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Assessment of serum free light chain levels in healthy adults immediately after marathon running

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Abstract

Background: Immunoglobulin κ and λ free light chains (FLC) are important serum biomarkers for diagnosing and monitoring plasma cell dyscrasias (via the κ:λ FLC ratio), and assessing immune competence and activation status (via ∑FLC). FLCs are produced, in excess of heavy chains, from healthy plasma cells during immunoglobulin production, but unlike intact immunoglobulins that are cleared by cellular catabolism over a number of weeks, FLC are rapidly cleared from the bloodstream by the renal glomerulus with a half-life of 3 (κ FLC)–6 (λ FLC) hours. Marathon running has been shown to acutely and transiently decrease renal function, however, the impact of prolonged aerobic exercise on FLC levels remains unknown.

Methods: We measured serum FLC levels in 60 runners before, and immediately after, the 2010 Eindhoven Marathon.

Results: A significant increase ($p<0.01$) in κ FLC levels was observed after the marathon, and κ FLC correlated positively with serum creatinine levels. No changes were observed for λ FLC, and thus, there were subtle elevations in the ∑FLC and FLC ratio in some participants. Indeed, we found that 13% of participants had an abnormally increased FLC ratio upon completion of the marathon; a phenomenon previously observed in renal diseases.

Conclusions: Abnormal FLC ratios observed after exercise reflected an increase in serum κ FLC levels, which may be due to acute and transient reductions in renal function during exercise, though we also observed an increase in serum IgG and IgA and thus cannot exclude exercise-induced immune stimulation or immunoglobulin redistribution.

Keywords: exercise; free light chains; immunoglobulins; renal function.

Introduction

Over the last decade, serum immunoglobulin free κ and λ light chains (FLC) have become a pivotal haematological biomarker for the screening and monitoring of plasma cell dyscrasias. FLCs are important for diagnosing, monitoring and prognosticating multiple myeloma, monoclonal gammopathy of unknown significance (MGUS) and an array of other associated disorders [1]. More recently, the prognostic utility of FLCs in non-neoplastic disorders has been highlighted [2–4]. Moreover, the sum of polyclonal κ and λ FLCs, expressed as ∑FLC, has been advocated as a global biomarker of immune activation [5], and is now regarded as a powerful prognostic marker for overall survival in the general population [6].

Additionally, FLCs are considered a more accurate 'real time' indicator of immune activation than intact immunoglobulins. This is because, unlike intact immunoglobulins that are cleared by cellular catabolism and have a relatively long half-life extending from 1 (IgA, IgM) to 3 weeks (IgG), FLCs are cleared from the bloodstream by the renal glomeruli and metabolised in the proximal tubules of the nephrons and have a considerably shorter half-life. κ FLCs, which are typically present in the bloodstream as

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monomers, have a half-life of 2–3 h, whereas λ FLC, which are typically dimerised have a longer half-life of 4–6 h. Thus, as a consequence of renal impairment, serum FLC are elevated and positively correlate with creatinine, cystatin-C and kidney disease staging [7].

Marathon running has been shown to induce a transient decrease in renal function evocative of acute kidney injury [8]. In the aforementioned study, it was shown that serum creatinine levels – supported by raised cystatin-C and urine biomarkers symptomatic of kidney injury – were elevated in nearly half of the participants who completed the marathon [8]. To date, the impact of aerobic exercise on FLC levels remains unknown. We recently investigated the effects of long duration walking exercise on FLC levels in the bloodstream of 37 elderly participants [9]. Long duration walking exercise did not significantly influence FLC levels, which is in line with the rationale that the physiological nature of walking exercise is not as likely to induce transient declines in renal function, as seen in marathon running [8]. Thus, it remains unknown how strenuous exercise affects κ FLC, λ FLC and ∑FLC levels, and whether acute alterations in kidney function during prolonged exercise affect the FLC ratio – a sensitive barometer of plasma cell neoplasm.

Thus, the aim of this study was to assess the effects of intensive aerobic exercise on κ FLC, λ FLC, ∑FLC and the FLC ratio. We hypothesised that previously reported acute and transient reductions in renal function during exercise [8], would result in an elevation in serum κ FLC, λ FLC and ∑FLC levels. As κ FLC are secreted by twice as many plasma cells as λ FLC, we hypothesised that a decline in renal clearance would increase the FLC ratio – as observed in renal disorders [7].

Materials and methods

Ninety-two moderately-to-highly trained runners (26–71 years of age) participated in the 2010 Eindhoven Marathon, as previously described [10]. Participants were recruited through the Eindhoven Marathons website, where an advertisement was placed prior to the event. Before participation, all participants provided informed consent and the Medical Ethical Committee of the Radboud University Medical Center approved the study which was conducted in line with the declaration of Helsinki.

All participants completed an online questionnaire about subject characteristics, including daily physical activity, marathon experience (e.g. previous completed marathons, personal best) and health (e.g. medical history and medication use). On the day of the marathon, participants underwent a series of measurements in our laboratory situated adjacent to the marathon start and finish area. After collecting demographic data, a venous blood sample was taken. In addition, heart rate was monitored continuously during the race using a chest band. Immediately after completion of the marathon (<5 min after exercise cessation), all measurements were re-taken. Finally, all participants reported their individual fluid intake, use of analgesics, physical complaints and rating of perceived exertion.

Ten millilitres of blood was drawn from an antecubital vein before and after the race. Whole venous blood was collected in a serum-gel vacutainer and allowed to clot for approximately 45 min before centrifugation. Serum was aliquoted and stored at −80 °C for later analysis.

Serum FLCs were measured on two established assays: a commercially available latex assay on the Roche Hitachi Modular utilising polyclonal antibodies (Freelite®, The Binding Site, Birmingham, UK), and an in-house multi-plex Luminex® assay utilising monoclonal antibodies (Abingdon Health Ltd©, Oxford, UK) as described previously [11]. Briefly, mouse anti-human FLC κ (clone # BUCIS 04) and anti-FLC λ (clone # BUCIS 09) monoclonal antibodies (Abingdon Health™) were covalently coupled to polystyrene Xmap® microspheres (BioRad, Hercules, CA, USA) and incubated – in a competitive inhibition format – with patient sera and biotinylated FLC. After appropriate wash steps to remove unbound FLC, streptavidin-rPhycoerythrin was then added before measurement on a Luminex® instrument where the fluorescent signal intensity was inversely correlated with κ or λ FLC concentration. The assay has good sensitivity and reproducibility, and correlates well with Freelite®™ (The Binding Site™) for polyclonal FLC [11]. In addition to FLCs, we also measured serum creatinine, IgG, IgA and IgM on a Roche Hitachi Modular. To minimise variation all Roche Hitachi Modular analyses were performed on a single day using the same calibration set-up, batches, and user.

Heart rate during the marathon was measured in 70 participants using a two-channel electrocardiograph (ECG) chest band system (Polar Electro Oy, Kempele, Finland). Mean heart rate (HRmean) was determined as the average heart rate between the start and finish of the marathon, Maximal predicted heart rate (HRmax=208–0.7*age) and exercise intensity (Ex Int=100*HRmean/HRmax) were calculated subsequently [12]. Exercise duration (start time – finish time) was obtained using the ChampionChip time registration (ChampionChip, MYLAPS, The Netherlands), from which mean running speed was calculated (Speed=42.195/exercise duration). After the marathon, participants completed a questionnaire indicating analgesic use and physical complaints. A visual analogue scale was used to measure rating of perceived exertion.

Participants were allowed to drink ad libitum during the marathon, but registered the time and amount (standard sized cups or bottles) of their individual fluid intake after the finish. An additional 2 mL of blood was drawn at baseline and directly after finishing to determine plasma levels of haematocrit (Hct) and haemoglobin (Hb) (RapidLab® 1265, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). In accordance with the method of Dill and Costill [13], Hct and Hb were used to assess plasma volume changes (PVC) as a proxy indicator of serum protein concentration changes due to exercise-induced dehydration. The equation is as follows:

$$\%\,\mathrm{PVC} = \frac{\left[\left(100\times\frac{\text{Hb baseline}}{\text{Hb exercise}}\right)-\left(\text{Hct exercise}\times\frac{\text{Hb baseline}}{\text{Hb exercise}}\right)\right]-(100-\text{Hct baseline})}{(100-\text{Hct baseline})}$$

PVC data was available for 39 participants; exact n for all PVC analyses is reflected in the reported degrees of freedom for each analysis.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Version 21, IBM Corp., Armonk, NY, USA). All data were reported as mean±SD unless stated otherwise, and statistical significance was assumed at a p-value <0.05. All data were normally distributed, as determined by the Kolmogorov-Smirnov test. Correlational relationships between assays and between variables were investigated using the Pearson's correlation coefficient, with significance adjusted to 0.01 level to account for multiple statistical testing. Differences between pre- and post-race levels for continuously distributed data were tested for significance with a Repeated Measures ANOVA.

Figure 1: Median (10th, 25th, 75th, 90th percentiles±outliers) serum creatinine levels before and immediately after the marathon (n=60 participants).
*Indicates significant difference (p<0.001) between pre- and post-marathon. This finding is maintained after controlling for plasma volume changes during exercise (n=39; p=0.002).

Results

Participant demographics and exercise parameters

Table 1 provides basic demographic and exercise characteristics of the participants who completed the marathon, with whom adequate serum sample volumes were available at baseline and post-marathon (n=69; 55 males, 14 females). No PVCs were observed from pre- to post-marathon (mean±SD: 0.7%±8.1%), but because some participants exhibited individual changes (range: –12% to 19.9%), we included PVCs in secondary analyses, described later.

Effects of marathon exercise on serum creatinine

Displayed in Figure 1 are the observed changes in creatinine following marathon exercise. As anticipated, Repeated Measures ANOVA revealed that marathon exercise resulted in a significant increase in serum creatinine from baseline to post-exercise ($F_{(1, 59)}$=220.026, p<0.001), and this finding was maintained upon inclusion of PVCs as a covariate ($F_{(1, 59)}$=11.312, p=0.002). Using the Acute Kidney Injury Network (AKIN) guidelines, we found that 42% of participants achieved AKI Stage 1, defined as a 50% rise in creatinine [14]. The percentage increase in creatinine from pre-marathon to post-marathon positively correlated with average exercise intensity (R^2=0.375; p=0.007; n=60) and average heart rate (R^2=0.367; p=0.009; n=60), but not race finish time or race speed (p>0.05).

Table 1: Mean±SD (100% range) demographics and marathon data for the n=69 participants enrolled in this study.

Demographics and marathon data	Mean±SD	100% range
Age, years	44.5±8.2	26–59
Body mass index, kg/m^2	23.0±2.3	16.3–29.0
Number of completed marathons	8.51±16.8	0–102
Average heart rate during marathon, beats/min	160.4±9.4	136–178
Average exercise intensity, % heart rate max	90.6±5.0	76.1–100.0
Marathon speed, km/h	11.4±1.4	8.5–15.0
Finish time, min	225.4±26.3	169–298

Serum FLC screening

Before assessing the effects of exercise on serum FLC levels, we first screened serum FLC results from all participants at baseline to exclude those with abnormal absolute κ and λ FLC levels which may be indicative of MGUS, immune stimulation or suppression, or renal impairment. To do this, we used the obtained Freelite® results, as the normal range for Freelite® is well established in the literature and is defined as 3.3–19.4 for κ FLC mg/L, 5.7–26.3 for λ FLC mg/L, and 0.26–1.65 for the FLC ratio [15]. At baseline, we found that nine of the 69 (12%) participants had an abnormal FLC ratio determined by Freelite®; these participants had no fundamental demographic or exercise physiological differences to those with a normal FLC ratio. Upon further investigation, three of the nine participants had a λ FLC result below the initial range of Freelite® sensitivity, i.e. located below the well characterised limit of detection

'gaps' [1]. These participants were thus, for data continuity, excluded from any further serology analyses. Despite apparently normal creatinine and serum immunoglobulin (IgG, IgM and IgA) levels, one of the nine participants with an abnormal FLC ratio had elevated κ FLC (start: 66.2 mg/L; finish: 71.06 mg/L) and λ FLC (start: 25.6 mg/L; finish: 23.9 mg/L) levels by Freelite® and was thus excluded on the grounds of abnormal absolute FLC levels. The remaining five of nine participants with abnormal FLC ratios by Freelite® were subsequently tested by immunofixation electrophoresis; none had detectable paraproteins indicative of plasma cell dyscrasias. However, unable to exclude light chain only MGUS, we conservatively excluded these participants from further serology analyses. Thus, a total of 60 participants with normal absolute κ and λ FLC levels, and a normal FLC ratio, were included in subsequent final serology analyses. We also confirmed that all 60 patients had an FLC ratio within the expected 100% reference range of the mAb assay, as previously cited [11]. Finally, to ensure the mAb assay and Freelite® quantified FLC in a similar manner, we affirmed that they correlated well at baseline for κ FLC (R^2=0.692; p<0.001; n=60), λ FLC (R^2=0.507; p<0.001; n=60), ∑FLC (R^2=0.653; p<0.001; n=60), and FLC ratio (R^2=0.534; p<0.001; n=60), supporting a prior report [11].

Next, we assessed whether demographic or physiological characteristics, reported in Table 1, were associated with resting FLC levels. Using results generated from Freelite® and the mAb assay, we found no significant associations between ∑FLC and age or BMI. Used as a proxy measure of physical fitness, we found that average exercise intensity and average heart rate were not associated with ∑FLC, and neither was marathon speed or finish time (data not shown). Eight participants reported hypercholesterolemia, but ∑FLC levels in these participants were not different to the other participants before or after the marathon. Finally, we assessed whether analgesics taken on the day of the marathon affected FLC levels. Five participants reported taking analgesics (n=3 paracetamol; n=2 paracetamol and ibuprofen), but these did not affect ∑FLC levels before or after the marathon.

Effects of marathon running on serum FLC levels

Illustrated in Figure 2 are the absolute κ FLC, λ FLC and ∑FLC levels before and after the marathon, measured on both the mAb assay and Freelite®, respectively. Repeated measures ANOVA indicated that both assays detected a significant increase in κ FLC after the marathon (mAb assay: $F_{(1, 59)}$=28.244, p<0.001; Freelite®: $F_{(1, 59)}$=9.344, p=0.003), which was maintained upon inclusion of plasma volume as a covariate (mAb assay: $F_{(1, 38)}$=7.214, p=0.011; Freelite®: $F_{(1, 38)}$=7.744, p=0.008). Furthermore, the percentage change in κ FLC correlated positively with percentage change in creatinine (mAb assay: R^2=0.631; p<0.001; Freelite®: R^2=0.716; p<0.001; both n=60), as well as average exercise intensity (mAb assay: R^2=0.361; p=0.010; Freelite®: R^2=0.342; p=0.015; both n=50), and average heart rate using results from the mAb assay (mAb assay: R^2=0.347; p=0.014) but not Freelite® (R^2=0.237; p=0.098 [NS]; both n=50); finish time and marathon speed showed no associations with κ FLC changes.

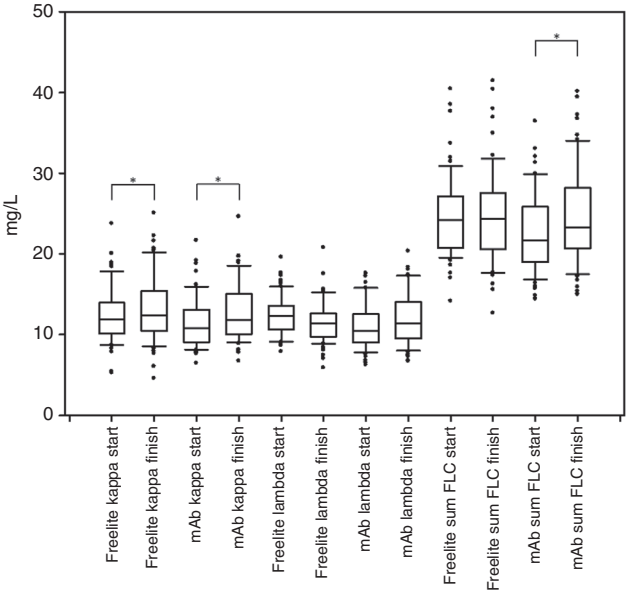
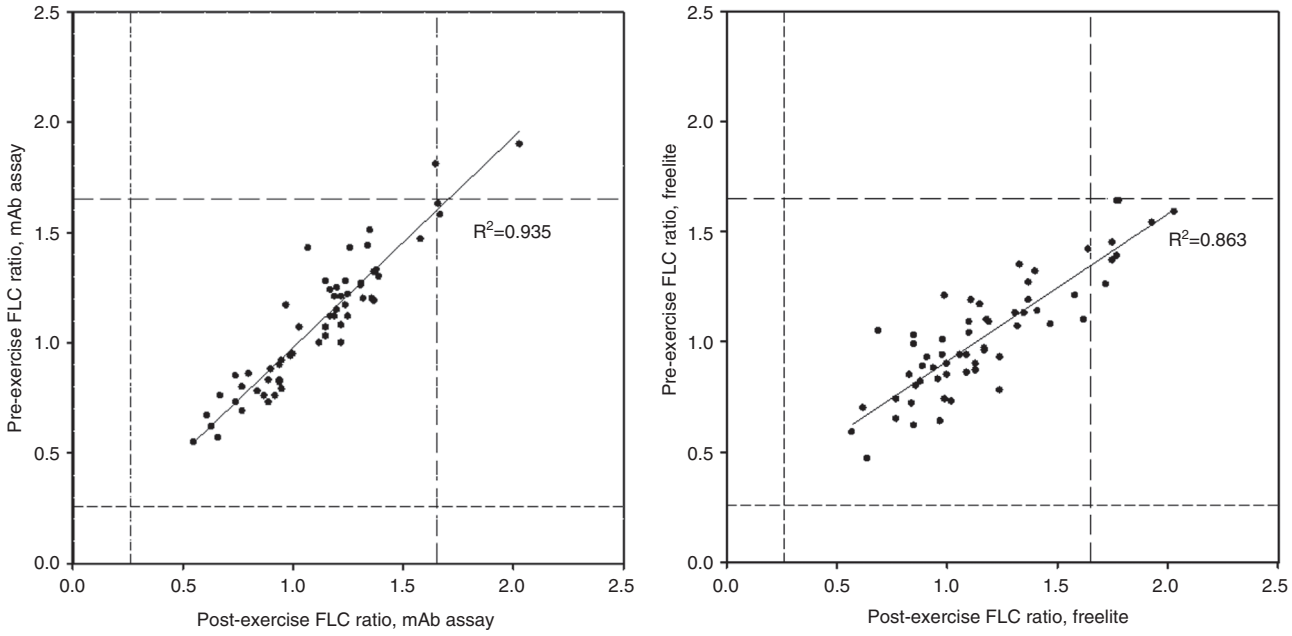


Figure 2: Median (10th, 25th, 75th, 90th percentiles) serum κ FLC, λ FLC and ∑FLC, measured by Freelite® and the mAb assay before and immediately after a marathon (n=60 participants).
*Indicates significant differences maintained during statistical analyses after controlling for plasma volume changes occurring during exercise (n=39, p<0.05).

Figure 2 displays λ FLC levels before and after marathon running. With PVCs included as a covariate, Repeated Measures ANOVA revealed no significant changes after exercise, as determined by mAb assay and Freelite® (both p>0.05). Thus, with PVCs taken into account, our results indicated that marathon exercise increased κ FLC, but not λ FLC levels.

Also illustrated in Figure 2 are the effects of marathon exercise on ∑FLC levels. Repeated Measures ANOVA revealed that ∑FLC levels increased significantly after exercise as determined on the mAb assay ($F_{(1, 59)}$=33.976,



FLC Ratio	Pre-exercise	Post-exercise
mAb assay	1.08±0.30	1.11±0.29
Freelite	1.03±0.27	1.17±0.35

Figure 3: Serum FLC ratio per participant (n=60) observed before and after marathon running, as measured on the mAb assay (left) and Freelite® (right).
When controlling for plasma volume changes occurring during exercise, no significant differences were observed on either assay between baseline and post-exercise (p>0.05). However, 8 (13%) of participants developed an FLC ratio above the normal Freelite® reference range (long dash=upper reference range, short dash=lower reference range) upon exercise cessation when measured by Freelite®. Pearson's correlation coefficients revealed that exercise introduced greater change to the FLC ratio on Freelite® compared to the mAb assay.

p=0.001), but not Freelite® (p=0.068); the mAb assay findings were maintained upon inclusion of PVCs as a covariate ($F_{(1, 38)}$=6.240, p=0.017) and correlated positively with percentage change in serum creatinine (R^2=0.698; p<0.001; n=60).

Illustrated in Figure 3 are the effects of marathon running on the FLC ratio, measured by both the Freelite® and mAb assays, respectively. Following exercise, we found a significant increase in the FLC ratio on Freelite® ($F_{(1, 59)}$=40.207, p<0.001), but not on the mAb assay (p=0.062). Interestingly, eight (13%) participants had an FLC ratio above the normal reference range on Freelite® after the marathon.

Effects of marathon running on serum immunoglobulin levels

Figure 4 displays the effect of marathon running on total serum levels of IgG, IgA, and IgM. We found a significant increase in IgG ($F_{(1, 59)}$=22.790, p<0.001) and IgA ($F_{(1, 59)}$=7.319, p=0.009) but not IgM (p>0.05) immediately after the marathon. This finding was maintained when accounting for plasma volume shifts (IgG: $F_{(1, 37)}$=14.710, p<0.001; IgA: $F_{(1, 37)}$=16.598, p<0.001).

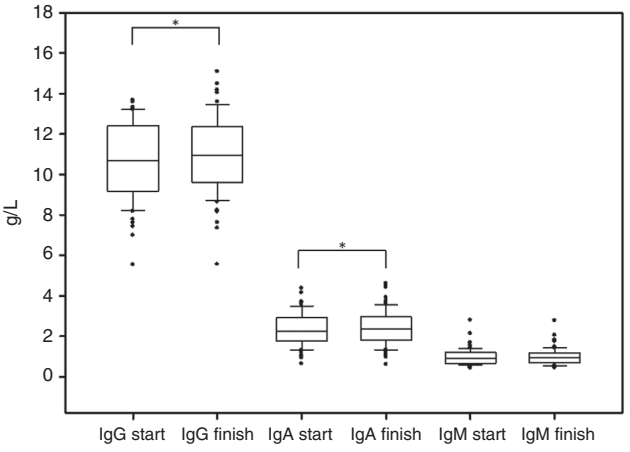


Figure 4: Median (10th, 25th, 75th, 90th percentiles) levels of serum IgA, IgM and IgG, measured before and immediately after a marathon (n=60 participants).
*Indicates significant differences maintained during statistical analyses when controlling for plasma volume changes occurring during exercise (n=39, p<0.05).

Discussion

Using two different FLC assays, we found that κ FLC levels in healthy participants were increased immediately after marathon exercise. Although the increase in κ FLC was modest (median to maximum increase: 7%–56% [Freelite®], 11%–52% [mAb assay]), and no changes to λ FLC were observed, we found that 13% of participants developed an abnormal FLC ratio upon exercise cessation. This is the first study to report changes to serum FLC levels in healthy participants following intensive aerobic exercise.

Generally, polyclonal serum FLCs can be elevated by two principle mechanisms: either immune stimulation or renal impairment. We are unable to exclude the possibility that the minor increases to FLCs – as well as IgG and IgA – seen in our study, reflects exercise-induced immune stimulation, or redistribution of immunoglobulins from peripheral sites during exercise. We previously demonstrated that long duration walking exercise did not significantly affect FLC levels [9]. The low intensity nature of the exercise task may not have elicited the pro-inflammatory effects typically seen in response to more intensive exercise modalities [16].

Marathon running may also induce acute and transient renal impairment [8]. In the aforementioned study it was shown that serum creatinine levels increased into territory normally diagnostic of kidney injury [7]. In our study, we report a large increase in serum creatinine [post-marathon median (range): 122 (72–198) mmol/L], which culminated in 42% of participants exhibiting creatinine levels indicative of kidney injury [14]. We also found that change in κ FLC levels positively correlated with increases in serum creatinine. This gave rise to an increase in the FLC ratio on the Freelite® assay, a phenomenon previously reported in kidney disease [7]. Interestingly, elevations to the FLC ratio in patients with renal impairment is an apparent feature of Freelite®, but is not replicated on mAb-based 'N Latex' [17] or 'Seralite assays [18]. Without the availability of an internationally accepted FLC reference method it remains unknown whether decreased kidney function affects the FLC ratio in serum. We also acknowledge that creatinine is not a gold standard biomarker for confirming renal impairment, and in this case is further limited by acute elevations in creatinine released from exercising muscle. However, McCullough et al. [8] showed that serum creatinine levels were positively correlated with cystatin-C post-exercise, and, kidney injury was confirmed in these participants via five-fold elevations to neutrophil gelatinase-associated lipocalin (NGAL) and a minor rise to kidney injury molecule-1 (KIM-1).

FLCs are biologically active molecules that can, under certain circumstances, induce renal inflammation and nephrotoxicity [4, 7]. The results from our study showed that an acutely strenuous bout of exercise induced only minor changes to absolute serum FLC levels, and thus, the effect on kidney function in healthy individuals is likely to be negligible. This study was unable to assess the effects of longer term exercise participation on FLC levels. Exercise training has been shown to reduce pro-inflammatory mediators, in both healthy and clinical populations [19], and, as such, future studies should investigate whether exercise training affects FLC levels. Importantly, exercise training has been shown to have benefits in chronic kidney disease patients [20–22], but as yet, it remains unknown whether this leads to a change in the levels of nephrotoxic FLCs.

In conclusion, we found κ FLC levels showed a minor increase after marathon running leading to an increase in the FLC ratio on the Freelite® assay; the FLC ratio was outside the normal reference range in 13% of participants at exercise cessation. As FLC are cleared by the kidneys, these findings may support prior reports showing acute and transient reductions in the renal function of healthy participants immediately after marathon distance running.

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