Effect of ultra-endurance exercise on left ventricular performance and plasma cytokines in healthy trained men

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ABSTRACT: The purpose of this study was to investigate the effect of ultra-endurance exercise on left ventricular (LV) performance and plasma concentration of interleukin (IL)-6, IL-10, IL-18 and tumour necrosis factor alpha (TNF-α) as well as to examine the relationships between exercise-induced changes in plasma cytokines and those in echocardiographic indices of LV function in ultra-marathon runners. Nine healthy trained men (mean age 30±1.0 years) participated in a 100-km ultra-marathon. Heart rate, blood pressure, ejection fraction (EF), fractional shortening (FS), ratio of early (E) to late (A) mitral inflow peak velocities (E/A), ratio of early (E') to late (A') diastolic mitral annulus peak velocities (E'/A') and E-wave deceleration time (DT) were obtained by echocardiography before, immediately after and in the 90th minute of the recovery period. Blood samples were taken before each echocardiographic evaluation. The ultra-endurance exercise caused significant increases in plasma IL-6, IL-10, IL-18 and TNF-α. Echocardiography revealed significant decreases in both E and the E/A ratio immediately after exercise, without any significant changes in EF, FS, DT or the E/E' ratio. At the 90th minute of the recovery period, plasma TNF-α and the E/A ratio did not differ significantly from the pre-exercise values, whereas FS was significantly lower than before and immediately after exercise. The increases in plasma TNF-α correlated with changes in FS (r=0.73) and DT (r=-0.73). It is concluded that ultra-endurance exercise causes alterations in LV diastolic function. The present data suggest that TNF-α might be involved in this effect.

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INTRODUCTION

Long-distance running such as the marathon or ultra-marathon are a very popular type of sports activity, although not always safe and beneficial to health. Numerous studies have shown that physical exertion can cause alterations in the cardiovascular, endocrine, nervous, immune and musculoskeletal systems [1, 2, 3]. The exercise-induced stress causes a number of physical changes that have short- or long-term effects on the body. Progressive activation of the sympathetic nervous system affects the circulatory system and neurotransmitters that serve as the brain messengers to the body. Exercise-induced muscle damage induces local inflammation with leucocyte accumulation and a systemic inflammatory response.

The systemic inflammatory response comprises leukocytosis and an acute-phase response [4, 5, 6, 7, 8], which is characterized by increased release of cortisol, adrenocorticotrophic hormone, cytokines and acute phase proteins such as C-reactive protein [2, 9, 10, 11]. Zheng et al. [12] reported significant increases in plasma IL-6 (125±), IL-10 (24x), IL-8, IL-1ra, granulocyte colony-stimulating factor (G-CSF), monocyte chemotactic protein 1 (MCP-1), and macrophage inflammatory protein 1beta (MIP-1beta) in ultra-marathon runners following a 160-km race event. The increases in plasma IL-6, G-CSF, IL-10, IL-1ra, and MCP-1 correlated positively with those of creatine phosphokinase and post-race perceptions of muscle soreness [3, 10, 11, 13, 14, 15, 16]. Makris et al. [17] found that strenuous exercise leads to the up-regulation of IL-6 production and enhanced nitric oxide (NO) release within the contracting skeletal muscles. The authors concluded that NO contributes to the induction of the inflammatory cytokine response. Bernecker et al. [18] found that the increase in plasma IL-6 and TNF-α during strenuous exercise is not attributable to blood mononuclear cells. Studies in isolated ventricular cardiomyocytes showed that IL-1, IL-2, IL-3, IL-6, IL-8, IL-10 and TNF-α have a direct negative inotropic effect [19, 20, 21]. The effect was concentration-dependent, reversible and mediated through myocardial nitric oxide synthase. It was suggested that the cytokine-induced negative inotropic effect may be due to the formation of peroxynitrite, which is generated via interaction of superoxide and NO in the heart [22]. It has been shown that cytokines and TNF-α...
produce potentially deleterious effects with progressive left ventricular dysfunction [23]. The association between increased plasma levels of IL-6 and TNF-α and left ventricular diastolic dysfunction was found in patients with stable coronary artery disease [24]. Several studies in endurance athletes have shown that strenuous prolonged exercise in the form of the ultra-marathon may result in reversible depressed left ventricular contractile function, minimal decreases in global left ventricular systolic function, transient alterations in diastolic function on echocardiography and a transient reduction in right ventricular function during the recovery period [25, 26]. Left ventricular systolic dysfunction usually was associated with increases in plasma levels of cardiac troponins and creatine phosphokinase [26, 27]. The plasma cardiac troponin T (cTnT) rise correlated significantly with a fall in left ventricular ejection fraction immediately after prolonged strenuous exercise. Healthy runners have been reported to show increased plasma cTnT in proportion to the reduction in global left ventricular function [28]. The purpose of this study was to investigate the effect of ultra-endurance exercise on left ventricular performance and to examine the relationships between the exercise-induced changes in plasma cytokine concentrations and those in echocardiographic indices of left ventricular function in ultra-marathon runners.

**MATERIALS AND METHODS**

**Subjects and Procedure.** Nine healthy trained male ultra-marathon runners (age 25 to 38 years), with experience in at least one long-distance competition, volunteered as subjects for this study. The information about the study was available on the website of the event (7 Valleys Run – a 100-km mountain ultra-marathon). Runners interested in participating in the study contacted us by e-mail. All were normotensive, non-smokers and not taking any medications. A comprehensive clinical evaluation was performed in all subjects by a physician, with testing including electrocardiography, echocardiography and haematological screening. All the subjects gave their informed consent to participate in the study. The investigation conformed with the principles outlined in the Declaration of Helsinki and was approved by the Local Ethics Committee. General characteristics of the subjects are presented in Table 1. The race started at 4:00 am, and runners had to reach the finish line within 17 hours. The highest point of the route was at 1262 m and the total altitude gain was 4500 m. The outdoor temperature ranged between 12°C and 24°C. Humidity was 55% to 60%. The runners were allowed to choose their own speed and had free access to fluids and carbohydrate-rich food available in support tents at the refreshment points. The research station was set up near the end of the race route.

**Measurements**

One week before the run body mass and height were measured, and subjects completed a questionnaire with information on health status and history of sports practice. Then, the subjects were submitted to an incremental, graded exercise test performed on a treadmill (SensorMedics T2000, USA) until volitional exhaustion to determine their maximal oxygen uptake (VO₂max). The treadmill speed was increased by 2 km · h⁻¹ every 3 min starting with 6 km · h⁻¹. Oxygen uptake, carbon dioxide production and heart rate were continuously recorded using a Vmax 29 (SensorMedics, USA) analyser.

**Echocardiographic examination**

A complete echocardiographic study was performed in all subjects prior to, immediately after the ultra-marathon and after 90 min of the recovery period, at the same place where the event was held. Standard two-dimensional mode (2D), pulsed-wave Doppler and tissue Doppler echocardiographic parameters were obtained from parasternal and apical acoustic windows. All echocardiographic images were acquired with a commercially available ultrasound system, MyLab 25 Gold (ESAOTE s.p.a. International Activities, Italy), equipped with a 1-4 MHz phased-array transducer (PA240E) and saved digitally in raw data format to magneto-optical disks for off-line analysis. Doppler echocardiograms were recorded on a strip chart recorder with a sweep speed of 100 mm · s⁻¹. All measurements were performed by the same investigator.

Left ventricular end-diastolic (LVEDd) and end-systolic (LVESd) diameters and wall thickness were measured by 2D targeted M-mode echocardiography according to the principal recommendations of the American Society of Echocardiography [29].

The left ventricular mass (LVM) was calculated using the Devereux formula [30]. Left ventricular end-diastolic (LVEDVd) and end-systolic (LVESVd) volumes and ejection fraction (EF) were determined by biplane Simpson’s method, as recommended by the American Society of Echocardiography [30]. Mean values for end-diastolic and end-systolic volumes were calculated from three consecutive cardiac cycles analysed. Fractional shortening (FS) was calculated according to the equation: $FS = \frac{LVEDVd - LVESd}{LVEDVd} \times 100$. For the pulsed-wave Doppler echocardiographic assessment of early (E) and late (A) diastolic filling peak velocities, the apical 4-chamber orientation was used with a 4 mm sample volume positioned at the tips of the mitral leaflets in diastole. From these data the E/A ratio was calculated. The deceleration time of early filling (DT) was measured from the maximum E-wave point to baseline.

| TABLE 1. Subject characteristics (n=9; mean values ± SEM; range) |
|-----------------------|-------|-------|
| Age (year)            | 30.0 ± 1.0 | 25-38 |
| Height (cm)           | 178.0 ± 2.0 | 169-186 |
| Body mass (kg)        | 72.0 ± 2.0 | 67-83 |
| Body mass index (kg · m⁻²) | 22.6 ± 0.4 | 20-25 |
| VO₂max (ml · kg⁻¹ · min⁻¹) | 54.1 ± 3.0 | 42-63 |
| Running history (year)| 4.9 ± 1.3 | 1-14 |
| Running distance (km · week⁻¹) | 92.2 ± 4.0 | 70-110 |
| Running time (h · week⁻¹) | 10.8 ± 0.7 | 9-15 |
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The isovolumetric relaxation time (IVRT) was measured from the Doppler spectral display as the time from the end of left ventricular outflow to the start of early transmural filling. For the tissue Doppler assessment of peak systolic (S'), early diastolic (E') and late diastolic (A') mitral annulus peak velocities in both the septal and lateral walls the apical 4-chamber view was used with a 2 mm sample volume positioned at the septal and lateral sides of the annulus. From these data the ratios E'/A' and E/E' were calculated. All measurements were made off-line on three consecutive cardiac cycles and averaged values are reported. Heart rate was calculated on the basis of the strip chart of Doppler echocardiography. Blood pressure (BP) measurements in the sitting position were performed using a standard sphygmomanometer before each echocardiographic evaluation.

Biochemical analyses

Before, immediately after and 90 minutes after completion of the ultra-marathon, blood samples were taken for determination of the plasma concentrations of IL-6, IL-10, IL-18, IL-1β and tumour necrosis factor alpha (TNF-α). The blood samples were collected in tubes containing EDTA. All samples were centrifuged within 15 minutes at 3000 rpm at 4°C, and stored at -70°C until further processed. High-sensitivity ELISA kits provided by R&D Systems (Minneapolis, MN, USA) were used for the pre-race blood samples for IL-6, IL-10 and TNF-α (Quantikine HS ELISA Human IL-6 Immunoassay, Quantikine HS ELISA Human IL-10 Immunoassay and Quantikine HS ELISA Human TNF-α Immunoassay, respectively) because these cytokines exist at very low levels in peripheral blood. Quantitative sandwich ELISA kits were used for the post-race and recovery blood samples for IL-6, IL-10, IL-18, IL-1β and TNF-α (R&D Systems Inc.; Minneapolis, MN, USA). Plasma concentration of IL-18 was determined using a Human IL-18 Instant ELISA kit (eBioscience, Bender MedSystems GmbH, Austria). All samples and provided standards were analysed in duplicate. Haematocrit was measured in duplicate. Whole blood samples (~9 µl) were transferred to heparinized microcentrifugation. Haematocrit was used to calculate percent microcapillary tubes and analysed by an automated system following high-speed centrifugation. The resting values of heart rate (HR) and systolic (SBP) and diastolic (DBP) arterial blood pressures were within normal limits. The characteristics of subjects are summarized in Table 1. This group of ultramarathoners was highly experienced, having trained for long distance races for an average of five years.

Statistics

The data are presented as means with standard errors (SEM). Comparisons of pre-race, post-race and recovery values were performed by one-way repeated-measures ANOVA analysis of variance followed by Student’s t-test for paired data with Bonferroni correction as a post-hoc test. The normality of variables was analysed by the Shapiro-Wilk test. The relationships between changes in plasma cytokines and those in echocardiographic parameters were analysed using Pearson’s correlation coefficient. A p value < 0.05 was accepted as the level of significance. For calculations, Statistica (2001) version 6 (StatSoft Inc., Tulsa, OK, USA) was used.

RESULTS

The pre-race parameters of both subjects’ characteristics (Table 1) and measured variables (Tables 2-4) were normally distributed.

Subject characteristics

The characteristics of subjects are summarized in Table 1. This group of ultramarathoners was highly experienced, having trained for long distance races for an average of five years.

Interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-18 (IL-18), tumour necrosis factor alpha (TNF-α) and haematocrit

The ultra-endurance exercise caused significant increases in plasma IL-6, IL-10, IL-18 and TNF-α (p<0.01) in all subjects. At 90 min of the recovery period, plasma concentrations of IL-6 and IL-10 were still higher (p<0.05) than before exercise, whereas plasma TNF-α did not differ significantly from the resting pre-exercise values (Table 2). The exercise-evoked increases in plasma TNF-α concentration correlated negatively with those in the DT (r=-0.73; p<0.05). A significant positive relationship was found between the changes in plasma TNF-α at 90 min of recovery and those in FS (r=0.73; p<0.05). No significant changes were seen in plasma IL-1β and the haematocrit.

Heart rate and blood pressure

The resting values of heart rate (HR) and systolic (SBP) and diastolic (DBP) arterial blood pressures were within normal limits. The

TABLE 2. Plasma concentration of interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-18 (IL-18), interleukin-1 (IL-1β) and tumour necrosis factor alpha (TNF-α) before and after the ultra-endurance exercise (n=9; the values are mean ±SEM).

|                  | Pre-race        | Immediately post-race | After 90 min of recovery |
|------------------|-----------------|-----------------------|--------------------------|
| TNF-α (pg·ml⁻¹) | 1.39 ± 0.09     | 1.63 ± 0.09*          | 1.54 ± 0.09              |
| IL-6 (pg·ml⁻¹)  | 0.54 ± 0.07     | 47.35 ± 8.48*         | 37.67 ± 7.94*††          |
| IL-10 (pg·ml⁻¹) | 0.31 ± 0.06     | 5.04 ± 1.34*          | 1.24 ± 0.35†             |
| IL-18 (pg·ml⁻¹) | 75.08 ± 9.46    | 96.74 ± 9.92*         | 101.25 ± 9.28            |
| IL-1β (pg·ml⁻¹) | 0.76 ± 0.24     | 1.30 ± 0.27           | 0.70 ± 0.12              |

Note: * - significant differences from the pre-race resting values: P < 0.05. †† - significant differences from the immediately post-race values: P < 0.01.
ultra-endurance exercise caused significant increases in HR (p<0.001) in all subjects. After 90 min of the recovery period HR was still significantly higher (p<0.05) and DBP significantly lower (p<0.05) than before exercise (Table 3).

**Echocardiography**

The echocardiographic parameters are reported in Table 4. The resting echocardiographic parameters were within normal limits. The ultra-endurance exercise caused significant (p<0.05) decreases in both early diastolic left ventricular filling peak velocity (E) and the ratio of early to late ventricular filling velocities (E/A) without significant changes in A. The ratio of early diastolic mitral inflow to early diastolic mitral annular tissue velocity (E/E') did not change significantly from rest to 90 min of the recovery period, as a consequence of similar reduction in E and E'. No significant changes in LVEF and FS data were noted immediately after exercise. However, a small, statistically insignificant reduction in left ventricular end-diastolic diameter (LVEDd), LVEF and mitral annular plane systolic excursion (MAPSE) as well as increases in deceleration time (DT) and isovolumic relaxation time (IVRT) were observed in five subjects immediately after the exercise. This left ventricular dysfunction was global, with no changes in segmental contraction. At 90 min of the recovery period, the E/A ratio did not differ significantly from the pre-exercise values, whereas E velocity was still lower than before exercise (p<0.05). Segmental analysis of myocardial velocities at the basal left ventricular walls demonstrated an increase in A' at the lateral segment at 90 min of the recovery period which resulted in a decrease of E'/lat/A'lat ratio. These changes were accompanied by a reduced LVEDd and FS (p<0.05).

Ultra-endurance exercise did not result in significant changes in the ratio of early diastolic mitral inflow to early diastolic mitral annulus velocity (E/E').

Significant negative correlations were found between the changes in plasma TNF-alpha and those in DT (r=-0.73; p<0.05) as well as positive correlations between the changes in plasma TNF-alpha and those in FS at 90 min of the recovery period (r=0.73; p<0.05).

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**TABLE 3.** Heart rate (HR) and blood pressure (BP) before and after the ultra-endurance exercise (n=9; the values are mean ±SEM).

|                     | Pre-race  | Immediately post-race | After 90 min of recovery |
|---------------------|-----------|-----------------------|--------------------------|
| HR (beats·min⁻¹)    | 58±3.0    | 84±2.0*               | 78±3.0†                  |
| SBP (mm Hg)         | 132±4.0   | 114±4.0               | 121±5.0                  |
| DBP (mm Hg)         | 85±2.0    | 74±2.0                | 78±3.0†                  |

Note: SBP – systolic blood pressure, DBP – diastolic blood pressure; * - significant differences from the resting values: P < 0.05. † - significant differences from the immediately post-race values: P < 0.05.

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**DISCUSSION**

The main finding of the present study is that the ultra-endurance exercise increased plasma levels of all measured cytokines and decreased the ratio of early to late diastolic mitral inflow peak velocities (E/A), without any changes in the ratio of early diastolic mitral inflow peak velocity to early diastolic mitral annulus peak velocity (E/E'). Additionally, at the 90th minute of the recovery period LVEDd and FS were significantly lower than before exercise, and the ratio of early to late diastolic mitral annulus peak velocities at the lateral side
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(E’lat./A’lat.) was reduced as a result of an increase in A’ velocity. No significant changes in left ventricular ejection fraction and fractional shortening data were noted immediately after exercise. The changes in both immune function and left ventricular relaxation observed in this study confirm the results of previous investigations in prolonged intense exertion such as the marathon or ultra-marathon [3, 11, 13, 14, 27, 31, 32, 33, 34]. Most authors believe that increased release of pro-inflammatory cytokines during strenuous exercise is associated with a number of interrelated factors such as muscle damage, glycogen deficiency, oxidative stress, releasing endotoxins or an increase in plasma cortisol and catecholamines [11, 13, 35, 36, 37]. Nieman et al. [10], who studied athletes competing in a 160-km race event, reported significant positive correlations between the exercise-induced increases in plasma cytokines (IL-6, IL-10, IL-1α, G-CSF, MCP-1, MIP-1β), perceptions of muscle soreness and plasma creatine phosphokinase, a marker of muscle damage. The strongest relationships were seen with IL-6 and G-CSF. Additionally, modest positive correlations were found between cytokines (IL-6, IL-8, IL-10) and F(2)-isoprostane, an indicator of oxidative stress, at 90 km of the 160-km race event when the greatest oxidative stress occurred [15]. The mechanisms explaining the inflammatory response to muscle damage are still poorly understood. It is believed that ultrastructural damage to skeletal muscle is associated with neutrophil infiltration and muscle IL-1 beta accumulation [7, 8]. It was found that IL-1 beta indirectly promotes chemotaxis of neutrophils by inducing production of IL-8 [38]. The positive relationship between muscle IL-1 beta and creatine kinase may indicate an indirect influence of IL-1 on neutrophil degranulation and superoxide release that subsequently affects muscle membrane permeability [7]. Since the exercise-induced percent rise in neutrophil count was correlated positively with cortisol, growth hormone, IL-6 and IL-8 kinetics, it was suggested that both stress hormones and cytokines may be important for inducing neutrophilia [9]. It was also demonstrated that IL-1 beta and TNF-alpha may act synergistically to induce myofibrillar proteolysis [39]. Some cytokines, such as L-6, can be released regardless of muscle damage. Several studies have shown that IL-6 is tightly regulated in response to exercise, being affected by factors such as exercise intensity and availability of energy substrates. Keller et al. [40] demonstrated that lowering muscle glycogen content prior to exercise as well as low muscle glycogen concentration during prolonged exercise enhanced the induction of IL-6 gene transcription and IL-6 mRNA in exercising skeletal muscle. The authors suggested that contracting skeletal muscle tissue is the main source of IL-6 production during this type of exercise. It should be noted that IL-6, besides antibody induction, may play a metabolic role by increasing hepatic glucose output and induction of lipolysis and fatty acids oxidation [36]. Wallberg et al. [41] investigated the kinetics of IL-6 in human plasma during ultra-endurance exercise and found that during exercise lasting over 12 hours the intensity is a major determinant of the IL-6 response. The present study demonstrated an over 80-fold increase in the plasma interleukin-6 concentration immediately after ultra-endurance exercise. As mentioned in the introduction, some cytokines, especially TNF-α, not only can act as immune-cell mediators but also can exert cardiodepressive action which results in progressive left ventricular dysfunction [19, 20, 21, 23]. This effect is concentration-dependent and reversible. The relationship between exercise-induced changes in plasma TNF-alpha and those in deceleration time shown in this study may indicate that the changes in plasma TNF-α during ultra-marathon running are related to cardiac performance. However, bearing in mind the complex network of cardiac reflexes and neurohumoral mediators involved in long duration exercise, the causal relationship between changes in plasma TNF-alpha and cardiac performance should be considered with caution. It cannot be excluded that the expected behaviour of this cytokine after ultra-marathon running may simply match in time the occurrence of left ventricular alterations. More research is needed to clarify the main source of TNF-alpha in response to this kind of exercise.

The alterations in echocardiographic indices of LV function after ultra-marathon running observed in this study are consistent with the findings of Passaglia et al. [27] and Hart et al. [42], who reported decreases in E/A and E’/A’ ratios after an ultra-marathon or marathon race in trained male runners. The authors concluded that the decrease in E/A ratio represents the complex of haemodynamic changes in the total blood volume and cardiovascular physiology. They believe that the decrease in E/A ratio after prolonged exercise may simply correlate with preload reduction [27]. On the other hand, the decrease in E’/A’ ratio may indicate that there is also an intrinsic impairment in myocardial relaxation. In the present study, immediately after the ultra-marathon, the E/A ratio was significantly decreased by 20% pre-race to post-race as a result of a 19% decrease in early mitral inflow peak velocity. At the 90th minute of the recovery period, the early left ventricular filling peak velocity was still lower than before running. The present study showed that in contrast to early mitral inflow peak velocity, early mitral annulus peak velocity in both the septal and lateral walls as well as the ratio of E/E’ that is used as an estimate of left ventricular filling pressure did not change significantly immediately after the ultra-marathon running. However, at the 90th minute of the recovery period the E’lat./A’lat. ratio was lower than immediately after running because of an increase in A’lat. This is consistent with the findings of Neilan et al. [33], who reported a greater decrease in E’/A’ ratio at the lateral segment compared with the septal segment. Some authors suggest that differences in regional decrements in diastolic function may be explained by alterations in the interaction between the left ventricle and right ventricle during diastole [32, 43, 44] and that E’ velocity is a relatively preload-independent variable in evaluating left ventricular diastolic function [45, 46]. It should be noted, however, that the lateral annular velocities can be affected by the translational movement of the heart and beam angle [45]. The changes in the E’lat./A’lat. ratio, observed in this study, were accompanied by decreases in diastolic blood pressure, left ventricular end-diastolic diameter and endocar-
dial fractional shortening. There was no change in haematocrit levels similar to what was found in previous studies with ultra-marathon runners [47].

CONCLUSIONS

In summary, the present study demonstrated that in well-trained young men, ultra-endurance exercise lasting 17 hours does not result in left ventricular systolic dysfunction. However, the exercise-induced changes in diastolic transmural flow velocities observed immediately after exercise suggest that ultra-endurance exercise may induce alterations in left ventricular diastolic function in healthy trained men. Additionally, the exercise-induced significant increases in plasma IL-6, IL-10, IL-18 and TNF-alpha may reflect both immunological changes in skeletal muscle and exercise-induced endotoxemia. The significant correlations between the changes in plasma TNF-α and those in DT and FS suggest that haemodynamic factors may be involved in the increased plasma TNF-alpha release during ultra-endurance exercise.

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