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Effect of pre-exercise single dose arginine ingestion on plasma amino acid profile during exhaustive exercise in elite male wrestlers

[Elit erkek güreşçilerde egzersiz öncesi tek doz arjinin alımının tüketici egzersiz sırasında plazma amino asit profiline etkisi]

Hasan Ulas Yavuz

Department of Sports Medicine, Near East University Medical School
Nicosia, Northern Cyprus, Mersin 10 Turkey

ABSTRACT

Aim: The aim of this study was to investigate the possible effects of pre-exercise single dose arginine ingestion on plasma amino acid profile during incremental exercise to exhaustion. **Methods:** Ten volunteer elite male wrestlers (age: 24.7±3.8) participated in this study. Test-retest protocol was done on the same subjects. Exercise protocol started at 90 watts on bicycle ergometry and the load was increased 30 watts in every 3 min till the exhaustion. Arginine (1.5gr/10 kg body weight) or placebo were given to the subjects after 12 h fasting (during the night) and venous blood samples were collected 1 h after the supplementation (right before exercise protocol) and right after the exercise protocol for both test and retest. Plasma amino acid profiles were determined by HPLC. **Results:** Pre- and post-exercise arginine, ornithine and citrulline concentrations were significantly higher in arginine vs. placebo trial ($p<0.05$). Glutamine, tyrosine, methionine, phenylalanine, isoleucine and leucine concentrations increased following exercise only in arginine trial ($p<0.05$). Citrulline and tryptophan levels decreased while alanine levels increased after exercise in both trials ($p<0.05$). There was an increase following exhaustive exercise in total branched chain amino acid concentrations in arginine trial ($p<0.05$) whereas there is no significant change in placebo trial. Total amino acid concentrations were increased with exercise in both trials but post-exercise concentrations was higher in arginine trial compared to placebo trial ($p<0.05$).

Conclusion: Results suggest that pre-exercise arginine supplementation can promote an amino acid profile that could be favorable for performance; however, further investigation is necessary to determine the effect of arginine supplementation on athletic performance.

Key Words: arginine, exhaustive exercise, amino acids, athletes

Conflict of interest: The author reports no conflicts of interest.

ÖZET

Amaç: Bu çalışmanın amacı egzersiz öncesi tek doz arjinin alımının, giderek artan iş yükünde tükenene kadar egzersiz sırasında plazma amino asit profiline olası etkilerinin incelenmesidir. **Gereç ve yöntemler:** Çalışmaya on elit güreşçi gönüllü olarak katılmıştır (yaş: 24.7±3.8). Aynı denekler üzerinde test ve tekrar testi protokolü uygulanmıştır. Bisiklet ergometride 90 watt iş yükünde egzersiz protokolüne başlanmış ve yük her 3 dakikada 30 watt arttırılarak tükenene kadar devam edilmiştir. Deneklere 12 saat (gece boyu) açlığı takiben arjinin (1.5gr/10 kg vücut ağırlığı) veya plasebo verilmiş; test ve tekrar testi protokollerinde venöz kan örnekleri suplemantasyondan bir saat sonra (egzersizin hemen öncesinde) ve egzersizin hemen sonrasında alınmıştır. Plazma amino asit seviyeleri HPLC ile belirlenmiştir.

Bulgular: Egzersiz öncesi ve egzersiz sonrası arjinin, ornitin ve sitrülin konsantrasyonları arjinin alındığında plaseboya göre anlamlı olarak yüksek çıkmıştır ($p<0.05$). Glutamin, tirozin, metionin, fenilalanin, izolösin ve lösin konsantrasyonları arjinin alındığında egzersizi takiben yükselmiştir ($p<0.05$). Her iki durumda da (arjinin veya plasebo) egzersiz sonrasında sitrülin ve triptofan seviyeleri düşerken, alanin seviyeleri yükselmiştir ($p<0.05$). Plasebo alındığında egzersizi takiben plasma toplam dallı zincirli amino asit konsantrasyonunda bir değişme gözlenmezken ($p>0.05$), arjinin alındığında anlamı bir artış görülmüştür ($p<0.05$). Her iki durumda da egzersizi takiben toplam amino asit konsantrasyonu yükselirken, egzersiz sonrası konsantrasyon arjinin alınması durumunda plaseboya göre daha yüksek bulunmuştur ($p<0.05$).

Sonuç: Sonuçlar, egzersiz öncesi arjinin alımının performans için avantajlı bir amino asit profili yaratabileceğini desteklemektedir. Bununla birlikte arjinin alımının sporcu performansı üzerine etkisinin belirlenmesi için ileri araştırmalar gereklidir.

Anahtar Kelimeler: arjinin, egzersiz, amino asit, sporcu

Çıkar çatışması: Yazar çıkar çatışması bulunmadığını bildirir.

Yazışma Adresi
[Correspondence Address]

Dr. H. Ulas Yavuz

Department of Sports Medicine
Near East University Hospital
Lefkosa, TRNC
Mersin 10 Turkey
Tel. +90 392 4440535 Ext.1059
Fax. +90 392 223 64 61
E-mail. uyavuz@neu.edu.tr

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Introduction

Dietary supplements containing arginine, a semi-essential amino acid, are one of the latest ergogenic aids intended to enhance strength, power and muscle recovery associated with both aerobic and resistance exercise [1]. It appears that there is an increasing interest in arginine-based supplements, and, therefore, more knowledge about its effects in healthy physically active subjects is needed [1]. Arginine administration has been claimed to promote an increase in blood perfusion in the active muscle [2], increasing substrates necessary for improving muscular recovery and protein synthesis during and/or after exercise. It also promotes greater removal of metabolites, such as lactate and ammonia [3], which are related to muscle fatigue during intense physical exercise. Review analysis [4] of the effect of both oral and intravenous arginine administration on metabolism at rest and during exercise emphasized that arginine supplementation appears to improve exercise capacity in individuals with cardiovascular disease, but had little impact on aerobic exercise capacity in healthy individuals [4]; while a very recent review claims that there is no clear evidence that arginine supplementation increases the performance regardless of the aerobic or anaerobic nature of the exercise and it is still premature to recommend dietary supplements containing arginine as an ergogenic aid for healthy physically active subjects [1]. There were only five acute studies retrieved from the literature that evaluated exercise performance after arginine supplementation [5-9], three of which reported significant improvements [5,6,9]. Arginine is the only substrate for endogenous synthesis of nitric oxide (NO). The acute effects of arginine supplementation supposedly promote vasodilatation due to enhanced NO synthesis in the active muscle during exercise and may increase the delivery and uptake of fuel substrates in the skeletal muscle [10]. Arginine is a potent stimulator of glucagon secretion at rest as well as during exercise [11]. Glucagon in turn stimulates gluconeogenesis in the liver by increasing the uptake of gluconeogenetic precursors into the liver. This could change plasma concentrations of some amino acids during exercise [12]. Free amino acids are metabolically important during exercise because they provide tricarboxylic acid (TCA) cycle intermediates which can be used as source of energy and have important functions in ammonia metabolism [13].

Studies in athletes have shown that intensive exercise causes a net rate of protein degradation in the muscle, which results in changes in their amino acid content and their release into the blood [14]. This net protein degradation could be modified by ingestion of amino acid mixtures or whey protein [15,16]. Generally, blood amino acid changes during exercise are described after prolonged and intense exercise protocols [17-19]. However, there is only one study showing the changes during intense short exercise protocol to exhaustion [20]. Although it has been shown that chronic arginine aspartate supplementation changes the plasma amino acid concentrations at rest and during a marathon run [12], to our knowledge, this is the first report in the literature examining the effect of pre-exercise arginine ingestion on plasma amino acid profile during the high intensity incremental exercise to exhaustion in elite athletes.

The aim of this study is to investigate the possible effects of pre-exercise arginine ingestion on plasma amino acid profile during incremental exercise to exhaustion.

Materials and methods

Subjects

Ten male national and international level wrestlers joined to the study. Key exclusion criteria included consuming any nutritional supplement in the last 2 months, smoking, a history of coronary heart disease, or any prior or current medical problems that would limit the subject's physical performance. The participants were apparently healthy and free of any significant medical problems. They were also not taking any medications during the time of the study.

Written informed consent was obtained from each subject, and the study protocol was approved by the Human Ethics Committee of Hacettepe University, Ankara, Turkey (Date:02.03.2006, No:LUT 06/75-4).

Study design

The study was carried out according to a cross-over design and lasted two weeks including a wash-out period of one week. The athletes performed an incremental bicycle ergometer test to exhaustion. Half of the athletes ingested the placebo before the first, and the other half before the second exercise protocol (test-retest protocol). The athletes followed their habitual eating and training patterns during the whole study. The subjects were advised to eat qualitatively the same carbohydrate-rich meals for dinner the day before the test and not to eat during the night (overnight fasting). Caffeine, alcohol, and dairy products were not allowed in the evening before the testing day. No physical activity was allowed the day prior to the test. All exercises were done in the same laboratory and same temperature and humidity conditions. Water intake was not allowed during the exercise protocol. The washout period for arginine used in the literature changes as 4 days [7], 7 days [12, 21, 22] and 10 days [23] in different studies. In this study, subject was randomly assigned to arginine or placebo trial separated by a week of washout period. Arginine trial consumed 1.5 g/10 kg body weight arginine capsules (General Nutrition, Pittsburgh, PA, USA) after an overnight fast and waited for sixty minutes for the arginine peak in plasma [24]. Placebo trial consumed equal number of capsules containing starch.

Exercise protocol

Subjects started to warm up at 60 rpm on a Monark cycle ergometer (894E, Monark, Varberg, Sweden) without any load and after 3 min, exercise protocol started at 90 watts and the load was increased 30 watts in every 3 min. Subjects were ask to maintain 60 rpm during the whole protocol. Exercise was continued till the subjects cannot go on cycling 60 rpm at the desired working loads or the subjects wanted to stop. Exercise protocol was modified from classical incremental exercise protocol [25] and was quite similar with the protocols used in the related literature [20,26]. The protocol was tested with two national level wrestlers who are not involved in this study before starting the actual study and the protocol was seen as appropriate for the subjects.

Blood collection

Blood samples were collected into 12 × 2 mL lithium heparin tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA) using a 21-gauge needle and vacutainer system from antecubital vein by a medical doctor. Samples were taken 60 min after the arginine or placebo ingestion (right before the exercise protocol) and right after the exercise protocol. The tubes were centrifuged at 3000 rpm for 10 min and the plasma immediately separated and stored at -80°C.

Plasma amino acid analysis

Subsequently, following thawing, plasma amino acids were extracted with ethanol, and then derivatized with AccQFluor (Waters, Milford, MA). Amino acid concentrations were then determined by reverse-phase high performance liquid chromatography (HPLC; Shimadzu corporation, Kyoto, Japan) with UV (250 nm) and fluorescence (excitation 250 nm, emission 395 nm) detection, using a method modified from van Wandelen and Cohen [27].

Statistics

Data in text and tables are presented as mean and 95%confidence interval of the mean. We compared the amino acid data after versus before exercise using the paired t test. The statistical comparison of the ratios between placebo and arginine trials was also made using the paired t test. The level of significance was set at $p < 0.05$.

Results

Effects of arginine supplementation on the plasma amino acid concentrations were measured before and after incremental exercise to exhaustion of ten elite male wrestlers; the age, height, and weight means of the subjects were as follows; 24.7 ±3.8 years, 174.2 ±5.2 cm, and 80.4 ±4.1 kg. Exercise protocol lasted approximately 23 min. Incremental exercise to exhaustion and arginine supplementation together and separately induced significant alterations in plasma amino acid concentrations (Table 1).

The plasma concentrations of aspartate, glutamate, asparagine, serine, histidine, glysine, threonine, valine, and cystine were not affected neither by arginine supplementation, nor exercise to exhaustion. Although there was no change with exercise in placebo trial, the plasma concentrations of glutamine, tyrosine, methionine, phenylalanine, leucine, and isoleucine found significantly higher following exercise in arginine trial ($p < 0.05$) (Table 1).

Figure 1 displays the plasma concentrations of pre- and post- exercise arginine, ornithine, and citrulline for placebo and arginine trials. Pre- and post- exercise concentrations of arginine and ornithine were found higher in arginine trial compared to placebo trial ($p < 0.05$), but not changed with exercise in neither trial ($p > 0.05$). The pre- and post-exercise concentrations of citrulline were significantly higher with arginine supplementation ($p < 0.05$) whereas exercise decreased the citrulline concentrations in both arginine and placebo trials ($p < 0.05$) (Figure 1). Figure 2 displays the pre- and post- exercise concentrations of tryptophan and alanine of placebo and arginine trials. The pre- or post-exercise concentrations of tryptophan and alanine were not affected by arginine supplementation ($p > 0.05$) but the plasma concentration of tryptophan decreased ($p < 0.05$), whereas alanine concentrations increased following exercise in both trials ($p < 0.05$) (Figure 2). Figure 3 shows pre- and post- exercise total BCAA and total amino acid concentrations for arginine and placebo trials. Although total BCAA concentrations did not significantly change following exercise in placebo trial ($p > 0.05$), there was an increase in arginine trial ($p < 0.05$). Total amino acid concentrations significantly increased following exercise in both placebo and arginine trials ($p < 0.05$), but post-exercise total amino acid concentration was higher in arginine trial compared to placebo trial ($p < 0.05$) (Figure 3).

Discussion

The effect of a single-dose supplementation with arginine on plasma amino acid profile was investigated during an incremental exercise bout to exhaustion. For the first time the total plasma amino acid response to such a supplementation and exhaustive exercise is reported. Besides a marked increase of circulating plasma arginine, many changes in the analyzed plasma amino acids were observed with the supplement.

It is obviously not possible to conclude from the present results if and to which extent the observed changes of the analyzed plasma amino acids affects the amino acid metabolism. However, an excessive supply with several grams of single amino acids, as is the case when supplementing single amino acids or peptides, can cause a large disproportion of the amino acid content of the regular diet [12].

Table 1. Changes in plasma amino acid concentrations (μmol/L) before and after the exercise protocol for placebo or arginine trials

	Arginine Trial		Placebo Trial	
Amino Acids (mmol/L)	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Taurine	43.8 ±7.2	51.1 ±9.4[b]	41.8 ±4.4	42.8 ±6.1
Aspartate	3.7 ±1.4	4.9 ±1.7	4.1 ±0.9	6.1 ±4.0
Glutamate	57.3 ±36.8	50.9 ±17.8	61.7 ±29.6	61.1 ±19.0
Asparagine	47.7 ±6.4	49.2 ±7.8	47.4 ±8.6	46.6 ±8.7
Serine	89.4 ±16.5	93.9 ±19.7	87.9 ±11.0	97.1 ±27.2
Glutamine	508.1 ±65.2	553.7 ±70.9[a,b]	470.7 ±99.2	472.9 ±70.9
Histidine	50.7 ±12.2	56.7 ±12.0	53.4 ±13.8	59.8 ±11.6
Glycine	211.5 ±35.9	217.3 ±34.7	206.7 ±29.5	196.4 ±27.5
Threonine	90.8 ±13.3	90.6 ±12.4	86.4 ±12.4	85.1 ±14.2
Arginine	225.9 ±32.9 [d]	215.3 ±51.4[b]	94.0 ±14.4	112.1 ±38.0
Alanine	346.4 ±77.7	574.6 ±110.3[a]	328.9 ±81.0	477.9 ±112.3[c]
Tyrosine	40.2 ±5.5	45.1 ±8.1[a]	44.3 ±6.2	48.9 ±8.3
Cirtulline	40.2 ±4.9 [d]	35.6 ±5.2[a,b]	31.4 ±5.9	28.6 ±5.2[c]
Tryptophan	34.9 ±7.2	27.6 ±5.9[a]	35.3 ±8.4	29.9 ±7.1[c]
Methionine	20.1 ±2.9	25.1 ±3.9[a]	21.6 ±4.6	26.4 ±6.0
Valine	186.6 ±27.2	193.2 ±25.4	194.6 ±40.1	195.3 ±39.7
Phenylalanine	37.2 ±6.2	40.8 ±5.8[a]	42.6 ±7.8	45.7 ±9.1
Isoleucine	47.8 ±10.1	51.7 ±9.5[a]	53.5 ±12.9	54.5 ±13.1
Leucine	95.4 ±17.0	104.7 ±14.1[a]	105.8 ±21.4	109.9 ±21.9
Lysine	103.7 ±38.0	131.7 ±35.9	107.2 ±31.0	138.9 ±45.3[c]
Cystine	18.6 ±6.9	18.4 ±3.7	21.0 ±9.2	18.4 ±6.6
Ornithine	81.0 ±17.9 [d]	76.4 ±22.0[b]	34.2 ±6.7	34.2 ±6.2
Total BCAA	329.8 ±52.9	349.6 ±47.1[a]	353.9 ±72.0	359.6 ±72.9
Total AA	2360.7 ±230.1	2717.8 ±245.3[a,b]	2174.3 ±240.1	2388.5 ±272.3[c]

Data are presented as mean and 95%confidence interval.
BCAA, Branched chain amino acids; AA, Amino acid
[a]p <0.05 vs. pre-exercise of arginine trial
[b]p <0.05 vs. post-exercise of placebo trial
[c]p <0.05 vs. pre-exercise of placebo trial
[d]p <0.05 vs. pre-exercise of placebo trial

Supplementation with single dose arginine prior to maximal exercise to fatigue we observed significant changes in plasma amino acid profile. It is clear that the cause of fatigue is complex, influenced by events occurring in both the periphery and the central nervous system [28]. The central fatigue hypothesis is a theory based on the observation that exercise promotes an increase in plasma free fatty acids and a decrease in plasma large neutral amino acids such as leucine, methionine, valine, phenylalanine and tyrosine due to uptake by skeletal muscle. As both conditions favor more tryptophan entering the CNS, increased production of brain serotonin might be expected and this could account for the decreased motor drive and increased sensation of fatigue, experienced after a period of strenuous exercise [29]. We observed significant increase in plasma concentrations of all these large neutral amino acids except for valine (it also increased but not statistically significant, probably because of low number of subjects) in arginine trial comparing to placebo trial. This change in amino acid profile may be in favor of delaying the central fatigue. It is also known that blood BCAA (branched chain amino acids –valine, leucine, and isoleucine) and free tryptophan compete for transport through the blood–brain barrier because they are carried by the same transport system [30]. The plasma BCAA increase produced by arginine administration may attenuate the feeling of fatigue. A similar increase

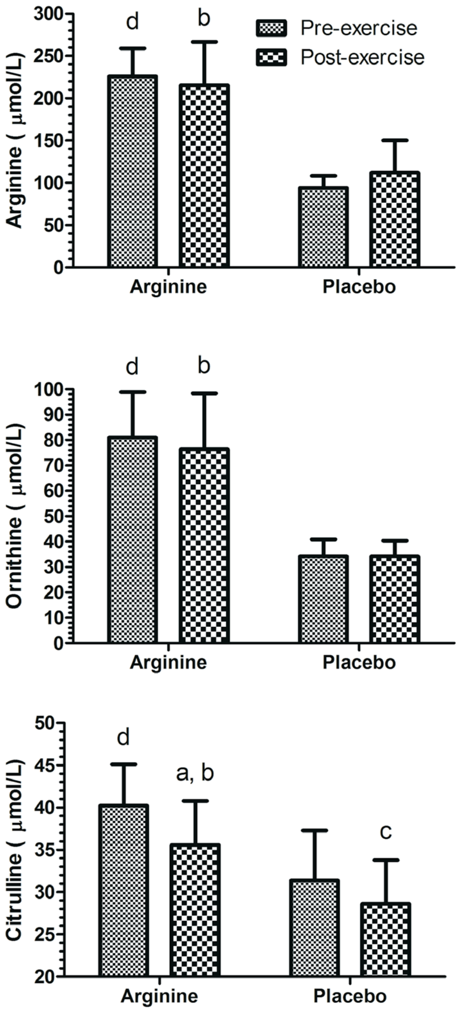

Figure 1. Pre- and post-exercise arginine, ornithine and citrulline concentrations of arginine and placebo trials. Data were presented as mean ±standart deviation.
[a]p <0.05 vs. pre-exercise of arginine trial.
[b]p <0.05 vs. post-exercise of placebo trial.
[c]p <0.05 vs. pre-exercise of placebo trial.
[d]p <0.05 vs. pre-exercise of placebo trial.

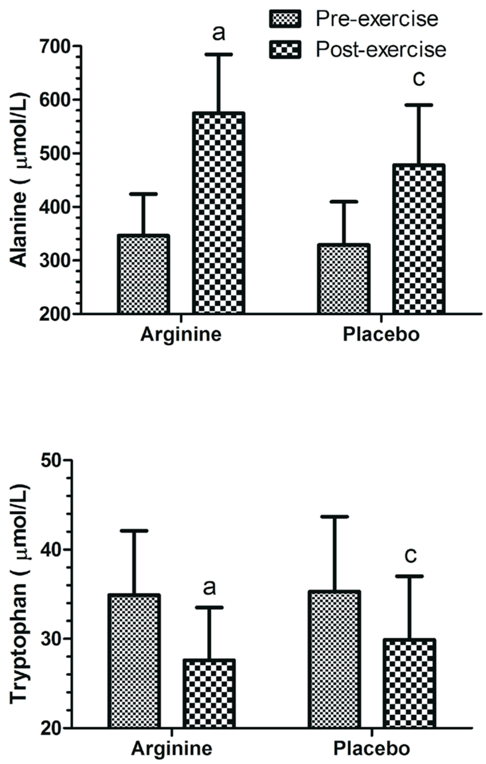

Figure 2. Pre- and post-exercise tryptophan and alanine concentrations of arginine and placebo trials. Data were presented as mean ±standart deviation.
[a]p <0.05 vs. pre-exercise of arginine trial.
[b]p <0.05 vs. post-exercise of placebo trial.
[c]p <0.05 vs. pre-exercise of placebo trial.
[d]p <0.05 vs. pre-exercise of placebo trial.

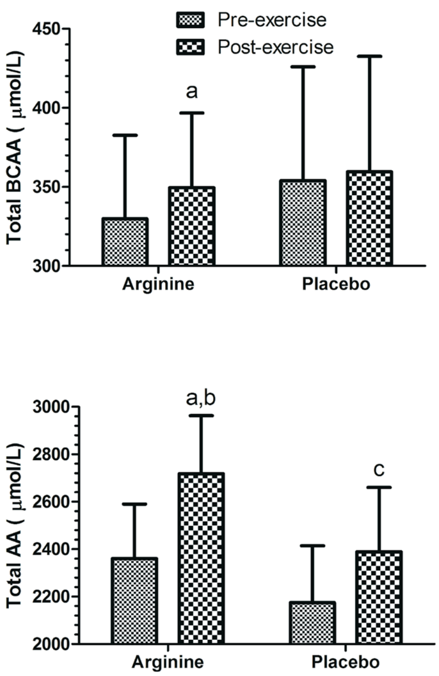

Figure3. Pre- and post-exercise total BCAA (Branched Chain Amino Acids) and total AA (amino acid) concentrations of arginine and placebo trials. Data were presented as mean ±standard deviation.
[a]p <0.05 vs. pre-exercise of arginine trial.
[b]p <0.05 vs. post-exercise of placebo trial.
[c]p <0.05 vs. pre-exercise of placebo trial.
[d]p <0.05 vs. pre-exercise of placebo trial.

in total BCAA was also obtained with ornithine (amino acid synthesized from arginine) supplementation in a recent study [31].

During short-term exercise, the BCAAs in plasma were found to be slightly increased (10 %) [32], decreased (-20%) [33] or unchanged [34] during the exercise period. After prolonged exhaustive exercise, serum BCAA levels have been shown to decrease 22%from values before exercise, whereas serum levels of free tryptophan increased by 74%[35]. It has also been reported that plasma BCAA levels decreased by 19%, whereas free tryptophan plasma levels increased by 15–17%after sustained exercise [36]. Tryptophan concentrations decreased following exercise in both arginine and placebo trials in our study and it is probably because of the different nature (short lasting, very high intensity) of the exercise protocol we used. The competition between tryptophan and BCAA for transport through the blood–brain barrier can be important for central fatigue during prolonged exercise but it may not have same effect for short-term exhaustive exercise.

The amino acid predominantly involved in energy metabolic processes is alanine along with the branched-chain amino acids (BCAA). During exercise, alanine is, among other tissue, synthesized in muscle from other amino acids or by transamination of pyruvate derived from [29]. Muscle protein turnover increases during strenuous physical activity [37] and exercise produces an approximately two- to threefold increase in the flux of alanine into the bloodstream [38]. During maximal exercise until fatigue, both alanine and glucose present high levels in blood, probably indicating an important operation of glucose-alanine cycle [20]. We also found significant increase in alanine concentrations following exercise in both trials but there was no significant effect of arginine supplementation on alanine concentrations.

We found higher pre- and post-exercise concentrations of citrulline in arginine trial and citrulline concentrations decreased following exercise in both groups. Arginine is converted into citrulline and nitric oxide (NO) by nitric oxide synthase (NOS). Since arginine is the only substrate for NOS, an increase in citrulline also means increase in NO. We did not observe exercise induced increase in citrulline reported in previous studies [7,39] Schaefer et al. [40] observed substantially higher concentrations of L-citrulline with intravenous L-arginine hydrochloride during incremental cycle ergometer exercise. Bailey et al [5] observed an increase in NO with oral arginine supplementation whereas Liu et al. [7] showed no differences neither in citrulline nor in NO between arginine and placebo trials during exercise in judo athletes. This variety of the citrulline concentrations can be the result of different exercise intensity, time or the different way of arginine administrations. Ornithine, another direct product of arginine and an intermediate of the urea cycle, was significantly elevated after the supplementation in our study. Similar increases in ornithine concentrations were shown with chronic [12] and short term supplementation with arginine [7].

Total AA concentrations were increased following exercise in both trails and this could be resulted from hemoconcentration but still pre- and post-exercise total AA concentrations were significantly higher in arginine trial. So this can be thought as the effect of arginine supplementation.

It was reported that low oral doses (<20g) of arginine are well tolerated and adverse effects are rare in healthy subjects [41] and we did not observe any side effects in our study.

Since this is the first study to our knowledge that has addressed the effects of arginine supplementation on plasma amino acid profile before and after exhaustive exercise, it is not possible to directly compare the results to other studies.

There are many variables that can affect the performance and it is not possible to say that arginine supplementation will improve performance with this study. Overall, this research only suggests that arginine supplementation can promote a metabolic profile that could be favorable for performance, however further investigation is necessary to determine the effects of arginine supplementation during different types of exercise and performance testing regimens.

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Declaration of interest: The author reports no conflicts of interest.

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