Abstract

Objectives

Endurance exercise is known to cause a rise in serum creatinine. It is not known to what extent this rise reflects renal stress and a potential acute kidney injury (AKI). Increases in Insulin Like Growth Factor Binding Protein 7 (IGFBP7) and Tissue Inhibitor of Metalloprotinases-2 (TIMP-2), urinary biomarkers of cell cycle arrest and renal stress, are associated with the development of AKI.

Design

Repeated measures study

Methods

Runners were recruited at the 2019 Brighton Marathon (UK) and provided urine and blood samples at baseline, immediately post-race and 24hrs post-race. Serum creatinine, urinary creatinine and urinary IGFBP7 and TIMP-2 were analysed from the samples.

Results

Seventy nine participants (23 females, 56 males), aged 43 ± 10yrs (mean ± SD), finish time 243 ± 40mins were included for analysis. Serum creatinine increased over the race by 40 ± 26% (p<0.001), TIMP-2 increased by 555 ± 697% (p<0.001) and IGFBP7 increased by 1094 ± 1491% (p<0.001) over the race. A subset of twenty-two participants supplied samples 24 hours post-race, reporting values similar to baseline at for all variables by this time. Significant increases (p<0.01) were seen in markers when corrected for urinary creatinine.

Conclusions

This study is the first to report large rises in IGFBP7 and TIMP-2 following marathon running. This suggests that rises in creatinine are not fully explained by changes in production and clearance and may reflect a state of kidney stress, or injury.

Key words:

Acute kidney injury, biomarker, endurance, running, IGFBP7, TIMP-2

Introduction

An acute rise in serum creatinine is the most widely used clinical measure of acute kidney injury (AKI), a diagnosis associated with significant short and long-term adverse outcomes in acute hospital settings [1]. Increased creatinine concentrations have also been reported in healthy and collapsed endurance events participants [2]. It has therefore been suggested that endurance running has the potential to impair kidney function and even cause AKI or AKD (acute kidney disease), the long-term sequelae of which may result in the development of chronic kidney disease (CKD) as seen in clinical practice [3,4].

Measuring changes in serum creatinine concentration in the context of exercise, as in acute care settings, is an imperfect measure of acute kidney function, given the multiple other factors that may elevate serum creatinine [5]. Exercise associated factors include impaired clearance due to reduced kidney blood flow secondary to intravascular volume depletion [6] and increased release as a consequence of muscle breakdown [7], processes that are augmented by exertional heat stroke [8] and exercise induced renal artery vasoconstriction [9]. These conditions are transient and resolve following cessation of exercise, reflected in a rapid reduction in creatinine concentration in healthy and collapsed endurance events participants [10]. Although transient AKI is observed clinically, it is relatively rare in pathological AKI. Given these observations, it is likely that the rise in creatinine during endurance exercise is multifactorial. It remains to be established to what extent a creatinine rise is physiological or indicative of impaired kidney function, stress, or even acute tubular injury.

Given serum creatinine is an imperfect "*gold standard*" of kidney function, clinical AKI studies have identified multiple alternative biomarkers of AKI [11]. In an exercise setting, the most frequently studied are neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule- 1 (KIM-1) [12]. These biomarkers are not exclusively produced by the kidney at times of acute inflammation and tissue ischaemia,[13] and may therefore be imperfect measures of kidney stress during exercise. Insulin Like Growth Factor Binding Protein 7 (IGFBP7) and Tissue Inhibitor of Metalloprotinases-2 (TIMP-2) are two proteins involved in G_1 cell cycle arrest. The product of urinary IGFBP7 and TIMP-2 ([TIMP-2] × [IGFBP7]) has been identified as the superior AKI prediction biomarker in critically ill patients in a study of 340 biomarkers,[13] and is FDA approved and commercially available as the NephroCheck® assay. Cell cycle arrest biomarkers are also of interest given sustained cell cycle arrest has been shown to lead to renal fibrosis and development of CKD [14,15].

To further understand the significance of exercise associated creatinine rise and whether it reflects kidney stress and injury, this study investigated changes in urinary IGFBP7 and TIMP-2 associated with marathon running in participants without evidence of kidney disease.

Method

Ninety-one runners (28 females, 63 males), aged 41±10yrs (mean ±SD), body mass 71.1±11.5 kg, height 174.9±10.0cm, were recruited at the Brighton Marathon, 14th April 2019. The study was advertised by email and social media to those who had entered and runners then volunteered to take part in the study. Participants provided written informed consent and institutional ethical approval was issued the University of Brighton Life Science Research Ethics Committee (0918). Seventy of the participants had previously completed a marathon. The average number of previous marathons was 5±7 (range 0 to 39). Participants self-reported 3-month training history prior to the marathon involved 43±17km per week (range 15 to 72 km per week).

Within 48 hours of the marathon, participants were invited to give resting venous blood and urine samples and complete a training diary. Immediately after the marathon, participants were escorted to the medical tent, for venous blood and urine samples. Weather conditions over the day of the marathon were 12-16°C, with 40-50% relative humidity.

Participants were asked to provide mid-stream urine sample both before and after the marathon. Urine was collected by participants in 30ml Universal Specimen Containers and was then decanted by research staff into 11ml vacuum containers (Vacutainer System, BD, New Jersey, USA). These containers were then placed into freezer containers cooled by liquid nitrogen before being transported to the laboratory.

Urine analysis was conducted using the NephroCheck® test (Astute Medical Inc, San Diego, USA) and VITROS analysers (Ortho Clinical Diagnostics, Raritan, USA). Urine from each sample was decanted into an individual NephroCheck® cartridge. These were then inserted into the Astute140 analysis device which provided measures of IGFBP7 and TIMP-2. This also provided the NephroCheck® AKIRisk® score calculated as the product of the 2 values ([TIMP-2]•[IGFBP7]) divided by 1000 to produce a single numerical test result with the units of $(ng.mL^{-1})^2.1000^{-1}$ (the units for all AKIRisk® results and values in this paper) [16,17]. Urinary Creatinine was measured using VITROS analysers. Urine was refrigerated until analysis and then inserted into the analyser, undiluted, using a CREA VITROS MicroSlide (Ortho Clinical Diagnostics, Raritan, USA).

Urine specific gravity and urine proteinuria was assessed using a urinalysis reagent strip (Multistix™ 10 SG, Siemens Healthcare GmbH., Erlangen, Germany). Proteinuria was defined, using a categorical scale: negative (0 $g.L^{-1}$), trace (0.15 $g.L^{-1}$), + (0.3 $g.L^{-1}$), ++ (1 $g.L^{-1}$), +++ (3 $g.L^{-1}$) and specific gravity using an interval scale: 1.000 to 1.030 in 0.005 increments.

Venous blood was collected by experienced phlebotomists using the Vacutainer System (BD, New Jersey, USA) in a serum separating tube. These samples were also frozen and then serum creatinine concentration was measured using the Abcam fluorometric assay (Abcam Ltd, Cambridge, UK).

Data was assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. Paired samples t-tests were used to compare pre and post-race values of the 79 runner pre to post-race. One way ANOVA was used to compare across the three time points for the sub-set of 22 runners tested 24hr post-race. Both Pearson's and Spearman correlation were used where appropriate to determine relationship between urine biomarkers and race variables. All statistical tests were completed using SPSS Statistics 26 (IBM, New York) and figures using DataGraph (4.7.1, Visual Data Tools). Significance was accepted at $p<0.05$. Values are reported as mean ± SD unless otherwise indicated.

Results

Eighty participants completed the marathon, 11 did not finish, and 1 finished but did not provide a post-race sample. Therefore 79 participants (23 females, 56 males, aged 43 ± 10yrs, finish time 243 ± 40mins) were included for analysis. Twenty-two of the participants, all of whom had finished and provided a post-race sample, returned for testing 24hrs post marathon. Average heart rate for the marathon was 153 ± 32$bts.min^{-1}$. Five participants reported minor medical issues over the race (*n*): muscle cramps (*3*), asthma exacerbation (*1*), gastrointestinal disturbance (*1*). Consumption of medications 48 hours pre-race included (*n*): paracetamol (1), ibuprofen (2), aspirin (2). Consumption of nutritional supplements 48 hours pre or during race included (*n*): carbohydrate gels (10), protein powder supplements (6). Consumption of medications 48 hours post-race included (*n*): paracetamol (9), ibuprofen (2), aspirin (2).

Table 1 and Figure 1 detail serum creatinine and urinary measures at the three sample points. From the 79 runners, serum creatinine increased over the race by 40 ± 26% ($p<0.001$), TIMP-2 increased by 555 ± 697% ($p<0.001$) and IGFBP7 increased by 1094 ±1491% ($p<0.001$) from baseline to post race. The subset of 22 runners also demonstrated the same significant increase ($p<0.01$) pre to post race, with biomarkers returning to near baseline at 24 hours

post-race. A similar trend but lower magnitude rise was seen in biomarker values when corrected for urinary creatinine concentration.

Forty three participants (54%) had a creatinine rise >26.5 μmol/l (AKI stage 1 criteria) and 1 had a rise over 2.0 times from their baseline level (AKI stage 2 criteria)[18] (range -4 to 107 $\mu mol.L^{-1}$). Sixty nine (86%) participants had a post-race [TIMP-2]•[IGFBP7] value >0.3 $(ng.mL^{-1})^2.1000^{-1}$ (range 0.08 to 40.14), the value indicative of high risk of imminent AKI in critically ill patients [19]. Thirty three (41%) of participants had a post race [TIMP-2]•[IGFBP7] value >2.0 $(ng.mL^{-1})^2.1000^{-1}$, a threshold associated with a 95% specificity for predicting AKI development. Mean serum creatinine and [TIMP-2]•[IGFBP7] returned to baseline at 24 hours post race (p<0.005). All participants' creatinine, TIMP-2 or IGFBP7 levels at 24 hours were lower compared to the immediate post-race value.

There was no association between measures of creatinine, TIMP-2 or IGFBP7 and participants' demographics, self-reported training volume, number of previous marathons, marathon finish time or average heart rate (*r<0.25, p>0.05*). TIMP-2 and IGFBP7 values were correlated (p<0.01) at all corresponding time points (pre-race r=0.65, post-race r=0.91). Figure 2 demonstrates the association (p<0.01) between pre to post race change in creatinine and [TIMP-2]•[IGFBP] (r=0.43), with no significant difference between TIMP-2 (r=0.43) and IGFBP7 (r=0.48). A trend that was maintained when correction for urinary creatinine was applied TIMP-2 (r=0.45), IGFBP7 (r=0.64) and [TIMP-2]•[IGFBP7] (r=0.63). There was no predictive relationship between pre and post-race [TIMP-2]•[IGFBP7].

Markers of urinary concentration (urinary creatinine and specific gravity) were associated with TIMP-2 and IGFBP7 at baseline and post-race (p<0.01). Baseline [TIMP-2]•[IGFBP7] was strongly associated with urinary creatinine (r=0.72), whilst moderate associations were present between post-race [TIMP-2]•[IGFBP7] and urinary creatinine (r=0.65). There was no association between serum creatinine and urinary creatinine (Figure 2).

Discussion

This study is the first to examine the effect of marathon running on TIMP-2 and IGFBP, two urinary biomarkers of cell cycle arrest that strongly predict development of AKI in clinical populations. TIMP-2 and IGFBP7 increased following marathon running and this rise was moderately correlated with the rise in serum creatinine. Adjusting the TIMP-2 and IGFBP7 values for urinary creatine, reduced the magnitude of the increase but maintained the association with serum creatinine rise.

TIMP-2 and IGFBP7 are proteins involved in the initiation of G_1 cell cycle arrest, a process that is a response to kidney epithelium stress and prevents mitosis occurring in an environment that is at higher risk of DNA damage [13]. They are exclusively produced within the kidney, therefore the increase levels relate to processes within the kidney alone. The rise in TIMP-2 and IGFBP-7 following marathon running supports the hypothesis that the serum creatinine rise that occurs with marathon running is, at least in part, related to kidney stress and potential damage and not only due to increased creatinine production or decreased clearance [2]. This may reflect the effects of systemic inflammation [20] or reduced renal blood flow [21] that are associated with exercise.

The return to baseline of creatinine, TIMP-2 and IGFBP7 at 24 hours following race completion is consistent with previous studies of exercise and kidney function markers [22]. The rapid normalisation of creatinine, TIMP-2 and IGFBP7 for most participants can be attributed to the cessation of exercise and return to baseline physiology. However, a significant number of participants who returned at 24 hours had one or more persistently elevated markers, with an increased spread of results when compared to the baseline measures. The cause of delayed normalisation of creatinine is likely multifactorial, although studies with follow up beyond 24 hours post cessation of exercise have demonstrated a return to baseline in all participants [23,24]. The significance of persistent evidence of renal tubular cell cycle arrest requires further investigation. It is reassuring that in all cases the levels were falling when compared with the immediate post-run values, however it should be considered that runners with persistently elevated values may be more susceptible to an exercise-mediated kidney insult.

Few studies have explored the relationship between creatinine and urinary biomarkers, with one marathon study reporting poor correlation between creatinine and uNGAL [25]. Elevations in TIMP-2 and IGFBP7 were moderately correlated with creatinine rise. This study supports the consensus that measuring the serum creatinine alone to detect kidney stress during exercise is inadequate. Participants with disproportionately small and large changes in serum creatinine relative to the cell cycle arrest biomarkers were identified in this study. A raised creatinine following exercise may represent increased solute load without significant kidney injury, whilst creatinine clearance could be maintained due to increased tubular flow despite the presence of some degree of kidney stress. No observational studies exist regarding the effects of chronic endurance exercise participation and kidney function, although associations between both recurrent AKI and cell cycle arrest and CKD have been suggested [14,15].

The increase in TIMP-2 and IGFBP7 was closely correlated, however the magnitude of increase was greater in IGFBP7. This variation in response is consistent with the only prior

study examining TIMP-2 and IGFBP7 during exercise that observed an increase in IGFBP7 but not TIMP-2 during a 2 hour walking bout in warm and humid conditions [26]. This variation may reflect the site of kidney stress given IGFBP7 is expressed in both proximal and distal tubular epithelial cells but secreted predominantly in the proximal tubule cells, whereas TIMP-2 is expressed and secreted primarily in distal tubule cells [27].

Unsurprisingly, correction for urinary creatinine concentration in this study reduced the magnitude of urinary biomarker rise but maintained the associations with serum creatinine. The validity of correcting urinary biomarkers to urinary creatinine to account for increased urine osmolality during exercise is not known given the dynamic fluctuations in creatinine production and glomerular filtration that occur. Variation in urine production during exercise influences biomarker values, with oliguria and increased urine osmolality causing an increase in urinary biomarker concentration. A finding observed in this study with a moderate association between rises in both IGFBP7 and TIMP-2 and specific gravity.

The optimal dose of physical activity for maximising health benefits is unknown. However, there is evidence that more extreme exercise could be harmful [28,29]. The Copenhagen City Heart Study findings of a U-shaped association between all-cause mortality and dose of jogging warrant further investigation of biomarkers of organ stress and injury and endurance exercise [29]. Although the benefits of exercise in patients with CKD are well established, there have been no studies on endurance event participation and long-term kidney function. As sports and exercise medicine increasingly explores personalised response to physical activity, the longitudinal use of a panel of biomarkers, including those of kidney function, should be of significant interest, particular for individuals with known susceptibility to specific organ dysfunction. This study indicates that marathon running has the potential to induce cell cycle arrest, a finding of significant relevance given the emerging evidence that recurrent cell cycle arrest is associated with increased DNA damage, a potential mechanism for the development of fibrosis and CKD [15].

Limitations of this study include a relatively low number of participants sampled at 24 hours post-race, however the distribution of results replicated previous work demonstrating a return toward baseline levels in the majority. Longer-term follow-up is desirable, including the investigation of the effects of repeated participation in endurance eventing. The participant retention was comparable to similar studies utilising a large city marathon as the subject of pre and post physiological variable analysis [30].

Conclusion

Increases in TIMP-2 and IGFBP7 kidney cell cycle arrest biomarkers following a marathon suggest that rises in serum creatinine previously associated with endurance exercise are, at least in part, related to kidney stress. The moderate association between these cell cycle arrest biomarkers and serum creatinine indicates that further consideration should be given to the development of tools for assessing kidney stress, function, and damage in the context of exercise.

Practical implications

- The magnitude of serum creatinine change following exercise does not necessarily correlate to the degree of kidney stress and potential injury incurred.
- Marathon running is associated with inducing cell cycle arrest in both proximal and distal tubular cells of the kidney.
- Cell cycle arrest is a process associated with DNA damage and the development of renal fibrosis and CKD, further study of the long-term consequences of exercise induced kidney stress is warranted.

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List of Tables

Table 1: Serum and urinary biomaker values values pre, immediately post and 24hrs post marathon. Data presented as mean (± SD). *denotes significant difference from pre-race values.

	Pre-race	Post-race	24hr post-race
Pre and post-race dataset	91	79	-
Pre, post and 24 hour post-race subset	21	21	21
Serum creatinine ($\mu mol.L^{-1}$)	80.1 (11.1)	112.2 (22.6)*	-
	80.4 (12.8)	118.8 (25.6)*	85.5 (15.9)
TIMP-2 ($ng.dL^{-1}$)	2.4 (1.2)	12.4 (9.1)*	-
	2.3 (0.9)	14.1 (10.1)*	3.2 (2.2)
TIMP-2/urinary creatinine ($ng.mg^{-1}$)	1.8 (1.2)	6.4 (3.16)*	-
	4.9 (3.3)	7.7 (4.6)*	4.0 (3.63)
IGFBP-7 ($ng.dL^{-1}$)	32.3 (25.5)	236.6 (218.9)*	-
	29.0 (22.7)	255.9 (203.7)*	36.5 (35.6)
IGFBP-7/urinary creatinine ($ng.mg^{-1}$)	18.6 (7.7)	114.5 (74.8)*	-
	51.7 (21.5)	49.1 (36.1)*	36.5 (20.5)
[TIMP-2]•[IGFBP] $(ng.dL^{-1})^2/1000$	0.10 (0.12)	4.74 (8.26)*	-
	0.08 (0.09)	5.38 (8.23)*	0.18 (0.26)
[TIMP-2]•[IGFBP]/urinary creatinine	2.8 (2.8)	1.80 (2.27)*	-
$((ng.dL)^2.100^{-1}/mg.dL^{-1})$	0.45 (0.37)	9.2 (11.8)*	1.9 (2.7)
Urinary creatinine ($mg.dL^{-1}$)	69.6 (50.7)	207.7 (124.9)*	-
	60.4 (51.6)	212.2 (139.3)*	113.9 (80.8)

List of Figures

Figure 1: Serum creatinine, TIMP-2 and IGFBP7 pre, post and 24 hours post marathon. Red lines indicate mean values for runner tested pre and post-race. Black lines indicate means for subset (n=21) completing pre, post and 24 hours post-race tests *Denotes significant difference from other time points.

Figure 2: Pre to post race change in serum creatinine and [TIMP-2]•[IGFBP7]. Coloured areas indicate AKI Risk levels (Green < 0.3, Yellow 0.3 - 2, Red > 2). Dashed black line indicates a serum creatinine increase of >26.5µmol.L^{-1}. Colour of the markers indicates scale of change pre to post-race urine creatinine (Green <68 (<IQR1), Blue 69-179 (IQR 1-3), Red >179 mg.dL^{-1} (>IQR 4)) with no association between serum creatinine and urine creatinine change (r=0.09).