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Original article

The effect of use of protein supplements on muscle damage

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Abstract. *Study objectives:* The purpose of this study was to examine the effect of ingested protein supplementation on muscle damage and delayed onset muscle soreness followed acute maximal weightlifting exercise. *Method:* Twenty-four males were included in this study who between the ages of 19-25 and were performing the physical activity for an average of 60 minutes 2 days a week. The volunteers were randomly divided into two groups as the experimental group that takes a protein supplement and control group that doesn't take a protein supplements. Blood samples were taken from all volunteers before exercise. Followed warming, 3 sets 10 repetitions weightlifting exercise were performed at 80% 90 and 100 of pre-determined maximal weights. The diets of athletes were determined by an expert dietitian as 1 g/kg per day of all participants by dividing into three meals. In addition, the experimental group was taken protein supplement also 35 grams of whey protein (total: 1.5g/kg per day) after the lunch by dissolving in 500 ml water. The blood samples of both groups were taken at the before, immediately after, 24th, 48th, and 72nd hours after exercise at the same time of the day and the VAS scores were recorded. *Results:* There was no significant difference between experimental and control groups before and immediately after exercise in terms of CK and Mb values when the group effect is considered ($p>0.05$). When, the VAS score were examined independent from the group effect, it was observed that the values of control group were higher than the experimental group in the 2nd, 3rd, and 4th comparisons ($p<0.05$). *Conclusion:* Consequently, although 1.5 g/kg protein supplementation did not significantly decrease the CK and Mb values after weightlifting exercise, it was observed that DOMS was decreased significantly.

Keywords: Weightlifting, Whey Protein, Myoglobin, Creatine kinase

Introduction

Nowadays beneficial effects of exercise and sports are known by everyone. This situation causes an increase in the number of people who perform exercise and sports. However, certain problems may occur in beginners. Most of the people experience a kind of pain or trouble due to performing an unusual activity. In the scientific literature, the symptoms of pain and loss of strength in the following hours and days of new exercises are usually attributed to the physical damage or tear of muscle structure and for this reason, they are called "muscle damage that starts with exercise" (1). As is known, unusual eccentric exercise induces muscle damage that is represented by the long-term loss of muscle function, delayed onset muscle soreness (DOMS), and increase in muscle proteins such as creatine kinase (Ck) and myoglobin (Mb) (2).

A proper and balanced diet is as important as training for athletes. However, active individuals use supplements in order to form muscle, gain strength, prevent future diseases, and increase performance in sports (3). The effect of most of the supplements was not scientifically proven. The main purpose of taking dietary supplements is to fill the gap in lack of nutrition, however, there are other reasons to consume supplements such as improving certain functions. For instance, a high-protein diet is required for burned vic-

tims and athletes who want to maximize their skills and potentials (4).

A healthy diet is regarded as an inseparable component of physical fitness. In addition to health reasons, people believe that a special diet based on the high intake of a certain nutrient such as protein increases their sports performance (5). It can be observed that the reason people incline towards the consumption of protein supplements is the belief of increased effects, increased competitive performance, or decreased risk of injury or disease (6).

The consumption of protein supplements among the people who perform physical exercise increased significantly (7). Protein supplements are taken for increasing the muscle mass and supporting the post-exercise regeneration. The purpose of this study was to examine the effect of ingested protein supplementation on muscle damage and DOMS followed acute maximal weight lifting exercise.

Material and Method

Participants

24 males were included in this study who were studying at the School of Physical Education and Sports, between the ages of 19-25 and were performing the physical activity for an average of 60 minutes 2 days a week. The voluntary participants underwent a medical examination at the Faculty of Medicine, Sports Medicine Outpatient Clinic for the fitness to the exercise. Volunteers who were inconvenient for heavy exercise were excluded from the study. Individuals who were using prescribed medication, smoking, drinking alcohol, and using other hazardous substances, and had chronic diseases were also excluded. Written consents were taken from athletes by giving information about the exercise procedure.

Experimental Design

The volunteers were divided randomly into two groups as the experimental group that takes protein supplement (15 individuals) and the control group that does not take protein supplements (9 individuals). By determining the weights that volunteers can lift in the following exercise equipment, 80%, 90%, and 100% of these weights were recorded (bench press, butterfly, butterfly reverse, shoulder press, triceps pushdown, biceps curl, sit-up, reverse sit-up, squat, leg press, leg extension, leg flexion, adductor, calf press). Then, the athletes did not perform any exercise and sports for one week. After a week, 5 ml of blood samples were taken from the antecubital vein of all participants at 09:00 am and the VAS scores were recorded. 10 minutes after taking blood samples, athletes performed warm-up and stretching exercises for 15 minutes on a treadmill. Then, the above-mentioned exercises were performed with 80%, 90%, and 100% of the maximal with 3x10 repetitions in each one. Stretching exercises were performed again after the exercises and blood samples were taken again after giving a break for 60 minutes. The blood samples were kept at -80 °C. The diets of athletes were determined by an expert dietitian as 1 g/kg per day of the participants by dividing into three meals. The experimental group was taken protein supplement also used 35 grams of whey protein after the lunch by dissolving in 500 ml water (Optimum Nutrition Gold Standard Whet Protein). The control group did not take protein supplements did not receive any supplement apart from the 1g/kg/day protein diet.

Collecting Blood Samples

The blood samples of both groups were taken at the before, immediately after, 24th, 48th, and 72nd hours after exercise at the same time of the day and the VAS scores were recorded as in Table 1. The volunteers did not eat anything apart from their diet and did not perform any exercise within this period. The blood

Table 1. The recording of blood samples and VAS scores

The times of bloodletting and VAS measurement	CK	Mb	VAS
Blood samples and VAS score at before exercise	CK0	Mb0	VAS0
Blood samples and VAS score at immediately after exercise	CK1	Mb1	VAS1
Blood samples and VAS score at the 24th hours after exercise	CK2	Mb2	VAS2
Blood samples and VAS score at the 48th hours after exercise	CK3	Mb3	VAS3
Blood samples and VAS score at the 72nd hours after exercise	CK4	Mb4	VAS4

samples were analyzed in the Biochemistry laboratory of Erciyes University. Mb was analyzed by 4 ELISA method and CK was analyzed by 4 enzymatic spectrophotometric method in Roche Cobas analyzers.

Statistical Analysis

The data were evaluated by using IBM SPSS 22 statistics package. The suitability of the data for normal distribution was evaluated by the histogram, Q-Q graphs, and Shapiro-Wilk test. Variance homogeneity was tested with the Levene test. For determination of the difference between groups and measurement times, Two-way repeated measures ANOVA was used Mauchly test for Sphericity was used to control whether the Sphericity hypothesis was provided or not. In the cases that Sphericity hypothesis was not provided, one of the Greenhouse-Geisser, Huynh-Feild, or Lower-Bound fixes was used. The significance level was taken as $p<0.05$.

Results

There was no significant difference between the control and experimental groups after the exercise in terms of CK and Mb values when the group effect is considered ($p>0.05$), (Tables 2, 3). In addition, there was a significant difference in the comparison of in-group variables when the CK and Mb variables both control and experimental groups ($p<0.05$), (Tables 2, 3).

When the VAS scores were examined, independent from the group effect, it was observed that the values of the experimental group were higher than the control group in the 2nd, 3rd, and 4th comparisons. There was a statistical difference in favor of the experimental group ($p<0.05$; Table 4). Moreover, there was a significant difference in the comparison of in-group variables when the VAS scores both the control and experimental groups ($p<0.05$), (Table-4).

Discussion

When the literature is examined, it can be observed that there aren't many studies about the effect of

Table 2. The change of Creatine Kinase (CK) values according to time and group

	The group that takes protein supplement	The group that does not take a protein supplement	p
CK0	160.62±102.86^{a}	138.80 ± 37.58^{a}	**0.464**
CK1	293.00±172.54^{b}	252.20 ± 96.71^{b}	**0.472**
CK2	489.37±245.48^{c}	1826.80 ± 292.20^{bc}	**0.099**
CK3	1358.62±152.27^{abc}	5372.73 ± 960.74^{ba}	**0.134**
CK4	4880.00±516.45^{d}	9728.93 ± 1516.82^{d}	**0.394**
***p**	**0.041**	**0.031**	

Ck: creatine kinase, 0: 1 hour before the exercise, 1: 1 hour after the exercise, 2: 24 hours after the exercise, 3: 48 hours after the exercise, 4: 72 hours after the exercise.
p<0.05; p<0.01; ***p<0.001*
There isn't a difference between the measurements with the same letters in the same column.

Table 3. The change of Myoglobin (Mb) values according to time and group

	The group that takes protein supplement	The group that does not take a protein supplement	p
Mb0	54.25±19.04^{a}	32.80 ± 12.38^{a}	**0.412**
Mb1	69.87±37.11^{ab}	188.20 ± 27.50^{b}	**0.122**
Mb2	168.87±12.14^{bcd}	101.06 ± 32.79^{c}	**0.188**
Mb3	342.25±32.29^{abcd}	719.26 ± 107.80^{de}	**0.226**
Mb4	631.25±62.18^{cd}	960.86 ± 120.42^{de}	**0.397**
***p**	**0.044**	**0.010**	

Mb: myoglobin, 0: 1 hour before the exercise, 1: 1 hour after the exercise, 2: 24 hours after the exercise, 3: 48 hours after the exercise, 4: 72 hours after the exercise.
p<0.05; p<0.01; ***p<0.001*
There isn't a difference between the measurements with the same letters in the same column.

Table 4: The change of VAS values according to time and group

	The group that takes protein supplement	The group that does not take a protein supplement	p
VAS0	0.00±0.00^{a}	0.00±0.00^{a}	-
VAS2	6.46±2.53^{bcd}	3.11±2.14^{b}	**0.003**
VAS3	8.00±1.88^{bc}	4.77±3.19^{c}	**0.018**
VAS4	5.46±2.03^{d}	3.44±2.96^{db}	**0.059**
***p**	**0.001*****	**0.001**	

VAS: visual analog scale score, 0: 1 hour before the exercise, 1: 1 hour after the exercise, 2: 24 hours after the exercise, 3: 48 hours after the exercise, 4: 72 hours after the exercise.
There isn't a difference between the measurements with the same letters in the same column.

protein supplements on muscle damage. Thus, the obtained results from the present study gain more importance. It is known that muscle damage emerges after an unusual or heavy exercise (9). It was demonstrated in several studies that muscle damage occurs within 24-48 hours after the eccentric exercise, peaks at 72 hours, and recovers within 5-7 days (10-12).

Muscle damage emerges as a result of myofibrillar damage depending on eccentric exercise and causes intracellular edema (10). The most important parameter of muscle damage is the measurement of CK and Mb values (13). These values reach a maximum within 24-48 hours and then start to decrease (14).

In the present study, muscle damage was formed in volunteers by performing concentric and eccentric weight exercises. Similar to the studies in the literature, CK and Mb values increased within 24-48 hours and peaked at 72 hours. Chen et al. (2019) stated that the CK which is the marker of muscle damage increased after the eccentric exercise that was performed by the athletes in their study (15). An increase was observed in CK and Mb values after the exercise in similar studies (16, 17).

In the present study, when the question of whether protein supplements decrease the muscle damage is examined, it can be observed that CK and Mb values were lower in the group that took protein supplement. This result can be interpreted as; the muscle damage in the group that took protein supplement was lower. However, the fact that the difference between the groups was not statistically significant weakens our hypothesis. It was observed that in the conducted studies, 2 g/kg protein was given to participants after acute exercises in order to decrease muscle damage (18).

However, participants in our study received 1.5 g/kg protein. 70% of this was provided with natural nutrients and the rest was provided with protein powder. The control group, on the other hand, received 1g/kg protein with natural nutrients. It is considered that the reason why there wasn't a statistically significant difference between the groups is that the amount of given protein powder was inadequate. The amount of protein powder was kept low intentionally by considering the health of athletes. In the study of Coochburn et al. (2010) reported that milk protein-derived supplements were effective in decreasing the CK increase. It is asserted that this inhibition and protein procurement is based on the increase of muscle-protein balance by increasing the synthesis and classifying the increases in degradation (19).

One of the most important parameters of muscle damage is DOMS and it is measured with the VAS (20). It is widely accepted that DOMS emerges when an individual is exposed to high successive eccentric muscle contractions or unusual exercises (21). DOMS usually continues to increase after the exercise and peaks within 24-48 hours after the exercise (22).

DOMS was also monitored in our study. In parallel to the data in the literature, after reaching the maximal value within 24-48 hours, DOMS started to decrease after 72 hours. Parallel to the CK and Mb values, VAS scores were lower in the group that took protein supplement. This indicates that muscle pain was lower in the group that took protein supplements. Furthermore, VAS scores were statistically lower in the group that took protein supplements than the control group at 24^{th} and 48^{th} hours.

Conclusion

Consequently, although taking 1.5 g/kg protein did not significantly decrease the CK and Mb values which are muscle damage parameters, a numerical decrease was observed in the group that took protein supplement. When the results about the DOMS are examined, it can be observed that DOMS was decreased significantly.

Limitations of the study

The low number of volunteers participating in the study weakens the strength of the study. Additionally, it is considered that giving 2 g/kg protein to the volunteers would affect the results more specifically. Another issue is that muscle damage parameters can be said that the increased more by performing the isolated eccentric exercise.

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