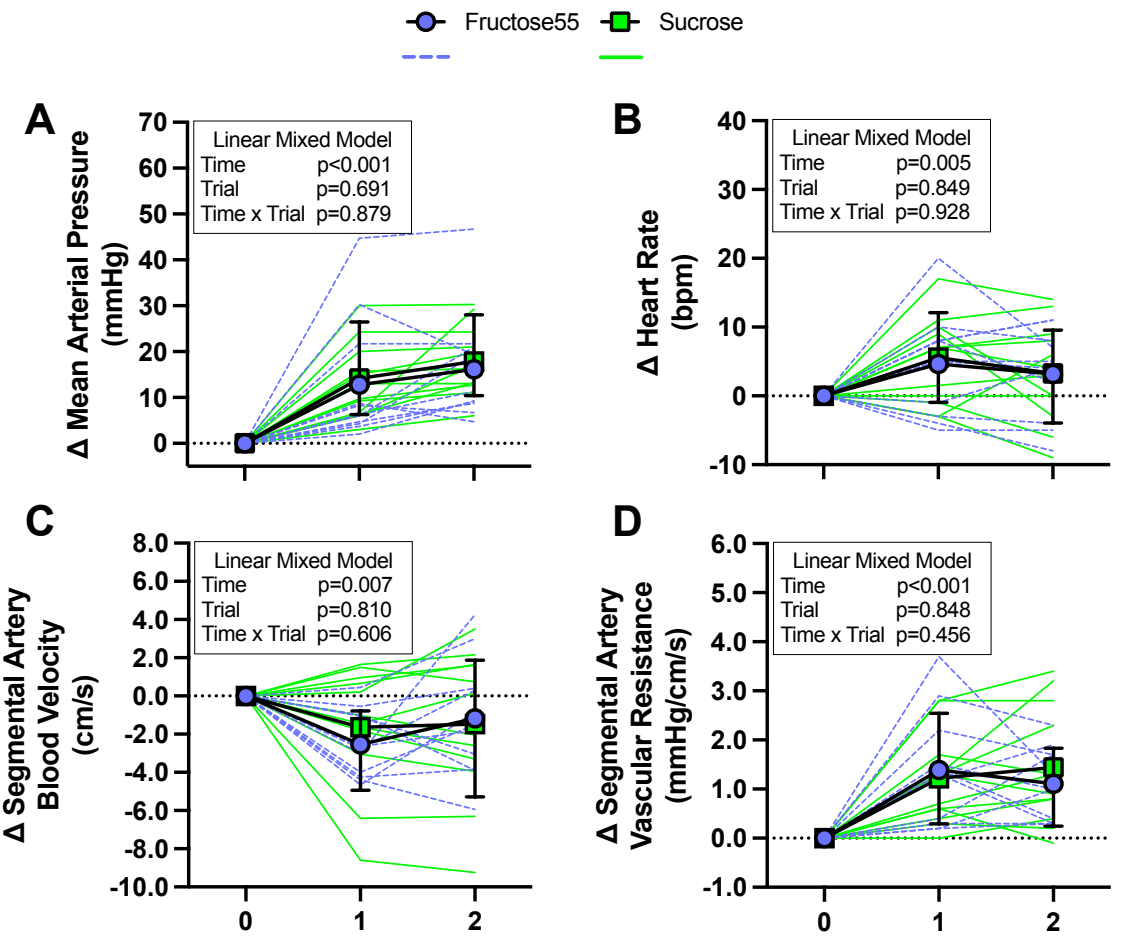








After WrHS



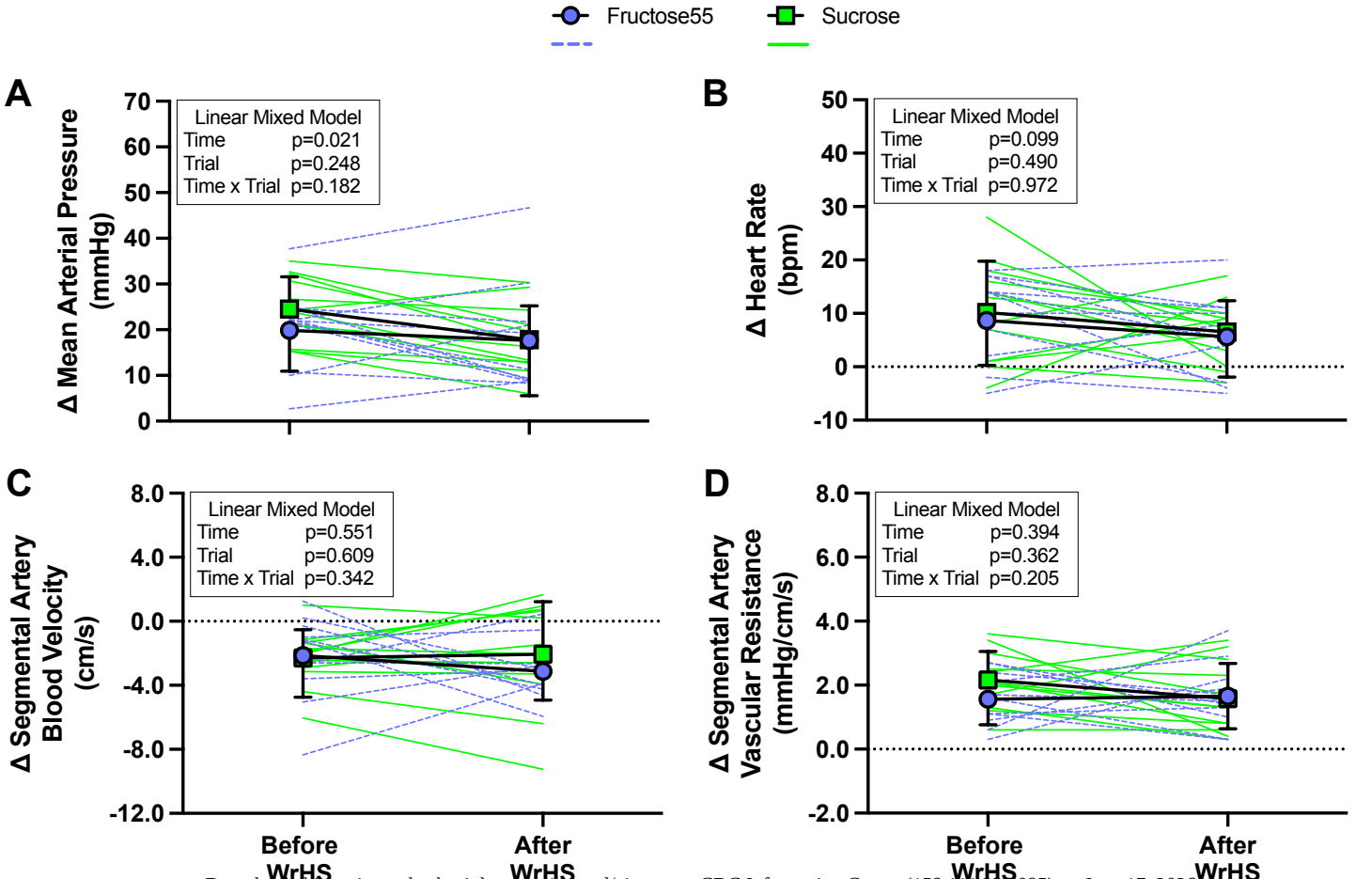


Table 1: Participant Characteristics (n=12; 6 women, 6 men)

	Mean ± SD	**[min, max]**
Age (y)	26 ± 7	[19, 41]
Height (cm)	175 ± 9	[156.0, 190.5]
Weight (kg)	77.9 ± 18.0	[50.2, 107.8]
Body surface area (m^2)	1.9 ± 0.3	[1.5, 2.29]
Body mass index (kg/m^2)	25.4 ± 4.4	[18.7, 33.5]
Resting heart rate (bpm)	65 ± 11	[49, 90]
Age-predicted heart rate max (bpm)	190 ± 5	[179, 195]
Systolic blood pressure (mmHg)	115 ± 8	[102, 126]
Diastolic blood pressure (mmHg)	69 ± 9	[49, 83]
Mean arterial pressure (mmHg)	84 ± 7	[69, 95]

Table 2: Sport drink formulation per 500 mL

		Fructose55	**Sucrose**
Total Carbohydrate (g)		28	28
Energy (kcal)		116	116
Electrolytes (mg)	Na^+	210	210
	K^+	65	65
Fructose load (g)		15.4	14
Osmolality (mOsm/L)		337 ± 6	204 ± 2

Table 3: Post drink #1 resting renal hemodynamics.

Measurement	Fructose55	Sucrose	p-value
			Paired t-test
Mean Arterial Pressure (mmHg)	76 ± 8	75 ± 7	0.530
Heart Rate (bpm)	56 ± 10	55 ± 12	0.749
Renal Artery Blood Velocity (cm/s)	31.7 ± 8.0	33.5 ± 6.4	0.523
Renal Artery Vascular Resistance (mmHg/cm/s)	2.5 ± 0.6	2.3 ± 0.5	0.377
Segmental Artery Blood Velocity (cm/s)	17.9 ± 2.8	18.1 ± 3.1	0.868
Segmental Artery Vascular Resistance (mmHg/cm/s)	4.3 ± 0.8	4.2 ± 0.6	0.682

Data are presented as means ± SD and analyzed using two-tailed paired t-tests with p-values reported. All data are n=12.

Table 4: Fluid regulation and kidney function markers following acute consumption of 500 mL Sport Drink

Measurement	Timepoint	Fructose55	Sucrose	*P-value*	
				Linear Mixed Model	
Plasma Volume (mL)	Baseline	4654 ± 1923	4493 ± 1820	Time:	p = 0.812
	Post Drink-1	4608 ± 2009	4538 ± 1900	Trial:	p = 0.993
				Time x Trial:	p = 0.372
Plasma Glucose (mg/dL)	Baseline	91 ± 13	87 ± 11	Time:	p = 0.014
	Post Drink-1	90 ± 12*	107 ± 20	Trial:	p = 0.223
				Time x Trial:	p = 0.011
Serum Osmolality (mOsm/kg)	Baseline	297 ± 23	302 ± 7	Time:	p = 0.893
	Post Drink-1	300 ± 6	301 ± 14	Trial:	p = 0.453
				Time x Trial:	p = 0.568
Serum Uric Acid (mg/dL)	Baseline	5.4 ± 1.2	5.3 ± 1.2	Time:	p = 0.122
	Post Drixnk-1	5.4 ± 1.2	5.1 ± 1.3	Trial:	p = 0.179
				Time x Trial:	p = 0.083
Serum Copeptin (pg/mL)	Baseline	19.3 ± 12.3	21.8 ± 13.1	Time:	p = 0.570
	Post Drink-1	23.0 ± 12.6	20.1 ± 15.0	Trial:	p = 0.917
				Time x Trial:	p = 0.033
Serum Creatinine (mg/dl)	Baseline	1.0 ± 0.2	0.9 ± 0.3	Time:	p = 0.004
	Post Drink-1	0.9 ± 0.2	0.9 ± 0.2	Trial:	p = 0.426
				Time x Trial:	p = 0.369
Urine Creatinine (mg/dl)	Baseline	99 ± 76	96 ± 80	Time:	p = 0.002
	Post Drink-1	40 ± 24	42 ± 26	Trial:	p = 0.966
				Time x Trial:	p = 0.860
Creatinine Clearance (mL/min)	Baseline	135 ± 105	115 ± 93	Time:	p = 0.046
	Post Drink-1	167 ± 37	161 ± 43	Trial:	p = 0.622
				Time x Trial:	p = 0.688
Urine Flow Rate (mL/min)	Baseline	1.4 ±0.7	1.3 ± 0.6	Time:	p < 0.001
	Post Drink-1	4.6 ± 1.7	4.6 ± 2.7	Trial:	p = 0.875
				Time x Trial:	p = 0.889
Serum sodium (mmol/L)	Baseline	140 ± 1	139 ± 1	Time:	p = 0.006
	Post Drink-1	139 ± 2	138 ± 2	Trial:	p = 0.318
				Time x Trial:	p = 0.999
Urine sodium (mmol/L)	Baseline	81 ± 51	77 ± 64	Time:	p = 0.001
	Post Drink-1	42 ± 29	40 ± 27	Trial:	p = 0.805
				Time x Trial:	p = 0.923
Serum potassium (mmol/L)	Baseline	4.8 ± 0.5	4.9 ± 0.7	Time:	p = 0.797
	Post Drink-1	4.8 ± 0.6	4.8 ± 0.6	Trial:	p = 0.846
				Time x Trial:	p = 0.303
Urine potassium (mmol/L)	Baseline	40 ± 31	29 ± 20	Time:	p = 0.002
	Post Drink-1	22 ± 17	16 ± 12	Trial:	p = 0.085
				Time x Trial:	p = 0.462

Data are presented as means ± SD and analyzed using two-way linear mixed models with Šidák's post hoc comparisons. All data are n=12. * Indicates significantly different from Sucrose trial (P ≤ 0.05).

Table 5: Markers of hydration during and after simulated work-related heat stress.

Measurement	Timepoint	Fructose55	Sucrose	*P-value*	
				Paired t-test	
Sweat Rate (L/h)	--	0.6 ± 0.2	0.7 ± 0.3	0.910	
Body Mass Loss (%)	--	-0.7 ± 1.0	-0.9 ± 1.0	0.466	
				Linear Mixed Model	
Nude Body Mass (kg)	Baseline	76.4 ± 18.3	76.9 ± 18.2	Time:	p = 0.003
	Post-WrHS	75.7 ± 17.9	75.9 ± 18.0	Trial:	p = 0.943
	Recovery	75.8 ± 18.1	75.9 ± 18.1	Time x Trial:	p = 0.271
Plasma Volume (mL)	Baseline	4654 ± 1923	4492 ± 1820	Time:	p = 0.130
	Post-WrHS	4608 ± 2009	4538 ± 1900	Trial:	p = 0.936
	Recovery	4571 ± 1842	4662 ± 1915	Time x Trial:	p = 0.762
Serum Osmolality (mOsm/kg)	Baseline	297 ± 23	302 ± 7	Time:	p = 0.223
	Post-WrHS	299 ± 8	292 ± 14	Trial:	p = 0.895
	Recovery	291 ± 23	292 ± 18	Time x Trial:	p = 0.341
	24 h	300 ± 10	300 ± 10		
Urine Osmolality (mOsm/kg)	Baseline	492 ± 298	380 ± 291	Time:	p = 0.025
	Post-WrHS	590 ± 202	572 ± 202	Trial:	p = 0.192
	Recovery	403 ± 289	405 ± 349	Time x Trial:	p = 0.615
	24 h	760 ± 229	664 ± 270		
Urine specific gravity	Baseline	1.013 ± 0.007	1.012 ± 0.008	Time:	p = 0.078
	Post-WrHS	1.018 ± 0.006	1.016 ± 0.006	Trial:	p = 0.191
	Recovery	1.014 ± 0.010	1.013 ± 0.011	Time x Trial:	p = 0.858
	24 h	1.022 ± 0.00 5	1.018 ± 0.008		

Data are presented as mean ± SD. Sweat rate and body mass loss were analyzed using a paired t-test, with all other data analyzed using two-way linear mixed models with Šidák's post hoc comparisons where necessary. Data are presented before WrHS (Baseline), immediately after (Post-WrHS), ~75 minutes after WrHS (Recovery), and at 24 h follow-up from baseline (24 h). Serum and urine data at all time points in the Fructose55 trial are n=12. In the Sucrose trial, urine data at the 24-hour follow-up (24 h) are n=11, and n=12 at all other timepoints. Serum data in the Sucrose trial at Recovery are n=11, while all other timepoints are n-12. Plasma volume data are n=7 in the Fructose (55%) trial and n=8 in the Sucrose trial. P-values are reported.

Table 6: Markers of kidney function during and after simulated work-related heat stress.

Measurement	Timepoint	Fructose55	Sucrose	*P-value* *Linear Mixed Model*	
Serum Creatinine (mg/dl)	Baseline	0.97 ± 0.23	0.94 ± 0.23	Time:	p = 0.007
	Post-WrHS	1.10 ± 0.30	1.09 ± 0.37	Trial:	p = 0.129
	Recovery	1.08 ± 0.31	1.06 ± 0.34	Time x Trial:	p = 0.763
	24 h	1.00 ± 0.20	0.91 ± 0.20		
Urine Creatinine (mg/dl)	Baseline	99 ± 76	96 ± 80	Time:	p = 0.114
	Post-WrHS	262 ± 161	212 ± 145	Trial:	p = 0.134
	Recovery	204 ± 246	199 ± 233	Time x Trial:	p = 0.530
	24 h	230 ± 100	193 ± 127		
Serum Copeptin (pg/mL)	Baseline	19.3 ± 12.3	21.8 ± 13.1	Time:	p = 0.531
	Post-WrHS	22.8 ± 12.3	21.4 ± 12.8	Trial:	p = 0.417
	Recovery	21.0 ± 13.4	18.01 ± 12.1	Time x Trial:	p = 0.164
	24 h	17.0 ± 9.4	21.8 ± 13.2		
Creatinine Clearance (mL/min)	Baseline	135 ± 105	115 ± 93	Time:	p = 0.013
	Post-WrHS	352 ± 212	309 ± 178	Trial:	p = 0.125
	Recovery	150 ± 36	161 ± 48	Time x Trial:	p = 0.409
	24 h	273 ± 144	203 ± 182		
Serum Albumin (g/dL)	Baseline	4.4 ± 0.2	4.4 ± 0.3	Time:	p = 0.003
	Post-WrHS	4.6 ± 0.4	4.5 ± 0.3	Trial:	p = 0.146
	Recovery	4.5 ± 0.3	4.4 ± 0.3	Time x Trial:	p = 0.541
	24 h	4.7 ± 0.3	4.8 ± 0.3		
Urine Albumin (g/dL)	Baseline	8.8 ± 6.2	7.4 ± 6.3	Time:	p = 0.310
	Post-WrHS	15.0 ± 9.5	10.7 ± 6.5	Trial:	p = 0.071
	Recovery	27.1 ± 53.0	21.3 ± 31.7	Time x Trial:	p = 0.578
	24 h	16.5 ± 16.6	9.1 ± 7.2		

Data are presented at before work-related heat stress (WrHS; Baseline), immediately after (Post-WrHS), ~75 minutes after WrHS (Recovery), and at the 24-hour follow-up (24 h). Data were analyzed using two-way linear mixed models with p-values reported. All data are represented as mean ± SD. Data at all time points in the Fructose55 trial are n=12. In the Sucrose trial, urine data at the 24-hour follow-up (24 h) are n=11, and n=12 at all other timepoints. Serum and plasma data in the Sucrose trial at Recovery are n=11, while all other timepoints are n-12.

Table 7: Kidney electrolyte and fluid balance markers during and after simulated work-related heat stress.

Measurement	Timepoint	Fructose55	Sucrose	*P-value* *Linear Mixed Model*	
Urine Flow Rate (mL/min)	Baseline	1.4 ± 0.7	1.3 ± 0.6	Time:	p = 0.070
	Post-WrHS	1.7 ± 0.6	2.0 ± 1.1	Trial:	p = 0.156
	Recovery	2.2 ± 1.8	3.3 ± 3.2	Time x Trial:	p = 0.068
	24 h	1.2 ± 0.5	0.9 ± 0.7		
Osmolar Clearance (mL/min)	Baseline	2.3 ± 1.9	1.5 ± 1.0	Time:	p = 0.027
	Post-WrHS	3.3 ± 1.8	3.7 ± 2.5	Trial:	p = 0.207
	Recovery	1.7 ± 0.7	1.7 ± 0.8	Time x Trial:	p = 0.212
	24 h	2.8 ± 1.2	1.9 ± 1.5		
Free water clearance (mL/min)	Baseline	-0.9 ± 1.4	-0.1 ± 1.0	Time:	p < 0.001
	Post-WrHS	-0.7 ± 1.3	-1.7 ± 1.8	Trial:	p = 0.039
	Recovery	0.5 ± 1.6	1.8 ± 2.9	Time x Trial:	p = 0.144
	24 h	-1.4 ± 1.2	-1.2 ± 1.0		
Serum Glucose (mg/dL)	Baseline	91 ± 13	87 ± 11	Time:	p = 0.030
	Post-WrHS	97 ± 16	109 ± 28	Trial:	p = 0.005
	Recovery	93 ± 11*	119 ± 27	Time x Trial:	p = 0.005
	24 h	90 ± 14	96 ± 10		
Serum sodium (mmol/L)	Baseline	140 ± 2	139 ± 1	Time:	p = 0.029
	Post-WrHS	139 ± 2	138 ± 1	Trial:	p = 0.675
	Recovery	138 ± 2	139 ± 2	Time x Trial:	p = 0.627
	24 h	140 ± 3	140 ± 2		
Urine sodium (mmol/L)	Baseline	81 ± 51	77 ± 63	Time:	p = 0.007
	Post-WrHS	50 ± 27	55 ± 32	Trial:	p = 0.631
	Recovery	29 ± 24	29 ± 22	Time x Trial:	p = 0.496
	24 h	67 ± 43	81 ± 60		
Serum potassium (mmol/L)	Baseline	4.8 ± 0.5	4.9 ± 0.7	Time:	p < 0.001
	Post-WrHS	4.2 ± 0.5	4.1 ± 0.4	Trial:	p = 0.771
	Recovery	4.4 ± 0.7	4.1 ± 0.4	Time x Trial:	p = 0.263
	24 h	4.3 ± 0.5	4.3 ± 0.3		
Urine potassium (mmol/L)	Baseline	40 ± 31	29 ± 20	Time:	p = 0.016
	Post-WrHS	97 ± 41	79 ± 33	Trial:	p = 0.063
	Recovery	67 ± 74	49 ± 46	Time x Trial:	p = 0.528
	24 h	77 ± 44	49 ± 43		
Fractional excretion of sodium (%)	Baseline	0.6 ± 0.2	0.6 ± 0.2	Time:	p < 0.001
	Post-WrHS	0.2 ± 0.1	0.3 ± 0.2	Trial:	p = 0.177
	Recovery	0.2 ± 0.1	0.2 ± 0.2	Time x Trial:	p = 0.116
	24 h	0.3 ± 0.1	0.4 ± 0.3		
Fractional excretion of potassium (%)	Baseline	8.7 ± 5.7	9.6 ± 9.6	Time:	p = 0.474
	Post-WrHS	11.5 ± 5.8	10.8 ± 3.9	Trial:	p = 0.364
	Recovery	10.2 ± 3.8	8.3 ± 2.1	Time x Trial:	p = 0.449
	24 h	10.6 ± 10.7	7.7 ± 6.1		

Data are presented at before work-related heat stress (WrHS; Baseline), immediately after (Post-WrHS), ~75 minutes after WrHS (Recovery), and at the 24-hour follow-up (24 h). Data were analyzed using two-way linear mixed models with p-values reported. All data are represented as mean ± SD. Data were analyzed using a two-way linear mixed models with p-values reported. All data are represented as mean ± SD. Serum, plasma, and urine data at all time points in the Fructose55 trial are n=12. In the Sucrose trial, urine data at the 24-hour follow-up (24 h) are

n=11, and n=12 at all other timepoints. Serum and plasma data in the Sucrose trial at Recovery are n=11, while all other timepoints are n-12.

Table 8: Urine and serum markers related to AKI risk during and after simulated work-related heat stress.

Measurement	Timepoint	Fructose55	Sucrose	*P-value* *Linear Mixed Model*	
Urine $NGAL_{USG}$ (Log_{10})	Baseline	1.2 ± 0.5	1.4 ± 0.8	Time:	p = 0.573
	Post-WrHS	1.4 ± 0.4	1.4 ± 0.4	Trial:	p = 0.372
	Recovery	1.3 ± 0.4	1.5 ± 0.6	Time x Trial:	p = 0.330
	24 h	1.4 ± 0.5	1.5 ± 0.6		
Urine $NGAL_{UFR}$ (Log_{10})	Baseline	1.1 ± 0.5	1.1 ± 0.7	Time:	p = 0.014
	Post-WrHS	1.1 ± 0.2	1.1 ± 0.3	Trial:	p = 0.519
	Recovery	1.2 ± 0.3	1.3 ± 0.5	Time x Trial:	p = 0.304
	24 h	1.7 ± 0.6	1.4 ± 0.7		
Urine Uric $Acid_{USG}$ (mg/dL)	Baseline	54 ± 16	47 ± 14	Time:	p = 0.266
	Post-WrHS	45 ± 10	46 ± 10	Trial:	p = 0.667
	Recovery	38 ± 13	45 ± 28	Time x Trial:	p = 0.238
	24 h	46 ± 10	52 ± 16		
Serum Uric Acid (mg/dL)	Baseline	5.4 ± 1.2	5.3 ± 1.2	Time:	p = 0.013
	Post-WrHS	6.0 ± 1.6	5.7 ± 1.8	Trial:	p = 0.158
	Recovery	6.0 ± 1.6	5.9 ± 1.7	Time x Trial:	p = 0.538
	24 h	5.6 ± 1.2	5.5 ± 1.3		
Urine $KIM\text{-}1_{USG}$ (ng/dL)	Baseline	0.4 ± 0.2	0.4 ± 0.2	Time:	p = 0.015
	Post-WrHS	1.2 ± 1.0	1.0 ± 0.7	Trial:	p = 0.073
	Recovery	0.9 ± 0.7	0.8 ± 0.6	Time x Trial:	p = 0.378
	24 h	0.9 ± 0.4	0.8 ± 0.4		
Urine $KIM\text{-}1_{UFR}$ (Log_{10})	Baseline	-0.6 ± 0.4	-0.7 ± 0.5	Time:	p < 0.001
	Post-WrHS	-0.4 ± 0.5	-0.3 ± 0.4	Trial:	p = 0.079
	Recovery	-0.3 ± 0.4	-0.4 ± 0.4	Time x Trial:	p = 0.135
	24 h	0.2 ± 0.3	-0.2 ± 0.6		
Serum $KIM\text{-}1_{USG}$ (pg/dL)	Baseline	29 ± 15	29 ± 16	Time:	p = 0.126
	Post-WrHS	28 ± 13	29 ± 15	Trial:	p = 0.561
	Recovery	29 ± 15	26 ± 13	Time x Trial:	p = 0.251
	24 h	31 ± 15	29 ± 15		
Urine $IGFBP7_{UFR}$ (Log_{10})	Baseline	1.4 ± 0.4	1.4 ± 0.6	Time:	p < 0.001
	Post-WrHS	1.9 ± 0.4	2.0 ± 0.3	Trial:	p = 0.337
	Recovery	2.0 ± 0.2	2.0 ± 0.3	Time x Trial:	p = 0.447
	24 h	2.0 ± 0.4	1.7 ± 0.5		
Urine $TIMP\text{-}2_{UFR}$ (Log_{10}	Baseline	0.1 ± 0.5	0.1 ± 0.5	Time:	p = 0.002
	Post-WrHS	0.4 ± 0.3	0.5 ± 0.2	Trial:	p = 0.371
	Recovery	0.5 ± 0.2	0.5 ± 0.3	Time x Trial:	p = 0.238
	24 h	0.8 ± 0.3	0.4 ± 0.6		

Data are presented at before work-related heat stress (WrHS; Baseline), immediately after (Post-WrHS), ~75 minutes after WrHS (Recovery), and at the 24-hour follow-up (24 h). Data were analyzed using two-way linear mixed models with p-values reported. All data are represented as mean ± SD. Data were analyzed using a two-way linear mixed models with p-values reported. If required, data were log transformed prior to statistical analyses. All data are represented as mean ± SD. Serum, plasma, and urine data at all time points in the Fructose55 trial are n=12. In the Sucrose trial, urine data at the 24-hour follow-up (24 h) are n=11, and n=12 at all other timepoints.

Serum and plasma data in the Sucrose trial at Recovery are n=11, while all other timepoints are n-12. * Indicates significantly different from Sucrose trial ($P \leq 0.05$).

Table 9: Markers of oxidative stress, inflammation and gut permeability during and after simulated work-related heat stress.

Measurement	Timepoint	Fructose55	Sucrose	*P-value* *Linear Mixed Model*	
Markers of oxidative stress					
Serum TRX-1 (Log_{10})	Baseline	1.9 ± 0.4	1.9 ± 0.4	Time:	p = 0.079
	Post-WrHS	1.9 ± 0.5	1.9 ± 0.4	Trial:	p = 0.040
	Recovery	1.9 ± 0.4	1.9 ± 0.4	Time x Trial:	p = 0.132
	24 h	1.9 ± 0.4	1.8 ± 0.4		
Urine $TRX\text{-}1_{USG}$ (Log_{10})	Baseline	1.7 ± 0.4	1.7 ± 0.5	Time:	p = 0.388
	Post-WrHS	1.8 ± 0.3	1.7 ± 0.3	Trial:	p = 0.937
	Recovery	1.8 ± 0.3	1.9 ± 0.5	Time x Trial:	p = 0.150
	24 h	1.8 ± 0.4	1.7 ± 0.4		
Urine $TRX\text{-}1_{UFR}$ (Log_{10})	Baseline	1.5 ± 0.4	1.5 ± 0.6	Time:	p = 0.013
	Post-WrHS	1.5 ± 0.3	1.5 ± 0.3	Trial:	p = 0.137
	Recovery	1.6 ± 0.3	1.7 ± 0.5	Time x Trial:	p = 0.069
	24 h	2.1 ± 0.5	1.6 ± 0.7		
Markers of inflammation					
Serum $MCP\text{-}1_{USG}$ (Log_{10})	Baseline	-0.6 ± 0.2	-0.6 ± 0.2	Time:	p < 0.001
	Post-WrHS	-0.4 ± 0.3	-0.5 ± 0.2	Trial:	p = 0.222
	Recovery	-0.4 ± 0.3	-0.5 ± 0.2	Time x Trial:	p = 0.371
	24 h	-0.6 ± 0.2	-0.6 ± 0.2		
Urine $MCP\text{-}1_{USG}$ (Log_{10})	Baseline	-1.1 ± 0.2	-1.1 ± 0.3	Time:	p = 0.026
	Post-WrHS	-0.7 ± 0.3	-0.8 ± 0.3	Trial:	p = 0.891
	Recovery	-0.9 ± 0.4	-0.8 ± 0.3	Time x Trial:	p = 0.131
	24 h	-0.9 ± 0.3	-0.9 ± 0.4		
Urine $MCP\text{-}1_{UFR}$ (Log_{10})	Baseline	-1.3 ± 0.4	-1.3 ± 0.4	Time:	p = 0.004
	Post-WrHS	-1.0 ± 0.4	-1.0 ± 0.3	Trial:	p = 0.196
	Recovery	-1.0 ± 0.2	-1.0 ± 0.3	Time x Trial:	p = 0.091
	24 h	-0.6 ± 0.3	-1.0 ± 0.7		
Serum IL-6 (Log_{10})	Baseline	-0.4 ± 0.2	-0.4 ± 0.2	Time:	p < 0.001
	Post-WrHS	0.5 ± 0.5	0.4 ± 0.5	Trial:	p = 0.387
	Recovery	0.4 ± 0.4	0.4 ± 0.4	Time x Trial:	p = 0.772
	24 h	-0.3 ± 0.3	-0.3 ± 0.3		
Serum TNF-α (Log_{10})	Baseline	0.2 ± 0.1	0.2 ± 0.2	Time:	p = 0.379
	Post-WrHS	0.3 ± 0.2	0.3 ± 0.2	Trial:	p = 0.556
	Recovery	0.3 ± 0.2	0.3 ± 0.2	Time x Trial:	p = 0.290
	24 h	0.3 ± 0.2	0.2 ± 0.2		
Markers of gut permeability					
Serum LBP (ng/mL)	Baseline	4396 ± 1576	4470 ± 1387	Time:	p < 0.001
	Post-WrHS	4076 ± 1352	4158 ± 1111	Trial:	p = 0.924
	Recovery	4160 ± 1698	4314 ± 1287	Time x Trial:	p = 0.387
	24 h	6093 ± 2361	5685 ± 2400		
Serum I-FABP (ng/mL)	Baseline	0.7 ± 0.4	0.7 ± 0.4	Time:	p = 0.086
	Post-WrHS	1.0 ± 0.4	0.9 ± 0.6	Trial:	p = 0.874
	Recovery	0.7 ± 0.3	0.7 ± 0.6	Time x Trial:	p = 0.452
	24 h	0.9 ± 0.4	0.9 ± 0.5		

Data are presented at before work-related heat stress (WrHS; Baseline), immediately after (Post-WrHS), ~75 minutes after WrHS (Recovery), and at the 24-hour follow-up (24 h). Data were analyzed using two-way linear

mixed models with p-values reported. All data are represented as mean ± SD. Data were analyzed using a two-way linear mixed models with p-values reported. If required, data were log transformed prior to statistical analyses. All data are represented as mean ± SD. Serum, plasma, and urine data at all time points in the Fructose55 trial are n=12. In the Sucrose trial, urine data at the 24-hour follow-up (24 h) are n=11, and n=12 at all other timepoints. Serum and plasma data in the Sucrose trial at Recovery are n=11, while all other timepoints are n-12. * Indicates significantly different from Sucrose trial ($P \leq 0.05$).

Table 10: After WrHS Resting Renal Hemodynamics

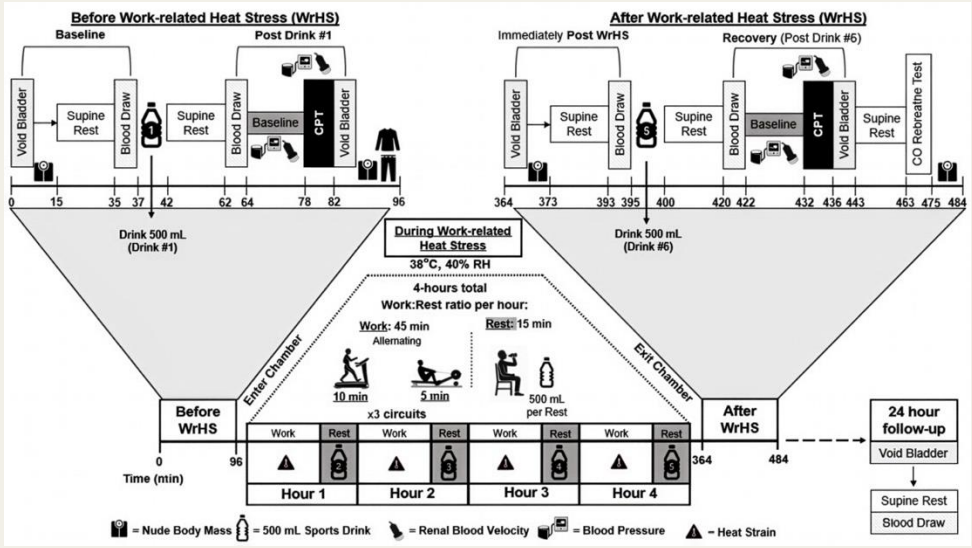
	Fructose55	Sucrose	p-value
			Paired t-test
Mean Arterial Pressure (mmHg)	76 ± 8	75 ± 7	0.530
Heart Rate (bpm)	71 ± 17	71 ± 20	0.854
Renal Artery Blood Velocity (cm/s)	34.4 ± 8.0	34.6 ± 5.4	0.956
Renal Artery Vascular Resistance (mmHg/cm/s)	2.3 ± 0.6	2.2 ± 0.4	0.446
Segmental Artery Blood Velocity (cm/s)	20.3 ± 3.6	19.3 ± 2.6	0.444
Segmental Artery Vascular Resistance (mmHg/cm/s)	3.9 ± 0.9	4.0 ± 0.6	0.761

Data were analyzed using a two-tailed paired t-test, and p-values are reported. All data are n=12 and presented as mean ± SD and individual values.

Hydrating with a higher-fructose sport drink does not worsen acute kidney injury risk during simulated work-related heat stress

METHODS

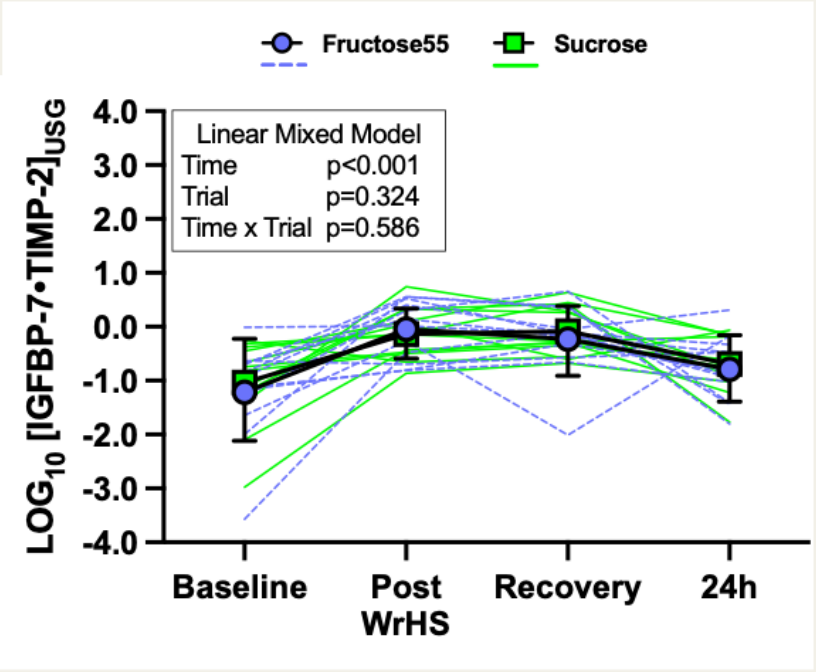
Study Design: Double blind, cross-over



Experimental Beverage Composition

		Fructose55	Sucrose
Total Carbohydrate (g)		28	28
Energy (kcal)		116	116
Electrolytes (mg)	Na^+	210	210
	K^+	65	65
Fructose load (g)		15.4	14
Osmolality (mOsm/L)		337 ± 6	204 ± 2

OUTCOME



Increases in biomarkers of acute kidney injury (AKI) risk did not differ between Fructose55 and Sucrose trials.

CONCLUSION Sugar type in the sport drink did not alter AKI risk, indicating that modest differences in fructose content does not meaningfully impact AKI risk.