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EFFECT OF ADAPTATION TRAINING ON THE IMMUNE SYSTEM

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SUMMARY

Studies have demonstrated that exercise induces considerable physiological changes including the immune system. The interaction between exercise stress and the immune system provides a unique opportunity to evaluate the role of underlying stress and immuno-physiological mechanisms. The purpose of the study was to examine the effect of adaptation exercise on innate, acquired, and humoral immunity following one bout of exhaustive exercise. A training group participated in an 8-week endurance training program, while the control group did not. Blood samplings of the two groups' members were obtained before and after performing the Bruce protocol test, before and following the 8-week period. Data was analyzed using the ANOVA test. There were no significant changes in cytokines and IgA concentrations, but the levels of CD4 decreased and those of CD8 increased significantly in the training group. It was concluded that endurance training may induce changes in lymphocyte subsets, but immune function was not suppressed following an exhaustive bout of exercise.

Key words: Interleukins, immune cells, exhaustive exercise, endurance training

ÖZET

ADAPTASYON ANTRENMANININ İMMÜN SİSTEM ÜZERİNE ETKİLERİ

Çeşitli çalışmalar egzersizin neden olduğu önemli fizyolojik değişikliklerin immün sistemde de gözlendiğini ortaya koymuştur. Egzersiz stresi ve immün sistem arasındaki ekileşimler, bu gözlemin altında yatan strese bağlı ve immünofizyolojik mekanizmaların rollerini açıklamak için eşsiz bir olanak sağlar. Bu çalışmanın amacı egzersize adaptasyon dönemi sonrası uygulanan bitkinleştirici egzersize doğuştan gelen, edinilmiş ve humoral immünitenin yanıtlarını incelemekti. Egzersiz grubu

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sekiz hafta boyunca dayanıklılık antrenmanı uygularken, kontrol grubu uygulamadı. Bu sürenin başında ve sonunda, test olarak Bruce protokolü uyulanmasının öncesi ve sonrası alınan kan örneklerinde ilgili parametrelere bakıldı. Veriler ANOVA testi ile değerlendirildi. Sitokin ve IgA düzeylerinde anlamlı farklılık gözlenmezken, CD4 derişimleri düştü, CD8 derişimleri ise yükseldi. Sonuç olarak, seiz haftalık dayanıklılık antrenmanının lenfosit subpopülasyonlarında değişikliklere yol açtığı, bitkinleştirici egzersizin ise immün fonksiyonu baskılamadığı ortaya kondu.

Anahtar sözcükler: *İnterlökinler, immün hücreler, bitkinleştirici egzersiz, dayanıklılık antrenmanı*

INTRODUCTION

Some studies suggest that exercise induces plasma cytokine response in human subjects during and following strenuous exercise (10,15,18,27). These indicate that strenuous exercise induces an increase in the pro-inflammatory cytokines TNFα (tumor necrosis factor) and interleukin IL-1, and a dramatic increase in the inflammation responsive cytokine IL-6. Other studies indicate that the level of IL-1 increases in response to exercise (4,11,15,24). An increase in IL-6 concentration has been reported immediately after a marathon run, but there was no detectable IL-1 (15). IL-6 was also shown to be elevated in response to various exercises (1,2,7,15). Whereas some studies report increased plasma TNFα concentrations (15,26), some have failed to detect TNFα following exercise (1,15).

Responses of blood leukocyte subpopulations to an episode of acute exercise are highly stereotyped. Lymphocyte concentrations increase during exercise and fall below resting levels after long duration physical work (15,21,24). Several reports describe exercise induced changes in subsets of blood mononuclear cells (15). During exercise the CD4 to CD8 ratio decreases, reflecting greater increase in CD8 lymphocytes than in CD4s (3,15). The initial increase in CD4 and CD8 cells after exercise appears not to be due to the repopulation by newly generated cells, but may result from a redistribution of activated cells. Simpson et al (23) examined the effects of intensive, moderate and downhill treadmill running on blood lymphocyte expression in trained subjects. No differences were found between the intensive protocol and the eccentric protocol at the same relative intensity. Analysis of lymphocyte subsets revealed that the total number of CD3, CD4 and CD8 lymphocytes increased after the intensive protocol before falling below pre-exercise values 1 h post-exercise.

In another study, healthy male subjects performed three bouts of bicycle exercise lasting 60, 45 and 30 min at 75% of VO_2max separated by 2 h of rest (15). The lymphocyte proliferation declined 2 h after each bout of exercise. It is believed that the diversity of results in various studies may reflect that enhancement or reduction of immune response depends on the intensity of exercise and the duration of rest between exercise sessions (15). IgA constitutes only 10-15% of serum immuno-globulins, but is the predominant class in mucosal secretions. Decreased salivary IgA was found after swimming, running and incremental treadmill running to exhaustion (15). The present study will provide measurements of the time course of TNF, IL-1, IL-6, TNFα, CD4, CD8 and IgA in the post-exercise period.

MATERIAL and METHODS

Eighteen active female healthy volunteers participated in the study. Subjects were assigned into the experimental (n=10, 21.6 ± 1.7 yrs old, 161.5 ± 2.7 cm of height, 57.3 ± 7.0 kg of body weight, and VO_2max of 34.2 ± 2.0 $ml.min^{-1}.kg^{-1}$), and control (n=8, 24.3 ± 4.3 yrs old, 159.8 ± 4.9 cm of height, 54.7 ± 3.8 kg of body weight, and VO_2max of 36.1 ± 3.8 $ml.min^{-1}.kg^{-1}$) groups. The experimental protocol was approved by the ethics committee, and all subjects were informed of the risks and purposes of the study before their written consent was obtained.

Blood samples were drawn from the antecubital vein before and immediately following an exhaustive Bruce protocol exercise. For each subject VO_2max was determined during an incremental exercise test on the treadmill. Ambient temperature during running was 17°C. The experimental group participated in an 8-wk incremental endurance training program. After the eight weeks, the subjects followed the same exercise and blood sampling protocols.

For analyzing cytokines and adaptive components of the immune system, 11 ml of blood sample was drawn into an EDTA containing glass tube. The tube was kept on ice for 15 min before centrifugation. Plasma was separated from the cells and stored until subsequent analysis for IL-1, IL-6, TNFα, CD4, CD8 and IgA. Plasma concentrations of TNF, IL-1 and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA). Determination of CD4 and CD8 subsets of leukocytes was accomplished by standard flow cytometry with three-color analysis. Cell surface molecule density was expressed as molecules of equivalent soluble fluorochrome.

RESULTS

While mean plasma concentrations of CD8, TNFα, IL-1 and IgA increased following exhaustive exercise; those of CD4 and CD4/CD8 ratios decreased, and IL-6 concentrations did not change at the start and the end of the eight weeks training program (Tables 1 and 2, Figure 1). ANOVA test revealed that CD4, CD8 and CD4/CD8 responses were significant (Table 1, $p<0.05$), while those of IL-1, IL-6, TNFα, and IgA were not (Table 2, $p>0.05$). The Tukey test proved significance.

Table 1. Changes in CD4 and CD8 cells before (1) and after (2) 8-wks of training, pre- and post-exhaustive exercise in experimental and control groups.

Group	Phase	CD4 ($n.10^{-3}$)	CD8 ($n.10^{-3}$)	CD4/CD8
Experimental	Pre 1	32.1 ± 5.4	22.8 ± 5.1	1.48 ± 0.36
	Post 1	26.8 ± 5.1	23.0 ± 4.7	1.19 ± 0.34
	Pre 2	44.7 ± 6.8	20.3 ± 2.6	1.52 ± 0.56
	Post 2	38.3 ± 6.5	21.7 ± 2.8	1.88 ± 0.44
Control	Pre 1	31.3 ± 3.2	20.5 ± 3.1	1.59 ± 0.37
	Post 1	24.3 ± 3.3	20.3 ± 3.2	1.23 ± 0.18
	Pre 2	43.6 ± 3.9	19.4 ± 1.9	2.29 ± 0.29
	Post 2	33.8 ± 4.1	19.5 ± 2.1	1.74 ± 0.26
ANOVA (F/p)		3.71/0.02*	4.88/0.007*	2.83/0.05*

Table 2. Changes in cytokines and IgA before (1) and after (2) 8-wks of training, pre- and post-exhaustive exercise in experimental and control groups.

Group	Phase	IL-1 (pg/ml)	IL-6 (pg/ml)	TNFα (pg/ml)	IgA (mg/dl)
Experimental	Pre 1	0.01 ± 0.00	0.013 ± 0.001	0.24 ± 0.07	210 ± 119
	Post 1	0.03 ± 0.02	0.016 ± 0.004	0.31 ± 0.03	224 ± 152
	Pre 2	0.11 ± 0.02	0.035 ± 0.007	0.19 ± 0.12	212 ± 129
	Post 2	0.20 ± 0.08	0.021 ± 0.004	0.27 ± 0.03	228 ± 138
Control	Pre 1	1.96 ± 0.56	0.012 ± 0.005	0.44 ± 0.06	188 ± 51
	Post 1	1.21 ± 0.32	0.019 ± 0.003	0.31 ± 0.02	202 ± 56
	Pre 2	0.11 ± 0.02	0.017 ± 0.001	0.35 ± 0.03	192 ± 51
	Post 2	0.13 ± 0.04	0.023 ± 0.007	0.41 ± 0.02	205 ± 47
ANOVA (F/p)		1.19/0.33	-/-	0.57/0.64	1.57/0.22

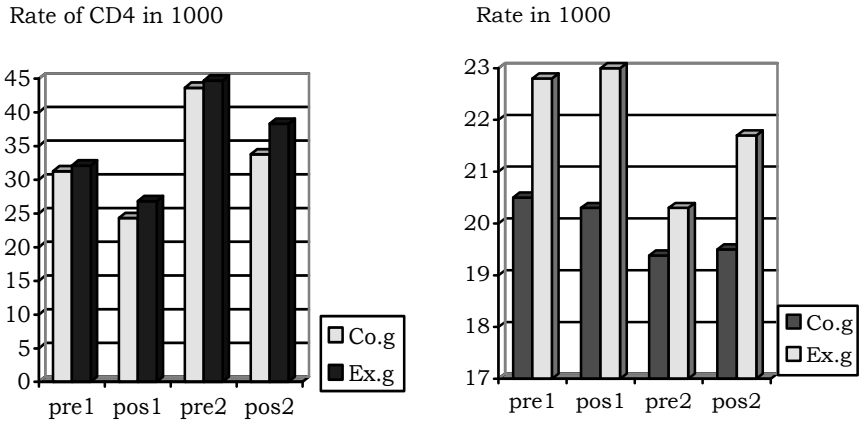

Fig.1 Changes in the CD 4 and CD8 cells before (1) and after (2) 8-wks of training, pre- and post-exhaustive exercise in experimental and control groups.

DISCUSSION

The present study provides information about changes in plasma concentrations of the cytokines TNFα, IL-1, IL-6, CD4, CD8, CD4/CD8 ratio, and IgA following an incremental endurance training program. The anti-inflammatory effect is among the physiological benefits of physical activity. Both cross-sectional and longitudinal studies support an inverse relationship between inflammatory cytokines and physical activity levels in healthy individuals. Whereas moderate exercise improves immune function, intense or prolonged exercise suppress it (6,12,28). By improving endothelial function, physical activity might reduce endothelial cell secretion of IL-1 and IL-6, both induced upon acute phase inflammatory response (8,14,16,17,19,25).

Previous studies demonstrated increased plasma levels of IL-1 post-exercise, depending either on assay sensitivity and specificity, or due to the fact of local cytokine production, which is rapidly cleared from the circulation. The recent finding of IL-1 mRNA in muscle biopsies obtained after strenuous exercise without increase in the IL-1 protein in plasma, and the finding of IL-1 in the urine of runners support this latter idea (10,18,24). Studies reported increased plasma TNFα 2h after completing a 2.5 h race and 1 h after a 5 km race, respectively, but other studies have failed to detect any TNFα increase following exercise (4,26).

It has been shown that carbohydrate loading diminishes the exercise-induced increase in IL-6 and IL-1 (5). Though carbohydrate intake was not controlled in the present study, subjects were assumed to be well loaded, according to the questionnaire. With carbohydrate restriction, an even more pronounced increase in plasma cytokine levels might have been found previously. Strenuous exercise induces an increase in the pro-inflammatory cytokines TNFα and IL-1 and a dramatic increase in the inflammation responsive cytokine IL-6. In this study, the exercise could not be considered as intense and strenuous.

Regular exercise may protect against diseases associated with chronic low-grade systemic inflammation (6,18). This long-term effect of exercise may be ascribed to the anti-inflammatory response elicited by an acute bout of exercise, which is partly mediated by muscle-derived IL-6. Physiological concentrations of IL-6 stimulate the appearance of the anti-inflammatory cytokines in the circulation, and inhibit the production of the proinflammatory cytokine TNFα (4,20,26). Local response to tissue injury involves the production of cytokines, which are released at the site of the inflammation (11,24). These cytokines facilitate an influx of lymphocytes, neutrophiles, monocytes and other cells, which participate in the clearing of antigens and healing of tissues (9,13,21,22).

To conclude, upon studying the effects of an 8-week adaptation training on the immune system in recreationally active women, no significant changes in cytokines and IgA concentrations were observed, but CD4 decreased and CD8 increased significantly. Endurance training may induce changes in lymphocyte subsets, but not in the suppression of immune function following an exhaustive bout of exercise.

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