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ORIGINAL ARTICLE

Effect of Oxidative Stress in Elite Athletes and Characterization of Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis

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ABSTRACT

Background: The aim of this study was to examine the protein characterization of oxidative stress effects in elite athletes with the SDS-Page method.

Material and Methods: 28 male athletes voluntarily participated in the study, competing in 4 different branches (Cycling – n = 7, Boxing – n = 7, Wrestling – n = 7, Teaekwondo – n = 7), with an average age of 21-25 years and actively doing sports. The study protocol was approved by the ethics committee of Selçuk University School of Physical Education and Sports. Blood samples were taken three times as a result of resting, anaerobic and aerobic strength tests. All serums obtained were protein isolated using the EURX brand Gene Matrix Universal DNA/RNA Protein Purifacition kit in the molecular biology laboratory of the Department of Biology, Faculty of Science, Selcuk University. Proteins were characterized by BIORAD Mini Protean Electrophoresis System SDS-Page method with THERMO PagerulerPrestained Protein Ladder marker.

Results: Bands with a size of 200 kDa, which were evaluated as immunoglobulins, were found in cycling rest, boxing resting and maximal, wrestling maximal and submaximal values. In addition, all three status values of the bicycle group are similar. Boxing group is the most distinctive group in terms of protein profile and content. While the maximal and submaximal values were similar in the wrestling group, fainter protein bands were observed in the resting states. Although the resting and maximal protein profiles and contents of the taekwondo group were similar, it can be said that protein bands are more prominent in submaximal exercise results. As a result; The serum protein concentrations of the athlete groups are similar. While the similarities of the cycling, boxing and taekwondo groups were more evident in terms of protein content and profiles, the maximal and submaximal post-exercise values of the wrestlers were similar.

Conclusions: We can associate the differences between the groups of athletes with the intensity and intensity of the exercise they have done. It can be said that the protein fraction is lower after maximal exercises for cyclists and submaximal for boxers and taekwondoers. The number of bands is higher and more prominent in the boxing group compared to the other groups. In the wrestling group, on the other hand, while the maximal and submaximal protein characterizations were similar, fainter bands were observed in the resting profiles. The reason for this change is thought to be due to oxidative stress as well as genetic differences and/or acute or chronic physiological changes specific to the branch.

Keywords: apoptosis, exercise, protein, oxidative stress

INTORUCTION

The physiological effects and metabolic differences of the energy systems used in parallel with the intensity and severity of physical activity are known. The aim of this study is to observe the serum protein profiles after maximal and submaximal exercises in branches with different energy systems and to compare them with the resting values of the athletes.

It is inevitable that oxidants are formed as a result of electron leakage that occurs in the cytochrome P450 system in the endoplasmic reticulum, during catabolic and anabolic reactions in the Electron Transfer System (ETS) during normal respiration with O2 in the mitochondria, one of the normal biological processes [1]. Antioxidants are substances that prevent and/or delay the effects of molecules that cause oxidation of essential elements in metabolism. Disruption of the balance between oxidant and antioxidant results in reactive oxygen species and oxidative damage. The resulting damage is expressed as oxidative stress [2,3]. The increase in muscle contraction during sports activities significantly accelerates metabolic activities due to more energy consumption. The increased metabolic rate as a result of sporting activity definitely increases oxygen consumption in the heart and active muscles, along with other tissues. Oxygen uptake to skeletal muscles increases 100-200 times. Depending on the increased metabolic activation, the amount of oxygen used increases and as electron leakage from the mitochondrial electron transport chain increases, many ROS are released, especially superoxide, hydrogen peroxide and hydroxyl radicals [4]. Oxygen-derived radical formation in the organism increases especially in intense sports loads. In this increase; Factors such as acceleration of electron flow in the electron transport chain in mitochondria, increased xanthine oxidase activity, local inflammation, release of iron from transferrin, and antioxidant consumption play a role [5].

The aim of this study is to compare the oxidative stress effects, serum protein concentrations, profiles and contents, which are thought to occur as a result of short-term exercises in which the submaximal and maximal capacities of the athletes competing in different sports branches are determined, with the findings in the resting state.

MATERIALS AND METHODS

Participants: 28 male athletes voluntarily participated in the study, competing in 4 different sports branches (Cycling – n=7, Boxing – n = 7, Wrestling – n = 7, Teaekwondo – n = 7), with an average age of 21-25 years and continuing active sports. Subjects were selected from athletes who had no neurological and auditory-visual disturbance in the past year and no serious injury to their lower and upper extremities in the last six months. Before the application, the athletes were told about the tests they would be exposed to within the scope of the research and they signed the document stating that they would participate voluntarily.

Research Design: Research Anaerobic Wingate power test in Selcuk University School of Physical Education and Sports performance laboratory, Aerobic power 20 m in Indoor Sports Hall. shuttle run test was performed. Blood samples were taken three times after resting, anaerobic and aerobic strength tests.

Data Collection Tools: Height and Body Weight

The subjects were weighed by wearing bare feet and shorts on a 20 g sensitive scale (Angel brand). Length (height) measurements were made with a (Holtain brand) scale, while the subjects were standing in an upright position, the caliper sliding on the scale was adjusted to touch the subject's head, and the length was read with an accuracy of 1 mm.

Wingate Anaerobic Strength Test: For the Wingate test, Monark 824 model (made in Sweden) foot bike ergonometer, connected to the computer set up and working with a compatible software program, was used. Height adjustments were made for each athlete before the tests. The test load for each athlete was calculated as 75gr/kg. The athletes were asked to reach the

highest pedal speed in the shortest possible time without resistance. When it was ensured that the maximum speed was reached (about 3-4 seconds later), the test was started by leaving a calculated load of 75gr/kg. Athletes pedaled at the highest speed for 30 seconds against the available resistance. Athletes were verbally encouraged throughout the test.

20 Meter Shuttle Run: 20 meter distance is run as a round trip by the subjects. The test was started at a slow running speed (8 km/h) and the subject ran in such a way that he had to start his run at the first signal tone and reach the line until the second signal tone. When he hears the second signal, he turns back to the starting line and this running speed increased by 0.5 km/h every minute and continued with the signals. When the subject hears the signal, he adjusts his tempo so that the second signal reaches the other end of the track. Slow speed in the beginning every 10 sec. also increases gradually. If subjects miss one beep and catch up with the second, they can continue with the test. If the subject misses two signals in a row, the test is terminated.

Analysis of Data: SPSS for Windows 15.0 package program was used to calculate and evaluate the data obtained as a result of age, height, body weight, 20m shuttle run and wingate power test. The measured variables are summarized by giving the mean and standard deviation (±).

Blood Analysis: Blood samples taken from all subjects were taken from the forearm elbow vein three times, 8 ml. Plasma and serum were separated by centrifugation at 3500 bp. Total Protein isolations were made from obtained serums in Selcuk University Science Faculty Molecular Biology laboratory. The purity and concentration of the proteins were determined by NANODROP 2000.

Protein Isolation Method from Serum: EURX brand Gene Matrix Universal DNA/RNA Protein Purifacation kit was used for protein isolation and isolation was performed as follows: 200 ml serum sample was taken from ependorpha. Leyse All buffer was prepared by adding 10 µl to 1 ml. DRP was prepared by adding 10 ml to 1 ml. It was mixed gently and centrifuged for 3 minutes. Supernatant was taken to DNAblinding column and centrifuged at 13000 rgm for 1 minute. The column was lifted at +4 °C and the lower filtrate was taken into a new tube. 300 ml of absolute ethanol was added and mixed with a pipette. The content was transferred to the RNA binding column and centrifuged at 12000 g for 1 minute. The column was lifted to +4 °C and the filtrate was taken into a new tube. 2 volumes of absolute ethanol was added and mixed. 30 min +4 °C removed. After centrifugation at 4500 rpm for 20 min at +4 +4 °C, the supernatant was poured slowly. After adding 70% ethanol, it was vartexed and centrifuged at +4 °C for 10 minutes. Supernatant 16 was poured and dried in a pallet desiccator for 5 minutes. After dissolving the pallet with 150 ml of PLB, the samples were incubated at 95°C for 5 minutes and then lifted to -20°C.

Determination of Protein Concentration by Brad-Ford Method: Bradford method was used to determine the concentrations of the proteins we obtained. First of all, Bradford dye was prepared at 5X concentration. For this purpose, 100 mg of Coomassie Brilliant Blue G-250 dye was dissolved in 47 ml of Methanol (100%), then 100 ml of Phosphoric Acid (85%) was added and made up to 200 ml with distilled water. It was used in the study by diluting to 1X concentration. To prepare the standard graph, 2% bovine serum albumin (BSA) solution was used as a stock as a standard protein. 5 different standards were prepared from the BSA solution as 2 mg/ml, 1 mg/ml 0.5 mg/ml 0.25 mg/ml and 0.125 mg/ml. Pure water was used for blanking. The protein samples we have were diluted 10-fold and made ready for measurement. Measurements were made based on the ratio of 2.5 ml of Bradford dye to 50 µl of protein. After adding Bradford dye and waiting for 5 minutes, measurements were made at a wavelength of 595nm. Protein concentrations were calculated by recording all measurements obtained.

SDS-PAGE (Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis): The contents and preparation of the stock solutions used in the SDS-PAGE experiment are as follows: 1.5 M Tris pH 8.8 Buffer; 18.16 g Tris base and make up to 100 ml with deionized water. The prepared solution was filtered with Whatman No: 1 filter paper, autoclaved and stored at room temperature. 1 M Tris pH 6.8 Buffer; Make up to 12.11 g Tris base and 100 ml with deionized water. Tris was dissolved in deionized water and the pH was adjusted to 6.8 with 3 N HCL. The prepared solution was filtered through Whatman No: 1 filter paper, autoclaved, and stored in the dark at room temperature. 10% Sodium Dodecyl Sulfate; 5 g of sodium dodecyl sulfate was made up to 50 ml with deionized water. It was stored in the dark at room temperature. 10% Ammonium persulfate; 0.1 g of 17 ammonium persulfate was made up to 1 ml with deionized water. It was prepared just before use and kept at +4°C. 5 X Running buffer pH 8.3; 3.75 g Tris base, 18 g Glycine, 5 g Sodium dodecyl sulfate were made up to 250 ml with deionized water. The pH of the prepared solution was adjusted to 8.3 using 1 N NaOH or 1 N HCL.

Since the buffer was prepared as 5 X, it was diluted 5 times before use and adjusted to 1 X. dyeing solution; 1 g of Coomassie Brilliant Blue, 500 ml of Methanol, 100 ml of Glacial acetic acid is completed to 1 liter with deionized water. After the dye was dissolved, it was filtered through Whatman No: 1 filter paper and stored in the dark at room temperature. Washing solution; 125 ml of Methanol, 175 ml of Glacial acetic acid was made up to 2200 ml with deionized water. The prepared solution was stored at room temperature in the dark. Two different gels were used for SDS-PAGE analysis. These are: separation and loading gels. The preparation of these gels was as follows: 10% Separation gel; It was prepared by adding 6 ml of distilled water, 5 ml of Acrylamide / Bis acrylamide (30%), 3.75 ml of 1.5 M Tris (pH 8.8), 150 µl of 10% SDS, 75 µl of 10% Ammonium Persulfate (APS), 7 µl of TEMED. 4% Loading gel; By adding 9 ml of distilled water, 1.98 µl Acrylamide/Bis acrylamide (30%), 3.78 µl 1 M Tris (pH 6.8), 150 µl 10% SDS, 75 µl 10% Ammonium Persulfate (APS), 15 µl TEMED was prepared. The following steps were followed in the SDS-PAGE analysis; Separation gel was prepared and poured into the cassette. To smooth the surface of the gel, isopropanol was poured from both sides of the gel cassette with the help of an injector. After the polymerization was completed, the upper level of isopropanol was poured and the gel surface was washed several times with distilled water. The loading gel was prepared and the comb was placed after pouring into the cassette. Waited for the gel to freeze. After freezing, the comb was carefully removed. Protein samples isolated from serum for analysis were taken to ice and diluted 1:10 with sterile deionized water after they were removed from -20°C. Protein samples were then loaded into the wells of the loading gel. PRESTAINED protein ledder was loaded into the marker well. After the loading process was completed, the lid of the tank was closed and the system was connected to the power supply. The power supply was set to 180 V for 60 minutes for the gel to run. After the execution process was finished, the gel was taken out from between the glass plates and placed in a plastic container with a lid and the staining process was started. The gel was stained by pouring the staining solution to completely cover the gel. The staining solution in the overnight stained gel was poured off and the gel was shaken in the wash solution for 1 hour. After 1 hour, the washing solution was poured and the washing solution was added again and the gel was washed for 1 more hour. This process was continued in this way until the paint on the background of the gel was completely removed. After the washing process was over, the gel was photographed with different brightnesses.

Statistical Analysis: No statistical program was used in the study. Data in the study analized by Proteins were characterized by BIORAD Mini Protean Electrophoresis System SDS-Page method with THERMO PagerulerPrestained Protein Ladder marker.

RESULTS

The averages of age, height, body weight, submaximal power (MaxVO2) and maximal power of the athletes are given below.

Table1: Average Age, Height and Body Weight of the groups

Groups	N	Age(years)	Height(cm)	Body Weight(kg)	Submax power (MaxVO2)	Max.power (W)
Bicycle	7	22.70±2.53	178.20±4.34	72.90±4.37	54.06±6.44	775.24±90.46
Boxing	7	22.20±2.64	178.50±5.20	74.20±10.46	48.58±3.94	735.08±93.90
Wrestling	7	21.70±2.34	174.20±7.02	77.50±13.34	51.37±3.58	857.94±127.47
Taekwondo	7	22.50±3.09	180.60±2.76	73.40±7.74	49.03±5.34	748.53±136.42

Table1. Groups; Age, height, body weight, submaximal power (MaxVO2) and maximal power averages of athletes doing Cycling, Boxing, Wrestling, Taekwondo branches are given in the Table 1.

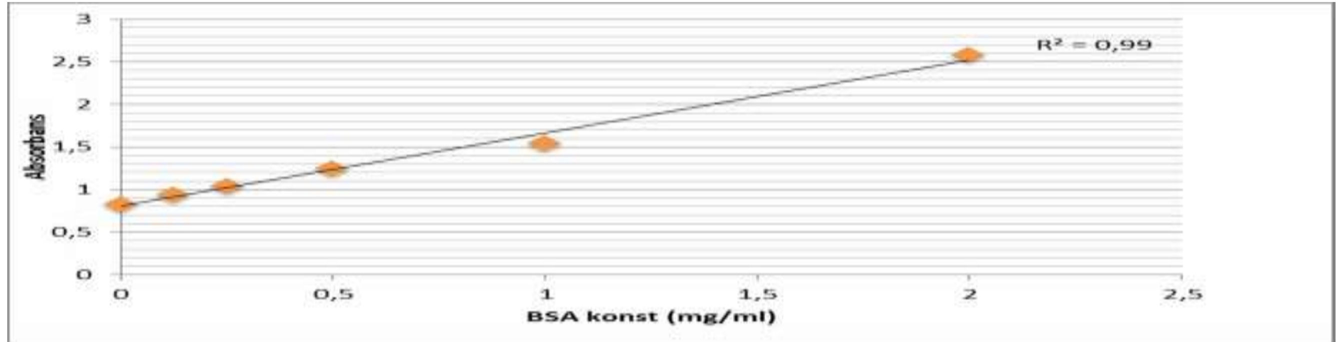


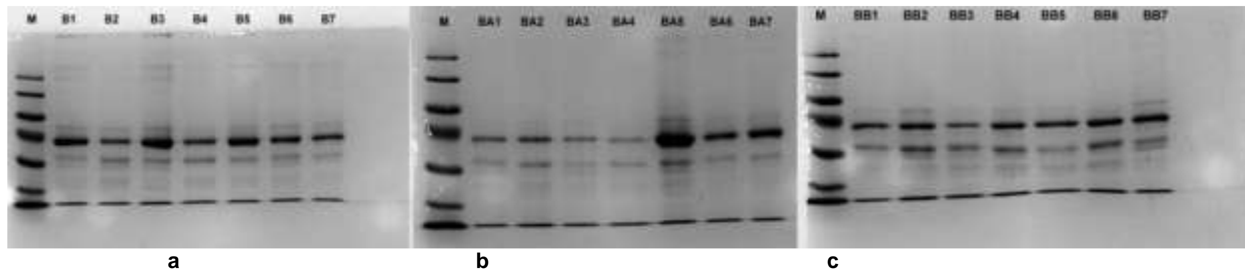
Figure1: BSA protein concentration (mg/ml).

Figure 1. Figure of the standard curve obtained using the Bradford method. The reliability of protein concentrations obtained from the athletes participating in the study is shown by finding R2=0.99 (figure 1).

Table 2: Cycling Group Protein Concentrations (Bradford).

Cycling athlets	1. athlet	2.athlet	3. athlet	4. athlet	5. athlet	6. athlet	7. athlet
Rest (mg/ml)	1,282738	1,143057	1,317658	1,282738	1,58538	2,237225	0,770574
Maximum Power (mg/ml)	0,433011	1,51554	2,691188	1,096496	2,598068	1,364218	1,794902
Submaximal Power (mg/ml)	3,017111	1,201257	1,306018	0,724014	-0,66116	1,317658	1,364218

The protein concentrations in mg/ml:milligram/milliliter obtained after the resting state, submaximal power and maximal strength training of cyclists are given in the Table 2.



a b c

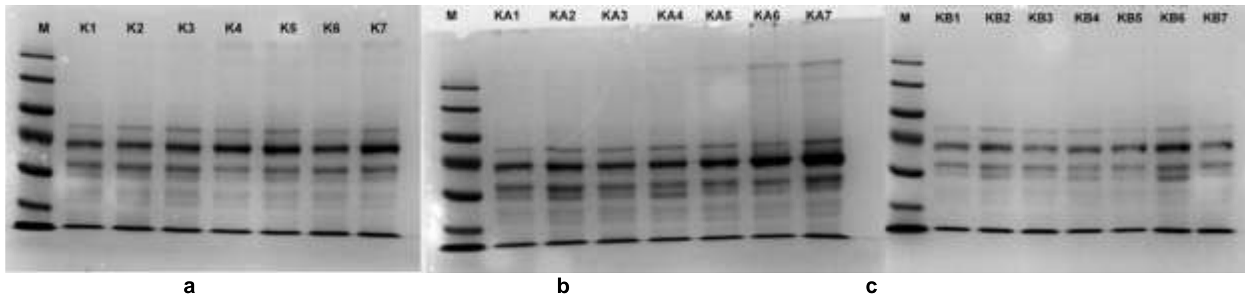
Figure 2: Cycling Group Resting (a), Maximal power (b), Submaximal power (c)SDSPage findings

In Figure 2; When the gel images we obtained as a result of SDS-PAGE are examined, there is a band of over 200kDa, a band of about 200kDa, 170kDa, and bands of 77kDa (transferrin), 67kDa, which we can describe as immunoglobulins in the cycling rest group. (albumin), 60kDa, 55kDa and 35kDa (Apolipoprotein) were detected, respectively. When the band profiles were examined in the cycling maximal group, in addition to a band of approximately 200kDa, which we can call immunoglobulin, 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa (α1-antitrypsin) and 35kDa (α1-antitrypsin) bands (Apolipoprotein) were detected, respectively. In this group, the 55kDa α1-antitrypsin band appears to be more indistinct compared to the other group. Bands of 170kDa and above in the bicycle 20m shuttle group (submaximal) group completely disappeared. In addition, bands of 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa and 35kDa (Apolipoprotein) sizes were detected.

Table 3: Boxing Group Protein Concentration (Bradford).

Boxing atlets	1. athlet	2. athlet	3. athlet	4. athlet	5. athlet	6. athlet	7. athlet
Rest (mg/ml)	1,003376	1,294378	1,212897	0,863695	0,630893	1,59702	2,761029
Maximum Power (mg/ml)	0,817134	1,375858	1,282738	2,92399	1,084856	1,049936	0,747294
Submaximal Power (mg/ml)	0,176929	2,388546	0,363171	0,409731	-0,07915	2,132464	1,445699

The protein concentrations in mg/ml:milligram/milliliter obtained after the resting state, submaximal power and maximal strength training of Boxing atlets are given in the Table 3.



a b c

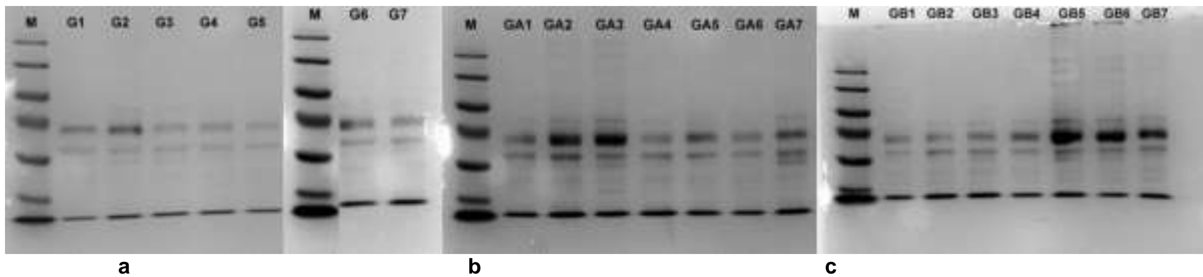
Figure 3: Boxing Group Resting (a), Maximal strength (b), Submaximal strength (c)SDSPage findings

In the Figure 3; Compared to the other groups, it was observed that the number of bands was higher in the gels of the boxing groups. In the boxing rest group, bands of approximately 200kDa, 160kDa, 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa, 2 bands between 55-40kDa and 35kDa (Apolipoprotein) were detected. Considering the boxing maximal group, it was observed that the bands became more pronounced in general. In this group, there are 200kDa, 21 160kDa, 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa, 2 bands between 55-40kDa and 35kDa (Apolipoprotein) bands. A significant increase was observed especially in albumin and α1-antitrypsin bands. In the boxing submaximal group, all the upper bands disappeared. In this group, the bands were generally indistinct and bands of 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa and 35kDa (Apolipoprotein) were detected.

Table 4: Wrestling Group Protein Concentration (Bradford).

Wrestling atlets	1. athlet	2. athlet	3. athlet	4. athlet	5. athlet	6. athlet	7. athlet
Rest (mg/ml)	1,049936	0,956815	1,480619	1,061576	1,422419	1,084856	1,946223
Maximum Power (mg/ml)	2,388546	0,933535	1,306018	1,51554	3,145152	1,63194	0,642533
Submaximal Power (mg/ml)	1,655221	1,969503	1,434059	2,190665	2,551507	1,119777	1,306018

The protein concentrations in mg/ml:milligram/milliliter obtained after the resting state, submaximal power and maximal strength training of Boxing atlets are given in the Table 3.



a b c

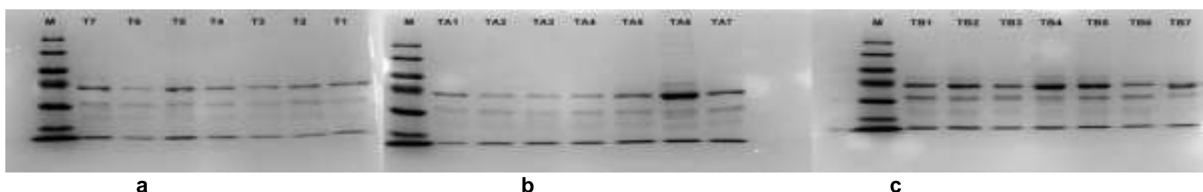
Figure 4: Wrestling Group Resting (a), Maximal strength (b), Submaximal strength (c)SDSPage findings

In the figüre 4; Looking at the resting wrestling group, less bands were observed in this group compared to the other groups. In this group, 77kDa (transferrin), 67kDa (albumin), 60kDa and 35kDa (Apolipoprotein) bands were detected. When the band profiles in the wrestling maximal group are evaluated, it is seen that the immunoglobulins increase in 3 athletes. In this group, bands of 77kDa (transferrin), 67kDa (albumin), 60kDa, 55 kDa and 35kDa (Apolipoprotein) were detected. In general, a sharpening is observed in the bands. It was observed that immunoglobulins increased in the wrestling submaximal group.The bands are 200kDa, 170kDa, 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa and 35kDa (Apolipoprotein). While the maximal and submaximal protein profiles were similar in the wrestling group, a 200kDa protein band was found after submaximal exercise. The resting protein bands of the athletes are lighter than the post-exercise conditions.

Table 5: group protein concentration (Bradford).

Taekwondo atlets	1.athlet	2.athlet	3. athlet	4. athlet	5. athlet	6. athlet	7. athlet
Rest (mg/ml)	1,445699	0,782214	0,817134	1,51554	2,458387	0,793854	0,817134
Maximum Power (mg/ml)	1,306018	3,599115	1,247817	1,655221	1,445699	1,026656	1,084856
Submaximal Power (mg/ml)	1,503899	2,609708	0,514492	0,619253	1,5621	3,249913	1,468979

The protein concentrations in mg/ml:milligram/milliliter obtained after the resting state, submaximal power and maximal strength training of Taekwondo atlets are given in the Table 5.



a b c

Figure 5: Teakwando Group Resting (a), Maximal strength (b), Submaximal strength (c)SDSPage findings

In the Figure 5; Looking at the resting wrestling group, less bands were observed in this group compared to the other groups. In this group, 77kDa (transferrin), 67kDa (albumin), 60kDa and 35kDa (Apolipoprotein) bands were detected. When the band profiles in the wrestling maximal group are evaluated, it is seen that the immunoglobulins increase in 3 athletes. In this group, bands of 77kDa (transferrin), 67kDa (albumin), 60kDa, 55 kDa and 35kDa (Apolipoprotein) were detected. In general, a sharpening is observed in the bands. It was observed that immunoglobulins increased in the wrestling submaximal group. The bands are 200kDa, 170kDa, 77kDa (transferrin), 67kDa (albumin), 60kDa, 55kDa and 35kDa (Apolipoprotein). While the maximal and submaximal protein profiles were similar in the wrestling group, a 200kDa protein band was found after submaximal exercise. The resting protein bands of the athletes are lighter than the post-exercise conditions.

DISCUSSION

The physiological effects and metabolic differences of the energy systems used in parallel with the intensity and severity of physical activity are known. The aim of this study was to observe the serum protein profiles after maximal and submaximal exercises in branches with different energy systems and to compare them with the resting values of the athletes. Most of the intracellular metabolism can be divided into two metabolic processes, protein breakdown, called catabolism, and protein synthesis, called anabolism. There are proteins that are central to both catabolism and anabolism signaling. Protein synthesis can be driven through a number of different intracellular signaling, all of which affect protein synthesis in a unique way. Many of these signals eventually combine to increase mTORC1 [6]. mTORC1 was created as the body's master regulator of protein synthesis.

Numerous upstream effectors have been identified to regulate activity status, such as amino acid flux, circulating growth factors, intracellular energy status (AMP/ATP ratio), and mechanical stimuli [7,6]. Following one or a combination of these inputs, the activity status of mTORC1 increases and high protein synthesis occurs [8].

In the electrophoresis gels of our study groups, 8 bands were detected in cyclists, 8 bands in boxers, 4 bands in wrestlers and 6 bands in taekwondo players. Although no bands with a molecular weight of 70-100kDa were found in the resting gels of the wrestling and taekwondo groups, there are bands with a molecular weight of 160kDa and 200kDa in boxers and cyclists. In addition, bands with molecular weights over 200 kDa were observed in cyclists. These differences may be an indication that different exercise types create different profiles on protein metabolism.

Three distinct mechanical transformative pathways that are suggested to increase following exercise. Following resistance exercise, muscle-specific α7β1 integrin binds to the extracellular matrix, recruits focal adhesion kinase (FAK) to its cytoplasmic tail, inhibits tuberous sclerosis complexes 1 and 2 (TSC1/2) and activates PI3K/AKT. It indirectly increases mTORC1. mTORC1 can then phosphorylate both 4E-BP1 and p70s6k, increasing protein synthesis [8].

Hornberger and arc directly investigated this differential effect of signaling kinetics by monitoring the type of muscle activity and found that the application of multiaxial and uniaxial muscle movements that mimic eccentric and concentric muscle movements, respectively, uniquely regulate muscle fiber signaling [9]. Specifically, multiaxial stress resulted in significantly higher phosphorylation of p70s6k in conjunction with ERK. While this increase in phosphorylation, particularly p70s6k, was found to decrease following pharmacological inhibition of the cytoskeleton, no such reduction in uniaxial muscle activity was found. Specifically, multiaxial stress resulted in significantly higher phosphorylation of p70s6k in conjunction with ERK. While this increase in phosphorylation, particularly p70s6k, was found to decrease following pharmacological inhibition of the cytoskeleton, no such reduction in uniaxial muscle activity was found. This indicates the ability of the cytoskeleton to sense different muscle movements, which subsequently alter protein phospho-kinetics [8].

No band with a molecular weight greater than 200kDa is observed in the resting state gels of the cycling group. The band with a weight of 200-170 kDa is only found in three athletes. The 170kDa band has disappeared. All protein bands in the resting state of boxers were more prominent after maximal exercise. Two bands with a weight of 200-170kDa and 50kDa, which were not observed at rest, were formed in the wrestling group, and all bands were observed to be more pronounced than at rest. In taekwondo players, on the other hand, a band with a weight of 100kDa, which is seen in all athletes at rest, was seen in only one athlete, while a band with a weight of 150kDa that was not found in any group and athlete was found in another athlete. It is a natural result that the aerobic performance MaxVO2 capacities of the athletes in the cycling branch are more developed than the athletes in boxing, wrestling and taekwondo. The lack of similarity between aerobic performance and resting state protein characterization in this group may be explained by adaptation to training and may suggest that the characteristic contraction types of the branches are indicative of differences in protein synthesis.

The cytoskeleton is in a constant state of flux and has been suggested to be central to muscle fiber mechanosensation. It should be noted that the above does not imply preference for one mode of muscle movement over another. Instead, the use of a wide range of muscle movement is recommended to regulate cellular anabolism, structural protein adaptations, and metabolic changes and is often required for sought-after training goals. A number of specific populations require that one mode of muscle movement be prioritized over another [8]. This explains the fact that the protein profile varies according to a certain sport branch.

Therefore, depending on the population, the use of one mode of muscle activity over another will inevitably vary [8, 9]. For example, the sport of American Football requires a series of sudden changes in direction that result in large eccentric forces working on the muscles in the lower body. These athletes may then place greater emphasis on exercise that imposes an eccentric overload [10].

In our study, submaximal serum protein profiles and contents of the groups were determined as 6 bands in cyclists, 5 bands in boxers, 7 bands in wrestlers and 7 bands in taekwondo players. In the cycling group, the band in the 200-170kDa range is only available in three athletes. The band in the range of 200 and 200-170 kDa, seen at rest, disappeared. In boxers, bands above 100-70kDa disappeared, while bands weighing 100-70kDa faded. A band over 200kDa was found in wrestlers, and a band over 160kDa was found in only three athletes. The protein band weighing 160kDa was not seen in the other two cases of wrestlers. In the taekwondo group, there were bands over 170kDa that were not seen after rest and maximal exercise. The intrinsic response to these various modes of muscle activity has been shown to be driven, in part, by differentially regulated mechanistically sensitive pathways that ultimately result in specific signaling cascades and gene expression.

Rindom's premise is that specific muscle action determines the relative activity of certain intracellular macromolecular structures; such that during eccentric muscle movements, transmembrane receptors are preferentially activated, while concentric muscle movements largely recruit sarcomeric proteins [11].

Nakada et al. reported that the degree of mechanical overload through varying degrees of synergistic ablation resulted in progressively increasing levels of markers of ribosome biogenesis. Specifically, 18S and 28S ribosomal RNA (rRNA) increased in parallel as the external stimulus increased [12]. In contrast, Romero et al. showed a marked increase in ribosome biogenesis following endurance exercise, which likely caused minimal

mechanical overload [13]. Fyfe et al. similarly found enhanced ribosome biogenesis after a combination of endurance and resistance exercise compared to resistance exercise alone [14]. These researchers clearly found that using different exercise modes with different levels of mechanical overload but yielding a similar elevation in ribosome activity.

Boxing, wrestling and taekwondo branches in our study groups have a training character in which alactic and lactic anaerobic energy systems are predominantly used. The similarity of the protein bands in the resting state with the maximal exercise state may be an indicator of the athletes' level of adaptation to the branches of protein metabolism and training. It can be thought that the trainings that improve anaerobic performance, which are exposed to severe loads, are not adversely affected by such loads in terms of protein concentration, content and profile of the athletes in this group in terms of oxidative stress. Submaximal trainings used to develop and determine the maximal oxygen utilization capacity (MaxVO2) are the loads in which the aerobic energy system is used. Recovery time in anaerobic processes is directly proportional to the adequate development of MaxVO2 capacity. While the protein concentrations of the boxers did not change after aerobic performance, the fact that they were lower in profile and content may be an indication that the protein fractions are at a lower level in this type of exercise.

During exercise, there is a negative balance between protein synthesis and degradation throughout the body. In addition to an increase in the rate of amino acid oxidation throughout the body, there is also a temporary catabolic state. The mechanisms leading to this condition differ according to the type of exercise. Exercises that require endurance reduce the rate of protein synthesis without affecting protein breakdown in the whole body (including skeletal muscle). In contrast, a prolonged resistance exercise causes the rate of protein breakdown in the whole body (including skeletal muscle) to be greater than the increase in the rate of protein synthesis. The magnitude of these changes also differs depending on the type of exercise. Even moderate exercise leads to a 25% increase in protein catabolism in the whole body of healthy adults. Therefore, in these individuals, the daily protein requirement should be ≥1 g/kg. In this case, if the protein taken through diet is not increased for the synthesis of muscle proteins, protein breakdown in the body will be more than protein synthesis. The result is a negative nitrogen balance. According to the results of a study, healthy adults who exercise intensely every day during the week and consume 1 g of protein per kg per day show a negative nitrogen balance throughout the training program [15, 16].

When dietary amino acids and protein are sufficient, a positive protein balance occurs in the whole body (including skeletal muscle) after exercise. For example, in adequately fed subjects, rates of muscle protein synthesis increased by 112%, 65%, and 34%, respectively, at 3, 24, and 48 hours post-exercise. However, muscle protein breakdown rates increased by 31% and 18% at the 3rd and 24th hours, respectively, and returned to resting levels at 48 hours. This suggests that the effective time for anabolic response after a single workout can take up to 48 hours [17]. The maintenance of muscle mass depends on the difference between muscle protein synthesis and muscle protein breakdown. Positive protein balance stimulates myofibril protein synthesis and mitochondrial protein synthesis in endurance training athletes. Measuring nitrogen balance is the most widely used method to determine the amount of protein needed [18]. Physical activity increases protein catabolism and decreases plasma essential amino acids concentrations. Determining plasma amino acids concentrations is the method of choice for assessment of amino acids of body organs [19, 20].

CONCLUSION

The serum protein concentrations of the athlete groups are similar. While the similarities of the cycling, boxing and taekwondo groups were more evident in terms of protein content and profiles, the maximal and submaximal post-exercise values of the wrestlers were similar. We can associate the differences between the groups of athletes with the intensity and intensity of the exercise they have done. It can be said that the protein fraction is lower after maximal exercises for cyclists and submaximal for boxers and taekwondoers. The number of bands is higher and more prominent in the boxing group compared to the other groups. In the wrestling group, on the other hand, while the maximal and submaximal protein characterizations were similar, fainter bands were observed in the resting profiles. The reason for this change is thought to be due to oxidative stress as well as genetic differences and/or acute or chronic physiological changes specific to the branch.

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