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THE EFFECT OF PROTEIN SUPPLEMENTS ON BLOOD PARAMETERS AFTER HEAVY EXERCISES

UČINEK BELJAKOVINSKIH DODATKOV NA KRVNE PARAMETRE PO ZAHTEVNEJŠI VADBI

ABSTRACT

It is claimed that protein taking among athletes has increased substantially. The aim of this study is to investigate the effect of the protein supplementation following heavy exercises on blood cells. 24 males between the ages of 19-25 were participated in the study. The participants were divided into two groups randomly as the protein group (n:15) and non-protein group (n:9). For all participants, 10 repetitive weight lifting exercises were performed with 80%, 90% and 100% of the maximum weight that they could lift to the main muscle groups. All volunteers were given 1g/kg protein daily diet divided into three meals. Additionally the protein group was given 35 grams of Whey protein dissolved in 500 ml water just after dinner as being 2 hours, 24 hours, 48 hours and 72 hours after the exercises. Blood samples of both groups were taken before exercise, after exercise, at the 24th hour, 48th hour and 72nd hour. Erythrocyte, leukocyte, platelets, hemoglobin neutrophil, lymphocyte analysis were performed. There was no significant difference between the protein group and non-protein group, but there were significant differences within both group by time. As a result, we can say that protein supplementation after heavy exercise has no effect on blood parameters.

Key words: heavy exercise, protein supplements, erythrocyte, leukocyte, platelets

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IZVLEČEK

Vse kaže, da se jemanje beljakovinskih dodatkov med športniki vse bolj povečuje. Cilj te raziskave je bil preučiti učinke beljakovinskih dodatkov na krvne celice po zahtevnejši vadbi. V njej je sodelovalo 24 prostovoljcev, starih od 19 do 25 let. Udeleženci so bili naključno razdeljeni v dve skupini, in sicer v beljakovinsko (n = 15) in nebeljakovinsko (n = 9). Vsi udeleženci so opravili 10 ponovitev vaj dviganja uteži z 80, 90 in 100 odstotkov maksimalne teže, kot so jo lahko dvignili z glavno skupino mišic. Dnevna prehrana udeležencev je vsebovala 1 g/kg beljakovin in je bila razdeljena na tri obroke. Poleg tega je beljakovinska skupina uživala še 35 g beljakovin sirotke, raztopljenih v 500 ml vode, takoj po večerji, kar je bilo 2 uri, 24 ur, 48 ur in 72 ur po vadbi. Vzorci krvi so bili odvzeti obema skupinama pred vadbo in po njej ter po 24, 48 in 72 urah. Analizirali smo levkocite, trombocite, hemoglobin, nevtrofilce in limfocite. Med beljakovinsko in nebeljakovinsko skupino ni bilo značilnih razlik, so bile pa značilne razlike znotraj obeh skupin glede na čas. Ugotovili smo, da beljakovinski dodatki po zahtevnejši vadbi ne vplivajo na krvne parametre.

Ključne besede: zahtevnejša vadba, beljakovinski dodatki, eritrociti, levkociti, trombociti

INTRODUCTION

The effects of regular exercises on health have been gaining more importance day by day. There are many beneficial effects of moderate exercises on health. It lowers blood glucose and insulin resistance of the patients with type 2 diabetes, helps the blood pressure regulation in hypertensive patients, reduces the risk of getting coronary diseases, prevents obesity, decreases the symptoms of depression, and reduces the risk of certain hormonal cancers (Akkurt, 2012; Eriksson & Lindgärde 1991; Martinsen, 2008;Cornelissen, 2013; Manson et al.,2002; Friedenreich & Orenstein 2002). It is also suggested that moderate exercises strengthens immune system (Shepherd & Shek 1999).

However, as the intensity and time of the exercises increase, some negative effects also appear. It is known that heavy exercises such as long-running or weight trainings performed at maximal causes muscle damages (Akkurt et al., 2015;Yanagisawa et al., 2010; Kanda et al., 2013). In addition, it is asserted that it leads immune system to be suppressed, and this effect is related to the elevated cortisol level (Ji et al., 2006; Gumus et al., 2016). Immune system is suppressed within 3-72 hours after heavy exercises, and this period is called as open window period (Nieman, 2015).

It is known that athletes use protein supplements in order to create muscle mass easier, to gain strength, to prevent future diseases and to support regeneration (Bianco et al., 2011; Lee, 2015). It is stated that protein taking has increased substantially (Harrison et al., 2004). There are many forms of protein supplements. Whey protein is developed by distillation in lactose and non-fat powder form and widely used among protein supplements (Akkurt, 2019).

It is known that proteins are used in the production of lymphocytes and immunoglobulin. Based on this, it has been thought that whey protein to be used in open window period can increase blood cell production. The aim of this study is to investigate the effect of the protein supplement given following heavy exercises on blood cells.

MATERIAL - METHOD

24 males between the ages of 19-25 and doing physical exercises for average 60 minutes 2 days a week participated in the study. The volunteer participants underwent a health check at the Faculty of Medicine Sports Medicine Polyclinic for the suitability for exercises. Those having chronic diseases, being on medication for any reasons, smoking, drinking alcohol and having substance abuse and not being suitable for weight trainings were not included in the study. The athletes were informed about the workout program and their written approvals were taken.

The participants were divided into two groups randomly as the protein group (15 participants) and non-protein group (9 participants). The subjects participated in the preparation test before the experiment. The test was designed to determine one-repetition maximum (1RM). In the framework of the planned program, the weights that participants could lift in one go on the exercisers below were determined, and 80%, 90% and 100% of these weights were recorded (bench press, butterfly, butterfly reverse, shoulder press, triceps pushdown, biceps curl, squat, leg press, leg extension, leg flexion, adductor, abductor, calf press). Afterwards, they were not allowed to do exercises and play sports for a week. At the end of the week, 5 cc blood samples were taken from the antecubital veins of the athletes. Ten minutes after blood collection, volunteers were given warm-up and stretching exercises on the treadmill for 15 minutes. Then, the above-mentioned exercises were first performed as 10 repetitions with 80% of maximal, then 10 repetitions with 90% and finally 10 repetitions with 100%. After the exercises, stretching exercises were repeated

and the athletes were allowed to have a rest for 30 minutes, then blood samples were taken again. Blood samples were centrifuged and stored at -80 °C. Diets designated by a dietician as being 1 g/day protein per kilogram of all volunteers were divided into three meals. The protein group was given 35 grams of Whey protein dissolved in 500 ml water just after dinner as being 2 hours, 24 hours, 48 hours and 72 hours after the exercises (Optimum Nutrition Gold Standard Whey Protein). The non-protein group was not given protein support product together with their 1 g/kg/day diet. Blood samples of both groups were taken at the 24th hour, 48th hour and 72nd hour at the same time of the day, and the volunteers were ensured not to take any other food except their diet and not to do the any exercises in the meantime.

Erythrocyte (RBC; Red Blood Cell), Leukocyte (WBC; White Blood Cell) and Thrombocyte (PLT; Platelet) blood cell analysis were performed in the biochemical central laboratory of Erciyes University using Siemens advia 2120 brand blood counting device.

STATISTICALLY ANALYSIS

The data were evaluated by using IBM SPSS 22 statistics package. The suitability of the data for normal distribution was evaluated by histogram, Q-Q graphs and Shapiro-Wilk test. Variance homogeneity was tested with Levene test. For determination of the difference between groups, t test was used in independent groups. two-way analysis of variance was used in iterative measurements. In comparing the groups according to the measurements within themselves, one-way analysis of variance was used in iterative measurements. Mauchly test for sphericity was used to control whether 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. Significance level was taken as $p<0.05$.

RESULTS

When group effect is considered, there is no significant difference in the comparisons of the intergroup variables after the exercises performed by control and experimental group ($p>0.05$) (Table 1). Considering the temporal variation of the WBC variable, an increasing significant difference was determined between the 1st and 2nd, 1st and 4th and 1st and 5th measurements of the experimental group ($p<0.05$). An increasing statistical difference was occurred between the 1st and 2nd measurements of the control group ($p<0.05$). There is no statistically significant difference between other measurements ($p>0.05$).

Table 1. The comparison of Leukocyte values in terms of time and groups

	Group U	**Group K**	**P**
WBC0	7.42±1.66 abc	7.17±1.05a	**0.687**
WBC1	11.67±3.3 a	10.38±1.70a	**0.225**
WBC24	8.39±1.73	8.67±2.80	**0.764**
WBC48	9.10±1.78 b	9.23±2.70	**0.884**
WBC72	9.04±1.62 c	8.96±2.39	**0.920**
P*	**0.002****	**0.001*****	

WBC: White blood cell (leukocyte), WBC0: 1 hour before the exercises, WBC1: 1 hour after the exercises, WBC24: 24 hours after the exercises, WBC48:48 hours after exercises, WBC72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter.

When group effect is considered, there is no significant difference in the comparisons of the intergroup variables after the exercises performed by control and experimental group (p>0.05) (Table 2). Considering the temporal variation of the RBC variable, an increasing significant difference was determined between the 1st and 4th, 2nd and 4th and 3rd and 4th measurements of the experimental group (p<0.05), and a decreasing significant difference was seen between the 4th and 5th measurements (*p<0.05). No statistical difference was found between the measurements of the temporal change of the variables of the control group (p>0.05).

Table 2. The comparison of erythrocyte values in terms of time and groups

Variable	Group U	Group K	P
RBC0	5.32±0.60a	5.28±0.25	**0.864**
RBC1	5.30±0.59b	5.32±0.23	**0.913**
RBC24	5.49±0.55c	5.21±0.23	**0.177**
RBC48	5.62±0.56abcde	5.24±0.25	**0.070**
RBC72	5.47±0.54e	5.20±0.21	**0.166**
P*	**0.001*****	**0.124**	

RBC: Red blood cell (Erythrocyte), RBC0: 1 hour before the exercises, RBC1: 1 hour after the exercises, RBC24: 24 hours after the exercises, RBC48: 48 hours after exercises, RBC72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter.

Table 3. The comparison of hemoglobin values in terms of time and groups

Variable	Group U	Group K	P
HGB0	15.71±1.29	15.80±1.24	0.873
HGB1	15.35±1.31ab	15.86±1.05	0.331
HGB24	15.90±1.24c	15.80±0.94	0.828
HGB48	16.42±1.26ac	15.70±1.02	0.160
HGB72	16.03±1.15b	15.47±1.00	0.244
P*	**0.001*****	**0.115**	

HBG: Hemoglobin, HBG0: 1 hour before the exercises, HBG1: 1 hour after the exercises, HBG24: 24 hours after the exercises, HBG48: 48 hours after exercises, HBG72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter.

When group effect is considered, there is no significant difference in the comparisons of the intergroup variables after the exercises performed by control and experimental group (p>0.05) (Table 3). Considering the temporal variation of the HGB variable, an increasing significant difference was determined between the 2nd and 4th, 2nd and 5th and 3rd and 4th measurements of the experimental group (p<0.05). No statistical difference was found between the measurements of the temporal change of the variables of the control group (p>0.05).

When group effect is considered, there is no significant difference in the comparisons of the intergroup variables after the exercises performed by control and experimental group (p>0.05)

(Table 4). No statistical difference was determined between the measurements of the temporal change of the variables of the experimental and control groups (p>0.05).

Table 4. The comparison of platelet values in terms of time and groups

Variable	Group U	Group K	P
PLT0	246.85 ± 76.17	275.00±64.15	0.364
PLT1	272.40 ± 36.90	283.66±72.48	0.617
PLT24	276.06 ± 44.77	275.33±65.52	0.974
PLT48	279.73 ± 42.90	274.88±70.69	0.835
PLT72	274.33 ± 40.29	285.00±68.95	0.635
P*	**0.218**	**0.172**	

PLT: Platelet (thrombocyte), PLT0: 1 hour before the exercises, PLT1: 1 hour after the exercises, PLT24: 24 hours after the exercises, PLT48: 48 hours after exercises, PLT72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter.

When group effect is considered, there is no significant difference in the comparisons of the intergroup variables after the exercises performed by control and experimental group (p>0.05) (Table 5). Considering the temporal variation of the WBC variable, a significant difference was determined in the within-group comparisons of the variables (p<0.05). In NE variable, an increasing statistically significant difference occurred between the 1st and 2nd, and 1st and 5th measurements (*p<0.05), and a decreasing difference was occurred between the 2nd and 3rd measurements (p<0.05). There was an increasing statistically significant difference between the 1st and 2nd measurements of the NE variable of the control group (p<0.05). There was no statistically significant difference between other measurements (p>0.05).

Table 5. The comparison of neutrophile values in terms of time and groups

Variable	Group U	Group K	P
NE0	4.28±1.53ab	4.61±0.95a	**0.571**
NE1	8.17±3.52ac	7.34±1.41a	**0.508**
NE24	4.88±1.34c	5.54±2.60	**0.422**
NE48	5.49±1.68	5.81±2.06	**0.686**
NE72	5.53±1.37b	5.60±2.01	**0.922**
P*	**0.001*****	**0.008****	

NE: Neutrophile, NE0: 1 hour before the exercises, NE1: 1 hour after the exercises, NE24: 24 hours after the exercises, NE48: 48 hours after exercises, NE72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter.

Considering the group effect was given Table 6, there is no significant difference between inter-group comparisons after the exercises performed by control and experimental groups (p>0.05). Considering the temporal variation of LY variable, an increasing significant difference was

determined between the 1st and 3rd measurements of the experimental group ($p<0.05$). An increasing statistically significant difference occurred between the 1st and 3rd, and 1st and 4th measurements of LY variable of the control group ($p<0.05$). No statistically significant difference was found between the other measurements ($p>0.05$).

Table 6. The comparison of lymphocyte values in terms of time and groups

Variable	Group U	Group K	P
LYM0	2.13±0.38a	1.96±0.51ab	**0.367**
LYM1	2.57±0.73	2.34±0.74	**0.454**
LYM2	2.68±0.59a	2.38±0.55a	**0.229**
LYM3	2.69±0.61	2.59±0.75b	**0.725**
LYM4	2.67±0.54	2.59±0.68	**0.780**
P*	**0.004****	**0.001*****	

LYM: Lymphocyte, LYM0: 1 hour before the exercises, LYM1: 1 hour after the exercises, LYM24: 24 hours after the exercises, LYM48: 48 hours after exercises, LYM72: 72 hours after the exercises

Group U (those taking protein support), Group K (those not taking protein support)

*p<0.05**;p<0.01;***p<0.001

There is a difference between the measurements in the same column and having the same letter

DISCUSSION

This study conducted to investigate the effect of the protein supplements after acute maximal weight exercises on blood parameters shows similarity with the results of the studies conducted related to exercises. However, since this study is associated with protein supplement use, the scarcity of the related studies in the literature increases the importance of this study and limits the discussion.

Considering the results of WBC values in the study in terms of temporal variations of the control and experimental group before and after the exercises, it was determined that the values were at the maximum level within the first 24 hours after the exercises, but a decrease was found within the 48 hour-period of time. In their study, İbiş et.al (2010) stated that there was an increase in WBC parameters in the volunteers after the anaerobic exercises. In addition, the increase in WBC was parallel with the increase in the intensity of the exercises. In many studies, an increase was observed in leukocyte values (Koç, 2011; Bezci & Kaya 2010; Koç et al.,2012). The increase in WBC values after the exercises is thought to be caused by getting into circulation of the leukocytes' that have adhered on blood vessel wall as a result of the increasing circulation (Karakus, 2017).

When the values before and after the exercises depending on the temporal variations of NE variable were considered, it was determined that the values reached at maximum level within the 2 hour-period of time after the exercises, and that they returned to normal within the 48 and 72 hour-period of time. Neutrophiles constitute 60% of the circulating leukocytes. They function as the first line of defense cells that use phagocytosis alone or in cooperation with antigen-specific defenses to eliminate infectious agents in plasma. Studies on the acute effects of exercise show that exercise usually reveals the first activation of neutrophiles evidenced by the release of cytoplasmic enzymes (degranulation) with secondary changes in key effector functions such as phagocytic

and respiratory burst activity (Pyne, 1994). In a similar study, Benoni et al. (1995) stated that NE values increased after exercises.

An increase occurred in LYM variable in both groups within the 48 hour-period of time after the exercises. In a study, Yapıcı (2006) stated similarly that an increase occurred in lymphocyte values after the exercises. Şarvan and Çinar (2014) indicated in their study that there was no difference between the values before and after the exercises. Krüger and Mooren stated in their study (2014) that exercises had a clear effect on the number and functions of LYM in the blood, and explained that the number of LYM in the circulation increased during the exercises and lymphopenia was observed after the exercises. Since the primary duty of lymphocytes is to perceive microorganisms and produce antibody against them, and to eliminate the damaged tissues by phagocytosis method, it is thought that the increase in lymphocytes is due to the muscle damage based on exercises (Hazar, 2004).

In this study, it was determined that RBC values before and after the exercises reached the maximum level 48 hours after the exercises, and a decrease was observed in HGB values 2 hours after the exercises, but it was determined that they reached at their maximum level 24 hours after the exercises. It was found that there was again a decrease in HGB values within the 48 and 72 hour-period of time after the exercises. It is considered that a decrease occurs in HGB values in individuals doing intensive exercises and that this situation is due to the fragmentation of old erythrocytes after the exercises. In the results of this study, it was observed that HGB values increased in the protein group. In this case, it can be said that protein support decreases erythrocyte destruction and thus maintains HGB values. Ronghui (2015) stated that protein powder intake after exercise increased the erythrocyte and hemoglobin values.

Eslami et al. (2010) stated that protein powder intake had no effect on hematological parameters in young men after resistance exercise. Considering the studies on this field, it can be seen that there is not a complete consensus on how exercises affect blood parameters, and increase or decrease is seen after exercises. It can be stated that this difference is due to the form and time of the performed exercises and the amount of protein taken.

In addition, as a result of the conducted study, no difference was determined between the groups in terms of blood parameters related to protein powder use of the individuals doing resistance exercises. In within-group comparisons, there are parallel decreases and increases in many results. No distinct effect of the protein powder was found depending on the results of the obtained findings. If the amount of protein given per kilogram had been increased, perhaps different effects might have been observed. However, a large amount of protein was not given to protect the athletes' health.

As a result, we can say that protein supplementation after heavy exercise has no effect on blood parameters

Conflicts of interest

The authors declare no conflict of interest.

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