Journal of Sports Sciences

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/rjsp20

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To cite this article: Emil List Larsen , Henrik Enghusen Poulsen , Cristina Michaelsen , Laura Kofoed Kjær , Mark Lyngbæk , Emilie Skytte Andersen , Christina Petersen-Bønding , Clara Lemoine , Matthew Gillum , Niklas Rye Jørgensen , Thorkil Ploug , Tina Vilsbøll , Filip Krag Knop & Kristian Karstoft (2020): Differential time responses in inflammatory and oxidative stress markers after a marathon: An observational study, Journal of Sports Sciences, DOI: 10.1080/02640414.2020.1770918

To link to this article: https://doi.org/10.1080/02640414.2020.1770918

JOURNAL OF SPORTS SCIENCES
https://doi.org/10.1080/02640414.2020.1770918

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PHYSIOLOGY AND NUTRITION

Differential time responses in inflammatory and oxidative stress markers after a marathon: An observational study

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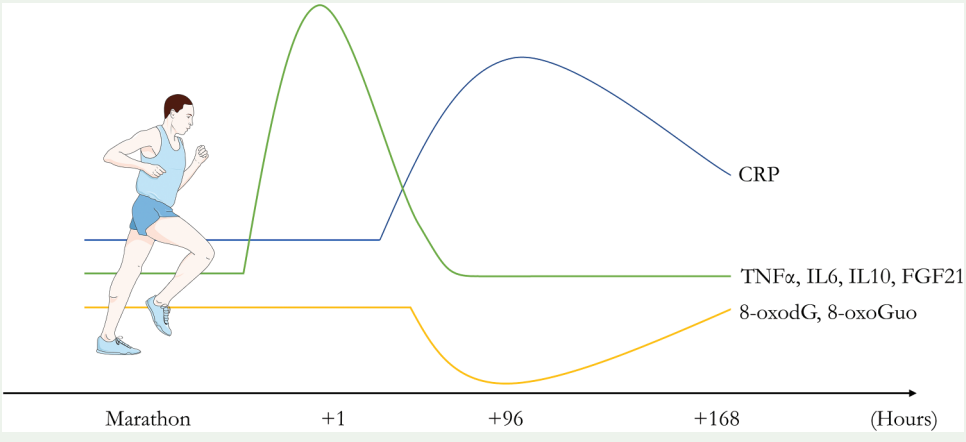
ABSTRACT

Acute and adaptive changes in systemic markers of oxidatively generated nucleic acid modifications (i.e., 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo)) as well as inflammatory cytokines (i.e., C-reactive protein, interleukin-6, interleukin-10, and tumour necrosis factor alpha), a liver hormone (i.e., fibroblast growth factor 21 (FGF21)), and bone metabolism markers (sclerostin, osteocalcin, C-terminal telopeptide, and N-terminal propeptide of type 1 procollagen) were investigated following a marathon in 20 study participants. Immediate changes were observed in inflammatory cytokines, FGF21, and bone metabolism markers following the marathon. In contrast, no immediate changes in urinary excretion of 8-oxodG and 8-oxoGuo were evident. Four days after the marathon, decreased urinary excretion of 8-oxodG (-2.9 (95% CI -4.8;-1.1) nmol/24 h, $P < 0.01$) and 8-oxoGuo (-5.8 (95% CI -10.3;-1.3) nmol/24 h, $P = 0.02$) was observed. The excretion rate of 8-oxodG remained decreased 7 days after the marathon compared to baseline (-2.3 (95%CI -4.3;-0.4) nmol/24 h, $P = 0.02$), whereas the excretion rate of 8-oxoGuo was normalized. In conclusion marathon participation immediately induced a considerable inflammatory response, but did not increase excretion rates of oxidatively generated nucleic acid modifications. In fact, a delayed decrease in oxidatively generated nucleic acid modifications was observed suggesting adaptive antioxidative effects following exercise.

Abbreviations: 8-oxodG: 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGuo: 8-oxo-7,8-dihydroguanosine; CI: confidence interval; CTX: C-terminal telopeptide of type 1 collagen; DXA: dual-energy X-ray absorptiometry; ELISA: enzyme-linked immunosorbent assay; FGF21: Fibroblast growth factor 21; h: hour; hsCRP: high sensitivity C-reactive protein; IL: interleukin; IQR: interquartile range; MS: mass spectrometry: P1NP: N-terminal propeptide of type 1 procollagen; TNFα: tumour necrosis factor alpha; UPLC: ultra-performance liquid chromatography



ARTICLE HISTORY
Accepted 10 May 2020

KEYWORDS
Bone metabolism; exercise; fibroblast growth factor 21; inflammation; oxidative stress

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Supplementary data for this article can be accessed here

Introduction

Like other cellular macromolecules, nucleic acids present a target of oxidation within the cell. Oxidatively generated nucleic acid modifications may cause disturbances in cellular signalling and control (Cheng et al., 1992; Tanaka et al., 2007; Wang et al., 2015) and are, thus, associated with several diseases (Larsen et al., 2019). The oxidatively generated modifications of DNA and RNA can be measured by urinary excretion rates of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), respectively (Poulsen et al., 2019). The urinary excretion rates present a measure of the clearance within the organism (Poulsen et al., 2019). The DNA modification, 8-oxodG, is associated with cancer (Valavanidis et al., 2009) and ageing (Evans & Cooke, 2004), whereas increased excretion rates of the RNA modification, 8-oxoGuo, are associated with increased all-cause- and cardiovascular mortality in patients with type 2 diabetes (Broedbaek et al., 2011, 2013, 2017; Kjær et al., 2017).

Physical activity alters the generation of both pro-oxidants and antioxidants in a complex manner that depends on exercise type, intensity, and duration (Powers & Jackson, 2008; Radak et al., 2008). Time courses of pro-oxidant and antioxidant generation following exercise are not well defined. It is hypothesized that a single bout of exercise induces a pro-oxidant state, whereas regular physical activity adapts the organism, hence, decreases oxidative stress (Radak et al., 2008). The concept "oxidative stress" describes both essential redox mechanisms (i.e., oxidative eustress), but also pathophysiological redox changes (i.e., oxidative distress) (Sies et al., 2017). Previously, antioxidant supplementation has been shown to blunt beneficial effects of exercise (Gomez-Cabrera et al., 2006; Ristow et al., 2009) suggesting and shown by others that exercise-induced oxidative stress seems to mediate beneficial effects of exercise (Margaritelis et al., 2018).

Oxidative stress is known to interact with inflammatory processes (Lugrin et al., 2014). Given that exercise promotes an inflammatory response (Pedersen & Febbraio, 2008) and antioxidant supplementation diminishes the circulating pro-inflammatory cytokines following exercise (Thompson et al., 2001; Vassilakopoulos et al., 2003), an interplay between oxidative stress and inflammation during and after exercise is likely, despite this has only been sporadically assessed (Neubauer et al., 2008). Especially, the potential interconnection and time courses of changes in oxidative stress and inflammation warrants further research. The liver hormone, fibroblast growth factor 21 (FGF21), seems to be modified during exercise (Hansen et al., 2015; Kim et al., 2013) and is associated with inflammatory processes and oxidative stress (Fisher & Maratos-Flier, 2016; Gómez-Sámano et al., 2017; Zhang et al., 2013). Additionally, the bone metabolism was investigated as an example of a target organ affected by exercise (Smith & Gilligan, 2013), that could be mediated through inflammatory and redox processes (Roy et al., 2016; Tian et al., 2017). To clarify the dynamic changes of these different but interlinked processes, we investigated their time courses and interplay following a marathon.

Material and methods

Study design and setting

This observational study was conducted in relation to Copenhagen Marathon 2018, 13th of May. The study was approved by the Biomedical Ethical Committee of the Capital Region of Denmark (H-17041877) and the Danish Data Protection Agency (VD-2018-76). The study protocol is available on request from the corresponding author.

Study participants underwent an information visit, a screening visit, and five study visits, Figure 1. At the *information visit* potential participants were verbally informed about the purpose, the practical execution, and potential risk of the study. Written information about the study was provided. Before the *screening visit*, each participant gave informed written and oral consent for participation in the study. Participants were asked questions regarding their lifestyle and disease history and a physical examination was performed. The *first study visit (baseline)* was conducted up to 7 days before the marathon. Participants handed in a 24-hour (h) urine collection (see section *"urine samples and analysis"*), and fasting blood samples were drawn from an antecubital vein. The *second study visit* was conducted at the finish area of Copenhagen Marathon 2018 where blood samples were collected within 75 minutes of the marathon completion. The *third and fourth study visits* were conducted 2–5 and 9–10 days after the marathon, respectively, and included collection of urine and a fasting blood sample as at the first study visit. Intervals instead of fixed days were planned in order to overcome logistic difficulties and increase compliance. The

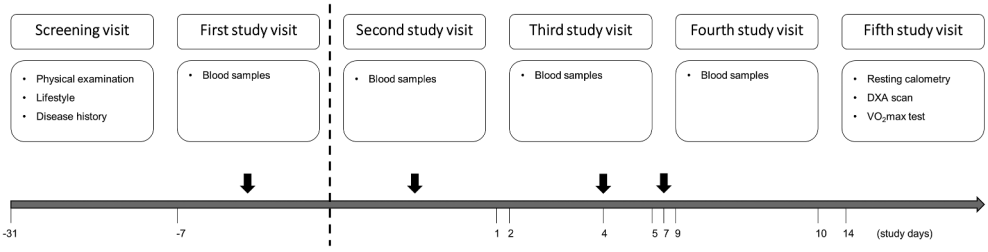

Figure 1. Figure 1 illustrates the study design. The dotted vertical line indicates participation in the marathon race. Black arrows indicate a 24 h urine collection. The third and fourth 24 h urine collection were collected ±2 days if participants could not collect on the exact day. DXA, dual x-ray absorptiometry.

fifth study visit was conducted approximately 2 to 3 weeks after the marathon. Here, fasting participants underwent a resting indirect calorimetry measurement with ventilated hood (CPET, Cosmed, Italy), a dual x-ray absorptiometry (DXA) scan (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA), and a standard cardiorespiratory fitness (VO_2max) test at a cycle ergometer (Monarch 739E) using indirect calorimetry with a mask (CPET). The VO_2max test was initiated with 5 minutes of warm-up at 70 W after which load was increased by 20 W every minute until exhaustion. All data were recorded in a study journal (paper form), and double-entered into REDCap electronic data capture tools hosted at the Capital Region of Denmark (Harris et al., 2009, 2019) after the final study visit was completed.

The study was conducted at the Department and Laboratory of Clinical Pharmacology, Bispebjerg-Frederiksberg Hospital, Copenhagen; the Centre for Physical Activity Research, Rigshospitalet, Copenhagen; and in the finish area of Copenhagen Marathon 2018.

Study participants

Inclusion criteria included male gender, age 18–50 years, and registered to run Copenhagen Marathon 2018. Participants were recruited through advertisements at online recruitment web pages, public places, and publicly available written material. The potential participants were excluded if they were diagnosed with certain diseases that may affect oxidatively generated nucleic acid modifications (i.e., haemochromatosis, schizophrenia, bipolar disease, diabetes, lactase deficiency, glucose-galactose malabsorption, hereditary galactose intolerance, or phenylketonuria), had a smoking history (i.e., current smoker, smoking within 1 month, or previously smoked more than one pack year), or evaluated non-compliant. All participants were compensated for the expenses of running Copenhagen Marathon if successfully completing the study.

Outcome variables

The primary endpoint was changed in 24 h urinary excretion of 8-oxodG and 8-oxoGuo following a marathon. Secondary endpoints included changes in plasma concentrations of inflammation markers, bone turnover markers, and liver hormones as well as changes in 24 h urine creatinine excretion following a marathon. Plasma albumin was determined to correct plasma outcome variables for variation in hydration state.

Urine sample collection and analysis

A total of four 24 h urine samples were collected during the study. The participants were comprehensively informed to discard the first urine void when starting the urine collection – and continuing urine collection for 24 hours ending with an empty bladder. The participants were provided written information about the urine collection procedure and received text message reminders to increase compliance of urine collections. As defined in the study protocol, if a participant lost a urine void, 150 mL was added to the diuresis volume. The lower limit of adequate compliance was 75%. The first 24 h urine collection was collected in the week before the marathon (i.e., starting the day before the first visit). The second 24 h urine collection was started 4 ± 3 hours after finishing the marathon, depending on logistics. The third and fourth urine collection were started 4 and 7 days after the marathon (as defined in the study protocol; if participants were unable to make the urine collection on specified days, the urine collection could be made ±2 days depending on logistics), Figure 1. Urine samples were stored at -20°C until analyses. Urine concentrations of 8-oxodG and 8-oxoGuo were determined using ultra-performance liquid tandem mass spectrometry (UPLC-MS/MS). Detailed description of the analysis method and quality control is available elsewhere (Rasmussen et al., 2016). In short, urine samples were thawed, mixed, and heated to 37°C for 5 minutes. Internal standards ($^{15}N_5$-8-oxoGuo and $^{15}N_5$-8-oxodG) were added. A reverse-phase chromatographic separation was applied using Acquity UPLC 1-class system with an Acquity UPLC BEH Shield RP18 column and a VanGuard precolumn (Waters, Milford, MA, USA). Detection was performed by tandem mass spectrometry with multi-reaction monitoring mode (MRM) using Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA) (Rasmussen et al., 2016). Urine creatinine was determined using an in-house Jaffe's method.

Blood sample collection and analysis

Blood samples were collected at each study visit from an antecubital vein. The blood samples were centrifuged at 2,000 g, 4°C for 15 minutes, and plasma samples were stored at -80°C until analyses. Plasma samples from the marathon visit (i.e., 'study visit 2') were stored on dry ice before and during transportation to the laboratory following the marathon before storage at -80°C. C-terminal telopeptide of type 1 collagen (CTX), and N-terminal propeptide of type 1 procollagen (P1NP), osteocalcin, sclerostin, FGF21, high sensitivity C-reactive protein (hsCRP), tumour necrosis factor alpha (TNFα), interleukin (IL)-6, and IL-10 were determined on plasma from EDTA tubes, albumin was determined on plasma from lithium heparin tubes. TNFα, IL-6, and IL-10 were determined using a multi-spot immunoassay (V-PLEX, Meso Scale Discovery, Rockville, MD, USA) in single determinations. HsCRP was determined using solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (CRP Human ELISA Kit, Thermo Fisher Scientific, MA, USA) in duplicate determinations. FGF21 was determined as full length 1–181 FGF21 by ELISA method (Human Intact Fibroblast Growth Factor (FGF-21) ELISA, Eagle Biosciences, NH, USA) in single determinations. CTX, P1NP, and osteocalcin were determined using IDS-iSYS CTX, IDS-iSYS intact P1NP, and IDS-iSYS N-mid Osteocalcin (Immunodiagnostic Systems, plc, Tyne and Wear, UK) in single determinations. Finally, sclerostin was determined in double determinations in one batch using the TECOMedical Sclerostin HS EIA assay (Quidel Corporation, San Diego, CA, USA).

Sample size

The study sample size was based on the primary endpoints, 24 h excretion rates of 8-oxoGuo and 8-oxodG, and was calculated based on a standard deviation of approximately 7.5 nmol/24 h (Rasmussen et al., 2016), a significance level of 0.05, a power of 80%, and an estimated effect size of 20%. The estimated sample size was 15. To avoid reduced power from potential drop-outs, we chose to include 20 participants.

Statistical methods

R version 3.6.1 (R Core Team, R: A Language and Environment for Statistical Computing, 2019) was used for statistical analysis and graphical illustrations. Clinical and biochemistry characteristics were evaluated for normal distribution graphically and presented as mean ± SD or median (interquartile range (IQR)) if Gaussian criteria were not met. The primary and secondary endpoints were analysed using a linear mixed-effect model (package 'lme4', function "lmer") with each study visit individually compared to the baseline visit and with "subject" as a random effect. Depending on the fit of the model the outcome variable was log-transformed or remained untransformed. In case of transformation, results were reversed before reporting. If the outcome variable was log-transformed, the estimate of change was reported as percentage change from baseline. Outcome variables measured in plasma are both analysed as absolute concentrations and normalized to plasma albumin (i.e., to adjust hydration state). Serum albumin has previously been identified as highly correlated to plasma volume following exercise (Miller et al., 2019). The normalization to plasma albumin was conducted on an individual basis (i.e., [outcome variable concentration]/[plasma albumin concentration]). A sensitivity analysis removing the observation with the highest Cook's distance was performed on the primary endpoints. Graphical illustrations and Pearson's correlation analysis were applied post-hoc to evaluate associations between changes in study endpoints following the marathon. If a relevant correlation was identified, a linear regression model was applied to quantify the association. All *P* values were two-sided with a significance level of 0.05.

Results

Participant flow and characteristics

Twenty-two potential participants were screened from 16 April 2018 through 9 May 2018. One potential participant was excluded due to a chronic disease and one participant withdrew before the first study visit due to an injury. A total number of 20 participants initiated the marathon. All participants completed each study visit. However, four participants missed one blood sample from either study visit 3 (n = 2) or study visit 4 (n = 2) due to technical difficulties during the sample collection. All participants were evaluated compliant with regard to 24 h urine collections. Total urine collection compliance (i.e., total collected urine volume compared to total expected urine volume collected) was 98.8%. Participant flow is illustrated in Figure 2. The clinical and biochemical characteristics of the participants initiating the study are shown in Table 1. The participants' median finish time of the marathon was 4 hours and 4 minutes, Table 1.

Oxidatively generated modifications of nucleic acids

The urinary excretion rates of 8-oxodG and 8-oxoGuo were unaffected immediately after the marathon. However, approximately 4 days after the marathon, a decrease in both urinary excretion of 8-oxodG (by -11%) and 8-oxoGuo (by -17%) was

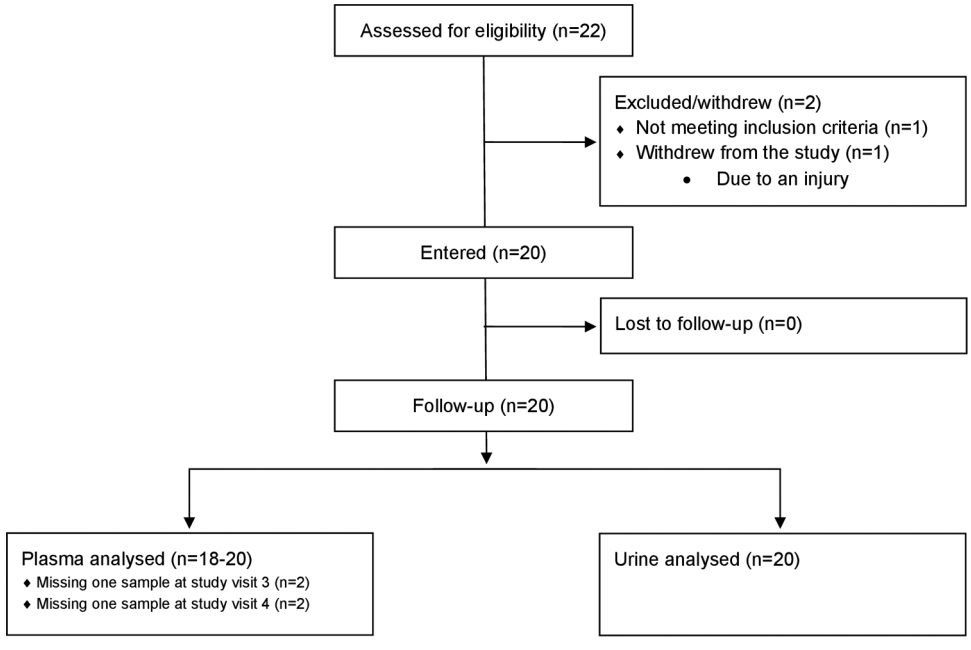

Figure 2. Participant flow during the study.

Table 1. Clinical and biochemistry characteristics of the participants as well as information regarding the marathon race. Data are presented as mean ± SD or median with interquartile range.

Clinical status	
N (males)	20
Age (years)	29.6 [24.3;37.2]
Systolic blood pressure (mmHg)	131 ± 7
Diastolic blood pressure (mmHg)	75 ± 7
Pulse (beats/min)	56 ± 9
Weight (kg)	81.2 ± 8.7
BMI (kg/m^2)	24.7 ± 2.0
Waist-hip ratio	0.89 ± 0.05
Lean weight (kg)	64.2 ± 7.8
Fat percentage (%)	15.9 ± 6.4
Resting metabolic rate (kcal/day)	1875 ± 254
Respiratory quotient at rest	0.78 ± 0.03
Exercise status	
Previous marathons (n)	1 [0;4]
Exercise last month (hours/week)	6.5 [4.0;9.6]
VO_2max (ml/min)	4176 ± 464
VO_2max (ml/min/kg)	52.5 ± 4.6
Biochemistry status baseline	
Plasma ferritin (µg/L)	97.0 [77.5;163.5]
Haemoglobin (mmol/L)	9.00 [8.8;9.3]
Plasma glucose (mmol/L)	4.8 ± 0.3
Plasma creatine kinase (U/L)	198 [143;250]
HbA1 c (mmol/mol)	32.0 ± 2.2
Plasma insulin (pmol/L)	32.5 [22.8;49.5]
Plasma C-peptide (pmol/L)	489.2 ± 113.2
Plasma cholesterol (mmol/L)	4.0 ± 0.7
Plasma HDL-cholesterol (mmol/L)	1.5 ± 0.3
Plasma LDL-cholesterol (mmol/L)	2.3 ± 0.7
Plasma triglyceride (mmol/L)	0.7 ± 0.3
Plasma albumin (g/L)	39.7 [38.8;40.7]
Marathon status	
Finish time (minutes)	244 [235;260]
Δ Creatine kinase (U/L)	292 [174;553]
Δ P-albumin (g/L)	6.4 [3.2;7.7]

observed compared to baseline excretion rates. The decreased excretion rate of 8-oxodG persisted at day seven (by -8%), whereas the excretion rate of 8-oxoGuo was similar at day seven to the baseline excretion rates. The urinary excretion rates of 8-oxodG and 8-oxoGuo are visualized in Figure 3. Table 2 presents the results of the linear mixed-effect models. The sensitivity analysis, where a potential outlier (the observation presenting highest Cook's distance) was removed, revealed a change in the interpretation of 8-oxoGuo immediate after the marathon. The sensitivity analysis showed an increase of 8-oxoGuo by 4.1 (95% CI 0.8;7.4) nmol/24 h, $P = 0.02$ immediately after the marathon. Furthermore, no significant change in the urinary excretion rate of 8-oxodG 7 days after the marathon was evident (change: -1.8 (95% CI -3.7;0.1) nmol/24 h, $P = 0.07$). The sensitivity analysis did not change the interpretation of the remaining results.

No significant changes in 24 h urine creatinine excretion were observed during the study period compared to baseline, Table 2.

Inflammation

An immediate increase was observed in the plasma concentration of the pro-inflammatory cytokines TNFα (by 45%) and IL-6 (a 43-fold increase) as well as the anti-inflammatory cytokine IL-10 (a 40-fold increase). All three markers returned to baseline concentrations 4 days after the marathon. No changes in plasma concentrations of CRP were observed immediately after the marathon, whereas a considerable increase was seen 4 days after the marathon (by 654%). No significant correlations were observed between changes in excretion rates of 8-oxoGuo/8-oxodG and changes of the pro-/anti-inflammatory cytokines, Supplementary file 1. The plasma concentrations of TNFα, IL-6, CRP, and IL-10 are visualized in Figure 4. Table 3 presents the results of the linear mixed-effect models.

FGF21

An increase in plasma concentration of FGF21 was observed immediately after the marathon race compared to baseline (by 100%). Approximately 4 days after the marathon, a minor decrease in plasma concentrations of FGF21 was observed (by -5%) compared to baseline. However, evaluating plasma albumin corrected concentrations the latter decrease was insignificant ($P = 0.053$). At day nine, the

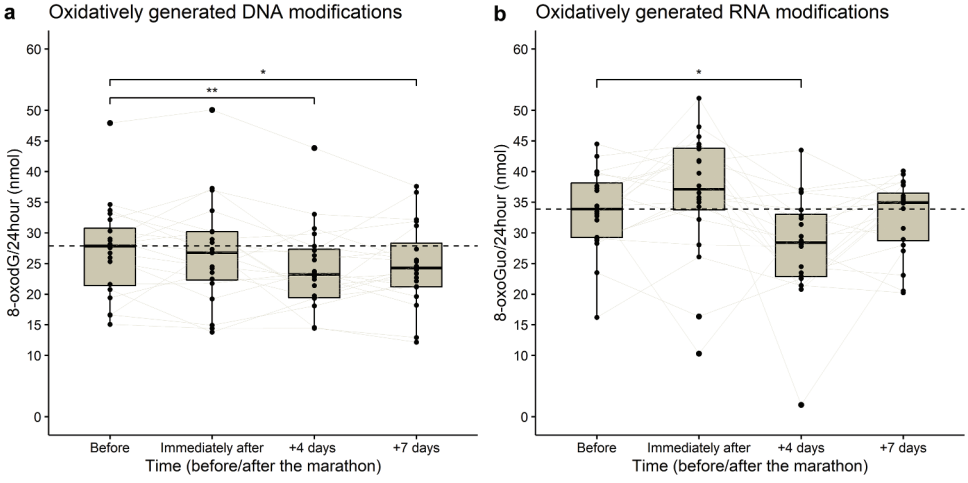

Figure 3. Urinary excretion of (a) 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and (b) 8-oxo-7,8-dihydroguanosine (8-oxoGuo) before and at various timepoints after the marathon illustrated as boxplots (horizontal lines indicate first quartile, median, and third quartile, respectively. The vertical line indicates minimum and maximum without outliers). Individual excretion rates are illustrated by black dots and grey lines (solid). Baseline median excretion rate is illustrated by a dashed horizontal line. *$P < 0.05$; **$P < 0.01$. Urine collections "+4 days" and "+7 days" were each allowed to be collected ±2 days if participants were unable to collect on specified days.

Table 2. Table 2 presents the results of the linear mixed effect models analysing changes in urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo), 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), and creatinine at various timepoints after the marathon each compared to the baseline visit. Data are presented as an estimate (95% confidence interval). Urine collections "Four days after" and "Seven days after" were each allowed to be collected ±2 days if participants were unable to collect on specified days.

			Immediate after	Four days after	Seven days after
8-oxodG	Fixed effects	Baseline (intercept) (nmol/24 h)	27.1 (23.5;30.7)	27.1 (23.9;30.3)	27.1 (23.9;30.3)
		Change from baseline (slope) (nmol/24 h)	−0.3 (-2.6;2.0)	−2.9 (-4.8;-1.1)	−2.3 (-4.3;-0.4)
	P value		0.77	**0.005**	**0.03**
8-oxoGuo	Fixed effects	Baseline (intercept) (nmol/24 h)	33.7 (29.9;37.5)	33.7 (30.2;37.1)	33.7 (30.8;36.5)
		Change from baseline (slope) (nmol/24 h)	2.7 (-1.5;7.0)	−5.8 (-10.3;-1.3)	−1.0 (-3.7;1.7)
	P value		0.21	**0.02**	0.47
Creatinine	Fixed effects	Baseline (intercept) (mmol/24 h)	11.2 (8.9;13.5)	11.2 (9.5;12.9)	11.2 (9.4;13.0)
		Change from baseline (slope) (mmol/24 h)	1.0 (-1.8;3.7)	−0.8 (-2.5;0.9)	−0.9 (-2.9;1.1)
	P value		0.50	0.34	0.37

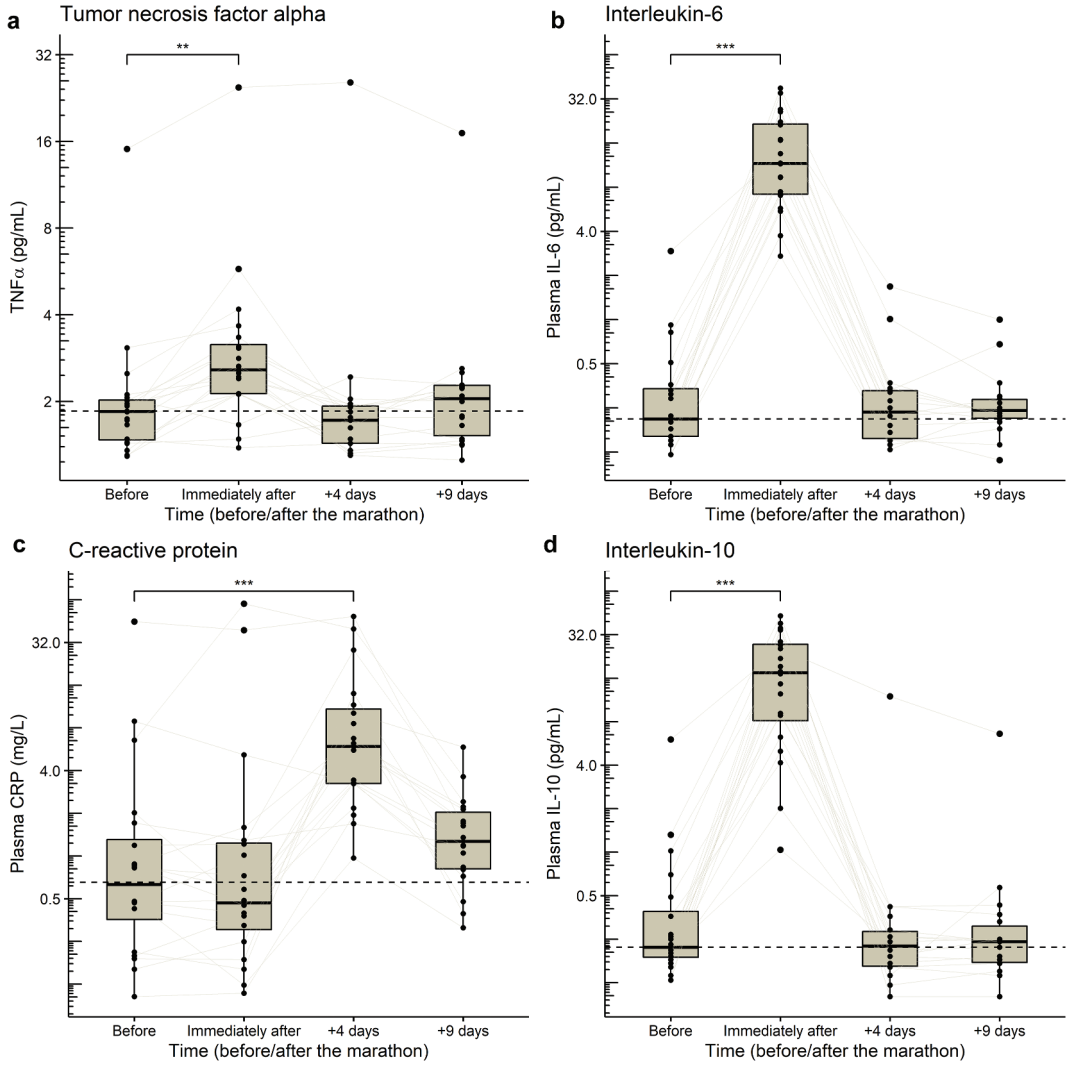

Figure 4. Unadjusted plasma concentrations of (a) tumour necrosis factor alpha (TNFα), (b) interleukin (IL)-6, (c) C-reactive protein (CRP), (d) IL-10 before and at various timepoints after the marathon illustrated as boxplots (horizontal lines indicate first quartile, median, and third quartile, respectively. The vertical line indicates minimum and maximum without outliers). Individual plasma concentrations are illustrated by black dots and grey lines (solid). Baseline median concentration is illustrated by a dashed horizontal line. The y-axis has been transformed into a log2-scale. $^{**}P < 0.01$; $^{***}P < 0.001$ of plasma albumin adjusted results. Plasma samples collected "+4 days" and "+9 days" were collected 2–5 days and 9–10 days after the marathon, respectively.

plasma concentration of FGF21 corresponded to baseline concentrations. The plasma concentrations of FGF21 are visualized in Figure 3. Table 2 presents the results of the linear mixed-effect models. A linear relationship between changes in the plasma concentrations of FGF21 and the changes in anti-inflammatory cytokine IL-10 was observed

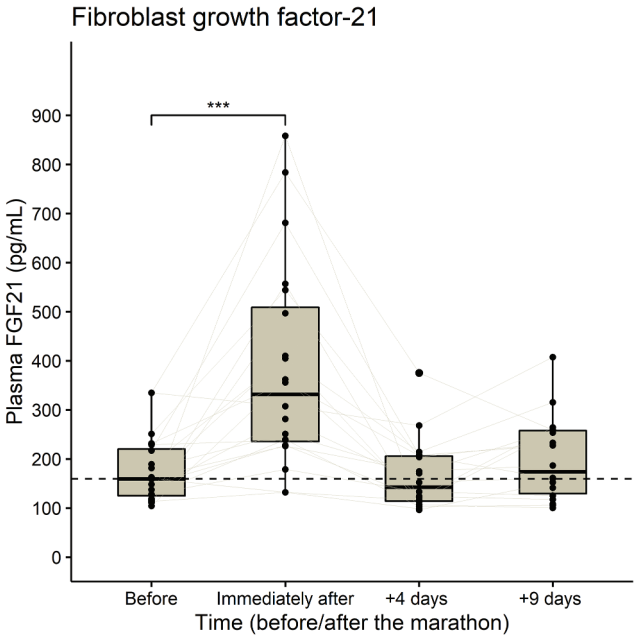

Figure 5. Unadjusted plasma concentrations of fibroblast growth factor 21 (FGF21) before and at various timepoints after the marathon illustrated as boxplots (horizontal lines indicate first quartile, median, and third quartile, respectively. The vertical line indicates minimum and maximum without outliers). Individual plasma concentrations are illustrated by black dots and grey lines (solid). Baseline median concentration is illustrated by a dashed horizontal line. ***$P < 0.001$ of plasma albumin adjusted results. Plasma samples collected "+4 days" and "+9 days" were collected 2–5 days and 9–10 days after the marathon, respectively.

(Figure 6). Each 1 pg/ml increase in plasma IL-10 increased FGF21 by 8.7 (95% CI 1.9; 15.5) pg/mL immediate after the marathon. FGF21 revealed no significant relationship with the pro-inflammatory cytokines or the markers of oxidatively generated nucleic acid modifications (data not shown).

Bone turnover markers

Immediate after the marathon differential effects on different bone metabolism markers were found. Plasma concentrations of sclerostin were increased (by 24%), whereas plasma concentrations of both P1NP and CTX decreased (by -8% and -19%, respectively) compared to baseline concentrations. Plasma concentrations of osteocalcin increased (by 24%), however, this change was insignificant when plasma concentrations were normalized to plasma albumin concentration. Four and nine days after the marathon no significant changes in plasma bone metabolism markers were evident compared to baseline concentrations. The bone turnover markers are visualized in Figure 7. Table 2 presents the results of the linear mixed-effect models. We did not find any correlation between plasma concentrations of sclerostin and plasma inflammation cytokines (data not shown).

Discussion

In this study, we investigated the time course of systemic levels of immunologic and metabolic variables in response to a marathon race. Overall, we found drastic changes in circulating markers of inflammation, FGF21, and bone metabolism immediately after the marathon, whereas systemic levels of oxidative stress were unaffected initially, but decreased some days after the marathon race. Observational studies have demonstrated that urinary excretion of 8-oxoGuo is a prognostic marker of all-cause mortality in patients with type 2 diabetes (K. Broedbaek et al., 2011, 2013; Kjær et al., 2017), and changes in excretion rates of 8-oxoGuo are associated with changes in mortality (K. Broedbaek et al., 2013). Thus, the present finding of decreased excretion rates of 8-oxoGuo following a marathon is of great interest and merits future investigations.

An exercise bout generates reactive oxygen species in muscle tissue. The effect of exercise on other tissues is not well defined given the difficulties investigating other tissues in humans (Powers & Jackson, 2008). Our study results show that urinary excretion rates of 8-oxodG and 8-oxoGuo are not increased immediately after a marathon. This suggests that muscle tissue can increase oxygen consumption considerably, without affecting systemic markers of oxidative stress. Previous studies examining the effects of excessive bouts of exercise on oxidatively generated DNA modifications have shown increased excretion rates of 8-oxodG immediately after running a marathon (or ultra-marathon) (Mrakic-Sposta et al., 2015; Radak et al., 2000; Tsai et al., 2001). One of the studies did not correct 8-oxodG concentrations to diuresis (Radak et al., 2000). Thus, hydration state may have biased their results. In contrast to previous studies, our results demonstrated very similar excretion rates of oxidatively generated DNA modifications immediately after the marathon compared to baseline values, whereas oxidatively generated RNA modifications tended towards a minor increase immediately after the marathon. Thus, we hypothesize that previous studies that have shown effects on oxidatively DNA modifications may be due to the analysing method (i.e., using ELISA kits). Most ELISA kits are unable to distinguish between oxidative DNA and RNA modifications (Larsen et al., 2019), and, thus, previous findings of increased urinary excretion rates of 8-oxodG-reactivity may be due to increased modifications of RNA (i.e., increased 8-oxoGuo excretion rates). This study does not imply that reactive species are not generated during exercise; however, under the current study conditions, the generation of reactive species following a marathon is insufficient to increase the urinary excretion rates of 8-oxodG and 8-oxoGuo.

Despite no immediate effects on oxidatively generated nucleic acids were observed, we found that the excretion rates of both oxidative DNA and RNA modifications were reduced 4 days after the marathon, which, for oxidative DNA modifications, persisted at day seven. A recent cross-sectional study showed that participants who regularly exercised had a lower excretion rate of oxidized RNA modifications (Kofoed Kjaer et al., 2019). These results agree with the present study, and suggest adaptive effects of exercise on oxidatively generated nucleic acid modifications, potentially through increased anti-oxidative defence mechanisms (Bloomer, 2008).

Even though inflammatory processes interplay with oxidative stress, the changes in inflammatory cytokines

Table 3. Table 3 presents the results of the linear mixed effect models analysing changes in plasma concentration of tumour necrosis factor alpha (TNFα), interleukin (IL)-6, C-reactive protein (CRP), IL-10, fibroblast growth factor 21 (FGF21), sclerostin, osteocalcin, C-terminal telopeptide of type 1 collagen (CTX), and N-terminal propeptide of type 1 procollagen (P1NP) at various timepoints after the marathon each compared to the baseline visit. Data are presented as an estimate (95% confidence interval). *2–5 days after the marathon. **9–10 days after the marathon.

			Immediate after	Four days after*	Nine days after**
TNFα	Fixed effects	Baseline (intercept) (pg/mL)	1.98 (1.53;2.55)	1.98 (1.52;2.58)	1.98 (1.56;2.52)
		Change from baseline (slope) (%)	45.4 (26.4;67.2)	1.1 (-7.2;10.0)	3.1 (-4.6;11.5)
	P value	Unadjusted	0.00004	0.80	0.44
		Adjusted to plasma albumin concentration	0.002	0.57	0.61
IL-6	Fixed effects	Baseline (intercept) (pg/mL)	0.27 (0.20;0.38)	0.27 (0.19;0.40)	0.27 (0.20;0.37)
		Change from baseline (slope) (%)	4176.0 (2829.7;6141.1)	8.0 (-13.9;33.9)	−8.6 (-35.8;32.5)
	P value	Unadjusted	$3e^{-14}$	0.49	0.62
		Adjusted to plasma albumin concentration	$6e^{-14}$	0.40	0.55
CRP	Fixed effects	Baseline (intercept) (mg/L)	0.81 (0.40;1.66)	0.81 (0.46;1.44)	0.81 (0.48;1.38)
		Change from baseline (slope) (%)	−4.6 (-32.9;35.6)	653.9 (276.9;1407.7)	51.9 (-24.1;211.5)
	P value	Unadjusted	0.79	0.00001	0.25
		Adjusted to plasma albumin concentration	0.30	0.00001	0.26
IL-10	Fixed effects	Baseline (intercept) (pg/mL)	0.32 (0.21;0.49)	0.32 (0.20;0.49)	0.32 (0.21;0.47)
		Change from baseline (slope) (%)	4023.1 (2333.0;6886.6)	−10.2 (-28.4;12.2)	−15.7 (-39.5;17.9)
	P value	Unadjusted	$2e^{-11}$	0.35	0.32
		Adjusted to plasma albumin concentration	$<2e^{-11}$	0.40	0.27
FGF21	Fixed effects	Baseline (intercept) (pg/mL)	166.7 (136.2;203.9)	166.7 (141.3;196.6)	166.7 (140.6;197.6)
		Change from baseline (slope) (%)	100.2 (54.4;159.4)	−8.0 (-14.7;-0.9)	8.0 (-5.9;24.4)
	P value	Unadjusted	0.00003	0.04	0.28
		Adjusted to plasma albumin concentration	0.00002	0.053	0.26
Sclerostin	Fixed effects	Baseline (intercept) (ng/mL)	0.68 (0.57;0.79)	0.68 (0.59;0.77)	0.68 (0.58;0.78)
		Change from baseline (slope) (ng/mL)	0.16 (0.10;0.23)	0.03 (-0.03;0.08)	0.05 (-0.00;0.10)
	P value	Unadjusted	0.00006	0.34	0.07
		Adjusted to plasma albumin concentration	0.03	0.17	0.14
Osteocalcin	Fixed effects	Baseline (intercept) (µg/L)	26.5 (20.8;32.2)	26.5 (22.3;30.6)	26.5 (22.4;30.5)
		Change from baseline (slope) (µg/L)	6.4 (1.5;11.3)	1.1 (-0.8;3.0)	0.3 (-1.5;2.3)
	P value	Unadjusted	0.02	0.26	0.72
		Adjusted to plasma albumin concentration	0.32	0.15	0.95
CTX	Fixed effects	Baseline (intercept) (ng/L)	740.4 (611.5;869.3)	740.4 (615.9; 864.8)	740.4 (623.9; 856.9)
		Change from baseline (slope) (ng/L)	−138.6 (-261.2;-15.9)	39.9 (-51.5;134.4)	−28.0 (-100.0;45.0)
	P value change	Unadjusted	0.04	0.40	0.45
		Adjusted to plasma albumin concentration	0.001	0.32	0.29
P1NP	Fixed effects	Baseline (intercept) (µg/L)	82.9 (71.5;94.3)	82.9 (71.3;94.5)	82.9 (72.2;93.5)
		Change from baseline (slope) (µg/L)	−6.4 (-11.6;-1.1)	−2.7 (-7.6;2.3)	−4.9 (-10.5;0.7)
	P value	Unadjusted	0.03	0.29	0.09
		Adjusted to plasma albumin concentration	0.00003	0.55	0.07

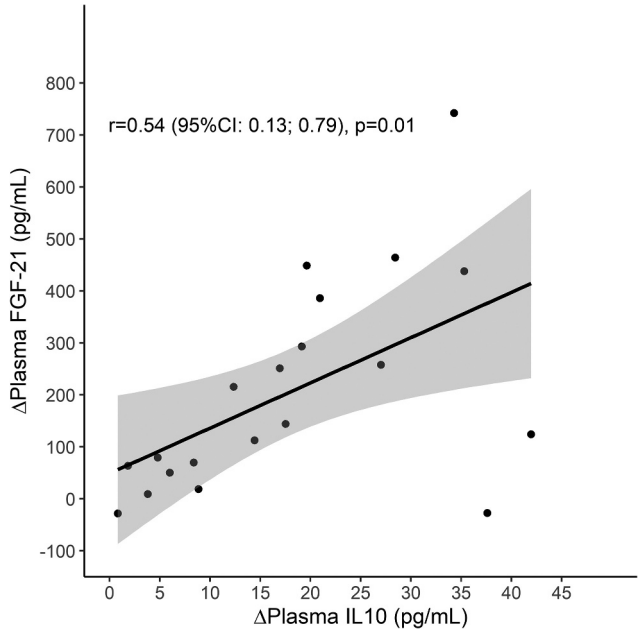

Figure 6. The relationship between changes in interleukin (IL)-10 and changes in fibroblast growth factor 21 (FGF21) immediate after the marathon. The linear regression line is denoted as a black line with a 95% confidence interval (CI) scattered grey and individual points as black dots. The Pearson's correlation coefficient is presented as r with 95% CI.

following the marathon did not correlate with changes in oxidatively generated nucleic acid modifications. In agreement with previous studies investing strenuous exercise, we observed immediate increases in plasma concentrations of IL-6, IL-10, and TNFα following the marathon (Pedersen et al., 2001), but these increases were transient and levels were back to baseline 4 days after the marathon. Hence, this increase in inflammatory markers was not mirrored by increased excretion rates of oxidatively generated nucleic acids, but rather a (delayed) reduction in these. As such, and despite it must be emphasized that other molecules than RNA/DNA may be more prone to acute changes in oxidative stress (Wagner et al., 2011), these data do not indicate that increased inflammation translates to increased oxidative stress *per se*. The plasma concentration of CRP was unchanged immediately after the marathon but was increased 4 days after the marathon where an ~7.5-fold increase was observed. Once again, the increase was transient and normalized 9 days after the marathon. A similar delayed response in CRP has previously been shown following a marathon (Santos et al., 2016).

FGF21 is a peptide mainly produced by the liver that possesses regulatory effects on glycaemic control, lipids, and energy balance (Fisher & Maratos-Flier, 2016; Kharitonenkov et al., 2005). Recently, an FGF21 analogue was shown to improve metabolic parameters and reduce hepatic fat in patients with non-alcoholic steatohepatitis (Sanyal et al., 2018). Additionally, FGF21 seems to be associated with sweet and alcohol preferences (Søberg et al., 2018, 2017). Thus, investigations aimed at modifying the plasma concentrations of FGF21 have emerged. We observed an ~2-fold increase in plasma concentrations and individual concentrations up to 858.2 pg/mL. A previous study revealed no immediate increases following 30 minutes of exercise, but an increase occurred 1 h post exercise. The study further suggested a dose–response relationship between exercise intensity and plasma concentrations (Kim et al., 2013). Interestingly, we found as a post-hoc analysis that the acute changes from baseline seemed to be associated with the changes of the anti-inflammatory cytokine IL-10. Others have suggested associations between FGF21 and inflammatory processes as well as oxidative stress (Fisher & Maratos-Flier, 2016; Gómez-Sámano et al., 2017; Zhang et al., 2013). Thus, the relationship between inflammatory processes and circulating FGF21 should be a topic of future investigations.

In this study, we observed transient effects on bone metabolism acutely after the marathon that did not persist 4 days after the marathon. We observed increased concentrations of sclerostin, which inhibits bone formation. Usually, sclerostin is downregulated upon mechanical loading to bone (Galea et al., 2017). However, the magnitude and duration of loading and the metabolic challenges during strenuous exercise such as a marathon may instead increase sclerostin production in bone resulting in an inhibition of bone formation. In line with this, we found that both markers of bone formation (P1NP) and bone resorption (CTX) were decreased. These changes indicate that bone turnover is inhibited following a marathon, which seems appropriate in a stressful situation. However, diurnal variations in plasma concentrations of CTX are known to occur (Qvist et al., 2002). Also, food intake is known to affect CTX concentrations (Qvist et al., 2002). The concentrations of CTX that promote bone resorption may be explained by differences in the timing of samples and fasting state. Therefore, CTX concentration from immediately after the marathon might not be directly comparable to concentrations from the other three timepoints. Adjusting to hydration state using plasma albumin, no changes in osteocalcin were evident compared to baseline. However, unadjusted plasma concentrations of osteocalcin revealed increased plasma concentrations. This underlines the importance of accounting for changes in plasma volume following exercise. None of the bone metabolism markers were associated with the inflammatory cytokines.

The strengths of the current study were the analysis method of oxidized nucleosides using UPLC-MS/MS that is considered the reference standard method due to high specificity towards the DNA and RNA forms. The repeated plasma samplings and 24 h urine collections enable the description of the time course following a marathon. Furthermore, we corrected plasma concentrations of outcome values to hydration state following the marathon using plasma albumin. The limitations of the study are the observational study design with no control group. Furthermore, due to logistic challenges and to improve compliance we had to separate some of the study visits into different days. The indirect calorimetry, DXA-scan, and VO_2max test were performed following the study period instead of before. The participants wore activity trackers 1 week before and 2 weeks after the marathon; however, many participants lost their activity trackers, so we were unable to make use of these data. Thus, we cannot account for changes in activity level in the study period. Additionally, we have no data regarding food intake in the study period.

In conclusion, this study revealed acute, but transient changes on several markers of immunometabolism, including IL-6, IL-10, TNFα, FGF21, sclerostin, CTX, and P1NP following a marathon in healthy, non-professional athletes. A delayed increase was

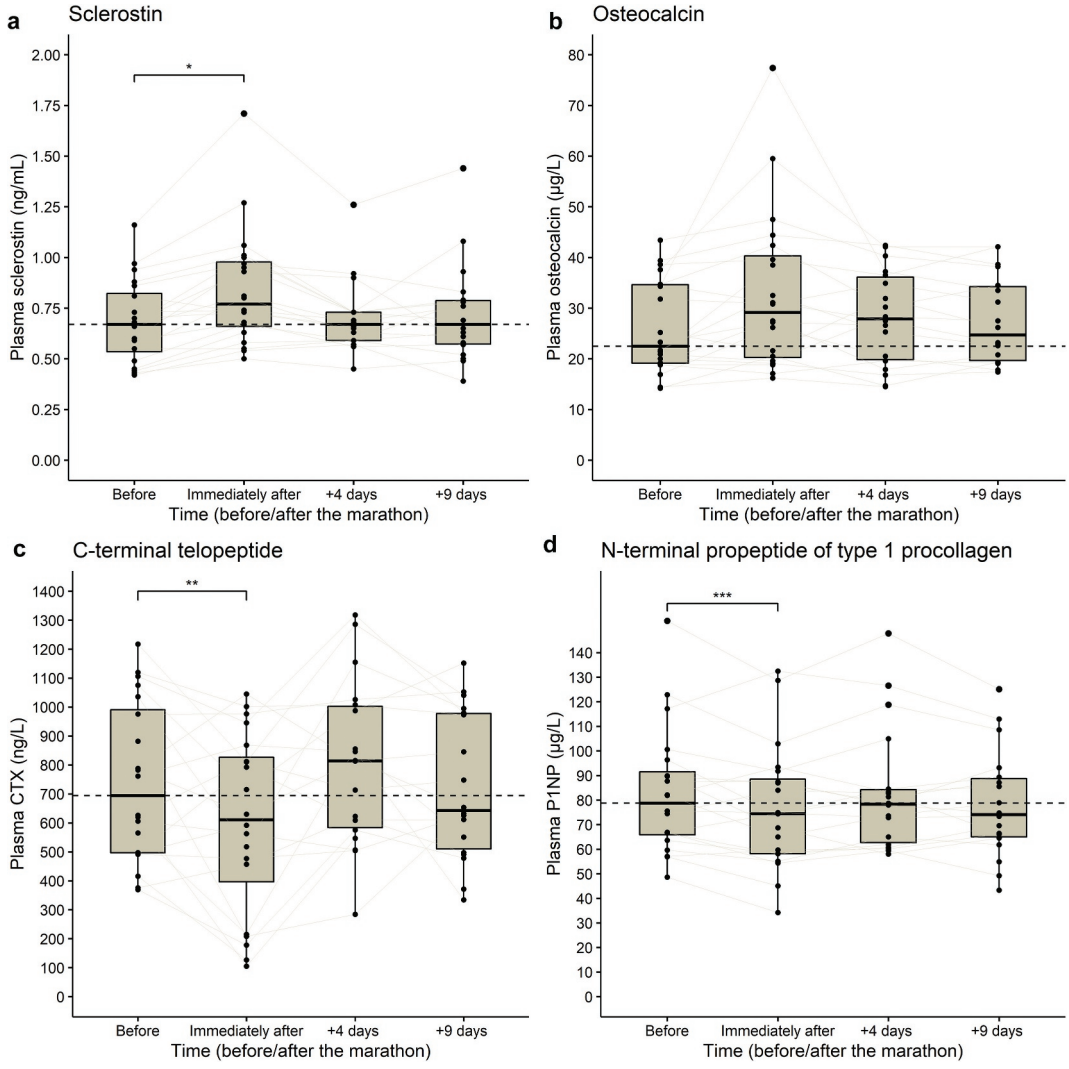

Figure 7. Unadjusted plasma concentrations of (a) C-terminal telopeptide of type 1 collagen (CTX), (b) N-terminal pro-peptide of type 1 procollagen (P1NP), (c) osteocalcin, and (d) sclerostin before and at various timepoints after the marathon illustrated as boxplots (horizontal lines indicate first quartile, median, and third quartile, respectively. The vertical line indicates minimum and maximum without outliers). Individual plasma concentrations are illustrated by black dots and grey lines (solid). Baseline median concentration is illustrated by a dashed horizontal line. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ of plasma albumin adjusted results. Plasma samples collected "+4 days" and "+9 days" were collected 2–5 days and 9–10 days after the marathon, respectively.

observed in CRP. Oxidatively generated DNA and RNA modifications were unaffected immediate after the marathon; however, both markers decreased 4 days after the marathon suggesting adaptive antioxidative effects following exercise.

Acknowledgments

We thank senior laboratorian technician Lis Kjær Hansen, laboratory technicians Stine W. Frederiksen and Agnethe Mehlsen for their tremendous help during this study. We thank chemist Trine Henriksen and laboratory technician Katja R. Christensen for conducting the urine analysis of 8-oxodG, 8-oxoGuo, and creatinine. We are grateful to Mathias Ried-Larsen and Cecilie Fau Brinkløv for helping with the accelerometer measurement although the data were not used in the current paper. We thank Sparta Atletik & Motion for providing a location and facility at the finish area of Copenhagen Marathon 2018, 'A.P. Møller Fonden - Fonden for Lægevidenskabens Fremme' for providing funding for this study and Centre of Active Research for letting us use their equipment at study visit 5, and www.smart.servier.com for providing an image for the graphical abstract. ML has received a research grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation. At last, but foremost, we thank the participants for making this study possible.

Disclosure statement

All authors declare no conflict of interest in relation to this study.

Funding

This work was supported by the A.P. Møller Fonden - Fonden til Lægevidenskabens Fremme (17-L-0305); Danish Diabetes Academy supported by Novo Nordisk Foundation.

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