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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).