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Muscle Damage and Immune-Endocrine Responses in 20-km Walking Race

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ABSTRACT

International Journal of Exercise Science 17(7): 1167-1182, 2024. The objective of the study was to monitor exercise-induced muscle damage (EIMD), inflammatory responses (IL-6, TNFα, and IL-10), and immune-endocrine balance (testosterone, cortisol, and salivary SIgA) in official 20 km walking race competitions. Eight 20 km professional walking racers (n = 6 women), 27 ± 9 years, underwent blood and saliva sampling, evaluation of delayed-onset muscle soreness (DOMS), and squat (SJ) and countermovement (CMJ) jump tests 2 h before (Pre), immediately after (Post), and 24 and 48 h after the competition. The rate of perceived exertion (RPE) was recorded 20 minutes after the race ended. The race evoked high competitive load (948.3 ± 268.0 a.u.), increased creatine kinase levels at 24 h ($p < 0.05$), and DOMS at 48 h ($p < 0.05$), but no significant changes in SJ and CMJ after the race. No significant changes in cytokines were detected. No changes in salivary SIgA secretion rate and inflammatory cytokines were detected ($p > 0.05$). The race induced increased testosterone ($p < 0.05$), and cortisol ($p < 0.01$) levels immediately after the race. Despite the high competitive load, 20-km walking racer athletes presented mild EIMD without impairment in immune-endocrine markers.

KEY WORDS: Physical endurance, muscle strength, endocrine disruptors, mucosal immunity

INTRODUCTION

Race walking is among the longest races in Olympic track and field competitions, requiring a high level of fitness from athletes (13, 39). The Olympic distances are 20 and 50 km, taking between 80 and 90 minutes to complete 20 km and from 3h 40 min to 4 h and 11 min for the 50 km race (19). Race walking has strict biomechanical rules; athletes must maintain continuous contact with the ground during displacement and keep one knee extended from the initial contact with the ground to the vertical position (13, 39). Competitive performance depends on stride biomechanical parameters, especially stride length, and frequency, reactive contact force and moment of force, and torque of upper and lower limb joints (18, 21, 39). The speed and biomechanical efficiency of running (flight time, posture phase, swing phase, contact time, stride

length, and frequency) are associated with running economy, demonstrating that both biomechanical and physiological (muscle strength) parameters contribute to the performance of walking racers athletes (18).

As a long-distance race, the walking race may lead to mechanical strain and metabolic alterations that can cause damage to muscle tissues, inflammatory responses, and fatigue during the race (7). Although walking races are performed at a slower speed than running races, large muscle groups are also recruited and may suffer some degree of fatigue and muscle damage during the competition. During the race, the hip extensor and plantar dorsiflexor muscles show high activity during the initial contact and displacement phase, while the quadriceps muscle maintains knee hyperextension, and the sural triceps are recruited in the final phase of the stride (17, 20). The eccentric contraction of these large muscles allows the storage of elastic energy and power generation using the knee as a lever (17, 20). However, eccentric contractions and long-term exercises can promote damage to muscle fibers, causing sarcomere disorganization and creatine kinase (CK) leakage, an important marker of muscle damage (7). In half-marathon and marathon runners, biomechanics were quite different than walking races. However, these long-distance races rely on concentric-eccentric contractions on the same muscular groups, displaying signs of muscle damage (4, 23). In long-distance running events, exercise-induced muscle damage (EIMD) is observed, with a serum increase in the CK enzyme and delayed-onset muscle soreness (DOMS) detected at 24 hours. (10, 43). The lower limb's power is also reduced after marathons and half marathon races (10, 43). Despite being an Olympic modality, it is unclear how much metabolic stress and mechanical damage are produced during race walking competitions. This is important for planning strategies for recovery and returning to a training routine. Walking races are performed over distances close to half marathon and marathon distances, in a longer time, which suggests that inflammatory symptoms and muscle damage may also occur in this modality.

Long-distance runners recruit large muscle groups in long-term exercises promoting an increase in the production of Interleukin-6 (IL-6), a cytokine that may have an anti-inflammatory function and that is necessary for glucose mobilization during exercise (5). However, the increase in IL-6 associated with the inflammatory cytokine Tumor Necrosis Factor-alpha (TNFα) is correlated with inflammatory responses observed in long-distance runs and marathons (5, 10). A study on elite athletes showed a moderate increase (5 to 10 times) in IL-6 levels after a simulated run in female (19 km) and male (25 km) athletes (31). However, the authors manipulated a ketogenic diet to assess the increase in IL-6. Bernecker and colleagues (5) suggest that the release of these cytokines is associated with the duration of endurance tests. However, it has not yet been established whether these inflammatory reactions occur in walking racers and their association with race performance. This is of concern since the inflammatory process has catabolic effects and may impair muscle performance.

Endurance exercises also cause modulation of the hormones testosterone and cortisol, promoting a significant reduction in testosterone for up to 72 h and an increase in cortisol levels during effort (1). Cortisol production can suppress the response of testosterone, an important anabolic hormone, essential for post-exercise recovery(9). Furthermore, in individual modalities

and runners the cortisol and testosterone levels before, during, and after the competition are associated with the athlete's performance and motivation (9). However, it is not clear whether walking racer athletes demonstrate changes in the testosterone: cortisol ratio in competitive events.

Prolonged high-intensity exercise, or training periods with high loads, can also promote impairment in mucosal secretory immunity, reducing salivary concentrations of secretory immunoglobulin A (SIgA) and increasing the risk of developing upper airway infections in athletes (40, 45). Long-distance running events promote a transitory reduction in SIgA and increase the risk of upper respiratory tract infection (URTI) (35, 37). However, the effects of walking race on mucosal immunity are not known.

Identifying the magnitude of muscle damage, inflammatory reactions, and immuno-endocrine imbalance in walking races can help to prescribe tapering and recovery methods that favor improvements in race performance and recovery of athletes, in addition to preventing the development of illness and injuries. Thus, the present study aimed to monitor EIMD, inflammatory responses, and immuno-endocrine alterations in official walking race competitions and to correlate the results with the internal competitive load and performance in a 20 km race. The study hypothesis was that a 20 km walking race promotes EIMD, associated with inflammatory reactions and immuno-endocrine changes, as observed in other endurance modalities.

METHODS

Participants

Eight high-performance athletes in the 20 km walking race, of both sexes (n = 6 women), 27 ± 9 years, 57 ± 08 kg, 170 ± 0.08 cm were evaluated. The athletes participated in the research voluntarily, being invited by telephone contact before the race. The athletes signed an informed consent form before the procedures. The study procedures were approved by the Ethics Committee Involving Human Beings of the Centro Universitário Integrado (4.280.972). This research was carried out fully under the ethical standards of the *International Journal of Exercise Science* (34).

Only athletes from the 20 km race, classified among the top 12 in the national ranking of the adult category, and who participated in official competitions of the CBAT were included in the study. Athletes who showed signs and symptoms of muscle-joint injuries that could compromise the physical tests or performance in the race and the presence of upper airway infections on the day of the race were excluded from the analysis. Athletes who used anti-inflammatory medications and nutritional ergogenic resources or recovery resources after the race were also excluded. The athletes rested during the observation period, after the competitions, were housed at the same accommodation, and followed the nutritional recommendations given by the technical staff.

The sample size was determined considering a large effect size (1.41) for an α error < 5% and statistical power (1−β) > 80%, in mean differences in circulating CK concentration in athletes 24 h after completion of a half marathon (30). A minimum of seven athletes was necessary, and then a group of the best-ranked (top 20) athletes of CBAT was invited to participate in the study. Nine athletes volunteered, but one was excluded based on exclusion/inclusion criteria.

Protocol

This is a cross-sectional observational study carried out in official competitions of 20 km walking races organized by the Brazilian Athletics Confederation (CBAT), held in the Brazil Athletics Trophy 2019 and Paraná Athletics Championship 2019. These official competitions are accounted for to define national athletes' ranking. The races were performed in outdoor tartan tracks, with temperatures ranging from 22–25 °C, relative air humidity between 76–80%, and wind flow speeds ranging from 5–7 km/h. The procedures of competitions followed the rules of World Athletics (https://worldathletics.org/).

Two hours before (Pre), immediately after (Post), and 24 and 48 hours post-race, blood and saliva samples were collected, muscle soreness in the lower limbs was evaluated, and countermovement (CMJ) and squat (SJ) jump tests were performed. Twenty minutes after the race, the RPE was reported using the Borg CR-10 scale. The athletes were familiar with all tests and procedures before data collection.

Twenty minutes after the end of the race, the rate of perceived exertion (RPE) was determined using the Borg-CR10 scale (14). The official total race time (minutes) was used to calculate the internal load intensity of the competition, multiplying the RPE by the time.

The EIMD was classified as mild, moderate, or severe based on criteria described by Paulsen and coworkers (38) that take into account the levels of circulating CK, loss of performance after physical efforts, and time to recovery.

Blood samples were collected in vacuum tubes containing EDTA (Vacutainer®, BD Biosciences, Franklin Lakes, USA), centrifuged at 4000 g, for 5 min, and the plasma was aliquoted and stored at −20 °C before use.

The concentrations of CK and circulating aspartate aminotransferase (AST) were determined using a biochemical analysis method in automated equipment (Dimension XL, Siemens, Munich, Germany) with commercial kits (Siemens, Munich, Germany), according to the manufacturer's recommendations.

Levels of TNFα, IL-6, and IL-10 were assessed by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA), according to the manufacturer's recommendations.

Unstimulated saliva samples were collected for 2 min, before being centrifuged at 4000 g for 5 minutes, and stored at −20 °C. The samples were diluted 1:1000 in PBS solution (pH 7.2) and the

concentration of salivary IgA (SIgA) was determined with a commercial ELISA kit (Bethyl Laboratories, Montgomery, USA), according to the manufacturer's recommendations. The SIgA secretion rate (µg/min) was determined by IgA concentration multiplied by saliva flow rate.

The salivary concentrations of cortisol (cat. 3002; Salimetrics, State Colege, PA, USA) and testosterone (cat. 2402; Salimetrics, State Colege, PA, USA) were determined with commercial kits, according to the manufacturer's recommendations.

Muscle soreness was assessed using a 10-point scale (33). The athletes were instructed to squat with their knees bent at 90° for 5 seconds, with their hands on their hips, and report the pain intensity in the lower limb muscles on the visual analog pain scale.

The squat (SJ) and countermovement (CMJ) jump tests were performed on a contact mat (Cefise, Nova Odessa, SP, Brazil), to assess explosive strength and reactive strength (6, 25). Three attempts were made, with a 1-minute interval between each jump, and the best attempt was used for statistical analysis. Jump heights were determined using software (Jump System, Cefise, Nova Odessa, SP, Brazil).

Statistical Analysis

The homogeneity of variances and normality distribution was assessed using the Bartlett test. Data with normal distribution are described as mean and standard deviation and data without normal distribution as medians and quartiles of 25–75% of value distribution. The percentual variation concerning pre-values (Δ) was calculated at Post, 24 h, and 48 h. Differences over time were determined using the repeated-measures ANOVA with Bonferroni's post hoc test for parametric measurements. Nonparametric data were evaluated by Friedman's test with Dunn's post hoc test. Differences between the moments were considered significant if $p < 0.05$. The correlation of the race time and RPE, with the study variables, was determined with Pearson's correlation coefficient (parametric data) or Spearman's rank correlation (nonparametric) tests. Cohen's *d* effect size (ES) and 95% confidence interval were calculated for differences between pre-values and Post, 24 h and 48 h. Sample size calculation was performed in G*Power v. 3.1.9.6 (Franz Faul, University of Kiel, Germany) and statistical analysis was performed in GraphPad Prism v.4 (GraphPad, Boston, USA).

RESULTS

The RPE in competitions was 8 ± 1.5 a.u. with an average race time of 117.5 ± 14 minutes and an estimated internal load of 926.3 ± 191.1 a.u. The athletes' age, CBAT ranking, 2019 best individual performance, race performance, and competitive training load were demonstrated in Table 1.

Table 1. Individual athletes' age, national ranking, best individual performance, race performance, and competitive training load.

Athlete	Age	Ranking CBAT	Individual Best Race Time	Race Time	RPE	Competitive Load (a.u.)
Female 1	25	2	1:33:09	1:42:15	10	1020
Female 2	24	4	1:55:27	1:57:06	7	819
Female 3	19	5	1:56:12	1:56:12	7	812
Female 4	19	9	2:01:31	2:02:33	8	976
Female 5	38	15	2:10:40	2:10:40	6	780
Female 6	27	17	2:12:10	2:12:10	10	1320
Male 1	23	11	1:37:20	1:37:55	9	823
Male 2	20	7	1:34:15	1:46:14	7	742

CBAT = Brazilian Athletics Confederation; RPE = rate of perceived exertion. a.u = arbitrary units.

The individual values of markers of EIMD were demonstrated as levels of CK (Figure 1a), AST (Figure 1b), SJ (Figure 1c), CMJ (Figure 1d), and DOMS (figure 1e).

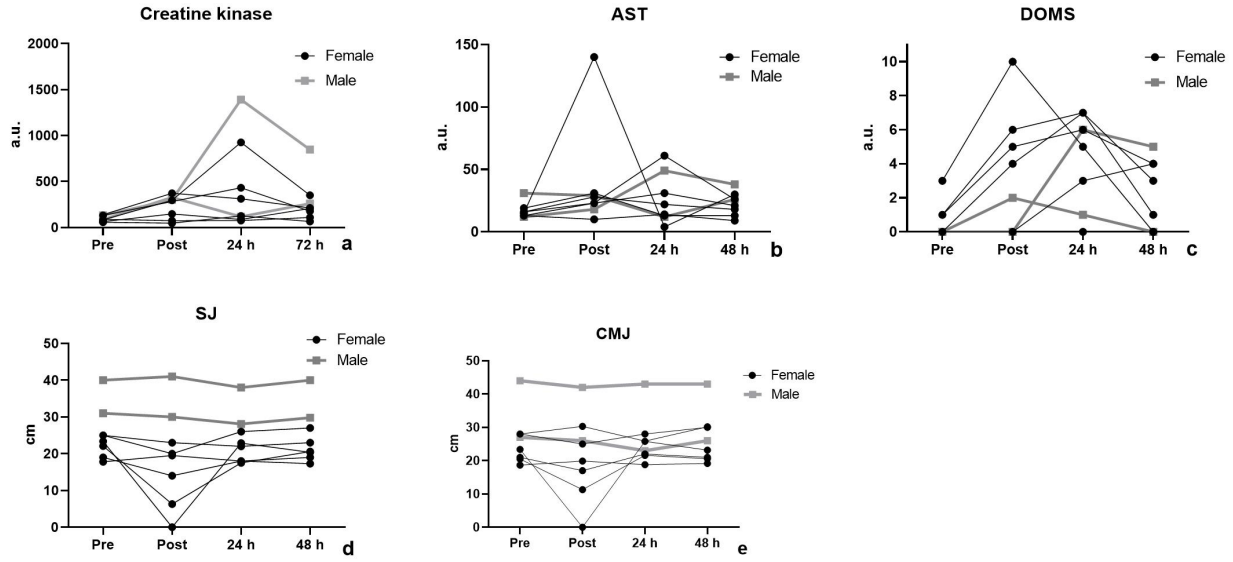

Figure 1. Individual values of markers of exercise-induced muscle damage before (Pre), immediately after (Post), and 24 and 48 h after the walking race competitions. a) Circulating levels of creatine kinase (CK). b) Circulating levels of glutamic oxalacetic transaminase (AST). c) Delayed-onset muscle soreness (DOMS). e) Squat jump performance (SJ). f) Countermovement jump performance (CMJ).

After 24 h, high levels of CK were observed in relation to the Pre values (Table 2). The effect size (EF) of CK increase in circulating levels was considered large to very large at Post, 24 h, and 48 h (Table 2). However, ΔCK were not significantly different (p = 0.20, F = 1.94) (Table 2).

The median AST values at Pre, Post, 24 h, and 48 h were not significantly different (p = 0.13; F = 5.58), but presented moderate EF for AST increase after all time points after the race (Table 2). The ΔAST values did not show statistical differences (p = 0.35; F = 2.38) (Table 2).

Median levels of DOMS were significantly high at 24 h in relation to Pre (Table 2). The EF for DOMS increase was considered large in relation to Pre values at all time points (Table 2). No significant differences in ΔDOMS (p = 0.19, F = 3.71) were detected (Table 2).

The mean values of the SJ at Pre, Post, 24 h, and 48 h were not significantly different (p = 0.11, F = 3.18, Table 2). The reduction in ΔSJ height was not significant (p = 0.11, F = 3.27), but presented a large EF at Post in relation to Pre and 48 h (Table 2). The mean values of CMJ (p = 0 .16, F = 3.40) and ΔCMJ (p = 0.13, F = 2.89) showed no significant differences, with a medium to trivial ES (Table 2). One athlete was very fatigated immediately after the race and tried to but could not jump onto the contact mat (Figure 1d and 1e). The Post value of this athlete was included in the analysis as zero.

The magnitude of muscle damage based on loss of function (jump height 24 to 48 h after the race) and CK levels (24 to 48 h) was considered mild in seven participants and moderate in one subject.

Table 2. Median or mean values and Cohen's effect size (95% confidence interval), and percentual variation (Δ), of creatine kinase (CK), aspartate aminotransferase (AST), delayed-onset muscle soreness (DOMS), squat jump (SJ), and countermovement jump (CMJ) at Pre and after walking race competitions.

	Pre	Post	24 h	48 h
CK (U/L)[1]	87 [64–132]	289.3 [92.5–321.3]	219 [94.7–802.0] *	209.5 [126–327.5]
ES Post	1.48 (0.31–2.49) VL	-	-	-
ES 24 h	0.99 (−0.10–1.96) L	0.58 (−0.45–1.54) M	-	-
ES 48 h	1.03 (−0.06–2.01) VL	0.24 (−0.75–1.21) S	−0.41 (−1.37–0.60) M	-
Δ CK (%Pre)[1]	-	129.6 [15.8–231.4]	120.3 [36.6–772.3]	129.2 [25.3–293.4]
AST (U/L)[1]	15.5 [13.0–18.2]	25.5 [19.2–30.5]	18.0 [12.2–44.5]	23.5 [14.4–29.0]
ES Post	0.70 (−0.34–1.67) M	-	-	-
ES 24 h	0.60 (−0.43–1.57) M	−0.37 (−1.33–0.64) S	-	-
ES 48 h	0.70 (−0.34–1.68) M	−0.50 (−1.46–0.52) S	−0.20 (−1.17–0.80) S	-
Δ AST (% Pre)[1]	-	56.5 [6.0–76.4]	22.6 [−53.8–254.7]	21.8 [27.1–100]
DOMS (A.U.)[1]	1.0 [0–3]	3.0 [0.0–5.7]	4.5 [1.5–6.7] *	2.0 [0–4.0]
ES Post	1.04 (−0.05–2.02) VL	-	-	-
ES 24 h	1.82 (0.57–2.86) VL	−0.31 (−0.69–1.28) S	-	-
ES 48 h (	0.80 (−0.17–1.88) L	−0.43 (−1.39–0.59) S	−0.93 (−1.90–0.15) L	-
ΔDOMS(%Pre)	-	100 [0 – 358]	400 [25 – 600]	150 [0 – 375]
SJ (cm)	25.4 ± 7.1	19.2 ± 12.9	23.8 ± 6.9	24.6 ± 7.4
ES Post	−0.60 (−1.56–0.44) M	-	-	-
ES 24 h	−0.23 (−1.20–0.77) S	0.44(−0.57–1.41) s	-	-
ES 48 h (	−0.11 (−1.08–0.88) T	0.51(−0.51–1.48) M	0.11(−0.87 – 1.09) T	-
Δ SJ (% Pre)	-	−30.5 ± 38.1	−5.7 ± 8.3	−3.2 ± 6.7
CMJ (cm)	26.3 ± 7.9	21.5 ± 12.4	26.0 ± 7.4	26.6 ± 7.8
ES Post	−0.46 (−1.43–0.56) s	-	-	-
ES 24 h	−0.04 (−1.02–0.94) T	0.44 (−0.57–1.41) S	-	-
ES 48 h	0.04 (−0.98–1.02) T	0.49 (−0.53–1.46) S	0.08 (−0.61–1.05) T	-
Δ CMJ (%Pre)	-	−25.2 ± 36.7	0.5 ± 7.9	0.5 ± 3.5

[1]Median [25 to 75% interquartile range]; *p < 0.05 in relation to Pre, Dunn's test. EF = effect size. VL = very large effect size; L = large effect size; M = medium effect size; S = small effect size; T = trivial effect size.

The individual values of inflammatory markers IL-6 (Figure 2a), TNFα (Figure 2b), IL-10 (Figure 2c), saliva flow rate (Figure 2d), salivary SIgA concentration (Figure 2e), salivary SIgA secretion rate (Figure 2f), testosterone (Figure 2g), cortisol (Figure 2h) and testosterone:cortisol ratio (Figure 2i) were demonstrated in Figure 2. Regarding inflammatory parameters, the mean values of IL-6 concentration ($p = 0.31$, $F = 1.20$) and ΔIL-6 ($p = 0.87$, $F = 0.45$) were not significantly different at any time point (Table 3). However, large ES were observed for differences in mean IL-6 concentration from Pre to Post, and 24 h (Table 3). No significant differences were identified in median TNFα concentrations ($p = 0.55$, $F = 0.57$) and ΔTNFα ($p = 0.47$, $F = 1.61$) at any time point (Table 3). A moderate ES was observed for a reduction in TNFα concentration at 24 h in relation to Pre and 48 h (Table 3). The median value of IL-10 concentration ($p = 0.07$, $F = 7.05$) and ΔIL-10 ($p = 0.07$, $F = 7.04$) were not significantly different (Table 3). The increase in Post IL-10 concentration presented a large EF in relation to Pre, 24-h, and 48-h values (Table 3).

The secretory salivary immunity did not present significant changes (Table 3). The mean value of salivary flow rate was higher ($p < 0.05$) at 24 h (1.2 ± 0.3 mL/min), compared to Pre (0.95 ± 0.3 mL/min) and Post (0.96 ± 0.2 mL/min), but with no difference in relation to 48 h (1.1 ± 0.37 mL/min). The median concentration of salivary SIgA ($p = 0,28$, $F = 3.75$) and ΔSIgA ($p = 0.23$, $F = 3.25$) showed no statistical differences (Table 3). The median SIgA secretion rate ($p = 0.41$, F= 2.85) and ΔSIgA secretion rate ($p = 0.23$, $F = 3.24$) did not show significant differences. Increased levels of SIgA concentration and secretion rate presented moderate effect sizes at Post and 24 h in comparison to Pre values (Table 3).

Endocrine markers present significant differences at Post moments (Table 4). Mean salivary testosterone levels were higher at Post compared to Pre and 24 h (Table 4). The Δtestosterone increased at Post compared to 24 h (Table 4). Very large effect sizes were observed for increased levels of testosterone Post in relation to Pre and 24 h (Table 4). Median cortisol values were also higher at Post compared to Pre and 48 h (Table 4). Very large effects were observed for the increase of cortisol at Post in relation to time points (Table 4). The mean testosterone: cortisol ratio and ΔT:C ratio did not show significant differences ($p = 0.56$, $F = 0.60$) at any moment (Table 4). Large effect sizes were observed for decreased T:C ratio at Post in comparison with Pre, 24 h, and 48 h (Table 4).

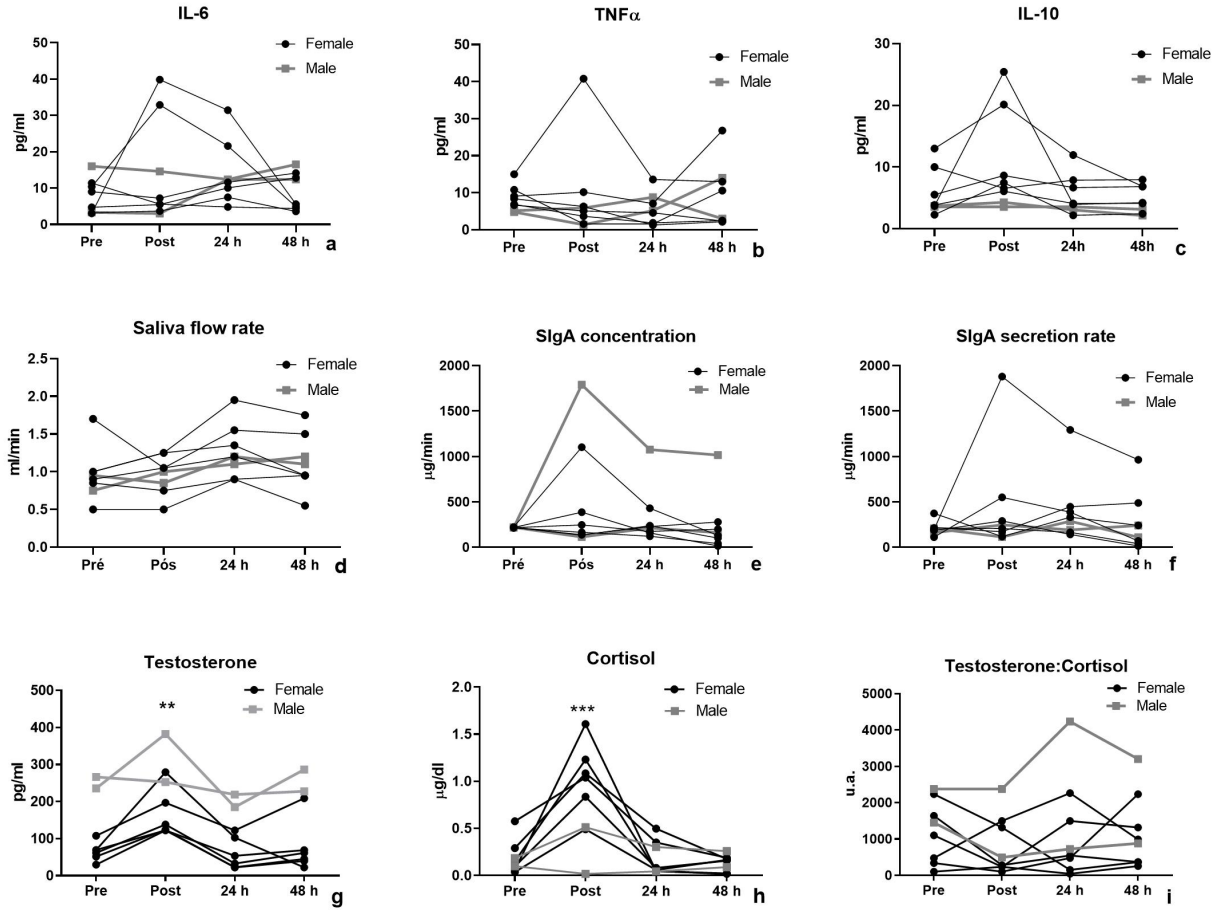

Figure 2. Individual plasma concentrations of serum cytokines, salivary immunity, and hormones before (Pre), immediately after (Post), and 24 and 48 h after a walking race competition**.** a) Interleukin-6 (IL-6). b) Tumor necrosis factor-alpha (TNF alpha). c) Interleukin-10 (IL_10). d) Saliva flow rate. e) Salivary SIgA concentration. f) Salivary SIgA secretion rate. g) Salivary testosterone. h) Salivary Cortisol. i) Testosterone:cortisol ratio.

Table 3. Median or mean values and Cohen's effect size (ES), and percentual variation (Δ), of Interleukin-6 (IL-6), Tumor Necrosis Factor-Alpha (TNF-α), Interleukin-10 (IL-10), concentration and secretion rate of SIgA.

	Pre	Post	24 h	48 h
IL-6 (pg/mL)	7.6 ± 4.7	14.0 ± 14.3	13.9 ± 8.5	9.2 ± 5.1
ES Post)	0.64 (−0.40–1.61) M	-	-	-
ES 24 h	0.92 (−0.16–1.89) L	−0.03 (−1.01–0.95) T	-	-
ES 48 h	0.33 (−0.68–1.29) S	−0.48 (−1.45–0.54) S	−0.67 (−1.64–0.37) M	-
Δ IL-6 (% Pre)	-	3.1 [−17.4 – 166.2]	110.9 [−9.86–237.8]	34 [−45.3–149.5]
TNFα (pg/mL)	7.6 [5.5–10.3]	5.5 [2.1–9.2]	4.9 [1.6–8.3]	6.8 [2.4–13.7]
ES Post	0.11 (−0.88–1.08) T	-	-	-
ES 24 h	−0.74 (−1.71–0.31) M	−0.39 (−1.36–0.62) S	-	-
ES 48 h	0.15 (−0.84–1.13 T	0.00 (−0.98–0.98) T	0.56 (−0.47–1.53) M	-
Δ TNFα (% Pre)	-	−24.4 [−64.7 – 12.2]	−26.6 [−81.5–3.9]	−27.8 [64–143.3]
IL-10 (pg/mL)	3.7 [3.6–8.8]	7.0 [4.7–17.2]	4.0 [3.1–7.5]	4.1 [2.6–6.8] #
ES Post	0.84 (−0.31–1.71) L	-	-	-
ES 24 h	0.09 (−1.06–0.90) T	−0.80 (−1.78–0.25) L	-	-
ES 48 h	−0.33 (−1.30–0.68) S	−0.95 (−1.93–0.13) L	−0.25 (−1.22–0.74) S	-
Δ IL-10 (% Pre)	-	55.6 [3.5 – 184.9]	−2.4 [−15.3 – 7.1]	−2.5 [−36.7–12.6]
SIgA (µg/mL)	218 [217–220]	206 [134–923]	221 [162–382]	146 [57–159]
ES Post	0.67 (−0.37–1.64) M	-	-	-
ES 24 h	0.50 (−0.52–1.47) M	−0.37 (−1.33–0.64) S	-	-
ES 48 h	0.11 (−0.88–1.08) T	−0.54 (−1.51–0.48) M	−0.27 (−1.24–0.73) S	-
Δ SIgA (% Pre)	-	−4.2 [−38.6 – 313.3]	0.9 [−25.3–71.4]	−34.2 [−73.3–19]
SIgA (µg/min)	202 [168–217]	227 [132–485]	309 [172–432]	176 [48–427]
ES Post	0.56 (−0.47–1.53) M	-	-	-
ES 24 h	0.73 (−0.32 – 1.70) M	−0.08 (−1.06 – 0.90) T	-	-
ES 48 h	0.27 (−0.73–1.24)S	−0.37 (−1.33 – 0.54) S	−0.38 (−1.35 – 0.62) S	-
Δ SIgA (%Pre)	-	24.4 [−39.5 – 308.1]	27.7 [−19.8 – 210.8]	−35.8 [−72.2 – 104.8]

# $p < 0.05$, compared to Post. VL = very large effect size; L = large effect size; M = medium effect size; S = small effect size; T = trivial effect size.

Table 4. Median or mean values and Cohen's effect size (ES), and percentual variation (Δ), of salivary testosterone, salivary cortisol, and testosterone:cortisol ratio.

	Pre	Post	24 h	48 h
Testosterone (pg/mL)	111.4 ± 89.2	202.3 ± 95.5*	95.0 ± 76.0##	120.1 ± 103.4
ES Post	1.02 (−0.07–2.00) VL	-	-	-
ES 24 h	−0.20 (−1.17–0.80) S	−1.29 (−2.29–2.06) VL	-	-
ES 48 h	0.09 (−0.90–1.07) T	−0.85 (−1.82–0.21) L	0.28 (−0.72–1.25) S	-
Δ Testosterone (%Pre)	-	136.5 ± 112.8	−16.1 ± 33.1#	7.5 ± 48.9
Cortisol (pg/dL)[1]	0.12 [0.06–0.26]	0.93 [0.49–1.19]*	0.07 [0.04–0.33]	0.12 [0.04–0.26]#
ES Post	1.83 (0.58–2.88) VL	-	-	-
ES 24 h	0.11 (−1.04–0.92) S	−1.85 (−2.91–−0.60) VL	-	-
ES 48 h	−0.38 (−1.34–1.63) M	−2.05 (−3.13–−0.75) VL	−0.30 (−1.27–0.70) S	-
Δ Cortisol (% Pre)	-	493 [105–1098]	−18.8 [−56.6–63.2] #	−24.7 [−65.5–56.7] #
T:C ratio (A.U.)	1215 ± 860.6	818.1 ± 822.7	1243 ± 1416	1202 ± 1036
ES Post	−0.80 (−1.67–0.34) L	-	-	-
ES 24 h	0.32 (0.68–1.29) S	1.18 (0.06–2.17) VL	-	-
ES 48 h	0.18 (−0.82–1.15) S	0.80 (−0.26–1.77) L	−0.07 (−1.04–0.92) T	-
Δ T:C (%Pre)[1]	-	−53.4 [−76.2–100.2]	−0.7 [−63.7–68.5]	27.3 [−67.9–143.2]

*$p < 0.05$ compared to Pre values. #$p < 0.05$, ##$p < 0.01$ compared to Post values. VL = very large effect size; L = large effect size; M = medium effect size; S = small effect size; T = trivial effect size.

DISCUSSION

The present study demonstrated that the 20 km walking race caused mild muscle damage, with a slight increase in CK at 24 h and DOMS at 48 h. Contrary to the study hypothesis, there was no significant loss of strength in the lower limbs or changes in inflammation markers and salivary secretory immunity. Despite the high load intensity reported at the end of the race, the endocrine modulation suggests that athletes presented a positive balance between stress and recovery after the race.

Increased CK values at 24 h occur in situations where physical exercise has caused muscle damage (7, 38). Although a significant increase in CK was observed at 24 h, CK values in the present study were lower than those observed in marathons and half marathon races (3, 10, 12, 42). Changes in CK levels in endurance sports are expected, with the highest level 24 h after the end of the exercise, and the magnitude of this increase seems to be proportional to muscle damage, being considered of low magnitude in the athletes of the present study (7, 38). Circulating AST levels are also associated with EIMD and marathon and half-marathon athletes presented increased levels of AST immediately after the race and for a period of 24 to 48 h (10, 12). Although the 20 km walking race is longer-lasting than running races, no significant changes in circulating AST levels were detected, suggesting a lesser degree of damage in this type of race modality.

The reduced ability to generate muscle strength and power is a key marker of muscle damage (38). The reduction in the capacity to generate strength and power in the lower limbs has been observed immediately after and for 24 h after marathon events (10, 22), although slight decreases are also seen in half-marathon recreational and amateur runners (11, 46). Although half-marathons cover distances close to walking races investigated in the present study, those studies may underestimate muscle damage in this distance of competition since investigated recreational athletes. In the present study, elite athletes were investigated during the most important national competitions, and higher demand and efforts were expected than in competitions held by amateurs and recreational athletes. Despite one athlete being so fatigated that could not jump after ending the race (Post), the mean vertical jump tests did not show any significant loss of function at any time after the race, suggesting that the level of muscle damage was very small (38). This is important since the improved capacity to store elastic energy and power generation in lower limb muscles during eccentric and concentric phases physical are requirements for muscle contraction in gait in a walking race (18, 20, 21). This suggests that well-trained high performance athletes could copy with 20-km walking races without been at high risk of muscle damage and underperformance.

Another sign of muscle damage is DOMS, characterized by mechanical hyperalgesia stimulated by neurotrophins, within 24 to 72 h after physical effort (36, 38). The magnitude of DOMS observed in the present study, associated with the findings of CK and jumping tests, suggests that mild muscle damage occurs in athletes after a 20 km walking race. DOMS symptoms in the present study were similar to those observed after half marathon races, in which DOMS symptoms were of small magnitude, occurring immediately after the race and 24 h after, and

returning to baseline values at 48 h (30, 47). Although we have investigated only two men, biochemical differences seems not to be relevant between both sexes. On the other hand, vertical jump is usually increased in men compared to women, but we decided to include both sexes in analysis since we adopted a repeated measures design in statistical analysis and there was no significant changes from pre values in pooled group. Despite literature highlights that estrogen could have some protective effects against muscle damage induced by eccentric contractions (24, 32), the magnitude of damage and reduced number of participants in the present study was small to evaluate any difference in men and women.

IL-6 is a cytokine produced by skeletal muscle during contraction, especially in long-term exercises (41). IL-6 has the function of mobilizing glucose and lipids to maintain energy homeostasis during aerobic efforts. Thus, increased levels of IL-6 were expected after the end of the 20 km walking race, since long-running races dramatically increase plasma levels of IL-6 after competition (3, 10, 12, 41, 42). The IL-6 Post levels in the present study are close to the values observed in half-marathon athletes, whose distance (21 km) and race time (108 minutes) were close to the present study (41). However, in the walking race, we observed different IL-6 responses after the race, with some athletes showing an increase, while others did not show significant changes. The values of TNFα, a cytokine that has an inflammatory action, did not show significant differences at any time after the race, suggesting that the 20 km race does not promote systemic inflammatory reactions. This result is similar to that observed in half-marathon events (41). IL-10 is an anti-inflammatory cytokine secreted in response to exercise, correlating with the individual's level of aerobic capacity (2). Considering the need for aerobic fitness for long-distance runs, high levels of IL-10 were observed after half marathon and marathon runs (10, 12, 42), and a very large effect was observed after the 20 km walking races evaluated in the present study. Differences in immunological parameters (cytokines and inflammatory cells) were not different between men and women after a fatiguing aerobic effort (28), so we included both sexes in the analysis. The results of the present study suggest that the walking race does not induce systemic inflammatory responses and is accompanied by the production of an anti-inflammatory mediator (IL-10). The absence of inflammatory mediators corroborates the results of muscle damage observed after the race, suggesting that there was little damage to the muscle fibers in 20 km walking race in professional athletes.

The effects of a 20 km walking race on testosterone and cortisol levels, and the testosterone:cortisol ratio are not described in the literature. However, the effects observed in the present study are similar to those seen in half marathons (27) and after high-intensity efforts in endurance athletes (33). The absence of significant changes in the testosterone: cortisol balance 24 and 48 hours after a walking race suggests that excessive muscle catabolism does not occur after the race. In sports, lower physical and technical performance was observed in athletes who had increased levels of cortisol and low testosterone concentrations before competition (26, 44). However, there was no association between cortisol and testosterone levels with the 20 km walking race performance.

The development of upper airway infections is associated with a reduction in salivary SIgA levels in response to very intense training periods or competitive events (8, 15, 29). However, a

study showed that endurance athletes submitted to a high-intensity effort present increased testosterone, cortisol, and SIgA after exercise (33). The results of the present study demonstrate that walking race athletes did not present significant changes in the mucosal immune response after the competition. It should be noted that none of the athletes had salivary SIgA levels below 60 µg/mL, considered a moderate risk, or 40 µg/mL, considered a high risk for the development of upper airway infections during the follow-up period (16).

The results of the study suggest that, despite the high internal load, the official 20 km walking race evoked mild EIMD, no circulatory inflammatory profile, and a negligible impact on immuno-endocrine modulation. Nonetheless, the recovery interventions that aim to reduce muscle damage may be useful during the recovery of elite athletes after the race. The secretory mucosal immunity also does not seem to be significantly affected by the physiological burden of the race. So, nutritional or immunostimulatory interventions to prevent airway infections in professional athletes who do not present low levels of SIgA immediately before races may be unnecessary.

This is the first study to describe the EIMD and immune-endocrine parameters of elite walking racers during official competitions. Despite similarities in distance and time between the half-marathon and the 20-km walking race, walking racers seem to display a lower level of EIMD and little impact of race in immune-endocrine markers than running races. In this way, coaches and athletes would benefit from a faster return to training routines and fewer risks of lesions than in other endurance long-distance sports. The results of this work have a direct impact on how and when to apply recovery strategies, such as cold-water immersions, immediately and thereafter races since athletes display a very low level of EIMD biochemical markers and recovered jump ability 24 h after the race. Once a walking race did not evoke significant inflammatory reactions and did not significantly impact the mucosal immunity of elite athletes, using dietary supplements and resources should be focused on improving physical performance rather than recovery.

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REFERENCES

1. Anderson T, Lane AR, Hackney AC. Cortisol and testosterone dynamics following exhaustive endurance exercise. Eur J Appl Physiol 116(8): 1503-1509, 2016.

2. Antunes BM, Campos EZ, Dos Santos RVT, Rosa-Neto JC, Franchini E, Bishop NC, Lira FS. Anti-inflammatory response to acute exercise is related with intensity and physical fitness. J Cell Biochem 120(4): 5333-5342, 2019.

3. Bernat-Adell MD, Collado-Boira EJ, Moles-Julio P, Panizo-Gonzalez N, Martinez-Navarro I, Hernando-Fuster B, Hernando-Domingo C. Recovery of inflammation, cardiac, and muscle damage biomarkers after running a marathon. J Strength Cond Res 1(35): 626-632, 2021.

4. Bernat-Adell MD, Collado-Boira EJ, Moles-Julio P, Panizo-Gonzalez N, Martinez-Navarro I, Hernando-Fuster B, Hernando-Domingo C. Recovery of inflammation, cardiac, and muscle damage biomarkers after running a marathon. J Strength Cond Res 35(3): 626-632, 2021.

5. Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M. Evidence for an exercise induced increase of TNF-alpha and IL-6 in marathon runners. Scand J Med Sci Sports 23(2): 207-214, 2013.

6. Bosco C, Luhtanen P, Komi PV. A simple method for measurement of mechanical power in jumping. Eur J Appl Physiol Occup Physiol 50(2): 273-282, 1983.

7. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull 81-82: 209-230, 2007.

8. Campos F, Molina Correa JC, Canevari VCM, Branco BHM, Andreato LV, de Paula Ramos S. Monitoring internal training load, stress-recovery responses, and immune-endocrine parameters in Brazilian jiu-jitsu training. J Strength Cond Res 36(3): 723-731, 2022.

9. Casto KV, Edwards DA. Testosterone, cortisol, and human competition. Horm Behav 82: 21-37, 2016.

10. Clifford T, Allerton DM, Brown MA, Harper L, Horsburgh S, Keane KM, Stevenson EJ, Howatson G. Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. Appl Physiol Nutr Metab 42(3): 263-270, 2017.

11. Del Coso J, Salinero JJ, Lara B, Abian-Vicen J, Gallo-Salazar C, Areces F. A comparison of the physiological demands imposed by competing in a half-marathon vs. a marathon. J Sports Med Phys Fitness 57(11): 1399-1406, 2017.

12. Faria FR, Gomes AC, Antunes A, Rezende KR, Pimentel GD, Oliveira CLP, Antunes BM, Lira FS, Aoki MS, Mota JF. Effects of turmeric extract supplementation on inflammation and muscle damage after a half-marathon race: A randomized, double-blind, placebo-controlled trial. Eur J Appl Physiol 120(7): 1531-1540, 2020.

13. Federations IAoA. IAAF competition rules. 2015. Available at: https://worldathletics.org/about-iaaf/documents/book-of-rules.

14. Foster C, Florhaug JA, Franklin J, Gottschall L, Hrovatin LA, Parker S, Doleshal P, Dodge C. A new approach to monitoring exercise training. J Strength Cond Res 15(1): 109-115, 2001.

15. Freitas CG, Aoki MS, Arruda AF, Franciscon C, Moreira A. Monitoring salivary immunoglobulin A responses to official and simulated matches in elite young soccer players. J Hum Kinet 53: 107-115, 2016.

16. Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA, Clancy RL. Salivary IgA levels and infection risk in elite swimmers. Med Sci Sports Exerc 31(1): 67-73, 1999.

17. Gomez-Ezeiza J, Santos-Concejero J, Torres-Unda J, Hanley B, Tam N. Muscle activation patterns correlate with race walking economy in elite race walkers: A waveform analysis. Int J Sports Physiol Perform: 1250-1255, 2019.

18. Gomez-Ezeiza J, Torres-Unda J, Tam N, Irazusta J, Granados C, Santos-Concejero J. Race walking gait and its influence on race walking economy in world-class race walkers. J Sports Sci 36(19): 2235-2241, 2018.

19. Hanley B. An analysis of pacing profiles of world-class racewalkers. Int J Sports Physiol Perform 8(4): 435-441, 2013.

20. Hanley B, Bissas A. Analysis of lower limb internal kinetics and electromyography in elite race walking. J Sports Sci 31(11): 1222-1232, 2013.

21. Hanley B, Bissas A. Ground reaction forces of Olympic and world championship race walkers. Eur J Sport Sci 16(1): 50-56, 2016.

22. Howatson G, McHugh MP, Hill JA, Brouner J, Jewell AP, van Someren KA, Shave RE, Howatson SA. Influence of tart cherry juice on indices of recovery following marathon running. Scand J Med Sci Sports 20(6): 843-852, 2010.

23. Inami T, Nakagawa K, Yonezu T, Fukano M, Higashihara A, Iizuka S, Abe T, Narita T. Tracking of time-dependent changes in muscle hardness after a full marathon. J Strength Cond Res 33(12): 3431-3437, 2019.

24. Kendall B, Eston R. Exercise-induced muscle damage and the potential protective role of estrogen. Sports Med 32(2): 103-123, 2002.

25. Komi PV, Bosco C. Utilization of stored elastic energy in leg extensor muscles by men and women. Med Sci Sports 10(4): 261-265, 1978.

26. Lautenbach F, Laborde S, Achtzehn S, Raab M. Preliminary evidence of salivary cortisol predicting performance in a controlled setting. Psychoneuroendocrinology 42: 218-224, 2014.

27. Levers K, Dalton R, Galvan E, O'Connor A, Goodenough C, Simbo S, Mertens-Talcott SU, Rasmussen C, Greenwood M, Riechman S, Crouse S, Kreider RB. Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals. J Int Soc Sports Nutr 13: 22, 2016.

28. Lobo LF, de Morais MG, Marcucci-Barbosa LS, Martins-Junior FAD, Avelar LM, Vieira ELM, Aidar FJ, Wanner SP, Silva LS, Noman MC, Camargos BM, Freitas KM, Goncalves WA, Pinho V, Nunes-Silva A. A single bout of fatiguing aerobic exercise induces similar pronounced immunological responses in both sexes. Front Physiol 13: 833580, 2022.

29. Lopes RF, Minuzzi LG, Figueiredo AJ, Goncalves C, Tessitore A, Capranica L, Teixeira AM, Rama L. Upper respiratory symptoms (URS) and salivary responses across a season in youth soccer players: A useful and non-invasive approach associated to URS susceptibility and occurrence in young athletes. PLoS One 15(8): e0236669, 2020.

30. Lynn A, Garner S, Nelson N, Simper TN, Hall AC, Ranchordas MK. Effect of bilberry juice on indices of muscle damage and inflammation in runners completing a half-marathon: A randomised, placebo-controlled trial. J Int Soc Sports Nutr 15: 22, 2018.

31. McKay AKA, Peeling P, Pyne DB, Welvaert M, Tee N, Leckey JJ, Sharma AP, Ross MLR, Garvican-Lewis LA, van Swelm RPL, Laarakkers CM, Burke LM. Acute carbohydrate ingestion does not influence the post-exercise iron-regulatory response in elite keto-adapted race walkers. J Sci Med Sport 22(6): 635-640, 2019.

32. Minahan C, Joyce S, Bulmer AC, Cronin N, Sabapathy S. The influence of estradiol on muscle damage and leg strength after intense eccentric exercise. Eur J Appl Physiol 115(7): 1493-1500, 2015.

33. Monje C, Rada I, Castro-Sepulveda M, Penailillo L, Deldicque L, Zbinden-Foncea H. Effects of a high intensity interval session on mucosal immune function and salivary hormones in male and female endurance athletes. J Sports Sci Med 19(2): 436-443, 2020.

34. Navalta JW, Stone WJ, Lyons TS. Ethical issues relating to scientific discovery in exercise science. Int J Exerc Sci 12(1): 1-8, 2019.

35. Nieman DC, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis JM, Nehlsen-Cannarella SL. Change in salivary IgA following a competitive marathon race. Int J Sports Med 23(1): 69-75, 2002.

36. Owens DJ, Twist C, Cobley JN, Howatson G, Close GL. Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? Eur J Sport Sci 19(1): 71-85, 2019.

37. Pacque PF, Booth CK, Ball MJ, Dwyer DB. The effect of an ultra-endurance running race on mucosal and humoral immune function. J Sports Med Phys Fitness 47(4): 496-501, 2007.

38. Paulsen G, Mikkelsen UR, Raastad T, Peake JM. Leucocytes, cytokines and satellite cells: What role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev 18: 42-97, 2012.

39. Pavei G, Cazzola D, La Torre A, Minetti AE. The biomechanics of race walking: Literature overview and new insights. Eur J Sport Sci 14(7): 661-670, 2014.

40. Pyne DB, Gleeson M. Effects of intensive exercise training on immunity in athletes. Int J Sports Med 19(Suppl 3): S183-191, Discussion S191-184, 1998.

41. Reihmane D, Jurka A, Tretjakovs P, Dela F. Increase in IL-6, TNF-alpha, and MMP-9, but not sICAM-1, concentrations depends on exercise duration. Eur J Appl Physiol 113(4): 851-858, 2013.

42. Santos VC, Sierra AP, Oliveira R, Cacula KG, Momesso CM, Sato FT, Silva MB, Oliveira HH, Passos ME, de Souza DR, Gondim OS, Benetti M, Levada-Pires AC, Ghorayeb N, Kiss MA, Gorjao R, Pithon-Curi TC, Cury-Boaventura MF. Marathon race affects neutrophil surface molecules: Role of inflammatory mediators. PLoS One 11(12): e0166687, 2016.

43. Shanely RA, Nieman DC, Zwetsloot KA, Knab AM, Imagita H, Luo B, Davis B, Zubeldia JM. Evaluation of Rhodiola rosea supplementation on skeletal muscle damage and inflammation in runners following a competitive marathon. Brain Behav Immun 39: 204-210, 2014.

44. Siart B, Nimmerichter A, Vidotto C, Wallner B. Status, stress and performance in track and field athletes during the European games in Baku (Azerbaijan). Sci Rep 7(1): 6076, 2017.

45. Walsh NP, Oliver SJ. Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress. Immunol Cell Biol 94(2): 132-139, 2016.

46. Wiewelhove T, Schneider C, Doweling A, Hanakam F, Rasche C, Meyer T, Kellmann M, Pfeiffer M, Ferrauti A. Effects of different recovery strategies following a half-marathon on fatigue markers in recreational runners. PLoS One 13(11): e0207313, 2018.

47. Withee ED, Tippens KM, Dehen R, Tibbitts D, Hanes D, Zwickey H. Effects of methylsulfonylmethane (MSM) on exercise-induced oxidative stress, muscle damage, and pain following a half-marathon: A double-blind, randomized, placebo-controlled trial. J Int Soc Sports Nutr 14: 24, 2017.