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Original article

Does nitric oxide intake affect post-exercise recovery in athletes? A study on cocoa, caffeine and nitric oxide supplement

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Summary. *Background:* The beneficial effect of popular supplements and use of nitric oxide (NO) precursor nutrients in athletes which aim at increase of sports performance. *Objective:* The aim of this study is to investigate the effect of nitric oxide precursor nutrients and some nutrients with the ability to increase NO levels in the body on increased NO production in metabolism and on the recovery after acute exercise. *Materials and Methods:* 8 volunteers, male athletes, in shape and trained participated into this study. Athletes were subject to research protocol every other day, a total of three times. Heart rate and lactate levels were determined at resting and recovery. *Results:* Statistically significant difference was detected between control and cocoa/caffeine (CC) groups in the 1 minute lactate levels. Lactate levels significantly decreased in the CC group compared to the control group in the 1 minute lactate levels ($p<0.05$). Statistical significance difference was found between the nitric oxide supplement (NOX) and CC groups in the 1 and 10 minutes heart rate levels. Heart rate levels significantly decreased in the CC group compared to the NOX group ($p<0.05$). Heart rate levels were significantly decreased in the CC group compared to the control and NOX groups in the 15 minute measurement ($p<0.05$). *Conclusions:* Nitric oxide consumed as a supplement in line with nutrition and recovery strategies in athletes improved the recovery by accelerating lactate excretion from the body after the exercise. All of these metabolic responses in the present study suggest that NO will have a positive effect on exercise performance and recovery.

Keywords: Exercise, sports nutrition, dietary supplements, nitric oxide, arginine, citrulline, ornithine, cocoa, caffeine, recovery.

Introduction

It is well known by all sports stakeholders that nutrition has a positive effect on exercise performance. Nutrition, which is as important as training and resting in the development of athletes, can affect many performance-related elements in a positive manner as well as rapid recovery after exercise (1). Although the appropriate nutrients taken before, during and after training can meet the nutritional requirements of athletes substantially, athletes, particularly with intense training programs, may not be able to meet their increased nutritional requirements with natural foods (2). Therefore, a wide range of nutritional supplements is produced to improve their performances (3). The reasons for using supplements are mainly energy requirements and fuel supply during exercise, liquid and electrolyte balance, adaptation to specific environmental conditions, physical activity, athletic performance, rapid recovery after exercise, general health, body weight, and composition (4).

In the study, cocoa and caffeine have been used along with a supplement enhancing the synthesis of NO. Being a different isoform of nitric oxide synthases

(NOS), NO is an important physiological stimulatory molecule and can change skeletal muscle function by taking part in blood flow regulation, muscle contraction, glucose and calcium homeostasis, mitochondrial biosynthesis, and breathing (5, 6). It is a gaseous signaling molecule with high spreading degree and synthesized by NOS, which is a calcium-dependent enzyme with a low molecular weight, as well as by arginine, citrulline, and ornithine. It plays a constant role in the regulation of blood flow and blood pressure during exercise and in skeletal muscle rest (7, 8). Nitric oxide has an impact on a wide-range area including physiological functions such as nerve conduction, platelet aggregation, and regulation of vessel expansion (9). Among the roles of NO, regulating the vasodilation and oxygen consumption in heart and skeletal muscle directly affect exercise performance (10). Nitric oxide is involved in many physiological functions including muscle vasodilation and oxidative metabolism control. In particular, it releases several vasodilator substances during exercise, affecting the regulation of vessel conductivity and muscle blood flow, and contributes to the redistribution of blood flow throughout the exercise. Increased NO production during exercise promotes vasodilatation in the heart and skeletal muscle and regulates the veins by playing a key role in the control of circulatory functions, and ensures the durability of the vessels. Endothelial cells are the source of NO, which changes vascular durability (11, 12, 13). Furthermore, NO playing an endothelial-dependent regulatory role during exercise and rest in skeletal muscle has a major effect on the rise of skeletal muscle glucose during exercise and may increase the secretion of insulin hormone, an important hormone for muscle growth and recovery (14, 15, 16).

Chocolate and coffee, consumption of which is very popular and common among athletes like other people, are believed to have positive effects on exercise performance, besides having a good taste (17, 18, 19, 20, 21). It is known that the active substances in these foods, which are cocoa and caffeine, are two powerful antioxidants (22, 23, 24). Cocoa and caffeine, which are known to increase NO production (19, 30, 31, 32, 33) and are frequently consumed together or separately, have been shown to have many positive effects on human health (25, 26, 27, 28, 29). The aim of this study is to investigate the effect of NO precursor nutrients and some nutrients with the ability to increase NO levels in the body on increased NO production in metabolism and on the recovery after acute exercise.

Materials and methods

Research Group

8 volunteers, male athletes, trained participated into this study. Athletes were subject to research protocol every other day, a total of three times (first day control group, second day nitric oxide supplement group, third day cocoa/caffeine group). 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: 55317723-604.01.01-E.).

Study Design

All athletes were subject to study protocol every other day (48 hours between each test day), a total of three times. First of all, athletes' heights were measured and the body of the athletes were analyzed. Then, athletes were subjected to RAST test. Heart rate and blood lactate levels were determined at resting, immediately after the RAST test, at the 1st, 5th, 10th, 15th, 30th, 45th and 60th minutes. On the first day, before the RAST test no supplements were given to the athletes (control group) but only water was given during the recovery period. On the second day after the 48 hours from first day, the measurements made at the beginning of the study were repeated but unlike first measurements, NO supplement were given to the athletes (NOX group) 30 minutes before RAST and water was given during the recovery period as given first day. Finally, on the third day after the 48 hours from second day, the measurements made at the beginning of the study were repeated but unlike first and second measurements cocoa and caffeine were given to the athletes (CC group) 30 minutes before RAST and water was given during the recovery period like first and second day. Measurements and tests were made in the same time period and physical conditions in all groups.

Table 1. Participant baseline characteristics (M±SD)

Characteristics	Mean ± SD	Range
Age (years)	21.50 ± 2.00	(20.00 – 26.00)
Height (cm)	180.00 ± 2.72	(176.00 – 183.00)
Body weight (kg)	75.03 ± 7.80	(65.50 – 89.50)
Skeletal muscle weight (kg)	38.01 ± 4.18	(31.80 – 43.10)
Fat mass (kg)	8.73 ± 2.84	(6.10 – 15.10)
Total body water (l)	48.60 ± 5.06	(41.30 – 54.80)
Body fat percentage (%)	11.61 ± 3.13	(7.50 – 16.90)
Body mass index (kg/m^2)	23.13 ± 2.24	(21.10 – 27.90)
Metabolic rate (kcal)	1801.37 ± 147.40	(1585.00 – 1977.00)

	Total Consumption		
Groups	**Control**	**NOX**	**CC**
Supplement (g/mg)	-	36	25 + 200
Water (ml)	500	500	500

Supplementation

Immediately after RAST test 8 athletes were subject to research protocol every other day, a total of three times. The study was conducted as a single blind application. Before the RAST test no supplements were given to the control group. NO supplement was given to the NOX group and cocoa/caffeine were given to the CC group and in both groups nutritional supplements were given 30 minutes before RAST test in accordance with administration and daily dosage (with 500 ml water). The supplementation was prepared beforehand and as a single dose that includes NOX supplement (arginine, citrulline, ornithine) 36 g and the CC supplements cocoa 25 g + caffeine 200 mg. The athletes were not informed about the substance given to them. So, the psychological effects may have occurred 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.

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). Athletes 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 athlete made six consecutive 35 meter sprints by giving 10 second breaks after each sprint. After a athlete started the test by making the first sprint and gave the first 10 second break, Power Timer device beeped and the athlete 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, 10th, 15th, 30th, 45th and 60th minutes after the RAST test, subjects' LA levels during recovery were determined.

Heart Rate (HR)

Polar watch (V-800) to measure HR was used. A transmitter was placed in the chest and HR in the resting and 1st, 5th, 10th, 15th, 30th, 45th and 60th minutes after the RAST test were determined.

Height, Weight and Body Composition Analyses

In the study, athletes' heights were measured through a Seca Height Measuring Device and the body of the athletes were analyzed using the Inbody 120 Bioimpedance Body Composition Analyzer.

Statistical Analyses

The research data obtained were given in the form of the mean ± standard deviation (M±SD) and median with interquartile (IQR). 95% confidence intervals (CI) are given for blood lactate and heart rates (HR). We assessed the distribution of the analyzed variables using a Shapiro-Wilk test. The results showed that the distributions deviated from normal distribution, so a detailed statistical analysis using nonparametric tests was necessary: a Wilcoxon matched-pairs test was completed to assess significantly difference between 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 statistical significant difference was detected between control and CC groups in the 1 minute blood lactate levels. Lactate levels significantly decreased in the CC group compared to the control group in the 1 minute lactate levels (p<0.05). There was no statistically significant difference all the other time periods when lactate levels were checked in all groups (p>0.05). When the heart rate levels of the three groups at all time periods were compared; statistical significance difference was found between the NOX and CC groups in the 1 and 10 minutes heart rate levels. Heart rate levels significantly decreased in the CC group compared to the NOX group (p<0.05). There was also significant difference between the control and CC heart rate levels as well as between NOX and CC heart rate levels in the 15 minute measurement. Heart rate levels were significantly decreased in the CC group compared to the control and NOX groups in the 15 minute measurement (p<0.05).

The blood lactate (m/mol) level of subjects linearly increased with no deviation from rest to 5min and then the data demonstrate a linear decrease until 60 min (Table 2, Figure 1). The heart rate (beat/min) peaked in 1 min and then the data demonstrate a linear decrease until 60 min (Table 3, Figure 2).

Table 2. Changes in the blood lactate levels at different time points

Group	Control	NOX	CC
Rest	1,95 (1,52-2,30)	2,00 (1,67-2,17)	1,95 (1,37-2,07)
1 min	5,75 (5,07-6,57)[+]	5,30 (4,70-5,97)	3,60 (3,27-3,85)*
5 min	9,90 (8,85-11,10)	8,50 (5,90-12,22)	8,75 (7,87-9,25)
10 min	9,95 (7,07-10,77)	9,00 (5,35-11,82)	8,55 (7,45-9,87)
15 min	8,35 (7,12-9,77)	8,50 (4,22-11,95)	7,70 (7,52-7,87)
30 min	5,50 (4,45-7,40)	5,95 (3,05-7,50)	4,80 (4,45-5,22)
45 min	3,75 (2,85-5,77)	4,40 (2,35-6,00)	2,95 (2,45-3,15)
60 min	2,80 (2,17-4,45)	3,30 (1,92-4,62)	2,15 (1,77-2,75)

Median (Interquartile Range).*Significant difference compared with control. [ı]Significant difference compared with NOX. [+]Significant difference compared CC.

Discussion and Conclusions

Athletes should pay attention to their nutrition to increase the quality of training, to improve the performance and to accelerate the recovery process. A balanced and proper diet combined with nutritional

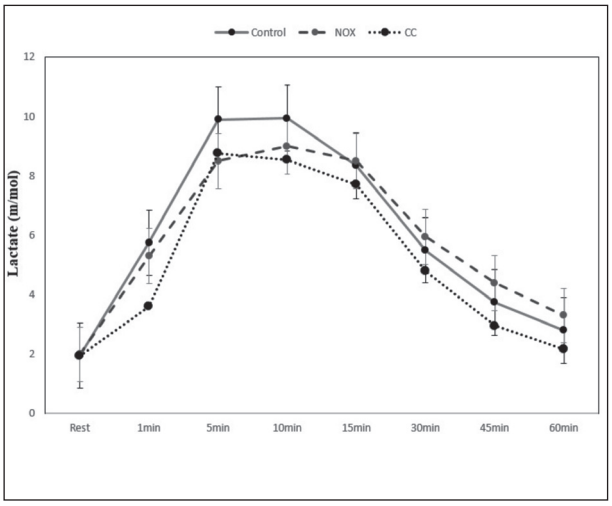

Figure 1. The blood lactate levels at different time points at resting and after exercise during recovery periods with and without supplementation. All values are median with interquartile (IQR). Statistical significance was set at p<0.05.

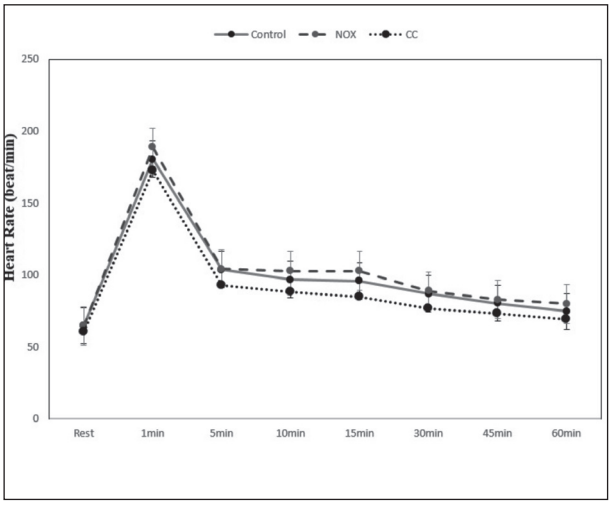

Figure 2. The heart rate levels at different time points at resting and after exercise during recovery periods with and without supplementation. All values are median with interquartile (IQR). Statistical significance was set at p<0.05.

Table 3. Changes in the heart rate at different time points

Group	Control	NOX	CC
Rest	65,00 (63,25-69,00)	64,66 (64,00-66,50)	60,50 (51,50-63,50)
1 min	180,66 (178,50-188,50)	189,00 (185,00-193,00)[+]	172,50 (162,75-174,75)[ǂ]
5 min	103,91 (98,45-105,00)	104,50 (100,00-109,00)	93,00 (87,25-101,75)
10 min	96,91 (90,20-100,75)	103,00 (99,00-107,00)[+]	88,50 (84,25- 92,75)[ǂ]
15 min	96,00 (92,75-96,75)[+]	103,00 (96,00-110,00)[+]	85,00 (81,25-88,00)*[ǂ]
30 min	87,00 (81,75-88,00)	89,00 (85,00-93,00)	77,00 (74,25-80,50)
45 min	80,50 (74,75-84,25)	83,00 (80,00-86,00)	73,50 (66,25-77,00)
60 min	74,75 (73,25-79,62)	80,00 (78,00-82,00)	69,50 (65,25-73,75)

Median (Interquartile Range). *Significant difference compared with control. [ǂ]Significant difference compared with NOX. [+]Significant difference compared CC.

strategies is important both for a rapid recovery and performance. The use of supplements, which are generally used to improve recovery after training, is one of the most important ones of these strategies (34). Nitric oxide is a common molecular communicator and exerts metabolic vasodilation and expands vessel diameter in straight muscles, contributing the exercise (35, 36, 37). It has various significant effects on health, performance during exercise, and physiological functions such as skeletal muscle and blood flow (38, 39, 40). It is an important substance in terms of the regulation of skeletal muscle and blood flow during physical activity and experts recommend NO intake (41, 42, 43). Besides regulating muscle metabolism, taking NO as a dietary supplement before the training accelerates blood flow, pumps blood to muscles, and improves performance (44, 45).

Supplements containing NO are used as ergogenic aids to improve performance in competitions and training and to improve recovery after the training. The scientific infrastructure of these supplements as ergogenic nutritional supplements has been revealed by recent researches showing that NO intake as a supplement has an enhancing effect on performance and accelerating effect on recovery (46). The literature review showed that there are different tools and methods related to supplements containing arginine, citrulline, and ornithine (7, 8), known as NO precursors, and nutrients containing cocoa and caffeine, known to increase NO production in metabolism (19, 31, 47, 48) and many different studies have been carried out on the effect of NO-containing food consumption on exercise performance and recovery (19, 20, 46, 49, 50).

In research on this subject, the participants were given 6 g L-arginine supplement per day for 14 days and their lactic acid levels and heart rate were checked at the 10th minute after the anaerobic exercise to monitor the recovery process before and after supplementation use. The results were compared with those in the placebo group; lactic acid levels were found to be higher in the experiment group in the 10th minute of the recovery period compared to the fifth minute before 14-day arginine supplement use, which were found to decrease in the 10th minute compared to the fifth minute after the supplement intake. In the placebo group, lactic acid levels at the 10th minute of the recovery period were found to be higher both before and after the supplement intake compared to the lactic acid levels in the fifth minute. Heart rate values were found to be low in the first minute of the recovery period in both groups compared to pre-supplementation values. However, a more significant decrease was observed in the heart rate values of the football players after the 14-day arginine supplementation use, compared to the placebo group and therefore, the recovery was more rapid. The lactate level of the experimental group was reported to decrease rapidly after the arginine supplementation, as well as accelerating the removal of lactic acid from the body and improving the recovery and researchers further emphasized that decrease in heart rate values would have a positive effect on recovery (46). In a similar study, healthy male athletes were divided into two groups namely experimental and placebo. During the course of 21 days, 5 g

of arginine and 5 g starch supplementation per day was given to the experimental group and placebo group, respectively. While no difference was observed in the pre-training measurements of the group receiving arginine in terms of pre- and post-test values, lactate level in the blood was found to be decreased in the experimental group after the training, compared to the placebo group (51). In a study including 24 male athletes, the experimental group was given 12 g arginine per day for seven days. Based on the results, after the arginine supplementation, there was a decrease in the heart rate measured immediately after the exercise in the experimental group, compared to the pre-supplementation heart rate measurements while there was an increase in the placebo group (52). In another study, where NG-nitro-L-arginine methyl ester (L-NAME) supplementation was given to healthy male volunteers, although blood lactate level of the L-NAME group at the beginning of exercise was found to be higher than the control group, no significant difference was found between the groups after the exercise. Blood lactate accumulation decreased significantly in the L-NAME group (13). In a different study including nine trained male athletes, in which volunteers were given dark chocolate produced from cocoa to increase the production of NO, performances of the athletes were analyzed. In that study, where many different performance tests were applied, researchers measured the heart rates of the participants, however, no difference was observed between the groups. Yet, they reported that dark chocolate supplementation was an effective ergogenic aid after they made an overall examination on the test results (53). In a similar study, 12 well-trained bikers were given cocoa supplementation and many parameters such as lactate level and heart rate were analyzed. As a result of this study, cocoa intake was found to increase the total antioxidant capacity during recovery and exercise, but acute cocoa intake did not improve the performance and recovery period (54). As cocoa consumption can improve performance, researchers have recommended that athletes should consume cocoa (48). In a study by Woolf et al. (2008) including 18 male athletes, the effect of caffeine consumption on anaerobic performance was investigated (55). Researchers did not identify a positive effect of caffeine consumption either on lactate levels, or on heart rate. However, it was stated that a normal dose of caffeine intake had a positive effect on anaerobic performance when other test results were evaluated (55). In another study, the research team divided the six male bikers into three groups, namely control, placebo, and caffeine supplementation and individuals in these groups underwent several performance tests. In another study examining the lactate levels and heart rate, a significant increase was observed in heart rate during exercise and in lactate levels after exercise, compared to the recovery period. However, no significant difference was observed between the study groups in terms of lactate levels and heart rate. Based on the performance test data, the researchers concluded that the use of caffeine supplementation improved the performance (56). Similarly, it was reported in another study that caffeine use was one of the effective ways to improve performance (21). In the present study, the intergroup comparison showed that there was a significant difference between control and CC groups in terms of blood lactate levels measured in the first minute. In the CC group, blood lactate level measured in the first minute was found to be significantly lower than the control group. Furthermore, no significant difference was found in other intergroup comparisons in terms of lactate levels. However, when the fifth and 10th-minute measurements, in which lactate reached the highest level, were examined, the lactate level of NOX and CC groups was observed to be low compared to the control group. Moreover, the blood lactate level of NOX and CC groups was found to be lower than the control group in terms of lactate values measured in other periods.

Many studies in the literature have shown that arginine, citrulline, and ornithine, as well as cocoa and caffeine supplements, have positive ergogenic effects on performance and recovery when used alone or in combination (17, 19, 46, 50, 57, 58). In a study by Wax et al. (2015), in which 8 g single dose citrulline malate was given to weight lifters, citrulline malate group was compared with the placebo group and as a result of this comparison, significant improvement was observed in the performance of the citrulline malate group (58). A significant increase was observed in blood lactate levels and heart rate after exercise, compared to the pre-exercise levels, however, no significant difference was

found between the supplement and placebo groups. Researchers concluded that citrulline malate intake may be useful in improving exercise performance during resistance exercise (58). Similarly, in another study, in which 8 g single dose of citrulline malate was given to trained male athletes, it was reported that citrulline malate supplementation improved the performance during resistance training but it had no effect on the blood lactate levels and heart rate (59). In the literature, the blood lactate levels of the individuals using citrulline were reported to be lower than those who did not use (60) and the use of citrulline did not have a positive effect on blood lactate levels (61) and on heart rate (49). In a different study, blood lactate levels were observed to be decreased after L-arginine supplementation and this was attributed to the increased levels of citrulline and NO (62). In a study in which L-arginine and L-ornithine supplementation was given to athletes, lactate level was found to be lower in the group received a supplement than the placebo group (63). In another study examining many data including blood lactate levels and heart rate, L-ornithine consumption was recommended against physical fatigue (64). In a study of acute L-arginine supplementation to increase NO levels, it was observed that oxygen intake decreased during moderate-intensity exercise in L-arginine consuming group. Furthermore, although blood lactate levels were found to be low during high-intensity exercise, no significant difference was found neither in blood lactate levels nor in heart rate values. L-arginine has been reported to prolong the exhaustion during high-intensity exercise by increasing high-intensity exercise tolerance (65). In a study by Demura et al. (2010), similar data was obtained between the group using L-ornithine supplement and placebo group in terms of blood lactate and heart rate values (66). In another study, the researchers also found similar data in blood lactate values in L-ornithine receiving group and placebo group (50). It has been stated in the literature that acute cocoa consumption has no effect on recovery (67). On the contrary, in a study, the use of cocoa was reported to have an ability to increase the cardiovascular benefits of exercise by facilitating vasodilation (68). In another study analyzing the heart rate, caffeine use was reported to improve performance, which was attributed to the increased central nervous system stimulation (69). In a study in which there was no significant difference in terms of heart rate and blood pressure, caffeine consumption was reported to have no effect on performance improvement in anaerobic exercise tests (70). In our study, the intergroup comparison showed that there was a significant difference between NOX and CC groups in terms of heart rate measured in the first minute and 10th minute. In the 15th minute, the heart rate of the CC group was found to be statistically and significantly different from that of NOX and control groups. In all of these measurement periods, the CC group was observed to have a lower heart rate level compared to the other groups. In all other time intervals, the CC group's heart rate was also low compared to other groups.

Since this study was conducted with limited resources, it did not reveal whether there were any changes in the blood lactate and heart rate levels 2 h after exercise of the subjects. Another limitation of the study can be said that the number of subjects were 8.

In conclusion, NO consumed as a supplement in line with nutrition and recovery strategies in athletes improved the recovery by accelerating blood lactate excretion from the body after the exercise. All of these metabolic responses in the present study suggest that NO will have a positive effect on exercise performance and recovery. In future studies on this subject, examination of establishing a controlled lifestyle for athletes, carrying out different measurement and tests, and following a regular nutrition program with chronic supplements as well as examining different metabolic changes may provide clearer information.

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