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Acute metabolic responses to a 24-h ultra-marathon race in male amateur runners

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Abstract The study was conducted to evaluate the metabolic responses to a 24 h ultra-endurance race in male runners. Paired venous and capillary blood samples from 14 athletes (mean age 43.0 ± 10.8 years, body weight 64.3 ± 7.2 kg, VO_{2max} 57.8 ± 6.1 ml kg^{-1} min^{-1}), taken 3 h before the run, after completing the marathon distance (42.195 km), after 12 h, and at the finish of the race, were analyzed for blood morphology, acid–base balance and electrolytes, lipid profile, interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), and serum enzyme activities. Mean distance covered during the race was 168.5 ± 23.1 km (range 125.2–218.5 km). Prolonged ultra-endurance exercise triggered immune and inflammatory responses, as evidenced by a twofold increase in total leukocyte count with neutrophils and monocytes as main contributors, nearly 30-fold increase in serum IL-6 and over 20-fold rise in hsCRP. A progressive exponential increase in mean creatine kinase activity up to the level 70-fold higher than the respective pre-race value, a several fold rise in serum activities of aspartate aminotransferase and alanine aminotransferase, and a fairly stable serum γ-glutamyl transferase level, were indicative of muscle, but not of liver damage. With duration of exercise, there was a progressive development of hyperventilation-induced hypocapnic alkalosis, and a marked alteration in substrate utilization towards fat oxidation to maintain blood glucose homeostasis. The results of this study may imply that progressive decline in partial CO_2 pressure (hypocapnia) that develops during prolonged exercise may contribute to increased interleukin-6 production.

Introduction

As ultra-endurance events have become increasingly popular, many athletes compete in single day races lasting 4 up to 24 h, or in multi-stage races performed over several days. Competing in ultra-endurance events imposes a severe metabolic stress and provokes acute cardio-respiratory responses to ensure adequate oxygen supply to the body. High level of physical demand during these events induces a wide range of metabolic changes, causes micro-injuries to the muscles and other tissues, which increases migration of white blood cells to the sites of injury, and induces acute phase inflammatory reactions (Kim et al. 2007; Tidball 2005; Bessa et al. 2008). Local response to tissue injury involves the production of a large number of acute phase proteins and cytokines. Among the latter, the muscle-derived interleukin-6 is considered important modulator of the immunological and metabolic responses to exercise (Fischer 2006; Pedersen and Febbraio 2008). Most studies on the metabolic responses to prolonged endurance effort have evaluated changes taking place immediately post-exercise and during the recovery period (Wu et al. 2004; Suzuki et al. 2003, 2006; Castell et al. 1997; Kratz et al. 2002), while only few reports focused on changes induced during ultra-endurance events (Kim et al. 2007, 2009; Wallberg et al. 2011), and very little attention has been devoted to arterial blood gas and [H$^+$]

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homeostasis during endurance exercise performed under competitive conditions in the field (Hanson et al. 1982). The aim of the present study was to evaluate complex metabolic responses that develop during a competitive 24-h ultra-marathon run in male amateur endurance-trained runners. A special focus was put on the changes in serum interleukin-6 and its role in modulating the acute metabolic responses to prolonged, endurance exercise.

Methods

Participants

Fourteen male amateur runners (mean age 43.0 ± 10.8 years, body weight 64.3 ± 7.2 kg, height 171 ± 5 cm, percent body fat $13.9 \pm 3.8\%$, weekly covered distance 81 ± 43 km, training history 8 ± 9 years) registered for a 24-h ultra-marathon, who volunteered to provide blood samples during the race, were enrolled for the study. Before the start of the race, after assessment of body composition by the bioimpedance method (InBody220, Biospace), the runners completed a questionnaire on their medical and training history, and signed their consent to participate in the study after being informed about the experimental procedures approved by the local ethics committee, in accordance with the Declaration of Helsinki. The runners competed on a 3,014 m long oval recreational walking trail located outside the town. Environmental conditions at the race start at 8 PM were 11°C and 67% relative humidity (RH), at 8 AM and 12 AM next day were 11°C (71% RH) and 15°C (55% RH), respectively, and at the completion of the race at 8:00 PM were 15°C (55% RH). The runners were allowed free access to food and liquids, but the actual food intakes were not recorded, which is a limitation of this study. Liquids consisted mainly of water, sport beverages, soft drinks and broth, while food supplements included sandwiches, cookies, bananas, and carbohydrate energy bars. Some days after the race, all competitors reported to the physical testing laboratory for an incremental running test on a treadmill (LE 200, Jaeger) to assess anaerobic threshold and peak oxygen uptake (VO_{2max}) using a stationary breath-by-breath metabolic unit (MetaLyzer 3B-R2, Cortex). Performance characteristics of the runners are presented in Table 1.

Blood sampling and biochemical analyses

Samples of venous and fingertip capillary blood simultaneously collected 3 h before the race, after completing the marathon distance (42.195 km), after 12 h, and not later than 10 min after the race, were immediately transported to a clinical laboratory for biochemical analyses including

Table 1 Performance characteristics of the runners ($N = 14$)

Variable	Mean $\pm$ SD	Min–Max
VO_{2max} (mL O_2/kg/min)	57.8 ± 6.1	49.0–71.0
VO_{2max} (mL O_2/kg$^{0.75}$/min)	164.7 ± 15.5	140.3–195.5
Individual anaerobic threshold (mL O_2/kg/min)	48.9 ± 5.1	39.0–57.0
Lactate threshold (%VO_{2max})	84.2 ± 4.0	77–92
Velocity at lactate threshold (km/h)	13.1 ± 0.9	12–14
VE_{max} (L/min)	140.1 ± 19.4	117.0–193.4
Peak heart rate HR_{max} (bpm)	182.1 ± 10.0	165–198
Peak lactate concentration (mmol/L)	8.64 ± 1.91	5.90–11.64

blood morphology, blood gases and electrolytes, lipid profile, C-reactive protein (hsCRP), and selected serum enzyme activities. Blood morphology was assessed in venous blood samples (anti-coagulated with EDTA) on an Automated Hematology Analyzer XS-1000i^{TM} (Sysmex), while measurements of blood gases, electrolytes, lactate and glucose in fingertip capillary blood were performed on the GEM Premier 3000 with IQM (Instrumentation Laboratory). Serum activities of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT), as well as serum concentrations of total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, and triglycerides (TG) were measured using the clinical chemistry analyzer SYNCHRON CX 9 PRO (Beckman-Coulter). The intra-assay coefficients of variation (CV) for these assays were 5.8, 6.70, 5.30, 5.60% and 3.50, 6.10, 4.50%, respectively. hsCRP was assessed in serum by a turbidimetric immunoassay using a Dade-Behring (Marburg, Germany) kit (intra assay CV 3.45%). Total serum concentration of interleukin-6 (IL-6) was determined using quantitative Human High Sensitivity ELISA kit (Gen-Probe Diaclone SAS, France) (sensitivity <0.81 pg/ml, intra assay CV 4.4%). Serum free fatty acids (FFA), glycerol and β-hydroxybutyrate contents were assessed using commercially available manual test kits NEFA, Glycerol (GLY) and RANBUT (CV: 2.7, 1.34, 4.71%, respectively) from Randox Laboratories (Antrim, UK). To estimate the risk of coronary artery disease (CAD) in the ultra-marathoners, we calculated the lipid ratios (TC/HDL, LDL/HDL, TG/HDL) and plasma atherogenic index [atherogenic index of plasma (AIP) $= \log_{10}(TG/HDL)$] (Dobiášová and Frohlich 2001).

Statistics

Data are presented as the mean (SD). The data were tested for homogeneity of variances by using the Levene test and then analyzed either by the nonparametric Wilcoxon test or by one-way repeated measures ANOVA followed, where

appropriate, by the Tukey post-hoc test. Spearman's rank order correlation analysis was used to assess relationships between selected variables. The level of significance of $P < 0.05$ was chosen for all statistical comparisons.

Results

Ultra-marathon performance and physiological profile of the competitors

The running performance data of the competitors are presented in Table 2. The highest running velocity was attained at the first stage of the race, i.e. over the marathon (42.195 km) distance, then it decreased markedly to reach a significantly lower level both over the mid-race and over the whole distance covered. There were marked differences in individual race performance measures; however, running velocity was not significantly related to age, body mass, BMI, or lean body mass (LBM). Noteworthy, running velocity appeared to be positively correlated with individual anaerobic threshold IAT ($R = 0.40$, $P < 0.01$), VO_{2max} ($R = 0.33$, $P < 0.05$) and with peak oxygen uptake expressed in relation to (body mass)$^{0.75}$ ($R = 0.38$, $P < 0.05$), which is considered a more reliable measure of oxygen uptake when comparing individuals with different body mass (Bergh et al. 1991). The mean values of VO_{2max} recorded in our younger (<40 years, $N = 6$) and older (>40 years, $N = 8$) runners during a progressive treadmill running test, which equaled 59.8 ± 6.7 and 56.3 ± 5.4 mlO$_2$/kg/min, respectively, were comparable to those reported by Wilmore and Costill (2005) for younger (18–39 years) or older (40–60 years) endurance-trained runners (60–85 and 40–60 ml/kg/min, respectively). The importance of a high VO_{2max} for performance in ultra-marathon events (Kreider 1991; Joyner and Coyle 2008; Millet et al. 2011) was additionally

supported by our finding of significant correlations between VO_{2max} or IAT and running velocity (see above) or distance covered during the race ($R = 0.57$, $P < 0.05$ or $R = 0.74$, $P < 0.005$, respectively), which proves that the higher is VO_{2max} and anaerobic threshold, the easier is to run at a given submaximal velocity in long-distance events (Noakes et al. 1990). Although a high VO_{2max} would contribute greatly to performance in endurance events at the highest level, other markers such as lactate threshold (LT) and running velocity at lactate threshold (v_{LT}) are considered to be more predictive. The mean values for IAT, lactate threshold expressed as $\%VO_{2max}$ and v_{LT} tended to be higher in younger compared to older runners (51 vs. 45 ml/kg/min; 85 vs. 80%, and 13.5 vs. 12.0 km/h, respectively), but in all cases they were comparable to those achieved by well-trained athletes (Wilmore and Costill 2005). Notably, compared to the mean running velocity at the anaerobic threshold (13.1 ± 0.9 km/h), which has been assessed during graded treadmill running test, expressed as a percent of maximal oxygen uptake equal, on average, 84.2 ± 4.0 %VO_{2max} (Table 1), the average speed sustained over the first 12 h or the whole 24 h race, corresponded to 51 or 40% VO_{2max}, which is comparable to that (40% VO_{2max}) found by Millet et al. (2011).

Biochemical data

Capillary blood gases and acid–base balance indices were within the normal ranges at rest, but they changed significantly with the progress of the race. A progressive respiratory alkalosis contributed to a mean increase in pH of 0.067 units from rest to the end of the competition, coincident with significant hypocapnia resulting from a 23% decrease in pCO$_2$ (Table 3). This was accompanied by a progressive increases in pO$_2$ of about 11 mmHg, oxygen saturation and plasma potassium, and a tendency towards lower free (ionized) calcium, while plasma sodium concentration remained unchanged (Table 3). It should be stressed that capillary blood gases accurately predict arterial carbon dioxide pressure, and arterial plasma lactate, glucose, pH, hemoglobin and electrolytes, but not arterial oxyhemoglobin saturation and arterial oxygen pressure (Zavorsky et al. 2005). Running the marathon distance led to a marked leukocytosis with significant increases in absolute neutrophil and monocyte counts and decreases in eosinophil count (Table 4). In contrast, no significant changes in any of the basic blood counts from baseline levels were found during the whole competition (Table 4).

Dramatic increases over the baseline values in muscle damage and inflammatory markers were observed (Table 5). Notably, serum activity of CK increased by 70 times, of AST—by 14 times, of ALT—by 4 times, while

Table 2 Ultra-marathon (24 h) race performance measures

Variable	Mean ± SD	Median	Min–Max
Marathon time (h)	4.6 ± 0.4	4.7	4.1–5.3
Marathon running velocity (km/h)	9.1 ± 0.7	9.0	8.0–10.2
12-h distance (km)	94.6 ± 11.6	94.0	73.0–109.0
12-h running velocity (km/h)	7.9 ± 1.0*	7.83	6.1–9.1
24-h distance (km)	168.5 ± 23.1	165.9	125.2–218.5
24-h running velocity (km/h)	7.0 ± 1.0*	6.9	5.2–9.1

* Significantly different ($P < 0.05$) from the running velocity achieved at the marathon distance

Table 3 Ultra-marathon-induced changes in acid–base balance indices and concentrations of electrolytes, lactate and glucose

Variable	Pre-race		Post-marathon		Post 12 h		Post-24 h	
	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
pH	7.396 (0.028)	7.350–7.430	7.405 (0.025)	7.360–7.450	7.445** (0.029)	7.390–7.510	7.463** (0.026)	7.400–7.510
pCO_2 (mmHg)	42.5 (2.6)	39–48	41.2 (3.5)	37–49	35.6** (3.8)	29–42	32.6** (4.0)	25–39
pO_2 (mmHg)	79.2 (10.9)	70–112	74.0 (7.4)	63–88	88.2 (7.2)	77–103	90.1* (13.2)	79–108
Standard bicarbonate (mmol/L)	25.6 (1.3)	23.7–27.4	25.6 (2.1)	22.4–29.4	25.4 (1.7)	22.7–27.7	25.1 (1.4)	23.4–28.0
BE (mmol/L)	1.0 (1.6)	−1.5 to +3.2	1.0 (2.7)	−3.1 to +5.9	0.7 (2.2)	−2.9 to +3.5	0.2 (1.9)	−1.9 to +4.0
O_2 saturation (%)	95.3 (1.2)	94–98	94.5 (1.6)	92–97	97.1** (2.6)	92–98	97.1** (1.7)	92–99
Sodium (mmol/L)	141.8 (4.1)	136–152	141.9 (2.5)	139–147	142.4 (2.6)	139–148	140.3 (2.8)	138–148
Potassium (mmol/L)	4.6 (0.4)	3.9–5.1	5.2* (0.6)	4.4–6.1	5.5** (0.5)	5.1–6.7	6.8** (1.1)	5.1–8.5
Ionized Ca (mmol/L)	1.25 (1.04)	1.17–1.42	1.21 (0.06)	1.08–1.31	1.17* (0.05)	1.06–1.23	1.19 (0.05)	1.08–1.26
Lactate (mmol/L)	3.1 (0.7)	2.3–4.4	3.4 (1.0)	2.5–5.6	3.4 (1.0)	2.0–4.9	4.1 (1.0)	2.0–5.9
Glucose (mmol/L)	6.6 (1.0)	5.1–8.9	6.0 (1.0)	5.0–7.7	6.5 (1.1)	4.6–8.5	7.5 (1.9)	5.2–12.0

* $P < 0.05$; ** $P < 0.005$ versus the corresponding pre-race value

Table 4 Ultra-marathon-induced changes in basic blood counts and circulating leukocytes and leukocyte subpopulations

Variable	Pre-race		Post-marathon		Post 12 h		Post-24 h	
	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Hb (g/dl)	14.2 (1.3)	11.9–16.4	14.6 (1.1)	12.8–16.1	14.4 (1.3)	12.2–16.7	14.3 (1.0)	12.6–16.5
Red cell count ($\times 10^{12}$/L)	4.74 (0.32)	4.24–5.22	4.87 (0.23)	4.51–5.23	4.79 (0.28)	4.38–5.29	4.76 (0.20)	4.46–5.27
Hct (%)	41.9 (3.0)	36–47	42.4 (2.2)	39–45	41.6 (2.9)	37–47	41.2 (2.3)	37.0–46.0
MCV (fl)	88.5 (3.1)	82.0–93.9	87.1 (2.7)	81.6–91.3	86.9 (2.8)	80.7–90.4	86.5 (2.7)	81–89.8
MPV (fl)	11.5 (0.6)	10.3–12.6	11.5 (0.8)	10.0–12.9	11.5 (0.7)	10–12.5	11.5 (0.7)	10.3–12.2
Platelets ($\times 10^9$/L)	262.4 (34.2)	215–317	291.9 (56.6)	129–365	289.6 (40.0)	235–364	282.1 (34.4)	227–362
White cell count ($\times 10^9$/L)	7.67 (2.49)	4.4–14.0	14.51** (3.23)	9.5–21.5	13.86** (3.42)	9.5–21.5	14.84** (2.54)	11.3–18.7
Neutrophils ($\times 10^9$/L)	4.30 (2.26)	2.01–10.21	9.93** (3.21)	5.63–17.35	10.45** (3.04)	5.88–16.39	10.40** (2.49)	7.54–14.93
Lymphocytes ($\times 10^9$/L)	2.55 (0.94)	1.51–4.64	3.20 (1.10)	1.75–6.13	1.90 (0.53)	1.08–2.88	2.51 (0.67)	1.33–3.50
Monocytes ($\times 10^9$/L)	0.68 (0.18)	0.42–0.98	1.25* (0.36)	0.68–1.85	1.45** (0.45)	0.69–2.59	1.86** (0.52)	1.25–2.77
Eosinophils ($\times 10^9$/L)	0.11 (0.08)	0.02–0.31	0.09 (0.08)	0.01–0.30	0.01** (0.01)	0.01–0.04	0.02** (0.02)	0.00–0.06
Basophils ($\times 10^9$/L)	0.03 (0.02)	0.01–0.09	0.05 (0.04)	0.01–0.13	0.04 (0.03)	0.01–0.12	0.04 (0.03)	0.01–0.11

* $P < 0.005$; ** $P < 0.0005$ versus the corresponding pre-race value

hsCRP level was over 20-fold and IL-6 concentration—30-fold higher at the finish than at the start of the race. In contrast, serum GGT activity and blood concentrations of lactate and glucose remained fairly stable (Table 5).

The ultra-endurance race was also associated with marked changes in circulating levels of FFA, β-hydroxybutyrate, glycerol, and TG (Table 6). This was accompanied by significant declines in serum total and LDL cholesterol levels and a significant increase in serum HDL cholesterol. Lipid ratios (LDL/HDL, TC/HDL, and TG/HDL), as well as AIP were significantly reduced post-race. In order to assess the impact of the ultra-endurance race on selected biochemical parameters, and on muscle damage and inflammatory markers, the Spearman rank correlation coefficients were calculated; selected statistically significant associations are shown in Table 7.

Discussion

The present investigation used 24 h foot race to study metabolic responses during prolonged submaximal exercise. The current study confirmed previous findings that continuous, prolonged, moderate-intensity running exercise is associated with severe muscle damage, markedly elevated IL-6 and hsCRP concentrations, and favorable changes in serum lipid profile. The results of this study may imply that hyperventilation-induced respiratory alkalosis with a progressive hypocapnia might contribute, at least to some extent, to increased IL-6 cytokine production.

Metabolic responses to the 24 h race

The extreme caloric demand during ultra-endurance competition requires an adequate supply of metabolic fuels (Kreider 1991; Zaryski et al. 2005). Because carbohydrate stores in the body are limited, the long-duration muscle activity raises the need for carbohydrates not only as an energy source for muscles, but also for their role both in the rapid metabolizing of fats for energy and in providing glucose for normal functioning of the central nervous. Therefore, the adequate supply of carbohydrates is considered crucial for endurance athletes. Since the runners in the present study had free access to food during the entire event, no significant changes in blood glucose concentration were found, which implies adequate carbohydrate supply during the competition. On the other hand, it could be easily predicted that the ultra-endurance race will have a major impact on fat metabolism, and in particular will enhance fat oxidation (Helge et al. 2007; Jeukendrup et al. 1998). Since the athletes were not fed with large amount of lipids, our findings of a more than 50% decrease in serum triacylglycerols, associated with significant, almost

Table 5 Ultra-marathon-induced changes in serum markers of muscle damage and inflammation

Variable	Pre-race		Post-marathon		Post 12 h		Post-24 h	
	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Creatine kinase (U/l)	249.2 (136.1)	96–559	786.9** (821.9)	169.0–3361	4,284.0** (3,544.9)	533–11,653	17,502** (15,040)	922–50,440
Aspartate aminotransferase (U/L)	28.7 (7.7)	19–54	–	–	–	–	388.5** (295.8)	45–978
Alanine aminotransferase (U/L)	24.1 (6.4)	14–35	–	–	–	–	84.4** (50.7)	27–179
Gamma glutamyltransferase (U/L)	29.6 (35.1)	12–145	29.6 (34.7)	11.0–143	29.6 (34.5)	13–143	27.9 (31.2)	13–130
hsCRP (mg/dL)	1.7 (2.7)	0.3–10.6	1.7 (2.5)	0.3–10.0	8.7** (4.6)	2.0–20.1	39.2** (16.7)	16.3–79.0
IL-6 (pg/ml)	0.87 (0.68)	0.01–1.91	20.29*** (7.77)	11.99–34.58	27.36*** (7.67)	12.77–36.47	28.49*** (11.99)	16.70–50.25

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$ versus the corresponding pre-race value

Table 6 Ultra-marathon-induced changes in serum lipid profile

Variable	Pre-race		Post-marathon		Post 12 h		Post-24 h	
	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Cholesterol (mmol/L)	5.07 (0.72)	3.8–6.4	–	–	–	–	4.04** (0.84)	2.7–6.1
HDL (mmol/L)	1.43 (0.30)	1.0–2.0	–	–	–	–	2.01** (0.32)	1.3–2.6
LDL (mmol/L)	2.86 (0.76)	1.71–4.21	–	–	–	–	1.74** (0.73)	0.70–3.70
Triglycerides (mmol/L)	1.73 (1.22)	0.2–5.1	–	–	–	–	0.80* (0.13)	0.56–0.98
Free fatty acids (mmol/L)	0.60 (0.24)	0.12–0.96	1.86** (0.96)	1.20–4.43	1.91* (1.25)	0.60–4.0	1.79** (1.06)	0.801–4.591
β-hydroxy-butyrate (mmol/L)	0.17 (0.06)	0.08–0.32	0.38 (0.41)	0.13–1.68	0.36* (0.31)	0.11–1.19	0.58** (0.36)	0.13–1.32
Glycerol (μmol/L)	88.4 (26.7)	27.7–122.6	339.5** (147.3)	110.9–591.7	298.9** (216.3)	73.24–841.2	270.2** (93.3)	133.9–457.8
TC/HDL	3.68 (0.84)	2.24–5.09	–	–	–	–	2.03** (0.38)	1.48–2.94
LDL/HDL	2.07 (0.63)	1.19–3.06	–	–	–	–	0.90** (0.40)	0.30–1.76
TG/HDL	1.34 (1.14)	0.12–4.64	–	–	–	–	0.41** (0.10)	0.27–0.61
AIP [$\log_{10}$(TG/HDL)]	−0.01 (0.39)	−0.93 to (0.66)	–	–	–	–	−0.40** (0.11)	−0.57 to (−0.21)

* $P < 0.05$; ** $P < 0.005$ versus the corresponding pre-race value

Table 7 Spearman rank correlation coefficients between the distance covered during the 24 h ultra marathon race and selected hematological and biochemical indices

Variables	R	P
Distance and pH	0.69	$<10^{-6}$
Distance and pCO_2	−0.75	$<10^{-6}$
Distance and ionized Ca	−0.41	<0.005
Distance and K^+	0.80	$<10^{-6}$
Distance and total WBC count	0.56	$<10^{-5}$
Distance and neutrophil absolute count	0.60	$<10^{-6}$
Distance and monocyte absolute count	0.74	$<10^{-6}$
Distance and eosinophil absolute count	−0.64	$<10^{-6}$
Distance and hsCRP	0.82	$<10^{-6}$
Distance and IL-6	0.68	$<10^{-5}$
Distance and CK	0.87	$<10^{-6}$
Distance and AST	0.82	$<10^{-6}$
Distance and ALT	0.81	$<10^{-6}$
Distance and total cholesterol	−0.42	<0.05
Distance and LDL-Cholesterol	−0.46	<0.05
Distance and HDL-Cholesterol	0.70	$<10^{-4}$
Distance and TG	−0.56	<0.005
Distance and FFA	0.50	<0.0005
Distance and β-hydroxybutyrate	0.46	<0.001
Distance and AIP [$\log_{10}$(TG/HDL)]	−0.66	<0.0005

threefold increases in circulating FFA, glycerol and β-hydroxybutyrate, reflect the use of fatty acids as fuels. These results support the view that marathon runners compared with normal individuals have a higher fat turnover rate at submaximal exercise intensities (Sjödin and Svedenhag 1985). It is worth to note that the decrease in TG was associated with significant decline in total and LDL cholesterol and a significant rise in HDL cholesterol. Moreover, highly significant negative correlations between the distance covered during the race and several lipid profile indices (serum TG, cholesterol total, LDL and HDL) strongly support the view of the beneficial effect of endurance effort on serum lipid profile. It should be stressed that not only concentrations of TG, total cholesterol and its fractions as the independent risk factors, but also the common cholesterol ratios (TC/HDL, LDL/HDL, and TG/HDL), which are considered better predictors of future coronary heart disease (Natarajan et al. 2003; Kinosian et al. 1994; Grover et al. 1999) were already relatively low at the start of the race, and reached the lowest levels at the finish. As expected, total and LDL-cholesterol were significantly positively related with age in the participating runners. Comparable changes in the lipid profile after prolonged exercise, suggesting reduction in cardiovascular disease (CVD) risk, were also observed by other authors (Gastmann et al. 1998;

Ginsburg et al. 1996). We are aware that there are more accurate methods to estimate the risk of CVD and atherosclerosis, e.g. determining lipoprotein(a) level or particle size distribution in LDL (Superko 1996). However, a surrogate method for evaluation of the atherogenicity of plasma lipoproteins based on the assessment of the AIP calculated as log(TG/HDL-C) (with TG and HDL-C expressed in molar concentrations) may also be useful (Dobiášová and Frohlich 2001). The most recent findings from studies in a cohort of adult subjects on the negative correlation between AIP and particle sizes of HDL and LDL support the view that AIP is a reliable predictor of the cardiovascular risk (Dobiášová et al. 2011). It should be stressed, therefore, that negative AIP values, which have been found in a half of the runners at the start of the race and in all of them—at the finish, may indicate a lowered risk of CVD. Taking into account that changes in concentration of small dense LDLs are significantly correlated with changes in TG (Baumstark et al. 1993), it may be postulated that the reduction in pro-atherogenic small dense LDLs induced by prolonged endurance effort would be larger in subjects with larger reductions in serum TG.

Another important aspect of metabolic response to ultra-endurance efforts is the role of IL-6. The concentration of this cytokine correlated positively with serum FFA ($R = 0.47$, $P < 0.005$), glycerol ($R = 0.42$, $P < 0.005$) and HDL-C ($R = 0.63$, $P < 0.005$) levels, and negatively—with serum TG ($R = -0.46$, $P < 0.05$). These findings strongly support the view that IL-6 enhances lipid turnover by stimulating lipolysis and fat oxidation (Bruce and Dyck 2004; Petersen and Pedersen 2005), thus it may be considered as one possible mediator of the beneficial effects of physical activity on fatty acid metabolism. Data from the literature provide also a strong evidence that IL-6 is involved in mediating glucose homeostasis during exercise (Petersen and Pedersen 2005), notably that activation of the IL-6 gene during exercise may be sensitive to muscle glycogen content. Low muscle glycogen substantially enhances IL-6 gene transcription and IL-6 protein production in skeletal muscles, whereas carbohydrate ingestion during exercise attenuates increases in plasma IL-6 (Keller et al. 2001; Li and Gleeson 2005). With regard to the results of the present study, one should notice that free access to carbohydrate-rich food during the race did not preclude the possibility that muscle glycogen stores in our runners were depleted, which could activate IL-6 expression. Sustained supply of exogenous sugars allowed the competitors to complete the race with no impairment of glucose homeostasis, although well maintained glucose concentration could blunt the response of serum IL-6 (Li and Gleeson 2005).

Ventilatory and immune responses to ultra-endurance exercise

It is well known that a continuous long-lasting run elicits a ventilatory response. The most distinctive feature of the response is a tachypnea associated with variable degrees of hypocapnia and respiratory alkalosis (Hanson et al. 1982). The hypocapnia develops when a strong respiratory stimulus causes the lungs to remove more carbon dioxide than is produced in metabolically active tissues. Indeed, in the present study a variable degree of hypocapnia and respiratory alkalosis did develop during the race. Alkalosis at the completion of the race was evident in nine subjects, showing above-normal pH values (7.46–7.51) and pCO_2 less than 35 mmHg. This was associated with a tendency towards a lowered free ionized calcium concentration. Hyperventilation-induced decreases in plasma concentrations of CO_2, $[H^+]$ and available calcium, acting as potent vasodilators during exercise, may limit O_2 delivery to locomotor skeletal muscle by causing vascular constriction and reducing blood flow (Chin et al. 2007). Of note, we have found in this study that serum IL-6 level correlated negatively with capillary blood pCO_2 ($R = -0.51$, $P < 0.001$), while it correlated positively with capillary blood pH ($R = 0.53$, $P < 0.0005$). These findings may imply that the respiratory-induced hypocapnic alkalosis might, at least to some extent, modulate in vivo production of this cytokine. To our knowledge, there is but a single study providing evidence that CO_2 concentrations modulated cytokine levels in endotoxin-stimulated human whole blood cell cultures (Kimura et al. 2008). Notably, those authors found that hypocapnic alkalosis stimulated IL-6 production, whereas the opposite effect, i.e. a reduced IL-6 concentration was observed after hypercapnic acidosis. Given that no detectable amounts of this cytokine were found by those authors in blood samples without endotoxin stimulation, one may presume that the elevated serum IL-6 evidenced during and after the 24 h race in all athletes in our study was, at least partly, due to exercise-induced muscle damage (Suzuki et al. 2006; Nieman 1997) and/or ensuing endotoxemia resulting from increased intestinal permeability (Jeukendrup et al. 2000). This presumption is strongly supported by positive correlations between serum IL-6 level and serum activities of CK ($R = 0.65$, $P < 10^{-4}$), AST ($R = 0.77$, $P < 10^{-4}$) and ALT ($R = 0.79$, $P < 10^{-4}$) that are considered indirect markers of work-induced muscle damage. Abnormally high serum activities of these enzymes are indicative of their leakage from skeletal muscle or other tissues into the bloodstream due to mechanical damage or increased membrane permeability.

Recent research demonstrated that plasma IL-6 increases exponentially with exercise intensity and duration, and

the mass of muscle recruited (Pedersen and Febbraio 2005, 2008). However, a fairly stable serum IL-6 concentration evidenced in the present study, as well as a significant correlation between IL-6 and distance covered ($R = 0.68$, $P < 10^{-5}$), seem to support most recent finding of Wallberg et al. (2011) that IL-6 does not increase after 12 h of exercise, which suggests that the main determinant of the IL-6 response is the intensity of exercise.

The results of our study demonstrate that running a 24 h ultra-marathon resulted in highly significant increases in serum activities of CK, AST and ALT, emerging in the second half of the race (between 12 and 24 h). Substantial increases in serum activities of AST and ALT following prolonged exercise, and fairly stable serum GGT are indicative of significant skeletal muscle and minor hepatic damage (Noakes 1987; Whitfield 2001; Rosales et al. 2008). In our study, the highest increase was recorded (Table 5) for the mean serum activity of CK that is widely accepted as an indirect marker of muscle damage (Noakes et al. 1983; Kim et al. 2007, 2009; Miles et al. 2008; Brancaccio et al. 2010). However, there were remarkable inter-individual differences in the relative magnitude of the increase, amounting to 27-fold difference between the highest and the lowest response. The reason for this variability is not clear; however, one may suspect that the extreme increases in serum CK activity may be related to the development of exertional rhabdomyolysis (Skenderi et al. 2006).

The body's response to tissue damage involves mobilization of immune cells and their migration towards sites of the injury (Tidball 2005; Kim et al. 2007; Bessa et al. 2008; Nieman 1997). In the present study, the immune response to muscle damage was characterized by a pronounced, almost twofold increase in total WBC count at the completion of the marathon distance, which persisted until the end of the race. The major components were neutrophils and monocytes, which reached counts almost three times those pre-race. It is known that increases in leukocyte counts during sustained moderate exercise, even in thermally comfortable environment (which was the case in the present study), are related mainly to elevations in plasma catecholamines, which induce a demargination of leukocytes (Brenner et al. 1998). It is well established that the release of leukocytes, and particularly neutrophils and monocytes, is stimulated by inflammatory mediators, such as IL-6 (Suzuki et al. 2003; Nieman 1997). This assumption is corroborated by our finding that IL-6 responses correlated significantly with ultra-endurance exercise-induced increases in total leukocyte ($R = 0.56$, $P < 10^{-4}$), absolute neutrophil ($R = 0.57$, $P < 10^{-4}$) and monocyte ($R = 0.71$, $P < 10^{-7}$) counts, which seems to support previous findings that IL-6 may mediate recruitment and activation of neutrophils and monocytes in response to

exhaustive exercise (Suzuki et al. 2003). However, it is well documented that IL-6 is produced in skeletal muscles also in non-inflammatory conditions, and that muscle contraction per se is a major stimulus for de novo synthesis of IL-6 in myocytes (Hiscock et al. 2004; Pedersen and Febbraio 2008).

One important feature of IL-6 is that it induces the production of hepatocyte-derived CRP (Fischer 2006) that is involved in the induction of anti-inflammatory cytokines in circulating monocytes, in suppression of the synthesis of proinflammatory cytokines in tissue macrophages (Pue et al. 1996) and is responsible for the recognition and removal of damaged cells (Plaisance and Grandjean 2006). In our study, plasma hsCRP concentration during the first 42.195 km distance was fairly stable. Changes in plasma hsCRP content occurred later, i.e. after finishing the marathon distance, when it rose in an exponential pattern to reach, at the finish of a 24 h race, the level more than 20 times higher than the pre-race value. Significant correlation between plasma CRP and IL-6 found in our study ($R = 0.51$, $P < 0.0005$) supports the presumption of the role of IL-6 as a primary inducer of hepatic production of acute phase proteins (Fischer 2006).

In summary, the main metabolic responses to the 24-h ultra-marathon race were: (i) a marked shift in substrate utilization towards fat oxidation to maintain blood glucose homeostasis, and (ii) favorable changes in serum lipid profile. The ultra-endurance run led to a substantial skeletal muscle damage and an acute inflammatory response evidenced by a wide range of changes in muscle injury-related indices and dramatic elevations in serum interleukin-6 and hsCRP. These effects were more pronounced during the second half of the race, and the primary determinants of these changes were both the duration and intensity of the exercise. The predominant ventilatory response to the ultra-endurance effort of the 24 h race was a tachypneic, respiratory alkalosis associated with hypocapnia and hyperkalemia, but not hyponatremia. Some results of this study suggest that the elevation in serum IL-6, which likely is due to increased production, might be related to hypocapnia that develops during prolonged endurance exercise. However, the correlation between these phenomena may as well be an indirect, non-causal association related to the effects of prolonged muscle effort on both pCO$_2$ and IL-6.

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