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EFFECTS OF BRIEF MAXIMAL EXERCISE ON INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-ALPHA

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Abstract. Acute bouts of prolonged strenuous exercise are often associated with immune modulation and an increased risk of infection. However, few studies have examined immunological responses to brief maximal exercise. We investigated the effects of brief maximal exercise on plasma Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) concentrations in both athletes and sedentary subjects. Seven athletes [mean (SEM)] [peak oxygen uptake = 55 (0.02) ml. kg^{-1}. mn^{-1}] and eight sedentary [peak oxygen uptake = 40 (0.11) ml. kg^{-1}. mn^{-1}] healthy volunteers performed an incremental exercise on an ergometer bicycle until $\dot{V}O_{2\,max}$ was attained. Cytokines plasma concentrations were measured before and immediately after exercise using an enzyme linked immunosorbent assay. Athlete's IL-6 plasma concentrations averaged immediately before and after exercise were [mean (SEM)] [4.85 (0.89) pg/ml] and [24.74 (0.64) pg/ml] respectively ($p<0.01$). However, no significant increase was observed in the sedentary group. Athlete's and sedentary plasma concentrations of TNF-α increased significantly immediately after exercise ($p<0.01$). We conclude that brief maximal exercise induce in athletes a moderate increase in both TNF-α and IL-6 secretion. This finding support hypothesis that plasma IL-6 concentrations increase with intensity and duration of exercise.

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Key words: Muscular exercise - Immune function – Cytokines - Infections

Introduction

Cytokines are glycosylated polypeptides that are secreted by most cells of the body [27]. Pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor

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necrosis factor-α (TNF-α), modulate immune cell function and migration, initiating and amplifying the acute phase, stress responses, and pyrogenesis [18,16]. Thus, cytokines are released at the site of inflammation caused by infectious pathogen or traumatic injury and facilitate an influx of neutrophiles, monocytes and other cells that participate at the clearance of the antigen and the healing of the tissue [16].

It is also known that muscular exercise enhances plasma levels of some cytokines [19]. Several studies demonstrated that strenuous exercise is accompanied by an increase in circulating pro-inflammatory responsive cytokines along with other bioactive stress molecules having some similarities with the response to sepsis and trauma [3,11]. However, the profile of cytokine production during exercise has not been yet well established.

Despite the difficulties inherent in measuring plasma cytokines concentrations [20], studies of subjects exercising intensively reported conflicting results. Some authors reporting increase [9] and others no changes [17] in TNF-α and IL-6 production after strenuous exercise. More information will be helpful in determining how far exercise can be characterized as a model of inflammation, and understanding the biologic significance of such observed phenomenon.

Increased cytokines levels have mostly been described after moderate or long duration exercise. However, to our knowledge, very little attention has been paid to the effects of brief maximal exercise on plasma concentrations of pro-inflammatory cytokines.

Thus, the purpose of this study was to examine the effects of brief maximal exercise on plasma concentrations of IL-6 and TNF-α in both athletes and sedentary subjects.

Materials and Methods

Subjects: After approval of experimental procedures by the Ethical Committee of Farhat Hached Hospital of Sousse, informed consent was obtained from 15 young adults. Before the admittance to the study, a medical examination was performed on each subject. Inclusion criteria were: male aged between 20 and 30 years. Exclusion criteria were as follows: 1) respiratory or cardiac disease, hypertension, or any other chronic disease; 2) being on regular medication; 3) a history of asthma or atopic disease or allergic disease and 4) smoking. The subjects were assigned into two groups: one performing regular physical exercise (athletes group) and the other was untrained volunteers (sedentary group).

Athletes: Seven highly trained athletes were admitted in the study. Inclusion criteria of selection of athletes were a maximal O_2 uptake ($\dot{V}O_{2\ max}$) > 50 $ml \cdot kg^{-1} \cdot mn^{-1}$. Athletes were basketball players. They had been participating regularly in regional competition.

Sedentary subjects: Eight untrained men who had not engaged in any type of muscular activity for at least one year prior to the study were admitted. From data on $\dot{V}O_{2\ max}$ corresponding to 40 $ml \cdot kg^{-1} \cdot mn^{-1}$, subjects were considered untrained.

Exercise protocol and blood sampling: Subjects performed an incremental exercise test on a cycle ergometer (Excalibur, Gruningen, Holland) until $\dot{V}O_{2\ max}$ was attained. After a 3 min rest, the initial power setting was 30W for untrained and 60W for highly trained during 3 min with successives increases of 30W every minute. Minute ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$), and CO_2 output ($\dot{V}CO_2$) were measured continuously using a breath-by-breath automatic exercise metabolic system (CPX Medical graphics, St Paul, MN). The data were averaged over an integral number of breaths during the last 20 sec of each minute and recorded during rest, exercise, and recovery. Prior the testing, gas analyzers were calibrated with standard gases of known concentrations. During exercise heart rate was continuously monitored with a Sports Tester (Polar, Finland). To ensure that $\dot{V}O_{2\ max}$ was attained, at least three of the following criteria had to be met: [1] a plateau of $\dot{V}O_2$ with the last increase in work rate ("leveling off" criterion), [2] attainment and stabilization of age-predicted maximal heart rate, [3] a respiratory exchange ratio (RER) value ≥1.1, and [4] an inability to maintain the required pedaling frequency (60 rpm) despite maximal effort and verbal encouragement.

Peripheral venous blood samples were drawn through a venal-catheter (Bio Fassy, France) at rest (Before Exercise) and immediately after the end of exercise (After Exercise).

Body mass index (BMI) was calculated as measured weight (kg)/height (m^2) (Table 1).

Total and differential leukocyte count: Total leukocyte count in Ethylene Diamine Tetra Acetic Acid (EDTA)-treated blood was measured by using a symex microcell counter (F-300). The cover slipped smears were prepared on freshly drawn whole blood immediately after blood sampling in the absence of anti-coagulant and Wright-Giemsa stained. The different leukocyte types were classified into band and segmented neutrophiles, lymphocytes, monocytes, basophiles by using oil-immersion magnification (x 1,000) from at least 200 cells / slide. The absolute number of each cell type was calculated from the total leukocyte count and the percentage of each differential count.

Plasma cytokines measurement: Venous blood was collected in pyrogen-free vacutainers containing EDTA (1.44 mg/5ml blood). Blood samples were

immediately centrifuged (1.000g, 15 min, 4°C), and the supernatant was harvested and stored at –80°C until process further.

Plasma concentrations of TNF-α and IL-6 were assayed using the quantitative high sensitivity ELISA technique in kit form according to the supplier's instructions (Immunotech, Marseille, France). The absorbance of the product (measured in optical density units), determined with an automated spectrophotometer-microtiter plate reader, was directly proportional to the amount of cytokine in the standard or sample. Absorbance was converted to concentration (in pg/ml), using standard curves. The limit detection of the assay was 5 pg/ml for TNF-α and 3 pg/ml for IL-6.

Statistical analysis: Data are expressed as means ± (SEM). Statistical analysis was carried out using Wilcoxon test to compare pre-post exercise plasma cytokines concentrations, leukocytes and lymphocytes counts. The level of statistical significance was set at p value <0.01.

Results

Anthropometric and physiological data are shown in Table 1 and 2.

Table 1
Subject's anthropometrics data

	Age (yr)	Weight (kg)	Height (m)	BMI (kg/m^2)
Athletes (n=7)	22.5	69.5	1.7	22.2
	(0.1)	(0.06)	(0.47)	(0.1)
Sedentary (n=8)	23.3	75.7	1.8	23.3
	(0.1)	(0.11)	(0.05)	(0.2)

Values are means (SEM)

Leukocytes subsets: The effects of maximal exercise on athlete's total leukocyte and lymphocyte counts are illustrated in Figs. 1 and 2. Athlete's total leukocyte counts were significantly increased immediately after exercise in comparison to the levels before attaining a cell count of 9.99 (0.057) $.10^9$ $cells \cdot l^{-1}$ (Fig. 1). However, no significant changes were observed in the sedentary group (results not shown).

Table 2
Subject's physiological data during maximal exercise

	$\dot{V}O_{2\ max}$	$\dot{V}E_{max}$
	($ml \cdot min^{-1} \cdot kg^{-1}$)	($l \cdot min^{-1}$)
Athletes (n=7)	55 *	118.34 *
	(0.02)	(0.3)
Sedentary (n=8)	40	107.61
	(0.11)	(0.3)

$\dot{V}O_{2\,max}$ - Maximal oxygen uptake; $\dot{V}E_{max}$ - Maximal minute ventilation
Values are means (SEM); *Significantly different from sedentary group: ($p<0.01$)

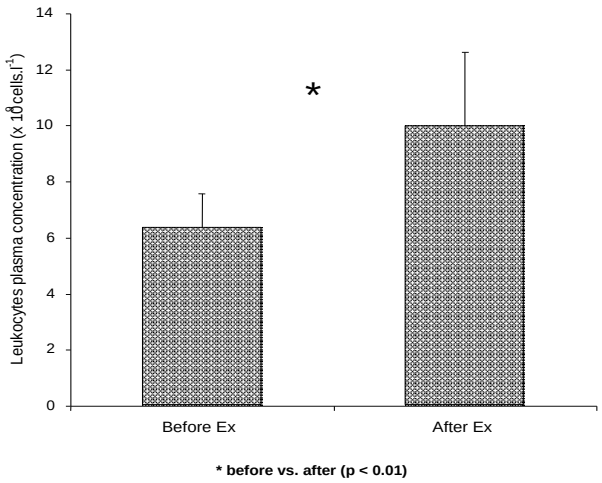

Fig. 1
Effects of exercise on athlete's leukocytes concentration;
*Significantly different from the pre-exercise measure

In athletes, brief maximal exercise induced a pronounced lymphocytosis, which was largely responsible for the changes in total white cell count. Significant increase in circulating lymphocytes was clear immediately after exercise ($p<0.01$). Circulating total lymphocytes count increased from 2.37 (0.08). 10^9 cells.l^{-1} at the beginning of exercise and peaked up to 4 (0.03). 10^9 cells.l^{-1} immediately after the end of exercise (Fig. 2). The circulating neutrophiles, eosinophiles, basophiles and monocytes also increased significantly for athletes (results not shown).

Like total leukocytes, the lymphocyte count did not peak within sedentary subjects (results not shown).

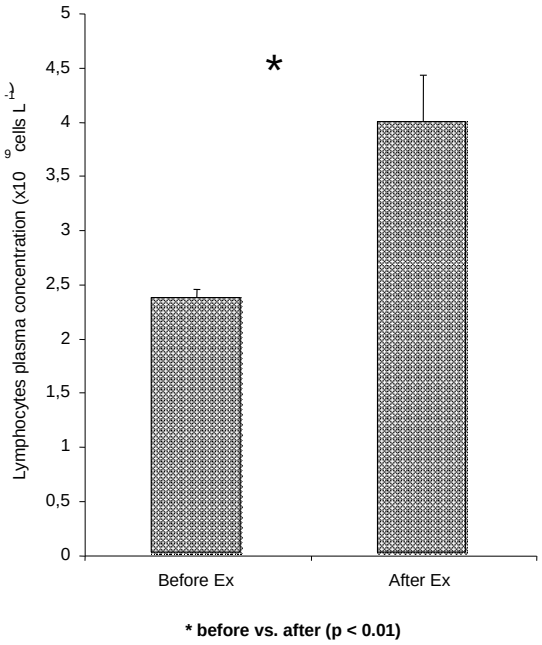

Fig. 2
Effects of exercise on athlete's lymphocytes concentration;
*Significantly different from the pre-exercise measure

Plasma cytokines response: Athlete's plasma concentrations of TNF-α increased significantly from 21.01 (1.2) pg/ml before to 123.57 (0.97) pg/ml immediately after exercise (P<0.01) (Fig. 3).

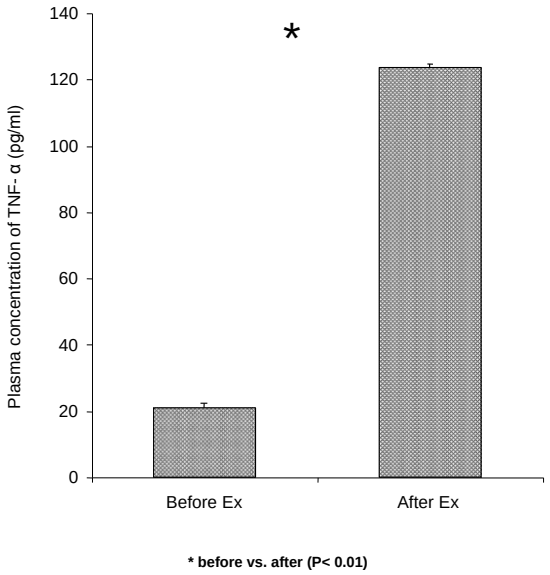

Fig. 3
Effects of exercise on athlete's plasma concentration of TNF-α;
*Significantly different from the pre-exercise measure

Sedentary plasma concentrations of TNF-α averaged 18.33 (1.07) pg/ml before and 54.95 (1.23) pg/ml after exercise (Fig. 4).In the athletic group, TNF-α levels increased significantly immediately after exercise (P<0.01). This increase is estimated at 6 folds the rest value compared with only 3 folds for sedentary.

Athlete's plasma concentrations of IL-6 increased from a pre-exercise concentration of 4.85 (0.89) pg/ml to 24.74 (0.64) pg/ml (6-fold) immediately after

(p<0.01) (Fig. 5). However IL-6 was undetectable within the sedentary group before the beginning of exercise (Fig. 6).

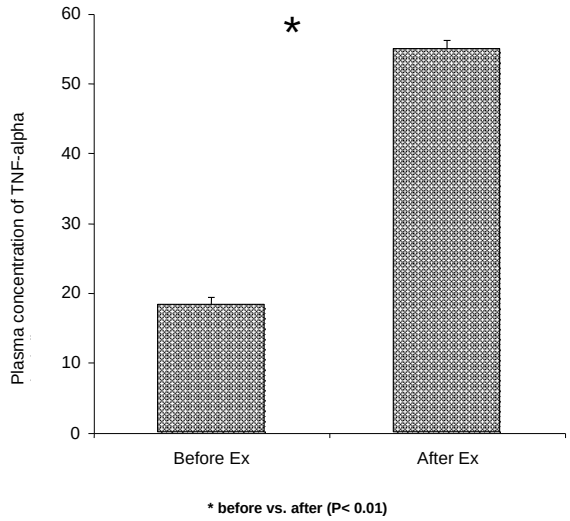

Fig. 4
Effects of exercise on sedentary plasma concentration of TNF-alpha
*Significantly different from the pre-exercise measure

Discussion

In agreement with other studies [15], we demonstrate that brief maximal muscular exercise induced a transient elevation in circulating leukocyte counts, driven largely by a lymphocytosis but also influenced by a decrease in monocytes, neutrophiles, eosinophiles and basophiles counts.

The stimuli leading to the increase in various leukocyte subsets during exercise seems to be understood. According to Shephard and Shek [21], Changes in plasma

concentrations of catecholamine and glucocorticoides potently modulate immune cell migration and activity during exercise. Catecholamine-induced changes in the interaction between lymphocytes and vascular endothelial cells are thought to increase circulating counts through a rapid demargination of immune cells.

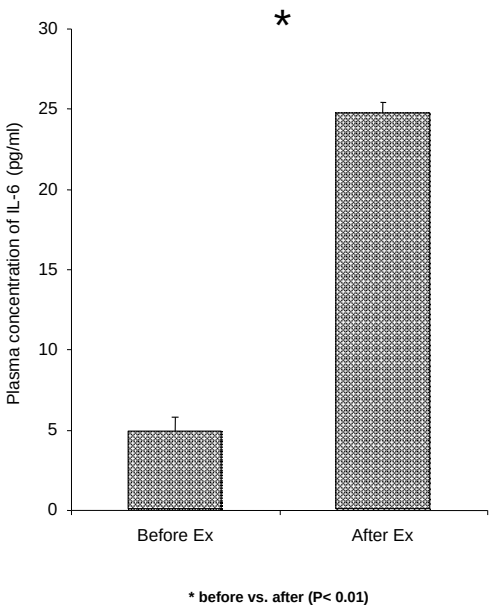

Fig. 5
Effects of exercise on athlete's plasma concentration of IL-6
*Significantly different from the pre-exercise measure

Glucocorticoids are depending of psycho-physiological stress; they are released during and after maximal exercise. Cortisol may counter the effect of β-adreno-receptor stimulation on circulating lymphocytes, inhibiting their entry into the circulation and promoting their exit into peripheral tissues [11].

We showed that athlete's plasma concentrations of TNF-α increased significantly immediately after brief maximal cycle ergometer exercise. Previous

studies decrypting changes in plasma levels of pro-inflammatory cytokines have yielded conflicting results, possibly in part due to differences in experimental design, timing of blood sampling, and cytokine assay sensitivity [1,26]. The increase in TNF-α secretion after maximal exercise could be related to the inflammatory reaction induced by mechanical muscle damage. Pro-inflammatory cytokines have been suggested both to induce and mediate local catabolic mechanisms [22]. Current opinion is that after acute exercise myofibers are mechanically damaged and, therefore, an inflammatory process occurs and local systemic production of cytokines is initiated [6]. The sequential release of cytokines resembles that observed in relation to trauma [7,8].

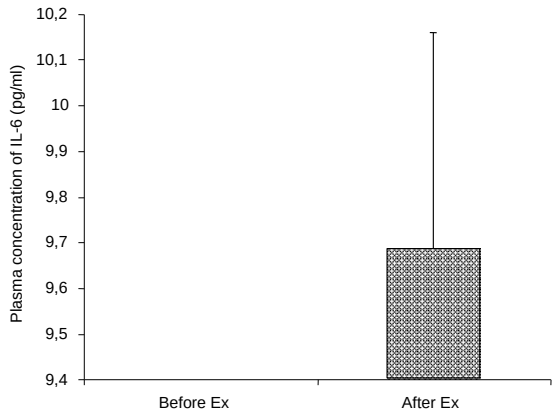

Fig. 6
Effects of exercise on sedentary plasma concentration of IL-6

This study also shows a significant increase in IL-6 concentrations for athletic subjects. An increase in IL-6 concentrations also occurred for sedentary although it

wasn't statistically significant. Theses increases are however lesser than those usually reported in the literature.

Thus, it has been demonstrated that plasma concentrations of IL-6 increases up to more than 100-fold during prolonged muscular exercise [14]. This increase is followed by the appearance of cytokines inhibitors such as IL-1ra, sTNF- R and the anti-inflammatory cytokine IL-10 [19].

The augmented IL-6 plasma concentrations following exercise was associated with muscle damage in an earlier study [12], but today it is very clear that exercise without any muscle damage also induces marked production of IL-6 and that IL-6 is produced as a direct consequence of contraction per se [13].

Plasma IL-6 during exercise increased with intensity and duration of exercise [10]. Theses observations are in agree with our results, showing only a 6-fold increase in IL-6 concentration after a brief and maximal exercise. Thus, it is interesting to determine the physiological source of IL-6 secretion. It has been demonstrated that monocytes in the blood are not the source of the elevated plasma IL-6 during exercise [23]. It was also shown that IL-6 was released from the contracting limb and from the resting limb of the same subjects [24].

Furthermore, it was recently demonstrated that pretendinous tissue is an IL-6 producing region during exercise and that connective tissue may contribute to the rise in IL-6 concentrations in plasma in response to exercise [5]. Interestingly IL-6 mRNA is up regulated in exercising human muscles [4].

The fact that IL-6 is produced locally in working muscles and released into the circulation in large amounts during exercise suggests that it has important biological roles. Thus, recent research regarding IL-6 during exercise suggests that working muscles produce and release IL-6 as a consequence of contraction and low intra muscular glycogen or altered energy turnover [2,24] Thus, it is tempting to suggest that IL-6 works as a hormone like fashion, exerting its effect on the liver and adipose tissue, thereby contributing to maintain glucose homeostasis during exercise and mediating exercise induced lipolysis. This hypothesis is sustained by a recent experience using recombinant IL-6 [25].

Physical exercise is believed to influence the immune function through the release of neuro-endocrine mediators and cytokines. IL-6 seems to be the main cytokine that was secreted in large amounts during exercise.

A major finding of the present study was that a 10 min duration maximal exercise induced a moderate increase in IL-6 secretion. Our data provides evidence that IL-6 plasma concentration increases with intensity and duration of exercise. Muscle derived IL-6 may contribute to mediate the beneficial metabolic effects of exercise.

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