Progress in Nutrition 2018; Vol. 20, N. 3: 329-337 DOI: 10.23751/pn.v20i3.6689

Original Article

The acute effects of combined supplementation of beta-alanine, carbohydrate and whey protein on biochemical parameters of athletes after exhaustive exercise

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Summary. *Background:* The beneficial effect of popular supplements and use of combined supplementation in athletes which purpose to increase sports performance. *Objective:* This study aimed to review biochemical responses that the athletes gave to combined supplementation received after exercise and some changes in hematological values. *Material and Method:* 16 volunteers, in shape, male athletes with ages between 18-25 participated into the study. Athletes were divided into two groups as experiment (supplement) (n=8) and control (placebo) (n=8). After the exercise made until exhaustion (shuttle run test), beta-alanine/vitargo(carbohydrate-electrolyte)/whey protein supplement was given to the experimental group while the control group received placebo (water). Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after ingestion supplement (PS); Urea, Creatinine, Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gamma Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) values have been analyzed. *Results:* Statistically significant differences in many biochemical parameters were found when comparisons of in-group basal, PE and PS time courses of supplement and control groups were reviewed ($p<0.05$). When inter-group comparison of triglyceride and cholesterol levels were reviewed, a significant difference was seen in basal levels ($p<0.05$) no difference was detected in values other than that ($p>0.05$). *Conclusions:* It is possible to say that acute combined supplementation used after exercise does not create a negative effect on biochemical parameters of athletes, on the contrary when the research result data were compared with control group, by looking at the basal, exercise and after supplementation values such as creatinine, cholesterol, triglycerides, HDL and LDL, the combined supplement intake showed positive results in terms of health.

Key words: sports nutrition, nutritional health, ergogenic aids, dietary supplements, carbohydrate, electrolyte, protein, amino acids, biochemical changes, exercise

Introduction

In recent years, the beneficial effects of nutrition on exercise performance were clearly documented with researches (1). The first and most important nutritional needs of athletes is adequate amounts of food and a balanced diet to supply their energy consumption. Then for training, competition and a successful recovery, nutrition strategy should be determined (2). Besides when looking at the reasons of nutrition consumption of athletes; the most important ones are to be healthy, optimum body weight and composition, fast recovery after exercise and, of course, to provide fuel for energy throughout the exercise together with high exercise performance (1). But of many athletes throughout the world even in elite level; level of nutrition knowledge is very low not being very different from the general population. Ergogenic aids are being used to eliminate inefficiencies and needs of nutrition or to maintain adequate intake of certain nutrients (3).

Nutrition is an important element in the training program of athletes. It also holds an important place in the training period of many athletes. Pre and post training proper nutrition intake can supply the nutritional needs of athletes to a large extent. However, it may not be possible to supply the increased nutritional need with natural foods at challenging and intense match traffic. Though it is thought that exercise and sports training increase the nutritional needs in some of the athletes, appropriate calories with balanced diet can substantially meet the nutrient requirements. Despite, for a variety of reasons it is not possible for all athletes to meet the increased nutritional needs with the natural diets, and so the natural supplements referred to overcome the deficiencies will improve the performance (4). Exercise capacity of people, as a result of involved regular physical activity combined with the advancement of age, incurs individual losses and regresses. But the diet supplements increase the capacity of exercise, protect physical compliance and improves general health (5). Athletes who want to succeed, go down the path of performance increasing by using physiological, nutritional and pharmacological factors. All these factors that help the athletes in developing the performance are referred to as ergogenic aid. Ergogenic aid includes drugs as well as normal nutrients consumed by people (6). It is thought that ergogenic aid was to use to provide help to the level of the athlete's performance. It is seen that some ergogenic aids safely increase the performance (7). As a result; optimum processing of human muscle energy systems depends on a variety of diet nutrients (8).

A wide range of dietary supplements are produced for the use of athletes to improve their performance (9). In the studies, it was determined that the use of supplement starts at college and university level and athletes over age of 18 use supplement (4). According to the statistics, many national, international and Olympic level elite athletes often prefer the use of supplement. When looking at reasons to use supplement; it is emerging that it is used for upgrading the percentage of sports performance impact, enhancing energy, improving performance, preventing nutrition deficiencies, preventing diseases, increasing muscle mass and improving recovery (4). When looking at the most popular dietary supplement that athletes use, the most common intake is aminoacids. This distribution is seen as; protein/aminoacid, electrolyte and carbohydrate (10). When looking at the causes of use of supplement of athletes; reasons such as energy requirement and providing fuel during exercise, fluid and electrolyte balance, adaptation to special environmental conditions, physical activity, athletic performance, recovery after exercise, general health, body weight and composition come in the first place. It is very important that these products are safe in terms of production, effective, potential/strong and legal (1).

In the study beta-alanine, vitargo (carbohydrate-electrolyte) and whey protein supplements were used as nutritional support. Beta-alanine that some athletes use to improve performance and increase exhaustion threshold at the same time, was qualified by experts as a nutritional support that would be useful and needs raising awareness about (11). Carbohydrate that is important in terms of providing sufficient energy and repletion of muscle glycogen stores, is also an important nutrient support for general health (1). Whey protein that athletes usually use for muscle development, and increasing strength and performance is known to improve general health as well (12).

In the study presented; it was aimed to investigate the effects of acute combined supplementation on physiological responses and some hematological values of athletes.

Materials and Methods

Research group

In this study, 16 volunteers, in shape, male athletes between the ages of 18-25, participated into this study. Athletes were divided into two groups as experiment (supplement) (n=8) and control (placebo) (n=8). In athletes, requirements of being healthy, not having chronic or acute disease and not having any movement limitation depending on disability occurred for any reason were looked for. For this study, by Sinop University Human Research Ethics Board it was decided that there was no inconvenience ethically and it was found appropriate (Number: 57452775-604.01.02-E.).

Study design

In the study firstly some biochemical blood parameters were analyzed by taking basal blood sam-

ples prior to shuttle run and supplementation. Later 20-metre shuttle run was applied to athletes and right after the exercise done until exhaustion venous blood was again taken from the athletes for tests and then drink products were given to the supplement and control groups. After athletes consumed the drinks given to them under the supervision of researchers and in allotted time venous blood was taken for the last time for biochemical blood analysis two hours after consumption and some hematological parameters of athletes were analyzed after acute supplementation. Measurements and tests were made at the same physical conditions in both the supplement and control groups.

Blood measurements

Venous blood was taken from the athletes by expert nurses for hematological tests and analysed by biochemistry specialists. Biochemistry analysis was studied from the serum samples acquired by 3500 cycle/minute and 15 minute centrifuge speed of venous bloods at Abbott Architect c16000 biochemistry autoanalyzer. The upper phases were transferred to eppendorf tubes and kept at –80 °C until the use. Blood was taken from the athletes three times as basal, post-exercise (PE) and 2 hours after supplement ingestion (PS); Urea, Creatinine, Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gamma Glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) values were analyzed.

Supplementation

Immediately after 20-metre shuttle run test 16 athletes were randomly divided into two groups as supplement (n=8) and control (n=8) group. The study was conducted as a single blind application. Beta-alanine/vitargo(carbohydrate-electrolyte)/whey protein supplements were given to the experiment group in accordance with administration and daily dosage (with 1000ml water), an equal amount of placebo (water) to the given nutritional supplement was given to the control group. The supplementation was prepared beforehand and as a single dose that includes beta-alanine 3 g, vitargo (carbohydrate-electrolyte) 75 g and whey protein 30 g. The athletes were not informed about the substance given to them. So the psychological effects that may occur in athletes were removed and the study was conducted in more reliable conditions. In addition, the athletes were warned about not consuming any alcohol and stimulants one day before the test, caring the nutrition and resting.

Shuttle run test

In the study, 20-metre shuttle run test was applied to increase level of fatigue of athletes. This test that is used to measure aerobic capacity test is frequently preferred since it is a method that can be applied easily. In addition, 20-metre shuttle run test is a test that its validity and reliability to measure aerobic capacity is proven (13). They do not need to warm up before starting to the test. Because the 20-metre shuttle run test is a multistage test, first stages are in warming tempo (14). This test; is a test starting with 8.5 km/h and in every 1 minute running speed increases by 0.5 km/h, 20-metre distance is run as round trips (15). Running speed is controlled with a tape that beeps at regular intervals. The subject begins to run from the signal heard first and has to reach the other line until the second beep. And when the second beep is heard, returns to the starting line and these running signals continue. When the subject hears the signal, he sets the tempo himself to be on the other end of the runway at the second signal. If the subject misses a signal and catches the second, he continues to the test. The test is over when the subjects cannot catch the line 3 times before the beep or they quit running due to exhaustion (14, 16).

Statistical analyses

The research data obtained were given in the form of the standard error of the mean (M±SEM). We assessed the distribution of the analyzed variables using a Shapiro-Wilk test. The results showed that the distributions deviated from normal distribution. The Mann–Whitney U test was used to compare basal, PE, PS values between the two groups. A Friedman rank test was undertaken to evaluate the statistical differences in time for each parameter. When a significant F-value in Friedmans' analysis was found, a post-hoc test with a Bonferroni correction was used to determine the between-means differences. Statistical significance was accepted as $p<0.05$. In making of statistical analysis derived from the study and comparing the results SPSS v.22 package program was used.

Results

A statistically significant difference was found when serum urea level after exercise (PE) was compared to urea level after supplement (PS) in the supplement group ($p<0.05$). There was no statistically significant difference at any phase when serum urea levels were checked in the control group ($p>0.05$). And when inter-group comparisons of urea levels were considered, there was no significance at any phase ($p>0.05$) (Figure 1a). Statistical significance was found when creatinine values of supplement group were compared in all processes (basal with PE, basal and PS, PE and PS) ($p<0.05$). And in the control group while there was a significant difference between basal creatinine level and PE and PE and PS ($p<0.05$), there was no between basal and PS ($p>0.05$). When inter-group comparisons of creatinine levels were considered, no statistically significant difference was found at any phase ($p>0.05$) (Figure 1b).

A statistically significant difference was found when basal cholesterol was compared to PS cholesterol level and PE level to PS level in the supplement group ($p<0.05$). There was statistical difference between basal and PE and PE and PS phases when cholesterol levels of the control group was considered ($p<0.05$). When inter-group comparisons of cholesterol levels were considered, there was significance at basal level ($p<0.05$) (Figure 2a). Statistical significance was found when PE triglyceride levels of supplement group was compared to PS level ($p<0.05$). When looking at the control group also there was a significant difference between the PE and PS levels ($p<0.05$). When inter-group comparisons of triglyceride levels were considered, there was significance difference at basal level ($p<0.05$) (Figure 2b).

There was statistical difference in both groups between basal and PE and PE and PS phases when HDL levels of the supplement and control group were considered ($p<0.05$). When inter-group comparisons of HDL levels were considered, there was no significance at any phase ($p>0.05$) (Figure 3a). A significant difference was detected only between PE and PS when levels of LDL of the supplement and control groups were compared at all phases ($p<0.05$) In inter-group comparisons of LDL levels, there was no statistical significance ($p>0.05$) (Figure 3b).

A statistically significant difference was found between basal and PE and PE and PS phases when GGT levels of supplement group were examined ($p<0.05$). There was no statistically significant difference at any

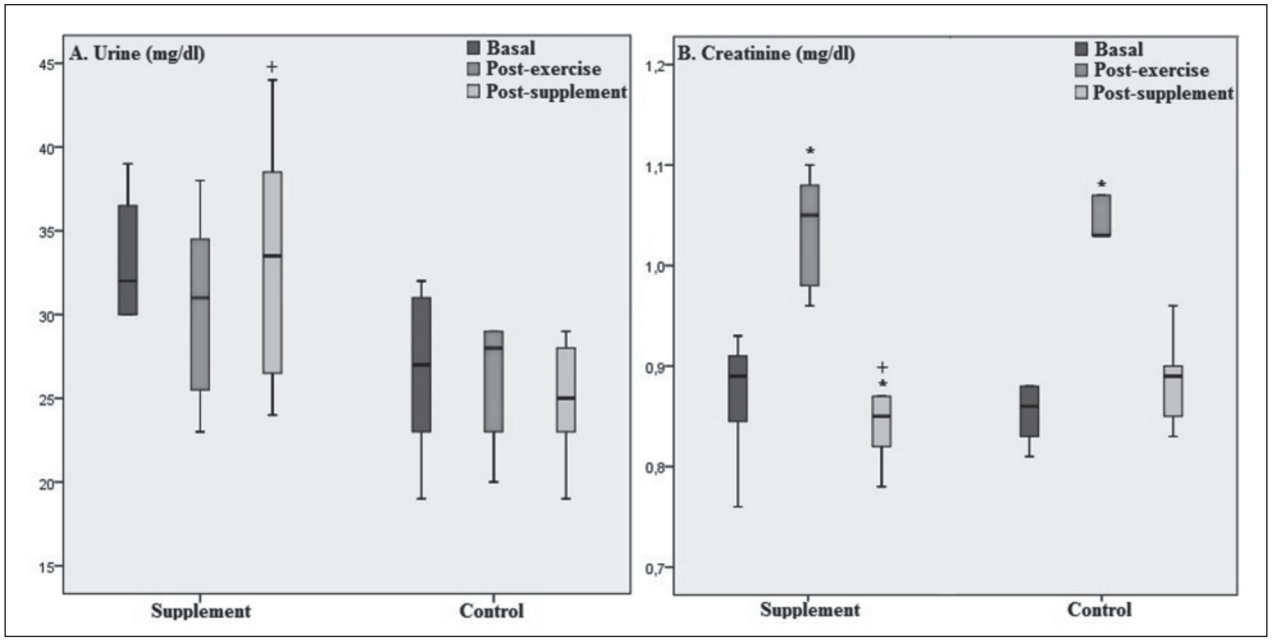

Figure 1. Changes in the serum urine (mg/dl) and creatinine (mg/dl). *Significant difference compared with basal ($p<0.05$). + Significant difference compared with post-exercise ($p<0.05$). #Significantly different between supplement and control groups ($p<0.05$) (M±SEM).

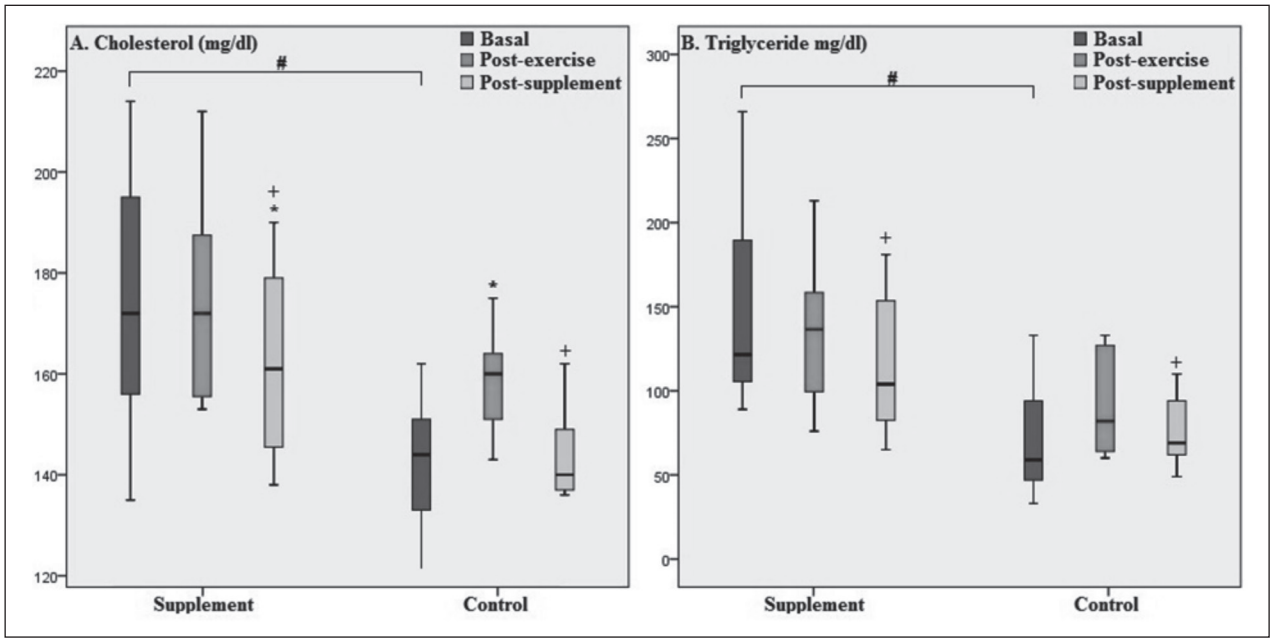

Figure 2. Changes in the serum cholesterol (mg/dl) and triglyceride (mg/dl). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

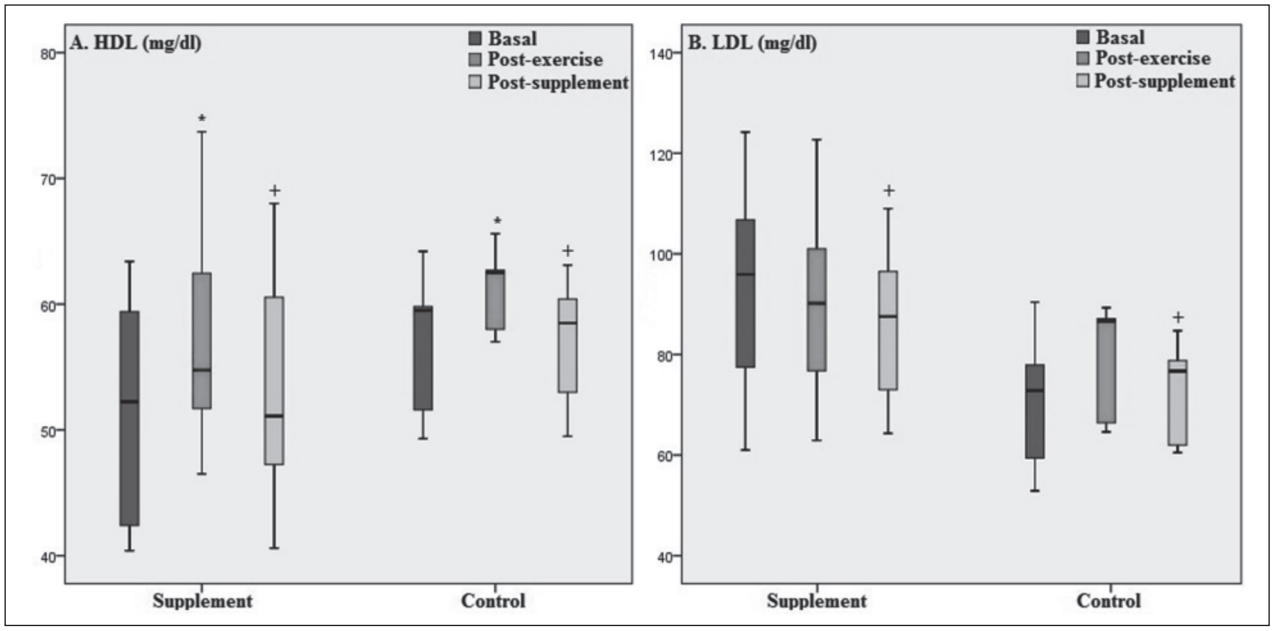

Figure 3. Changes in the serum HDL (mg/dl) and LDL (mg/dl). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

phase when serum GGT levels of control group were considered (p>0.05)(Figure 4a). In addition, when ALP values of both the supplement and control group were compared in groups statistical significance was found in all phases (basal with PE, basal with PS, PE and PS) (p<0.05). When inter-group comparisons were considered, no statistically significant difference was found at any phase (p>0.05) (Figure 4b).

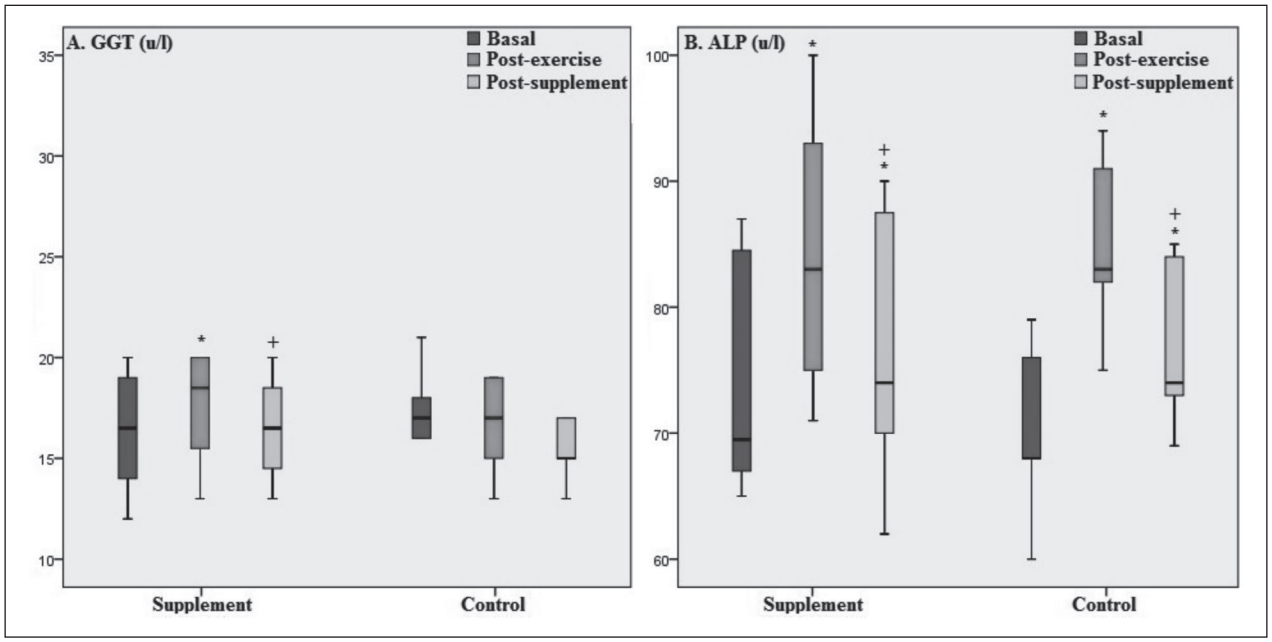

Figure 4. Changes in the serum GGT (u/l) and ALP (u/l). *Significant difference compared with basal (p<0.05). + Significant difference compared with post-exercise (p<0.05). #Significantly different between supplement and control groups (p<0.05) (M±SEM).

Discussion and Conclusions

All athletes need body fuel, liquid and nutrition to bring their performance levels to the best. Roles of sports nutritionist here are, choosing products that would protect general health and make it fine while recommending proper supplements needed before, during and after the training (1). Because health has a place like 16% when popular reasons of supplement usage are examined (17). Also, there are many conditions that nutritional supplements play very important role for health and performance (18). While this is the case, supplements athletes use to improve their performance should create no threat in terms of health. On the contrary, the expectations of athletes from supplements consumed to enhance performance are to also upgrade general health.

In the study presented; acute combined beta-alanine, vitargo (carbohydrate-electrolyte) and whey protein supplementation was made to the athletes after the exercise made until exhaustion. When physiological responses and hematological values received after supplementation were considered; biochemical changes were within the body's normal level ranges after intake of the supplement, while creating no risk in terms of metabolic balance, positive results in terms of overall health have emerged in some biochemical values in the group taking nutritional supplement.

In a study, it was established that 88% of college athletes in America are using at least one natural supplement, 58% of them are using 2 or more supplements (4). The contents and the effects of consumed supplements on metabolism are usually evident. However the use of 2 or more supplements which is quite common in athletes, brings the question of what impact would they do on metabolism when supplement are used in combination.

Many combined supplements of which their effects are proved in a scientific way are offered to consumers. Competition, effort to be superior and taking action for maximizing personal potential of athletes together with increased awareness about nutrition preferences to effect athletic performance caused an increase in combination of supplements that boost sportive performance. And manufacturers produce these products usually thinking that combined products would benefit more than a single product. Besides, the efficiencies of very few combined products given to athletes are proven scientifically (19).

In the results obtained from the study, it was seen that similar results were present when the basal, after exercise and after supplement hematological values of the supplement and control groups were compared. This circumstance shows that the acute supplementation does not cause a noxious change in biochemical values of athletes. Besides, the results suggest that rise and fall in hematological values are physiological effects of the workout. In addition, it was shown that supplementation could lead to positive results in terms of health when basal, training and post supplement creatinine, cholesterol, triglyceride, HDL and LDL values were compared between the two groups. These positive changes revealed that the combined supplements consumed with a good timing after the training brought the metabolism from the catabolic state to the anabolic state. If rise in sportive performance and a rapid recovery after exercise are added to besides these results, the net effect of conscious use of supplement on the athletes' life will be clearly seen.

It is already known that intake of important nutrients before, during and after exercise improves health, performance and recovery (20, 21). From a literary perspective; while some studies have found that the use of combined supplements has a positive effect on athlete's health and performance (22, 23, 24), the results of some studies suggest that combined supplementation does not benefit performance or metabolism (25, 26). When looking at other acute and chronic supplementation studies in literature; in the study Flakoll et al. (2004) made with U.S. Marine recruits, they gave protein/carbohydrate/fat to the experiment group, carbohydrate/fat to the control group and none to the placebo group for 54 days right after exercise. According to the emerging results, when three groups were compared, in the experimental group consuming the combination supplement a 33% reduction in referring to health facilities for general reasons, a 28% decrease in referring for bacterial / viral infections, a 37% decrease in referring for muscle and joint problems and a 83% reduction in referring for heat exhaustion. Flakoll et al. (2004) reported that combined supplementation improved overall health, reduced muscle pain, and had positive results on tissue hydration according to the emerging results.(27). Jialal and Grundy (1993) gave combined α-tocopherol, ascorbate, and beta-carotene supplements to 12 male subjects in their study and examined LDL changes in metabolism. In the obtained data, it was found that supplement intake had a blocking role on LDL. In the study done with rats, Maxwell et al. (2001) supplemented rats with L-arginine with drinking water for 4-8 weeks and measured their cholesterol levels. The researchers found that the supplements of L-arginine had no effect on the cholesterol levels of rats (29). In the study done with rats, Suzuki (2009) exercised the rats and performed L-arginine / L-ornithine loading. As a result of the study, supplementation facilitated the development of blood vessels in the feet of the rats, which is an additional effect in exercise; led to the development of capillary vessels in the sole and soleus muscles of both feet (30). In the study Jang et al. (2011) made with 9 training wrestlers, they divided wrestlers into three groups as placebo, carbohydrate and carbohydrate+BCAA+arginine and looked at some biochemical parameters. In the obtained data, they found an increase in glucose and insulin levels, decrease in glycerol, and non-esterified fatty acid concentration in carbohydrate and carbohydrate+BCAA+arginine groups according to the placebo group (31). In the study El-Kirsh et al. (2011) did with rats, they investigated the effect of combined L-arginine or L-citrulline supplements on biochemical parameters taken with high-fat and cholesterol diets. In the study subjects were divided into 6 groups. Those groups were determined as; group 1-control, group 2-basal diet+L-arginine, group 3-basal diet+L-citrulline, group 4-high fat and cholesterol diet (HFC), group 5-HFC diet+L-arginine, group 6-HFC diet+L-citrulline. They found that the combined supplementation caused a significant decrease in ALT and AST enzymes and stated that the supplementation had a protective effect for metabolism. In the study, there was significant increase in urea levels of group 3 and group 4 compared to that of group 1 and group 2, significant decline in urea levels of group 5 and group 6 compared to group 3 and group 4. Researchers explained this situation as that the supplement does not generate any damage in the kidney. In addition, the study shows that high-fat and cholesterol diet resulted in a significant decrease in HDL cholesterol, but caused the increase of all the other lipid parameters when compared with a control group. In

the study, biochemical parameters such as total cholesterol, creatinine, LDL cholesterol and HDL cholesterol of the subjects were maintained and in this results cholesterol, creatinine, LDL cholesterol values of subjects using supplement in high fat and cholesterol diet were found low and HDL cholesterol was found high. These results showed that supplementation caused in reduction of serum lipid profile and brought about the rise in HDL cholesterol. In addition, it showed the supplements prevented the damage that high fat and cholesterol diets give to the body (32).

As a result, it is possible to say that acute combined supplementation used after the exercise does not create a negative effect on biochemical parameters of athletes, on the contrary when the research result data were compared with control group, by looking at the basal, exercise and after supplementation values such as creatinine, cholesterol, triglycerides, HDL and LDL, the combined supplement intake showed positive results in terms of health. In addition, it showed that the combined supplements consumed consciously and with a good timing after the training could bring the metabolism from the catabolic state to the anabolic state and these generated biochemical responses would have positive effects on athletic performance and recovery. In the future works on the subject; a controlled lifestyle that can be created on athletes, chronic supplementation with a regular nutritional program and watching the changes in different biochemical mechanisms may reveal more clear information in terms of the result of the study.

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