Soft Drink Consumption During and Following Exercise in the Heat Elevates Biomarkers of Acute Kidney Injury

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Abstract

The purpose of this study was to test the hypothesis that consuming a soft drink (i.e., a high fructose, caffeinated beverage) during and following exercise in the heat elevates biomarkers of acute kidney injury (AKI) in humans. Twelve healthy adults drank 2 L of an assigned beverage during 4 h of exercise in the heat (35.1 ± 0.1°C, 61 ± 5% relative humidity) in counterbalanced soft drink (Soft Drink) and water (Water) trials, and ≥1 L of the same beverage after leaving the laboratory. Stage 1 AKI (i.e., increased serum creatinine ≥0.30 mg/dL) was detected at post-exercise in 75% of participants in the Soft Drink trial compared to 8% in Water (P=0.02). Furthermore, urinary neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of AKI, was higher during an overnight collection period after the Soft Drink trial compared to Water in both absolute concentration (6 ± 4 ng/dL vs. 5 ± 4 ng/dL, P<0.04) and after correcting for urine flow rate (6 ± 7 [ng·dL^{-1}]/[mL·min^{-1}] vs. 4 ± 4 [ng·dL^{-1}]/[mL·min^{-1}], P=0.03). Changes in serum uric acid from pre-exercise were greater in the Soft Drink trial than Water at post-exercise (P<0.01) and 24 h (P=0.05). There were greater increases from pre-exercise in serum copeptin, a stable marker of vasopressin, at post-exercise in the Soft Drink trial (P<0.02) than Water. These findings indicate that consuming a soft drink during and following exercise in the heat induces AKI, likely via vasopressin mediated mechanisms.

Keywords: Heat stress; exercise; dehydration; soda; chronic kidney disease

Introduction

In healthy kidneys, the normal physiological response to physical work (e.g., exercise) in a hot environment is a reduced renal blood flow (44). This acute reduction in renal blood flow promotes blood pressure regulation and fluid conservation, and is thought to be transient and clinically benign. However, in clinical settings, severe reductions in renal blood flow can result in acute kidney injury (AKI) due to decreased oxygen delivery and the high oxygen demand within the renal microvasculature (26). Indeed, increased hospital admissions for AKI have been reported in higher ambient temperatures (24). Laboratory experiments in humans have shown increases in biomarkers of AKI after exercise both with (22) and without (42) the addition of heat stress. However, elevations in these biomarkers appear to be transient and are resolved after a recovery period.

Our laboratory has previously shown that increases in core body temperature and dehydration brought about by exercise in the heat elevates biomarkers of AKI, and that these elevations are exacerbated with greater increases in the magnitude of core body temperature and dehydration (51). García-Arroyo et al. recently demonstrated in rats that the type of beverage consumed following recurring heat-induced dehydration has implications on renal health, such that consuming a soft drink-like beverage (i.e., a drink with a high fructose content) increased AKI compared to a noncaloric sweetened control beverage (14). It is believed that this AKI is mediated by vasopressin release and the activation of the polyol-fructokinase pathway, both of which are elicited by dehydration and can be exacerbated by drinking a hyperosmotic beverage with high fructose content (14, 45). Collectively, both vasopressin and activation of the polyol-

fructokinase pathway can bring about oxidative stress, inflammation, and uric acid production in the renal tubules, which may ultimately lead to AKI (46, 47).

Soft drink consumption is common in people who perform manual labor in hot environments (13). However, it is not known if consuming a soft drink (i.e., a high fructose, caffeinated beverage) during and following exercise in the heat elevates biomarkers of AKI. Therefore, the purpose of our study was to test the hypothesis that consuming a soft drink during and following exercise in the heat elevates biomarkers of AKI compared to a water control trial.

Methods

Participants

Twelve healthy adults (3 females) participated in this study. Participants characteristics were: age: 24 ± 5 years, height: 177 ± 12 cm, weight: 76.0 ± 11.6 kg, and body mass index: 24 ± 2 kg/m^2. Each participant was fully informed of the experimental procedures and possible risks before giving informed, written consent. All participants were physically active, nonsmokers, and reported to be free from any known cardiovascular, renal, metabolic, neurological, or gastrointestinal diseases. This study was conducted during the winter season in Buffalo, NY. Thus, participants were assumed to not be heat acclimatized. Female participants were not pregnant, which was confirmed via a urine pregnancy test before each trial, and self-reported to be normally menstruating. Females were tested during the first 10 days after their self-identified menstruation, a period in which estrogen and progesterone are at their lowest concentration. The study was approved by the Institutional Review Board at the

University at Buffalo and performed in accordance with the standards set by the latest revision of the Declaration of Helsinki. Participants visited the laboratory on three separate occasions. In visit one, participants were screened and familiarized with the exercise protocol. The final two visits were the experimental trials.

Instrumentation and measurements

Participants ingested a telemetry pill (HQ Inc., Palmetto, FL) 6-8 h prior to arriving at the laboratory for measurement of core temperature. Heart rate was measured via a wireless monitor (Polar Electro, Inc., Bethpage, NY). Nude body weight and height were measured using a stadiometer and scale (Sartorius Corp., Bohemia, NY). Urine specific gravity was measured in duplicate using a refractometer (Atago USA, Inc., Bellevue, WA). Systolic blood pressure and diastolic blood pressure were measured manually in duplicate by an experienced member of the research team.

Hemoglobin was measured using the HemoPoint H2 (Alere, Orlando, FL). Hematocrit was measured in triplicate using microcentrifugation. Serum and urinary osmolality were measured via osmometry using freezing point depression (Model 3250; Advanced Instruments, Inc., Norwood, MA). Serum measurements of creatinine, blood urea nitrogen (BUN), uric acid, potassium and sodium, and urinary measurements of creatinine, potassium, sodium, and urea nitrogen were measured via standard clinical techniques by Kaleida Health, Department of Pathology and Laboratory Medicine (Williamsville, NY), using an Architect Clinical Chemistry Analyzer (Abbott Diagnostics, Abbott Park, IL). Neutrophil gelatinase associated-lipocalin (NGAL) was measured in plasma and urine using a commercially available human NGAL ELISA kit (Toronto

Bioscience, Toronto, Canada). Plasma NGAL is generally interpreted to provide an indication of the degree of potential renal ischemia and urinary NGAL provides an indication of the magnitude of renal tubular injury (50). Vasopressin was indirectly assessed by measuring serum copeptin, a stable and sensitive surrogate for vasopressin release (36). Serum copeptin was measured using a human copeptin ELISA kit (LifeSpan BioSciences, Inc., Seattle, WA). All blood (whole blood, serum, and plasma) and urine samples were analyzed in duplicate.

Experimental Protocol

A counterbalanced crossover design was employed, with each participant consuming either a soft drink (Mountain Dew®, PepsiCo, Inc., Purchase, NY; Soft Drink) or water (Water) during and following 4 h of exercise in the heat. Mountain Dew® was specifically chosen on the basis of the documented use of soft drinks in heat-exposed workers (13) and its high fructose content relative to other soft drinks (58). The Mountain Dew® had a measured osmolality of 834 ± 2 mOsm/kg, and on-label contents of ~154 mg/L caffeine, ~480 calories/L, ~130 g/L sugar, ~170 mg/L sodium, and ~20 mg/L potassium. Participants performed both interventions on separate days and with at least 7 days between each trial to minimize any potential effects of heat acclimation. Participants also came into the laboratory 24 h after the start of each trial for additional data collection.

Participants arrived at the laboratory having refrained from strenuous exercise, alcohol and caffeine for 12 h, and food for 2 h. Upon arrival, participants voided their bladder and euhydration was confirmed via a urine specific gravity of <1.020 (Table 1)

(1). Participants were then weighed nude and instrumented with a heart rate monitor. Then, participants dressed in the provided clothing which included a cotton t-shirt, long-sleeve work shirt, and work pants (~0.8 clo) (32). Participants then assumed a supine position for 20 minutes in a 23 ± 1˚C, 24 ± 6% relative humidity environment for pre-exercise measurements of core temperature, heart rate and blood pressure, after which a venous blood sample was obtained. The ambient temperature and relative humidity were not statistically different between trials during pre-exercise measurements (paired t-test, $P \geq 0.51$). All venous blood samples were taken after 20 min supine rest to control for compartmental fluid shifts associated with changes in body posture (17). After measuring hemoglobin and hematocrit in the whole-blood, blood samples were separated via centrifugation, aliquoted, and stored in a -80˚C freezer for future analysis.

Following pre-exercise measurements, participants entered the 35 ± 0°C, 61 ± 5% relative humidity environmental chamber, a typical outdoor work thermal environment encountered by workers at risk for AKI (16, 29). In both trials, participants completed four 1 h work-rest cycles each consisting of 45 min of exercise and 15 min seated rest, resulting in a total of 4 h in the environmental chamber. A total duration of 4 h was chosen to simulate half of an 8 h work day. The exercise component consisted of 30 min treadmill walking at 4.8 kph. During the first experimental trial (independent of whether it was the Soft Drink or Water trial), the grade of the treadmill was set to a grade eliciting 55% of estimated heart rate maximum within 3 min. This relative heart rate was chosen because this is the average relative heart rate that has been recorded during outdoor physical work in the heat in workers at risk of AKI (29). Once target heart rate was reached, the grade was maintained constant for the remainder of the first trial,

and the same grade was used for the second trial. The remaining 15 min of exercise was further divided into 5 min of a simulated tool lifting task, 5 min of a dexterity task, and 5 min of a sledgehammer swing. The tool lifting task consisted of picking one object from the floor and setting it onto a 73 cm tall table every 12 s, after which the participants set the objects back onto to the floor, and this process was repeated for 5 min. Three of the objects were barbells weighing 4.1 kg, 5.4 kg, and 6.8 kg, and the two remaining objects were rolled fire hoses each weighing 21.1 kg. The dexterity task has previously been used in our laboratory (52). In the present study, participants were instructed to secure and disassemble as many nuts and bolts as possible in 5 min. Following the dexterity task, participants swung a 4 kg dead blow sledgehammer against a 72 kg I-beam every 10 s (Keiser Corporation, Fresno, CA). Participants were instructed to apply maximum effort during these swings to move the I-beam the furthest possible distance. These non-treadmill based exercises were chosen to mimic some of the lifting, upper body, and manual handling tasks undertaken by manual laborers.

Upon completing each of the 45 min periods of exercise, participants rested in a seated position in the environmental chamber. During this rest period, participants consumed 500 mL of soft drink or water. Both drinks were provided at a cool temperature (~11°C). At 15 min into each rest period, heart rate, blood pressure, and core temperature measurements were taken. After 15 min of rest, participants returned to the exercise protocol, and repeated this work-rest cycle for a total of 4 h unless ethically mediated stopping criteria were met. Stopping criteria were a core temperature measurement ≥39.5˚C, two consecutive heart rate measurements exceeding estimated maximum heart rate (56), or volitional fatigue. If stopping criteria were met, participants

were given the remaining volume to immediately consume, so that in total all participants drank 2 L of Soft Drink or Water by the end of exercise in the heat. Four participants in the Soft Drink trial (*n*=3 due to core temperature reaching 39.5˚C and *n*=1 due to volitional fatigue), and one participant in the Water trial (core temperature) were unable to complete all 4 h of exercise in the heat. Notably, all of the trials in which stopping criteria were reached occurred on the first experimental trial. Exercise in the heat during the second trial was terminated at the same time point as the first trial, independent of the heart rate, core temperature and/or a willingness to proceed. Thus, participants completed the same duration and volume of exercise in both trials. The average time of exercise in the heat in both trials was 211 ± 40 min.

Following completion of the final 15 min rest period measurements, participants returned to a moderate environment and rested in the supine position for 20 min where post-exercise core temperature, heart rate and blood pressure measurements were taken, after which venous blood and urine samples were obtained. Participants were returned to a moderate environment for post-exercise measurements for safety, in the case when core temperature reached our ethical cutoff (39.5°C), consistent with our previous work (51). Participants were then given 1 L of the assigned beverage or a volume equal to 115% of body mass lost (whichever was greater) and were instructed to drink the given beverage (1.1 ± 0.1 L) before having any additional beverages. During the overnight period, participants were not given specific instructions except to relax and to not undertake any exercise until arriving at the laboratory the following morning. Participants were instructed to record all food and beverage items and volumes to replicate their overnight diet for their next trial. Furthermore, participants were given a

24 h urine collection container for measurement of overnight urine volume and flow rate. A small portion of this urine was also aliquoted and frozen for subsequent measurement of overnight urinary NGAL. Participants then returned to the laboratory 24 h from the beginning of their trial for additional measurements (24 h), which consisted of measurements of heart rate and blood pressure, as well as urine and venous blood sample collections.

Data & Statistical Analysis

All data were collected at pre- and post-exercise, and 24 h. Data are presented as absolute values and the absolute change (Δ) from pre-exercise. Analyzing the change from pre-exercise data allows us to isolate the effect of beverage consumption on changes in the dependent variables. Core temperature, heart rate, and mean arterial pressure are also reported from measures taken at the end of the final rest period during exercise in the heat or immediately upon exiting the environmental chamber (for those participants whose trials were terminated for ethical reasons). Fluid and food intakes from overnight food diaries were analyzed for fluid volume and measures of total energy, fat, protein, carbohydrate, sugar and sodium using online software (37). Core temperature is reported as n=10 due to technical difficulties with the telemetry pill. Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure. Whole body sweat rate was estimated from changes in nude body weight, after correcting for urine output and the weight of the 2 L of beverage consumed (Soft Drink: 2.19 kg, Water: 2.00 kg), and dividing by the length of time for each trial. Percentage changes in plasma volume were estimated using standard equations (11). Due to errors

234 associated with the measurement of pre-exercise urine flow rate, creatinine clearance
235 (i.e., a marker of glomerular filtration rate) could not be calculated. Thus, glomerular
236 filtration rate was estimated (eGFR) from serum creatinine using the following eGFR
237 equations: males = [175 x serum creatinine$^{-1.154}$ x age$^{-0.203}$] or females = [175 x serum
238 creatinine$^{-1.154}$ x age$^{-0.203}$ x 0.742] (28). The fractional excretions of sodium and
239 potassium were calculated from concentrations in the serum and urine, and the change
240 in serum and urinary creatinine concentrations. The ratio of BUN to serum creatinine
241 (BUN:creatinine) was calculated to provide a general indication of the potential cause of
242 the AKI, with ratio being >20 interpreted as suggestive of a pre-renal etiology (39). The
243 incidence of Stage 1 AKI was quantified using the Acute Kidney Injury Network (AKIN)
244 criteria as a change in serum creatinine ≥0.3 mg/dL above pre-exercise concentrations
245 or a urine output of <0.5 mL/kg/h for >6 h (33), as has been employed previously (22,
246 51). Despite the limitations associated with using the change in serum creatinine during
247 exercise, because the amount of work performed was not different between trials, it was
248 deemed acceptable to use the change in serum creatinine as a biomarker of AKI as the
249 only difference that occurred during the trials was the type of beverage consumed. It is
250 noteworthy that these criteria are used in clinical settings independent of the limitations.
251 Urinary NGAL is reported as *n*=10 due to inexplicably high concentrations measured in
252 two participants at 24 h. Overnight urinary NGAL was normalized to urine flow rate
253 resulting in overnight urinary NGAL flow rate, as has been recently suggested (22).
254 Serum copeptin is reported as *n*=11 due to a single measurement being inexplicably
255 outside of the normal physiological range at pre-exercise.

Data were analyzed using a two-way repeated-measures ANOVA (trial x time) using GraphPad Prism (Version 7, La Jolla, CA). If the ANOVA revealed a significant *F* value, *post hoc* two-tailed Sidak's test pairwise comparisons were made. Two-tailed paired t-tests were used to analyze differences between trials for overnight fluid and food intake data, and overnight urine flow rate. Overnight urinary NGAL data were analyzed using one-tailed paired t-tests. One-tailed tests were deemed acceptable given our directional hypothesis. The incidence of stage 1 AKI at post-exercise and 24 h were analyzed using independent two-tailed Wilcoxon matched-pairs signed rank tests. Statistical significance was set *a priori* at $P \leq 0.05$, and actual P values are reported where possible. Data are reported as mean ± SD.

Results

Dietary control & performance tasks

Participants arrived at the laboratory euhydrated for both trials (Table 1). Additionally, during the overnight period there were no statistical differences in dietary intakes of fluid volume, fat, protein, and sodium intakes from food and beverages (Table 2). However, during the overnight period, there were greater intakes of total energy and carbohydrate in the Soft Drink trial, which were likely due to increased sugar intake from the Soft Drink consumed after leaving the laboratory compared to Water.

There were no statistical differences between trials in the total number of bolts secured and disassembled for the dexterity task (Soft Drink: 32 ± 16 points, Water: 31 ± 13, P=0.77), or total distance the I-beam was moved in the sledgehammer swing task (Soft Drink: 530 ± 278 cm, Water: 479 ± 209 cm, P=0.15).

279

280 *Thermal and cardiovascular response to exercise in the heat*

281 Core temperature (Figure 1A) did not statistically differ between trials at any time
282 point ($P \geq 0.33$). Elevations in heart rate (Figure 1B) were not statistically different
283 between trials at the end of exercise ($P=0.61$) or at post-exercise ($P=0.11$). Heart rate
284 returned to pre-exercise levels at 24 h in both trials ($P \geq 0.64$). Mean arterial pressure
285 (Figure 1C) was higher in Soft Drink at the end of exercise (increase of 9 ± 10 mmHg
286 vs. 3 ± 6 mmHg, $p<0.03$). Mean arterial pressure was elevated above pre-exercise
287 levels at post-exercise in Soft Drink (by 5 ± 8 mmHg, $P<0.05$), although it did not
288 statistically differ between trials ($P=0.33$) and returned to pre-exercise levels in both
289 trials at 24 h ($P \geq 0.92$).

290

291 *Hydration status*

292 Following exercise in the heat, absolute body weight and the relative change in
293 body weight (Table 1) were not statistically different between trials at post-exercise
294 ($P \geq 0.44$). However, when correcting for the weight of the 2 L beverage consumed (Soft
295 Drink: 2.19 kg, Water: 2.00 kg), the relative change in body weight was greater at post-
296 exercise in Soft Drink compared to Water ($P=0.05$), but there were no statistical
297 differences in absolute body weight ($P=0.99$). By extension, estimated whole body
298 sweat rate was slightly higher in Soft Drink (0.9 ± 0.2 L/h) compared to Water (0.8 ± 0.2
299 L/h, $P<0.02$). Plasma volume (Table 1) decreased in Soft Drink at post-exercise
300 ($P<0.02$), but was not statistically different between trials ($P=0.08$). Plasma osmolality
301 and serum sodium (Table 1) were elevated in Soft Drink compared to Water at post-

exercise (P<0.01). At 24 h, plasma osmolality and serum sodium were elevated above pre-exercise in Soft Drink (P<0.04) despite not statistically differing from Water (P=0.43). Serum potassium was reduced at post-exercise in the Soft Drink trial (by -0.5 ± 0.3 mmol/L, P<0.04), but was not statistically different than Water at this time point (P=0.34). Urine specific gravity and urinary osmolality, sodium, and potassium (Table 1) did not statistically differ between trials at any point (P≥0.18). It is important to note that changes in urinary sodium at post-exercise during Soft Drink (-23 ± 34 mmol/L, P=0.053) and Water (-10 ± 43 mmol/L, P=0.62) were likely underestimated because 4 values in the Soft Drink trial and 5 values in the Water trial were measured at or below our sensitivity for detection (i.e., 20 mmol/L). In these instances, a conservative approach was utilized and values of 20 mmol/L were assigned.

Renal function and biomarkers of acute kidney injury

There were greater reductions in eGFR (Table 3) from pre-exercise to post-exercise in Soft Drink (P<0.01). At 24 h, eGFR remained reduced in Soft Drink (P<0.01) but returned to pre-exercise levels in Water (P=0.11). Urinary creatinine (Table 3) was not statistically different between trials at pre- and post-exercise, and at 24 h (P≥0.62), but was elevated in both trials from pre-exercise at post-exercise (P≤0.01) and 24 h (P<0.01). BUN (Table 3) was not statistically different between trials at pre-exercise (P=0.99), but at post-exercise was reduced in Soft Drink compared to Water (P=0.03). Reductions in the fractional excretion of sodium (Table 3) were attenuated in Soft Drink (P<0.03) at post-exercise. The fractional excretion of potassium (Table 3) was not statistically different between trials at any time point (P≥0.94). Overnight urine volumes

(Soft Drink: 1.6 ± 0.6, Water: 2.0 ± 0.7 L, P=0.01) and flow rates (Figure 3A, P=0.01) were lower in Soft Drink.

Serum creatinine (Table 3) increased ≥0.30 mg/dL in 8 out of 12 participants in Soft Drink (change in serum creatinine: 0.30 ± 0.08 mg/dL, P<0.01) and in 1 out of 12 participants in Water (change in serum creatinine: 0.16 ± 0.03 mg/dL, P<0.01). Urine output during the overnight period was lower in Soft Drink compared to Water (1.2 ± 0.4 vs. 1.4 ± 0.5 mL/kg/h, P<0.02). However, neither met the classification for Stage 1 AKI. In total, based on the change in serum creatinine data, the incidence of Stage 1 AKI (Figure 2A) was greater in Soft Drink at post-exercise (P=0.02), but any AKI from this classification was resolved in both trials at 24 h. At pre-exercise, BUN:creatinine (Table 3) was not statistically different between trials (P>0.99). However, there were greater reductions in BUN:creatinine at post-exercise in Soft Drink compared to Water (P<0.01). In both trials, plasma NGAL (Figure 2B) increased at post-exercise (P<0.01) and returned to pre-exercise concentrations at 24 h (P≥0.91). Urinary NGAL (Figure 3C) did not statistically differ between trials at post-exercise (P=0.99) or 24 h (P=0.99), but was elevated above pre-exercise concentrations in Soft Drink at 24 h (P<0.03). Additionally, correcting urinary NGAL concentrations to urine osmolality or urinary creatinine did not produce any changes in these findings (data not shown). Overnight urinary NGAL (Figure 3B) was elevated in Soft Drink compared to Water (6 ± 4 ng/dL vs. 5 ± 4 ng/dL, P=0.03). Furthermore, when correcting for urine flow rate, overnight urinary NGAL flow rate (Figure 3C) remained elevated in Soft Drink (4 ± 4 $[ng \cdot dL^{-1}]/[mL \cdot min^{-1}]$ vs. 6 ± 7 $[ng \cdot dL^{-1}]/[mL \cdot min^{1}]$, P=0.03).

Potential mechanisms of acute kidney injury

Serum copeptin (Figure 4A) was lower in Soft Drink pre-exercise ($P<0.01$). However, the increase in serum copeptin (Figure 4B) was greater in Soft Drink at post-exercise ($P<0.02$) and 24 h ($P<0.01$). Increases in serum uric acid (Figure 4D) were greater in Soft Drink at post-exercise ($P<0.01$) and 24 h ($P=0.05$).

Discussion

In support of our hypothesis, the present study provides evidence in humans that consuming a soft drink during and following exercise in the heat reduces renal function and increases markers of AKI compared to when consuming water. Specifically, renal function was reduced to a greater extent in the Soft Drink trial, as demonstrated by larger increases in serum creatinine and reduced overnight urine flow rate compared to water. Furthermore, the incidence of stage 1 AKI and overnight urinary NGAL, a marker of renal tubule injury, were both higher in the Soft Drink trial. Our data also indicate that elevations in vasopressin (i.e., copeptin) and serum uric acid may play important roles in the mechanisms that lead to the development of AKI evoked by consuming a soft drink during and following exercise in the heat compared to when consuming water.

Mild dehydration invoked by soft drink consumption

Consuming a soft drink during exercise in the heat worsened some markers of dehydration compared to water. A greater reduction in relative body weight occurred during the Soft Drink trial. Plasma osmolality and serum sodium did not increase from pre-exercise in the Soft Drink trial at post-exercise. However, in the Soft Drink trial,

plasma osmolality and serum sodium were elevated above concentrations in the Water trial. Previous work in rats indicates that consuming a soft drink-like beverage after 30 d of heat induced dehydration results in greater increases in plasma osmolality (14). Our data do not support that this occurs acutely in humans consuming soft drinks during and following exercise in the heat. The reason for the differences between our work and that conducted in rats is not inherently clear. However, we speculate it may be related to acute versus chronic soft drink consumption. Plasma volume was reduced in the Soft Drink trial at post-exercise but not in Water. Based on previous evidence, this reduction in plasma volume occurring with soft drink consumption is not likely a function of caffeine content. This is supported by findings that moderate caffeine intake during exercise in the heat has minimal effects on body fluid regulation (2) and that the relatively small diuretic effect of moderate caffeine intake at rest is negated during exercise (64). Rather, we believe that the decrease in plasma volume with soft drink consumption was likely because of the increased net secretion of water into the intestinal lumen that occurs when ingesting a hypertonic beverage (12). Our data are also indicative of an increased state of water conservation with soft drink consumption as vasopressin (copeptin) concentrations increased to a greater extent from pre-exercise in the Soft Drink trial. Thus, soft drink consumption during and following exercise in the heat induced a mild state of dehydration compared to relative maintenance of euhydration in the Water trial.

Mechanisms of AKI with soft drink consumption

Exercise in the heat increases sympathetic activation and circulating vasopressin (35), which subsequently reduces blood flow to the kidneys (44), creating a potentially deleterious pre-renal state (40). Exercise (23), heat strain (44), and dehydration (38) independently reduce renal perfusion, and results in even greater decrements when these states are combined (53). Reductions in renal perfusion can create localized ischemia within the kidneys resulting in a low ATP environment that promotes elevations in uric acid, oxidative stress and inflammation, which ultimately results in AKI (4). In the present study, renal perfusion was not assessed. Despite this consideration, due to the mild dehydration, elevated blood pressure response, and increased serum uric acid concentration elicited during the Soft Drink trial, we speculate that increased sympathetic activity and greater increases in circulating vasopressin (copeptin) likely reduced renal perfusion to a greater extent in Soft Drink compared to Water during exercise in the heat. Although relatively high amounts of caffeine in soft drinks (~150 mg) acutely increase blood pressure (57), there appears to be no additive effect of caffeine on the relative hypertension evoked by prolonged exercise in the heat (10). Thus, the relative hypertension in the Soft Drink trial may be contributed to by the consumption of fructose, which elevates blood pressure acutely (5) and may be caused by increases in serum uric acid (27, 41). In contrast to the evidence supporting a pre-renal etiology, BUN:creatinine is often used clinically to distinguish the etiology of AKI (i.e., pre-renal or intrarenal), where a ratio >20 is indicative of AKI of pre-renal origin (39). BUN:creatinine in the present study was approximately 10:1 in the Soft Drink trial at post-exercise, suggestive of an intrarenal derived AKI. However, recent evidence suggests that diagnostic efficacy of BUN:creatinine is unclear (31), particularly

416 considering that there currently is no consensus definition of pre-renal failure (30).
417 Despite this consideration, the intrarenal derived AKI is also supported by the higher
418 urine NGAL flow rate, which is suggestive of tubular injury, in the Soft Drink trial.

419 Vasopressin is believed to play a key role in the mechanism by which exercise in
420 the heat elicits AKI. Renal vasopressin receptor 2 agonism (48) and antagonism (15)
421 exacerbates and protects, respectively, against AKI during recurrent heat-induced
422 dehydration in non-human animals. In the present study, vasopressin (copeptin)
423 concentration increased to a greater extent during the Soft Drink trial compared to
424 Water. Because plasma osmolality was not elevated from pre-exercise in the Soft-Drink
425 trial, this observation was likely in response to the modest reduction in plasma volume
426 and/or greater circulating levels of fructose, which has been shown to independently
427 increase vasopressin release (54). This latter contention is speculative because we did
428 not measure circulating fructose. However, prior studies in humans suggest that
429 fructose stimulates vasopressin independent of osmolality (63).

430 It has been shown that rehydration with water in mice undergoing chronic heat-
431 induced dehydration is protective against AKI (14). Our data support this contention in
432 that the maintenance of fluid balance by consuming Water during and following exercise
433 in the heat prevented AKI in humans. Our laboratory has previously identified that the
434 magnitude of increases in core temperature and dehydration contributed to the
435 incidence and severity of AKI occurring during exercise in the heat (51). The findings
436 from the Water trial of the present study, together with these previous studies in human
437 (22) and non-human animals (14, 15), indicate that hydration status may be a more
438 important factor in dictating AKI compared to increases in core temperature. The

potential importance of body fluid status is particularly notable because, unlike our study, the aforementioned non-human animal studies demonstrating the efficacy of water rehydration did not involve exercise during heat exposure. This is important because prolonged exercise alone has been shown to increase markers of AKI (3). Clearly, formal studies are required to delineate the relative effects of exercise, increases in core temperature, and dehydration on AKI.

Increased circulating vasopressin and dehydration activate the polyol-fructokinase pathway, which may induce AKI by promoting reductions in ATP and increases in uric acid, oxidative stress and inflammation when fructokinase metabolizes fructose in the renal tubules (48). Importantly, knockout of the fructokinase enzyme is protective against AKI during heat stress and dehydration (45), suggesting that an increased fructose load may promote AKI. In support of this, endogenous fructose production, which is an end-product of the sorbitol produced to protect the renal medullary cells from a hypertonic environment (21), can amplify the polyol-fructokinase pathway (45). Furthermore, this pathway is exacerbated and the resultant AKI is more severe when fructose is consumed exogenously with high fructose beverages (e.g., soft drinks) following heat-induced dehydration in animals (14). To our knowledge, it is not possible to measure renal fructokinase and aldose reductase activity in humans. Thus, the effect of these pathways in humans exercising in the heat remains unclear, although we speculate that a potential role is likely.

Fructose markedly decreases renal cortical and medullary ATP (6) and raises serum uric acid concentration (55). Uric acid is not only a by-product of the polyol-fructokinase pathway, but increased concentrations in the blood stimulate aldose

462 reductase (19). Treatment with allopurinol (a xanthine oxidoreductase inhibitor) to blunt
463 elevations in serum uric acid abates the increase in renal cortical derived-fructose
464 during recurrent heat-induced dehydration in mice and decreases AKI (49). In these
465 animals, fructose and uric acid concentrations in the renal cortex increase independent
466 of (49), and when consuming (14), exogenous fructose following recurrent heat-induced
467 dehydration. As previously mentioned, this model commonly employed in mice does not
468 consider exercise. Importantly, in humans, exercise in the heat decreases renal
469 perfusion (44) and elevates serum uric acid concentrations (25). Moreover, uric acid is
470 believed to be a mediator for AKI in the epidemic of chronic kidney disease in outdoor
471 workers (46), and has been shown to be elevated across a work shift in these workers
472 (16, 59, 60, 62).
473
474 *Considerations*
475 There are a few considerations that warrant discussion. Due to an error during
476 the data collection process, we were unable to establish pre-exercise urine flow rates.
477 Thus, we were unable to report glomerular filtration rate as a function of creatinine
478 clearance. In the present study glomerular filtration rate was estimated from serum
479 creatinine, which has been previously shown to underestimate the extent of reductions
480 in glomerular filtration rate during exercise (43). Urinary NGAL was not different at post-
481 exercise, but was increased above pre-exercise at 24 h in the Soft Drink trial. Moreover,
482 recovery data were not collected in the present study. Thus, it is possible that acute
483 changes in urine NGAL may have been detected with an increased sample frequency
484 after the post-exercise data collection period (such as following a recovery period). The

485 use of water as a control for a soft drink does not allow interpretation for which
486 constituents of the drink are responsible for the increases in AKI when consumed during
487 and following exercise in the heat. Further research is warranted to establish the degree
488 to which beverage osmolality, caffeine, the concentration of fructose, and/or the dose of
489 these constituents bring about AKI. It has been suggested that phosphorus in soft drinks
490 (e.g., colas) could exacerbate AKI during exercise in the heat (9). However, Mountain
491 Dew® has a relatively low phosphate concentration compared to colas (<1 mg/serving
492 vs. ~58 mg/serving) (61). This suggests phosphorus is an unlikely contributor to the
493 increases in biomarkers of AKI in our study. A negative control (i.e., a trial without
494 rehydration) was not performed in the present study. However, we believe this to be
495 justified based on the ethics of not allowing rehydration during prolonged exercise in the
496 heat, which would be contrary to current occupational heat stress recommendations (1,
497 20). Also, the exercise protocol simulated a 4 h workday, and may not reflect the
498 changes in AKI brought about by a longer work shift. While the environmental conditions
499 in our experiment, with a wet bulb globe temperature (WBGT) of 30.5˚C, were similar to
500 a morning work shift, these conditions could be underestimating the total heat load
501 experienced throughout a work day by laborers at risk for AKI. WBGT's have been
502 recorded up to 33.8˚C during afternoon outdoor exercise (16), far exceeding the
503 occupational exposure limits for acclimatized workers (26-28˚C) (20). Subclinical muscle
504 damage and/or rhabdomyolysis has been shown to increase the extent of AKI during
505 exercise in the heat (22). Markers of muscle damage (e.g., creatine kinase) were not
506 measured in the present study. However, it is unlikely that there was differential muscle
507 damage occurring with soft drink consumption compared to water as both trials were the

same duration and intensity, and participants were appropriately familiarized during the screening visit. Further the results of this study are constrained to an acute bout of exercise in the heat. Thus, it is possible the results may vary from chronic exercise in the heat and subsequent heat acclimatization. Finally, the present study is limited to the acute effects of 3 L of soft drink consumption during and following exercise in the heat. As such, the dose-response relationship and effects of chronic consumption on biomarkers AKI in humans remains unknown. However, long-term consumption of sugar-sweetened soft drinks (i.e., high fructose) is associated with a higher risk of chronic kidney disease, and could be worsened with the addition of regular exercise in the heat (7).

Conclusions

We have demonstrated that soft drink consumption during and following exercise in the heat increases markers of AKI. These increases were likely due to the hyperosmolality and fructose content of the soft drink, which increased circulating vasopressin and resulted in elevations in serum uric acid. The results of this study highlight a potential role of vasopressin, uric acid and the polyol-fructokinase pathway in the etiology of AKI when consuming soft drink during and following exercise in the heat.

Perspectives and significance

There is an epidemic of chronic kidney disease occurring in people who regularly work in hot environments that is believed to be due to an accumulation of repetitive subclinical AKI (8). In the present study, the exercise protocol and environmental

conditions were specifically chosen to reflect the typical physical and outdoor demands of workers at risk for chronic kidney disease (16). AKI was not detected when consuming Water during and following 4 h of exercise in the heat. However, transient elevations in biomarkers of AKI were detected with soft drink consumption and were resolved at 24 h. Our findings suggest that consuming a soft drink (i.e., a hypertonic, high fructose drink) during and following exercise in the heat may elicit AKI in humans through an augmented vasopressin response and likely increased polyol-fructokinase response that elevated uric acid production. Our data also indicate that consumption of soft drink during and following exercise in the heat does not rehydrate (Table 2). Thus, consuming soft drinks as a rehydration beverage during exercise in the heat may not be ideal. Future work will need to discern the long-term effects of soft drink consumption during exercise in the heat, and its relation to the risk of AKI.

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Conflict of Interest

There are no conflicts of interest to report.

554

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557

558 **Figure and Table Legends**

559

560 Figure 1 – Thermal and cardiovascular responses to exercise in the heat when
561 consuming a soft drink (Soft Drink) or water (Water). Absolute values of core
562 temperature (*n*=10) before (Pre), at the end (End), and immediately after (Post) exercise
563 in the heat during Soft Drink and Water trials (A). Absolute values of heart rate (B, *n*=12)
564 and mean arterial pressure (C, *n*=12) at Pre, End, Post and 24 h from pre-exercise (24
565 h) during Soft Drink and Water trials. Statistical analyses from post hoc two-tailed
566 Sidak's test pairwise comparisons completed following a two-way repeated measures
567 ANOVA: * = different from Water (P<0.02), P = different from Pre (P<0.04). Mean ± SD.

568

569 Figure 2 – Biomarkers of acute kidney injury (AKI) when consuming a soft drink (Soft
570 Drink) or water (Water) during and following exercise in the heat. Incidence of stage 1
571 AKI (A, *n*=12) at post-exercise (Post) and 24 h from pre-exercise (24 h). Absolute
572 values of neutrophil gelatinase-associated lipocalin (NGAL) in plasma (B, *n*=12) and
573 urine (C, *n*=10) at pre-exercise (Pre), Post and 24 h in Soft Drink and Water trials.
574 Statistical analyses from two-tailed independent Wilcoxon matched-pairs signed rank
575 test at Post or 24 h (A), or post hoc two-tailed Sidak's test pairwise comparisons

576 completed following a two-way repeated measures ANOVA (B, C): * = different from
577 Water (P<0.02), P = different from Pre (P<0.03). Mean ± SD.
578
579 Figure 3 – Analysis of the overnight urine collection taken between the time period of
580 post-exercise in the heat and 24 h from pre-exercise (~18 h) where participants
581 consumed either soft drink (Soft Drink) or water (Water) during and following exercise.
582 Absolute values of urine flow rate (A, *n*=12) and neutrophil gelatinase-associated
583 lipocalin (NGAL) in the urine (B, *n*=12) in the Soft Drink and Water trials. Absolute
584 values of urinary NGAL normalized to urine flow rate (C, *n*=12) in the Soft Drink and
585 Water trials. Statistical analyses from one-tailed (A, C) and two-tailed (B) paired t-tests.
586 Mean with individual data.
587
588 Figure 4 – Potential mechanisms of acute kidney injury (AKI). Absolute values and
589 changes (Δ) in serum copeptin – a stable proxy of vasopressin (A and B, *n*=11), and
590 serum uric acid (C and D, *n*=11) before (Pre), immediately after (Post), and 24 h after
591 pre-exercise (24 h) in the heat. Soft Drink (Soft Drink) or water (Water) was consumed
592 during and following exercise. Statistical analyses from post hoc two-tailed Sidak's test
593 pairwise comparisons completed following a two-way repeated measures ANOVA: * =
594 different from Water (P<0.02), P = different from Pre (P≤0.05). Mean ± SD.
595
596 Table 1 – Markers of hydration status before (Pre), immediately after (Post), and 24 h
597 after pre-exercise (24 h) in the heat when consuming a soft drink (Soft Drink) or water
598 (Water) during and following exercise. Statistical analyses from post hoc two-tailed

Sidak's test pairwise comparisons completed following a two-way repeated measures ANOVA: * = different from Water ($P \leq 0.05$), P = different from Pre ($P < 0.05$). Mean ± SD, *n*=12.

Table 2 – Fluid and food intakes during the overnight period (~18 hrs) between post-exercise and 24 h from pre-exercise in the heat when consuming a soft drink (Soft Drink) or water (Water) during and following exercise. Statistical analyses from two-tailed paired t-tests. Mean ± SD, *n*=12.

Table 3 – Absolute values and change (Δ) from pre-exercise of indices of renal function before (Pre), immediately after (Post), and 24 h after pre-exercise (24 h) in Soft Drink and Water trials. eGFR = estimated glomerular filtration rate, FENa = fractional excretion of sodium, FEK = fractional excretion of potassium, BUN:creatinine = the ratio of blood urea nitrogen to serum creatinine. Statistical analyses from post hoc two-tailed Sidak's test pairwise comparisons completed following a two-way repeated measures ANOVA: * = different from Water ($P < 0.05$), P = different from Pre ($P \leq 0.04$). Mean ± SD, *n*=12.

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Table 1. *Markers of Hydration*

Parameter	Water			Soft Drink		
	Pre	*Post*	*24 h*	*Pre*	*Post*	*24 h*
Fluid volume loss						
Absolute body weight (kg)	76.7 ± 11.8	76.6 ± 11.3	76.3 ± 12.2[P]	77.4 ± 12.4*	77.1 ± 11.9*	76.6 ± 12.5[P]
(corrected for drink weight)		74.6 ± 11.3[P]			74.9 ± 11.9[P]	
Δ Relative body weight (%)	-	-0.0 ± 0.7	-0.6 ± 1.0[P]	-	-0.3 ± 0.8	-1.1 ± 1.1[P]
(corrected for drink weight)		-2.7 ± 0.4[P]			-3.2 ± 0.5[P]*	
Blood samples						
Δ Plasma volume (%)	-	-2 ± 7	-1 ± 8	-	-5 ± 6[P]	-1 ± 5
Plasma osmolality (mOsm/kg)	280 ± 4	274 ± 4[P]	281 ± 2	278 ± 4	280 ± 4*	281 ± 3[P]
Serum sodium (mmol/L)	139 ± 2	137 ± 2[P]	139 ± 1	139 ± 2	140 ± 2*	140 ± 1[P]
Serum potassium (mmol/L)	4.3 ± 0.5	4.1 ± 0.4	4.3 ± 0.5	4.2 ± 0.3	3.7 ± 0.3[P]	4.4 ± 0.3
Urine samples						
Specific gravity	1.008 ± 0.006	1.015 ± 0.008[P]	1.020 ± 0.006[P]	1.011 ± 0.008	1.017 ± 0.011[P]	1.021 ± 0.007[P]
Osmolality (mOsm/kg)	292 ± 255	478 ± 246[P]	697 ± 207[P]	376 ± 279	445 ± 308[P]	662 ± 256[P]
Sodium (mmol/L)	44 ± 40	34 ± 23	62 ± 40	56 ± 40	33 ± 16	66 ± 51
Potassium (mmol/L)	13.1 ± 9.6	57.0 ± 37.6[P]	45.4 ± 16.8[P]	20.7 ± 16.3	46.2 ± 37.5[P]	55.7 ± 27.8[P]

Table 2. *Overnight fluid and food intake.*

	Water	Soft Drink	P-Value
Fluid volume (mL)	2362 ± 1242	2709 ± 1053	0.09
Total energy (kcal)	1736 ± 837	2480 ± 769	<0.01
Fat (g)	61 ± 40	52 ± 42	0.28
Protein (g)	70 ± 33	65 ± 37	0.44
Total carbohydrate (g)	205 ± 122	474 ± 135	<0.01
Sugar (g)	79 ± 70	340 ± 88	<0.01
Sodium (mg)	2356 ± 983	2594 ± 1159	0.39

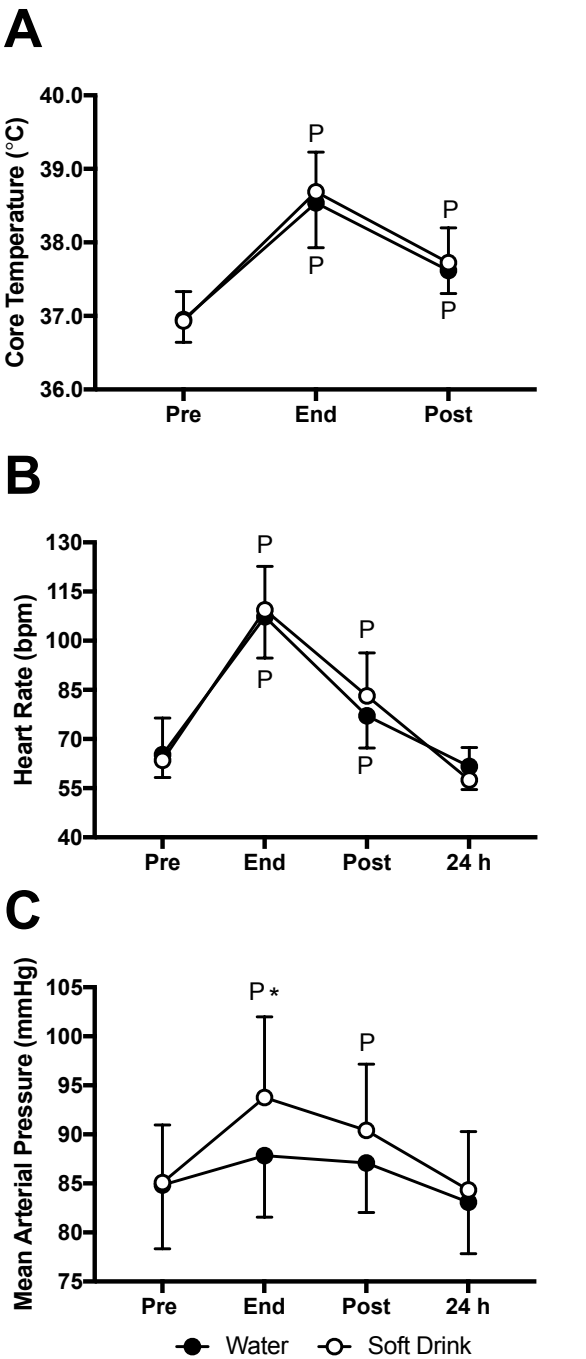
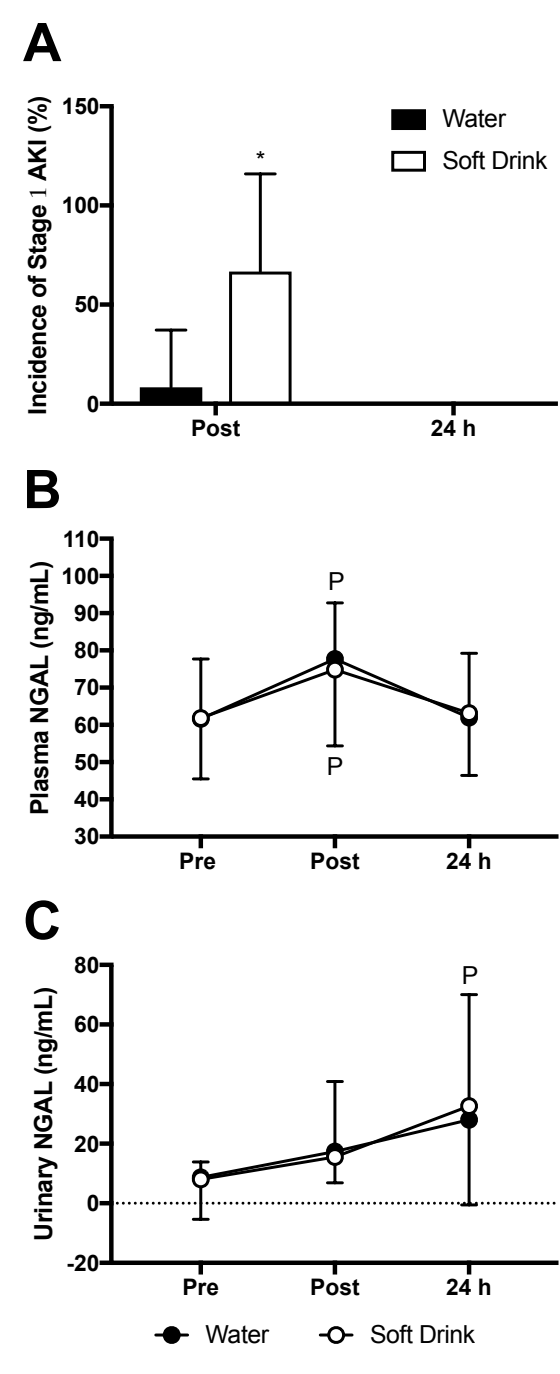
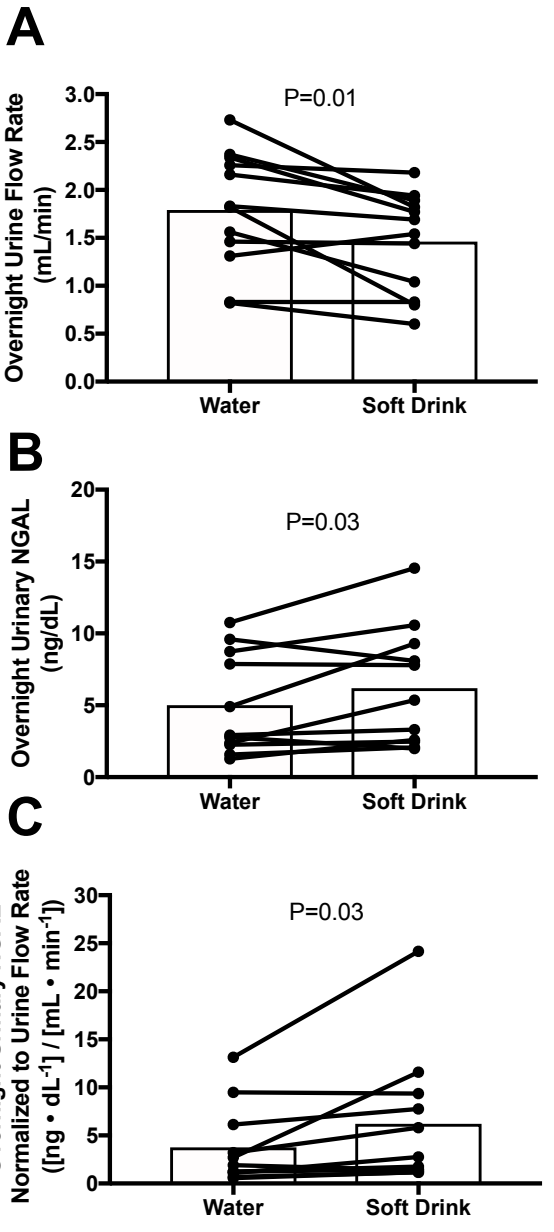
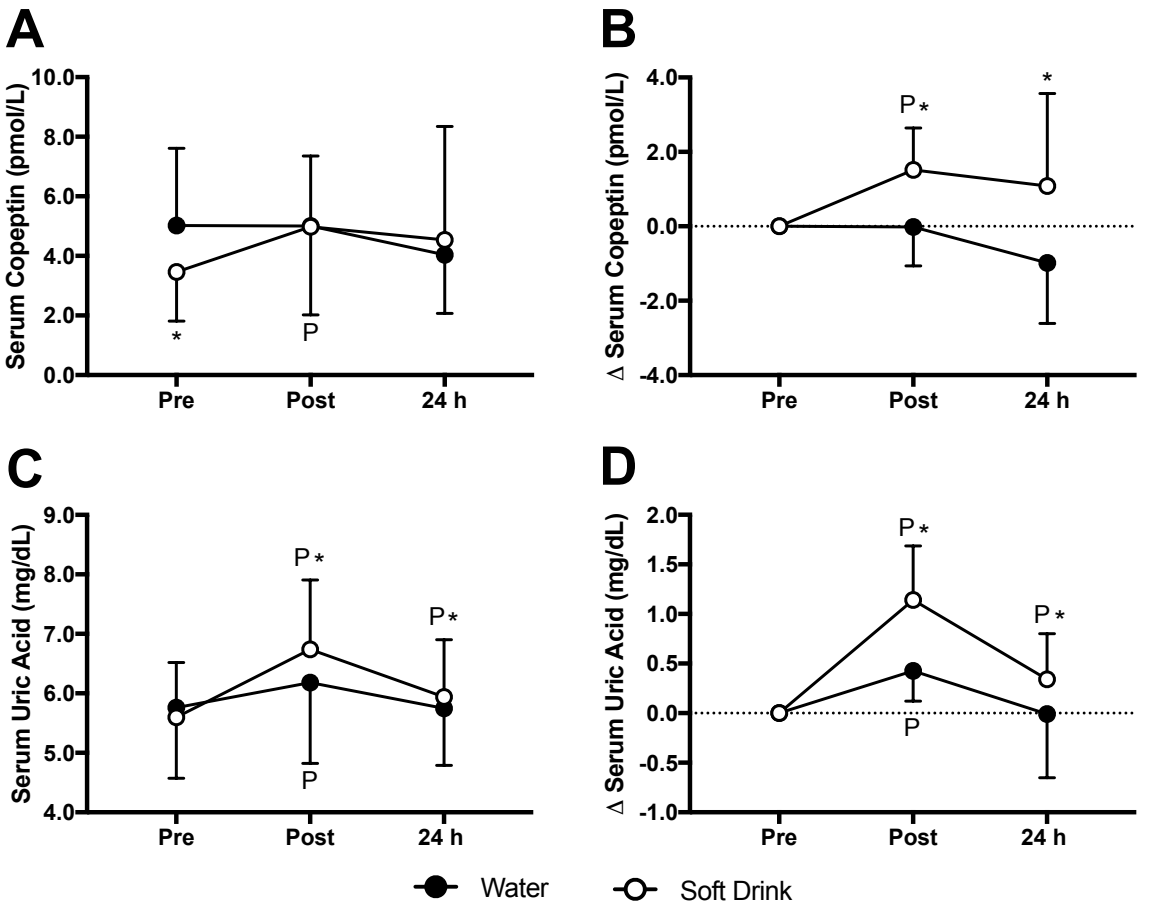

A
Core Temperature (°C)
40.0
39.0
38.0
37.0
36.0
P
P
P
P
Pre
End
Post
B
Heart Rate (bpm)
130
115
100
85
70
55
40
P
P
P
P
Pre
End
Post
24 h
C
Mean Arterial Pressure (mmHg)
105
100
95
90
85
80
75
P *
P
Pre
End
Post
24 h
Water
Soft Drink

Table 3. *Indices of renal function*

Parameter	Water			Soft Drink		
	Pre	*Post*	*24 h*	*Pre*	*Post*	*24 h*
Serum creatinine (mg/dL)	0.96 ± 0.15	1.11 ± 0.21^{P}	0.98 ± 0.17	0.94 ± 0.13	1.24 ± 0.18^{P*}	1.02 ± 0.16^{P}
Δ Serum creatinine (mg/dL)		0.16 ± 0.08^{P}	0.03 ± 0.05		0.30 ± 0.08^{P*}	0.08 ± 0.09^{P*}
Urinary creatinine (mg/dL)	62 ± 57	211 ± 148^{P}	108 ± 112^{P}	79 ± 66	176 ± 142^{P}	236 ± 143^{P}
Δ Urinary creatinine (mg/dL)		149 ± 124^{P}	145 ± 115^{P}		97 ± 140^{P}	156 ± 158^{P}
Blood urea nitrogen (mg/dL)	13 ± 3	15 ± 3	14 ± 3	13 ± 3	12 ± 4*	12 ± 4*
Δ Blood urea nitrogen (mg/dL)		1 ± 1	1 ± 2		-1 ± 1	-0 ± 2
Urinary urea nitrogen (mg/dL)	410 ± 377	683 ± 365^{P}	1039 ± 404^{P}	518 ± 439	595 ± 412	1008 ± 440^{P}
Δ Urinary urea nitrogen (mg/dL)		273 ± 438^{P}	629 ± 454^{P}		77 ± 520	490 ± 527^{P}
eGFR (mL/min/1.73m^2)	92 ± 12	78 ± 12^{P}	89 ± 13	93 ± 11	69 ± 11^{P*}	85 ± 13^{P}
Δ eGFR (mL/min/1.73m^2)		-14 ± 5^{P}	-3 ± 5^{P}		-24 ± 5^{P*}	-8 ± 8^{P*}
FENa (%)	0.7 ± 0.5	0.2 ± 0.1^{P}	0.2 ± 0.2^{P}	0.6 ± 0.2^{P}	0.3 ± 0.3^{P}	0.3 ± 0.2
Δ FENa (%)		-0.6 ± 0.5^{P}	-0.5 ± 0.5^{P}		-0.3 ± 0.4^{P*}	-0.3 ± 0.2^{P}
FEK (%)	7.5 ± 4.5	8.6 ± 3.3	6.3 ± 2.9	8.3 ± 6.0	10.4 ± 8.4	7.4 ± 6.0
Δ FEK (%)		1.1 ± 3.5	-1.2 ± 5.0		2.1 ± 5.3	-1.0 ± 5.4
BUN:creatinine (a.u.)	14 ± 3	12 ± 3	14 ± 3	13 ± 3	10 ± 2^{P*}	12 ± 4*
Δ BUN:creatinine (a.u.)		-1 ± 1	0 ± 2		-4 ± 1^{P*}	-1 ± 3*


A
Incidence of Stage 1 AKI (%)
150
100
50
0
*
Water
Soft Drink
Post
24 h
B
Plasma NGAL (ng/mL)
110
100
90
80
70
60
50
40
30
P
P
Pre
Post
24 h
C
Urinary NGAL (ng/mL)
80
60
40
20
0
-20
P
Pre
Post
24 h
Water
Soft Drink


A
Overnight Urine Flow Rate (mL/min)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P=0.01
Water
Soft Drink
B
Overnight Urinary NGAL (ng/dL)
20
15
10
5
0
P=0.03
Water
Soft Drink
C
Overnight Urinary NGAL Normalized to Urine Flow Rate ([ng • dL-1] / [mL • min-1])
30
25
20
15
10
5
0
P=0.03
Water
Soft Drink


A
Serum Copeptin (pmol/L)
10.0
8.0
6.0
4.0
2.0
0.0
*
P
Pre
Post
24 h
B
Δ Serum Copeptin (pmol/L)
4.0
2.0
0.0
-2.0
-4.0
P*
*
Pre
Post
24 h
C
Serum Uric Acid (mg/dL)
9.0
8.0
7.0
6.0
5.0
4.0
P*
P*
P
Pre
Post
24 h
D
Δ Serum Uric Acid (mg/dL)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
P*
P*
P
Pre
Post
24 h
Water
Soft Drink