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Effect of arginine supplementation on footballers' anaerobic performance and recovery

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Summary. *Background:* The use of supplements has increased in recent years. L-arginine is popular supplement in athletes and this supplement stimulates nitric oxide which purpose to increase sports performance. *Aims:* This study aims to determine the effects of L-arginine supplement on anaerobic performance and recovery. *Materials and Methods:* 28 male active football players who play in amateur leagues, get training regularly, between the ages of 18 and 30 participated the study. The subjects were randomly assigned to experimental and placebo group. During 14 days, experimental group consumed 6 grams of arginine and the placebo group consumed 6 grams of wheat bran. Both before and after the supplementation anthropometric, biochemical and anaerobic capacity levels were measured. In order to search recovery after the anaerobic test, the level of Lactic Acid (LA) and Heart Rate (HR) were observed up until the 10th minute of recovery. *Results:* The research results suggest that after supplementation, the experimental group's body mass index (BMI) decreased compared to pre-supplementation (Pre= 23,60±2,28kg/m^2 vs. Post= 23,39±2,12kg/m^2)($p<0.05$). On the other hand, the anaerobic performance measurements of both groups do not demonstrate any statistically significant difference before and after supplementation. The post supplementation recovery LA levels showed more rapid reduction from 5th min up to 10th min in experimental group. After the supplementation, 1st minute recovery HR levels were lower than pre in both groups but the experimental group experienced a higher decrease than placebo group. This suggests that suplementation of arginine helps to excrete LA from the body. The comparison of the HR values measured before and after the 14 day long supplementation period shows that both the experimental and the placebo groups experienced a decrease in the resting HR values as well as the HR values during the 1st minute of recovery. The experimental group experienced a higher decrease. After 14 day supplementation Aspartat Aminotransferaz (AST), Alanin Aminotransferaz ALT and Laktat Dehidrogenaz (LDH) (LDH; Pre= 229,41±47,23 vs. Post= 176.08±45.62) levels significantly decreased compared to the pre-supplementation in arginine group ($p<0.05$). *Conclusion:* Consequently, the findings suggest that supplementation of L-Arginine accelerates the excretion of lactic acid from the body and decreases the amount of fat in the body. It also rapidly recovers the muscle injuries caused by the decrease in LDH enzyme levels after training and has a positive impact on anaerobic performance. Finally, it accelerates recovery.

Key words: Footballer, L-Arginine, anaerobic performance, lactic acid, recovery

Introduction

In the last two decades an increasing interest can be observed for the matter of athlete's diet which is supported by research into biochemical and physiological aspects of training (1). Athlete cannot meet their increasing needs for nutrition through only organic diet. Therefore, natural supplements are assumed to satisfy their needs (2).

After strength training, active muscle fibers are damaged. It is proved, however, that a balanced and regular intake of protein reduces damage to muscles and it prevents muscle weakness which decreases physical power. It also improves recovery after strength training. Besides, supplementing a diet with protein is necessary for the improvement and recovery of muscles since protein has a key role in balancing anabolic hormones (3).

When training gets more intensive and it exceeds a certain limit, aerobic mechanism is unable to produce sufficient energy and anaerobic mechanism takes control (4). Anaerobic threshold is defined as the point at which lactate starts to accumulate in the blood stream (about 4 mmol) (5). Concentration of lactate in muscles and blood appears as a function of the intensity of training (6). The amount of lactate in the blood increases at an accelerating rate due to intense training (7). Since Lactic Acid (LA) is one of the metabolites that causes fatigue, the accumulation of it in muscles will decrease training performance (1). The body can recover by renewing the energy burnt and removing LA which has accumulated in the body (8).

One of the recent supplements; L-arginine triggers vasodilation because it increases the production of Nitric Oxide (NO) in muscles during training. When blood flow increases due to vasodilation, active tissues are supplied with a large amount of nutrition and oxygen. Similarly, increasing blood flow raises protein synthesis and eases the recovery of muscles (9). L-arginine is a semi-essential amino acid and it is a basic source for the production of NO (10). NO is a gas molecule which emits high signals and it is synthesized by NO synthase -a calcium dependent enzyme which has a low molecular weight- , L-arginine and L-citrulline. NO plays a crucial role in regulation of blood flow and blood pressure during training and in relaxing skeletal muscles (11,12). Among many roles of NO, two of them directly affect training performance; these are balancing vasodilation in cardiac and skeletal muscles and oxygen consumption (13). Little et al. (14) reported that L-Arginine is a supplement which improves training performance. It has been observed that it increases maximal strength and repeated sprint performance and improves physical endurance.

Effects of arginine on anaerobic performance and recovery were studied by different designs. But, this study is the only one investigating the impact of arjinin on anaerobic performance and recovery by a different test, which was Running Anaerobic Sprint Test (RAST). The aim of this study is to examine the effects of oral arginine supplement -which is given in daily amount of 6 grams during 14 days- on anaerobic performance and recovery.

Materials and Methods

Research group

The study was carried out with 28 male footballers between the ages of 18 and 30 who volunteered to take part in the study. They currently play football in amateur leagues. They are active football players who get training regularly. The footballers were assigned to one of two groups: experimental/arginine (n=14) and placebo (n=14). The subjects were chosen on condition that they were healthy, had no chronic diseases or movement disorder caused by any sort of reason. In order to increase the reliability of the study, it was conducted with only one football team and all the subjects were chosen from the same football team considering that different teams might have different training programs which would affect the study negatively. The study was approved and granted permission by Ondokuz Mayıs University, Clicnical Research Center, Ethical Foundation (Number: B.30.2.ODM.0.20.08/211).

Study Design

Before supplementation, the subjects' blood samples were analyzed to determine some biochemical parameters. Their height, weight and anaerobic capacity values were measured through RAST. Besides, in order to examine recovery after RAST, their LA levels and Heart Rate (HR)s were measured. After these measurements, the experimental group consumed arginine, and the placebo group consumed wheat bran. After this 14 day period, the measurements made at the beginning of the study were repeated. In other words, each footballer who took part in the study were measured twice (before and after supplementation). The measurements were made under the same physical conditions.

Performance Testing and Biochemical Analyses

Height and Weight
In the study, subjects' height and weight were measured through a device (Seca) and their body mass indexes (BMI) were calculated.

Blood Measurements

Nurses drew five ml of venous blood from footballers and it was analysed by biochemical specialists. During the study, subjects' urea, creatinine, cholesterol, triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Gama Glutamil Transferaz (GGT), Alanin Aminotransferaz (ALT), Aspartat Aminotransferaz (AST), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) levels were analysed.

Running Anaerobic Sprint Test (RAST)

To measure the anaerobic capacity RAST test was done by using a New Test-Power Timer 1.9.5. (Newtest, Oulu, Finland). Footballers were asked to warm up before the test so that they can get mentally and physically ready and they were given 15 minutes to do so. During the RAST test, each footballer made six consecutive 35 meter sprints by giving 10 second breaks after each sprint. After a footballer started the test by making the first sprint and gave the first 10 second break, Power Timer device beeped and the footballer made the second sprint. The test was completed after six consecutive sprints were made in the same way.

Blood Lactate Test

For the lactate test, a lactate analyzer (Lactate Scout) was used. In order to meaure the LA level, blood samples were taken from ear lobe. Each time blood was taken for measurements, needles and test strips were changed. By measuring resting LA levels and LA levels in the 1st, 5th and 10th minutes after the RAST test, subjects' LA levels during recovery were determined.

Heart Rate (HR)

Polar watch (RS 800) to measure HR was used. A transmitter was placed in the chest and HR in the 1st, 3rd, 6th and the 9th minutes after the RAST test were determined.

Supplementation

One day after the pre-tests, 28 participants were randomly divided into two groups as arginine and placebo. The study was conducted as single blind.

The experimental group was given L-arginine supplement and the placebo group was given wheat bran during 14 days under the supervision of the researcher. They consumed three grams of supplement before training (one gram before breakfast and two grams 30 minutes before training) and three grams after training (two grams one hour after training and one gram before sleep). During the rest days, footballers were given three grams (two grams before breakfast and one gram before sleep) of their assigned supplements.

It was only known by the researcher and the person who made the measurements which group consumed which supplement. Therefore, psychological influences were eliminated and the study was carried out in a reliable atmosphere. Footballers were given explanations about their diets and asked to carry on with their daily routines and existing training programs.

One day after the 14 day period, biochemical tests, antropometric measurements and the RAST test were repeated.

Statistical Analyses

All values are expressed as means ± standard deviation. SPSS 19.0 version was used for the statistical analysis. Data sets were normally distributed. Values betwen pre and post supplementation were compared by paired sample t test.

Results

As a result of the study, we look at the statistical analysis of the results of tests and measurements, in terms of body weight, BMI, lactate, HR, biochemical test data and RAST values significant differences were found in some parameters, while no significant difference was brought out by some values.

Supplementation of arginine caused a decline in weight and BMI levels. Pre and post BMI levels showed statistically significant differences in arginine group (p<0,05). No such differences were recorded, however, in the BMI measurements of the placebo group (p>0,05).

The pre and post supplementation anaerobic performance values of neither group show any statistically significant differences (p>0,05).

In table 3, pre and post supplementation LA values were given. Both the resting lactate levels of placebo and arginine groups demonstrated a decrease after supplementation but only the placebo group showed a significant difference (p<0,01). Post-suplementation 1th min recovery lactate values were higher than pre-suplementation values in both groups (p<0,05).

Before suplementation of arginine, 5th min of recovery LA value was 10,67±2,83 mmol/L, and 10th min value was increased to 10,99±1,67mmol/L in experimental group. After 14 day-long supplementation of arginine, however, 5th min of recovery LA value was 12,07±2,92. Whereas, 10th min value was decreased to 11,23±1,97.

Before supplementation, the LA value in the 5th min was 9,66±3,38, it increased to 10,5±2,09 in the

Table 1. Physical features of subjects

			Arginine Group		Placebo Group	
Variable	**Period**	**n**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
Weight (kg)	Pre- supplementation	14	71,85	9,34	74,71	10,10
	Post-supplementation	14	71,26	8,67	74,77	10,00
BMI (kg/m²)	Pre-supplementation	14	23,60 ♦ (p=0,031)	2,28	24,22	2,32
	Post-supplementation	14	23,39 ♦ (p=0,031)	2,12	24,31	2,40

p<0,05 ♦

Table 2. Anaerobic performance values before and after supplementation

RAST Values			Arginine Group		Placebo Group	
Analysis	**Period**	**n**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Peak Power (W)	Pre-supplementation	14	360,80	83,32	388,87	69,67
	Post-supplementation	14	375,04	68,82	403,95	48,99
Minimum Power (W)	Pre-supplementation	14	286,56	70,31	287,85	55,83
	Post-supplementation	14	285,88	65,58	294,80	44,64
Average Power (W)	Pre-supplementation	14	319,01	69,67	341,05	61,69
	Post-supplementation	14	333,95	60,84	348,12	43,56
Fatigue Index Average (W/s)	Pre-supplementation	14	40,65	2,97	38,40	3,58
	Post-supplementation	14	39,06	3,65	38,10	4,77

Table 3. Comparison of LA values between before and after supplementation

	ARGININE (n=14)				PLACEBO (n=14)			
		Recovery				Recovery		
LA(mmol/L)	Resting	Minute 1	Minute 5	Minute 10	Resting	Minute 1	Minute 5	Minute 10
Pre supplementation	2,32±,56	6,88±1,77	10,67±2,83	10,99±1,67	2,23±,71	5,53±2,37	9,66±3,38	10,5±2,09
Post supplementation	2,15±,93	8,95±3,42	12,07±2,92	11,23±1,97	1,72±,43 (p=,007)	8,28±3,07 (p=,046)	11,41±2,26	12,72±2,78
P	0.526	0.020 ♦	0.219	0.681	0.007 ♦♦	0.046 ♦	0.053	0.073

p<0,05 ♦ *p<0,01* ♦♦

10th min of recoveryin the placebo group. After 14 days, the LA value in the 5th min was 11,41±2,26 and it increased to 12,72±2,78 in the 10th min.

Table 4 shows the recovery HR values before and after 14 day-long supplementation. It is observed that the 1th min of recovery and resting HR levels of the arginine group decreased after supplementation ($p<0,05$ and $p<0.01$). Placebo group also showed a decrease after supplementation in their resting HR and HR level in the 1th min after training and a significant difference was recorded ($p<0,05$). Other HR findings of arginine and placebo groups did not show a significant difference ($p>0,05$).

AST, ALT and LDH values of the footballers who received arginine supplementation were found lower, Triglyceride value was found higher when compared with pre-supplementation values ($p<0,05$, $p<0.01$ and $p<0.001$). As for the placebo group, a decrease was found in the alkaline phosphatase values and a statistical difference was found ($p<0,05$). No statistically significant difference was found in other findings ($p>0,05$).

Discussion and Conclusions

The purpose of this study was to examine the effect of oral arginine supplementation on anaerobic performance and recovery. In the study, arginine supplementation caused a decrease in body weight and BMI values. L-arginine supplementation may also be effective on fat loss. No such difference was found in the placebo group. Researching the reason of this reduction in BMI, it is thought that arginine, which may have acute effect, provides the body fat's usage of energy resource by increasing the lipolysis level in metabolism. In their study with the footballers, Angeli et al. (15) gave daily 1 gram vitamine C supplementation and 3 gram arginine to the experimental group and 1 gram vitamine C to the control group. In the arginine group, at the end of 8 week-long weight training, the researchers found an increase in body weight and muscle mass and a decrease in body fat percentage. In their study with athletes, Burtscher et al. (16) gave L-arginine L-aspartate in saccharose to the experimental group and only saccharose to the placebo group during 3-weeks exercise. No physical feature difference was found between two groups at the end of the 3-weeks long supplementation. The results of Angeli et al. (15)'s study is similar to our study in terms of its results.

In our study, a significant difference was found in the 1st minute post-training lactate measurements of both groups; however, this difference was in the form of increase in the lactate levels just after exercise when compared with the pre-test findings. The reasons for this may be the content and intensity of the training before the second (post) test, too many anaerobic training methods in the exercises or the high difficulty level of the game played that week.

In our study, post exercise 1st, 5th and 10th minute LA levels were measured in order to track recovery in pre and post supplementation period. In the arginine group, LA levels of the 10th minute were higher than those of the 5th minute in the pre supplementation period while LA levels of the 10th minute were lower than those of the 5th minute in the post supplemen-

Table 4. Comparison of HR values between before and after supplementation

	ARGININE (n=14)					PLACEBO (n=14)				
		Recovery					Recovery			
HR values (heartbeat/min)	**Resting**	**Minute 1**	**Minute 3**	**Minute 6**	**Minute 9**	**Resting**	**Minute 1**	**Minute 3**	**Minute 6**	**Minute 9**
Before supplementation	74,33±9,31	176,50±6,47	104,16±10,70	99,83±9,54	96,58±8,99	79,33±11,73	173,22±11,44	112,55±10,80	106,11±12,11	104,44±12,99
After supplementation	67,66±12,35	169,25±10,73	104,41±8,07	98,00±8,22	97,66±8,40	74,77±12,44	167,44±13,00	108,55±13,21	100,00±13,84	100,33±13,67
P	0.037 ♦	0.008 ♦♦	0.920	0.448	0.553	0.036 ♦	0.025 ♦	0.241	0.083	0.315

p<0,05 ♦ *p<0,01* ♦♦

Table 5. Comparison of Biochemical values between before and after supplementation

	ARGININE (n=14)										
Biochemical tests Mg/dL - U/L	**Urine**	**Creatinine**	**Cholesterol**	**Triglyceride**	**HDL Cholesterol**	**LDL Colesterol**	**AST**	**ALT**	**GGT**	**Alkaline Phosthapase**	**LDH**
Before supplementation	29,81±6,17	0,88±,09	159,41±37,19	65,08±21,26	47,58±11,75	98,81±29,76	36,66±13,29	24,25±8,75	17,83±6,43	84,25±30,86	229,41±47,23
After supplementation	27,58±7,09	0,88±,08	165,25±38,56	92,00±28,43	46,33±9,64	99,91±30,34	21,33±3,72	17,75±8,00	16,91±6,52	83,83±33,46	176,08±45,62
P	0.273	1.000	0.293	0.003 ♦♦	0.292	0.802	0.001 ♦♦♦	0.017 ♦	0.231	0.775	0.000 ♦♦♦
	PLACEBO (n=14)										
Biochemical tests Mg/dL - U/L	**Urine**	**Creatinine**	**Cholesterol**	**Triglyceride**	**HDL Cholesterol**	**LDL Colesterol**	**AST**	**ALT**	**GGT**	**Alkaline Phosthapase**	**LDH**
Before supplementation	25,68±4,75	0,87±,09	143,55±18,35	77,88±17,57	41,44±4,53	86,53±16,88	25,66±5,36	25,22±11,34	17,11±3,88	107,77±51,67	184,33±27,05
After supplementation	25,55±4,38	0,93±,13	141,33±19,35	91,44±33,84	41,00±3,77	82,04±15,56	23,22±4,65	22,66±13,36	16,55±4,06	102,00±51,86	167,11±28,42
P	0.896	0.212	0.652	0.285	0.525	0.292	0.338	0.331	0.247	0.020 ♦	0.068

p<0,05 ♦ *p<0,01* ♦♦ *p<0,001* ♦♦♦

tation. However, this result was not the same for the placebo group. In the placebo group, LA levels of the 10th minute of recovery were higher than those of the 5th minute in both pre and post supplementation. In the placebo group, LA level was found to increase and thus, LA removal was found to be slow. In short, post arginine supplementation lactate levels of the experimental group were found to decrease faster. This result shows that arginine supplementation accelerates the removal of LA from the body and improves recovery.

In their study with 30 body builders, Imanipour et al. (17) gave arginine to the first of the experimental groups, BCAA (branched-chain amino acid) to the second experimental group and they did not give any substance to the control group. At the end of the 6-week long training, statistical test results showed that although basal levels of lactate in the arginine supplementation group had decreased slightly after 42 days supplementation, the difference was not statistically significant. Muazzezzaneh et al. (18) grouped male athletes in two as the experimental and placebo group. They gave daily 5 grams of arginine to the experimental group and daily 5 grams of wheat supplementation to the placebo group for 21 days. As a result of their study, they did not find a difference between the pre-training measurements of two group while the reduction of LA after exercise has been found to be more in arginine group than placebo. Wilkerson et al. (19) gave NG-nitro-L-arginine methyl ester (L-NAME) supplementation to the healthy male volunteers and although the blood lactate levels of the L-NAME supplementation group were found to be higher than those of the control group at the beginning of the exercise, there was no significant post exercise difference between them. In L-NAME group, blood lactate accumulation showed significant decrease. All these studies support the findings of our study and they also show that arginine supplementation accelerates the removal of LA.

In our study, HR levels were analyzed to track post anaerobic exercise recovery. Post supplementation rested and 1.min of recovery HR levels of both groups were found to decrease when compared with pre supplementation ($p<0,05$ and $p<0.01$). Similar characteristics of groups that have the same primary phase and the same training program bring to mind that arginine supplementation does not have any effect on HR. However, when the results were analyzed, it can be seen that the decrease in the post supplementation HRs of footballers who used arginine when compared with the pre supplementation were more obvious when compared with the placebo group; thus, recovery occured faster. It is thought that this decrease in the post arginine supplementation HR will also be effective on recovery. Willoughby et al. (20) gave daily 12 grams of arginine to 24 male athletes for 7 days. According to their results, post supplementation HRs of the experimental group just after exercise were found to be lower than those of the pre supplementation while an increase was found in the placebo group. In their study with 16 athletes, Burtscher et al. (16) gave L-arginine L-aspartate in saccharose (daily 3 grams) to the experimental group during 3-week exercise and only saccharose to the placebo group. As a result of their study, Burtscher et al. (16) found a decrease in the HRs of the L-arginine L-aspartate group and they found a statistically significant difference. These studies bring to mind the idea that L-arginine supplementation will improve performance and recovery.

Post arginine supplementation AST, ALT and LDH values were found lower, Triglyceride value was found higher when compared with pre-supplementation ($p<0,05$, $p<0.01$ and $p<0.001$). As for the placebo group, a decrease was found in the ALP values and a statistical difference was found ($p<0,05$). No statistically significant difference was found in other findings ($p>0,05$). AST enzyme, which was over the normal value range before supplementation in footballers who used arginine, got back to normal value range after supplementation. This result brings to mind that arginine use does not cause a disadvantage on liver enzymes and moreover it heals the enzymes. In addition, a decrease was seen in LDH -an intracellular enzyme responsible for the conversion of pyruvate to lactate- that increases as a result of muscle damage after intense and extreme exercise due to arginine supplementation. This decrease in LDH enzyme levels will accelerate recovery since it heals the muscle damage fast. LA causes intracellular acid and thus, the cell will be damaged. Thus, a fast decrease in LDH enzyme is important for recovery and renewal. Cells should become alkaline instead of acidic. These enzymes, which were critically high in the arginine group before supplementation, decreased after supplementation and became normal. As a result of the decrease in LDH enzyme values caused by argininie supplementation, the muscle damage that occurs after intense exercise will be healed and accelerate recovery. In the placebo group, there was a decrease only in the ALP. The fact that all these differences did not occur in the placebo group brings to mind that arginine supplementation does not cause a harm to bodily biochemical values, arginine supplementation has positive effects on metabolism, performance and recovery and it is a reliable dietary supplementation. In their study with 12 athletes, Sales et al. (21) grouped these athletes in three groups as arginine, placebo and control and gave daily 4,5 g arginine supplementation to the arginine group. The researchers checked the pre and post supplementation urea and creatine levels of the athletes. According to their results, arginine supplementation did not cause a significant difference in urea and creatine values. Thus, their study supports the reliability of arginine.

In our study we analyse LDH one day after exercise, namely not right after the exercise. In the studies it was found increase because they analyse LDH 5 or 10 min after exercise or following acute exhaustive exercise. But they found less increase in LDH levels in arginine group than control. In the study of Taylor et al. (22) L-NAME hydrochloride appeared to lower LDH release independent of exercise. Exercise plus L-NAME resulted in less LDH release at both 5 and 10 min than exercise without L-NAME. In the study of Lin et al. (23), the activities of plasma Creatine Kinase (CK) and LDH were significantly decreased in Arginine supplemented plus exercised rats compared with exercised rats. These findings suggest that Arginine supplementation reduces the oxidative damage and inflammatory response on the myocardium caused by exhaustive exercise in rats. In the study of Gupta et al. (24) L-arginine administered orally 30 min prior to cold (5C)–hypoxia (428 mmHg)–restraint (C-H-R) exposure. The C-H-R exposure of control rats on attaining rectal temperature (Trec 23)C, resulted in a significant increase in LDH. On recovery (Trec 37) of control rats, there was an increase in LDH too. But L-Arginine supplementation resulted in a lower increase in LDH compared with controls (45.3 versus 58.5% and 21.5 versus 105.2%) on attaining Trec 23C during C-H-R exposure and on recovery to Trec 37C. The results suggested that L-arginine possesses potent anti-stress activity during C-H-R exposure and recovery from C-H-R-induced hypothermia. In the rats treated with L-arginine (100 mg/kg body weight), the increase in LDH levels both on attaining Trec 23C and on recovery of Trec 37C were less than in control rats. This suggested that L-arginine supplementation was acting at a cellular level in maintaining the energy-dependent process of membrane permeability. Their results supported the view that arginine supplementation will decrease the LDH levels and improve recovery. In the light of these findings, low levels of LDH bring to mind that it will improve performance and recovery.

As a conclusion, it was found that in footballers, L-argininie supplementation accelerated the removal of LA from the body, decreased the amount of fat in the body, healed the post training muscle damage caused by the decrease in LDH enzyme level, enabled muscle renewal accelerated recovery.

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