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Hypohydration Augments the Acute Increase in Urinary Biomarkers of Kidney Injury Following the 100-mile Western States Endurance Run

Braxton A. Linder^{1,2}, Soolim Jeong^{1,2}, Nina L. Stute², Omar B. El-Kurd³, Joseph Vondrasek⁴, Andrew Pasternak⁵, James R. Bagley³, Joseph C. Watso⁴, Zachary J. Schlader¹, Matthew C. Babcock⁶, Gregory J. Grosicki^{7,†}, Austin T. Robinson^{1,2,†}

¹Department of Kinesiology, Indiana University School of Public Health - Bloomington, IN, USA 47405

²School of Kinesiology, Auburn University, Auburn, AL, USA 36849

³Department of Kinesiology, San Francisco State University, San Francisco, CA, USA 94132

⁴Department of Health, Nutrition and Food Sciences, Florida State University, Tallahassee, FL, USA 32306

⁵Silver Sage Center for Family Medicine, Reno School of Medicine, University of Nevada, Reno, NV, USA 89521

⁶Virginia Commonwealth University, Richmond, VA, USA, 23284

⁷WHOOP, Inc., Boston, MA, USA 02215

Abstract

Purpose: Ultra-endurance races may transiently increase acute kidney injury (AKI) risk, yet data on contributing factors are sparse. Therefore, we examined the acute effects of the Western States Endurance Run (WSER; [100 miles; 161 kilometers]) on urinary AKI biomarkers and kidney blood flow, while also assessing the influence of race pace and hydration status.

Methods: Blood and urine samples were collected to assess urine specific gravity (USG), creatinine, and AKI risk (nephritin, neutrophil gelatinase-associated lipocalin [NGAL], insulin like growth factor binding protein 7 [IGFBP7], tissue inhibitor of metalloproteinase 2 [TIMP2], and IGFBP-7●TIMP-2). Renal blood velocity and conductance were measured via Doppler ultrasonography.

Results: Pre and post measures were obtained from 36 runners (29Males/7Females, age: 44±9 years, mean finish time: 25:45:55). Urinary AKI biomarkers increased post-race, with nephritin,

Address for correspondence: Austin Robinson, PhD, Associate Professor, Department of Kinesiology, Indiana University Bloomington School of Public Health, Bloomington, IN 47405, ausrobin@iu.edu.

[†]Co-senior author contributions

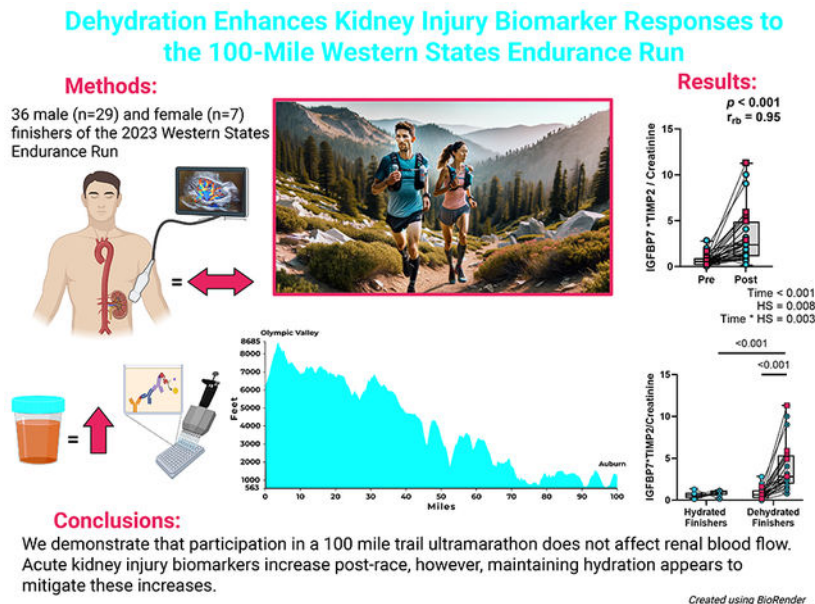
Disclosures

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IGFBP7, and IGFBP7•TIMP-2 remaining elevated after indexing to creatinine ($p < 0.008$). Hydration status declined, with only 8 of 35 runners remaining euhydrated post-race. Finishing euhydrated attenuated increases in urinary creatinine and creatinine-indexed IGFBP-7•TIMP-2 ($p \leq 0.006$). Faster race pace correlated with increases in USG ($\rho = 0.338$, $p = 0.048$) and urinary creatinine ($\rho = 0.443$, $p = 0.010$), but not with creatinine-indexed AKI biomarkers ($p \geq 0.382$). Kidney blood velocity and conductance were unchanged ($p \geq 0.186$).

Conclusion: Finishing hydrated appears to help mitigate transient increase in kidney injury following an ultra-endurance race.

Graphical Abstract



New and Noteworthy:

Urinary concentrations of several acute kidney injury (AKI) biomarkers increased after completing the 2023 Western States Endurance Run, however, there were no changes in renal blood flow in a subset of runners. When normalized to creatinine, several AKI biomarkers including IGFBP7•TIMP2 (an FDA approved biomarker of AKI) remained elevated. Notably, finishing the race euhydrated appeared to have a protective effect, mitigating increases in IGFBP7•TIMP2.

Keywords

Ultramarathon; Acute kidney injury; renal blood flow; hydration; performance

1. Introduction

Ultramarathons (i.e., footraces over 42 kilometers) have become increasingly popular, with interest and participation rising exponentially over the past decade [1]. Beyond prolonged physical exertion, ultramarathon runners often face extreme temperatures, hypohydration, and altitude changes, contributing to a relatively high incidence of mild-to-moderate acute

kidney injury (**AKI**) [2]. Importantly, findings from epidemiological and experimental studies suggest that AKI may serve as a trigger for the development of chronic kidney disease [3, 4].

Prolonged exercise transiently increases serum creatinine (proxy for decreased kidney function) and urinary AKI biomarkers [5-14]. Indeed, data from marathon [15-18] and ultramarathon finishers [5, 9, 14, 19-21] indicate that up to 80% of finishers experience transient elevations in AKI biomarkers. The clinical significance of elevated AKI biomarkers and whether repeated participation predisposes runners to long-term sequelae remains unclear [5, 9, 22]. Nonetheless, many studies have not accounted for hydration status or race intensity (i.e., running pace) when assessing post-race AKI. Given the rise in popularity of ultra-endurance footraces, it is important to investigate strategies, such as maintaining euhydration, to mitigate AKI risk. Regarding potential mechanisms of AKI, blood flow is redistributed during exercise, thus reducing renal perfusion 7 – 8-fold during maximal exertion [23, 24]. The reduction in renal perfusion during exercise, mediated mainly by renal sympathetic nerve activity [23] and catecholamine release [25], can be exacerbated by heat stress, and hypohydration [26-28], which are common during ultramarathons. Specifically, shifts in blood flow towards working muscles for oxygen delivery [29, 30] and skin for cooling [31] and reduced blood volume with exercise and heat-induced hypohydration lead to reduced kidney perfusion. Thus, adequate hydration, which can help prevent blood volume reductions, may attenuate AKI risk during ultramarathons. Another concern with these events is post-race increases in circulating creatine kinase (**CK**) [32, 33], a biomarker of muscle damage, and exertional rhabdomyolysis, both of which are linked to AKI in endurance athletes [34]. However, their role in AKI development during ultramarathons is uncertain.

The purpose of this study was to examine whether completing the 161 km (100.2-mile) Western States Endurance Run (**WSER**) ultramarathon 1) increases AKI risk, evaluated using a comprehensive panel of urinary biomarkers; and 2) reduces renal perfusion. We also investigated whether maintaining euhydration blunts increases in AKI biomarkers or reductions in renal perfusion, and whether race pace influences these changes. We indexed urinary AKI markers to creatinine and osmolality to account for variations in urine concentration and dilution. We hypothesized that runners with a post-race urine specific gravity(**USG**) ≤ 1.020 , whom we presumed maintained euhydration during the WSER [35], would experience smaller increases in urinary AKI biomarkers [36]. We also hypothesized that faster race pace would correlate with greater changes in AKI biomarkers [23, 25].

2. Methods

Participants were recruited from athletes entered to run the 2023 WSER, an ultra-endurance event traversing the Sierra Nevada mountains that includes 5486 m of ascent and 7010 m of descent [37]. As we have described [33], the race is held annually in the last week of June, with typical ambient temperatures ranging between 4 and 37°C. The race begins at 0500 Pacific time, and runners have 30 hours to complete the distance. There are 20 aid stations on the course all supplied with beverages and runners are encouraged to maintain *ad libitum* food and fluid intake for the duration of the event. We collected information

on environmental conditions within our field data collection laboratory using a Davis Instruments Vantage Vue weather station console (DAVIS 6351M).

2.1 Study Design

The present investigation was conducted in 2023, a year in which 379 runners (296 male/83 female, ~22% female) began the race, of which 328 (260 male/68 female, ~21% female) finished the race (86.5% finish rate) [33]. Temperature ranged from 10 to 27°C and relative humidity ranged from 30-75%. All athletes were provided written informed consent before participation and all procedures involving human research participants were approved by the Institutional Review Board at Georgia Southern University (H23253).

Participants were recruited up to six months prior to the 2023 WSER, all of which completed virtual questionnaires (training, nutrition, race preparation details) before any testing. Data included in this investigation was collected up to 72 hours before the beginning of the event (**pre**), and within 1 hour (30 ± 14 minutes, max = 59 minutes) of individuals finishing the race (**post**). At each time point, renal blood velocity was assessed as an applied measure of kidney perfusion, and spot urine and capillary blood samples were collected.

2.2 Urine collection and hydration assessment

Participants were provided sealed, sterilized urine collection cups at the pre- and post-race data collections. From the provided urine samples, we measured USG (ATC 3-in-1 Scale Clinical Refractometer) at our field laboratory and stored aliquots of urine in cryogenic tubes for future analyses on dry ice prior to longer term storage at -80°C [38, 39]. While freezing can influence stability of certain biomarkers [38, 39], frozen samples were thawed simultaneously to minimize variability and subsequently analyzed at Auburn University School of Kinesiology for electrolyte concentrations (sodium, potassium, chloride) using a Smartlyte Easylyte Electrolyte Analyzer and osmolality using an Advanced Instruments Advanced 3D3 Osmometer model 3250 [40-43]. Notably, USG and urine osmolality are well accepted biomarkers of hydration status for athletes [35, 44]. Changes in urine electrolyte concentrations and ratios (e.g., sodium: potassium) have also been reported after endurance exercise events [45, 46]. As we have previously described [40-43], we measured all analytes in triplicate, and using these methods has resulted in a $<1\%$ intra-sample coefficient for urine electrolytes and urine osmolality [41].

2.3 Biomarkers of kidney function and AKI

Capillary blood was collected using Touch-Activated Phlebotomy device (TAP Micro, YourBio Health, Medford, MA). Blood was centrifuged at $735 \times g$ for 10 minutes to separate serum, which was aliquoted and stored on dry ice prior to storage at -80°C . Serum CK was measured with a Roche Integra 400+ analyzer at the Indiana University School of Medicine Center for Diabetes and Medical Disease's Translational Core Laboratory. We also measured serum creatinine as a proxy for glomerular function via the University of Alabama at Birmingham Biochemical Genetics Laboratory. Serum and urine creatinine samples were measured using liquid chromatography-mass spectrometry (LC-MS). Briefly, LC-MS was performed using tandem mass spectrometry using multiple reaction monitoring and quantified via stable isotope dilution [47]. For LC-MS experiments, 20 μL of the sample was

deproteinated and diluted with a stable isotope in a single step. The diluted samples were subjected to isocratic HPLC with 10 mM ammonium acetate in 65% acetonitrile. Creatinine and d₃-creatinine were detected by electrospray ionization tandem mass spectrometry. For general kidney function we also measured pre- and post-race urine cystatin C as measures of glomerular filtration [43, 48].

Urine samples were also shipped to the Indiana University School of Medicine Center for Diabetes and Medical Disease's Translational Core Laboratory for multiplex analysis to assess kidney function. Multiplex assays were performed in duplicate using Meso Scale Discovery assay kits (Meso Scale Diagnostics, Rockville, MD) according to the manufacturer's instructions. The assays were selected based on the specific analytes of interest, ensuring compatibility with the matrix being tested. The assay plates were pre-coated with capture antibodies specific to the target analytes. Regarding our selection of specific analytes, nephrin is a protein expressed in the glomerulus and is responsible for maintaining the glomerular barrier, and the excretion of nephrin being associated with glomerular damage [49]. As for biomarkers related to the renal tubules, neutrophil gelatinase-associated lipocalin (**NGAL**) is a protein expressed in the thick ascending loop of Henle and the collecting ducts and is detectable up to three hours after toxic kidney injury [50]. Insulin like growth factor binding protein 7 (**IGFBP7**) and tissue inhibitor of metalloproteinase 2 (**TIMP2**) induce cell cycle arrest [51]. While it remains unclear precisely where IGFBP7 and TIMP2 are secreted and what their specific function is surrounding kidney injury, the product of the two urinary biomarkers (**IGFBP7●TIMP2**) is a sensitive biomarker for the prediction of AKI [27]. Indeed, urinary IGFBP7●TIMP2 is the only *United States Food and Drug Administration*-approved biomarker for the clinical screening of AKI risk (proprietary name NephroCheck, Astute Medical, San Diego, CA) [27]. We chose this array of analytes based on our experience [40, 43, 50-54], the Indiana University Translation Core's experience measuring them, their relatively high expression along the nephron such as the proximal tubule [28, 55] and ascending loop of Henle [56], as well as their documented effectiveness at representing sub-clinical kidney injury [28, 55-57].

Similar to our prior work, we indexed all urinary biomarkers of AKI to urine creatinine concentration [40, 43], which was an important methodological consideration since we collected spot urine samples. We indexed the AKI biomarkers to account for fluctuations in urine concentration and ensure more accurate comparisons across samples [58-61]. In clinical populations elevated spot urine creatinine alone has utility in identifying kidney injury-related risk of all-cause mortality [62] and hospitalization [63]. We anticipated indexing samples to creatinine would attenuate the magnitude of pre- to post-race changes [14]. However, we suspected elevations would persist, a finding supported by recent dehydration and endurance race studies [18, 27]. Nonetheless, urinary creatinine may be affected by prolonged exercise where it could be a function of changes in glomerular filtration rate or muscle breakdown. Therefore, we also indexed AKI biomarkers to urine osmolality and urinary cystatin C.

2.4 Renal Blood Velocity

Renal and segmental artery blood velocity (**RBV**) were collected using doppler ultrasound (GE Logiq e) with a 3-5 MHz phased array transducer [65, 66]. Participants were positioned in the decubitus position on a massage table during each collection (pre- and post-race) and familiarized with a brief 10-second breath holding protocol to minimize the movement of the kidneys [65, 66]. The coronal approach was used to visualize both renal and segmental arteries. Color flow was used to confirm the internal structure of the renal vasculature. Once the renal vasculature was confirmed, each measure lasted between three and five cardiac cycles. Measures were collected in triplicate for both renal and segmental arteries for both timepoints. Semi-automated waveform tracing (GE software) within the ultrasound was used to analyze individual mean RBV and we report the average of replicate measures here.

Regarding a technical limitation, the diameter of the vessel walls of the renal vasculature cannot be accurately measured due to the resolution of Doppler imaging with the relatively deep kidney tissue depth. Using measures such as MRI for a field study would not be feasible therefore, we used Doppler ultrasound to quantify blood velocity [65]. The use of Doppler ultrasound to quantify hemodynamics in the renal and segmental arteries operates under the assumption that vessel diameter does not change during physiologically induced changes in blood flow. This assumption is based on evidence that, during infusion of a vasoconstrictor (adenosine) and vasodilators (isosorbide dinitrate, papaverine, dopamine, and fenoldopam), changes in renal blood flow are dependent on changes in blood velocity rather than changes in the diameter of the renal artery [67, 68]. Thus, changes in Doppler ultrasound-derived RBV are often interpreted as changes in renal blood flow [27, 66, 69, 70].

Additional hemodynamic measures were obtained from this study, which we have reported on previously [33, 71]. We used estimated aortic blood pressure to calculate renal vascular conductance (velocity ÷ pressure), as this more closely represents the pressure within the renal arteries, as opposed to brachial pressures. During pre- and post-race, aortic blood pressure was estimated via pulse wave analysis (SphygmoCor XCEL, AtCor Medical, Naperville, USA). Briefly, brachial artery pressure waveforms were transformed using a validated generalized transfer function to estimate an aortic pressure waveform and derive aortic blood pressure [72, 73].

2.5 Statistics

All data were assessed for normality using a Shapiro-Wilks test. If data were normally distributed, paired samples t tests were used to determine differences between pre- and post- race measures; If data were not normally distributed, Wilcoxon ranked test was used. We used Cohen's *d* to assess the effect size for normally distributed data [74] and interpreted effects as small ($d=0.2-0.49$), medium ($d=0.5-0.79$), and large ($d>0.8$). We used the rank-biserial correlation (r_{rb}) to assess effect size for non-normally distributed data [75], interpreted as small (0-0.19), medium (0.2-0.29), large (0.3-0.39), and very large (0.4-1.0). Creatinine-indexed AKI biomarker change scores were calculated to correlate the relative change in AKI biomarkers, RBV and performance measures as $[\% \Delta = ((post - pre) / pre) * 100]$ and were only calculated in participants with complete AKI

and RBV data. Spearman's rho was used for all correlations as neither pace, nor any biomarkers were normally distributed. Finally, participants were split based on hydration status at the end of the race (Euhydrated: USG ≤ 1.020 , Hypohydrated: USG > 1.020) [35, 44] to assess whether finishing euhydrated attenuated the effect of the race on elevating urinary AKI biomarker concentrations. Since residuals for AKI biomarkers were not normally distributed, data were natural log-transformed ($\ln$) before running a mixed model ANOVA (Hydration status * Timepoint), however we visually depict the raw data. We used Fisher's exact test to compare proportions between groups. Since this was a field study we decided to not screen for outliers unless we observed clear visual outliers ($\sim 3SD$) outside of the range of the data distribution. Specifically, given the exploratory, field-based nature of the study, we did not conduct a formal power analysis [76], and due to the small sample size we used a deliberately lenient outlier criterion to preserve physiological variability and avoid introducing bias through arbitrary data exclusion, particularly female athletes who are historically underrepresented in this research area [77]. There were two data points that were removed with this criterion (one from NGAL/creatinine-pre and one from IGFBP7/TIMP2/creatinine-pre; **Supplemental Figure 1**). *Post hoc* pairwise comparisons with Bonferroni adjustment were completed where appropriate. Statistics were run in jamovi (v2.6.19) and figures were generated in Graphpad Prism (v10.4.1). Significance was set *a priori* to $p \leq 0.05$. All data are presented as mean $\pm$ standard deviation for normally distributed data or median (interquartile range) for non-normally distributed data in the tables or as individual data points superimposed on box and whisker plots in Figures. Interquartile ranges are reported as single numerical values (Q3 – Q1) to reflect variability from pre to post race.

3. Results

3.1 Demographics

A schematic of participant recruitment and enrollment is presented as Figure 1. Thirty-six participants were included in the analyses investigating the effects of completing the WSER on biomarkers of AKI. Twenty-nine (29) of those 36 participants completed RBV for at least one artery and were included in investigating the effects of completing the WSER on RBV. Thirteen participants of the 29 who completed RBV for at least one artery, had complete renal (Paired; $n=12$) or complete segmental (Paired; $n=12$) artery velocity data and were included in exploratory correlations between RBV and biomarkers of AKI as both pre and post measures are needed to calculate change scores. Participant demographics for both subsets of participants can be found in Table 1.

Due the small number of female participants and unequal sample sizes ($F=7$, $M=29$), we did not separate participants by biological sex but we depict them with different symbols in figures. Additionally, we did complete a sex comparison for pre-race hydration given a prior study demonstrating sex differences in the change in hydration during an endurance cycling event [44]. Nonetheless, we observed no baseline sex differences in USG (male participants = 1.010 ± 0.006 , female participants = 1.011 ± 0.008 , $p = 0.741$) or urine osmolality (male participants = 460 ± 245 , female participants = 421 ± 268 mOsm/kg, $p = 0.667$). Furthermore, we also observed a decrease in aortic mean blood pressure ($p = 0.016$, $d = 0.423$, pre: 93 ± 8 mmHg, post: 89 ± 9 mmHg) and an increase in serum CK

concentration ($p < 0.001$, $d = 1.582$, pre: 132 ± 85 , post: $13,373 \pm 8414$ U/L) in all the runners, similar to our previous findings [33].

3.2 Post-race hypohydration is associated with pace

Upon completion of the 2023 WSER, USG (Figure 2A; $p < 0.001$) and osmolality (Figure 2B $p < 0.001$) were increased. Using USG to classify hypohydration status (USG > 1.020), only 11% of participants (4/35) were hypohydrated at their pre-race collections whereas 77% were hypohydrated (27/35) upon completion Figure 2C (Fishers exact test $p < 0.001$). Race pace was positively correlated with changes in both USG (Figure 2D $\rho = 0.34$, $p = 0.048$) and urine osmolality (Figure 2E; $\rho = 0.53$, $p = 0.002$). The larger range in percent change for urine osmolality compared to USG reflects differences in scale. For example, an increase in urine specific gravity from 1.020 to 1.030 represents only a ~1% change, whereas an increase in urine osmolality from 200 to 800 mOsm/kg reflects a 300% change. A more extreme shift from 150 to 950 mOsm/kg represents a 533% increase. These differences explain the broader y-axis range for osmolality in Figures 2D & 2E. Of note, one participant had missing USG data that was not realized until all urine had been used for other measures, hence $n = 35$ as opposed to $n = 36$.

3.3 Post-race increases in biomarkers of hydration, electrolytes, and AKI

Comparisons between pre- and post-race serum creatinine, urine electrolyte concentrations, and raw urinary AKI biomarker concentrations are reported in Table 2. We observed pre- to post-race increases in urine potassium concentration ($p < 0.001$, $r_{tb} = 0.84$) and all raw urinary AKI biomarker concentrations ($ps < 0.001$); and decreases in urine sodium ($p < 0.001$, $r_{tb} = 0.75$) and urine chloride concentration ($p < 0.001$, $r_{tb} = 0.97$). As presented in Figure 3A-F, we also observed significant increases in urine creatinine concentration and all urine AKI biomarkers when indexed to urine creatinine ($ps < 0.008$), except for creatinine-indexed NGAL ($p = 0.334$) and TIMP2 ($p = 0.287$). **Supplemental Figure 1** depicts the data with outliers included. The only change in interpretation is the interaction term for our mixed model ANOVA for time * hydration status on IGFBP7•TIMP2 (before outlier removal $p = 0.104$; after outlier is removed $p = 0.003$). Respective qq-plots have been included in Supplemental Figure 1 to demonstrate why these outliers were removed with the data points highlighted by a crossed-out box. In addition to the creatinine-indexed data, we indexed all AKI biomarkers to osmolality and cystatin C as presented in Table 4. These findings were generally consistent with the data presented in Figure 3. When indexed to osmolality, all AKI biomarkers were elevated from pre- to post-race except NGAL. When indexed to cystatin C, all AKI biomarkers were elevated from pre- to post-race except IGFBP7.

3.4 Hypohydration linked to greater post-race increases in IGFBP7•TIMP2

Comparisons between euhydrated ($n = 8$) and hypohydrated ($n = 27$) finishers are presented in Figure 4. Urinary creatinine (Figure 4A; $p = 0.006$) and creatinine-indexed IGFBP7•TIMP2 (Figure 4F; $p = 0.003$), exhibited significant time * hydration status interaction terms, but not creatinine-indexed nephrin, NGAL, IGFBP7, or TIMP2 ($ps > 0.136$). Regarding the creatinine and IGFBP7•TIMP2/creatinine (biomarkers demonstrating

time x hydration status interactions), we also observed pre- vs. post-race pairwise differences in within the hypohydrated finishers' group only (Figures 4A and F). Additionally, hypohydrated finishers exhibited higher post-race urinary creatinine and IGFBP7•TIMP2/creatinine than euhydrated finishers (Figures 4A and F). We observed no baseline differences between euhydrated and hypohydrated finishers in any AKI biomarker. Interestingly, we noted that all euhydrated finishers were males (0/7 females euhydrated vs. 8/28 males; Fishers exact test $p = 0.166$).

3.5 No reductions in renal or segmental artery blood velocities

As presented in Figure 5, we did not detect any pre- vs. post-race differences in renal artery blood velocity (Figure 5A; $p = 0.186$, $d = 0.41$), renal artery conductance (Figure 5B; $p = 0.339$, $r_{tb} = 0.33$), segmental artery blood velocity (Figure 5D; $p = 0.483$, $d = 0.21$), nor segmental artery conductance (Figure 5E; $p = 0.622$, $r_{tb} = 0.18$). Representative images of the renal ultrasound assessment of RBV are presented in Figure 5C and 5F.

3.6 Fast race pace associated with greater changes in electrolytes and creatine kinase

We present simple bivariate correlations in our variables of interest, primarily correlations between race pace (km/hr) and pre- vs. post-race differences in urinary AKI biomarkers, race pace and pre- vs. post-race differences in hydration, and pre- vs. post-race differences in hydration and urinary AKI biomarkers, in Table 4. We observed correlations between pace and changes in serum CK ($\rho = -0.596$, $p = 0.028$), urine sodium ($\rho = 0.543$, $p = 0.004$), urine potassium ($\rho = 0.341$, $p = 0.049$), urine chloride ($\rho = 0.128$, $p = 0.468$), and urine creatinine concentration ($\rho = 0.433$, $p = 0.010$). We observed no correlations between pace and changes in renal artery blood velocity ($\rho = -0.109$, $p = 0.755$) or conductance ($\rho = -0.027$, $p = 0.946$) or changes in segmental artery blood velocity ($\rho = 0.373$, $p = 0.261$) or conductance ($\rho = 0.382$, $p = 0.248$). There were no correlations between changes in any AKI biomarker and changes in renal artery velocity ($ps > 0.297$), renal artery conductance ($ps > 0.366$), segmental artery velocity ($ps > 0.067$), or segmental artery conductance ($ps > 0.075$). Additionally, changes in serum CK were negatively associated with changes in urine osmolality ($\rho = -0.571$, $p = 0.045$), but there were no correlations between serum CK and any urinary AKI biomarker ($ps > 0.202$). We were unable to correlate serum CK and measures of RBV due to insufficient complete data across both measures.

4. Discussion

We investigated the acute effects of ultra-endurance competition on urinary biomarkers of AKI and surrogate measures of kidney blood flow (RBV and conductance). Our main findings were that completing the 161 km footrace increased select urinary AKI biomarkers, with nephrin and IGFBP7•TIMP2 remaining elevated regardless of creatinine, osmolality, or cystatin C indexing. Importantly, finishing euhydrated appeared to attenuate increases in urinary creatinine and creatinine-indexed IGFBP7•TIMP2.

Preliminary reports suggest that completing the WSER ultramarathon may leave participants at a higher risk of needing prolonged dialysis treatment for AKI with exertional

rhabdomyolysis compared to other ultramarathons [37]. Similarly, we observed elevations in serum creatinine, however, all changes in AKI biomarkers were of subclinical magnitude and did not require hospitalization (Tables 2 and 4). We observed increases in all unadjusted concentrations of urinary AKI biomarkers, and select creatinine-indexed AKI biomarkers (nephritin, IGFBP7, and IGFBP7•TIMP2), which aligns with the prior work done in competitors in marathons and ultramarathons (Figure 3). For example, findings from the Boston Marathon indicated transient increases in urinary creatinine and NGAL at the conclusion of the race [15]. However, 24 hours after the race, the two biomarkers diverged with NGAL remaining elevated and urinary creatinine decreasing to below pre-race concentrations. Another ultramarathon study (80km race) reported increased urinary creatinine, NGAL, and kidney injury marker-1 [14]. In our study, when NGAL was indexed to creatinine or osmolality, the differences were abolished. The NGAL discrepancy may be due, in part, to the runners completing the 80km race [14] running 1.1 kph faster than the runners in our present study, or differences between the event environments (e.g., changes in elevation, temperature/time of year), although we acknowledge these are speculative reasons. Nonetheless, all biomarkers returned to basal concentrations in a 9-day post-race sample after the 80km ultramarathon in the prior study. We replicated the transient increases in raw urinary NGAL and creatinine and extended this finding to additional AKI biomarkers that provided a more comprehensive evaluation of kidney injury (Figure 3). Indeed, the AKI biomarkers we selected enable us to infer that kidney injury occurred within the glomerulus (nephritin) and along the tubules (IGFBP7•TIMP2). Future mechanistic studies may further interrogate specific structures within the nephron that may be most impacted by ultra-endurance competition.

Interestingly, changes in creatinine-indexed IGFBP7•TIMP2, were attenuated in the participants who finished the race euhydrated (Figure 4). These findings align with prior studies demonstrating that maintaining euhydration attenuates changes in urine AKI biomarkers in the context of thermal stress [28, 36]. They also support findings demonstrating short-term water restriction, leading to mild-to-moderate hypohydration without concomitant heat stress or exertion, leading to elevation in urine AKI biomarkers [27]. These data suggest that maintaining adequate hydration throughout the event, an assumption based on post-race hydration status, is an effective strategy to attenuate developing acute AKI after ultra endurance competition. Future investigators should aim to capture individual nutrition data to determine patterns of fluid and electrolyte balance that adequately prevent AKI development while not increasing risk of hyponatremia during ultramarathons.

We observed no correlations between race pace and changes in urine AKI biomarkers, contrary to our hypothesis that faster race pace would be positively correlated with greater changes in urine AKI biomarkers (Table 3). We speculated that running the race faster may create greater renal ischemia and subsequent kidney injury. Relative exercise intensity is likely a better correlate of renal ischemia and subsequent kidney injury [25] than simply using race pace. While our study was not designed to assess the relative intensity at which runners completed the event, this is an interesting future direction to examine in future ultramarathon races, potentially using heartrate data and metabolomic profiling. However, several factors may explain why our hypothesis was not fully supported. Slower runners are

on the course for longer periods, leading to extended exposure to dehydration, electrolyte imbalances, and other environmental stressors. Conversely, the faster runners may have been somewhat protected due to greater fitness and/or resiliency from higher training volumes. Indeed, a prior ultramarathon study (100 km) reported that those who experienced AKI after an ultramarathon exhibited slower running velocities over the course of the race [20]. These findings highlight the complex interplay between race pace, environmental exposure, and physiological responses in determining AKI risk, while suggesting that faster race pace does not inherently exacerbate this risk.

Understanding that RBV decreases in response to exercise [25], we expected to observe reductions in RBV that may substantiate prior observations of AKI in response to ultra endurance events [5, 9, 14, 19-21]. However, we did not observe reductions in RBV or conductance in response to completing the 2023 WSER (Figure 5). An important methodological consideration is that we measured RBV after the cessation of exercise which reflects post-exercise recovery, when RBV would be expected to return to resting values, unlike the more pronounced reductions observed during exercise [69, 70]. We suspect RBV returned to baseline values quicker than we were able to capture these measures. We know that there are many mechanisms by which blood flow is redistributed both at the onset and cessation of exercise [29, 30] though this has predominantly been assessed in skeletal muscle.

We observed significant increases in serum creatinine [33], as well as increases in USG and osmolality, reflecting higher urinary solute concentrations that are commonly observed after endurance exercise [45] (Tables 2 and 4). Race pace was correlated with urine osmolality which corresponds with previous marathon literature reporting race pace to be correlated with fluid loss measured by changes in body mass [78]. We also observed the WSER influenced electrolyte excretion, with urine sodium and chloride concentrations being reduced and potassium concentration being elevated after the race (Table 2). Therefore, the potassium:sodium excretion increased. These findings are consistent with prior reports demonstrating urine potassium:sodium increases after ultra endurance races [46]. While outside the scope of our investigation, others have speculated this response is may be driven by renin angiotensin aldosterone system activation during exercise, resulting in altered electrolyte excretion [46]. We did not assess serum electrolytes due to limited blood sample volume. However, prior WSER investigations have examined pre- and post-race blood sodium concentrations to assess exercise associated hyponatremia [79]. Coupling urine and serum electrolyte data is an interesting future direction that could provide additional complementary insight.

There were no correlations between changes in RBV and AKI biomarkers, which we speculate may result from methodological limitations, as our post-race measures of RBV may not accurately reflect the changes in blood flow during the actual exercise (Table 3). Interestingly, race pace was negatively correlated with changes in CK, potentially due to slower runners being on the course longer and therefore accumulating more muscle damage and/or permitting more time for serum CK to accumulate. This finding could also reflect lower training levels among slower runners, which may exacerbate muscle damage during such events. Similarly, the WSER could also have been a greater relative insult for runners

with lower training volumes, resulting in greater physiological responses. Furthermore, serum CK concentrations begin to peak between 24 and 48 hours after bouts of exhaustive and submaximal exercise [80, 81]. Therefore, we may have missed CK peak windows in our participants, though it is worth considering that our participants had been active for over 24-hours on average and could have finished as serum CK was starting to peak. There was no correlation between race pace and serum creatinine. The kidneys maintain relatively stable glomerular pressure over a wide range of arterial pressures through autoregulation to reduce risk of kidney damage [82], and that could be one reason why there was no influence of race pace.

Limitations and Future Directions

We would like to acknowledge the limitations of the present study. First, these data were collected in the field. Specifically, pre (~1890m) and post (~385m) measures were taken at different altitudes due to the spaces provided to collect data near the start and finish lines. Similarly, WSER 2023 was a relatively temperate year and resulted in the highest completion rate on record (per WSER website). Therefore, we hesitate to use our data to compare to other years (e.g., years where temperatures may have been substantially more elevated for the duration of the event) or other ultramarathons where environmental demands can vary widely. Additionally, most participants dispersed very shortly after the race, hence we could not collect recovery samples to determine the timeline of resolution for the increase in AKI biomarkers. In future iterations of the WSER, researchers should attempt to have participants provide a post-race sample perhaps 24 or 48 hours after the race and arrange for shipping to complete these measures as presentation of emergency symptoms may range between 17 and 32 hours post-race [37]. Lastly, it is possible that the timing of the last meal prior to the urine sampling could have influenced urine concentration of certain biomarkers such as osmolality and creatinine which are commonly used to assess urine concentration, potentially creating an artifact suggestive of hypohydration despite overall euhydration [83, 84]. We asked participants to refrain from eating for at least 3 hours before pre-race data collection; however, this was not strictly enforced, and adherence likely varied. Additionally, we did not control meal timing for the post-race measures.

The field study nature of the investigation also affected our RBV measures. We collected these data as close to immediately after the race as we could. Some participants finished the race in the evening and in the middle of the night, which we consequently collected RBV data at potentially near opposite times of day resulting in inconsistent light exposure, temperature, control of external stimuli, etc. between collections. Additionally, participant fatigue and shivering made obtaining post-race recordings of the renal and segmental artery difficult, hence the smaller paired sample size for these measures. Finally, to more comprehensively understand the physiological impact of these events, it is important to examine organ crosstalk. For instance, exercise is associated with both acute [85-87] and chronic [88] changes to the gut microbiome, which produces metabolites that may contribute to kidney injury (e.g., indoxyl sulfate, p-Cresyl sulfate). Future studies should examine these interactions to provide a more holistic understanding of the effects of ultramarathon on inter-related organ systems.

Conclusions

Taken together, our results suggest that a 161 km footrace increases concentrations of excreted urinary AKI biomarkers and that completing the race euhydrated attenuates increases in creatinine-indexed urinary IGFBP7•TIMP2. With the growing popularity of ultra-endurance footraces, monitoring participant health post-race is important. While endurance competition elevates post-race urinary AKI biomarkers, evidence suggests that transient AKI after an ultramarathon does not necessarily increase the risk of subsequent AKI in future races. Within the select group of competitors in which we were able to collect RBV, we observed no changes in RBV in response to the 2023 WSER and also observed no correlations between change in RBV and changes in AKI or hydration biomarkers. Nonetheless, the long-term implications of repeated exposures to elevations in AKI biomarkers remain unclear. Our findings suggest that maintaining euhydration may be one strategy to reduce AKI risk, although future controlled hydration studies are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data will be made available upon reasonable request.

Abbreviations:

AKI	Acute Kidney Injury
CK	Creatine Kinase
IGFBP7	Insulin Growth Factor Binding Protein 7
NGAL	Neutrophil Gelatinase-Associated Lipocalin
RBV	Renal Blood Velocity

TIMP-2	Tissue Inhibitor of Metalloproteinases-2
USG	Urine Specific Gravity
WSER	Western States Endurance Run

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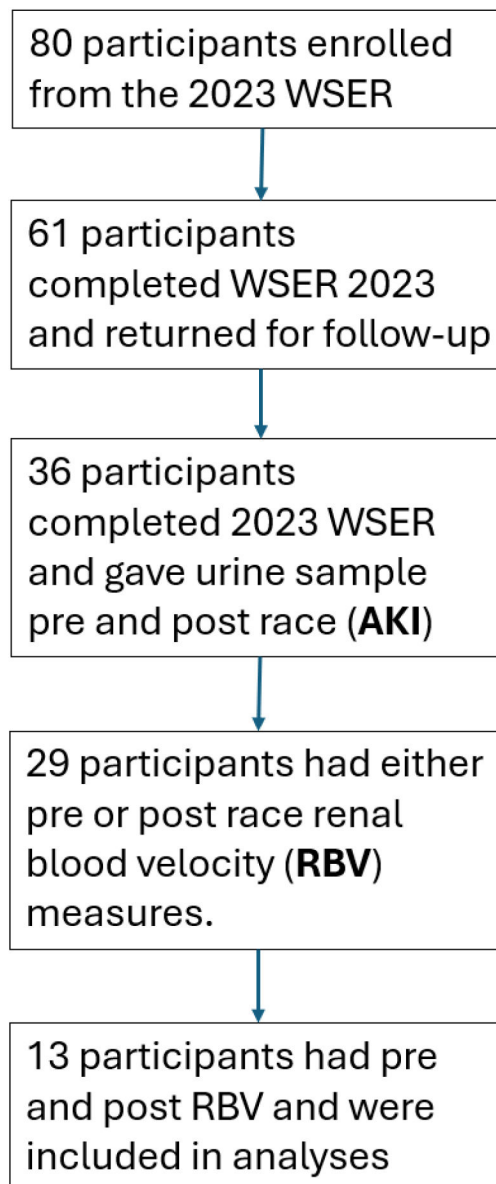


Figure 1:
Outline of participant selection

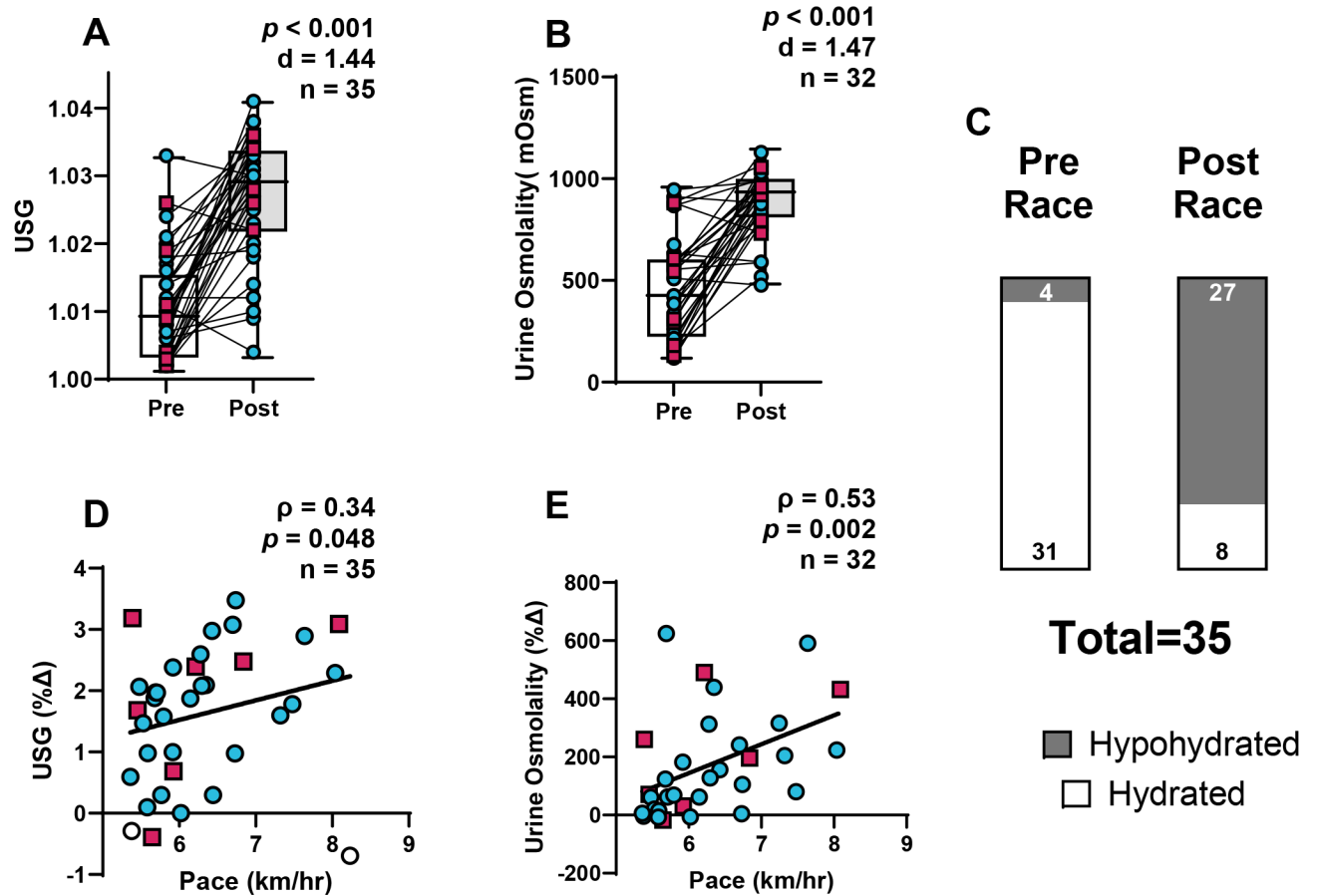


Figure 2:

Markers of hydration (A and B) compared between pre- and post-race time points. C) Hydration status (USG > 1.020 is dehydrated; USG ≤ 1.020 is hydrated) of participants at pre- and post-race timepoints. Fisher's exact test results ($p < 0.001$) indicate a significant association between timepoints and spot hydration status. Change in hydration markers (D and E) correlated to overall pace of participants who completed 2023 WSER. Blue circles indicate male participants; pink squares indicate female participants.

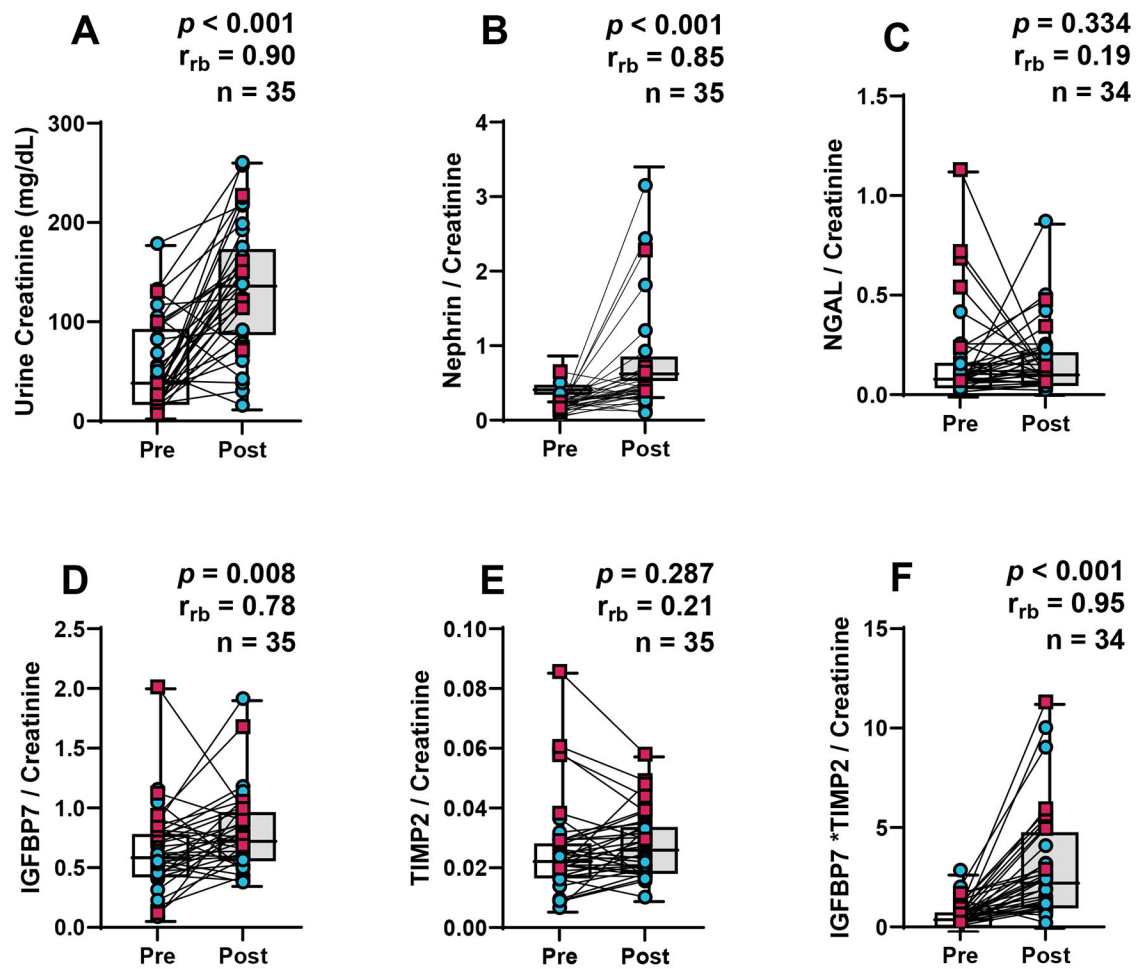
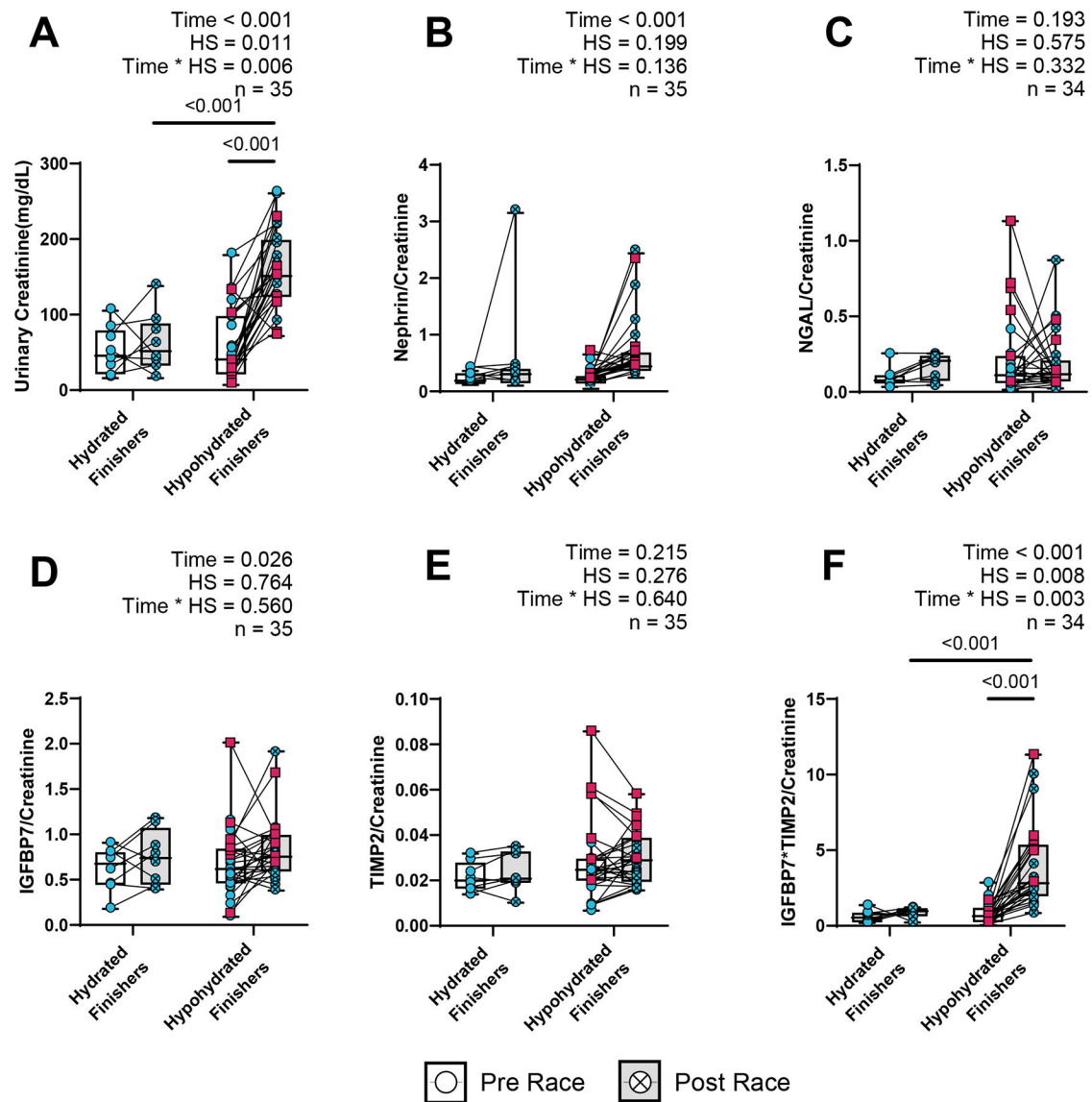


Figure 3:

Creatinine indexed kidney injury markers before and after completing WSER 2023. Blue circles indicate male participants; pink squares indicate female participants.

**Figure 4:**

Creatinine indexed kidney injury biomarker responses on completing 2023 WSER by post-race hydration status. Blue circles indicate male participants; pink squares indicate female participants. Statistics are reported from the log-transformed data used for ANOVA for all biomarkers; data are plotted as raw values.

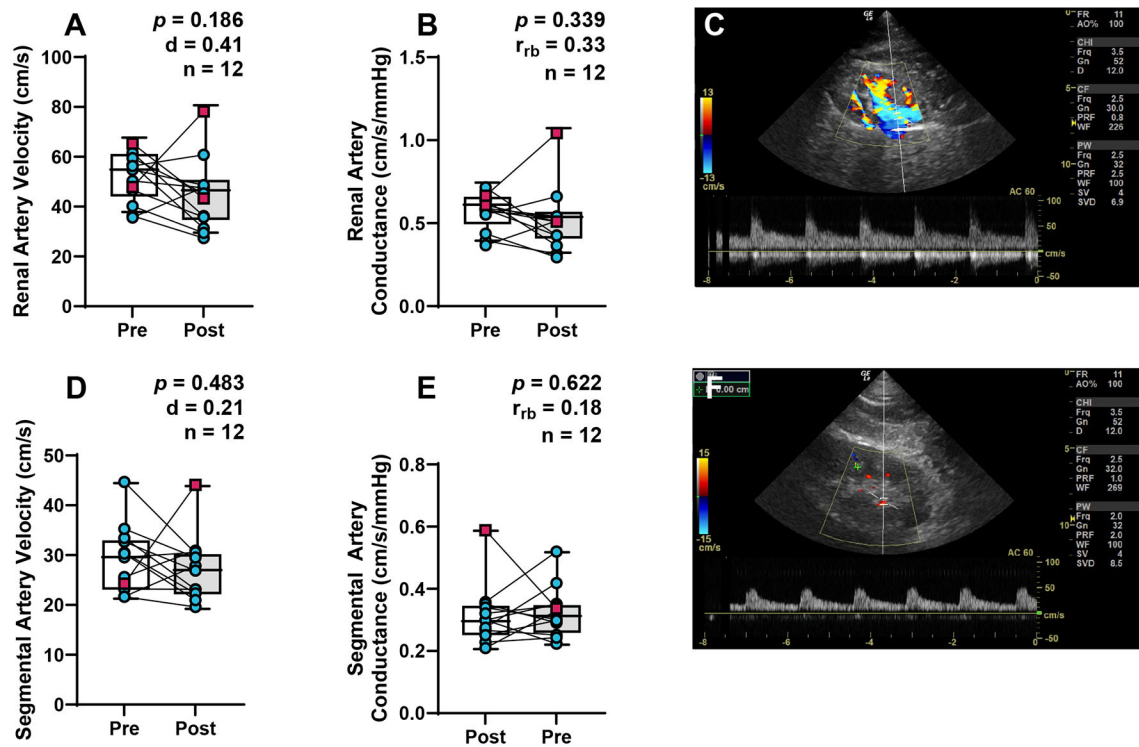


Figure 5:

Renal (A-C; $n = 12$) and segmental (D-F; $n = 12$) blood velocity data. Blue circles indicate male participants; pink squares indicate female participants. Reference image of renal (C) and segmental (F) artery blood velocity collections.

Table 1.

Demographics for urine collections (AKI) and renovascular assessments (RBF; includes all participants who had). M=male, F= female, W = white, A = asian, N = American Indian or Alaskan Native, NW = American Indian or Alaskan Native and White, PNA = prefer not to answer.

	AKI	RBV (total)	RBV (paired)
N	36	29	13
Sex	29M/7F	25M/4F	11M/2F
Height (cm)	175.9 ± 8.8	176 ± 8.2	174.3 ± 7.2
Body mass (kg)	70.4 ± 10.0	71.5 ± 9.3	69.1 ± 10.4
BMI (unit)	22.6 ± 1.8	22.9 ± 1.8	22.6 ± 2.1
Age (years)	44 ± 9	44 ± 9	45 ± 10
Race	28W/3A/1N/1NW/3PNA	26W/2A/1PNA	12W/1A
Finishing time (hh:mm:ss)	25:45:55 ± 3:05:50	25:39:12 ± 3:09:07	24:40:48 ± 3:22:57

Table 2.

Raw values of serum creatinine, urine point of care measures and raw AKI biomarker concentrations. Serum creatinine and eGFR were normally distributed and are presented as mean $\pm$ SD. All urinary biomarkers were not normally distributed and are therefore presented as median (IQR). d = Cohen's d ; rrb = rank biserial correlation.

Biomarker	Spot units	n	Pre	Post	p	d	rrb
Serum							
Creatine Kinase (U/L)		14	109.5 (61.8)	13232 (11216)	<0.000	-	1.00
Creatinine (mg/dL)		15	0.74 $\pm$ 0.14	0.95 $\pm$ 0.23	<0.001	1.38	-
Urine							
Sodium (mmol)		31	62.1 (48.3)	27.4 (23.8)	<0.001	-	0.75
Potassium (mmol)		34	41.4 (58.9)	132.8 (71.3)	<0.001	-	0.84
Chloride (mmol)		34	95.0 (75.9)	31.4 (25.5)	<0.001	-	0.97
Creatinine (mg/dL)		35	42.4 (73.6)	138.5 (79.5)	<0.001	-	0.90
Cystatin C (ng/mL)		36	22.7 (39.2)	87.5 (102.9)	<0.001	-	0.87
Nephritin (ng/mL)		36	7.2 (17.6)	60.6 (72.4)	<0.001	-	0.88
NGAL (ng/mL)		36	4.50 (6.36)	16.60 (18.96)	<0.001	-	0.72
IGFBP7 (ng/mL)		36	21.9 (24.6)	96.8 (74.7)	<0.001	-	0.84
TIMP2 (ng/mL)		36	1.03 (1.26)	3.49 (2.51)	<0.001	-	0.87
IGFBP7*TIMP2 (ng/mL)		36	23.4 (63.5)	326.6 (518.5)	<0.001	-	0.87
eGFR (mL/min/1.73m ²)		15	112 $\pm$ 114	93.6 $\pm$ 89.8	<0.001	1.22	-

Table 3:

Correlations of applied measures of intensity, kidney function, urine point of care measures, and AKI biomarkers. Spearman's rho (ρ) was used for all correlations, and correlations were conducted on normalized change scores. Statistics are presented as Spearman's ρ (p -value). Bolded cells indicate significance. " - " indicates measures where there was insufficient sample to investigate relations between variables.

	Race	Renal Artery Velocity	Segmental Artery Velocity	Renal Artery Conductance	Segmental Artery Conductance	Urine specific gravity	Urine Osmolality	Serum Creatinine Kinase
Serum								
Creatine Kinase	-0.596 (0.028)	-	-	-	-	-0.473 (0.090)	-0.571 (0.045)	-
Creatinine	-0.032 (0.913)	-	-	-	-	0.059 (0.844)	0.055 (0.856)	-0.077 (0.817)
Urine								
USG	0.338 (0.048)	-0.109 (0.755)	0.373 (0.261)	-0.027 (0.946)	0.382 (0.248)	-	0.815 (<0.001)	-0.473 (0.090)
Osmolality	0.533 (0.002)	0.200 (0.584)	0.233 (0.552)	0.103 (0.785)	0.333 (0.385)	0.815 (<0.001)	-	-0.571 (0.045)
Sodium	0.543 (0.004)	-0.033 (0.948)	0.381 (0.360)	0.017 (0.982)	0.405 (0.327)	0.456 (0.018)	0.292 (0.157)	-0.252 (0.430)
Potassium	0.341 (0.049)	-0.182 (0.595)	0.000 (1.000)	-0.127 (0.714)	0.091 (0.797)	0.780 (<0.001)	0.834 (<0.001)	-0.305 (0.288)
Chloride	0.128 (0.468)	0.136 (0.694)	0.436 (0.183)	0.255 (0.451)	0.491 (0.129)	0.361 (0.037)	0.396 (0.031)	0.068 (0.820)
Creatinine	0.433 (0.010)	-0.049 (0.886)	-0.007 (0.991)	0.119 (0.716)	0.021 (0.956)	0.903 (<0.001)	0.819 (<0.001)	-0.379 (0.202)
Nephtrin/Creatinine	0.101 (0.562)	0.021 (0.956)	0.175 (0.588)	0.084 (0.800)	0.119 (0.716)	0.670 (<0.001)	0.549 (0.002)	-0.154 (0.617)
NGAL/Creatinine	0.033 (0.855)	-0.118 (0.734)	-0.173 (0.614)	0.118 (0.734)	-0.155 (0.654)	-0.147 (0.411)	-0.154 (0.416)	-0.055 (0.863)
IGFBP7/Creatinine	-0.066 (0.706)	0.126 (0.700)	0.378 (0.227)	0.273 (0.391)	0.322 (0.308)	-0.114 (0.519)	-0.341 (0.061)	-0.093 (0.765)
TIMP2/Creatinine	-0.152 (0.382)	-0.329 (0.297)	-0.035 (0.921)	-0.287 (0.366)	-0.147 (0.651)	-0.151 (0.393)	0.294 (0.109)	0.000 (<0.999)
IGFBP7*TIMP2/Creatinine	0.164 (0.352)	-0.063 (0.852)	0.552 (0.067)	0.084 (0.800)	0.538 (0.075)	0.549 (0.001)	0.249 (0.184)	-0.245 (0.444)

Table 4:

Urinary AKI biomarkers normalized to urine osmolality and urine creatinine. Urinary nephrin/cystatin C is normally distributed and presented as mean $\pm$ SD, No other urinary biomarkers were normally distributed and are therefore presented as median (IQR). d = Cohen's d ; r_{rb} = rank biserial correlation.

Biomarker	n	Pre	Post	p	d	r _{rb}
Osmolality normalized						
Nephrin/Osmolality	32	0.018 (0.024)	0.076 (0.063)	<0.001	-	0.80
NGAL/Osmolality	32	0.011 (0.021)	0.022 (0.020)	0.224	-	0.25
IGFBP7/Osmolality	32	0.065 (0.039)	0.114 (0.070)	<0.001	-	0.75
TIMP2/Osmolality	32	0.003 (0.001)	0.004 (0.003)	<0.001	-	0.70
IGFBP7*TIMP2/Osmolality	32	0.065 (0.086)	0.417 (0.509)	<0.001	-	0.80
Cystatin C normalized						
Nephrin/Cystatin C	36	0.402 $\pm$ 0.135	0.581 $\pm$ 0.200	<0.001	0.76	-
NGAL/Cystatin C	36	0.268 (0.303)	0.155 (0.146)	0.048	-	0.38
IGFBP7/Cystatin C	36	1.243 (0.844)	1.015 (0.422)	0.119	-	0.30
TIMP2/Cystatin C	36	0.045 (0.020)	0.037 (0.021)	0.006	-	0.52
IGFBP7*TIMP2/Cystatin C	36	1.274 (1.440)	3.365 (4.126)	<0.001	-	0.72