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Effect of a four-week exercise program on the secretion of IFN-γ, TNF-α, IL-2 and IL-6 cytokines in elite Taekwondo athletes

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Abstract. The aim of the present study was to examine how a 4-week exercise program affects the serum levels of certain cytokines in Taekwondo athletes. The study involved 10 elite male Taekwondo athletes (mean age, 20.67±0.24 years; mean weight, 65.45±1.69 kg) who were studying at the Physical Education and Sports High School of Selçuk University (Konya, Turkey) in June 2014. The subjects were involved in a Taekwondo exercise program on every weekday for 4 weeks. The subjects were also engaged in an exercise to exhaustion session twice; once before starting the 4-week exercise program and once upon completion of the program. Blood samples were collected from the subjects in four rounds: During rest, upon fatigue, and before and after the 4-week exercise program. These samples were analyzed to establish the serum levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-2 and IL-6 using enzyme-linked immunosorbent assay test kits. Pre- and post-exercise program, the IFN-γ and TNF-α levels did not show any significant difference. When compared with the pre-exercise levels, serum IL-2 levels of the subjects were found to be elevated after the 4-week exercise program. The highest serum IL-6 values were established after the subjects were exercised to fatigue before the exercise program was initiated ($P<0.05$). The 4-week exercise program resulted in a decrease in IL-6 levels ($P<0.05$). The findings of the study indicate that a 4-week exercise program did not result in significant changes in IFN-γ and TNF-α levels, but led to an increase in IL-2 levels. The notable finding of the present study is that a 4-week exercise program reduces cellular immune functions and, thus, the levels of IL-6, which negatively influences performance.

Introduction

Described as a set of activities that improve one's health and help one maintain good health, sports or exercise, in addition to the protective function, serve as a treatment tool (1-3). However, despite its ameliorative effects, exercise (which is also considered to be a controlled catabolic process) leads to the destruction of muscle and even bone tissue, to a certain extent (4,5). Given the mechanisms of immune responses, it can be argued that tissue injury in the skeletal muscle resulting from exercise paves the way for activation of immune reactions (6). However, previous studies indicated that a cytokine response may be observed even after exercise that is not strenuous (7). The decisive factor distinguishing elevation of cytokines in response to the immune response to infections from the post-exercise elevation of cytokines is the increase observed in tumor necrosis factor-α (TNF-α) levels in inflammation. In response to exercise, however, the first cytokine to increase is interleukin (IL)-6, although the extent of the increase will depend on the type of exercise and the level of effort exerted (8). The major reason behind the increase is that muscle injury caused by exercise serves as a strong signal for IL-6 response (8,9). While developing appropriate training programs to increase performance in physical activity, the dietary alternatives in athlete nutrition are also given greater emphasis (10-12). However, when strategies for athlete performance and diet are devised, one of the major issues that requires consideration is the elimination of factors that may negatively impact the health of an athlete (12). It was reported that regular and mild exercise had a favorable effect on immune responses (13,14), whereas slow-paced and extended exercise may impair the T-lymphocyte cell functions and natural killer cell activation, thus altering the cytokine balance and negatively influencing immune reactions (13,15). In this context, the association between exercise and immune system becomes a significant research topic (13). Consequently, the present study aims to investigate how a 4-week exercise program affects the serum values of certain cytokines in individuals performing Taekwondo.

Subjects and methods

Subjects. The current study involved 10 elite healthy male Taekwondo athletes (mean age, 20.67±0.24 years; mean weight, 65.45±1.69 kg) studying at the Physical Education and Sports High School of Selcuk University (Konya, Turkey). The athletes were informed about this study and they participated in the study with consent. The study protocol was approved by the ethics committee of Selçuk University's Physical

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Table I. Serum cytokine levels of the subjects.

Time-point	IFN-γ (IU/ml)	IL-2 (U/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
Before exercise program				
Rest	0.25±0.05	0.53±0.02	20.58±4.13	4.04±0.98
Exhaustion	0.29±0.16	0.55±0.07	25.05±5.03[a]	3.43±0.73
After exercise program				
Rest	0.26±0.32	0.71±0.10[a,b]	16.72±4.72[a,b]	4.01±1.07
Exhaustion	0.27±0.12	0.74±0.15[a,b]	16.96±4.62[a,b]	4.35±1.97

Values are presented as mean ± standard deviation. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. [a]P<0.05 vs. before exercise program, rest; [b]P<0.05 vs. before exercise program, exhaustion.

Education and Sports High School. The study was performed in June 2014.

Methods

Four-week Taekwondo exercise program. The athletes attended Taekwondo training every day of the week for 4 weeks. The exercise program began with a 20-min warm-up. Following the warm-up, each athlete was involved in a hand target practice. Spending maximal effort, the athletes practiced all techniques on the hand target up to complete exhaustion. The practice was repeated three times and the training session ended with a cool-down exercise.

Exhaustion exercise (Bruce protocol). The athletes were worked to exhaustion on two occasions; once before and once after the 4-week exercise program. A common clinical exercise assessment is the Bruce protocol, which was used as the exhaustion test in the current study. The incline and speed of the treadmill (Cosmed T150 treadmill) were adjusted every 3 min until the subject was unable to continue (16).

Collection of blood samples from subjects. Blood samples (5 ml) were collected from the subjects in four rounds: During rest and upon exhaustion before the 4-week exercise program, and again during rest and upon exhaustion after the 4-week exercise program. Samples were taken from the forearm vein at 9:00 a.m. on an empty stomach. The samples were then centrifuged at 2,000 x g for 10 min at 4˚C to separate the sera and stored at -80˚C until the time of analysis.

Biochemical analyses. Interferon-γ (IFN-γ) (LOT:121101/A), IL-2 (LOT:122301), IL-6 (LOT:124802) and TNF-α (LOT:121902A) parameters were analyzed in the sera using ELISA kits which were purchased from DIAsource ImmunoAssays (Louvain-la-Neuve, Belgium).

Analyses were performed according to the manufacturer's instructions. The results are presented in units of IU/ml for IFN-γ and IL-2, and pg/ml for IL-6 and TNF-α.

Statistical analysis. SPSS 16.0 software package (SPSS, Inc., Chicago, IL, USA) was used for the statistical evaluation of data. Following calculation of the arithmetic means and standard deviations of all parameters, repeated measures variance analysis was used to detect the differences between the measurements taken at different times, and the least significant difference test was employed to identify the time period from which the difference arose. P<0.05 was considered to indicate a statistically significant difference.

Results

The IFN-γ levels of the subjects did not vary before and after the 4-week exercise program. When compared with the levels measured prior to exercise, IL-2 levels were found to be increased subsequent to the 4-week exercise program (P<0.05). The highest serum IL-6 value was obtained after exercise to exhaustion prior to initiation of the 4-week program (P<0.05). The 4-week exercise program resulted in a decrease in IL-6 levels (P<0.05). In addition, the TNF-α levels before and after the 4-week exercise program did not differ (Table I).

Discussion

Levels of IFN-γ and TNF-α did not differ significantly in the measurements during rest and exhaustion before and after the 4-week exercise program. The results of studies regarding the associated between exercise and cytokines are inconsistent. It has been shown that a 32-week exercise program did not affect the TNF-α levels in older individuals (17). Consistently, it was reported that acute aerobic exercise in humans did not alter the cytokine activity of T cells (18), and that combined strength and endurance exercise did not change IFN-γ and TNF-α levels in individuals with type II diabetes (19). However, it was shown that 12 weeks of swimming exercise elevated IFN-γ and TNF-α levels in mice (20), and that exercise increased the release of IFN-γ in horses (21). Additionally, it was demonstrated that intense physical activity could disrupt immune responses (22), and that an 8-week swimming exercise program (duration, 150 min per session; frequency, 6 days per week) reduced IFN-γ and IL-2 levels in rats (23). Thus, the results of the studies investigating the association between exercise and cytokines are inconsistent. These differences may be attributed to the variances in exercise type, the duration and intensity of exercise and the subjects, as well as other aspects of implementation. The IFN-γ and TNF-α levels of the subjects in the current study remained unaffected before the exercise

program and after the 4-week Taekwondo exercise program. This result is consistent with the reports of Marques *et al* (17), LaVoy *et al* (18) and Touvra *et al* (19) all of whom noted that the TGF-β1 cytokine was not affected by exercise.

In comparison to the levels measured before the program, the IL-2 levels of the subjects after the 4-week Taekwondo exercise program were found to be elevated. Suzuki *et al* (24) reported that there was no increase in the secretion of cytokines following endurance training in athletes, and it was demonstrated in another study that IL-2 levels of exercised rats remained unchanged (25). Similarly, Kara *et al* (6) noted that exercise did not cause any change in the IL-2 levels of individuals performing wrestling as a sport. The results of these studies are not consistent with the elevated IL-2 levels that were observed after the 4-week Taekwondo training program. However, one-hour strenuous training was shown to have a marginal effect on cytokine production in male rowers (26) and it was suggested that exercise in energy-restricted rats may increase IL-2 production (27). Another study reported elevated cytokine levels in cyclists (28), while Petersen *et al* (29) demonstrated that exercise significantly increased CD^{4+} and CD^{8+} lymphocytes, and cytokine levels in individuals who received antioxidant supplementation. Another study examining the interaction between exercise and cytokines revealed that exercise in a cold environment elevated IL-2 levels (30). All of these reports suggesting that exercise increases IL-2 levels are consistent with our finding of elevated IL-2 levels following a 4-week Taekwondo training program.

The highest serum IL-6 values in the current study were identified during the exercise to exhaustion test before the program; the 4-week Taekwondo exercise program appeared to reduce the IL-6 levels. Although the extent of the increase may vary depending on the type and physical load of exercise, the first cytokine to be elevated in response to exercise is IL-6 (31). It was established that plasma IL-6 level showed an ~100-fold increase following long-term and strenuous exercise (32). Studies investigating the factors that cause the post-exercise IL-6 elevation showed that the major factors were the length of exercise and the muscle mass involved in exercise (31,32). Consequently, it has been proposed that muscle damage caused by exercise is a warning signal for IL-6 response (31,32). The abovementioned studies suggest that the increase that was established in the present study in the IL-6 values, during exercise to exhaustion, is an expected outcome. Furthermore, Yfanti *et al* (33) demonstrated that exercise to exhaustion elevated IL-6 levels. Notably, in the current study, however, the 4-week Taekwondo exercise program suppressed the IL-6 levels. This finding may be novel, as the studies cited above indicate that the muscular damage in physical activity causes an increase in IL-6 levels (31-34). However, our study demonstrates that a 4-week Taekwondo training program reduced the IL-6 levels, which had become elevated as a result of exercise to exhaustion. In addition, it has been reported in a previous study that regular exercise exerts a regulatory effect on immune responses (18). In conclusion, the results of the present study indicate that long-term regular physical activity serves a regulatory function in immune responses by elevating serum IL-2 levels and suppressing serum IL-6 levels. Howevever, this exercise program had no affect on IFN-γ and TNF-α.

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