



## The Comparison between Acute Effect of Citrulline Malate Different Doses on Aerobic and Anaerobic Power as Well as Lactate and Blood Urea Level in Young Soccer Players

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### Abstract

**Background:** It has been suggested that citrulline malate (CM) is one of the effective supplements to improve exercise performance and fatigue resistance. The present study aimed to investigate the effect of consumption of CM at various doses on aerobic power, anaerobic power, as well as plasma levels of lactate and urea in young soccer players.

**Methods:** In this semi-experimental study, performed at Ferdowsi University of Mashhad (2018), through targeted sampling, 40 trained young soccer players with were selectively classified into 4 homogenized groups based on their  $Vo_{2max}$  value including low dose (LD) (4g), medium dose (MD) (8g), high dose (HD) (12g), and sugar solution control (C). The supplements were taken slowly within 15 minutes 1 hour prior to the tests. Then, the yoyo recovery test-level 1 and the Repeated-Sprint Ability (RSA) were used to evaluate their aerobic and anaerobic capacity, respectively. Blood biomarkers were also measured in the first 10 seconds after RSA test. Further, one-way ANOVA test was employed for data analysis. Significance level was set at 0.05.

**Results:** The results showed that among the groups, the higher and lower values belonged to (respectively)  $Vo_{2max}$  in HD and LD ( $51.13 \pm 6.22$ ,  $47.62 \pm 4.16$ ), for RSA in HD and MD ( $34.11 \pm 6.34$ ,  $30.11 \pm 5.6$ ), for urea in HD and LD ( $4.64 \pm 1.3$ ,  $4.16 \pm 1.46$ ) and for lactate in C and MD ( $107.27 \pm 5.08$ ,  $103.66 \pm 7.34$ ) groups (mean  $\pm$  S.D), respectively. There was no significant association between the groups for  $Vo_{2max}$  value, RSA records as well as plasma levels of urea and lactate in response to taking different doses of CM (Pvalue  $>$  0.05).

**Conclusions:** The results indicated that acute supplementation of CM at different doses did not affect aerobic and anaerobic power, or blood levels of lactate and urea.

**Keywords:** Citrulline malate, Aerobic power, Anaerobic power, Lactate, Soccer.

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phosphorylation during exercise training,<sup>4</sup> and finally decrease ammonium and lactate production.<sup>5</sup> Throughout intense physical activity, impaired muscle function occurs due to increasing anaerobic glycolysis and limited access to oxygen causing accumulation of glycolysis metabolites and H<sup>+</sup> production.<sup>6-8</sup> It has been assumed that CM consumption may positively affect anaerobic and aerobic performance.<sup>1</sup> CM may indirectly be related to NO-induced vasodilation and increased aerobic power. Furthermore, it can improve anaerobic capacity via malate metabolism and decreased lactate accumulation.<sup>1,2,4,9,10</sup> Meanwhile, it seems that CM elevate the rate of ammonia elimination and help lactate removal during physical activity.<sup>11</sup> There are conflicting reports related to ergogenic effects of short-term CM supplementation. In this regard, 14 days of CM consumption significantly reduced fatigue perception and increased oxidative ATP production by 14% during 3 minutes of submaximal finger flexion.<sup>4</sup> On the other hand, there was no effect of 14 days CM (6 grams daily) on  $Vo_{2max}$  value during ergometer pedaling.<sup>12</sup> No effects were observed either for acute intake of 12 grams citrulline malate 1 hour before 10 sets of 15 seconds of cycling with maximum efforts and 30 seconds active rest between sets on lactate levels, acidosis and high intensity pedaling-induced fatigue.<sup>6</sup> Again, supplementation with 8 grams of CM 40 minutes before weight training workout (5 sets, 15 repetition of bench press with 75% of 1RM) had no effect on performance capacity.<sup>13</sup> In contrast, enhanced performance capacity by 19% has been reported following taking 8 grams of CM 1 hour before bench press exercise with 80% of 1RM.<sup>14</sup>

Because of lack of knowledge about the effectiveness of acute CM supplementation and no optimal recommendation of acute dosage taken, the present study aimed to compare the effect of CM supplementation with different doses on aerobic and anaerobic power as well as plasma levels of lactate and urea serum in young soccer players.

### Materials and Methods

This semi-experimental study was carried out in Ferdowsi University of Mashhad during summer 2018. In order to conduct the research, 40 trained young soccer players with targeted sampling were selected with at least 2 years of playing soccer background. The sample size was determined based on previous related studies. Inclusion criteria were not taking any supplements or drugs with any possible interactions with CM or affecting the dependent variables, no smoking for at least the latest 6 months, doing vigorous activity 48 hours prior to the tests and any injury or problems affecting the subject

## Introduction

In order to improve their sport performance, athletes adopt taking supplements.<sup>1</sup>

Recently, citrulline malate (CM) has been considered as a supplement to postpone fatigue and increase power output of skeletal muscles.<sup>2</sup> CM consists of two components including citrulline as an unnecessary amino acid and malate driven from malic acid<sup>1-3</sup> which may be effective to enhance muscle function, aerobic and anaerobic activity due to improved oxygen delivery to active muscles, increase oxidative

performance, on the other hand, the exclusion criteria were failure to perform any tests correctly or inability to complete them. Before the first session, all the steps such as functional and laboratory tests were described for the subjects and they signed a written consent letter. An overall schematic view of the research process is illustrated in figure 1.

Forty-eight hours before the first session of exercise, all subjects were called for the anthropometric measurements. The anthropometric measurements included weight, height, and BMI. Next, the  $VO_2\text{max}$  of subjects was determined by doing Yo-Yo intermittent recovery test level 1 as explained further. The subjects were then selectively divided into four homogenized groups (10 in each group) based on their  $VO_2\text{max}$  including low dose supplementation (LD, 4gr), medium dose supplementation (MD, 8gr), high dose supplementation (HD, 12gr), and sugar solution as control (C). The supplementation was conducted based on a double-blind trial. All the subjects took the supplement related to their own group along with 250 ml of water 1 hour before undergoing tests. The control group merely received 250 ml of a 6% sugar solution.

The test involved running in a 20-meter track based on a round trip. Specifically, the subjects stood behind the starting line and with a beeping sound at a given time, they had to pass the 20-meter distance and get back to the starting point. Further, the subjects took a rest actively in a 5-meter route in a round-trip way and kept repeating by hearing the beeping sound. The test started with a 5-kilometer speed per hour with the speed growing rapidly with each step. At the minute the test taker could not reach the finish line twice, the test was terminated. After the test, the aerobic power of the test taker was calculated using the following formula in the form of maximum oxygen consumption.<sup>15</sup>

The test included round-trip running a 20-meter distance and there was a 20-second inactive rest between the shuttles. Overall, the subjects should have repeated it six times and finally the percentages of reduction in their score in the ability to repeat the speed running was calculated by the following formula.<sup>16</sup>

Blood sampling (5 ml) was done immediately after RSA test by a laboratory expert, centrifuged at 4°C, with the plasma samples stored at -70°C used to analyze lactate and urea.

All subjects were asked to record their diet 24 hours before the first session test and follow it in the next sessions. Further, all subjects were informed to avoid alcohol and caffeine consumption along with doing any intense activity 24 hours before the tests. In order to optimize the hydration in all subjects, they were asked to drink 500 ml of water every morning to implement each test.

All data were analyzed using one-way ANOVA test for determining the level of significance. All data were represented as mean ± SD. SPSS19 was used for data analysis, and the significance level was set at 0.05.

### Results

The mean and standard deviation of the anthropometric characteristics (age, height, weight and BMI) of all four groups are reported individually in table 1.

As shown in table 2, the highest and lowest values were observed for  $VO_2\text{max}$  in the groups with high dose and the low dose, respectively. However, the results of one-way ANOVA showed that there was no significant difference between the groups in  $VO_2\text{max}$  values (Pvalue > 0.05). Blood urea values of the subjects immediately after RSA showed that the minimum and maximum urea level were in the low dose and high dose group, respectively. However, there was no significant difference between the groups (Pvalue > 0.05, table 2). The lowest and highest percentages of RSA reduction were seen in the low dose and the high dose groups, respectively. However, no significant difference was observed (Pvalue > 0.05, table 2). The results of one-way ANOVA indicated that there was no significant difference in lactate levels immediately after RSA test between the groups. Meanwhile, the lowest levels of lactate were seen in the medium dose group (Pvalue > 0.05, table 2).

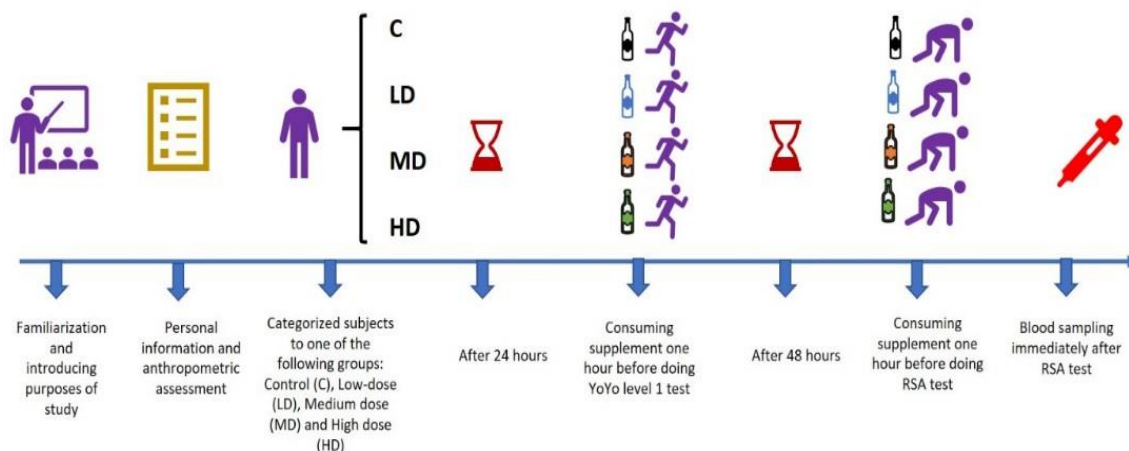


Figure 1. An overall schematic view of the research process

**Table 1. Anthropometric characteristics of subjects from the four groups**

	C (Mean ± SD)	HD (Mean ± SD)	MD (Mean ± SD)	LD (Mean ± SD)	Pvalue
Age (Year)	18.24 ± 0.39	18.17 ± 0.64	18.25 ± 0.34	18.24 ± 0.30	0.98
Height (cm)	173.55 ± 3.77	172.88 ± 4.07	177.66 ± 8	177.11 ± 5	0.16
Weight (kg)	62.66 ± 3.84	66.55 ± 9.24	67 ± 7.90	66.88 ± 9.29	0.60
BMI (kg/m <sup>2</sup> )	20.81 ± 0.34	21.35 ± 3.24	20.92 ± 1.38	20.81 ± 2.07	0.91

**Table 2. Comparison of Vo2max, RSA, urea, and lactate between the groups**

	C (Mean ± SD)	HD (Mean ± SD)	MD (Mean ± SD)	LD (Mean ± SD)	Pvalue
VO <sub>2</sub> max (ml/kg/Min)	50.11 ± 4.50	51.13 ± 6.22	50.83 ± 4.85	47.62 ± 4.16	0.44
RSA (% of reduction rate)	33.72 ± 6.69	34.11 ± 6.34	30.11 ± 5.6	32.33 ± 4.27	0.46
Urea (mg/dl)	4.57 ± 1.94	4.64 ± 1.3	4.62 ± 1.6	4.16 ± 1.46	0.9
Lactate (mg/dl)	107.27 ± 5.08	106.11 ± 7.07	103.66 ± 7.34	105.88 ± 6.97	0.49

## Discussion

Previous studies have tested different dosages of CM supplementation on physical performance and reported contradictory association between CM supplementation and athletic performance.<sup>4,6,12-14</sup> The present study aimed to investigate the effect of acute supplementation of CM at low, medium and high doses on aerobic and anaerobic performance, as well as serum levels of lactate and blood urea of young soccer players. Based on the results of this study, no association was found between different dosages of CT and probably acute CM supplementation does not have obvious effects on aerobic power, anaerobic power, lactate and blood urea levels. Previous studies have reported that citrulline supplementation increases the production of aerobic energy when doing exercise. A probable mechanism for this is the NO-induced vasodilation. Oral supplementation of CM increases conversion of citrulline, the main substrate for NO synthesis, to arginine in blood during the exercise. NO causes vasodilatation and increases blood flow to the activated muscles. It also enhances the availability of oxygen and nutrients to the muscle fibers, while the wastes from metabolism, including lactate, can be removed from muscle cells.<sup>1,9,17,18</sup> On the other hand, malate is one of the mediators of the tricarboxylic acid cycle which can potentially increase H<sup>+</sup>+NADH+in the cycle. Therefore, increased accessibility to malate can indirectly enhance ATP production.<sup>1,6,11</sup>

However, our results demonstrated that acute CM supplementation does not increase aerobic power. In contrast, some studies have reported that short-term consumption of 8gr CM supplements from 7 to 14 days increased the oxidative phosphorylation energy production and thus increased aerobic capacity.<sup>4,14,19</sup> It seems that duration of supplementation is a limiting factor and acute consumption is not enough alone to affect the aerobic capacity of athletes.

There are contradictory reports for anaerobic power, lactate, and blood urea levels. Some studies concluded that 6, 8 or 12gr of CM 1 hour before intensive exercises did not affect lactate levels and exercise performance capacity.<sup>2,6,13,20</sup> On the other hand, consumption of 8gr of the CM proved to be effective on anaerobic power in female tennis players.<sup>18</sup> It also increased the submaximal resistance exercise performance of upper and lower body with intensity equal to 80% of 1RM until fatigue in well-trained females.<sup>21</sup>

The urea cycle in the liver is responsible for converting ammonia into urea. Throughout the intense activity, the conversion of AMP to IMP as well as subsequent production of

ammonia increases, which stimulates the presence of acidosis caused by intensive activity during this process which intensifies hyperammonemia. Therefore, the increase in available citrulline can enhance liver urea and create a protective effect against ammonia poisoning. On the other hand, by increasing the bicarbonate renal reabsorption, the effects of exercise-induced acidosis diminish especially from high-intensity activity.<sup>1,14,17,22</sup> In spite of proposed mechanisms mentioned above for the effectiveness of CM on physical fitness factors, in the present study, acute consumption was not effective at different doses. Probably, factors such as diet and physical fitness, as well as type and severity of activity exercise by the subjects should be considered when examining the effects of CM intake.<sup>14,21,23</sup>

The results of the present study revealed no associations between different dosages of CM where physical and acute supplementation of CM may not be an effective strategy to improve aerobic and anaerobic power performance in young soccer players.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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