EFFECTS OF PROTEIN SUPPLEMENTS ON LIVER ENZYMES LEVELS IN ATHLETES

Lejla Čano Dedić
Department of Medical-Biochemical Diagnostics, Atrijum Sarajevo Polyclinic, Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina, lejla.cano.dedic@fzs.unsa.ba
Arzija Pašalić
Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina, arzija.pasalic@fzs.unsa.ba
Sabina Šegalo
Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina, sabina.segalo@fzs.unsa.ba
Nafija Serdarević
Clinical Center University of Sarajevo, Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina, nafija.serdarevic@fzs.unsa.ba
Amna Vefić
Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina, amna.vefic@fzs.unsa.ba

Abstract: Athletes have an increased physiological need for protein, which they satisfy by taking various synthetic supplements. Although numerous benefits of protein supplementation are known, the purpose of this study is to investigate whether serum levels of liver enzymes (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and lactate dehydrogenase) decrease in athletes after a seven-day break from protein intake. The study was designed as a cohort, longitudinal, and intervention study. It was conducted at the Department of Medical-Biochemical Diagnostics, Atrijum Sarajevo Polyclinic, after approval by the Ethics Committee of the Faculty of Health Studies and the health facility. In the study, 190 subjects were divided into groups based on their professional status, supplement use, training duration, and type of exercise. The personal data of the subjects were protected according to the principles of the Declaration of Helsinki. Spectrophotometric determination of liver enzymes was performed using a Mindray BS 480 biochemical analyzer. Descriptive and inferential statistical tests were used for statistical analysis, and the p-value was set at the conventional level of 0.05. According to the results of the study, the average age of the subjects in the control group was 27.9 years, while in the studied groups it was 34.8, 35.1, and 19.8 years, respectively. At the first measurement, the highest mean values of alanine aminotransferase (74.83 U/L), gamma-glutamyl transferase (26.43 U/L), and lactate dehydrogenase (421.56 U/L) were obtained in the group of subjects with low-intensity training and protein supplementation, and aspartate aminotransferase (56.83 U/L) in the group with high-intensity training and supplementation. After a seven-day break from supplementation, a greater decrease in alanine aminotransferase (57.96%), aspartate aminotransferase (59.58%), and lactate dehydrogenase (3.37%) was found in the group with high-intensity training. A greater decrease in gamma-glutamyl transferase (18.31%) was registered in the low-intensity training group. A statistically significant difference was found between the studied groups at the level of $p < 0.05$ for all studied variables. From the above results, it can be concluded that the use of protein supplements is an independent predictor of short-term liver load reflected in increased values of liver enzymes in athletes. A significant decrease in the levels of most liver enzymes after short-term interruption of supplementation indicates the onset of hepatocyte regeneration. Insufficient education about the importance of establishing a nutritional plan and an expert, individualized supplementation program for athletes based on laboratory test results is a major problem in our country. More research is needed to provide relevant and detailed information on the effects of protein supplementation not only on the liver but also on the entire metabolic system.
Keywords: protein supplements, training, liver enzymes, athletes

1. INTRODUCTION

Physical training is a stimulus that elicits numerous psycho-physiological responses in the organism, which are responsible for the adaptations of various organ systems (Malm et al., 2019). A properly programmed training stimulus causes a precise acute response of each organ system, while their repetition leads to chronic adaptation (Impellizzeri et al., 2019). Training and nutrition are closely related, because intense training causes increased metabolic, physical and psychological activity, so the energy requirements of athletes are higher than those of people who do not actively participate in sports (Bytomski J. R, 2018). The purpose of nutrition of athletes is to improve their strength and endurance, and it depends on the type of sport, gender, age, and dietary habits (Šatalić, Z. & Sorić, M. 2016). Athletes have an increased physiological need for protein to maintain adequate protein synthesis and energy production, as well as a sufficient immune function under conditions of increased stress due to frequent, intense, and prolonged training cycles (Jäger et al., 2017). The need for protein increases with increasing intensity and duration of athletic performance. Therefore, protein is consumed in meals before and after the performance and regularly throughout the day to ensure an efficient supply of essential amino acids (Thomas et al., 2016). To meet the specific nutritional needs of athletes, various synthetic supplements have been developed to be taken orally to fortify the diet and increase endurance, strength, and muscle mass, but with the proviso that they are not medicine (Maughan et al., 2018). Active athletes take supplements to meet increased nutritional needs, improve physical performance, increase strength and endurance, reduce recovery time, alleviate discomfort due to strenuous exercise, and improve rehydration (Maughan, R. & Burke, L. 2012). The most commonly used protein supplement is whey protein. It contains essential amino acids, β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin, and blood serum albumin. The advantage of whey protein over other proteins is its rapid absorption in the intestine, which ultimately leads to the synthesis of muscle-building proteins (Mansour et al., 2015). Ingestion of protein supplements increases the flux of amino acids into liver tissue, where catabolic conversion of amino acids into nitrogenous end products excreted from the body increases. This is caused by an increase in the activity of liver enzymes involved in these processes. In this way, excess amino acids are removed from the organism because, unlike fat and carbohydrate depots, the organism does not have a special system for depositing proteins (Tomé, D. & Bos, C. 2000). Recent research indicates that supplement use is widespread among professional athletes (Maughan et al., 2018; Garthe, I. & Maughan, MRJ. 2018; Larson-Meyer et al., 2018). According to the literature, the prevalence of supplement use is 59.8%, depending on age, gender, and sport (Shoshan, T., & Post, E. 2021). In recent years, numerous studies have examined the advantages and disadvantages of taking dietary supplements, which is a major challenge for researchers given the conflicting conclusions in the literature. A particular problem is the complex nature of unregulated supplement use, as well as the refusal of recognized clinical trials to provide data on the established safe dose of supplements, in that real-world cases, continue to emerge showing supplements being taken in excess of the recommended safe dose. Also, a major problem is the practice of taking multiple dietary supplements together, which increases the possibility and frequency of various adverse effects. The issue of protein supplementation in athletes has not been studied in Bosnia and Herzegovina. Due to the possible risks associated with the intake of protein supplements, we considered it important to conduct a study aimed at determining whether there is a significant difference between the enzyme levels of the studied groups and to prove whether the increased activities of liver enzymes in the serum of athletes are reduced after a seven-day break in the intake of protein supplements.

2. MATERIAL AND RESEARCH METHODS

The longitudinal cohort study was performed after obtaining approval from the Ethics Committee of the Faculty of Health Studies and Atrijum Sarajevo Polyclinic. In total, 190 male subjects with complete medical documentation and informed consent to participate were enrolled in the study. Participants' personal data were coded and protected in accordance with the principles of the Declaration of Helsinki. The subjects were divided into three studied groups according to the professional status of the athlete, use of dietary supplements, duration, and type of training. Group I, as a control group, consisted of healthy subjects (n=80) who do not use dietary supplements and are not in the process of training. Group II (n=80), consisting of athletes with low-intensity training, was divided into two equal subgroups: IIa participants who do not use protein supplements, and IIb athletes with supplements support. Group III consisted of professional athletes with high-intensity training using protein supplements (n=30). Low-intensity training was performed twice daily for 60 minutes and included strength and endurance training. High-intensity training was performed twice daily for 90 minutes and included strength, speed, plyometrics, endurance, and flexibility training. In both groups with supplementation, the prescribed dose of 30 grams of Whey protein for 28 days was taken half an hour before exercise and immediately after exercise. Laboratory determination of liver enzymes was performed in the Department of Medical-Biochemical Diagnostics of the Polyclinic Atrium Sarajevo. In all participants, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were determined. The sample for analysis was blood collected by venepuncture from the cubital vein into tubes containing separating gel. Transparent serum samples were obtained by centrifugation and prepared for spectrophotometric determination of liver enzymes on a Mindray biochemical analyzer BS-480. For groups IIb and III, enzyme determination was performed at two-time points, during supplement intake, and after a seven-day break. Data preparation and storage for statistical analysis were performed in Excel (Microsoft), and the software package used for data processing was IBM SPSS Statistics 20.0. Descriptive statistics tests were used to calculate means and standard deviations. The Kolmogorov-Smirnov and Shapiro-Wilkov tests were applied to test the normality of the distributions, and the Mann-Whitney U test to examine the statistically significant difference between the test groups. The p-value was set at the conventional level of 0.05.

3. RESULTS

The average age of subjects in the control group was 27.9 years, while in the groups IIa, IIb and III it was 34.8, 35.1, and 19.8 years, respectively. In the first laboratory determination of liver enzyme viability, the highest mean values were obtained for ALT (74.83 U/L) in group IIb and AST in athletes in group III (56.83 U/L). In subjects with low training and protein supplementation, the values of ALT were significantly double compared with the same group without supplementation, and a threefold increase was found compared with the control group. In the group of subjects with high-intensity training and protein supplementation, a double increase of AST was found compared to the subjects with low-intensity training without protein supplementation, and a significantly more than double increase was found compared to the control group. The highest mean values of GGT (26.43 U/L) and LDH (421.56 U/L) were found in group IIb, while the lowest GGT values (16.80 U/L) were found in the group with high-intensity training and supplementation. In the control group, ALT, AST, and LDH were the lowest (27.60 U/L, 21.46 U/L, and 337.40 U/L, respectively).

Table 1: Mean values of the tested parameters in the first laboratory test according to groups

Variables	Groups of respondents			
	Group I (Mean ± SD)	**Group IIa (Mean ± SD)**	**Group IIb (Mean ± SD)**	**Group III (Mean ± SD)**
AGE (YEARS)	27.92 ± 1.21	34.87 ± 1.55	35.10 ± 1.46	19.80 ± 1.27
ALT (U/L)	27.60 ± 8.62	31.22 ± 11.59	74.83 ± 26.60	68.37 ± 21.75
AST (U/L)	21.46 ± 5.85	26.83 ± 8.56	49.04 ± 10.82	56.83 ± 17.36
GGT (U/L)	20.89 ± 8.56	25.98 ± 8.94	26.43 ± 8.62	16.80 ± 7.26
LDH (U/L)	337.40 ± 60.16	370.59 ± 46.63	421.56 ± 28.18	395.57 ± 33.63

Table 2 shows a comparison of the mean values of the tested parameters at the first laboratory examination and after a seven-day break, with the percentage reductions of the individual liver enzymes indicated. In both groups, regardless of the type of training, a significant decrease in ALT and AST values was observed. For the above parameters, a decrease of more than 50% was found in the group with high-intensity training (57.96% and 59.58%). In about one fifth (18.31%) the decrease in GGT was registered in the low-intensity training group, while in the high-intensity training group about one tenth (10.54%). The percentage decrease in LDH enzymes was statistically lower (1.58% and 3.37%) than the decrease in aminotransferase levels in both tested groups.

Table 2: Comparison of the differences between the mean values of the liver enzymes after a seven-day break

Variables	Low-intensity training group			High-intensity training group		
	Values during supplementation (U/L)	**Values after a seven-day break (U/L)**	**Decrease in mean value (%)**	**Values during supplementation (U/L)**	**Values after a seven-day break (U/L)**	**Decrease in mean value (%)**
ALT	74.83	36.31	51.48	68.37	28.74	57.96
AST	49.04	32.06	34.62	56.83	22.97	59.58
GGT	26.43	21.59	18.31	16.80	15.03	10.54
LDH	421.56	414.92	1.58	395.57	382.24	3.37

The investigation of the existence of statistically significant differences in the values of the tested parameters between subjects with and without supplementation was performed using the Mann-Whitney U test. Statistically significant differences were found for ALT, AST, GGT and LDH at the level $p < 0.05$.

Table 3. Differences in liver enzyme levels compared with the use of dietary supplements

Variables	Groups	N	Mann-Whitney U test	Z	Asymp. Sig. (2-tailed)
ALT	Use supplements	70	88.00	-8.15	0.001
	Do not use supplements	40			
	Total	110			
AST	Use supplements	70	115.00	-7.985	0.000
	Do not use supplements	40			
	Total	110			
GGT	Use supplements	70	1016.50	-2.38	0.017
	Do not use supplements	40			
	Total	110			
LDH	Use supplements	70	729.500	-4.166	0.000
	Do not use supplements	40			
	Total	110			

4. DISCUSSION

Our study showed that subjects with protein supplementation had significantly higher levels of liver enzymes ALT, AST, GGT and LDH than the control group and subjects in the training process without supplementation. We investigated the effects of training on the value of liver enzymes in athletes training at low intensity and compared them with the values of athletes in the same category with protein supplementation in addition to training. The results showed that athletes of the same category who took protein supplements in addition to the training process had twice as high levels of ALT and AST enzymes compared to the group of subjects with low-intensity training who did not take protein supplements. Thus, we excluded the training factor as a cause of the elevated liver enzyme levels.

Comparison of our results with those of other studies that have evaluated the effects of protein supplement intake on liver enzymes is limited given the lack of similar studies. Most of the available studies are based on an experimental animal model. A study by Gürgen S. et al. (2015) included 30 rats divided into three groups: a control group, a short-term protein supplementation group (252 g/kg, 5 days), and a long-term protein supplementation group (252 g/kg, 4 weeks). In addition to hepatotoxicity markers (ALT and AST), apoptosis and inflammation markers were measured among study participants. The results showed that the long-term protein intake group had significantly higher ALT and AST activities (> 35%) compared to the other groups. Chen WC. et al. (2014) conducted a study with 40 rats divided into four groups. Two groups received Whey protein supplementation (4.1 g/kg body weight) for six weeks. The levels of AST, ALT, ALP, LDH, CK and bilirubin were monitored. It was demonstrated that the activities of AST, ALT and LDH were higher in the rats that received supplementation and exercise than in the group that did not use Whey protein. The study showed that there was a statistically significant difference between the tested parameters ALT ($p < 0.001$), AST ($p = 0.0342$) and LDH ($p < 0.0001$). The results of the two experiments are consistent with our results.

In a case study published by Whitt KN. et al. (2008), protein supplement intake was associated with liver damage in a healthy 27-year-old man who developed hepatic cholestasis due to prolonged intake of protein supplements. Pillai A. et al. (2015) showed in a case study that protein supplement intake was associated with elevated levels of liver enzymes ALT (1939 U/L) and AST (1737 U/L) in a 44-year-old man with a severe clinical presentation of toxic liver injury. In a 2015 study by Deminice R. et al., Whey protein intake in a non-exercise group resulted in increased ALT and AST levels as a sign of liver dysfunction. The results of our study showed that the increased activity of the tested enzymes in the serum of athletes decreased after a seven-day break in the intake of protein supplements. This area is relatively unexplored, and to our knowledge there are no studies that measure the activity of liver enzymes after a break in the intake of protein supplements.

5. CONCLUSIONS

Research findings suggest that protein supplement intake is an independent predictor of short-term hepatic load, reflected by increased levels of liver enzymes in athletes. After a seven-day break in protein supplement intake, a significant decrease in liver enzyme activity was observed, suggestive of hepatocyte regeneration. Insufficient education about the importance of creating a nutritional plan and an individual supplementation program for athletes, along with regular analysis of laboratory parameters, is a major problem in our country. Considering the positive effects of protein supplements found in the literature, we believe that the dose of supplementation should be individually adjusted to athletes and that it is partly responsible for the obtained results of laboratory tests. It is necessary to conduct more research to obtain relevant and detailed information about the effect of protein supplements not only on the liver but on the whole metabolic system.

REFERENCES

Bytomski J. R. (2018). Fueling for Performance. *Sports health*,*10*(1), 47–53. https://doi.org/10.1177/1941738117743913

Chen, W. C., Huang, W. C., Chiu, C. C., Chang, Y. K., & Huang, C. C. (2014). Whey protein improves exercise performance and biochemical profiles in trained mice. *Medicine and science in sports and exercise*, *46*(8), 1517–1524. https://doi.org/10.1249/MSS.0000000000000272

Deminice, R., Comparotto, H., & Jordao, A. A. (2015). Whey protein supplementation increases methionine intake but not homocysteine plasma concentration in rats. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, *40*(1), 46–50. https://doi.org/10.1139/apnm-2014-0175

Garthe, I., & Maughan, R. J. (2018). Athletes and Supplements: Prevalence and Perspectives. *International journal of sport nutrition and exercise metabolism*, *28*(2), 126–138. https://doi.org/10.1123/ijsnem.2017-0429

Gürgen, S. G., Yücel, A. T., Karakuş, A. Ç., Çeçen, D., Özen, G., & Koçtürk, S. (2015). Usage of whey protein may cause liver damage via inflammatory and apoptotic responses. *Human & experimental toxicology*, 34(7), 769–779. https://doi.org/10.1177/0960327114556787

Impellizzeri, F. M., Marcora, S. M., & Coutts, A. J. (2019). Internal and External Training Load: 15 Years On. *International journal of sports physiology and performance*, 14(2), 270–273. https://doi.org/10.1123/ijspp.2018-0935

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., Purpura, M., Ziegenfuss, T. N., Ferrando, A. A., Arent, S. M., Smith-Ryan, A. E., Stout, J. R., Arciero, P. J., Ormsbee, M. J., Taylor, L. W., Wilborn, C. D., Kalman, D. S., Kreider, R. B., Willoughby, D. S., … Antonio, J. (2017). International Society of Sports Nutrition Position Stand: protein and exercise. *Journal of the International Society of Sports Nutrition*, *14*(1). https://doi.org/10.1186/s12970-017-0177-8

Larson-Meyer, D. E., Woolf, K., & Burke, L. (2018). Assessment of nutrient status in athletes and the need for supplementation. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(2), 139–158. https://doi.org/10.1123/ijsnem.2017-0338

Malm, C., Jakobsson, J., & Isaksson, A. (2019). Physical Activity and Sports-Real Health Benefits: A Review with Insight into the Public Health of Sweden. *Sports (Basel, Switzerland)*, *7*(5), 127. https://doi.org/10.3390/sports7050127

Mansour, D. F. S., Nada, S. A., El-Denshary, E. S., Omara, E. A., Asaad, G. F., & Abdelrahman, R. F. (2015). Milk whey proteins modulate endotoxemia-induced hepatotoxicity in rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*, 65–71.

Maughan, R., & Burke, L. (2012). *Nutrition for Athletes, Nutrition Working Group of the International Olympic Committee*.

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., Rawson, E. S., Walsh, N. P., Garthe, I., Geyer, H., Meeusen, R., van Loon, L. J. C., Shirreffs, S. M., Spriet, L. L., Stuart, M., Vernec, A., Currell, K., Ali, V. M., Budgett, R. G. M., … Engebretsen, L. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *British Journal of Sports Medicine*, *52*(7), 439–455. https://doi.org/10.1136/bjsports-2018-099027

Pillai, A., & Thapar, M. (2015). Drug-Induced Liver Injury After Soy Protein Supplement Use. *ACG case reports journal*, *2*(3), 178–180. https://doi.org/10.14309/crj.2015.47

Shoshan, T., & Post, E. (2021). Prevalence of Protein and Pre-Workout Supplement Use among High School Football Players and Potential Product Contamination *Global pediatric health*, *8*, 2333794X211031202. https://doi.org/10.1177/2333794X211031202

Šatalić, Z., Sorić, M., & Mišigoj-Duraković, M. (2016). *Sportska prehrana*.

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the Academy of nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the Academy of Nutrition and Dietetics*, *116*(3), 501–528. https://doi.org/10.1016/j.jand.2015.12.006

Tomé, D., & Bos, C. (2000). Dietary protein and nitrogen utilization. *The Journal of nutrition*, *130*(7), 1868S–73S. https://doi.org/10.1093/jn/130.7.1868S

Whitt, K. N., Ward, S. C., Deniz, K., Liu, L., Odin, J. A., & Qin, L. (2008). Cholestatic liver injury associated with whey protein and creatine supplements. *Seminars in liver disease*, *28*(2), 226–231. https://doi.org/10.1055/s-2008-1073122