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ORIGINAL ARTICLE

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Moderate and exhaustive endurance exercise influences the interferon-γ levels in whole-blood culture supernatants

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Abstract The aim of this study was to investigate whether moderate or exhaustive endurance exercise influences cytokine levels in whole-blood culture supernatants after stimulation. Therefore, eight healthy subjects were first exposed to moderate exercise on a cycle ergometer for 30 min at 70% of their 4-mmol/l lactic acid (anaerobic) threshold, and 1 week later to exhaustion (for 90 min) at their anaerobic threshold. Blood samples were taken before, 30 min after and 24 h after each exercise bout. The following lymphocyte subpopulations were determined: CD14-positive(+)/CD45+, CD4+, CD8+, and CD16+. Cytokine levels in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA). Production of interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α were induced with lipopolysaccharides (LPS), and that of IL-2 and interferon (IFN)-γ with staphylococcal enterotoxin B (SEB) and phytohaemagglutinin (PHA). Cortisol levels were also determined by ELISA. The lymphocyte subset distribution was observed to be unchanged after moderate exercise. Thirty minutes after exhaustive exercise, the CD16+ count was found to be significantly lower, whereas 24 h later the CD4+ count was significantly higher than pre-exercise counts. Moderate exercise influenced the IFN-γ production (PHA-stimulated), which increased significantly from 974 (391) pg/ml before exercise to 1450 (498) pg/ml 24 h later. Thirty minutes after exhaustive exercise the IFN-γ level in the supernatants (SEB-stimulated) was significantly decreased (from 14470 (11840) pg/ml before exercise to 6000 (4950) pg/ml after exercise). The IL-1β and TNF-α production per monocyte was also significantly reduced.

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Introduction

Cytokines play an important role with regard to the regulation of specific and non-specific immune responses. Virtually all cells that are involved in the immune response depend on cytokines for their growth, differentiation and functional activation (Balkwill 1993). Somatic cells are also influenced by cytokines. An optimal immune reaction also depends on an adapted cytokine synthesis. Insufficient or delayed production of cytokines is followed by a weakened immune reaction and, therefore a higher risk of infection. Excessive cytokine production can have fatal effects in septic shock. Epidemiological studies have demonstrated a higher rate of infections after strenuous exercise (Nieman 1994; Nieman et al. 1990; Peters and Bateman 1983). Apart from changes in the distribution of white blood cells in the tissues and in innate immunity (Espersen et al. 1990; Pedersen 1991), this higher rate of injection that occurs after strenuous exercise might be caused by the influence of exercise and high levels of stress hormones (e.g. catecholamines and cortisol) on cytokine production. Several authors (Haahr et al. 1991; Lewicki et al. 1988; Northoff et al. 1994; Tvede et al. 1993) have described a reduced phytohaemagglutinin (PHA)-stimulated interleukin (IL)-2 production in cell culture after exhaustive exercise. However, an increased synthesis (concanavalin-A-stimulated) has also been reported (Northoff et al. 1994). Furthermore, Northoff et al. (1994) reported suppressed interferon (IFN)-γ production in lipopolysaccharide (LPS)-stimulated whole-blood cultures, while Haahr et al. (1991) measured no significant changes. The results for IL-1β are contradictory (Cannon et al. 1991; Drenth et al.1996; Haar et al. 1991; Lewicki et al.1988; Northoff et al. 1994). We hypothesize that moderate and exhaustive endurance exercise influence post-exercise, in vitro cytokine synthesis in different ways.

Methods

Following informed consent, eight healthy subjects (seven male, one female) participated in this study. The biometric data of these subjects are shown in Table 1. About 1 week prior to the scheduled research this working capacity at 4 mmol/l lactic acid (anaerobic threshold) was determined while they performed exercise on a cycle ergometer. The subjects started at a workload of 50 W, with the workload subsequently increasing stepwise by 50 W every 5 min until exhaustion. Lactic acid was sampled from the left earlobe after each step. The working capacity of the subjects at 4 mmol/l lactic acid was defined as 100% of exercise intensity. On the 1st day of the study, the subjects exercised for 30 min (again on a cycle ergometer) at 70% of their anaerobic threshold workload (moderate exercise). About 1 week later they exercised for 90 min at 100% intensity (exhaustive exercise). Blood samples were taken by venous puncture before, 30 min after and 24 h after exercise. The sampling time of 30 min after exercise was chosen to overcome the problem of a large change in the lymphocyte subset distribution that occurs immediately after exercise.

Differential blood picture and lymphocyte subpopulations

The leucocyte counts were determined using a counter (Sysmex, Digitana, Hamburg, Germany). The differential blood picture (percentage of granulocytes, monocytes and lymphocytes) was determined from the physical characteristics of the cells observed (lymphocytes, granulocytes) and the expression of the CD14 antigen (monocytes). Degranulated granulocytes and monocytes in the lymphocyte gate were identified using the CD14 and CD45 antibodies.

The following lymphocyte subpopulations were identified by flow cytometry (Facscan, Becton Dickinson, Heidelberg, Germany): CD14-positive(+)/CD45+, CD4+, CD8+, CD16+. Auto-fluorescence was determined using mouse IgG antibodies; cells showing a fluorescence intensity above that of the auto-fluorescence were classified as positive. The samples were prepared according to the directions of Becton-Dickinson. The data were evaluated using Consort 30 software (Becton-Dickinson).

Whole-blood assay

Within 2 h of collection the blood samples were cultured in a whole-blood assay, a technique which has been described previously (Kirchner et al. 1982). Fifty microlitres of blood was added to 400 µl RPMI 1640 (Biochrome KG, Berlin, Germany) in a polystyrol tube. For the induction of IL-1β, IL-6 and tumour necrosis factor (TNF)-α, LPS (*E. coli* 0111:B4; Sigma, Munich, Germany) was added. The IL-2 and IFN-γ were induced using PHA (Murex, Burgwedel, Germany) and Staphyloccocal enterotoxin B (SEB, Sigma). The final concentrations in culture were 1 µg/ml LPS, 5 µg/ml PHA and 100 µg/ml SEB. Incubation times were 48 h for IL-2 and TNF-α, 120 h for IL-1β, IL-6, IFN-γ and sIL2-R soluble interleukin 2-receptor (Kirchner et al. 1982). The supernatants of the cultures were collected and frozen at −80°C. Cytokine concentrations were determined by standard enzyme-linked immunosorbent assay (ELISA) technique (Laboserv, Gießen, Germany for IL-2, IL-1β and IFN-γ; R&D, Wiesbaden, Germany for IL-6, sIL2-R and TNF-α). Cortisol was also determined by ELISA technique (Biermann, Bad Nauheim, Germany).

Table 1 Biometric data of the subjects ($n = 8$; 7 male, 1 female)

Parameter	Value
Age (years)	30 (13)
Height (cm)	183 (4)
Weight (kg)	76.0 (9.7)
Anaerobic threshold (4 mmol lactic acid/l)	213 (47) W

Statistics

The significance of the data was validated by the Friedmann (nonparametric analysis of variance) and Wilcoxon test, using a significance level of $P \leq 0.05$ (significant). Correlations were determined using Spearman's correlation analysis. The level of significance was set at $P \leq 0.01$.

Results

After moderate exercise no significant changes were observed in white blood cell count, differential blood picture and distribution of lymphocyte subpopulations (percentage and absolute counts). The exhaustive exercise was followed by a significant leucocytosis, mainly due to a granulocytosis. Monocytes were elevated 30 min and 24 h after exercise (Table 2). Thirty minutes after exhaustive exercise the CD16-positive(+) count [133 (91) vs 255 (197)/µl] and percentage [11.4 (5.4) vs 20.0 (8.3)] were significantly lower compared with the pre-exercise values. The CD4+ cell count was significantly higher 24 h after this exercise bout [587 (241) vs 439 (187)/µl before exercise].

The cytokine synthesis of whole-blood cultures was influenced significantly for IFN-γ production. Twenty-four hours after submaximal exercise the PHA-induced synthesis of this cytokine was increased (Fig. 1). After the exhaustive exercise SEB-induced IFN-γ production was suppressed (Fig. 1). Twenty-four hours later no further suppression was seen. Furthermore, the sIL2-R levels (SEB-stimulated) in the whole-blood culture were

Table 2 White blood cell counts and differential blood picture. After exhaustive exercise leucocyte, granulocyte and moncyte counts were significantly elevated. The monocyte count was also elevated 24 h later

Cell type	Number of cells per microlitre					
	Before moderate exercise	30 min after moderate exercise	24 h after moderate exercise	Before exhaustive exercise	30 min after exhaustive exercise	24 h after exhaustive exercise
Leucocytes	5800 (1000)	6300 (1600)	5900 (1200)	5800 (1100)	10800 (3900)*	6100 (1600)
Lymphocytes	1292 (408)	1360 (507)	1600 (487)	1182 (453)	1177 (430)	1467 (423)
Monocytes	317 (174)	378 (152)	420 (68)	268 (116)	474 (282)*	403 (129)*
Granulocytes	4166 (632)	4279 (1407)	3831 (782)	4375 (748)	8871 (3377)*	4194 (1271)

Significance: $^{*}P \leq 0.05$

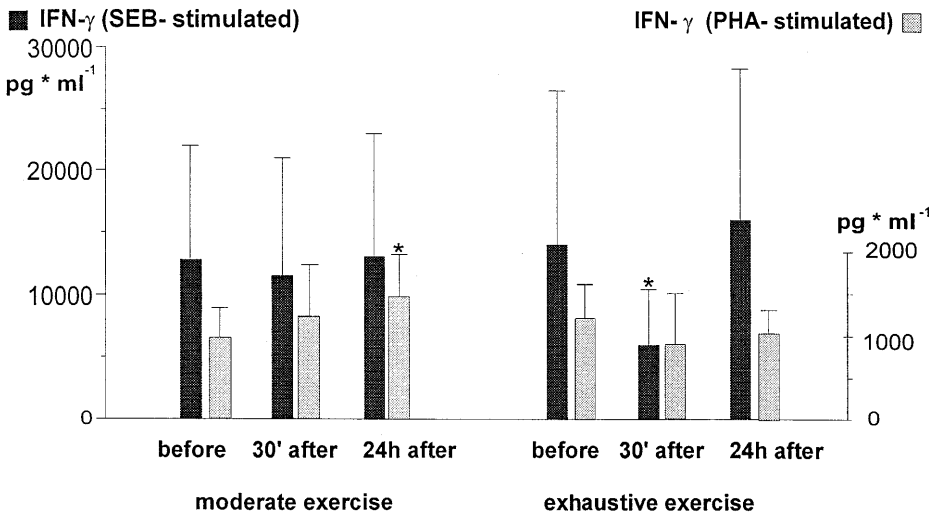

Fig. 1 INF-γ levels in culture supernatants after stimulation with phytohaemagglutinin (*PHA*) and staphyloccocal enterotoxin B (*SEB*). Twenty-four hours after moderate exercise, the PHA-stimulated interferon (INF)-γ production was significantly elevated. In contrast, 30 min after exhaustive exercise the SEB-stimulated INF-γ production was significantly impaired ($P \leq 0.05$)

significantly lower 30 min after exhaustive exercise [4158 (1098) vs 4762 (1063) IU/ml before exercise]. For TNF-α and IL-1β, which are produced mainly by monocytes, the quotient of the whole-blood production and the monocyte count was calculated. A similar calculation was performed for the CD4+ cell count and the synthesis of IFN-γ. After exhaustive exercise TNF-α and IL-1β synthesis by monocytes were significantly impaired (Fig. 2). The IFN-γ production per CD4+ cell was suppressed after the exhaustive exercise (Fig. 3). Cortisol levels were significantly lower 24 h after the exhaustive exercise as compared with the levels before this bout of exercise ($P \leq 0.05$); no further significant changes of this hormone were observed.

Correlations were calculated between cortisol levels and cytokine synthesis. Before moderate exercise a significantly negative correlation between IL-2 synthesis (SEB-stimulated) and cortisol was determined ($r = -0.9286$, $P \leq 0.001$; before exhaustive exercise the correlation was $r = -0.8001$, $P \leq 0.017$). After the exercise bouts (and for the other cytokines) no significant correlations with cortisol levels were observed.

Discussion

The present study demonstrates that 1.5-h exhaustive endurance exercise leads to a suppression of cytokine synthesis by white blood cells. In particular, there seems to be a strong suppression of SEB-stimulated IFN-γ synthesis in whole-blood cultures. On a per CD4+ cell basis this decrease was also significant. We conclude that changes in the lymphocyte subset distribution cannot be the reason behind this observation. This result is in accordance with the findings of Northoff et al. 1994, who found a pronounced refractoriness of whole-blood cultures to the production of INF-γ in response to LPS stimulation after a 1-h triathlon. Haahr et al. (1991) measured no significant changes of INF-γ production in response to PHA after 1 h of cycle ergometry at 75% of maximal oxygen consumption. However, in this study PHA-stimulated IFN-γ production was lower after maximal exercise, but a level of significance was not reached. On a per CD4+ cell basis we also measured a significant suppression of IFN-γ production 24 h after

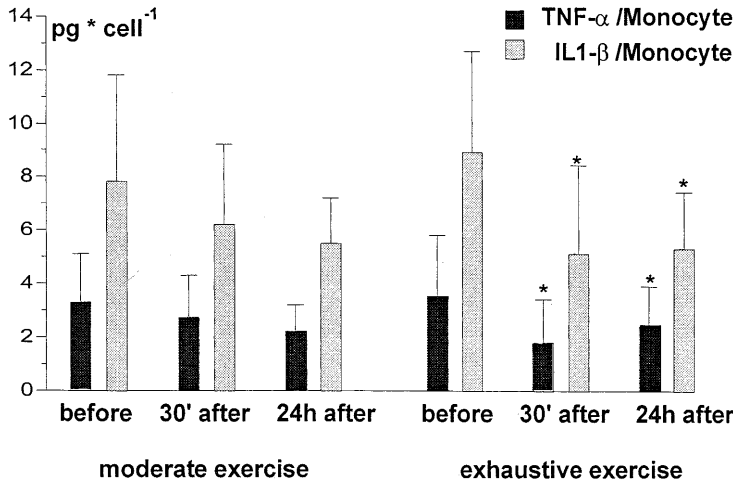

Fig. 2 Interleukin (*IL*)-1β and tumour necrosis factor (*TNF*)-α levels per monocyte in culture supernatants after stimulation with lipopolysaccharides (*LPS*). After exhaustive exercise, the LPS-stimulated IL-1β and TNF-α production per monocyte was significantly reduced ($P \leq 0.05$)

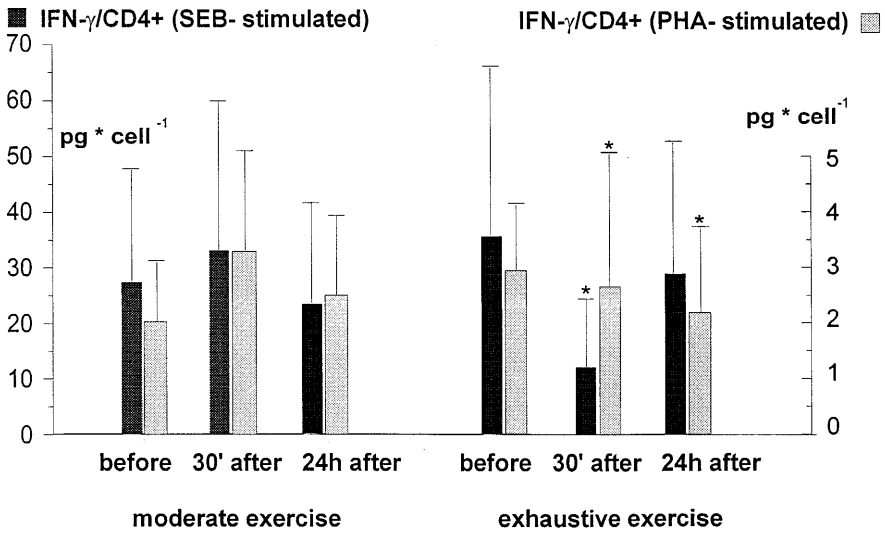

Fig. 3 INF-γ levels per CD4+ cell in culture supernatants after stimulation with PHA and SEB. After moderate exercise, the *PHA-* and *SEB-stimulated* INF-γ production on a per CD4+ cell basis were unchanged. Thirty minutes after exhaustive exercise the SEB-stimulated INF-γ production per CD4+ cell was significantly impaired. For the PHA-stimulated production this quotient was significantly lower at both sampling times after exercise ($P \leq 0.05$)

the exercise. This indicates a more prolonged impairment of IFN-γ synthesis. In vivo serum levels for INF-γ were not influenced by exhaustive exercise (Northoff et al. 1994; Viti et al. 1985), nevertheless the impaired IFN-γ synthesis we observed might play a role in the significantly higher rate of upper respiratory tract infections observed after performance of competitive and exhaustive exercise (Niemann et al. 1990; Peters and Bateman 1983). Such infections are caused predominantly by viruses, and the impaired IFN-γ synthesis, therefore, might be one reason for the disadvantageous effects of exhaustive exercise. The reason behind the impaired IFN-γ synthesis remains unclear. No significantly elevated cortisol level was measured after intensive exercise, nor could correlations between cortisol levels and IFN-γ synthesis be determined. Therefore, a functional inhibition of lymphocytes by cortisol seems unlikely. A strong shift from T helper type 1 cells to T helper type 2 cells in the CD4+ subpopulation is unlikely since IL-2 synthesis was unimpaired. The result that IL-2 levels in the whole-blood culture were not influenced by maximal exercise is in contrast to the results of other investigations in which a reduced or increased IL-2 production after exhaustive exercise was described (Northoff et al. 1994; Tvede et al. 1993). We found significantly lower sIL-2R levels (SEB-stimulated).

The synthesis of IL-6, IL-1β and TNF-α in whole-blood culture was not significantly influenced by any exercise bout. For TNF-α and IL-1β the synthesis on a per monocyte basis was calculated, because they are mainly produced by these white blood cells. At both sampling times after the exhaustive exercise, a significantly reduced synthesis of TNF-α and IL-1β per monocyte was observed. These results are in accordance with studies by Drenth et al. (1996) and Northoff et al. (1994), who found a decreased synthesis of these cytokines, not on a per cell basis, but for the whole blood. In contrast, other authors have described an elevated synthesis of IL-1β (Cannon et al. 1991; Haahr et al. 1991; Lewicki et al. 1988) and an unimpaired (Haahr et al. 1991) or higher (Cannon et al. 1991) production of TNF-α. These contradictory results might be attributable to differences in the intensity and duration of exercise. It has been reported that competitive exercise (e.g. a 6-h run or a 1-h triathlon) is followed by a depressed ex vivo production of TNF-α on a whole-blood basis, while after eccentric exercise (45 min) a higher IL-1β production has been described. Therefore, the depression of monocyte function might occur after exhaustive (competitive) endurance exercise. Due to the fact that no correlations could be determined between cortisol levels and the synthesis of TNF-α and IL-1β, an influence of this hormone seems to be unlikely. Other mediators, like prostaglandins, might play a role in the inhibition of monocyte function (Pedersen 1991; Tvede et al. 1993). In addition, monocytes that are mobilized after exercise might be in a different state of maturity; in the post-exercise phase leucocyte-function-antigen-1-positive monocytes decrease preferentially (Gabriel et al. 1994). Monocyte subsets circulating before and after exercise might have a different ability to produce cytokines.

In conclusion, the present study demonstrates a modulation of TNF-α, IL-1β and IFN-γ synthesis by exhaustive exercise. Furthermore, moderate exercise was followed by an improved INF-γ production. However, training studies are necessary to investigate whether IFN-γ synthesis is improved permanently by regular exercise.

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