



# CHANGES OF TSH, IMMUNE, INFLAMMATORY AND TUMOR MARKERS AFTER ANAEROBIC EXERCISE IN ELITE KARATE ATHLETES

Tihomir Vidranski<sup>1</sup>, Valentina Vidranski<sup>2</sup> and Tomislav Jukić<sup>2,3,4</sup>

<sup>1</sup>Faculty of Kinesiology, University of Zagreb, Zagreb, Croatia;

<sup>2</sup>Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center, Zagreb, Croatia;

<sup>3</sup>School of Medicine, University of Zagreb, Zagreb, Croatia;

<sup>4</sup>Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

**SUMMARY** – Exercise causes considerable changes in the number and function of immune cells and hormones in circulation. The aim of this study was to assess the impact of training regime on immune and inflammatory markers and parameters of complete blood count, thyroid function and tumor markers, immediately after intense, mostly anaerobic training in elite karate athletes. There are no similar studies that would determine changes in the concentration of tumor markers immediately after intense exercise. Study population included seven elite trained athletes, members of the Croatian National Karate Kata female team and Kata male team. Our data provided evidence that the white blood cell and segmented neutrophil granulocyte counts were significantly decreased and lymphocytes significantly increased with no major changes in interleukin-6 immediately after anaerobic exercise. We also noticed a strong increase of thyrotropin and no significant change in the levels of tumor markers. It was concluded that immune response and thyroid hormone status were in correlation with exercise duration and intensity. According to our results, a larger study is needed to define the impact of training and interrelation between sports and laboratory medicine as a perfect combination in terms of discovering new mechanisms and biochemical strategies in training of elite athletes.

*Key words: Karate; Exercise; Blood cell count; Interleukin-6; Thyrotropin; Tumor marker*

## Introduction

Body exposure to exercise of various intensity and duration leads to acute and chronic changes in blood composition, as well as structural and biochemical adaptation to it<sup>1-3</sup>. While some sports disciplines have already been extensively studied, the effects of training in elite karate athletes are scarce and quite unreliable due to heterogeneity of the studied groups, especially those on female karate athletes, which barely exist at all<sup>4</sup>.

Karate with millions of trainees worldwide is one of the most attractive combat sports with a small number of elite karate performers that require an exceptionally high trainee level. Modern non-contact karate tournaments of the World Karate Federation consist

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Correspondence to: *Prof. Tihomir Vidranski, PhD*, Faculty of Kinesiology, Savski gaj II. 37, HR-10000 Zagreb, Croatia  
E-mail: vidranskit@gmail.com

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of two equally important karate disciplines, i.e., the kumite and kata. Kumite is a synonym for karate fight, while kata is often described as a set sequence of karate moves organized into a prearranged fight against imaginary opponents. A kata team consists of three people performing synchronously<sup>3</sup>.

Croatian male and female kata teams are elite athletes who won 3<sup>rd</sup> place at the European championship at the time of this study. Their annual training of 720 hours (42 weeks; 7-10 of training units *per* week; two hours *per* training) was mostly anaerobic. As these athletes make a unique group of individuals, the impact of their long-term, lifelong exercise on their blood parameters is an important analytical and/or preanalytical factor and surely deserves attention<sup>5</sup>.

Epidemiologic studies have shown that athletes have a reduced incidence of cardiovascular diseases and cancer mortality, and live longer compared to the general population. On the other hand, there is a widespread theory of induced immunosuppression and high mental stress. Is it due to a lifestyle that includes frequent travel to contests and exposure to many people, or some other factors that perfectly fit the central dogma that extremely difficult exercise and long-term periods of intense training impair humoral and cellular immunity, making the body susceptible to infections? A crucial factor would be the duration and type of exercise due to its effects on hemostasis and neuroendocrine system, as well as anthropomorphic differences among elite athletes that influence, among other parameters, hormonal profiles<sup>6-11</sup>.

The aim of this study was therefore to assess the impact of training exercise regime on immune and inflammatory markers (interleukin 6, IL-6) and complete blood count (CBC) parameters, thyroid function (thyrotropin, TSH) and tumor markers immediately after intense, mostly anaerobic training of elite karate athletes.

## Subjects and Methods

### Subjects

Study population included seven elite trained athletes, three male aged 26-29, mean 27 years and four female aged 19-29, mean 24 years, members of the Croatian National Karate Kata male team and

Kata female team, their training lasting for 15 years on average, having won 3<sup>rd</sup> place at the last European championship at the time of the study. The study sample was small because the respondents make up the total sample in the Republic of Croatia, and such respondents are few worldwide.

They had five training sessions a week, yielding 300 days of training *per* year. At the time of survey, the athletes had no clinical signs of disease. Coach's comment referred to the presence of constant complaint on a year-round viral cold with symptoms of runny nose. The athletes performed exercise training in their usual time while the duration of exercise was 97 (female) and 100 minutes (male). Training took place in the evening, and the athletes were not fasting, with the last meal three hours before training. The athletes gave their written consent for this study.

### Methods

Before training, the athletes were measured on an Omron HBF-500 body composition monitor with scale that works on the principle of bioelectrical impedance method, whereas pulse and blood pressure were measured on an Omron HEM-RML 31 blood pressure monitor. Anthropological and descriptive data were known data on body height, body weight, body mass index (BMI), percentage of muscle tissue, fat tissue, visceral fat, and basal metabolic rate. Menstruation was not present in female athletes.

All study athletes were non-smokers and not currently taking any medication (e.g., cardiovascular drugs, oral contraceptives, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs) or supplements.

Throughout the training session, the athletes wore a Polar smart watch around their wrist and Polar H7 Bluetooth Heart Rate Sensor & Fitness Tracker (Polar, Kempele, Finland) around the chest which monitors heart rate (frequency *per* minute), counts burned calories in kcal, and shows the time spent in the aerobic or anaerobic state in minutes.

Blood samples were obtained 10 minutes before and 5-10 minutes after training session to determine CBC, white blood cells (WBC), subsets of segmented neutrophil granulocytes (SNG), lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean

corpuscular hemoglobin concentration (MCHC), platelets, mean platelet volume (MPV), TSH, carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), cancer antigen 15-3 (CA 15-3), cancer antigen 19-9 (CA 19-9), and IL-6.

Peripheral blood samples were collected by venipuncture of cubital vein, in the sitting position by one laboratory staff person in 6-mL Vacuette® serum tubes with clot activator (red cap) and then in 6-mL Vacuette® hematology tubes (K<sub>3</sub>EDTA tubes, lavender cap) (Greiner Bio-One, Kremsmünster, Austria) according to the Croatian national recommendations for venous blood sampling<sup>12</sup>.

Hematology parameters were analyzed in fresh samples within appropriate time for analysis (4 hours). The tubes with serum samples were centrifuged within 4 hours and serum was isolated and stored in a refrigerator at +4 °C, and all parameters were analyzed within 24 hours of centrifugation. Blood for serum testing was centrifuged for 10 minutes at 2150 xg at 4 °C on a Hettich ROTINA35 centrifuge (Hettich, Germany).

Serum samples were bright, without the presence of hemolysis, icterus and lipemia. Blood count was analyzed on a UniCel DxH800, Beckman-Coulter (Beckman Coulter, Los Angeles, CA, USA) using Beckman-Coulter reagents and original controls.

Analysis of IL-6 was performed in serum samples on Immulite 1000 (Siemens Healthcare Diagnostics, Erlangen, Germany) automated immunochemistry analyzer with analytical principle of chemiluminescent reaction, using original Siemens assays, calibrators and controls; TSH on Immulite 2000xpi (Siemens Healthcare Diagnostics, Erlangen, Germany) automated immunochemistry analyzer with analytical principle of chemiluminescent reaction, using original Siemens assays, calibrators and Bio-Rad Lyphochek Immunoassay (Bio-Rad Laboratories, USA) assayed chemistry controls; and CA 125, CA 15-3, CEA, AFP and CA 19-9 on Cobas e411 (Roche Diagnostics GmbH, Penzberg, Germany) automated immunochemistry analyzer with analytical principle of electrochemiluminescence reaction, using original Roche assays, calibrators and controls.

Table 1. Anthropometric measures in female and male elite karate athletes

Parameter	Male	Female	Recommended range, male	Recommended range, female
Age (years)	27 (26-29)	24 (19-29)	-	-
Body height (cm)	181 (172-187)	164 (152-173)	-	-
Body weight (kg)	85 (75-99)	55 (50-66)	-	-
BMI (kg/m <sup>2</sup> )	27 (24-30)	22 (19-25)	18.5-25.0	18.5-25.0
MUS (%)	39 (35-45)	30 (27-34)	33.3-39.3	24.3-30.3
FAT (%)	22 (11-29)	30 (22-36)	8.0-19.9	21.0-32.9
VIS (%)	9 (5-11)	4 (2-5)	≤9	≤9
BMR (kcal)	1835 (1710-2027)	1262 (1160-1394)	-	-
AHF (beats/min)	132 (129-134)	128 (125-130)	-	-
MHF (beats/min)	220 (217-222)	210 (209-211)	-	-
Tae (min)	33 (31-35)	30 (29-31)	-	-
Tanae (min)	67 (65-69)	67 (66-68)	-	-
Kcal	858 (831-889)	607 (556-677)	-	-
Training (min)	100	97	-	-

Data are expressed as median (interquartile range), age is expressed as median (minimum-maximum); BMI = body mass index; MUS = muscle tissue; FAT = fat tissue; VIS = visceral fat; BMR = basal metabolic rate; AHF = average heart frequency; MHF = maximum heart frequency; Tae = time of aerobic type of training; Tanae = time of anaerobic type of training; kcal = kilocalories

### Statistical analysis

Since the participants were rare elite karate athletes and the participant sample was small, all data were expressed as median and 95% confidence interval (CI), except for age that was expressed as median (minimum-maximum). The level of difference significance was set at  $p < 0.05$ . Difference between analyzed time points (before and after training session) was calculated using non-parametric Wilcoxon's matched paired test because of the small ( $n < 15$ ) sample size, where the results were expressed as median (95% CI). Statistical analyses were performed using MedCalc Software (Ostend, Belgium) version 20.106 (2022).

### Results

Table 1 shows anthropometric measures of female and male karate athletes, Table 2 shows values of blood pressure and pulse, whereas Table 3 indicates values of hematology parameters before and after training. Table 4 presents tumor marker concentrations in karate athletes, Table 5 shows TSH and IL-6 parameters in all athletes, and Table 6 compares individual values of CA 125 in all seven karate athletes before and after training.

Table 1 shows demographic and anthropometric data of study athletes. Male athletes had slightly

Table 2. Blood pressure and pulse in all athletes, female and male athletes before and after training

Parameter	Before training	After training	Reference interval	p
Systolic blood pressure (mm Hg)	117 (103-130)	128 (120-141)	<120	0.016*
Diastolic blood pressure (mm Hg)	69 (64-77)	74 (69-82)	<80	0.016*
Pulse (beats/min)	69 (55-76)	79 (70-97)	60-80	0.016*

\*Difference statistically significant

Table 3. Hematology parameters in all athletes before and after training

Parameter	Before training	After training	Reference interval (female)	Reference interval (male)	p
WBC ( $\times 10^9/L$ )	8.5 (7.2-11.5)	5.9 (5.0-8.0)	3.4-9.7	3.4-9.7	0.016*
SNG (%)	67 (60-72)	57 (47-60)	32-68	32-68	0.016*
Ly (%)	25 (18-29)	32 (29-41)	20-46	20-46	0.016*
Mo (%)	8 (6-9)	8 (7-9)	2-12	2-12	0.688
Eo (%)	2 (0-3)	2 (1-5)	0-7	0-7	0.013*
Ba (%)	0.5 (0.1-0.8)	0.5 (0.1-0.9)	0-1	0-1	0.375
RBC ( $\times 10^{12}/L$ )	4.47 (4.23-4.84)	4.39 (4.30-4.90)	3.86-5.08	4.34-5.72	0.469
Hb (g/L)	135 (131-147)	135 (130-148)	119-157	138-175	0.688
Htc (L/L)	0.402 (0.382-0.426)	0.400 (0.392-0.439)	0.356-0.470	0.415-0.530	0.156
MCV (fL)	90.1 (85.3-93.9)	90.0 (86.3-94.7)	83.0-97.2	83.0-97.2	0.031*
MCH (pg)	29.9 (29.1-32.7)	29.9 (29.0-32.6)	27.4-33.9	27.4-33.9	1.000
MCHC (g/L)	339 (328-351)	335 (326-348)	320-345	320-345	0.438
RDW (%)	12.6 (12.0-13.4)	12.6 (12.0-13.6)	9.0-15.0	9.0-15.0	0.688
Plt ( $\times 10^9/L$ )	234 (184-284)	224 (172-282)	158-424	158-424	0.156
MPV (fL)	8.6 (7.4-9.9)	9 (7.5-9.9)	6.8-10.4	6.8-10.4	0.156

\*Difference statistically significant; WBC = white blood cells; SNG = segmented neutrophil granulocytes; Ly = lymphocytes; Mo = monocytes; Eo = eosinophils; Ba = basophils; Hb = hemoglobin; Htc = hematocrit; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; Plt = platelets; MPV = mean platelet volume

Table 4. Tumor marker parameters in all athletes before and after training

Parameter	Before training	After training	Reference interval	p
CEA ( $\mu\text{g/L}$ )	0.5 (0.9-2.7)	1.5 (0.8-2.8)	<5.2	0.688
CA 125 (kIU/L)	15 (12-25)	15 (12-27)	<35	0.109
CA 15-3 (kIU/L)	22 (13-28)	21 (13-27)	<25	0.688
CA 19-9 (kIU/L)	7 (4-28)	8 (5-29)	<39	0.563
AFP (kIU/L)	1.9 (1.1-2.4)	1.9 (1.0-2.3)	<5.2	0.375

CEA = carcinoembryonic antigen; AFP = alpha-fetoprotein

Table 5. TSH and IL-6 parameters in all athletes before and after training

Parameter	Before training	After training	Reference interval	p
IL-6 (pg/mL)	<2	<2	0-28	NS
TSH (mIU/L)	2.3(0.7-3.3)	3.0(1.4-5.2)	0.4-4.0	0.016*

\*Difference statistically significant; NS = nonsignificant; IL-6 = interleukin 6; TSH = thyrotropin

Table 6. CA 125 in all athletes before and after training

Parameter CA 125 (kIU/L)	Before training	After training	Reference interval
Male athlete 1	13.63	14.95	<35
Male athlete 2	14.51	14.57	<35
Male athlete 3	12.95	13.07	<35
Female athlete 1	29.08	30.76	<35
Female athlete 2	10.63	10.60	<35
Female athlete 3	19.83	22.24	<35
Female athlete 4	19.62	19.52	<35

higher BMI and visceral fat level, muscle percentage at the upper limit according to the Omron Healthcare, and fat percentage at the lower limit according to the National Institutes of Health/World Health Organization guidelines. Measurements on Polar equipment also revealed that most of the training time was anaerobic in both male and female athletes (67 [65-69] min and 67 [66-68] min, respectively). The number of calories burned by female and male athletes was 607 (556-677) kcal and 858 (831-889) kcal, respectively.

The significance of differences recorded before and after training was assessed using Wilcoxon's matched paired test. Table 2 shows statistical differences in blood pressure and pulse before and after training, which were not out of reference ranges; these results could be interpreted as well-trained athletes.

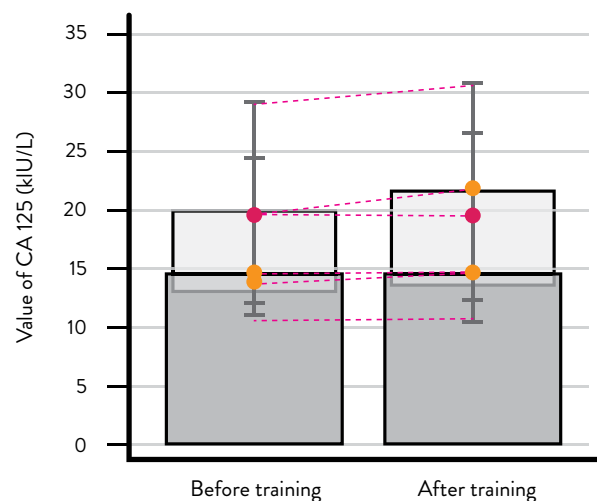


Fig. 1. Box-whisker graph of CA125 before and after training.

Table 3 displays differences recorded before and after training, assessed by Wilcoxon's matched paired test. Statistically significant difference was observed in the values of WBC ( $p=0.016$ ), SNG ( $p=0.016$ ), lymphocytes ( $p=0.016$ ), eosinophils ( $p=0.013$ ) and MCV ( $p=0.031$ ), i.e., lower values of WBC and SNG and higher levels of lymphocytes and MCV.

There was no statistically significant difference in serum tumor markers as acute response to exercise. Although there was no statistically significant difference ( $p=0.109$ ) in CA 125, there was a slight increase in this parameter in nearly all study subjects (Table 6 and Fig. 1).

Serum TSH concentration was significantly higher after exercise (3.0 [1.4-5.2] mIU/L) compared to the values before training (2.3 [0.7-3.3]) ( $p=0.016$ ), while there was no difference in IL-6 values which were below detection limit of the method ( $<2$  pg/mL).

## Discussion

Interrelation between sports and laboratory medicine is a perfect combination in the field of science in terms of discovering new mechanisms and biochemical strategies in athlete training. Exercise causes considerable changes in the number and function of immune cells and hormones in the circulation and exerts positive impact both on healthy individuals and people with health problems. Our understanding of the relationship between prolonged, intensive exercise, the immune system, hormones, tumor markers and host protection against viruses and bacteria, as well as the question of establishing reference ranges for elite athletes increases with every new study.

Complete blood count profile is a great tool to access information on the shifts in immune cells because inflammatory cells, particularly macrophages, are essential in skeletal muscle regeneration. It has been demonstrated that physical exercise, generally, induces changes in the number of leukocytes and their subsets in the circulating blood<sup>13,14</sup>. Skeletal muscle damage during exercise provokes sterile inflammation, and considering the characteristics of exercise in combat sports, the degree of tissue damage is more frequent and larger than in other sports<sup>15-17</sup>. Changes in the immune and inflammatory response can be influenced

by many factors such as catecholamines, increase in cardiac output, or decreased adherence of leukocytes to endothelial tissues<sup>18</sup>.

Our data provided evidence that WBC and SNG counts were significantly decreased and lymphocytes significantly increased, with no significant changes in IL-6 immediately after anaerobic exercise and at rest in well-trained elite karate athletes. Many studies have reported similar findings; however, some studies report neutrophil increase or total increase in leukocytes, neutrophils, lymphocytes and monocytes<sup>18-22</sup>. Kostrzewa-Nowak *et al.* describe a similar work in elite karate athletes during the European Senior Karate Championships, Montpellier, France, 2016, where they noticed opposite results with significant change in IL-6 five minutes after exercise<sup>23</sup>. On the other hand, Walsh *et al.*<sup>20</sup> recorded lymphocyte increase and no significant changes in IL-6, as reported in our study. Fischer has good explanation for low post-exercise IL-6, where the duration of exercise, i.e., training is a key factor. The apparent discrepancy among tissues regarding response to IL-6 may be due to downstream IL-6 signaling in different tissues. IL-6 released from the contracting muscles may induce an anti-inflammatory response without concomitant increases in pro-inflammatory mediators<sup>24</sup>.

According to the known data, we can conclude that immune response is in correlation with exercise duration and intensity. Literature supports the idea that post-exercise immune depression is most pronounced when the exercise is of moderate to high intensity, continuous, prolonged over one hour and a half, and performed without food intake. Periods of intensified training lasting for one week or more can result in longer lasting immune dysfunction. Although elite athletes are not clinically immunodeficient, it is possible that the combined effects of small changes in several immune parameters may compromise resistance to common minor illnesses. Protracted immune depression linked with prolonged training may determine susceptibility to infection, particularly at times of major competitions. This is obviously a concern because of the potential impact of an infectious episode on exercise performance. It is not really a concern for the general population, as individuals do not need to indulge in heavy training loads to obtain health benefits of exercise that may well be proven to

be due, in large part, to its anti-inflammatory effects<sup>25</sup>. In accordance with other literary citations, immune, inflammatory and oxidative stress markers can be used to assess cardiopulmonary transformation, physical performance, and recovery during training<sup>26,27</sup>.

In our study, we also noticed a strong TSH increase, which is consistent with the fact that maximal aerobic exercise greatly affects the level of circulating thyroid hormones, and TSH continued to rise at 90% of maximum heart rate<sup>28</sup>. Vera *et al.* showed that karate athletes had lower values of TSH<sup>29</sup>.

An overview by the International Agency for Research in Cancer and World Cancer Research Fund brings knowledge of physical exercise as a factor preventing colon cancer and breast cancer in premenopausal women. The literature shows the impact of exercise on endometrial, lung and pancreatic tumors. The biological mechanism of the interrelation of cancer and exercise is not understood although the potential mediators include reduction in body weight or visceral fat, decreased levels of sex hormones, and therefore the effect of antioxidant defense, anti-inflammatory effect, and anti-tumor immune response. A considerable number of studies have been made in animal models; however, their results were inapplicable to human samples<sup>30</sup>.

Our study was designed on the presumption that there were no similar studies that would determine changes in the concentrations of the most widely used tumor markers immediately after intense exercise, in particular CA 125 the role of which has been recognized as a parameter of heart failure and heart damage. CA 125 is shed from the surface of cardiac mesothelial cells in response to mechanical stress such as fluid overload and inflammatory stimuli, and the pathophysiologic mechanism of this phenomenon is still unclear<sup>31-33</sup>. There are only few studies on the correlation between exercise and tumor marker concentration. Yoon *et al.* showed that CEA, CA 125 and CA 15-3 were decreased but not significantly and AFP was significantly increased in postmenopausal obese women after exercise<sup>33</sup>. We assume that AFP increase was due to the fact that it is an acute-phase protein. A similar study showed that 8-week aerobic exercise was ineffective in inducing changes in CEA and CA 15-3 in breast cancer patients<sup>34</sup>.

Therefore, we aimed to examine the effect of intensive training with a significant increase in pulse and

blood pressure, its effect on the heart muscle in trained athletes and on CA 125 change, which has not been investigated so far. Our results indicated that there was no statistically significant difference in the values of CA 125 recorded before and after exercise; however, statistical analysis with box-whisker plot clearly demonstrated CA 125 increase. As for the impact of exercise on the concentration of tumor markers, it is necessary to examine it more extensively, especially CA 125 increase, during different kinds of exercise regarding increased heart rate.

The present study had some limitations. Since only seven patients were included in the study, three of them male athletes, this sample size was too small to draw relevant conclusions regarding the impact of exercise on the study parameters. According to our results, a larger international study is needed to define the impact of training on hematologic, biochemical and hormonal status of elite karate athletes, the impact of long-term training process on these parameters, and maybe also to standardize preanalytical phase with the possibility of defining reference values in these specific populations.

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### Sažetak

## PROMJENE VRIJEDNOSTI TSH, IMUNO-UPALNIH I TUMORSKIH BILJEGA NAKON ANAEROBNOG VJEŽBANJA KOD VRHUNSKIH KARATISTA

*T. Vidranski, V. Vidranski i T. Jukić*

Tjelesna aktivnost uzrokuje značajne promjene u broju i funkciji imunskih stanica i hormona u cirkulaciji. Cilj ovog istraživanja bio je procijeniti utjecaj režima treninga na imuno-upalne biljege i parametre kompletne krvne slike, funkciju štitnjače i tumorske biljege neposredno nakon intenzivnog, uglavnom anaerobnog treninga vrhunskih karatista. Ne postoje slične studije koje bi utvrdile promjenu koncentracije tumorskih biljega neposredno nakon intenzivnog vježbanja. Ovu studijsku populaciju činilo je 7 vrhunski obučanih sportaša, članova hrvatske nacionalne karate reprezentacije ženskog i muškog Kata tima. Naši podaci pružaju dokaz da je koncentracija bijelih krvnih stanica i segmentiranih neutrofilnih granulocita značajno smanjena, a limfociti značajno povećani bez značajnih promjena u interleukinu-6 neposredno nakon anaerobne vježbe. Također smo primijetili snažan porast tireotropina, bez značajne promjene vrijednosti tumorskih biljega. Zaključeno je da je imunski odgovor i status hormona štitnjače u korelaciji s trajanjem i intenzitetom vježbanja. Prema našim rezultatima potrebna je veća studija kako bi se utvrdio utjecaj treninga i međuodnos sporta i laboratorijske medicine kao savršene kombinacije u smislu otkrivanja novih mehanizama i biokemijskih strategija u treningu vrhunskih sportaša.

**Ključne riječi:** *Karate; Tjelesna aktivnost; Kompletna krvna slika; Interleukin-6; Tireotropin; Tumorski biljeg*