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Acute Effect of Repeated Sprint and Aerobic Endurance Training on Foxp3+ Regulatory T Cells and Cytokine Levels

Tekrarlı Sprint ve Aerobik Dayanıklılık Antrenmanının Foxp3+ T Regülatör Hücreler ve Sitokin Düzeylerine Akut Etkisi

Orkun Akkoç[1], Deniz Genç[2], Hakan Özel[3], Recep Fatih Kayhan[4], Muazzez Gökalp[2]
Özlem Kırandı[1], İlker Yücesir[1], Tunç Akkoç[3]

[1]Department of Sport Science, İstanbul University-Cerrahpasa, İstanbul, Turkey
[2]Department of Pediatric Allergy and Immunology, Marmara University, İstanbul, Turkey
[3]Department of Sport Science, Marmara University, İstanbul, Turkey
[4]Department of Sport Science, Recep Tayyip Erdogan University, Rize, Turkey

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Corresponding Author: Orkun Akkoç, İstanbul University-Cerrahpasa, Department of Sport Science Faculty, 34063 Avcılar, İstanbul, Turkey
orkunakkoc@hotmail.com

Abstract

Objective: The aim of our study was to investigate the acute effect of repeated sprints and aerobic endurance training on Foxp3+ T regulatory cells and cytokines.
Materials and Methods: The study population consisted of 16 sprinters and 16 long distance runners. Each subject was divided into his/her own branch as sprinting and distance training. Within the scope of the study, the height, body weight, sporting age, Foxp3+ T regulatory cells and cytokine values of subjects were recorded. Immunity subparameters were compared in venous blood samples taken before and after training. The Wilcoxon test was used to compare the values before and after training with level of statistical significance accepted as $p<0.05$.
Results: A statistically significant change was not observed for Foxp3+ T regulatory cells before and after training in sprinter ($p=0.47$) and distance runners ($p=0.52$). Sprinters had increased IL-2 ($p=0.00$), IL-4 ($p=0.00$), IL-10 ($p=0.02$), IL-17 ($p=0.000$) and TNF-α ($p=0.000$), decreased IL-6 ($p=0.000$) and unchanged IFN-γ levels ($p=0.81$). Distance runners had increased IL-4 ($p=0.000$), IL-10 ($p=0.000$), IL-17 levels ($p<0.00$), decreased TNF-α ($p=0.00$), IL-2 ($p=0.05$) and unchanged IFN-γ ($p=0.15$) and IL-6 ($p=0.15$).
Conclusion: Immune system is affected by the intensity and type of exercise. It can be said that anaerobic exercises like sprinting with high intensity supress the immune system more severely.

Keywords: Immune response to exercise, cytokines, Foxp3+ T regulatory cells

Öz

Amaç: Çalışmanın amacı, tekrarlayan sprint ve aerobik dayanıklılık antremanının Foxp3+ T regülatör hücreleri ve sitokinler üzerindeki akut etkisini araştırmaktır.
Gereç ve Yöntem: Çalışmaya 16 sprinter ve 16 uzun mesafe koşucusu katıldı. Sprinter ve mesafe antrenmanı yapmak üzere her denek kendi branş grubuna ayrıldı. Çalışma kapsamında deneklerin boy, vücut ağırlığı, spor yaşı, Foxp3+ T regülatör hücre ve sitokin değerleri kaydedildi. Antrenman öncesi ve sonrası alınan venöz kan örneklerinde bağışıklık alt parametreleri karşılaştırıldı. Wilcoxon testi eğitim öncesi ve sonrası değerleri karşılaştırmak için kullanıldı ve anlamlılık $p<0.05$ olarak kabul edildi.
Bulgular: Sprinter ve mesafe sporcularının antrenman öncesi ve sonrasında Foxp3+ T regülatör hücrelerinde düzeylerinde artış, istatistiksel seviyelerinde değişiklik gözlenmemiş, (sprinterlerde $p=0.47$, mesafecilerde $p=0.52$). Sprinter seviyelerinde artış ($p=0.00$), seviyesinde değişiklik gözlenmemesine karşılık ($p=0.02$), IL-17 ($p=0.000$) ve TNF-α artmış, IFN-γ değişmemiş, IL-6'da azalma saptandı ($p<0.05$). Mesafecilerde IL-4, IL-10, IL-17 artmış, IL-6 ve IFN-γ değişmemiş, TNF-α ve IL-2 seviyelerinde azalma saptanmıştır ($p<0.05$).
Sonuç: Sonuç olarak immün sistem egzersiz şiddeti ve egzersiz türünden etkilenmektedir. Yüksek şiddette yapılan sprint tarzı anaerobik egzersizlerin immün sistemi daha çok baskıladığı söylenebilir.

Anahtar kelimeler: Egzersize bağışıklık tepkisi, sitokinler, Foxp3+ T regülatör hücreler

ORCID: O. Akkoç 0000-0003-0718-6883, **D. Genç** 0000-0003-0351-2805, **H. Özel** 0000-0002-0422-5376, **R. F. Kayhan** 0000-0002-1022-2892, **Ö. Kırandı** 0000-0001-5690-4576, **İ. Yücesir** 0000-0002-0003-867X, **T. Akkoç** 0000-0001-9179-2805

Introduction

Physical activity has a protective effect against many chronic diseases.[1] Physical inactivity increases the risk of diseases like heart diseases, diabetes, metabolic syndrome and depression.[1] The immune system is the body's protective mechanism. It protects the body from harmful microorganisms like microbes, viruses, bacteria, fungi and parasites.[3] The response of the immune system to exercise is important for the development of inflammation and infection after exercise in addition to understanding acute and chronic diseases that may develop after exercise. However, the ideal intensity and duration of exercise is controversial.[3,4] The immune system has different acute and chronic responses to exercise. The response of the immune system to exercise varies according to the intensity, time and type of training.[2] Moderate and low-intensity exercises increase immunity, while high-intensity exercises are proposed to suppress the immune system and increase the risk of infection and inflammation.[3,4] This period of reduced immunity is called the open window.[3]

The immune system creates a self-reaction of the body to itself with a range of protective methods to prevent harm. Suppressive T cells are actually determined to be T regulator cells (Foxp3+ T regulatory cells). In peripheral blood CD4+ T lymphocytes carry 5-10% of IL-2R (CD25) chains[5,6] and these cells are called Foxp3+ T regulatory cells that aid in maintaining resistance against self-antigens. TGF-β is an immunosuppressive cytokine[7] and Foxp3+ T regulatory cells interacting with this cytokine express the Foxp3+ T regulatory cell gene and cause abnormal development of suppressive Foxp3+ T regulatory cells. IL-6 suppresses induction of TGF-β-mediated Foxp3+ T regulatory cells.[5] When studies investigating exercise and Foxp3+ T regulatory cells levels are examined, it is seen that the concentration of Foxp3+ T regulatory cells increased after acute and chronic exercise and training without regard to intensity of exercise.[6-9]

Cytokines belong to a low-molecular weight protein group secreted by white blood cells in response to a stimulant. Cytokines regulate the relationships between cells in many biological events like development,[10] differentiation and activation of immune system cells, antigen presentation, expression of adhesion molecules, acute phase responses like the immune response and all stages of inflammation, cell death, hematopoiesis and wound healing.[10] The cytokine response forming after exercise is similar to the response developed after trauma and it was proposed that the exercise is a trauma model.[11] After intense exercise the levels of cytokines like IL-1, IL-6, TNF-αR1-2, IL-8 and MIP-1b increase in circulation.[3,11,12] Bruunsgaard et al. compared concentric and eccentric exercise forms and identified a correlation between the increase in muscle injury as assessed with analysis of creatine kinase (CK) and increased IL-6 levels.[13] Ostrowski et al. identified local IL-6 mRNA in muscle biopsies of runners and IL-2 ra-mRNA in mononuclear cells in blood samples obtained after a marathon race. This locally-produced IL-6 was shown to induce a systemic antiinflammatory response.[14] When studies performed after exercises and competitions are examined, without regard to intensity of the exercise, increased cytokine levels were detected after exercise and competition.[15-17]

In the literature, there are studies about how the levels of Foxp3+ T regulatory cells and cytokines change after exercises of different intensity; however, the Foxp3+ T regulatory cells and cytokine response after sprints and aerobic endurance exercises as a competition model have not been investigated so far. We think that sprint training will cause greater suppression of the immune system compared to aerobic endurance exercises. The aim of our study was to investigate the acute effect of repeated sprints and aerobic endurance trainings on Foxp3+ T regulatory cells and cytokines.

Materials and Methods

Subjects

Thirty-two elite male (n=16) and female (n=16) athletes participated in the study. Of these runners, 16 were short distance (100 m, 200 m and 400 m) and 16 were long distance (5000 m, 10,000 m) runners. Sixteen control (8 male, 8 female) subjects were selected from healthy volunteers aged between 19-27 years. The control group was sedentary people who did not do regular physical activity. Subjects were given information about the content and aims of the study and they signed voluntary consent forms. Necessary ethics committee permission for the study was obtained from Marmara University Faculty of Medicine Ethics Committee (protocol no: 09.2016.175).

Study Design

The athletes were divided into two groups as sprinters and distance runners. Sprinters and distance runners participated in the study on different days. Before applying the training protocol, first venous blood samples were taken from subjects and training began half an hour later. Similarly, a half an hour after the end of training, blood samples were taken again and pretest and posttest results were compared.

Training Protocol

Sprinters performed repeated speed training, while distance runners ran 10,000 meters. Distance runners attempted to run the distance to their best degree. Before training, both groups had 10 min general warm-up, fol-

lowed by 5 min dynamic and 5 min static stretching. The group with sprint training performed 60% speed 100-meter sprint, 1.5 min rest, 70% speed 100-meter sprint, 2 min rest, 80% speed 100-meter sprint, 3 min rest, 90% speed 100-meter sprint and full rest. After the full rest interval, they did thrice 100-meter sprints at 100% tempo. After exercising, both groups had a 10 min cooling run and 5 min static stretching.

Lymphocyte Isolation

Venous blood samples (30 mL) of 32 runners were placed in heparinized blood collection tubes (BD Vacutainer, US) and lymphocytes were isolated from these samples. Lymphocyte isolation was performed with Ficoll gradient solution (Fisher Scientific, US). Briefly, venous blood samples were diluted 1:1(v/v) with PBS solution, and 8 mL of diluted blood sample was added on 4 mL of Ficoll solution. Cells were centrifugated at 2000 rpm for 20 minutes, and buffy coat layer was collected from the center of the falcon tubes. Cells were washed twice with PBS and homogenized cell suspension was placed on a Thoma slide for cell counting.

Analysis of Foxp3+ T regulatory cells

After lymphocytes were cultured for 72 hours with the stimulation of anti-CD3/anti-CD28 (Thermofisher, US) specific for T lymphocytes, the percentage of CD4+/Foxp3+ T regulatory cells was analyzed via flow cytometry. Cells were stained according to the protocol stated in the Foxp3+ T regulatory cells buffer kit (BD Biosciences, US). In brief, at the end of the 72 hours of culture period, cells were collected and washed twice with PBS buffer. Approximately $1x10^5$ cells were initially stained with 10 μl of anti-CD4 (FITC) (BD Biosciences, US) and 10 μl of anti-CD25 (APC) (BD Biosciences, US) for cell surface markers, and incubated for 15 minutes at room temperature. After incubation period permeabilization buffer was added on the cells and incubated for 45 minutes at room temperature. Then, cells were stained with anti- Foxp3+ T regulatory cells antibody to reveal the intracellular expression of the transcription factor Foxp3+ T regulatory cells. Analysis was performed with BD FACS Calibur Software. Gating strategy was performed as follows: Lymphocytes were gated from total cell population (P1). CD4+cells were gated (R1) from P1-lymphocyte population and analyzed for the presence of CD25+ Foxp3+ T regulatory cells. Upper right quadrant was analyzed for CD25+ Foxp3+ T regulatory cells (Figure 1).

Analysis of Cytokines

The PBMC isolated from each runner was cultured for 72 hours with the stimulation of anti-CD3/anti-CD28 (Thermofisher, US) specific for T lymphocytes. At the end of 72 hours of culture period of PBMC, amount of cytokine in the culture supernatant was analyzed via flow cytometry using a cytokine bead array (CBA) kit (Human Th1/Th2/Th17 kit, BD Biosciences, US). Microbeads found in the CBA kit were prepared as standards in accordance with the manufacturer's protocol and samples were compared with the standard amounts of cytokine, analyzed and the results were expressed in pg/mL.

Statistical Analysis

Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) program. For descriptive statistics of subjects, mean, standard deviation, minimum, and maximum values were given. Normal distribution of the groups was determined with the Kolmogorov-Smirnov test and histogram. The difference between immune values in the groups before and after training was determined with the Wilcoxon Signed Ranks Test. Significance level was accepted as $p<0.05$.

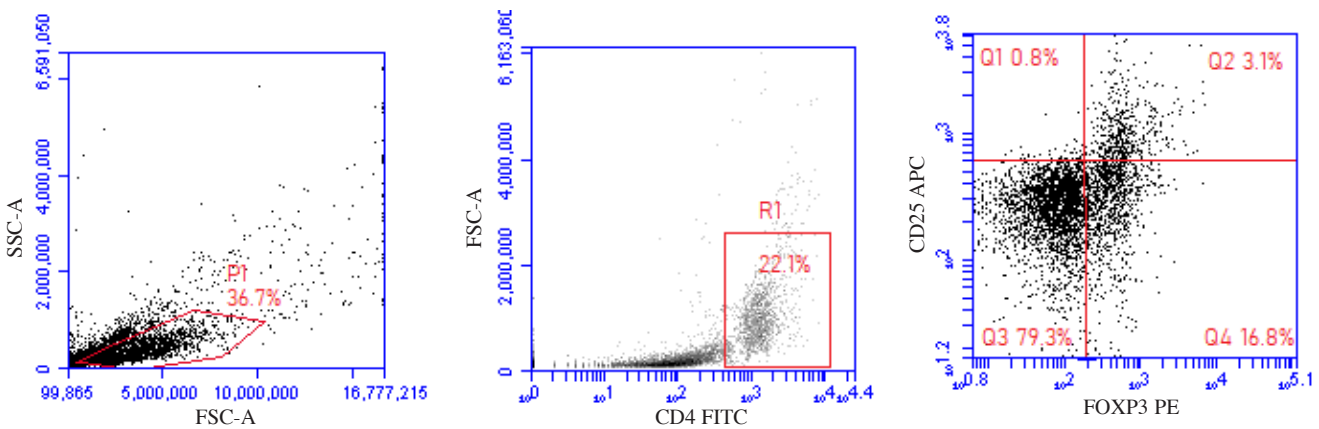

Figure 1. Gating strategy for CD4+CD25+ Foxp3+ T regulatory cells.

Descriptive Characteristics of The Subjects

For sprinters, mean values for age (19.4±2.1 years), height (1.7±0.4 m) body weight (63.5±9.2 kg), body mass index (21.7±2.0 kg/m^2) and years as an athlete (5.5±2.24 years) were determined. For distance runners, mean values for age (19.8±2.3 years), height, (1.7±0.2 m), body weight (61.3±8.7 kg), body mass index (21.3±2.2 kg/m^2) and sporting age (5.5.±1.62 years) were also determined.

Foxp3+ T regulatory cells

The mean concentration of Foxp3+ T regulatory cells of sprinters before and after training were 0.9±0.2 pg/mL, and 1.2±0.3 pg/mL, respectively. The Foxp3+ T regulatory cell levels before and after training of sprinters had a tendency to increase; however, this increase was not found to be statistically significant (p=0.47). For distance runners, Foxp3+ T regulatory cell concentration before, and after training were 0.8±0.2 pg/mL and 0.6±0.1 pg/mL, respectively. Foxp3+ T regulatory cells before and after training of distance runners had a tendency to decrease; however, this reduction was not statistically significant (p=0.52). The mean pre- test concentration of Foxp3+ T regulatory cells of the control group was 2.6±0.2 pg/mL. Foxp3+ T regulatory cell concentration of the control group was higher than the other groups (p=0.03). The Foxp3+ T regulatory cell values of subjects are summarized in Table 2.

Cytokines

The mean, standard deviation, minimum and maximum values before and after training and the differences in cytokine levels are given in Table 3. Sprinters had increased IL-2 (p=0.00), IL-4 (p=0.00), IL-10 (p=0.02), IL-17 (p=0.000) and TNF-α (p=0.000), decreased IL-6 (p=0.000) and unchanged IFN-γ levels (p=0.81). Distance runners had increased IL-4 (p=0.000), IL-10 (p=0.000), IL-17 levels (p<0.00), decreased TNF-α (p=0.00), IL-2 (p=0.05) and unchanged IFN-γ (p=0.15) and IL-6 (p=0.15). Statistical results of the pre-training cytokine levels (IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α and IFN-γ and Foxp3+ T regulatory cells) of both groups are shown in Figures 1 and 2, respectively. IL-2 and TNF-α- values were higher in distance runners (p=0,00). IL-4, IL-6 and IFN-γ values were higher in sprinters and distance runners (p=0.00). IL-10 and IL-17 levels were higher in sprinters (p=0.00).

Positive correlation was found between IL-2 cytokine and Foxp3+ T regulatory cells in sprinters (r= 0,15, p=0.02). Positive correlation was also found between IL-2 secretion and Foxp3+ T regulatory cell numbers in distance runners (r= 0,17, p=0.03) (Table 4).

Discussion

The effect of intensity, duration and type of exercise on the immune system has been intensely debated.[18] It has been proposed that high intensity, prolonged exercise suppresses the immune system, causing the body open to a variety of diseases led by infection.[19] Previous studies stated that moderate-intensity exercise preserved the immune system with its antiinflammatory effect.[18,19] However, long-lasting endurance exercises, like high-intensity exercises and marathons, suppress the immune system and all was revealed to be major risk factors for asthma and lung diseases.[4,20] In our study, the response of cytokines, as regulating hormones in the immune system, and Foxp3+ T regulatory cells responsible for immune regulation to repeated sprint training and distance running

Table 1. Relevant demographic data for sprinters and distance runners.

Parameters	Sprinters Mean±Standard Deviation	Distance runners Mean±Standard Deviation	Control Group
Age (years)	19.38±2.13	19.75±2.32	22.21±2.33
Height (m)	1.73±0.39	1.69±0.21	1.71±0.23
Body weight (kg)	63.5±9.16	61.33±8.68	65.13±5.68
Body mass index (BMI kg/m^2)	21.71±2.04	21.33±2.21	22.33±2.21
Years as licensed athlete (years)	5.50±2.24	5.50±1.62	-

Table 2. Foxp3+ T regulatory cell values (pg/mL) before and after training for sprinters and distance.

Group	Parameters	N	Mean±Standard Deviation	Min-Max	z	p
Sprinters	Before training Foxp3+ T regulatory cells	16	0.91±0.24	0.29-1.62	-.628[b]	.470
	After training Foxp3+ T regulatory cells	16	1.21±0.31	0.26-2.84		
Distance runners	Before training Foxp3+ T regulatory cells	16	0.81±0.23	0.31-1.84	-.983[c]	.525
	After training Foxp3+ T regulatory cells	16	0.60±0.12	0.05-1.54		
Control subjects	Basline Foxp3+ T regulatory cells	16	2.64±0.22	2.42-2.86		

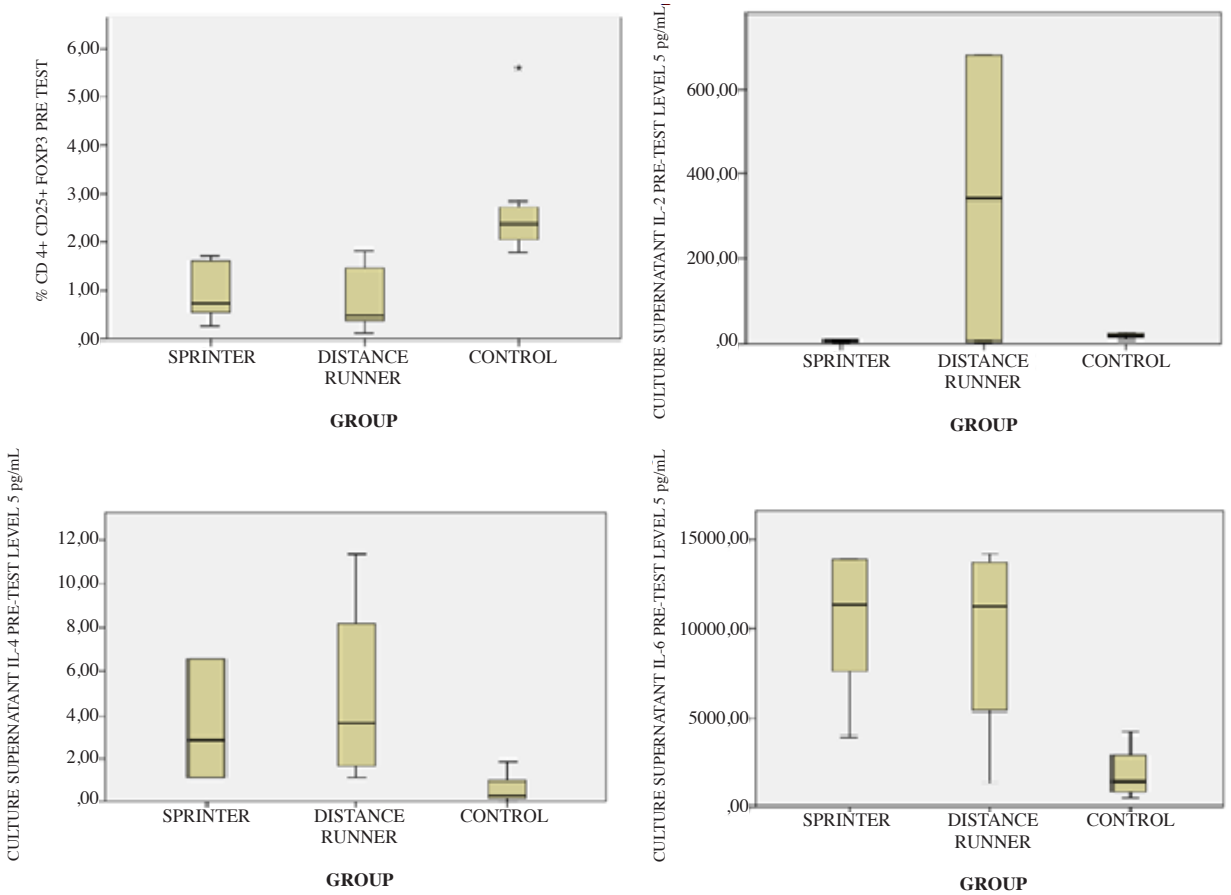

Figure 2. IL-2, IL-4, IL-6 levels in culture supernatants and % CD 4+CD25+Foxp3+ T regulatory cell frequency.

was investigated. The results of our study showed that Foxp3+ T regulatory cells did not change in both groups. For sprinters, IL-2, IL-4, IL-10, IL-17 and TNF-α concentrations increased whereas, IFN-γ levels did not change and IL-6 levels decreased. For distance runners, IL-4, IL-10, and IL-17 levels increased, IL-6 and IFN-γ levels did not change and TNF-α and IL-2 levels decreased.

Yeh et al. found the capacity of Foxp3+ T regulatory cells increased in a 12-week exercise program performed for health and treatment purposes.[6] In a study on mice Lowder et al. found that 3-4 weeks of aerobic exercise increased the levels of Foxp3+ T regulatory cells regulatory cells.[8] In a study on runners doing 42 long-term endurance sports, Perry et al. found that Foxp3+ T regulatory cell levels did not change before and after a race.[21] Wang investigated the effect of 6 weeks of moderate and high- intensity exercises on Foxp3+ T regulatory cell levels. At the end of the study, exercise of moderate intensity did not change these levels, while in mice on high intensity exercise program number of Foxp3+ T regulatory cells increased. In line with these results, the authors emphasized that high- intensity exercise may cause higher rate of infection in mice.[9] Wilson et al. investigated the effect of high- intensity exercise on Foxp3+ T regulatory cell levels among young swimmers and found that acute high-intensity exercise increased the levels of Foxp3+ T regulatory cells. Michal compared IL-10 and T regulatory cells in sprinters, distance runners, people doing recreational exercises and sedentary persons. IL-10 production was found in endurance-trained athletes compared with the other groups. Endurance-trained athletes had a higher Foxp3+ T regulatory cell percentage of total lymphocyte counts compared with sedentary individuals. Also IL-10 production significantly correlated with the proportion of T regulatory cells.[28] In our study, among sprinters and endurance-trained trained athletes, Foxp3+ T regulatory cells tended to increase in sprinters, while it tended to decrease, though not statistically significantly in distance runners.

In sprinters, IL-2, IL-4, IL-10, IL-17 and TNF-α, levels were increased, unchanged, IFN-γ and decreased IL-6 levels were observed. IL-4, IL-10, and IL-17, unchanged IL-6 and IFN-γ levels were unchanged, whereas TNF-α and IL-2 levels were found to be decreased in distant runners. These results show that exercises with different intensities induce different acute responses in cytokines. Nieman et al. detected increased levels of cytokines among

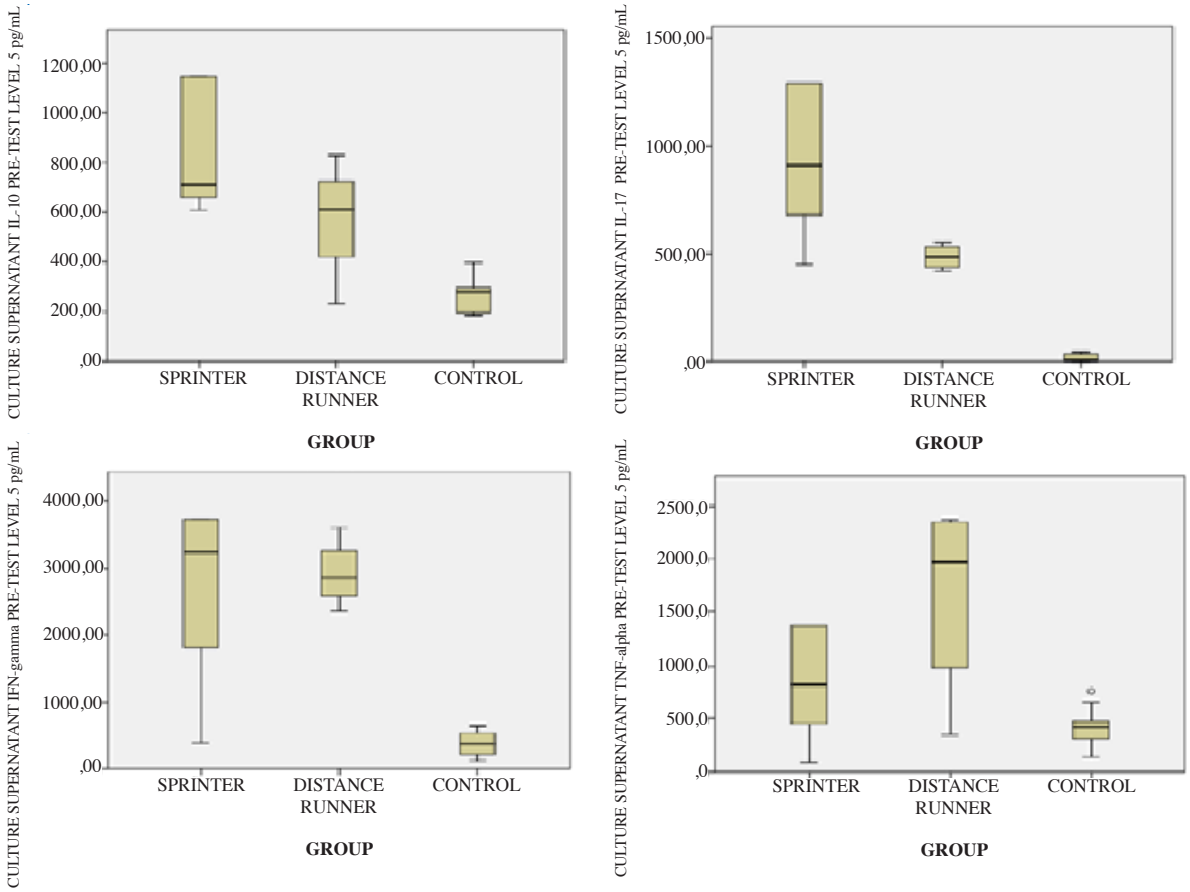

Figure 3. IL-10, IL-17, IFN-γ and TNF-α levels in culture supernatants of T lymphocytes.

marathon runners after marathon races.[15] In a study of people performing endurance sports and running as recreational activity, Scott et al. found that levels of cytokines increased in both groups after running exercises-groups.[16] Pedersen et al. reported that cytokine levels increased with high- intensity exercise.[22] Cullen et al. investigated the cytokine response to acute interval exercises with low and high intensity and revealed that the group practicing high-intensity exercises had greatest cytokine increase.[17] Helge et al. acutely in response to acute endurance levels of IL-6 expression.[23] Reihmane et al. reported that IL-6 levels were increased to a great extent conpared to half-marathon runners.[24]

Scott et al. found increased IL-6 in recreational activity performers and endurance runners.[16] Christiansen et al. found that IL-6 levels increased after moderate- intensity exercise, with no change after low intensity exercise.[25] Middelbeek et al.compared moderate- intensity endurance training and high- intensity interval training in sedentary men.[29] Exercise training reduced IL-6 concentrations in healthy men by 49% in sprint interval training and by 11% in moderate- intensity training.[29] Rose et al. found that sprint and endurance training groups the levels of IL 6 and TNF-α were similar in both groups, while IL-10 levels were higher in sprinters.[30] IL-17 is a cytokine which aids T-cells in the inflammatory response. It plays an important role in scavenging extracellular pathogens. After intense exercise, IL-17 levels increase.[21] In our study IL-17 levels increased after repeated sprint and endurance training. These results show that IL-17 regulates the immune system after speed and endurance training. IFN-γ did not change in both groups after exercise which demonstrated that IFN-γ was not affected by exercise. Rhind et al. found that IL-2 levels increased 33% after moderate- intensity exercise.[26] Pery et al. found that IL-2 levels fell after triathlon and marathon races. In the periphery, T regulator cell levels change linked to IL-2 concentration.[27] In our study, IL-2 levels increased in sprinters while they decreased in distance runners. Our results showed that IL-2 levels increase after training in sprinters in accordance with the increase in Foxp3+ T regulatory cell expression. Similarly, the reduction in IL-2 levels after training in distance runners is associated with Foxp3+ T regulatory cell expression. IL-10 is an antiinflammatory cytokine.[22] IL-10 levels were found to be increased after exercise.[22] In our study, IL-10 levels increased in both

Table 3. Cytokine levels (pg/mL) in the culture supernatants before and after training for sprinters and distance runners.

Groups	Parameters	N	Mean±Standard Deviation (pg/mL)	Min-Max	z	p
Sprinters	Before training IL-2	16	10.45±4.22	1.42-22.64	-3.100[b]	**.002**
	After training IL-2	16	636.28±240	6.18-1920		
Distance runners	Before training IL-2	16	340.39±151.47	3.49-676.91	-1.894[c]	**0.05**
	After training IL-2	16	41.67±12.37	1.35-148.25		
Control subjects	IL-2	16	16.34±5.36	7.65-23.87		
Sprinters	Before training IL-4	16	3.36±1.42	1.13-6.56	-4.297[b]	**.000**
	After training IL-4	16	10.23±3.82	3.23-19.17		
Distance runners	Before training IL-4	16	4.95±2.26	1.13-11.40	-3.078[b]	**.002**
	After training IL-4	16	27.81±6.16	6.34-38.55		
Control subjects	IL-4	16	0.64±0.71	0.15-1.87		
Sprinters	Before training IL-6	16	10343.00±4054.41	3895-13911	-2.623[c]	**.009**
	After training IL-6	16	5827±1354.06	4327-7974		
Distance runners	Before training IL-6	16	9531±1279.25	1344-14231	-1.421[c]	.155
	After training IL-6	16	7972±2872.33	1712-11358		
Control subjects	IL-6	16	1902.62±1335.11	458.79-4194.77		
Sprinters	Before training IL-17	16	927.05±230.15	456-1292	-3.100[b]	**.002**
	After training IL-17	16	1263.95±142.33	1027-10342		
Distance runners	Before training IL-17	16	485.91± 51.74	422-554	-3.078[b]	**.002**
	After training IL-17	16	767.11±56.69	630-903		
Control subjects	IL-17	16	15.91±20.39	5.45-47.37		
Sprinters	Before training IFN-γ	16	2680.64±405.4	389-3734	-.238[c]	.816
	After training IFN-γ	16	1861.2±153.92	35-3434		
Distance runners	Before training IFN-γ	16	2920.18±465.03	2358-3602	-1.421[c]	**.155**
	After training IFN-γ	16	2556.16±337.31	1413-3645		
Control subjects	IFN-γ	16	406.67±185.12	125.78-653.32		
Sprinters	Before training TNF-α	16	866.93±523.67	102-1367	-3.100[b]	**.002**
	After training TNF-α	16	1223.56±269.29	334-1695		
Distance runners	Before training TNF-α	16	1664.05±250.82	351-2398	-2.605[c]	**.009**
	After training TNF-α	16	750.41±174.02	164-1289		
Control subjects	TNF-α	16	440.25±175.51	138.12-765.00		
Sprinters	Before training IL-10	16	830.98±237.25	608-1147	-2.305[b]	**.021**
	After training IL-10	16	1283.25±165.16	1113-1465		
Distance runners	Before training IL-10	16	572.50±124.2	234-831	-3.078[b]	**.002**
	After training IL-10	16	1289.22±183.75	521-2310		
Control subjects	IL-10	16	279.11±73.77	186.23-397.69		

groups independent of the intensity and duration of exercise.

In our study, TNF-α was found to be increased after training in sprinters while they decreased in distance runners. TNF-α is an important proinflammatory cytokine.[24] Its higher levels in short-distance runners were shown to be an immune system response to inflammation. Another proinflammatory cytokine, IL-6 was suppressed after training showing that the tendency of Foxp3+ T regulator cells to in order to modulate the inflammation. The regulation of Foxp3+ T regulatory cells and changes in IL-6 levels were not observed before and after training in dis-

Table 4. IL-2 cytokine and The Foxp3+ T regulatory cells.

Superman correlation analysis			The Foxp3+ T regulatory cells
Sprinters	IL-2 cytokine	Superman Correlation	0.155
		Sig. (2-Tailed)	0.02
		N	16
Distance Runners	IL-2 cytokine	Superman Correlation	0.17
		Sig. (2-Tailed)	0.03
		N	16

tance runners. Decreased levels of proinflammatory cytokines that were observed in distance runners were correlated with the increase in the immunosuppressive cytokine IL-4 levels after training. However, increases in IL-4, IL-10 and IL-17 levels in both groups show that these cytokines that were have a protective role against exercise without regard to the intensity and duration of exercise.

In conclusion, the immune system demonstrates different responses to different intensity of exercise in aerobic and anaerobic environments. Number of Foxp3+ T regulatory cells did not change after sprinting and aerobic endurance exercises. IL-4, IL-10 and IL-17 increased without regard to intensity of exercise which indicates that these cytokines are regulatory elements in the immune system after exercise.

Ethics Committee Approval: Necessary ethics committee permission for the study was obtained from Marmara University Faculty of Medicine Ethics Committee (protocol no: 09.2016.175).

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