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Original Investigation

Kidney Injury and Repair Biomarkers in Marathon Runners

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Background: Investigation into strenuous activity and kidney function has gained interest given increasing marathon participation.

Study Design: Prospective observational study.

Setting & Participants: Runners participating in the 2015 Hartford Marathon.

Predictor: Completing a marathon.

Outcomes: Acute kidney injury (AKI) as defined by AKI Network (AKIN) criteria. Stage 1 AKI was defined as 1.5- to 2-fold or 0.3-mg/dL increase in serum creatinine level within 48 hours of day 0 and stage 2 was defined as a more than 2- to 3-fold increase in creatinine level. Microscopy score was defined by the number of granular casts and renal tubular epithelial cells.

Measurements: Samples were collected 24 hours premarathon (day 0), immediately postmarathon (day 1), and 24 hours postmarathon (day 2). Measurements of serum creatinine, creatine kinase, and urine albumin were completed, as well as urine microscopy analysis. 6 injury urine biomarkers (IL-6, IL-8, IL-18, kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, and tumor necrosis factor α) and 2 repair urine biomarkers (YKL-40 and monocyte chemoattractant protein 1) were measured.

Results: 22 marathon runners were included. Mean age was 44 years and 41% were men. 82% of runners developed an increase in creatinine level equivalent to AKIN-defined AKI stages 1 and 2. 73% had microscopy diagnoses of tubular injury. Serum creatinine, urine albumin, and injury and repair biomarker levels peaked on day 1 and were significantly elevated compared to day 0 and day 2. Serum creatine kinase levels continued to significantly increase from day 0 to day 2.

Limitations: Small sample size and limited clinical data available at all time points.

Conclusions: Marathon runners developed AKI and urine sediment diagnostic of tubular injury. An increase in injury and repair biomarker levels suggests structural damage to renal tubules occurring after marathon. The results of our study should be validated in larger cohorts with longer follow-up of kidney function.

INDEX WORDS: Acute kidney injury (AKI); injury biomarkers; repair biomarkers; marathon running; urine microscopy; acute tubular injury; strenuous exercise; serum creatinine; urine albumin; creatine kinase; tubular injury; renal damage.

There is limited knowledge regarding the possible deleterious effects of vigorous activity and heat stress on kidney function. Marathon running serves as a human model of strenuous physical exertion due to the intense 26.2-mile run and heat stress involved.[1,2]

The relationship between marathon running and kidney injury has not been thoroughly evaluated in the literature, but given the increase in marathon participation—with a record high of 550,600 participants in 2014 in the United States—this relationship may become consequential.[3] Despite this increasing participation in marathons, the association between marathon running and kidney function has largely been overlooked because runners are generally regarded as healthy athletes with trained physiology to tolerate high states of energy expenditure.[4] For example, it has been shown that marathon runners can maximize their oxygen uptake nearly 50% more than healthy nonrunners who are half their age.[4] Synergistic to this increase in oxygen uptake, cardiac output typically increases 3- to 5-fold above levels at rest to meet the physical demands of marathon running.[5] However, although blood flow to the skeletal muscles and skin significantly increases, renal blood flow may decrease to 25% of levels at rest during strenuous activity.[5] It is hypothesized that this reduction in blood supply to the kidneys may lead to ischemic tubular damage because normally kidneys receive 20% of cardiac output.

Another possible mechanism of tubular damage in runners could be the increase in core body temperature, which could induce heat stress leading to kidney injury. It has been shown that runners' rectal

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temperatures may increase to ~102°F (39°C) in cool running temperatures of ~50°F (10°C), but may exceed 104°F (40°C) in hotter running temperatures of ~95°F (35°C).[1] Such an increase in core body temperatures for at least 2 hours in a standard marathon raises concern for cellular kidney damage. Army recruits, mine workers, and men who exercise vigorously in warm climates have all been noted to develop acute kidney injury (AKI).[6-8] In general, AKI induced by heat stress resolves in complete recovery, but in one study, ~10% of those with heat stress−induced AKI went on to develop chronic interstitial nephritis.[9]

Lastly, although volume depletion might be another reasonable explanation, research indicates that kidney injury can occur even with adequate hydration during running.[10] One study suggested that marathon running induces actual structural damage in the kidneys with an increase in levels of serum creatinine and injury biomarkers leading to AKI.[11] However, because most studies are using serum creatinine level, which is a marker of filtration, the type of structural injury to the kidneys remains unclear and the hypothesis of ischemic damage is yet to be supported by evidence. Because urine microscopy is a hallmark of acute tubular injury, its use in combination with other conventional and research biomarkers could help elucidate the cause of kidney injury associated with marathon running.[12] Thus we present a prospective observational study evaluating the kidney function of runners participating in the Hartford Marathon using both conventional and novel renal biomarkers of injury and repair to illuminate the relationship between vigorous activity and kidney function.

METHODS

Study Design and Participants

Marathon runners participating in the 2015 Hartford Marathon (Connecticut) were enrolled in the study. Recruitment in this prospective observational cohort study was achieved via a survey posted on the Hartford Marathon Registration website and through local running clubs. Runners who were aged 22 to 63 years and consented for research were included. Other inclusion criteria included normal body mass index of 18.5 to 24.9 kg/m^2, at least 3 years of running experience, minimum of 15 miles of training per week on average for the last 3 years, completed at least 4 races that were >20 km in distance, and completed a previous marathon within the last 5 years within 50% to 70% of their World Association of Veteran Athletes performance limit.[13] Runners were excluded from the study if they sustained any major running injuries over the last 4 months, participated in another marathon within 4 weeks prior to the race, used nonsteroidal anti-inflammatory drugs within 48 hours prior to or 24 hours after the marathon, used statins or anabolic steroids, donated blood within 8 weeks prior to the race, or had a history of hypothyroidism, kidney disorders, coronary artery disease, or convulsive seizures.

Sample Collection and Measurement

Urine and blood samples were collected at 3 different times: 24 hours premarathon (day 0), immediately (within 30 minutes) postmarathon (day 1), and 24 hours postmarathon (day 2). Six injury biomarkers (interleukin 6 [IL-6], IL-8, IL-18, kidney injury molecule 1 [KIM-1], neutrophil gelatinase-associated lipocalin [NGAL], and tumor necrosis factor α [TNF-α]) and 2 repair biomarkers (human cartilage glycoprotein 39 [YKL-40] and monocyte chemoattractant protein 1 [MCP-1]) were measured. Serum creatinine and creatine kinase, urine albumin, and urine microscopy were also evaluated at each time point. Only 2 participants refused to provide urine samples on day 1.

Urine and blood samples were transported on ice to the Yale University biorepository within 2 hours after collection at Quinnipiac University (days 0 and 2) and the Hartford Marathon (day 1). Upon arrival to the biorepository, samples were centrifuged at 5,000*g* for 10 minutes at 4°C, separated into 1-mL aliquots, and immediately stored at −80°C until biomarker measurement. All laboratory personnel were blinded to runner information.

Conventional Biomarker Measurement

Blood pressure, heart rate, pulse oximetry, and respiratory rate were measured on days 0 and 2, but only heart rate and pulse oximetry were measured on day 1. EDTA plasma samples were used as inputs for the measurement of serum creatine kinase and serum creatinine. Serum creatinine was measured via spectrophotometry using the Jaffé reaction by Quest Diagnostics Laboratory, and serum creatine kinase was also measured via spectrophotometry by the Yale New Haven Hospital Laboratory. Urine albumin, urine sodium, and urine creatinine were measured enzymatically via Randox technology by Yale New Haven Laboratory. Urine test strips/dipsticks were used for urinalysis via an automated analyzer by Siemens Clinitek diagnostics.

Novel Urinary Biomarker Measurement

Urinary biomarker measurements were analyzed as concentrations in nanograms per mililiter for NGAL (intra-assay coefficient of variation [CV], 5.2%) and in picograms per milliliter for the following injury and repair biomarkers: IL-6 (CV, 3%), IL-8 (CV, 2.6%), IL-18 (CV, 5.5%), KIM-1 (CV, 8%), TNF-α (CV, 6.1%), YKL-40 (CV, 6.2%), and MCP-1 (CV, 5.8%). All were measured using the Meso Scale Discovery platform (Meso Scale Diagnostics), which uses electrochemiluminescence detection combined with patterned arrays.

Urine Microscopy

Urine microscopy was performed within 2 hours after sample collection. After centrifugation and aliquoting, about 0.5 mL of urine was left in the test tube. Test tubes were gently agitated manually and a pipette was used to transfer 1 drop on a glass slide followed by application of a cover slip with minimal trapping of air bubbles. Samples were examined under low power (original magnification, ×10) followed by high power (original magnification, ×40) on bright field microscopy. Examining at least 10 fields per each power field, urine sediments were analyzed for the presence and number of renal tubule epithelial cells and granular casts. Granular casts and renal tubule epithelial cells per high-power field were quantified, if present, as 1 to 5, 6 to 10, and more than 10 and as 1 to 5, 6 to 20, and more than 20, respectively. Urine sediment pictures were taken using an Apple i-phone 6s camera. An experienced second-year nephrology trainee (S.G.M.) prepared and examined the microscopy slides and captured images of identified pathology. A nephrology attending physician, expert in urine microscopy (M.A.P.), and the nephrology trainee jointly discussed and determined the final urine microscopy findings (using procedures outlined in http://patr.yale.edu/resources/#page3).

Outcome Definitions

AKI was defined using AKI Network (AKIN) criteria.[14] Stage 1 AKI was defined as a 1.5- to 2-fold increase or 0.3-mg/dL increase in serum creatinine level from day 0 to peak creatinine value on either day 1 or day 2, and stage 2 AKI was defined as a more than 2- to 3-fold increase in serum creatinine level.

Urine microscopy score was based on the number of renal tubule epithelial cells and granular casts seen under high power. These scores were used to differentiate acute tubular injury from pre-renal AKI due to decreased kidney perfusion.[12,15] A score ≥ 2 on day 1 or 2 was defined as having positive urine microscopy findings. The scoring system is shown in Table S1 (provided as online supplementary material).

Statistical Analysis

All analyses were 2 tailed, and $P < 0.05$ was considered significant. Descriptive statistics for continuous variables were reported as mean $\pm$ standard deviation or median and interquartile range (IQR), and for categorical variables, as frequency and percentage. Marathon runners' characteristics were analyzed using Mann-Whitney Wilcoxon tests for continuous variables and χ^2 tests for categorical variables. Pearson correlations were used to evaluate bivariate relationships between clinical and novel biomarkers. Analyses were performed using SAS, version 9.4, software for Windows (SAS Institute).

This study was approved by the Yale Human Investigation Committee (HIC protocol number: 1509016483), and all participants gave informed consent.

RESULTS

Study Participants

Of 132 individuals who responded to the online survey, 68 met the inclusion criteria and 22 runners agreed to participate and consented to the study. The study cohort is shown in Fig 1. A total of 9 (41%) men and 13 (59%) women were included; mean age was 44.2 $\pm$ 12.9 years and mean body mass index was 22.4 $\pm$ 2.4 kg/m^2 (Table 1). Runners had a median running experience of 12 (IQR, 5.0-15.0) years and participated in a median of 5 (IQR, 2-16) prior marathons. Runners trained an average of 31.8 $\pm$ 10.4 miles per week, and overall, the cohort was fairly healthy without comorbid conditions, except for 2 runners with hypertension and 1 with type 1 diabetes. Six runners (27%) consumed nonsteroidal anti-inflammatory drugs within 2 weeks of the race, but not within 48 hours of race day, and 11 (50%) were taking herbal supplements. On the day of the marathon, the weather was sunny with an average temperature of 62°F. All runners completed the 26.2 miles with an average marathon finishing time of 4.02 $\pm$ 0.64 hours.

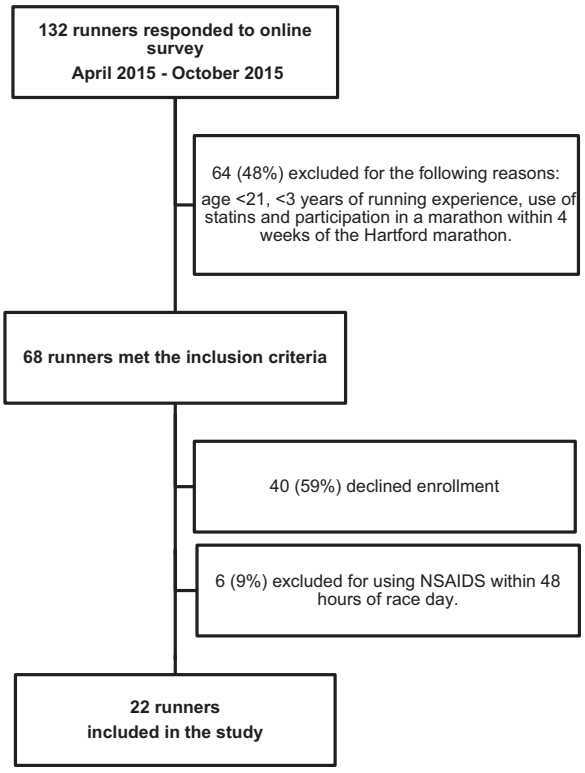

Figure 1. Enrollment chart of runners in the study cohort. Abbreviation: NSAID, nonsteroidal anti-inflammatory drug.

Conventional Biomarkers

Vital signs of runners across the 3 study time points are shown in Table 2. Serum creatinine concentration peaked on day 1 in all runners, as shown in Fig 2. Median creatinine values on days 0, 1, and 2 were 0.81 (IQR, 0.76-0.95) mg/dL, 1.28 (IQR, 1.09-1.54)

Table 1. Characteristics of Marathon Runners and Postmarathon Outcomes

Characteristics and Outcomes	Value
Baseline characteristics	
Age, y	44.2 $\pm$ 12.9
Male sex	9 (41)
Height, cm	169.7 $\pm$ 7.8
Weight, kg	65.0 $\pm$ 11.6
BMI, kg/m^2	22.4 $\pm$ 2.4
Hypertension	2 (9)
DM type 1	1 (5)
Self-reported NSAID use	6 (27)
Herbal supplements use	11 (50)
Running experience, y	12.0 [5.0-15.0]
Average miles per week	31.8 $\pm$ 10.4
Best marathon finishing time, h	4.2 $\pm$ 0.6
No. of prior marathons	5 [2-16]
Postmarathon outcomes	
Hartford Marathon finishing time, h	4.02 $\pm$ 0.64
AKI $\geq$ stage 1[a]	18 (82)
Positive urine microscopy findings (score $\geq$ 2)	16 (73)

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as mean $\pm$ standard deviation if normally distributed or median [interquartile range] if non-normally distributed.

Abbreviations: AKI, acute kidney injury; BMI, body mass index; DM, diabetes mellitus; NSAID, nonsteroidal anti-inflammatory drug.

[a]Only one runner developed stage 2 AKI.

Table 2. Vital Signs of Marathon Runners by Time Points

Vital Signs	Day 0: Premarathon	Day 1: Immediately Postmarathon	Day 2: Postmarathon	Overall *P*[a]
Systolic BP, mm Hg	118 ± 6	NA	113 ± 6	0.01
Diastolic BP, mm Hg	75 ± 8	NA	72 ± 7	0.05
Heart rate, beats/min	62 ± 9	126 ± 5	63 ± 12	<0.001
Respiratory rate, breaths/min	16 ± 3	NA	15 ± 2	0.03
Pulse oximetry, %	98 ± 1	98 ± 1	99 ± 1	0.09

Note: Unless otherwise indicated, values are given as mean ± standard deviation.
Abbreviations: BP, blood pressure; NA, not applicable (indicates that vital sign was not measured).
[a]Overall *P* values were obtained via Kruskal-Wallis test.

mg/dL, and 0.90 (IQR, 0.80-0.90) mg/dL, respectively (Table 3). Eighty-two percent of runners developed at least stage 1 AKI by AKIN criteria, and 1 runner developed stage 2 AKI. Along with serum creatinine, urine albumin level also peaked on day 1, as shown in Table 3. However, serum creatine kinase level continued to increase 24 hours postmarathon, with day 2 values significantly higher compared to days 0 and 1 (Table 3). Fractional excretion of sodium was <1% across all 3 time points, but significantly decreased from day 0 to days 1 and 2 (Table 3). There were no significant correlations between levels of creatine kinase and both conventional and novel biomarkers across the time points except for YKL-40, which positively correlated with creatine kinase level on day 1 ($r = 0.51$; $P < 0.02$), and IL-8, which negatively correlated with creatine kinase level on day 1 ($r = -0.51$; $P < 0.03$; Table S2).

Urine microscopy revealed minimal findings on day 0, but days 1 and 2 revealed a significant increase in scores among runners, with 9%, 65%, and 59% having positive scores on each day, respectively ($P < 0.001$). A total of 16 (73%) runners were scored as having positive microscopy findings on day 1 or day 2. No crystals, including urate crystals, were seen on urine sediment. Representative urine microscopy images taken from runners on days 1 and 2 are shown in Fig 3.

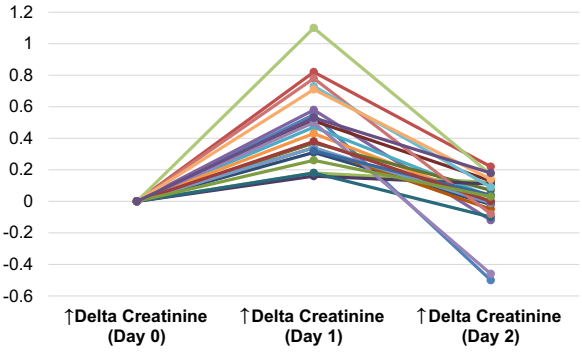

Figure 2. Absolute change in serum creatinine level per runner compared to baseline value on day 0.

Injury Biomarkers

Levels of urine biomarkers of injury were significantly elevated on day 1 compared to days 0 and 2 (Table 4). The highest-fold increases on day 1 as compared to baseline (day 0) values were seen in IL-6, KIM-1, and IL-8, as seen in Fig 4A.

Repair Biomarkers

Both YKL-40 and MCP-1 levels peaked on day 1, as seen in Table 3. MCP-1 levels had a 7.5-fold increase, and YKL-40 levels had an 8.2-fold increase on day 1 after marathon running as compared with baseline levels (Fig 4B).

DISCUSSION

In this prospective study with sample collection along with urine microscopy, we discovered that both serum creatinine and urine albumin levels significantly increased after marathon participation, with most (82%) runners developing at least stage 1 AKI. Given that serum creatinine level can be affected by multiple nonrenal factors, such as muscle breakdown and volume shifts, we also assessed injury biomarkers in runners.[16-18] There are several injury biomarkers that are being developed in the research setting. The biomarker NGAL has been identified to be an early injury biomarker of AKI by genomic, transcriptomic, and proteomic techniques.[19,20] It is a neutrophil-derived marker, which is also produced by renal tubular cells in the setting of various types of injury.[21] Cutoff values ranging from 50 to 100 ng/mL have been used to accurately diagnose AKI in multiple different clinical settings, such as after cardiac surgery and kidney transplantation.[22-24] Another injury biomarker, KIM-1, is extensively expressed in renal proximal tubular cells after injury and at much lower levels in lymphocytes.[25,26] It has been shown to have considerable predictive value in early detection of AKI in multiple clinical settings.[27] Similarly, specific interleukins, which are mainly produced by macrophages but also expressed in renal tubular cells, have been identified to detect AKI with substantial accuracy.[28,29] In intensive care patients, IL-18 was found to be a marker of AKI and mortality.[30] Alongside

Table 3. Levels of Conventional Biomarkers by Time Point

Conventional Biomarker	Day 0: Premarathon (n = 22)	Day 1: Immediately Postmarathon (n = 22[a])	Day 2: Postmarathon (n = 22)	Overall *P*[b]
Serum creatinine, mg/dL	0.81 [0.76-0.95]	1.28 [1.09-1.54][c]	0.90 [0.80-0.90]	<0.001
Serum creatine kinase, U/L	86 [74-159]	268 [242-344]	722 [434-844][c]	<0.001
FE_{Na}, %	0.82 [0.61-1.11][c]	0.23 [0.11-0.29]	0.22 [0.18-0.41]	<0.001
Urine albumin, mg/dL	0.50 [0.50-0.50]	3.50 [1.69-6.53][c]	0.54 [0.50-0.68]	<0.001
Urine albumin-creatinine ratio, mg/g	0.01 [0.01-0.01]	0.02 [0.02-0.05][c]	0.01 [0.00-0.01]	<0.001
Urine microscopy score ≥ 1	2 (9%)[c]	13 (65%)	13 (59%)	<0.001
Urinalysis				
Specific gravity	1.02 [1.01-1.02]	1.02 [1.01-1.03]	1.02 [1.01-1.02]	0.2
pH	7.0 [6.0-7.0][c]	5.5 [5.5-7.0]	6.0 [5.5-6.5]	0.002
Blood				0.3
0	19 (86%)	13 (65%)	18 (82%)	
1	2 (9%)	4 (20%)	3 (14%)	
2	0 (0%)	1 (5%)	0 (0%)	
3	0 (0%)	2 (10%)	0 (0%)	
4	1 (5%)	0 (0%)	1 (5%)	
Nitrite positive	0	1	0	0.3
Protein				0.03
0	21 (95%)	12 (60%)	18 (82%)	
1	1 (5%)	2 (10%)	4 (18%)	
2	0 (0%)	4 (20%)	0 (0%)	
3	0 (0%)	1 (5%)	0 (0%)	
4	0 (0%)	1 (5%)	0 (0%)	
Ketones				<0.001
0	22 (100%)	9 (45%)	17 (77%)	
1	0 (0%)	8 (40%)	5 (23%)	
2	0 (0%)	3 (15%)	0 (0%)	
Leukocyte esterase				0.7
0	16 (73%)	15 (75%)	18 (82%)	
1	4 (18%)	4 (20%)	1 (5%)	
2	1 (5%)	0 (0%)	1 (5%)	
3	1 (5%)	1 (5%)	2 (9%)	

Note: Values for categorical variables are given as number (percentage); for continuous variables, as median [interquartile range].
Abbreviation: FE_{Na}, fractional excretion of sodium.
[a]Only 20 urine samples were available on day 1 because 2 runners refused to provide urine.
[b]Overall *P* values were obtained via Kruskal-Wallis test for continuous variables and χ^2 test statistic for categorical variables.
[c]Value is significantly different compared with the other 2 time points.

IL-18, both IL-6 and IL-8 have been identified as markers for predicting AKI after cardiac surgery.[31,32] Last, TNF-α, which is mainly produced by macrophages, has also been associated with AKI in patients with severe sepsis.[33]

Similar to creatinine, these injury biomarker levels were significantly increased among marathon runners in the present study. This suggests that there is parenchymal damage (primarily tubular) in the kidney secondary to strenuous exercise. However, given nonrenal sites of production for several of these biomarkers, it is also plausible that this increase in biomarker levels is not specific to kidney injury because increased systemic production could lead to increased levels in urine.[21,26,28,29]

Interestingly, despite the increase in creatine kinase level, as would be expected from muscle breakdown during running, there was no significant positive correlation between creatine kinase levels and injury or conventional biomarker levels. Given that kidney injury does not correlate with muscle breakdown, we hypothesize that heat stress and increase in core body temperature along with systemic inflammation are likely associated with AKI in marathon runners. It has been shown that runners have significant increases in rectal temperatures after marathon running.[34] However, because we did not measure core body temperatures before and after running, our hypothesis regarding heat stress needs to be evaluated in future studies. Despite prior work showing that most marathon runners maintain adequate hydration, fractional excretion of sodium values in our cohort suggest that dehydration leading to decreased kidney perfusion could have also contributed to the increase in creatinine levels.[10] However, it has also been shown that low fractional excretion of sodium states are present in other conditions, such as acute tubular injury, given the presence of vasoconstriction in early acute tubular injury.[35]

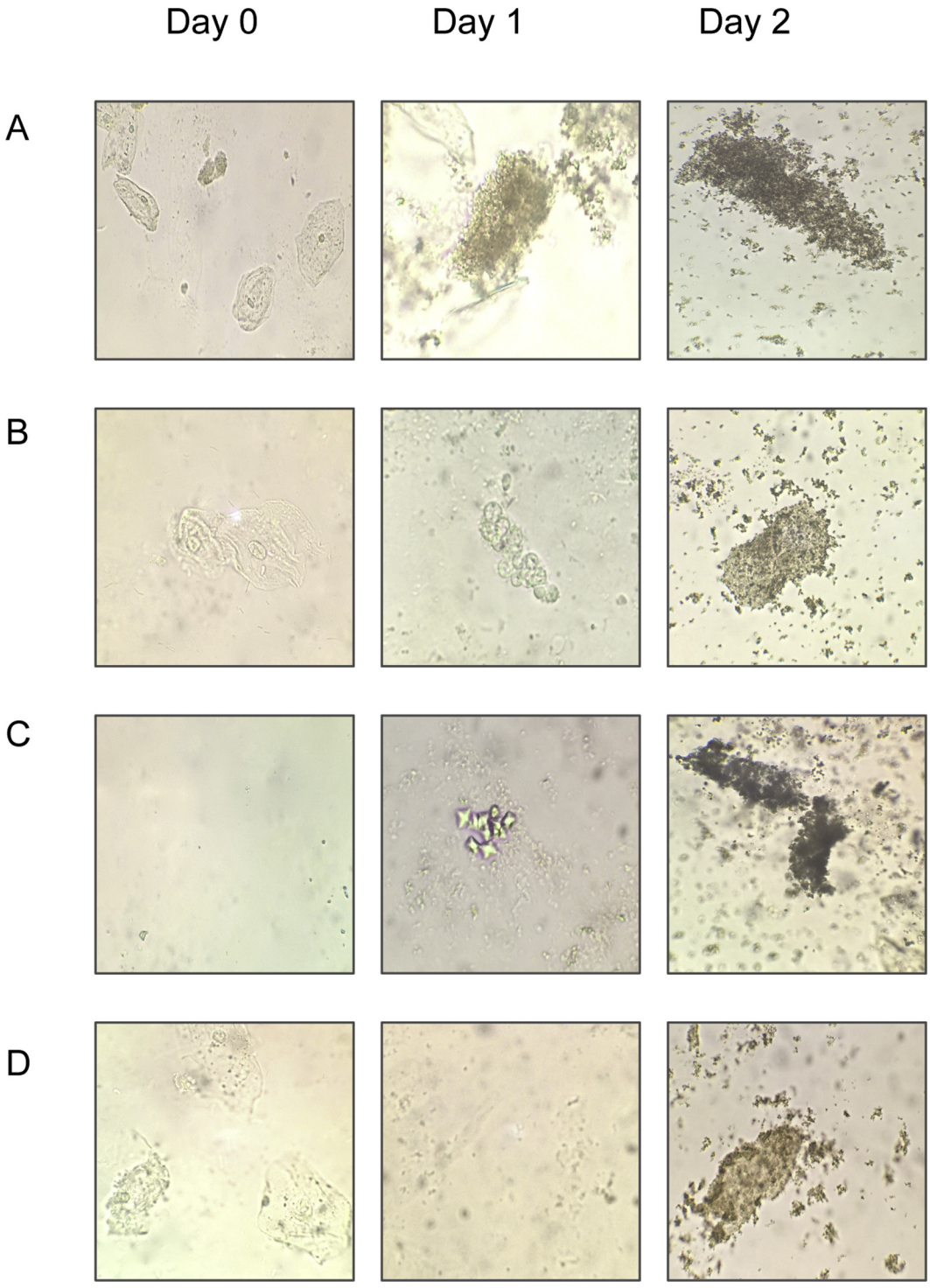

Figure 3. Representative urine microscopy images of samples from 4 runners by time point.

In this study, urine microscopy reveals that the main cause of AKI in marathon runners is acute tubular injury. Given that acute tubular injury occurs due to ischemia, which usually occurs in the clinical context of background comorbid conditions or multiple organ failure, runner acute tubular injury is distinct in that it is in healthy individuals.[36] Despite a reduction in cystatin C–estimated glomerular filtration rate directly after marathon running, one study showed a return to baseline in renal parameters 2 weeks after running, suggesting that despite the development of acute injury after

Table 4. Levels of Injury and Repair Biomarkers by Time Point

Urinary Biomarker	Day 0: Premarathon	Day 1: Immediately Postmarathon	Day 2: Postmarathon	Overall *P*[a]
Injury biomarkers				
KIM-1, pg/mL	132.59[b] [67.61-219.98]	723.32 [459.36-1,970.64]	702.42 [123.27-1,098.67]	<0.001
TNF-α, pg/mL	0.02 [0.01-0.04]	0.09[b] [0.04-0.18]	0.02 [0.00-0.04]	0.001
IL-18, pg/mL	6.43 [4.24-12.26]	45.89[b] [23.42-63.45]	16.95 [4.84-29.98]	<0.001
IL-6, pg/mL	0.05 [0.03-0.15]	0.96[b] [0.38-2.24]	0.19 [0.08-0.30]	<0.001
IL-8, pg/mL	4.80 [0.92-20.65]	43.87[b] [9.93-123.00]	9.31 [2.88-23.11]	0.01
NGAL, ng/mL	8.00 [4.15-30.48]	37.64[b] [19.03-84.61]	18.49 [9.25-33.69]	0.001
Repair biomarkers				
YKL-40, pg/mL	96.25 [43.96-124.31]	865.13[b] [466.84-1764.28]	202.97 [55.91-398.81]	<0.001
MCP-1, pg/mL	39.56 [26.12-79.29]	264.47[b] [131.12-702.01]	186.28 [55.91-366.74]	<0.001

Note: Unless otherwise indicated, values are given as median [interquartile range].

Abbreviations: IL-18, interleukin 18; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase-associated lipocalin; TNF-α, tumor necrosis factor α; YKL-40, human cartilage glycoprotein 39.

[a]Overall *P* values were obtained via Kruskal-Wallis test.

[b]Value is significantly different compared with the other 2 time points.

running, there is no persistent reduction in filtration function.[37] However, there are no studies to evaluate renal structure and damage in runners beyond 2 weeks, and hence it is unclear whether runners develop chronic sequela from repetitive renal insults while running. However, despite rigorous training with about 32 miles per week and a median of 12 years of running experience of those in our cohort, there was no evidence of chronic kidney disease (CKD) in our participants. In addition, there is no published evidence of an increase in CKD prevalence among marathon runners. The possibility of ascertainment bias also exists because CKD is an asymptomatic disease, with most runners being healthy individuals who may not undergo routine annual health examination.

In parallel to the strenuous physical state and heat stress that is experienced by athletes such as marathon runners, agricultural workers in Central America may have similar working environments, but with notable differences that may explain the variance in the prevalence of CKD in both populations.[38] On average, sugarcane workers spend about 4 hours per day in 95°F to 108°F temperatures.[39,40] The intensity of the work is magnified because workers are paid not by the hour, but rather by the number of sugarcanes they cut at the end of the day.[41] Similarly in this study, runners completed the Hartford marathon in an

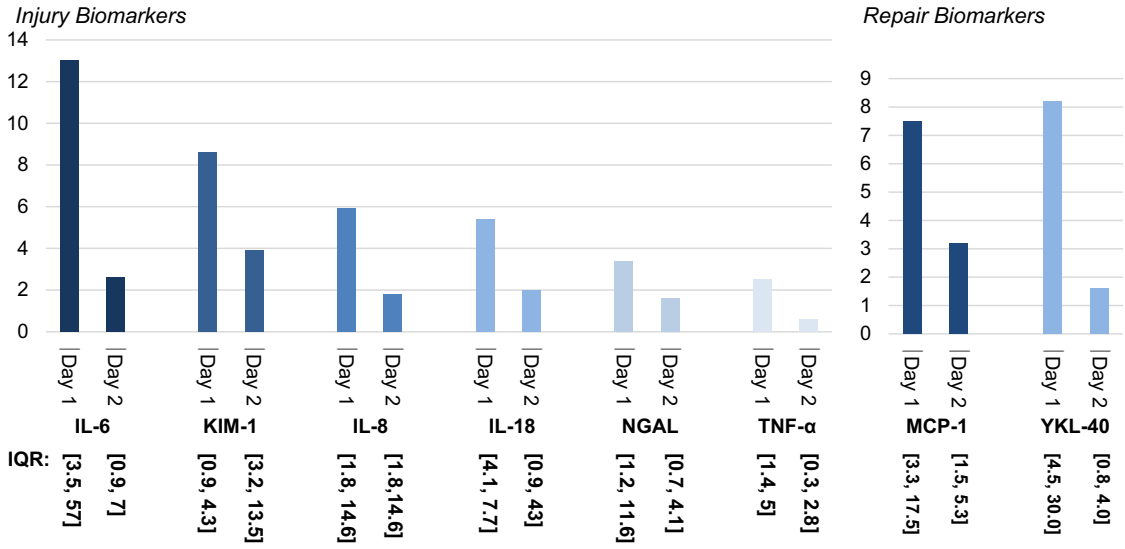

Figure 4. Fold increase in biomarker levels compared to baseline on day 0. Abbreviations: IL-6, interleukin 6; IQR, interquartile range; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase-associated lipocalin; TNF-α, tumor necrosis factor α; YKL-40, human cartilage glycoprotein 39.

average of 4 hours, with likely comparable levels of intensity of physical labor but under much cooler temperatures of 62°F. Hence, the increase in prevalence of CKD in Central America might be secondary to field workers' exposure to higher external ambient temperatures, as well as more frequent heat stress injury. Agricultural workers have been shown to have acute decreases in kidney function and progression to CKD associated with dehydration, systemic inflammation, and oxidative stress.[42] It is also possible that compared with agricultural workers, marathon runners have controlled ischemic preconditioning throughout their training, which may improve the kidney's ability to better tolerate repeated injury.[43] However, agricultural workers have ischemic injury in a more uncontrolled setting with limited access to hydration and health resources, which may hinder their ability to adapt to recurrent injury.

Given our small sample size and lack of multivariable analyses, we can only speculate that marathon runners adapt well to injury because AKI duration was only transient. This is despite 23% of runners in our cohort having NGAL levels > 90 ng/mL, which approach levels traditionally seen in critically ill patients such as those with hepatorenal syndrome or those immediately following cardiac surgery (Table S3).[44-46] This highlights that elevation in biomarker levels is only part of the story of renal effects of running because NGAL represents the level of injury and inflammation, but does not highlight the cascade of repair processes triggered in response to injury. The latter was captured in runners by the significant increases in levels of both repair biomarkers, YKL-40 and MCP-1, immediately post-marathon. A glycoprotein, YKL-40 has been shown to play an important role in cytoprotection and repair, especially in recovery from AKI.[47-49] Also, MCP-1 has been shown to be essential for macrophage recruitment and healing, especially after ischemia-reperfusion injury.[50,51] It is possible that in contrast to American runners, repetitive injury in Mesoamericans is met with inadequate reparative responses, leading to sequela of fibrosis and progression from acute to chronic injury.

Our study is unique in that to our knowledge, it is the first to evaluate urine microscopy in parallel with conventional and novel biomarkers of injury and repair in marathon runners. We have shown that AKI in runners is secondary to structural injury, mainly acute tubular injury, as evidenced by serum creatinine levels, urine microscopy analysis, and levels of novel biomarkers of injury and repair. We acknowledge the limitations of our study, namely that our sample size is small and hence is subject to confounding. In addition, our study had a short follow-up time of 48 hours and no long-term outcomes are captured. Lastly, the presence of organic compounds such as ketones could have led to false-positive serum creatinine measurements because ketones produce similar color changes as creatinine when using the Jaffé methodology to determine creatinine values.[52]

In this study, we have shown that marathon runners develop an increase in creatinine level that is equivalent to AKI stages 1 and 2 based on AKIN criteria, with urine sediments that are diagnostic of acute tubular injury. This is accompanied by an increase in levels of injury and repair biomarkers, further indicating structural damage in the kidney. The results of our study are mainly hypothesis generating and should be further validated in larger cohorts.

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Contributions: Research idea and study design: SGM, RWP, TGM, CRP; data acquisition: SGM, MAP, CRP; data analysis and interpretation: SGM, CRP; statistical analysis: SGM, CRP, GV; supervision and mentorship: CRP. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. CRP takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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SUPPLEMENTARY MATERIAL

Table S1: Microscopy scoring system.

Table S2: Pearson and Spearman correlation between biomarker levels and creatine kinase levels for each time point.

Table S3: Distribution of NGAL levels and training of marathon runners.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2017.01.045) is available at www.ajkd.org

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