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ORIGINAL ARTICLE

Effects of Endurance Training on the Serum Levels of Tumour Necrosis Factor-α and Interferon-γ in Sedentary Men

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Physical activity could be considered one of the factors that affect the immune system status and function. To find the relation between exercise and cytokines, we examined the possible effects of an 8-week endurance training program on the serum levels of cytokines, including tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in sedentary men. A total of 30 healthy young male volunteers were randomly divided into an endurance training group and a control group. The training group followed a specific exercise protocol (running on a treadmill for 15～30 min at 50～70% maximal heart rate) for 8 weeks and the control group did not participate in any exercise program. Venous blood samples were collected from both the groups 24 h before and 24 h and 48 h after the exercise. Repeated ANOVA was used for statistical purposes. The serum levels of TNF-α and IFN-γ were determined by ELISA. Significant ($p<0.05$) and non-significant ($p>0.05$) decreases were observed in the serum levels of IFN-γ and TNF-α, respectively, after the 8-week endurance training program. Our findings indicated that an 8-week endurance exercise may affect the serum levels of some inflammatory cytokines, suggesting the beneficial role of this training protocol in elderly population and people with certain conditions (inflammation of the vertebrae or other inflammatory diseases).

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Keywords: Cytokine, Endurance exercise, IFN-γ, TNF-α

INTRODUCTION

Cytokines are one of the most important products of the immune system, which mediate the interactions of the cells involved in the immune responses and are secreted by the cells of the innate and acquired immune system, endothelial cells and fat storage cells. There are different types of cytokines, among which interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) have fundamental roles (1). IFN-γ plays critical roles in cell-mediated innate and acquired immunity, and is produced by helper T cells, cytotoxic T cells and natural killer (NK) cells. TNF-α, which is mostly released from the monocytes, macrophages and NK cells, is a major pro-inflammatory cytokine that produces many effects on various cell types and immune responses (2).

It has been recognised that the immune system is affected by different types of psychological and physiological stressors. Physical activity is considered as one of the many factors that affect the immune system status and function (3). Moreover, various studies have shown that cytokines are produced by a range of physiological stimuli such as intense exercise, stress hormones, oxidative stress and energy crisis (4).

Nowadays, the immune system and its response to endurance exercise and activities has become a favourite topic of health and exercise. Moderate and regular exercises increase

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Abbreviations: IFN-γ, interferon-gamma; NK cell, natural killer cell; ELISA, enzyme linked immunosorbent assay; Ab, antibody; TNF-α, tumour necrosis factor-alpha; BMI, body mass index; IFN-γ, interferon-γ

the body's resistance against infections, such as upper respiratory tract infection, by enhancing the activity of the immune system. However, it has been observed that the body's resistance to infections significantly decreases after an intense exercise. Endurance exercise affects the secretion of pro-inflammatory cytokines (5). Moreover, many studies have indicated that short-term exercise produces different effects on the circulating levels of the pro-inflammatory cytokines, when compared with prolonged exercise (6).

With regard to the beneficial (e.g. reduced risk of cardiovascular disease) and harmful (e.g. implications of asthma mediated through the activity of circulating leukocytes) effects associated with exercise, interests on studies focusing on the relation between the immune response status and exercise are increasing among researchers (7). Exercise or physical activity, based on the type, intensity, duration and various physical conditions, induces different effects on various factors of the immune system (8). Hence, the aim of this study was to investigate the effects of an 8-week endurance activity on the serum levels of IFN-γ and TNF-α in sedentary men.

MATERIALS AND METHODS

Subjects

A total of 28 healthy young men, aged 18～24 years, were randomly divided into two groups, namely, endurance training group (ET group, n=15) and control group (C group, n=13). Subjects with a history of heart disease, hypertension, diabetes, smoking and use of drugs were excluded from the study. Furthermore, the subjects were asked not to participate in any activities except the training program during the study period.

Research design and exercise program

The subjects belonging to the ET group underwent an 8-week endurance training program followed by a recovery period (Fig. 1) (9). In brief, the subjects exercised on a treadmill three times a week for 8 weeks. Their running on the treadmill lasted for 15～30 min at 50～70% maximal heart rate. Venous blood samples were collected 24 h before and 24 and 48 h after the exercise.

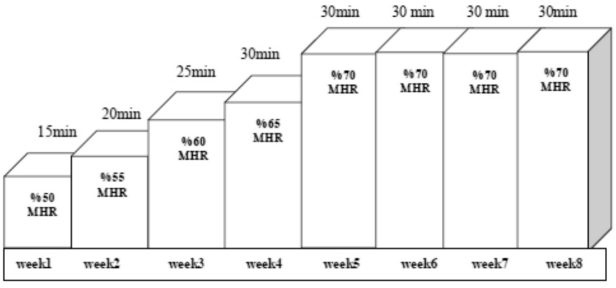

Figure 1. Eight-week endurance-training program.

Anthropometric characteristics, including height, weight and body mass index (BMI) of all the participants, were measured using standard procedure before the beginning of the study, and resting blood samples were collected for cytokine assessment. In detail, 24 h before the beginning of the training program and after a 10-h fasting period at 10:00 am, all the participants were asked to remain seated for 10 min, and first blood samples were collected. Subsequently, second and third blood samples were collected 24 h and 48 h after recovery (end of the exercise training program) (Fig. 2). All the participants were allowed to warm up for 5 min, and then they started to run on the treadmill (Table I).

Maximal heart rate

The heart rates of all the participants were recorded for the

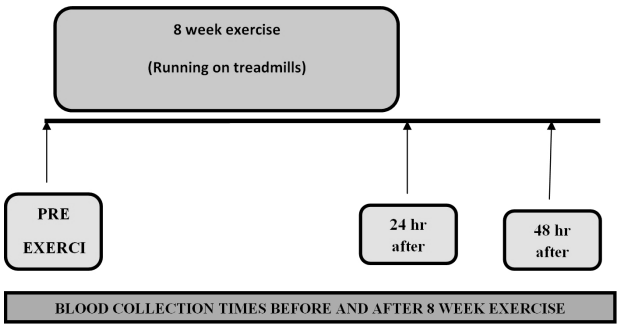

Figure 2. Blood collection times before and after the 8-week exercise.

Table I. Duration and intense of training in an 8-week endurance training

	Duration of running	Intense of training
Week 1	15 minutes	%50 maximal Heart rate
Week 2	20 minutes	%55 maximal Heart rate
Week 3	25 minutes	%60 maximal Heart rate
Week 4	25 minutes	%65 maximal Heart rate
Week 5	30 minutes	%70 maximal Heart rate
Week 6	30 minutes	%70 maximal Heart rate
Week 7	30 minutes	%70 maximal Heart rate
Week 8	30 minutes	%70 maximal Heart rate

maximal heart rate calculation using Karvonen method (10) as follows:

$$HR_{target}=\%\ Intensity(HR_{max}-HR_{rest})+HR_{rest}$$

Cytokine concentrations tests

During each blood sampling, 2 ml of blood sample were collected from the brachial vein. The separated sera were tested using commercial ELISA kits for the measurement of serum cytokine concentrations.

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Serum TNF-α was measured by commercial ELISA kit (Bendermedsystems, Austria: Cat. No. BMF 223) follows company instruction. It is based on the direct sandwich technique with biotin-Streptavidin, in which two monoclonal antibodies (Ab) are directed against human TNF-α. Human TNF- present in the sample or standard binds to antibodies adsorbed to the microwells. A biotinconjugated anti-human TNF- Ab is added and binds to human TNF- captured by the first Ab. Following incubation unbound biotin-conjugated anti-human TNF- Ab is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human TNF- Ab. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of human TNF- present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human TNF- standard dilutions and human TNF- sample concentration determined.

Serum IFN-γ was measured by ELISA (Bendermedsystems, Austria: Cat. No. BMS279INST), According to company instruction as the same method for serum TNF-α.

Statistical analysis

For data analysis, one-way ANOVA with repeated measures test was employed using SPSS-12. The measurements were considered as statistically significant if $p<0.05$. All the values were expressed as mean and standard deviation.

RESULTS

Subjects

There was no significant difference in the baseline characteristics, including age and BMI, between the ET group (21.1±1.8 years and 21.2±2.4 kg/m^2, respectively) and C group (19.3±1.2 years and 20.5±2.2 kg/m^2, respectively) ($p>0.05$).

Cytokine response to exercise

The serum levels of the inflammatory cytokines IFN-γ and TNF-α decreased after the 8-week endurance training program. However, the reduction in the TNF-α level, when compared with the pre-exercise levels, was not statistically significant ($p>0.05$) (Fig. 3), whereas a significant decrease in the serum IFN-γ level was noted after the 8-week endurance training program ($p<0.05$) (Fig. 4).

DISCUSSION

There are only limited reports on the effects of low-intensity and low-duration aerobic exercise on inflammatory cytokines. The present study is the first to indicate the influence of

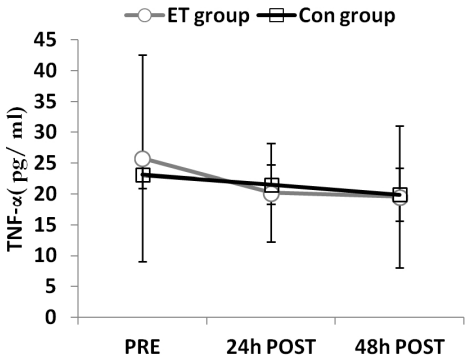

Figure 3. TNF-α serum level pre-exercise, 24 h and 48 h post exercise. Open bars, exercisers; solid bars, controls.

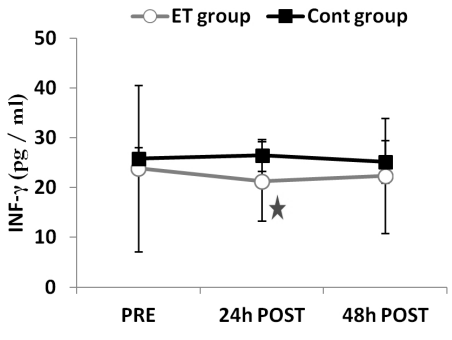

Figure 4. IFN-γ serum level pre-exercise, 24 h and 48 h post exercise. Open bars, exercisers; solid bars, controls. *Significant difference comparing pre-exercise value ($p<0.05$).

low-intensity and low-duration exercise on the serum levels of inflammatory cytokines, including TNF-α and IFN-γ, and it was observed that an 8-week endurance training program could result in a non-significant decrease in the TNF-α level. Previous studies on the effect of exercise on inflammation showed controversial results. Conraads reported that a combination of endurance and resistance exercises did not affect the plasma levels of TNF-α (11). However, Ryan demonstrated that weight loss and exercise program (aerobic+resistance) decreased the concentrations of TNF-α (12). Furthermore, an increase in the plasma and urinary concentrations of TNF-α after exercise (13) and up to 72 h post-exercise (8) has also been reported. In agreement with the findings of the present study, many previous studies have reported that the TNF-α level decreased after physical activities and exercises (14,15). Nevertheless, there are several reports demonstrating significant increases in the TNF-α level after exercise (3,16). In addition, it has been indicated that the sport field can affect the changes in the level of TNF-α (17).

Some studies have shown that the presence of high levels of TNF-α for longer duration could cause tissue damage and some complications, whereas low levels of TNF-α may contribute to tissue repair (18). In addition, high concentrations of TNF-α may produce negative effects on heart function (19). Therefore, the endurance training program may be useful for people with high levels of TNF-α.

The results of the present study showed significant decreases in the serum IFN-γ concentration following the 8-week endurance training program. This finding is in agreement with those reported in previous studies, which demonstrated a decreased level of IFN-γ after physical activity and exercise program (20,21). However, the results of several studies are in contrast to our findings. Some investigators reported a significant increase in the IFN-γ level after exercise (9,16). Heesen reported that the TNF-α and IFN-γ plasma levels increased after an intensive exercise with bicycle ergo metre (9), while Suzuki showed that the concentrations of IFN-γ and TNF-α did not alter after a marathon match (14). Moreover, several studies have reported that the production of IFN-γ from T cells is inhibited by cortisol and epinephrine, which are increased in response to exercise (22). Therefore, this mechanism may be responsible for the decrease in the IFN-γ levels, observed in the present study and previous studies (3).

The response of cytokines to exercise is complex and related to the intensity of the exercise, training conditions, location of cytokine measurement (e.g. tissue, plasma or urine) and specificity and sensitivity of the methods of measurement. Furthermore, the duration of the time between exercise and cytokine response may differ depending on the type of cytokines (8). As we could not find any other studies with a research design similar to that employed in the present study, it is necessary to distinctly examine the effects of each type of exercise on the immune system.

In summary, the results of the present study revealed that 8 weeks of aerobic exercise with low to medium intensity and duration could affect the production of inflammatory cytokines such as IFN-γ and TNF-α.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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