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The Relationship Between Maximal Exercise-Induced Increases in Serum IL-6, MPO and MMP-9 Concentrations

D. Reihmane*†, A. Jurka† & P. Tretjakovs†‡

*Xlab, Center for Healthy Aging, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; †Institute of Clinical and Experimental Medicine, University of Latvia, Riga, Latvia; and ‡Pauls Stradins Clinical University Hospital, Riga, Latvia

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Correspondence to: D. Reihmane, Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark and Institute of Clinical and Experimental Medicine, University of Latvia, Ojara Vaciesa str. 4, LV-1004, Riga, Latvia. E-mail: dace.reihmane@sund.ku.dk; dace.reihmane@lu.lv

Abstract

The aim of this study was to test the hypothesis that exercise would induce inflammatory response characterized by increased pro-inflammatory cytokines – interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), adhesion molecule, matrix metalloprotease-9 (MMP-9) and myeloperoxidase (MPO) levels. Additional aim was to elucidate the possible source of maximal exercise-induced increase in MMP-9 concentration. To examine our hypothesis, 26 professional male ice hockey players [age 25 ± 1 (mean ± SEM) years; BMI 25.8 ± 0.4 kg/m^2] performed an incremental bicycle test until exhaustion, when maximal oxygen consumption was recorded. Venous blood samples were collected 30 min before and 2 min after exercise. There was an increase in the count of leucocytes (8.7 ± 1.8 versus 5.7 ± 1.3 × 10^9 cells per l) and IL-6 (1.24 ± 0.17 versus 0.69 ± 0.13 pg/ml), MPO (72 ± 7 versus 50 ± 4 ng/ml) and MPP-9 (139 ± 9 versus 110 ± 6 ng/ml) concentrations ($P < 0.05$) comparing post- and pre-exercise levels. Maximal exercise-induced increase in MPO correlated with the increases in IL-6 ($P < 0.05$, $R = 0.54$) and MMP-9 ($P < 0.01$, $R = 0.62$) concentrations. Furthermore, increase in IL-6 correlated with the increase in MMP-9 concentrations ($P < 0.05$, $R = 0.60$). Maximal exercise induces an inflammatory response characterized by leucocytosis and increased IL-6, MPO and MMP-9 concentrations. Correlations between increased MPO (marker of neutrophils degranulation) and both increased IL-6 and MMP-9 concentrations may suggest that neutrophils could be the main source of these inflammatory biomarkers during maximal exercise. Furthermore, correlation between increases in serum IL-6 and MMP-9 concentrations may suggest that IL-6 could exert modulatory effects on MMP-9 release during maximal exercise.

Introduction

Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are pro-inflammatory cytokines that have been considered as a key orchestrator of the acute phase inflammatory response [1, 2]. Moreover, increases in IL-6 [3, 4] and TNF-α [5] plasma and serum levels play an important role in the host response to acute exercise [6], for example, promoting leucocyte adherence and chemotaxis capacities, by increased expression of adhesion molecules on the surface of leucocytes and endothelial cells [7, 8]. Further on, leucocytes participate in aseptic muscle inflammation associated with muscle fibre injury caused by intensive exercise, and neutrophils reinforce muscle tissue injury assisting in its clearance [9]. Exercise-induced activation of the secretory function of neutrophils results in their degranulation that leads to an increase in plasma concentration of marker neutrophil proteins, including myeloperoxidase (MPO) [10]. Recent studies suggest that also MMP-9 plays a critical role in cleaving muscle-specific proteins and contributing to extracellular matrix formation, remodelling and regeneration in skeletal muscle [11], but it remains unclear which tissues are a basic source of the increased MMP-9 concentration [12] induced by physical exercise. It has been shown that a single bout of exercise can induce MMP-9 expression in skeletal muscle [13] which is stimulated by

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increased levels of TNF-α [14]. Moreover, increased level of IL-6 during inflammation augments MMP-9 expression in leucocytes [1].

The aim of this study was to test the hypothesis that exercise would induce inflammatory response characterized by increased pro-inflammatory cytokines – interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), adhesion molecule, matrix metalloprotease-9 (MMP-9) and MPO levels. Additional aim was to elucidate the source of maximal exercise-induced increase in MMP-9.

Methods

Subjects. After approval of experimental procedures by the Ethical Committee of the Institute of Experimental and Clinical Medicine, University of Latvia, informed consent was obtained from 26 professional male ice hockey players (Table 1). All subjects were asked to fast 3 h before the maximal exercise test, to refrain from caffeine for 12 h, and from alcohol, nicotine and any medication for 24 h, as well as to avoid physical overload or other stressors. Body composition was assessed in all subjects using bioelectrical impedance analyser T Scan (Jawon Medical, Kyungsan, Korea).

Maximal exercise protocol. We measured maximal oxygen consumption on a cycle ergometer Monark Ergomedic 839E (Monark, Sweden) because the muscle groups used in ice skating are quite similar to those used in cycling. Cortex Metalyzer 3B system (Cranlea & Company, Birmingham, UK) was used to evaluate cardio-respiratory functions (ventilation, respiratory exchange ratio, electrocardiogram, heart rate, blood pressure). Athletes achieved their maximal oxygen consumption, when three of four commonly accepted criteria were met: (1) volitional exhaustion; (2) maximal heart rate measured at exhaustion was superior to 90% of the age-predicted maximal heart rate; (3) respiratory exchange ratio was above 1.10; and (4) capillary blood lactate concentration was >8 mM.

Blood sampling and analysis. Venous blood samples for IL-6, TNF-α, sE-selectin, sICAM-1, sVCAM-1, MMP-9, MPO and standard blood tests (leucocyte formula etc.) were taken 30 min before and 2 min after maximal exercise. As these professional athletes had an active training season, time limitation was the main reason for the lack of data during recovery phase. Blood samples for the determination of cytokines, adhesion molecules and other bioactive molecules were collected without anticoagulant and were allowed to coagulate for 20–30 min at room temperature. This was followed by centrifugation for 10 min, at $1600 \times g$ in room temperature. All specimens were immediately aliquoted and put in the freezer at −80 °C. Commercially available multiplex immunoassay kits (MILLIPLEX MAP kit Cat. No.: HCVD1-67 AK; MILLIPLEX MAP kit Cat. No.: HADK2-61K-B) were used for quantitative determination of IL-6, TNF-a, sE-Selectin, sVCAM-1, sICAM-1, MMP-9 and MPO by Luminex 200 analyzer (Luminex Corp., Austin, TX, USA). Cortisol and high sensitivity C-reactive protein (hsCRP) were measured by Immulite 2500 analyzer (Siemens Medical Solutions, USA). Capillary blood lactate concentration was determined using lactate analyzer Biosen 5030 AutoCal (EKF Diagnosticb GmbH, Magdeburg, Germany). Other blood tests were performed in clinical laboratory 'E.Gulbja laboratorija', Riga, Latvia.

Haemoglobin and haematocrit were also measured (Table 2) to take into account the possible influence of exercise-induced plasma volume change. Correction of plasma concentrations for blood cells and inflammatory molecules was made according to the method described previously [15].

Statistical analysis. The levels of measured mediators below the detection limit were arbitrarily assumed to be one-half of the detection limit value [16]. Data were analysed by SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, USA). After testing normality (Shapiro–Wilk test), data with normal distribution were analysed using paired *t*-test. Wilcoxon signed-rank test was used as nonparametric method (data marked as †). Data were expressed as mean ± standard error of the mean (SEM). Data for correlation analysis (Pearson correlation or Spearman rank *R* test) were expressed as difference between absolute values of measured parameters before and after exercise and further labelled in the article as delta (Δ). A value of $P < 0.05$ was considered to be significant.

Table 1 Subject characteristics.

Age (years)	25 ± 1
Body mass index (BMI) (kg/m^2)	25.8 ± 0.4
Fat mass (%)	22 ± 1
Muscle mass (%)	74 ± 1
LDL cholesterol (mM)	2.7 ± 0.2
HDL cholesterol (mM)	1.5 ± 0.1
Triglyceride (mM)	1.2 ± 0.1
VO_{2max} (ml/kg/min)	50.3 ± 1.0
Maximal heart rate (bpm)	188 ± 2
Maximal respiratory exchange ratio	1.12 ± 0.01
Maximal lactate concentration (mM)	10.3 ± 0.4

Values are means ± SEM of 26 male subjects.

Results

Athletes' maximal oxygen consumption at incremental bicycle test was 50 ± 1 ml/kg/min (Table 1). There was a significant increase in haemoglobin concentration and haematocrit (Table 2), probably, because of the reinforced sweating during maximal exercise. Maximal exercise induced significant changes in absolute counts of all leucocyte subsets. Athletes' total leucocyte count was significantly increased after maximal exercise (Table 2).

Table 2 Pre-exercise and post-exercise blood mediators' measures and haematological parameters.

	Pre-exercise	Post-exercise	*P* value	*n*
IL-6 (pg/ml)†	0.69 ± 0.13	1.24 ± 0.17	<0.001	20
TNF-α (pg/ml)	6.31 ± 0.49	6.68 ± 0.56	NS	25
sE-selectin (ng/ml)	29 ± 2	29 ± 2	NS	25
sICAM-1 (ng/ml)†	120 ± 13	111 ± 15	NS	25
sVCAM-1 (ng/ml)	1103± 45	1105 ± 42	NS	25
MMP-9 (ng/ml)†	110 ± 6	139 ± 9	<0.001	25
MPO (ng/ml)	50 ± 4	72 ± 7	<0.001	26
hsCRP (mg/l)	0.55 ± 0.08	0.51 ± 0.08	<0.05	26
Cortisol (nM)	446 ± 27	420 ± 29	NS	26
Insulin (μU/ml)†	7.3 ± 0.9	7.4 ± 0.8	NS	26
Glucose (mM)	5.5 ± 0.1	6.4 ± 0.2	<0.001	26
Haemoglobin (mM)	9.4 ± 0.1	10.0 ± 0.1	<0.001	22
Haematocrit (%)	46 ± 1	50 ± 1	<0.001	22
Leucocytes × 10^9 cells per l	5.7 ± 0.3	8.7 ± 0.4	<0.001	22
Neutrophils × 10^9 cells per l†	2.86 ± 0.2	3.91 ± 0.4	<0.001	22
Eosinophils × 10^9 cells per l	0.17 ± 0.01	0.20 ± 0.02	<0.05	22
Basophils × 10^9 cells per l	0.05 ± 0.00	0.07 ± 0.00	<0.001	22
Lymphocytes × 10^9 cells per l	2.1 ± 0.1	3.8 ± 0.1	<0.001	22
Monocytes × 10^9 cells per l	0.50 ± 0.03	0.73 ± 0.03	<0.001	22

Values are means ± SEM. NS, non-significant.
†Data nonparametric methods were used.

Elevation of circulating lymphocytes and neutrophils was the main reason for the change in total white cell count. There was a significant increase in glucose ($P < 0.001$), decrease in high sensitivity C-reactive protein ($P < 0.001$), but there was no change in cortisol or insulin concentrations after maximal exercise (Table 2).

Sera IL-6 concentration increased significantly between the rest and the end of maximal exercise ($P < 0.001$, see Table 2). Likewise, there were significant increases in MMP-9 and MPO concentrations ($P < 0.001$, see Table 2). Sera concentrations of TNF-α, sE-selectin, s-ICAM-1 and sVCAM-1 did not differ from pre-exercise levels ($P > 0.05$, see Table 2).

There were a number of correlations between maximal exercise-induced increases in determined variables. Maximal exercise-induced increase in MPO concentration correlated with Δ neutrophils abs count ($P < 0.05$, $R = 0.52$, $n = 22$) and Δ IL-6 concentration ($P < 0.01$, $R = 0.64$, $n = 19$, see Fig. 1). Furthermore, Δ MMP-9 correlated with Δ MPO ($P < 0.01$, $R = 0.62$, $n = 24$, see Fig. 2) and Δ IL-6 concentrations ($P < 0.05$, $R = 0.60$, $n = 17$, see Fig. 3).

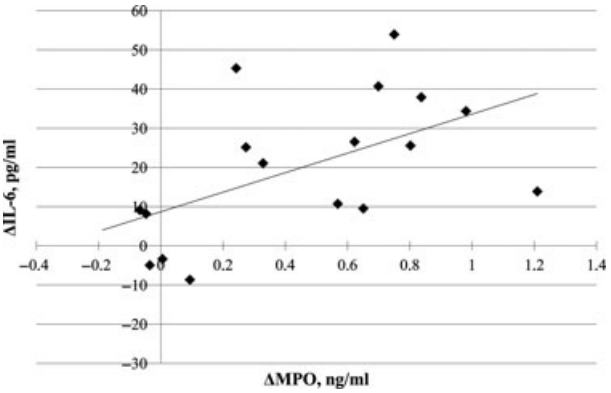

Figure 1 The correlation between maximal exercise-induced increases in MPO and IL-6 concentrations. $P < 0.05$, $R = 0.54$, regression equation: $y = 8.6949 + 24.91*x$.

Discussion

Exercise is associated with temporary changes in the immune system, for example, count of immune cells [17] and concentrations of cytokines [18], adhesion molecules [19], MMPs [12] and MPO [9]. The present study shows that maximal exercise induced significant changes in all subpopulations of leucocytes, especially neutrophils and lymphocytes ($P < 0.05$). Moreover, our data are in agreement with other studies that have shown exercise-induced degranulation of neutrophils and subsequent release of MPO ($P < 0.05$), confirmed by correlation between these two biomarkers ($P < 0.05$) [10].

Muscle damage often caused by eccentric exercise [20] and low plasma glucose or muscle glycogen levels [21] increase IL-6 concentrations during exercise. In fact, we observed an increase in serum glucose concentrations, most likely due to exercise-induced sympatho-adrenergic hepatic stimulation and increase in IL-6 concentration after maximal exercise ($P < 0.05$). It appears that the increase in IL-6 concentration in this study is less

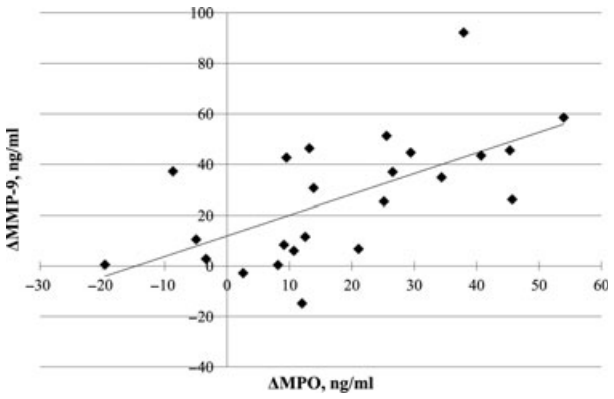

Figure 2 The correlation between maximal exercise-induced increases in MPO and MMP-9 concentrations. $P < 0.01$, $R = 0.62$, regression equation: $y = 11.8963 + 0.8147*x$.

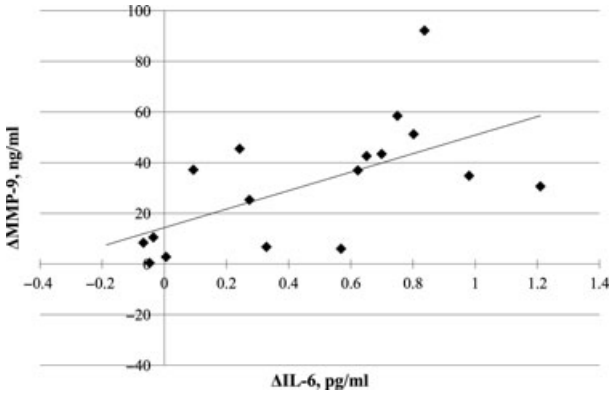

Figure 3 The correlation between maximal exercise-induced increases in IL-6 and MMP-9 concentrations. $P < 0.05$, $R = 0.60$, regression equation: $y = 14.3641 + 36.5221*x$.

connected with glucose homoeostasis. Moreover, our data showed that maximal exercise-induced MPO expression correlated with the increase in IL-6 concentration ($P < 0.05$). With the release of MPO, marker of neutrophil degranulation, it is plausible that also IL-6 at least to some extent is released from the neutrophils during maximal exercise.

There are data that show pro-inflammatory cytokine (e.g. IL-6, TNF-α) incentive effect on the adhesion molecule up-regulation on the endothelial cells [7]. However, we did not observe sICAM-1, sVCAM-1 or sE-selectin changes during exercise despite the maximal exercise-induced increase in IL-6 concentration [22]. Our data are in agreement with other studies and show no change in TNF-α levels in healthy male athletes following maximal exercise [2]. Gokhale *et al.* [23] also found that majority of the athletes and non-athletes demonstrated a rise in IL-6 and a fall in TNF-α levels. This relation is in agreement with the opinion that IL-6 also exerts anti-inflammatory effects by inhibiting production of TNF-α, possibly also during maximal exercise.

MMP-9

Exercise can cause a damage of skeletal muscles and connective tissue, which leads to activation of tissue MMPs. MMPs, including MMP-9, are the major components of neutrophilic tertiary granules and are also expressed by other types of leucocytes including monocytes and lymphocytes [1]. Furthermore, a single bout of exercise as cycling can induce an increment in concentration of total MMP-9 protein and its mRNA in human skeletal muscle [24]. In addition, the studies on the effect of exercise on plasma concentrations of MMP-9 are equivocal, showing no change [25] or increase after maximal exercise [26]. It has been shown that there is no correlation between exercise-induced increase in MMP-9 and creatine kinase activity, suggesting that the rapid and transient increase in the serum MMP-9 concentration may reflect accelerated release of MMP-9 to circulation because of exercise-induced changes in leucocyte number rather than an increased extracellular matrix breakdown [27]. Our data showed that maximal exercise-induced MPO expression correlated with the increase in MMP-9 concentration ($P < 0.05$), supporting the previous mentioned hypothesis, that neutrophils could be the major contributors for increased MMP-9 levels. Moreover, our data showed that maximal exercise-induced MMP-9 expression correlated with the increase in IL-6 concentration ($P < 0.05$), suggesting a close interplay between inflammatory and proteolytic processes. There is no substantial evidence in scientific literature showing IL-6 induced MMP-9 release from neutrophils; however, it is known that IL-6 stimulate MMP-9 expression in different cell types, for example, fibroblasts [28]. The correlations found in this study possibly could point out IL-6 modulatory effects on MMP-9, which needs to be clarified in further studies. It has been suggested that IL-6 might also regulate mobilization of neutrophils into circulatory system [29], which could be another way of IL-6 contribution to increased MMP-9 levels after maximal exercise.

In conclusion, maximal exercise induces an inflammatory response characterized by greater count of all subpopulations of leucocytes and increased IL-6, MPO and MMP-9 concentrations. Although study design does not allow estimating precise source of increased IL-6 and MMP-9, their correlations with increased MPO (maker of neutrophils degranulation) levels may suggest that neutrophils could be the main source of these inflammatory biomarkers during maximal exercise. Furthermore, correlation between increases in serum IL-6 and MMP-9 concentrations may suggest that IL-6 could exert modulatory effects on MMP-9 release during maximal exercise.

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