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ORIGINAL ARTICLE

Oxidative stress, inflammation, and muscle soreness in an 894-km relay trail run

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Abstract We describe the effects of multi-day relay trail running on muscle soreness and damage, and systemic immune, inflammatory, and oxidative responses. 16 male and 4 female athletes ran 894 km in 47 stages over 95 h, with mean (SD) 6.4 (1.0) stages per athlete and 19.0 (1.7) km per stage. We observed post–pre run increases in serum creatine kinase (qualified effect size extremely large, $p = 0.002$), IL-6 (extremely large, $p < 0.001$), urinary 8–isoprostane/creatinine (extremely large, $p = 0.04$), TNF-α (large, $p = 0.002$), leukocyte count (very large, $p < 0.0001$) and neutrophil fraction (very large, $p < 0.001$); and reductions in hemoglobin (moderate, $p < 0.001$), hematocrit (moderate, $p < 0.001$), and lymphocyte fraction (trivial, $p < 0.001$). An increase in ORAC total antioxidant capacity (TAC, small, $p = 0.3$) and decrease in urinary 8-OHdG/creatinine (small, $p = 0.1$) were not statistically significant. During the run, muscle soreness was most frequent in the quadriceps. The threshold for muscle pain (pain-pressure algometry) in the *vastus lateralis* and *gastrocnemius* was lower post-run (small, $p = 0.04$ and 0.03). Average running speed was correlated with algometer pain and leukocyte count (large, $r = 0.52$), and TAC was correlated with IL-6 (very large, $r = 0.76$) and 8-isoprostane/creatinine (very large, $r = -0.72$). Multi-day stage-racing increases inflammation, lipid peroxidation, muscle damage and soreness without oxidative DNA damage. High TAC is associated with reduced exercise-induced lipid peroxidation, but is not related to immune response or muscle damage.

Keywords TNF-α · IL-6 · ORAC · 8-isoprostane · 8-OHdG · DOMS

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Introduction

Ultra-endurance relay-racing is increasing in popularity world-wide, with athletes of all levels competing in 24-h relay events and long-distance stage races. Competing in such races imposes special demands on human physiology. In races lasting multiple days a portion of the race must be performed while the body is affected by physiological perturbations usually only observed during recovery, such as tissue damage as indicated by delayed onset muscle soreness (DOMS, Smith 1992) and elevations in serum concentrations of inflammatory markers (Suzuki et al. 2006) and oxidative damage (Radák et al. 2000). The physiological effects of performing strenuous exercise in such a state are not well described.

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DOMS is an exercise-induced muscle tenderness which usually peaks between 24 and 48 h after exercise with an eccentric component such as running (Smith 1992). It is associated with disruption to skeletal muscle ultrastructure (Newham et al. 1983) and reductions in muscle strength (Cheung et al. 2003; Smith 1992). Despite the likelihood that performing exercise with tender, weakened muscles will impair performance, there are no published descriptions of the frequency, severity or location of DOMS throughout the course of a multi-day ultra-endurance race.

The occurrence of DOMS after prolonged exercise is associated with increases in serum concentrations of cytokines such as interlukin-6 (IL-6, Neubauer et al. 2010; Suzuki et al. 2006). Several studies have also reported increased serum concentration of tumor necrosis factor α (TNF-α) after less prolonged exercise (Suzuki et al. 2006; Wallberg et al. 2010) but not after ultra-endurance exercise (Kim et al. 2007). It has been postulated that the elevation of serum TNF-α depends primarily on the intensity of endurance exercise, compared to IL-6 where exercise duration plays a relatively more important role (Packer 1997), implying that during ultra-endurance exercise the intensity achieved is not sufficient to elevate serum TNF-α.

Exercise increases the rate of myocellular metabolism, increasing the rate of production of reactive oxygen species (ROS) in muscle mitochondria (Powers et al. 1999). During moderate intensity exercise there is a concomitant increase in myocellular antioxidant capacity enabling ROS to be reduced without increased oxidative stress (Knez et al. 2006; Mastaloudis et al. 2001). However, there is evidence that during severe unaccustomed exercise such as ultra-endurance running the increase in ROS production may overwhelm myocellular ability to increase antioxidant capacity (Dohi et al. 2007; Schwedhelm et al. 2004; Wu et al. 2004) causing oxidative damage to both lipids and DNA. As such, and in contrast to performing regular habitual exercise, frequently participating in extreme ultra-endurance events may cause a slight increase in risk of developing non-communicable diseases such as cancer and cardiovascular disease (Knez et al. 2006).

In the current study, we took advantage of an opportunity to observe for the first time the physiological and perceptual responses of athletes competing in a 4-day, 894-km relay running race. We describe changes in perceived muscle soreness during the race and a variety of biochemical outcomes following the race in twenty athletes. In addition to those parameters modified by continuous ultra-endurance exercise, after the high-intensity ultra-endurance relay exercise performed in the current study we also observed elevations in serum TNF-α, a progressive increase in muscle soreness with subsequent run stages and a significant relationship between serum antioxidant capacity and exercise-induced lipid peroxidation.

Methods

Subjects

A total of 20 athletes (16 men and 4 women) volunteered to provide blood and urine samples before and after a multi-day stage race completed in June, 2009 (http://www.adventurescience.ca/blaze/). The physical characteristics of the subjects are presented in Table 1. All of the athletes had many years of experience in trail running, orienteering, adventure racing, and nordic skiing with racing experience at national or international level. One female athlete was unable to continue after experiencing a 2nd degree ankle sprain on the third leg of the race and her data were subsequently excluded from analysis, leaving a total of 19 athletes for subsequent analysis. Each of the subjects provided written informed consent form prior to testing. The study was approved by the Hamilton Health Sciences Research Ethics Board and complied with the declaration of Helsinki.

Design

The study comprised: subject physical and physiological characterization, pre-race physiological collection (from urine and blood) and psychometric-based muscle soreness measures, followed by the race and post-race collection and psychometric measures. During the race, additional muscle soreness measures were taken before and following each run stage.

Pre-race measurements. Each of the subjects completed their habitual work-out schedule in the week prior to the initial testing session (last exercise bout ~24 h before testing) and did not exercise on the day prior to testing. Subjects arrived in the laboratory ~2 h after their last meal and completed a dual x-ray absorptiometry scan (Lunar Prodigy Advance, Madison, WI, USA) for two-compartment body composition analysis. The runners then had a blood sample taken from the antecubital vein into EDTA and heparin treated evacuated tubes that were placed on ice and analyzed within 2 h by the core laboratory at McMaster University Medical Center for complete blood count, and creatine kinase activity (CK). Plasma from the heparin tubes was aliquoted and frozen at −86°C for subsequent analysis of IL-6 and TNF-α (see below). The anti-oxidant capacity of the plasma was determined using the oxygen radical absorbance capacity (ORAC) assay (Cao et al. 1993). To determine the rates of lipid peroxidation and DNA oxidation, a urine sample was obtained and was analyzed by the McMaster University Medical Center core laboratory for 8-isoprostanes and 8-OH-2-deoxyguanosine (8-OHdG, see below) respectively.

Table 1 Subject Characteristics

	Mean (SD)	Minimum	Maximum
Age (year)	37.0 (6.7)	27	46
Height (cm)	177.2 (7.8)	159	186
Weight (kg)	72.2 (9.1)	52	86
VO_2 maximum (mL kg^{-1} min^{-1})	60.6 (4.9)	53.8	68.5
Lean body mass (kg)	59.7 (9.5)	41.9	74.2
Body fat (%)	14.5 (5.3)	6.6	27.8

Lower limb soreness was assessed before and after the run (within 30 min of the blood draw) using an algometer (Force Dial FDK 60, Wagner Instruments; Greenwich, CT) to apply pressure and measure changes in muscle pain sensitivity (Ali et al. 2007). Three anatomical landmarks were selected to quantify muscle soreness: *vastus lateralis* muscle 20 cm above distal end of the lateral aspect of the femur, *vastus medialis* muscle 10 cm above distal end of the medial aspect of the femur, and centre of the medial *gastrocnemius* muscle belly. Up to 20 kg cm^{-2} (200 N cm^{-2}) of pressure was applied to each site using the algometer with a metal probe covered by a rubber tip. Subjects were asked to verbally indicate when the force became *uncomfortable* and this value was recorded. If no indication of discomfort was given, soreness at that site was considered not present (Ali et al. 2007). Each site was pressure tested for muscle soreness twice and the mean taken. If measurements were different by greater than 1 kg cm^{-2} a third measurement was completed and the median taken.

Following this testing, the subjects completed a symptom limited maximal aerobic capacity test (VO_{2peak}) test on a treadmill (Life Fitness 95Ti, Schiller Park, IL, USA) using an online gas collection system (Moxus modular oxygen uptake system, AIE technologies, Pittsburgh, PA, USA). Following a 5 min warm up at a self-selected pace, subjects ran at an indicated speed of 8.0 mph (males) or 7.5 mph (females). The incline was raised by 2% every 1 min until volitional fatigue. Mean VO_{2peak}, was based on the highest value averaged over 30 s for each subject. The athletes started the stage race within 2 weeks of testing (see below).

Trail run. The athletes completed a continuous relay race consisting of 45 legs ranging from 9.0 to 24.5 km in length. Our study followed two teams of 10 athletes each (8 men and 2 women). Athletes ran the legs individually during daylight hours or in pairs if the leg started within 30 min before official sundown or <30 min before official sunrise. The average temperature was 17.4°C (range, 12–24) with a mean relative humidity of 78.3% (41–99). The athletes followed the Bruce Trail (894 km) and had to follow the official white blazes (20–200 m apart). The trail was predominantly single-track trail with short sections along gravel roads. Athletes were allowed to eat and drink ad libitum during the course of the race. Athletes provided a ready supply of carbohydrate–electrolyte sports drink (PowerBar Endurance Formula, Nestle, Vevay, Switzerland) and high-carbohydrate sports bars (PowerBar, Nestle, Vevay, Switzerland) to consume while running, and a formulated high protein-carbohydrate recovery drink for ingestion following each run (PowerBar Performance Recovery Drink, Nestle, Vevay, Switzerland) as well as nutritional advice to help maintain optimal hydration and carbohydrate stores. Athletes were asked not to consume any other nutritional supplements during the race. Due to the relay nature of the race and staggered finish times for each individual, the final blood and urine samples were taken between 30 min and 16 h after the final leg at the same time of day (1,000–1,130 h). Finally, within 15 min prior to and within 60 min following each leg, the athletes were instructed to make a pen mark on a continuous scale rating their perception of soreness on identified regional body parts. Location and rating of perception of muscle soreness was quantified using scales modeled from Borg's CR10 (Borg 2001). Verbal descriptors were associated with the scale: 0, no ache/soreness; 0.5, just noticeable; 1, very weak; 2, weak; 3, moderate; 5, strong; 7, very strong; 10, extremely strong; and 13.5, absolute maximum/unbearable.

Analyses

Commercially available high sensitivity ELISA kits (Cat. No. HS600B, sensitivity 0.039 pg·ml^{-1}, and HSTA00D, sensitivity 0.106 pg·ml^{-1}, R&D Systems, Minneapolis, MN, USA), were used to measure plasma levels of IL-6 and TNF-α, according to the manufacturer's instructions. In our laboratory the intra- and inter-assay CVs for these assays are $\leq$5 and $\leq$12%, respectively (Timmons et al. 2006). The urine was analyzed for 8-isoprostanes, and 8-OHdG using commercially available ELISA kits (Kit #516351, sensitivity 10 pg ml^{-1} and #589320, sensitivity 100 pg ml^{-1}, Cayman Chemicals, Ann Arbor, MI, respectively). Total antioxidant capacity (TAC) was measured using TAC-Peroxyl Assay (TAC01 from Northwest Life Science Specialties, Vancouver, WA, intra-assay CV 3.2%) following manufacturer's instructions. RLU readings were taken using GloMax 20/20 Luminometer (Promega, Sunnyvale, CA). Final TAC (in seconds) was calculated by constructing graphs for the induction time of samples against the induction time for different concentrations of Trolox standards. Each runner's average speed was calculated according to their total distance run divided by their total time.

Statistics. Blood, urine and algometer variables were analysed for pre vs. post differences using two-tailed Student's paired *T* test. The magnitude of the post–pre change was qualified using a modified Cohen effect size scale: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, extremely large >4.0 (Hopkins et al. 2009). Potential gender effects were investigated by repeating the pre versus post analysis with female subjects excluded. Pearson correlation analysis was performed on average running speed; post-race TAC; and pre-post changes in neutrophil and lymphocyte counts; serum creatine kinase activity, IL-6 concentration and TNF-α concentration; urinary 8-isoprostane and 8-OHdG content; and *vastus lateralis* and *vastus medialis* algometer pain scores. The strength of correlation was qualified using modified Cohen scale: 0.1, 0.3, 0.5, 0.7, and 0.9 for small, moderate, large, very large, and extremely large for the correlation coefficients (Hopkins et al. 2009). Correlations identified as large (i.e. $r > 0.5$) were plotted for visual identification of outliers. Identified outliers were removed, the analysis re-run, and any correlations with $r < 0.4$ were discarded. All data were plotted and visually inspected to ensure no obvious violations of the normality assumption. All analysis was performed using Excel V.14.0.0 (Microsoft Corporation, Seattle, WA, USA). Inference was via effect size and reference to the null, where a $p < 0.05$ was considered significant.

Results

Race characteristics

The previous record for the race was halved with the winning time of 3 days and 23 h and 10 min. The two teams finished within 15 min of each other with the lead changing 3 times. Each runner ran between 4 and 8 legs for a total distance of between 77.6 and 158.5 km (mean 119.6 km) with a mean distance covered per leg of 19.0 km (range 7 to 26.6 km). Athletes ran at an average pace of between 5:15 and 8:37 min km^{-1} [average speed 9.2 (1.1) km h^{-1} mean (standard deviation)]. As data collection was performed in the field continuously for over 95 h there were occasional technical difficulties preventing some samples from being collected as planned. The number of complete data sets for each variable is noted in the results.

Blood and Urine Markers of Health Status, Immune and Inflammatory, and Oxidative Stress

Blood and urine samples were successfully obtained from 15 athletes (12 male and 3 female) and the results are presented in Tables 2 and 3. There were extremely large increases in serum creatine kinase and IL-6 concentrations and a large increase in serum TNF-α concentration but only a small, non-significant decrease in serum sodium concentration. The decrease in blood hemoglobin and hematocrit was moderate but the small increase in mean cell hemoglobin concentration (MCHC) was not significant. There was a very large increase in the total number of leukocytes, primarily due to a very large increase in the number of neutrophils. There was a large increase in urinary creatinine and consequently all other urinary excretion measurements were expressed relative to creatinine [units of pg (μmol creatinine)$^{-1}$]. The increase in urinary 8-isoprostane content was extremely large, but the decrease in urinary 8-OHdG was small and not significant. The small increase in total antioxidant capacity as determined by ORAC assay was also not significant.

Muscle soreness

Pressure algometer. Algometer pain scores were successfully obtained from 17 athletes (14 male and 3 female). Pain scores for the *vastus lateralis* and *gastrocnemius* were significantly lower following the race (indicating increased muscle soreness); and the values for the *vastus medialis* also trended lower following the race (Table 3).

Site-specific limb soreness. Site-specific limb soreness measurements were obtained for 16 athletes (13 male and 3 female). The location and relative frequency of limb aches or soreness before and after each run leg is illustrated in Fig. 1 and the magnitude of change in soreness for four main sites is provided in Table 4. The frequency of limb soreness was most prevalent in the legs, particularly in the quadriceps. The frequency of quadriceps soreness increases with run number as did the severity of reported soreness in all leg muscle areas (Table 4).

Gender effects

There were no changes in the qualified effect size for any comparison when the analysis was repeated with female subjects excluded. The increase in *vastus lateralis* and *gastrocnemius* sensitivity to pain stimulus after the race no longer reached the 5% significance threshold once female subjects were excluded, although the *p* values remained of similar magnitude (0.78 and 0.12, respectively).

Correlation analysis

Complete data for all variables included in the correlation analysis was available for 15 subjects. Data from other subjects were excluded. Changes in each of *vastus lateralis* ($r = -0.81$, very large, $p = 0.0025$) and *vastus medialis*

Table 2 Effect of ultra-endurance trail running on markers of immune function, inflammation, and muscle damage

Parameter	Pre run	Post run	Outcome			
	Mean (SD)	Mean (SD)	Effect size	Effect size 95% CL±	Qualified effect[a]	*p*
Hemoglobin (g L^{-1})	149.1 (9.3)	139.6 (10.5)	−1.02	0.47	Moderate	0.0003
Mean cell hemoglobin concentration (g L^{-1})	345 (4)	347 (5)	0.50	0.61	Small	0.10
Hematocrit	0.43 (0.03)	0.40 (0.03)	−1.00	0.13	Moderate	0.0002
Leukocytes ($\times 10^9$ L^{-1})	5.7 (1.5)	9.0 (2.3)	2.20	0.095	Very large	$<10^{-5}$
Lymphocytes ($\times 10^9$ L^{-1})	1.93 (0.56)	1.89 (0.56)	−0.07	0.58	Unclear	0.80
Lymphocytes (fractional %)	0.34 (0.09)	0.21 (0.07)	−0.14	0.067	Trivial	0.0004
Neutrophils ($\times 10^9$ L^{-1})	3.26 (1.13)	6.37 (2.09)	2.75	1.1	Very large	$<10^{-5}$
Neutrophils (fractional %)	0.57 (0.10)	0.70 (0.07)	1.30	0.65	Large	0.0006
Creatine kinase (iU)	214 (134)	2,339 (2281)	15.86	9.1	Extremely large	0.002
Sodium (mmol L^{-1})	139.9 (1.5)	139.2 (1.7)	−0.47	0.78	Small	0.22
Interleukin-6 (pg L^{-1})	0.78 (0.75)	4.1 (2.8)	4.37	1.9	Extremely large	0.0002
TNF- α (pg L^{-1})	0.48 (0.14)	0.70 (0.22)	1.57	0.9	Large	0.002
Serum ORAC total antioxidant capacity[b]	570 (162)	612 (188)	0.26	0.51	Small	0.30

[a] Qualifiers of effect size: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, extremely large >4.0

[b] Total antioxidant capacity as determined by the oxygen radical absorbance capacity assay and quantified against Trolox standards

Table 3 Effect of ultra-endurance trail running on markers of oxidative stress, muscle damage, and muscle soreness

Parameter	Pre run	Post run	Outcome			
	Mean (SD)	Mean (SD)	Effect size	Effect size 95% CL ±	Qualified effect[a]	*p*
Urine						
Creatinine (mmol·L^{-1})	6.9 (6.2)	16.8 (6.1)	1.60	0.62	Large	$<10^{-5}$
8-isoprostanes [pg (μmol creatinine)$^{-1}$]	84.9 (28.6)	112.6 (52.7)	−6.91	6.6	Extremely large	0.040
8-OHdG [pg (μmol creatinine)$^{-1}$]	11,166 (5613)	9,045 (4813)	−0.38	0.39	Small	0.055
Pain scores						
Vastus lateralis (%)[b]	62.0 (19.6)	51.7 (18.6)	−0.53	0.48	Small	0.033
Vastus Medalis (%)[b]	58.7 (18.8)	52.1 (17.6)	−0.35	0.39	Small	0.075
Gastrocnemius (%)[b]	72.4 (23.6)	62.0 (29.3)	−0.44	0.41	Small	0.039

[a] Qualifiers of effect size: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, extremely large >4.0

[b] Pain scores are expressed as percentage of maximum algometer force required to feel uncomfortable

($r = -0.66$, large, $p = 0.0074$) algometer pain scores were significantly correlated with the change in lymphocyte count, while change in *vastus medialis* pain score was significantly correlated with running speed ($r = 0.52$, large, $p = 0.047$) and change in neutrophil count ($r = 0.52$, large, $p = 0.047$). Running speed was also significantly correlated with changes in lymphocyte ($r = -0.68$, large, $p = 0.005$) and neutrophil ($r = 0.72$, very large, $p = 0.002$) counts and urinary 8-isoprostane ($r = 0.59$, large, $p = 0.02$). Post-race TAC showed very large correlations with change in serum IL–6 concentration ($r = 0.76$, very large, $p = 0.001$) and urine 8-isoprostane ($r = -0.72$, very large, $p = 0.002$). However, exclusion of visually identified outliers revealed that the observed correlations between running speed and neutrophil count, vastus medialis pain and urinary 8-isoprostanes, as well as the correlation between neutrophil count and vastus medalis pain, were excessively dependent on the values from a single subject (Fig. 2) so these results were excluded from further analysis. There were no significant correlations involving creatine kinase activity, TNF-α concentration, or urine 8-OHdG. Scatter plots illustrating each

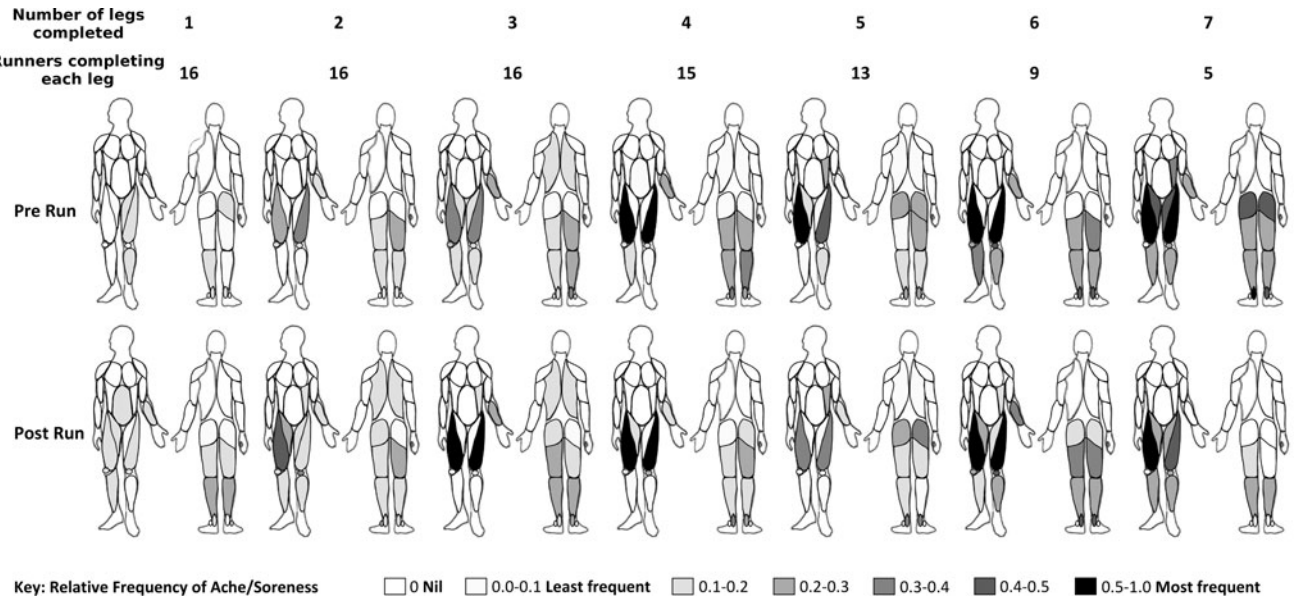

Fig. 1 Relative frequency of site-specific limb soreness over the course of the run. Data are the frequency of a soreness rating >1.0 (very weak) reported by athletes after they had completed each leg. "Number of legs completed" indicates the number of legs each runner had completed before giving the pain rating presented below. "Runners completing each leg" indicates how many runners completed at least the corresponding number of legs

Table 4 Limb soreness rating for the top four selected sights most affected during the multi-stage trail run

Location[a]	*n*	Quadriceps		Hamstrings		Calves		Achilles	
Measure and run number		Count[b]	Median (range)[c]	Count[b]	Median (range)[c]	Count[b]	Median (range)[c]	Count[b]	Median (range)[c]
Pre									
1	16	0	0	1	2	1	1.5	2	1.5 (1–2)
2	16	4	2.5 (1–7)	4	1.5 (1–3)	3	2 (2–3)	3	2 (1–2.5)
3	16	6	2.3 (1–6)	3	1 (1–3.5)	4	1.3 (1–3)	5	2 (1–7)
4	15	8	2 (1–6)	3	3 (2–3)	5	2 (1–3)	4	1.8 (1–2)
5	13	7	3 (1–8)	3	2 (2–3)	2	1.8 (1.5–2)	3	3 (1–4)
6	9	6	3 (1–9)	3	3 (2–3)	2	4 (3–5)	4	3 (1–6)
7	5	4	7 (1–10)	1	5	1	8	2	1.8 (1.5–2)
8	1	1	7	0					
Post									
1	16	3	3.3 (2–6)	2	6.5 (6–7)	4	2.3 (1.5–5)	1	3
2	16	7	2.5 (1–7)	3	3 (2–4)	3	2 (1–4)	3	3 (1–5)
3	16	8	3 (1–1.5)	3	2 (2–3)	4	2.3 (1.5–4)	4	2.3 (1.5–3)
4	15	9	3 (1–7)	3	3 (3–4)	2	3	4	2.3 (2–6)
5	13	4	5 (1–8)	2	3.5 (3–4)	1	3.5	4	3.5 (1–6)
6	9	6	5.5 (1–9)	3	3.5 (3–5)	2	5 (3–7)	4	2.8 (1–4)
7	5	3	8 (1–8)	0		1	2	0	
8	1	0		0					

[a] Data are for the anatomical right side

[b] Number of runners reporting weak soreness (scale unit >1.0) or higher

[c] Where no value for range is presented, the value for range is nil or equal to the mean. Scale values relate to the magnitude-based descriptors: 0, no ache/soreness; 0.5 just noticeable; 1, very weak; 2, weak; 3, moderate; 5, strong; 7, very strong; 10, extremely strong; and 13.5, absolute maximum/unbearable

significant correlation are presented in Fig. 2. Although of moderate effect size correlations with $r < 0.5$ or $p > 0.05$ were excluded for brevity.

Discussion

In the current report, we describe changes in muscle soreness and markers of muscle damage, oxidative stress, immunity and inflammation in response to a 894-km relay running race. The relay format allowed runners to complete an ultra-endurance distance (mean 119.5 km) at a much higher intensity than could be achieved during a continuous race. We demonstrate a progressive increase in muscle soreness after completing each relay leg and a corresponding large increase in markers of inflammation, lipid peroxidation and muscle damage after completing the race, suggesting that athletes competing in multi-day ultra-endurance events may benefit from specific interventions to prevent DOMS. Furthermore, we show that high post-race TAC is associated with a smaller increase in lipid peroxidation, suggesting that blood antioxidant capacity may be an important factor in the ability to compete in ultra-endurance racing without detrimental health effects.

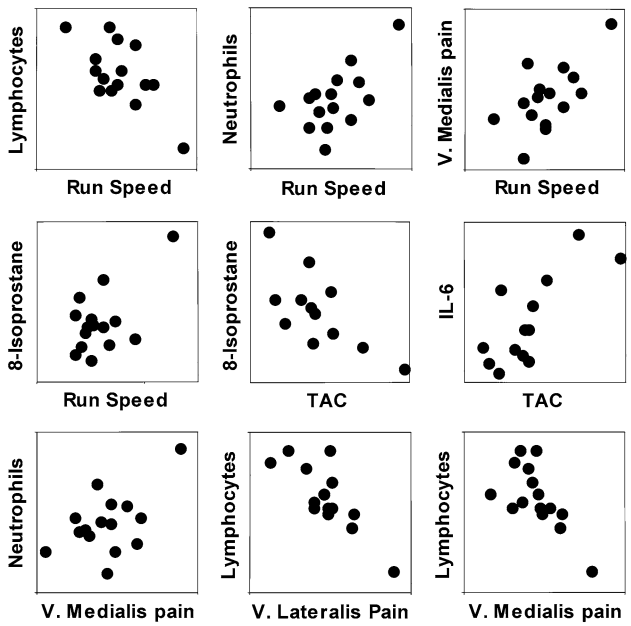

Fig. 2 Scatter plots of significant correlations. $N = 15$, $r > 0.50$, $p < 0.05$ for all correlations. Individual correlation coefficients are provided in the text. *V. medialis* and *V. lateralis* = pre-post changes in *vastus medialis* and *vastus lateralis* pain scores measured using algometer. 8-isoprostane = pre-post changes in serum 8-isoprostane normalized to serum creatinine. TAC = post-race total antioxidant capacity using ORAC assay. Lymphocytes and neutrophils = pre-post changes in blood cell counts

The multi-day relay race observed in the current study allowed us to record the location and severity of muscle soreness as it developed throughout an ultra-endurance race. By the start of their 4th leg, the majority of runners reported quadriceps muscle soreness (Fig. 2). DOMS induced by a bout of downhill running has been associated with various alterations in running biomechanics and physiology which have the potential to reduce running performance: reduced stride length (Braun and Dutto 2003; Harris et al. 1998) altered lower-limb range of motion during running (Hamill et al. 1991), reduced knee extensor concentric and eccentric torque (Eston et al. 1996), and reduced running economy (Braun and Dutto 2003). Importantly, when Eston et al.(1996) exposed some runners to an eccentric exercise bout 2 weeks prior to the downhill run, those runners experienced significantly less DOMS and a significantly smaller reduction in peak torque compared to runners who did not perform the prior eccentric exercise. As the athletes in our study were experiencing substantial DOMS during the course of the race, it seems likely that DOMS had some impact on their performance. It would be of interest to examine the effect on ultra-endurance running performance of a prior exercise bout designed specifically to protect against DOMS, such as a downhill run performed 2–3 weeks prior to the race.

We observed large increases in inflammatory markers after the run. The increase in plasma IL-6 that we observed at the end of the relay (30 min to 16 h after each individual runner's last leg) is similar to increases previously observed after continuous exercise of a similar duration; i.e. smaller than observed immediately after exercise (Neubauer et al. 2010; Suzuki et al. 2006; Wallberg et al. 2010), but greater than 24 h after exercise (Neubauer et al. 2010; Suzuki et al. 2006). The increase in plasma TNF-α that we observed after the race has not previously been observed after continuous exercise of a comparable duration to the current study (Kim et al. 2007, 2011; Suzuki et al. 2006; Wallberg et al. 2010) but has been observed after continuous exercise of shorter duration and higher intensity (Brenner et al. 1999; Starkie et al. 2001). Our observations are therefore consistent with the proposal of Kim et al. (2007) that plasma TNF-α elevation only occurs when exercise is performed above some threshold intensity that is greater than can usually be sustained during ultra-endurance exercise. We also observed an increase in leukocytes, particularly neutrophils, confirming the effect previously described following ultra-endurance running (e.g. Gundersen et al. 2006) and an ultra-endurance cycle relay (Bessa et al. 2008; Neubauer et al. 2008).

In addition to increases in markers of inflammation, there was also an increase in the urinary 8-isoprostane content. Such an increase is consistent with previous reports (Alessio et al. 2000; Mastaloudis et al. 2001) and

indicates an increase in the rate of whole body lipid peroxidation (Kim et al. 2011). While the long-term health effects of an acute increase in lipid peroxidation are difficult to determine, relatively short-term changes in urinary isoprostane concentration (8 weeks) are associated with changes in the rate of formation of atherosclerotic plaques in mice (Praticò et al. 1998) and chronic elevation of urinary isoprostane is associated with increased risk of atherosclerosis in humans (Dohi et al. 2007; Schwedhelm et al. 2004). As such, our results raise the possibility that a multi-day ultra-endurance event may cause a small increase in the rate of atherogenesis. Given the increasing popularity of ultra-endurance racing this possibility deserves further evaluation.

In contrast to our 8-isoprostane results, we also observed a non-significant trend toward a small decrease in urine 8-OHdG ($p = 0.055$). Such a decrease is consistent with previous research involving stage races over 4 days (Radák et al. 2000) despite reports of an increase in urinary 8-OHdG after a single day of ultra-endurance exercise (Miyata et al. 2008; Radák et al. 2000). As decreased urinary 8-OHdG concentrations indicate a decrease in oxidative damage to DNA (Valavanidis et al. 2009), these results suggest that multi-day ultra-endurance exercise does not is not likely to increase the risk of mutation based health disorders such as cancer (Wu et al. 2004).

At first glance the increase in markers of oxidative damage to lipids but decrease in markers of oxidative damage to DNA appears paradoxical. However, recent studies have demonstrated an increase in lipid membrane stress (Kim et al. 2011) and a slight decrease in DNA damage (Neubauer et al. 2010; Wagner et al. 2010) after ultra-endurance exercise of comparible distance to the current study. We speculate that these observations could be accounted for either by better antioxidant protection of DNA than lipids, or by the location of lipid membranes more proximal to the source of oxidative free radical generation.

Our study is the first to describe a very large correlation between post-race TAC and change in urinary 8-isoprostane concentration in humans, although a similar relationship has been previously observed in horses (Kinnunen et al. 2005). We did not find a substantial correlation between pre-race TAC and 8-isoprostane concentration. However, as TAC is increased by even a short bout of aerobic exercise (Alessio et al. 2000), TAC over the course of a 4-day relay could be in a post-exercise state for much of the race. This correlation suggests that serum antioxidant capacity plays an important role in limiting oxidative damage to lipids, a suggestion which is strengthened by experiments showing that exercise-induced lipid peroxidation is attenuated when TAC is increased by antioxidant supplementation (Lafay et al. 2009; Mastaloudis et al. 2004). In light of the potentially important health effects of lipid peroxidation (Dohi et al. 2007; Praticò et al. 1998; Schwedhelm et al. 2004), athletes may wish to consider short-term antioxidant supplementation to acutely increase TAC during and after an ultra-endurance event. Athletes should also note that there is evidence suggesting prolonged antioxidant supplementation may decrease endogenous antioxidant defenses (e.g. Ristow et al. 2009), so a prolonged supplementation strategy may be ineffective or even detrimental.

The current study has some potentially confounding factors common to field-based assessment that were not able to be controlled. The temporal variation between the end of exercise and post-race measurements due to the relay nature of the race (between 30 min and 16 h post-run) would be expected to increase variability in parameters that continue to increase following exercise, such as plasma CK activity and IL-6 concentration and TAC (Michailidis et al. 2007; Neubauer et al. 2008; Yamada et al. 2002), which may have masked relationships between muscle soreness and these variables (Malm et al. 2004; Nieman et al. 2005). Likewise, it seems probable that the observed correlation between plasma IL-6 concentration and TAC is due to the similar time-course of changes in these variables following exercise (Michailidis et al. 2007; Yamada et al. 2002) rather than an important mechanistic relationship. However, as the Cohen's effect size for the change in these variables was extremely large it is clear that the variability introduced does not preclude the use of these data for within-subject pre-post comparisons. Furthermore, post-race measures were collected at the same time of day (1000–1130 h) for all subjects, diurnal variation and ambient environmental conditions were consistent across all subjects, which will have reduced variability in all measurements.

The sample size ($n = 15$) used in our correlation analysis has relatively low statistical power, and necessitates the exclusion of correlations with highly influential outliers (Fig. 2), so may have masked real relationships between some variables. However, despite these limitations, the large correlation coefficients identified in the current study still provide a useful description of the linear relationships between paired variables.

The BLAZE race involved running over stages of variable distance and gradients so it was not possible to control for running intensity, time, distance or elevation change. However, as our subjects were highly motivated and competitive athletes, it is likely that they all exercised to the full extent they were capable. The timing of the night legs meant each athlete experienced a unique disruption to their normal sleep patterns. Nevertheless, as subjects each ran less than 4 h per day, there was adequate rest time to obtain their normal amount of sleep and deprivation was not expected to have a major impact on physiological or pain measurements.

Conclusions

Multi-day stage racing places unique demands on the body, as demonstrated by our finding that post-race serum TNF-α concentration was elevated despite very long exercise duration. Muscle soreness increased progressively throughout the race, and later stages were performed in a state of considerable physiological perturbation. The severity of muscle soreness and immune response appears to be strongly related exercise intensity, whereas the severity of exercise-induced lipid peroxidation is related to blood antioxidant capacity. Our results suggest that an ultra-endurance relay race might increase future risk of cardiovascular disease, but that this effect may be attenuated if serum antioxidant capacity is high. Athletes competing in multi-day ultra-endurance running may benefit from strategies designed to limit DOMS and acutely increase antioxidant capacity for the duration of the race.

Ethical standards: The experiments described in this report comply with the current laws of Canada, the country in which they were performed.

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