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Investigation of Acute Effect of Citrulline Malate Supplementation on Maximal Anaerobic Power in Elite Boxers

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Keywords	ABSTRACT
Citrulline Malate Boxing Maximal Peak Power Maximal Peak Velocity	This study aimed to determine the acute effect of 8 grams of citrulline malate supplementation 1 hour before exercise on the maximal anaerobic power level of elite boxers by measuring upper extremity peak power and velocity through bench press throwing and landmine punching (right-left arm) tests. The study group consists of 13 elite boxers aged between 18-22 years (19.8 \pm 1.28), studying at Istanbul Gelisim University, who have at least 3 years (7.5 \pm 3.59) of regular boxing experience and actively participate in competitions. The study was designed with a single group pretest-posttest experimental design. The effects of citrulline malate supplement on elite boxers was examined with comparison of the results recorded with and without citrulline malate supplement. As a result of the study, it was found that acute use of 8 grams citrulline malate supplement 1 hour before exercise in elite boxers showed a statistically significant difference (p<0.05) in peak velocity (p=0.024) and peak power (p=0.048) of bench press throw, right arm landmine punch throw peak velocity (p<0.01) and peak power (p=0.007). As a result, it was determined that the use of 8 grams of citrulline malate supplementation 1 hour before exercise in elite boxers contributed to a significant increase in the measured anaerobic power parameters

1. INTRODUCTION

The effects of food and nutritional supplements on physical performance are not new. We can say that humanity has been experimenting with different nutrients to increase physical strength since ancient times of history. Nowadays, health professionals and coaches are trying to better understand the effects of different nutrients and nutritional supplements on performance with the spread of scientific approach [1].

It is well known that athletes who cannot pay attention to their nutrition cannot maintain their optimal performance in training or competition. In addition to adequate and balanced nutrition, many athletes resort to various nutritional strategies in order to complete high athletic performance and recovery phase quickly and to be ready for the next training or competition [2]. Small changes in the performance of elite level athletes can directly affect the results of the competition $[\underline{3}]$.

Citrulline is a type of non-essential can be synthesized in the body and does not have to be taken from outside with food or supplements) amino acid. The main dietary source of citrulline is watermelon [4]. Citrulline increases plasma larginine, which is necessary for nitric oxide (NO) synthesis [5]. It is stated that citrulline supplementation increases the l-arginine level higher than arginine supplementation, thus providing higher NO biomarkers in plasma compared to arginine supplementation [6,7].

Boxing is one of the combat sports that require maximum strength [8]. In order to have high competition performance in boxing, aerobic and anaerobic power levels should be high [9]. Anaerobic energy system provides energy for attacks that require short duration and maximum power [10]. The aerobic energy system helps the

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recovery process between rounds and especially the recovery of the high-energy phosphate system [11].

It has been explained that citrulline malate supplementation can improve maximal anaerobic power parameters by affecting the high-energy phosphate system [11] and increasing mitochondrial efficiency [6,7,11].

2. MATERIALS AND METHODS

2.1. Research Group

The research group of the study consisted of 13 elite boxers studying at University Istanbul Gelisim, aged between 18-22 years (19.8 \pm 1.28), with at least 3 years of regular boxing experience (7.5 \pm 3.59) and actively participating in competitions.

Participants were randomly selected from boxers who had not used citrulline malate supplementation before. After the participants were informed about the study, participation in the study was voluntary.

This study was carried out after the approval of the Ethics Committee of Istanbul Gelisim University. Participant provided informed consent, with the volunteer form covering research details, risks, benefits, confidentiality, and participant rights. The research strictly adhered to the ethical principles of the Declaration of Helsinki, prioritizing participant's rights and well-being in design, procedures, and confidentiality measures.

2.2. Study Design

This study was designed with a one-group pretest-posttest experimental design to investigate the acute effect of 8 grams of citrulline malate supplementation orally 1 hour before exercise on maximal anaerobic power parameters in 13 elite boxers. The measurements and tests applied were; age calculation, height and weight measurements, 1 repetition maximum bench press, bench press throwing, landmine punching. Participants were randomly selected among athletes who did not use any supplements to avoid any effect on the study. During the study, each athlete used only the supplement given to him/her and did not use any other supplements. All participants were informed about the procedures of the study and then signed an informed consent form. In the familiarisation phase before the study, the purpose of the study was explained to the athletes and all tests were checked twice. Then, on the first day of the study, age calculation, height, weight and 1TM bench press measurements of the athletes were performed. On the second measurement day 48 hours later, only bench press throwing and landmine punching tests were performed. On the third measurement day 48 hours after the second measurement day, all athletes consumed citrulline malate supplementation 10 minutes apart and were measured in turn. Since it was aimed to measure the effect of the supplementation at the peak point, the participants were kept under observation for 40 minutes. Each participant started the warm-up protocol at 40 minutes and then completed the bench press throw and landmine punch tests at 55 and 65 minutes. Two days rest period was given between the measurement days. The next measurement was taken after the resting phase was completed. Finally, the level of effect of citrulline malate supplementation on elite boxers was analysed by comparing the results recorded before and after citrulline malate supplementation.

2.2.1. One rep max bench press test

The test was performed on a Smith machine with a starting weight of 20 kg for all participants. To standardise the bench press technique, the participants used the same hand placement in each trial, determined by the distance between the index finger and the barbell lines. The test was performed with the participants' shoulders and hips in contact with the bench bench. A full arm extension was performed after the barbell lightly touched the centre of the chest. During the test, all trials that did not meet the standardised technique criteria were considered invalid. The weight lifted for each trial was increased by 2.5-10 kg with free weight plates until failure occurred. The 1 repetition maximum load that participants could lift was performed using 1-2 min rest periods between trials [12].

2.2.2. Becnh press throw test

To measure upper extremity peak velocity and power, the bench press throwing exercise was used on a Smith machine with a starting weight of 20 kg. The applied load was determined to be 30% of the athletes' 1 repetition maximum bench press exercise [<u>13</u>]. Participants were asked to explosively throw the barbell in a hand position of their choice for maximum height in the vertical direction with the elbow at an angle of approximately 90° [14,15]. After the barbell reached its maximum height, it was grabbed by 2 "spotters" and slowly lowered into the participant's hands as it transitioned to the down phase. Each participant was given 3 attempts and the highest value was recorded. Peak power and peak velocity produced during each bench press throw repetition were measured using the Gymaware device [16].

2.2.3. Landmine punch throw test

The peak power and peak velocity that the participants could produce during the punching action were measured using the Gymaware device with the Landmine punching test. The test was performed by adding 5 kg load to an Olympic barbell with a starting weight of 20 kg for all participants. The participant was placed in the guard position and in a position where he/she could throw the free end of the barbell grasped with one hand, grasping it in the centre with his/her hand and holding it at shoulder level. While the participant was preparing for the throwing action, he/she kept his/her chest upright and flexed slightly towards the side that would perform the throw by transferring the weight to the back leg. In the starting position, the leg, hip and shoulder were strongly rotated to allow optimal power transfer from the lower to the upper extremity. The command "3-2-1 start!" was given and 3 repetitions of the throwing action were performed for each arm as soon as the participant felt ready. When the participant threw the free end of the barbell, the barbell was caught by the observer in front of the participant and handed to the participant for the next throwing action. The best result obtained during the landmine punch throw repetition for each arm was considered the highest value [17,18].

2.2.4. Citrulline malate supplementation procedure

Protein Ocean brand Citrulline Malate (2:1 citrulline malate ratio, each serving contains 1.5 grams citrulline malate; 1 gram l-citrulline contains 0.5 grams malic acid), which is sold over the counter, was used in the study. The product is produced in accordance with the Turkish Food Codex Communiqué on Supplementary Foods. The supplementary food approval number is specified as 012050-02.12-2021. There is a net amount of 250 grams in a box. Participants were given 8g of citrulline malate supplement orally after mixing with 400 ml of water and thoroughly homogenised. Participants tolerated the citrulline malate supplement well and no participants reported adverse effects. Since it is known that the peak concentration of citrulline occurs at approximately 1 h and decreases rapidly 15-30 min after the peak. regardless of the dose taken [19], all participants consumed the supplement 10 min apart and were measured sequentially. Each participant was kept under observation for 40 minutes and started the warm-up protocol at the 40th minute. After supplementation was used for each participant, measurements were completed at 55 and 65 minutes on average.

2.3. Statistical Analysis

Statistical analysis of the data in the study was evaluated using SPSS 25 package programme. Arithmetic mean and standard deviation values are given for the characteristics of the participants.In reliability analysis, Intraclass Correlation Coefficient (ICC) values of all variables were calculated. The normality distribution of the data was checked by Shapiro-Wilk test. Before and after performance measurements of the group were analysed using Paired T-Test. Effect size was calculated to examine the differences between pretest and post-tests. Significance were set at p <0.05.

3. RESULTS

Table 1. Age, height, weight, experience mean andstandard deviation values of athletes

Variables	Ν	$\bar{x} \pm SD$	Min	Max	
Age	13	19.8±1.28	18	22	
Height (cm)	13	173.1±6.4	162	184	
Weight (kg)	13	68.5±13.04	45	94	
Experience (year)	13	7.5±3.59	3	14	
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cm: centimeter, kg: kilogram

According to Table 1, the mean values of the 13 athletes participating in the study were as follows: age 19.8 ± 1.28 , height 173.1 ± 6.4 , weight 68.5 ± 13.04 , experience 7.5 ± 3.59 .

Tablo 2. 1 TM BP, 1 TM BP-Mean Velocity, BPT mean and standard deviation values of athletes

Variables	Ν	π̄±SD	Min	Max
1 RM BP (kg)	13	78.65±17.98	45	100
1 RM BP - MV (m/s)	13	0.17 ± 0.04	0.12	0.25
BPT %30 (kg)	13	23.6±5.39	13.5	30
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kg: kilogram, m/s: meter/second, BPT: Bench Press Throw, 1 RM BP: 1 Rep Max Bench Press

According to Table 2, the mean values of the 13 athletes participating in the study were as follows: maximum 1 repetition bench press 78.65 \pm 17.98, maximum 1 repetition bench press average speed 0.17 \pm 0.04, bench press throw %30 23.6 \pm 5.39.

Variables	Tests	CI %95	Pre-Test ICC	CI %95	Post-Tests ICC
	BPT	990-999	996	973-996	989
Peak Velocity (m/s)	LPT-Right	930-993	978	947-993	979
	LPT-Left	892-993	976	972-997	989
	BPT	806-974	922	824-976	929
Peak Power (w)	LPT-Right	921-990	970	755-967	902
	LPT-Left	916-913	976	649-953	859

Tablo 3. Intra-Class Correlation Coefficient (ICC)

m/s: meter/second, w: watt

Table 3 shows that in the reliability analysis of the study, Intraclass Correlation Coefficient (ICC) values of all variables were calculated: <0.5: poor reliability, 0.5-0.75: moderate reliability, 0.75-0.90: good reliability and >0.90: excellent reliability. Bench press throwing peak power and speed pretest-posttest, landmine throwing right arm peak power and speed pretest-posttest, landmine throwing left arm peak speed pretestposttest were classified as excellent reliability (>0.90), and landmine throwing left arm peak power pretest was classified as excellent reliability (>0.90) and posttest was classified as good reliability (0.75-0.90).

Tablo 4.	Pre-test post-test	comparisons
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Variables	Ν	Test	Time	x± SD	р	ES
Peak Velocity (m/s)	13	BPT	Pre Post	2.04±0.2 2.09±0.2	0.024*	-0.717
	13	LPT-Right	Pre Post	2.14±0.4 2.48±0.4	0.001*	-1.553
	13	LPT-Left	Pre Post	2.13±0.3 2.34±0.3	0.001*	-1.377
Peak Power (w)	13	BPT	Pre Post	712.8±245.4 752.4±266.7	0.048*	-0.610
	13	LPT-Right	Pre Post	804.8±217.8 1025,3±290.1	0.001*	-1.327
	13	LPT-Left	Pre Post	827.5±271.7 949.5±270.1	0.007*	-0.898

*p<0.05, p: Significance Value, w: watt, m/s: meter/seconds, BPT: Bench Press Throw, LPT/Right: Landmine Punch Throw/Right Arm, LPT/Left: Landmine Punch Throw/Left Arm, ES: Effect Size

Table 4 shows that the pre-test and post-test performance measurements of the 13 athletes participating in the study were analyzed using paired t-test (p<0.05). When the effect size was analyzed, it was determined that there was a medium effect size (0.5 < ES < 0.8) for peak speed and peak power in the bench press throwing test and a large effect size (ES > 0.8) in the landmine punching test. In addition, there were statistically significant differences in peak speed (p=0.024) and peak power (p=0.048) in bench press throwing test, peak speed (p<0.01) and peak power (p<0.01) in right arm landmine throwing, and peak speed (p<0.01) and peak power (p=0.007) in left arm landmine throwing.

4. DISCUSSION

Gonzalez et al. (2018) [20] reported that they did not obtain a significant difference as a result of their study by applying barbell bench press exercise to evaluate performance on resistance exercise with the use of 8 grams of citrulline malate supplementation 40 minutes before exercise (p>0.05). Trexler et al. (2019) [21] explained that as a result of their study by applying leg extension exercise to evaluate performance on resistance exercise with the use of 8 grams of citrulline malate supplementation 2 hours before exercise, the nitric oxide level in the body increased compared to the placebo group, but they did not achieve a significant difference in leg extension exercise performance (p>0.05). Wax et al. (2015) [22] stated that the use of 8 grams of citrulline malate supplementation 1 hour before exercise increased upper extremity performance as a result of their study by applying pull-up, reverse grip pull-up and push-up exercises to evaluate performance on resistance exercise (p<0.05). While our current study and the study conducted by Wax et al. (2015) [22] are similar in terms of improving exercise performance, it differs

from the study conducted by Gonzalez et al. (2018) [20] and Trexler et al. (2019) [21]. We think that the reason for these differences is due to the timing of consumption of the supplement.

In the literature, there are studies indicating that nitric oxide stimulating dietary supplements (e.g. citrulline, arginine and dietary nitrate) improve performance in high-intensity exercise [23,24]. In another study, Coggan et al. (2015) [25] reported that the acute use of 140 ml of beetroot juice (dietary nitrate) 2 hours before exercise in healthy men and women increased the maximum velocity and power of the muscle during exercise. In another study, Cuenca et al. (2018) [26] examined the effects of the use of 70 ml beetroot juice supplements on performance and fatigue in resistance-trained individuals and reported that they observed an increase in performance in the 30-second wingate test, but did not observe an effect on fatigue. Although the statistically significant difference (p<0.05) seen on bench press throwing and landmine punching (right and left arm) tests as a result of our current study was not included in the evaluation by showing similarity with these studies, we think that citrulline malate supplementation benefits performance because it increases nitric oxide level in the body.

Chappell et al. (2018) [4] reported that the grams of citrulline of 8 malate use supplementation 1 hour before exercise did not cause a significant difference in anaerobic performance as a result of the study in which the effects on German volume training performance and muscle soreness were examined. In their study, Chappell et al. [4] also included the chemical analysis of 5 different citrulline malate supplement products. As a result of the chemical analysis of 5 different brands of citrulline malate supplements (Trade Ingredients, Peak Supps, Bodybulding WarehouseTM, MyproteinTM and Bulk Powders), it was explained that the citrulline malate rates stated by the manufacturers do not reflect the truth. In addition, Chappell et al. (2018) [4] explain that the citrulline malate supplement (Bulk Powders) they used in the study contained a lower ratio of citrulline malate than they planned (approximately 4.2 g instead of 5.3 g according to the 2:1 ratio). Glenn et al. (2016) [27] investigated the effects of acute use of 8 grams of citrulline malate supplementation 1 hour before the test on grip strength, vertical power and anaerobic cycle power in female tennis players and reported that it increased peak power in the wingate test and increased average and maximum grip strength in the grip strength test (p<0.05). No differences existed between trials for peak or average vertical power. Glenn et al. [27] stated that consuming 8

grams of citrulline malate supplements 1 hour before a tennis match may provide a strategic advantage over female, well-trained tennis athletes. The statistically significant difference (p<0.05) on the maximal anaerobic parameters (peak velocity, peak power) measured by landmine punching and bench press throwing tests in our current study is similar to the peak power value that increased in the wingate test in the study conducted by Glenn et al. (2016) [27], while it differs from the study conducted by Chappell et al. (2018) [4]. We think that the reason for this difference is that they could not reach the citrulline malate rate they planned due to the brand they preferred.

5. CONCLUSION

As a result, it was determined that the use of 8 grams of citrulline malate supplementation 1 hour before exercise in elite boxers contributed to a significant increase in the measured anaerobic power parameters. In line with these data, it can be said that the acute use of citrulline malate supplementation in sports branches where the phosphogen system is predominantly used provides an advantage for training and competition performance for athletes. Therefore, the increase in anaerobic power parameters may affect the competition performance of a boxer in the ring and increase the possibility of winning the match.

Acknowledgement

We thank the athletes who voluntarily participated in our study.

Conflict of Interest

No conflict of interest is declared by tehe authors. In addition, no financial support was received.

Ethics Committee

The study protocol was approved by the Ethics Committee of Istanbul Gelisim University. (Ethics Committee Approval: 2022/14).

Author Contributions

Study Design, FÖ, AK; Data Collection, FÖ; Statistical Analysis, AK; Data Interpretation, FÖ, AK; Manuscript Preparation, FÖ, AK; Literature Search, FÖ, AK. All authors have read and agreed to the published version of the manuscript.

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