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Systemic pro-inflammatory molecule response to acute submaximal exercise in moderately and highly trained athletes

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Abstract

The effect of training status on acute exercise-induced release of pro-inflammatory biomarkers in circulatory system was studied in 10 moderately trained and seven highly trained athletes. The subjects performed an hour of submaximal bicycle exercise at workload equal to ~70% of heart rate reserve. Venous blood samples were collected 15 min before and immediately after the exercise. Intereukin-6, tumor necrosis factor-α, and monocyte chemotactic protein-1 response to exercise was similar in moderately trained and highly trained athletes (2.5 ± 2.4 vs. 1.0 ± 1.0 pg mL^{-1}; 0.4 ± 0.6 vs. 0.8 ± 0.8 pg mL^{-1}; 50 ± 67 vs. 74 ± 116 pg mL^{-1}, respectively), suggesting that training status had no impact on cytokine and monocyte chemotactic protein-1 post-exercise concentrations with this experimental model.

Key words: acute exercise, pro-inflammatory molecules, training.
Abbreviations: CK, creatine kinase; E-selectin, endothelial-leukocyte adhesion molecule-1; HRR, heart rate reserve; HT, highly trained athletes; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; LDH, lactate dehydrogenase; MCP-1, monocyte chemotactic protein-1; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; MT, moderately trained athletes; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule..

Introduction

Exercise and physical strenuous activity are associated with an inflammatory response involving the activation of several types of blood cells (Chaar et al. 2011), increase in pro-inflammatory cytokines (Kinugawa et al. 2003), adhesion molecules (Nielsen, Lyberg 2004), chemokines (Peake et al. 2005; Smith et al. 2007), myeloperoxidase (MPO) (Melanson et al. 2006) and matrix mettaloproteinase-9 (MMP-9) (Rullman et al. 2009). It has been shown that these molecules to some extent are linked in common pathways when inflammatory response is generated. For example, it has been suggested that interleukin-6 (IL-6) exerts modulatory effects on MMP-9 release from skeletal muscle cells (Srivastava et al. 2007), fibroblasts (Dasu et al. 2003), and neutrophils (Chen et al. 2006). Furthermore, both IL-6 and tumor necrosis factor-α (TNF-α) induce adhesion molecule (Weber et al. 1995; Karatzis 2005), and MCP-1 (Biswas et al. 1998; Sobota et al. 2008; Ahmed et al. 2009) expression from various tissues. Thus, one of the aims of this study was to test whether there are relationships between these mediators that are induced by exercise.

There are many factors that can influence systemic levels of cytokines and other blood biomarkers. Exercise-induced local muscle damage, bioavailability of carbohydrates, and high intensity and long duration of the exercise can be the cause of increased blood IL-6 (Helge et al. 2003; MacDonald et al. 2003; Nieman et al. 2003; Nieman et al. 2007; Toth et al. 2011; Wallberg et al. 2011), TNF-α (Kimura et al. 2001; Bernecker et al. 2011) adhesion molecules (Akimoto et al. 2002; Nielsen, Lyberg 2004), MMP-9 (Danzig et al. 2010; Madden et al. 2011), MPO (Morozov et al. 2003), and monocyte chemotactic protein-1 (MCP-1) (Suzuki et al. 2003) concentrations. Studies describing the impact of training status on cytokine responses to acute exercise are not conclusive. It has been suggested that athletes have an attenuated cytokine response to acute exercise (Gokhale et al. 2007). However, higher post-exercise responses in IL-6, but not in TNF-α, were shown to be positively related to performance improvement in highly trained male rowers

(Maestu et al. 2010). Finally, an acute exercise-induced plasma IL-6 concentration response was shown to remain unchanged after 10 weeks of endurance training (Fischer et al. 2004). Thus, the second aim of this study was to investigate the release of cytokines, e.g. IL-6 and TNF-α, adhesion molecules, MMP-9, MPO and MCP-1 during submaximal exercise, and to test if there are differences in release between moderately and highly trained athletes.

Materials and methods

Subjects

After approval of experimental procedures by the Ethical Committee of the Institute of Experimental and Clinical Medicine, University of Latvia, written informed consent was obtained from 17 young healthy males (age 21 to 35 years; Table 1). The subjects were divided in moderately trained (MT) and highly trained (HT) athelets based on weekly training hours (MT < 7 h; HT > 10 h) and average physical load (MT < 2.5 W kg^{-1} > HT) during the exercise. Before the exercise, anthropometric characteristics (weight, height etc.) were collected by investigators using standard procedures.

Submaximal exercise protocol

On the experimental day, the subjects arrived at the laboratory at 14:00 to 16:00. All subjects performed one bout of prolonged submaximal recumbent cycling exercise on a veloergometer Ergoselect 600P (Ergoline GmbH, BLTZ, Germany) for 1 h. Intensity of 70% of heart rate reserve (HRR) was reached during the first 10 min of cycling and was maintained during the whole exercise. Target heart rate for each subject was calculated by the use of the Karvonen formula (Karvonen et al. 1957). Before the exercise, maximal heart rate was determined using the Polar Fitness Test provided by Polar S810. The Polar Fitness Test is based on precise detection of heart rate and heart rate variability at rest (Gamelin et al. 2006) and personal information, e.g. age, height and weight. Heart rates were monitored by the 12 lead Stress Test ECG system (AMEDTEC ECGpro®, GmbH, Aue, Germany) throughout the experiment.

Table 1. Subject characteristics in the present study. Values are means ± SD. #, significant difference between the groups

Parameter	Moderately trained athletes	Highly trained athletes
Age (years)	26 ± 5	26 ± 2
Body mass index (kg m^{-2})	22.6 ± 1.7	22.7 ± 2.0
Moderate intensity training (h $week^{-1}$)	5.4 ± 1.6	11.1 ± 4.3#
70% HRR (beats min^{-1})	150 ± 5	149 ± 5
Load (W kg^{-1})	2.2 ± 0.2	2.7 ± 0.3#

Blood sampling and analysis

Venous blood samples for IL-6, TNF-α, MMP-9, MCP-1, soluble forms of endothelial-leukocyte adhesion molecule 1 (sE-selectin), intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule (sVCAM-1), MPO and standard blood tests (e.g., leukocyte formula) were taken 15 min before exercise and immediately after the exercise. Resting venous blood samples were taken after 3 h fast (subjects were asked to refrain from high fat or carbohydrate diet, instead having a balanced meal for lunch), and all subjects were asked to refrain from caffeine, alcohol, nicotine and medication for 24 h before the blood sampling, as well as to avoid physical overload or other stressors.

Blood samples were collected without anticoagulant and were allowed to coagulate for 20 to 30 min. Serum was separated by centrifugation and all specimens were aliquoted, frozen, and stored at –80 °C. Commercially available multiplex immunoassay kits (MILLIPLEX MAP kit Human Adipocyte 96 Well Plate Assay Cat. No. HADCYT-61K; MILLIPLEX MAP kit Human Cytokine/Chemokine 96-Well Plate Assay Cat. No. MPXHCYTO-60K; and MILLIPLEX MAP kit Human Cardiovascular Disease Panel 1 96 Well Plate Assay Cat. No. HCVD1-67-AK) were used for quantitative determination of IL-6, IL-8, TNF-a, sE-Selectin, sVCAM-1, sICAM-1, MCP-1, MMP-9 and MPO by a Luminex 200 analyzer (Luminex Corp., Austin, TX, USA).

To avoid inter-assay variation both measurements (15 min before and after exercise) were analysed in the same assay for each subject. Manufacturer provided intra-assay coefficients of variation for particular parameters were 7.9% (IL-6), 7.3% (TNF-α), 6.8% (MMP-9), 7.9% (sICAM-1), 6.1% (MCP-1), 11.2% (sE-Selectin), 4.5% (sVCAM-1), and 12.3% (MPO). Cortisol was measured by an Immulite 2500 analyzer (Siemens Medical Solutions, USA).

Other blood tests [haemoglobin, haematocrit, leukocyte formula, creatine kinase (CK), lactate dehydrogenase (LDH), glucose, lactate] were performed in a certified clinical laboratory "E. Gulbja laboratorija", Riga, Latvia.

Correction of plasma concentrations for blood cells and inflammatory molecules were made according to the method described previously (Dill, Costill 1974).

Statistical analysis

The levels of measured biomarkers below the detection limit were assumed to be the detection limit value. Data were analysed by Sigma plot 11.0 software (Systat Software Inc., San Jose, CA, USA). Two-way repeated measure ANOVA and t-tests were performed as appropriate. Data for correlation analysis were expressed as differences between absolute values of measured parameters before and after exercise (Δ) and assessed by Pearson´s correlation. Data were expressed as mean ± standard deviation. A value of $P < 0.05$ was considered to be significant.

Results

All athletes performed exercise with similar relative intensity – 70% of HRR (149 ± 5 beats min^{-1}). Absolute workload for the HT group was significantly higher than that for the MT group (Table 1, $P < 0.05$).

The haemoglobin concentration and haematocrit were at higher levels after exercise (Table 2). The subjects were not allowed to drink any liquid during the exercise, and thus the reduction of blood and subsequently plasma volume can be explained by dehydration. Concentrations of blood cells are shown in Table 2. All levels of subpopulations of leukocytes, except eosinophils and monocytes ($P < 0.05$), increased with exercise across both groups. Both leukocytes and neutrophil levels increased significantly in the MT group compared to the HT group ($P < 0.05$).

There was no indication of skeletal muscle damage, as CK, LDH and lactate concentrations did not change with exercise (Table 3, $P > 0.05$). Peak concentrations of cortisol were significantly elevated in both groups ($P < 0.05$, Table 3). There was a decrease in glucose concentration with exercise across both groups ($P < 0.05$, Table 3).

There was also an increase in IL-6, TNF-α and MCP-1 concentrations with exercise across both groups ($P < 0.05$, Table 3). When compared to the pre-exercise values (Table 3), only the MT group demonstrated an increase in the post-exercise IL-6 concentration. In contrast, exercise-induced increase in TNF-α concentration was seen only in the HT group, but not in the MT group (Table 3). There were no exercise-induced changes in MMP-9, MPO, sE-selectin, sICAM-1 and sVCAM-1 concentrations ($P > 0.05$, Table 3). Furthermore, there were no differences in post-exercise biomarker concentrations between the two groups ($P > 0.05$, Table 3).

Statistical analysis revealed a significant relationship between maximal exercise-induced changes in MMP-9 and MPO concentrations ($P < 0.05$, $R = 0.57$, $n = 17$).

Discussion

The present study showed that the elevated levels of IL-6, TNF-α and MCP-1 after acute exercise were not associated with the training status, as there were no differences between submaximal exercise-induced increases in the concentration of these mediators in the MT group and in the HT group ($P > 0.05$). This does not, however, rule out the possibility that training status might have influenced the exercise-induced pro-inflammatory molecule response in other experimental models, previously shown by others (Gokhale et al. 2007; Maestu et al. 2010).

Exercise is associated with temporary changes in the immune system, e.g. in concentrations of immune cells (Lippi et al. 2010), cytokines and chemokines (Suzuki et al. 2003). Two major mechanisms appear to drive the immune response to exercise: neuroendocrine factors and muscle damage. In the present study the serum concentrations of lactate and the muscle enzymes indicating microtrauma – CK and LDH, remained unchanged during submaximal exercise. However, the study showed that submaximal exercise (1 h) induced leukocytosis mainly by neitrophilia, suggesting that neuroendocrine factors, e.g. adrenaline, are responsible for the acute exercise effects on lymphocytes (Pedersen, Toft 2000). This study also showed that submaximal exercise increased cortisol concentration.

Previous studies have shown that exercise-induced activation of the secretory function of neutrophils results in their degranulation, which leads to an increase in plasma concentration of marker neutrophil proteins, including MPO (Morozov et al. 2003). The present study showed that MPO concentration remained unchanged, suggesting that the intensity of the exercise was not high enough to elicit muscle damage and subsequent activation of the secretory function of neutrophils. It has been suggested that leukocytes (Chen et al. 2006) and human skeletal muscle (Rullman et al. 2007) express MMPs, including

Table 2. Pre-exercise and post-exercise haematological parameters. Values are means ± SD. *, significant difference compared to pre-exercise. #, significant difference between the groups

Parameter	Moderately trained athletes		Highly trained athletes		Two way repeated measure ANOVA	
	Pre-exercise n = 10	Post-exercise n = 10	Pre-exercise n = 7	Post-exercise n = 7	Main effect of group	Main effect of exercise
Haemoglobin (nmol L^{-1})	9.1 ± 0.6	9.5 ± 0.5*	9.0 ± 0.7	9.3 ± 0.8	NS	P < 0.001
Haematocrit	45 ± 3	47 ± 2*	45 ± 3	47 ± 3*	NS	P < 0.001
Leukocytes (× 10^9 cells L^{-1})	6.1 ± 1.1	8.3 ± 2.1*	5.0 ± 1.1	5.8 ± 0.7*#	P < 0.01	P < 0.001
Neutrophils (× 10^9 cells L^{-1})	3.5 ± 1.0	4.9 ± 1.8*	2.8 ± 0.8	3.2 ± 0.5#	P < 0.05	P < 0.01
Basophils (× 10^9 cells L^{-1})	0.07 ± 0.03	0.08 ± 0.02	0.04 ± 0.02	0.06 ± 0.03*	NS	P < 0.05
Eosinophils (× 10^9 cells L^{-1})	0.19 ± 0.07	0.19 ± 0.09	0.14 ± 0.08	0.16 ± 0.13	NS	NS
Monocytes (× 10^9 cells L^{-1})	0.50 ± 0.21	0.56 ± 0.22	0.32 ± 0.10	0.33 ± 0.08#	P < 0.05	NS
Lymphocytes (× 10^9 cells L^{-1})	1.86 ± 0.46	2.54 ± 0.67*	1.67 ± 0.36	2.08 ± 0.32	NS	P < 0.001

Table 3. Pre-exercise and post-exercise blood mediator measures. Values are means ± SD. *, significant difference compared to pre-exercise; #, significant difference between the groups

Parameter	Moderately trained athletes		Highly trained athletes		Two way repeated measure ANOVA	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Main effect	Main effect
	n = 10	n = 10	n = 7	n = 7	of group	of exercise
IL-6 (pg mL^{-1})	1.1 ± 1.3	3.7 ± 3.2*	0.6 ± 0.0	1.6 ± 1.0*	NS	P < 0.01
TNF-α (pg mL^{-1})	4.2 ± 1.6	4.6 ± 2.0	4.9 ± 0.7	5.7 ± 0.8*	NS	P < 0.01
MMP-9 (ng mL^{-1})	89 ± 54	146 ± 102	98 ± 77	118 ± 41	NS	NS
MCP-1 (pg mL^{-1})	279 ± 137	329 ± 97	273 ± 58	346 ± 137	NS	P < 0.05
sE-selectin (ng mL^{-1})	20 ± 6	20 ± 5	26 ± 6	25 ± 6	NS	NS
sICAM-1 (ng mL^{-1})	97 ± 31	92 ± 23	129 ± 41	117 ± 17#	P < 0.05	NS
sVCAM-1 (ng mL^{-1})	1301 ± 206	1294 ± 192	1296 ± 299	1382 ± 288	NS	NS
MPO (ng mL^{-1})	47 ± 32	45 ± 24	33 ± 21	39 ± 16	NS	NS
Creatine kinase (IU L^{-1})	177 ± 80	181 ± 83	172 ± 84	175 ± 84	NS	NS
Lactate dehydrogenase (IU L^{-1})	314 ± 103	309 ± 105	345 ± 60	359 ± 71	NS	NS
Glucose (mmol L^{-1})	4.8 ± 0.5	4.1 ± 0.3*	5.1 ± 0.9	4.5 ± 0.5	NS	P < 0.01
Lactate (mmol L^{-1})	2.3 ± 1.3	2.6 ± 0.9	1.7 ± 0.3	1.8 ± 0.3#	NS	NS
Cortisol (nmol L^{-1})	271 ± 114	458 ± 128*	306 ± 62	546 ± 112*	NS	P < 0.001

MMP-9, as a response to local damage of skeletal muscles and connective tissue, in order to cleave muscle-specific proteins and contribute in extracellular matrix formation, remodelling, and regeneration in skeletal muscle (Urso et al. 2009). There was no significant change in MMP-9 concentrations, supporting the notion that exercise intensity and duration applied in this study did not elicit muscle damage.

Muscle damage that often is caused by eccentric exercise increases IL-6 concentrations during exercise (Bruunsgaard et al. 1997; Toft et al. 2002). It has been suggested that IL-6 released by the contracting muscles may improve skeletal muscle energy supply and assist in the maintenance of stable blood glucose levels during exercise (Pedersen 2009). However, the previously obtained results on the effect of low carbohydrate bioavailability on IL-6 release during exercise are not conclusive. Some studies have shown that low plasma glucose and muscle glycogen levels increase IL-6 release (Helge et al. 2003; Nieman et al. 2003), whereas others did not confirm a relationship between IL-6 and carbohydrate turnover (Nieman et al. 2004; Helge et al. 2011). It appears that the observed increase in IL-6 concentration in this study is less connected with muscle damage, as there were no changes in CK and LDH levels, nor in activation of neutrophil degranulation. In fact, we observed a decrease in glucose concentrations and an increase in cortisol concentration, confirming activation of the hypothalamo-pituitary-adrenal axis in order to ensure growing energy demands to the organism (de Vries et al. 2000). Thus, it is plausible that IL-6 can be released from working muscles in order to maintain glucose homeostasis.

It has been suggested that athletes have an attenuated cytokine response to acute exercise (Gokhale et al. 2007). This notion was based on the fact that intermittent running exercise induced greater magnitude of change in venous plasma IL-6 and TNF-α concentrations in non-athletes compared to athletes (Gokhale et al. 2007). In contrast, significant improvement in acute 6000 meter rowing resulted in an increase of post-exercise venous plasma IL-6 concentrations in highly trained athletes (Maestu et al. 2010). Although there was a tendency for higher post-exercise IL-6 concentrations in the MT group compared to the HT group ($P = 0.085$), we did not observe a significant difference between the two groups. This is in agreement with previous findings, which showed that the arterial plasma IL-6 response to acute exercise remained unchanged after 10 weeks of endurance training, despite markedly lower skeletal muscle IL-6 mRNA expression (Fischer et al. 2004).

It is well known that IL-6 and TNF-α promote expression of adhesion molecules in leukocytes and endothelial cells (Weber et al. 1995; Romano et al. 1997; Karatzis 2005; Monchanin et al. 2007). Similarly to other studies, our data showed a significant increase in the concentration of IL-6 and TNF-α after submaximal exercise (Kimura et al. 2001; Suzuki et al. 2003) but not in adhesion molecules. Both IL-6 and TNF-α also induce MCP-1 expression from various tissue (Biswas et al. 1998; Sobota et al. 2008; Ahmed et al. 2009). In agreement with other studies (Suzuki et al. 2003; Peake et al. 2005), we demonstrated a significant increase in MCP-1 concentration after submaximal exercise. Not only proinflammatory cytokines, but also increased shear stress during exercise activates endothelium and monocytes, both of which may release MCP-1.

In conclusion, acute submaximal exercise resulted in greater leukocyte counts and increased release of serum IL-6, TNF-α and MCP-1. The response of IL-6, TNF-α and MCP-1 to exercise in moderately trained athletes was similar to that in highly trained athletes, suggesting that

training status had no impact on cytokine and MCP-1 post-exercise concentrations in this experimental model.

However, this study lacks a control group not performing exercise, thus we cannot exclude that along with the exercise some of the observed changes could be due to day-to-day fluctuations etc. Blood sampling during recovery would provide more information about the kinetics of measured systemic variables.

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