

No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes[☆]

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Abstract

Arginine supplementation has been shown to alleviate endothelial dysfunction and improve exercise performance through increasing nitric oxide production in patients with cardiopulmonary diseases. In addition, arginine supplementation could decrease accumulations of lactate and ammonia, metabolites involved in development of muscular fatigue. The aim of this study was to investigate the effect of short-term arginine supplementation on performance in intermittent anaerobic exercise and the underlying mechanism in well-trained male athletes. Ten elite male college judo athletes participated with a randomized crossover, placebo-controlled design. The subjects consumed 6 g/day arginine (ARG trial) or placebo (CON trial) for 3 days then performed an intermittent anaerobic exercise test on a cycle ergometer. Blood samples were collected before supplementation, before and during exercise and 0, 3, 6, 10, 30 and 60 min after exercise. ARG trial had significantly higher arginine concentrations than CON trial at the same time point before, during and after exercise. In both trials, nitrate and nitrite concentration was significantly higher during and 6 min after exercise comparing to the basal concentration. The increase in nitrate and nitrite concentration during exercise in both trials was parallel to the increase in plasma citrulline concentrations. There was no significant difference between the 2 trials in plasma nitrate and nitrite, lactate and ammonia concentrations and peak and average power in the exercise. The results of this study suggested that short-term arginine supplementation had no effect on nitric oxide production, lactate and ammonia metabolism and performance in intermittent anaerobic exercise in well-trained male athletes.

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1. Introduction

Arginine, a substrate for nitric oxide (NO) synthase, has drawn significant attention for its potential role in alleviating endothelial dysfunction and improving exercise performance through increasing NO production [1]. The

vasodilation effect of arginine has been shown in both central and peripheral circulation. Oral arginine supplementation could improve coronary endothelial function in patients with nonobstructive coronary artery disease [2]. Arginine given orally or intravenously could also improve endothelium-dependent vasodilation in the forearm [3–5]. NO also plays a role in exercise-induced vasodilation in patients and healthy subjects [6–8]. The impairment of NO production and the resulting endothelial dysfunction are the major factors that limit exercise capacity in patients with various cardiopulmonary conditions. As the result, arginine supplementation has been shown to improve exercise

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capacity in patients with hypercholesterolemia [9], chronic heart failure [10–12], pulmonary hypertension [13] and stable angina pectoris [14–16].

Arginine supplementation could also improve exercise capacity by altering the exercise-induced accumulations of lactate and ammonia, metabolites which have been shown to be involved in the development of muscular fatigue due to the increased muscular acidity [17–19]. In addition, ammonia has also been suggested to play a role in fatigue at the central nervous system [20]. It has been shown that 3 g of arginine hydrochloride given intravenously resulted in significantly lower blood lactate and ammonia concentrations compared to a placebo after maximal graded exercise on a cycle ergometer in recreationally active subjects [21]. The reduction in exercise-induced ammonia accumulation may be associated with the increased ureagenesis as ornithine, an intermediate of the urea cycle, was significantly elevated after the supplementation. The reduction in lactate accumulation may have partly resulted from increased peripheral muscle perfusion as the increase in citrulline, a by-product of NO synthesis, was negatively correlated with the increase in lactate. Oral supplementation for 10 days [22] or prior to the exercise [23] of arginine aspartate or arginine glutamate complex could also reduce the elevation in blood lactate and ammonia after strenuous exercise.

The effect of arginine on exercise capacity in healthy subjects and well-trained athletes is less clear. It has been suggested that oral supplementation of arginine, along with glycine and α -ketoisocaproic acid prior to exercise, may increase work output in exhaustive anaerobic exercise in healthy young males [24,25]. The supplementation of arginine and α -ketoglutarate for 8 weeks could also increase peak power in Wingate test in resistance-trained men [26]. However, the role of arginine in these studies was difficult to identify because the two other components may also contribute to the delay of fatigue. On the other hand, it has been revealed that a 14-day supplementation of arginine aspartate did not affect the performance in the subsequent marathon in endurance runners [27].

Despite the numerous studies in cardiopulmonary patients, the role of arginine supplementation on NO production, exercise capacity and exercise metabolism in athletes is still not clear. As arginine could increase exercise-induced vasodilation and remove the metabolites that may inhibit exercise performance, we hypothesized that oral

supplementation of arginine may improve exercise performance in well-trained athletes. The aim of this study was to investigate whether short-term arginine supplementation could improve performance in intermittent anaerobic exercise and the underlying mechanism in well-trained male athletes. Plasma lactate and ammonia concentrations were measured to examine the metabolic effect. Furthermore, plasma concentrations of nitrate and nitrite (NO_x), the major NO metabolites, and citrulline, the by-product of NO synthesis, were measured to determine the role of NO.

2. Methods and materials

2.1. Subjects

Ten elite male college judo athletes recruited from National Taiwan College of Physical Education participated in this study. The subjects with similar body weight were selected to avoid potential difference in exercise performance and supplementation dosage. The potential subjects with known cardiovascular disease risks, with musculoskeletal injuries, or who have taken any protein supplement in the previous 3 months were excluded. The potential subjects with abnormally high or low plasma arginine concentrations in the preliminary screening were also excluded. All athletes have undergone regular judo training for at least 3 years and have competed in national or international level. The subjects were 20.2 ± 0.6 years old. The height were 1.72 ± 0.02 m. The body weight in arginine (ARG) and control (CON) trials were 73.3 ± 2.1 and 73.6 ± 2.0 kg, respectively. The body weight was not significantly different between the 2 trials.

The subjects were asked to maintain their regular training schedule and diet habits during the study period. The subjects were asked to consume the same food on the day prior to each trial. All subjects gave their written informed consent after the experimental procedure, and possible risks were explained. The study protocol was approved by the Human Subject Committee of National Taiwan College of Physical Education.

2.2. Experimental design

This study used a randomized cross-over, placebo-controlled design. Each subject was randomly assigned to ARG or CON trial separated by a 4-day washout period. ARG trial consumed 6 g/day arginine tablets (General

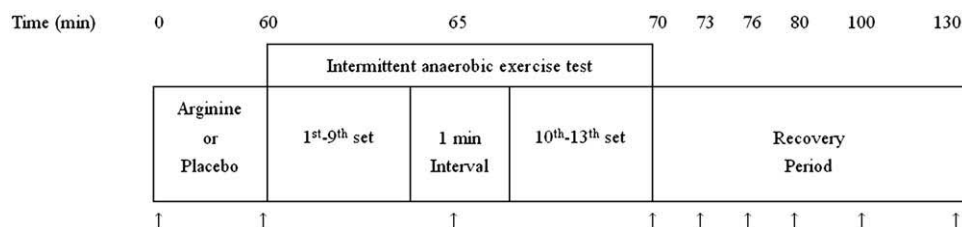


Fig. 1. The schedule of exercise test and blood sampling. ↑, blood sampling.

Nutrition, Pittsburgh, PA, USA) for 2 days. In the third day, the subjects consumed 6 g of arginine after an overnight fast then performed the intermittent anaerobic exercise test 60 min later. CON trial consumed equal number of tablets containing starch. The schedule of exercise test and blood sampling is shown in Fig. 1.

2.3. Intermittent anaerobic exercise test

The intermittent anaerobic exercise test was designed to mimic the actual judo competition. All subjects practiced the test at least once before participating in the study. The subjects alternated 20-s all-out exercise and 15-s rest periods for 13 sets on a Monark cycle ergometer (894E, Monark, Varberg, Sweden). There was a 1-min rest after the ninth set for blood sampling. The load was 0.05 kp/kg body weight. The subjects were asked to pedal as fast as possible with vocal encouragement by research personnel. In the rest periods the load was removed and the subjects were asked to pedal at 60 rpm. The peak and average power of each set was recorded.

2.4. Blood collection

Blood samples were obtained from a cannula in the antecubital vein by licensed personnel. Blood samples were collected before supplementation, before and during exercise and 0, 3, 6, 10, 30 and 60 min after exercise. Hemoglobin content in whole blood was measured to correct potential plasma volume change resulted from the exercise. Plasma samples were stored at -70°C for later analysis.

2.5. Measurement of arginine

Plasma arginine concentration was analyzed with capillary electrophoresis as previously reported [28,29], with slight modifications. Plasma was deproteinized by incubating with equal volume of acetone for 30 min at room temperature. The supernatants were collected after centrifu-

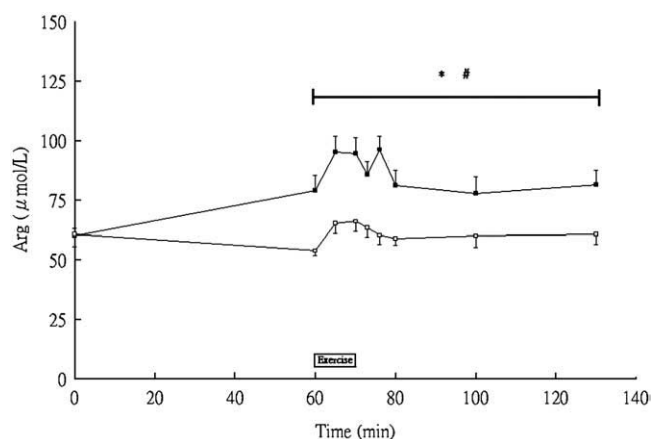


Fig. 2. Plasma arginine concentrations at each sampling time point in ARG (■) and CON (□) trials. * $P < .05$, ARG vs. CON; # $P < .05$ vs. 0 min in ARG trial.

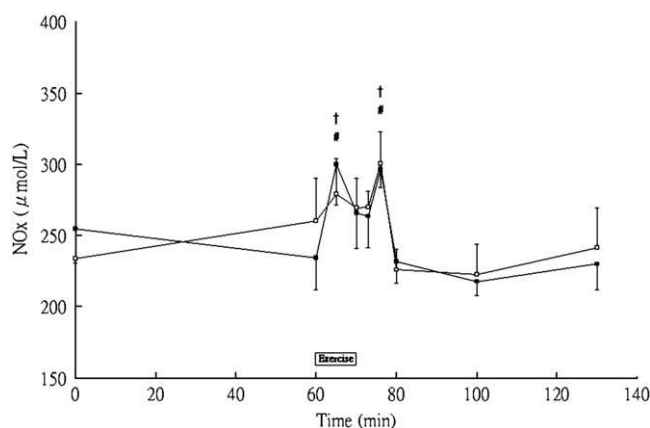


Fig. 3. Plasma NO_x concentrations at each sampling time point in ARG (■) and CON (□) trials. # $P < .05$ vs. 0 min in ARG trial; † $P < .05$ vs. 0 min in the CON trial.

gation at $5600\times g$ for 10 min at 4°C . An aliquot of 100 μL deproteinized plasma was incubated with 50 μL 20 mmol/L sodium borate, pH 10.0, containing 200 $\mu\text{mol/L}$ Nor-leucine and 100 μL 5.5 mmol/L freshly prepared fluoresceine isothiocyanate in acetone in darkness overnight. Nor-leucine served as an internal standard to correct the fluorescence change of amino acid derivatives. After centrifugation at $5600\times g$ for 10 min at 4°C , the amino acid derivatives were diluted 100 times with 20 mmol/L sodium borate for direct injection and analyzed by capillary electrophoresis equipped with argon ion laser-induced fluorescence (LIF) detector (P/ACE MDQ CE, Beckman, Fullerton, CA, USA). The LIF detection was carried out with excitation at 488 nm and emission at 525 nm. An uncoated fused-silica capillary (total length 110 cm and effective length 100 cm; 50 μm internal diameter) was used. Separation buffer was 120 mmol/L sodium borate, pH 9.2, containing 45 mmol/L α -cyclodextrin. Samples were injected into the capillary at 0.5 psi for 5 s and separated at 25 kV for 120 min at 25°C . Arginine concentration was determined by comparing to the standard curve constructed with known amounts of standard. The inter- and intra-assay coefficients of variation of arginine measurement in our lab were 5.20% and 3.57%, respectively.

2.6. Measurement of citrulline

Plasma citrulline concentration was analyzed as previously reported [30] with minor adjustment to allow the measurement by a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). The absorbance was measured at 540 nm.

2.7. Measurement of lactate, ammonia and hemoglobin

Commercial kits were used to measure plasma lactate (Randox, Antrim, United Kingdom) and ammonia (Kanto Chemical, Kanagawa, Japan) concentrations by an automatic analyzer (Hitachi 7020, Ibaraki, Japan). Hemoglobin content in 5 μL of whole blood was diluted with 1495 μL deionized

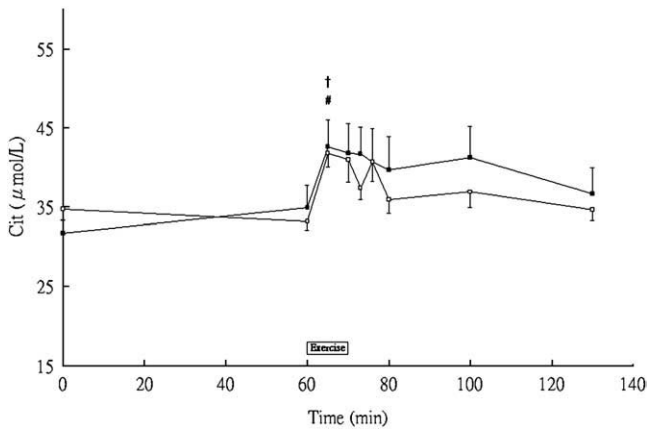


Fig. 4. Plasma citrulline concentrations at each sampling time point in ARG (■) and CON (□) trials. #*P*<.05 vs. 0 min in ARG trial; †*P*<.05 vs. 0 min in CON trial.

water and subsequently determined with a commercial kit (Randox) using the automatic analyzer.

2.8. Measurement of NO_x

Plasma NO_x concentrations were determined by Griess reaction [31] with slight modifications. Nitrate was reduced to nitrite by incubating 100 μl plasma with 10 μl 50 mU nitrate reductase and 100 μl 80 μmol/L NADPH in 20 mmol/L Tris, pH 7.6, for 3 h at 35°C. The supernatants were subsequently deproteinized with 400 μl methanol: diethylether (3:1 v/v) in the dark overnight at room temperature. After centrifugation, 200 μl supernatants were incubated with 50 μl 6.5 mol/L HCl and 50 μl 37.5 mmol/L sulphanic acid for 10 min at 4°C, followed by addition of 50 μl 12.5 mmol/L *N*-(1-naphthyl)ethylene-diamine. After incubation for 30 min at 4°C, absorbance was measured at 540 nm by a microplate spectrophotometer. The results were compared with a standard curve constructed with known concentrations of nitrite.

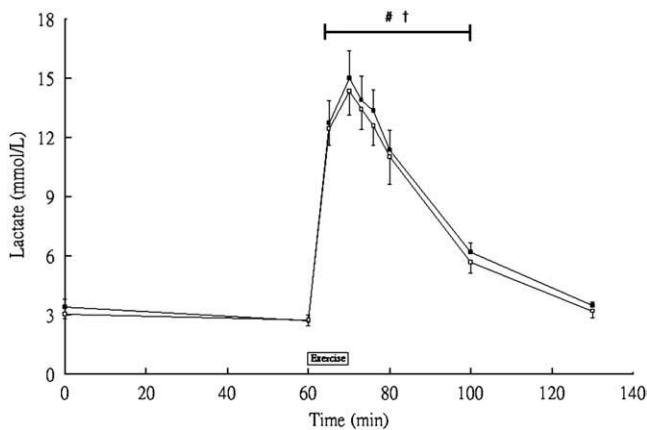


Fig. 5. Plasma lactate concentrations at each sampling time point in ARG (■) and CON (□) trials. #*P*<.05 vs. 0 min in ARG trial; †*P*<.05 vs. 0 min in CON trial.

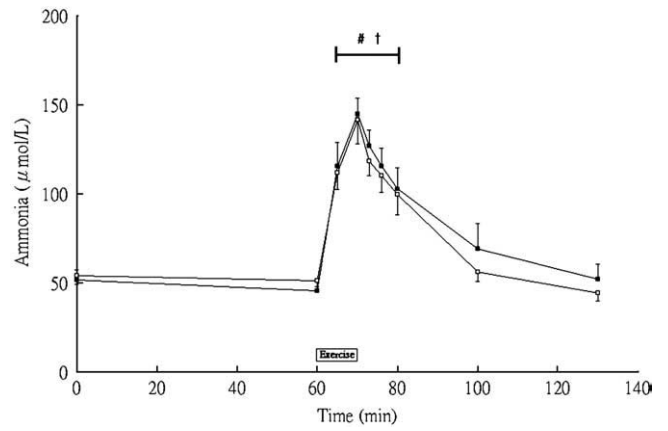


Fig. 6. Plasma ammonia concentrations at each sampling time point in ARG (■) and CON (□) trials. #*P*<.05 vs. 0 min in ARG trial; †*P*<.05 vs. 0 min in CON trial.

2.9. Statistical analysis

All values were expressed as means±S.E.M. The area under the curve was calculated from before exercise to 60 min after exercise. Changes in plasma metabolites concentrations were analyzed by a two-way analysis of variance with repeated measures. The difference between baseline and other time points in plasma metabolites were analyzed by post hoc Tukey’s test. The biochemical and intermittent exercise performance parameters at each time point were analyzed by Student’s paired *t* test. The difference between area under the curve of the two trials was analyzed by Student’s paired *t* test. The analysis was performed with SPSS for Windows 10.0 (SPSS, Chicago, IL, USA). A *P* value less than .05 was considered statistically significant.

3. Results

Plasma arginine concentrations in each sampling time in ARG and CON trials are shown in Fig. 2. The supplement-

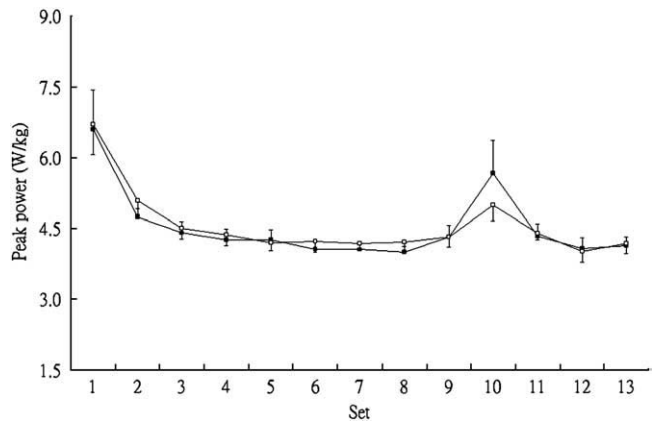


Fig. 7. Peak power in each set of intermittent anaerobic exercise in ARG (■) and CON (□) trials.

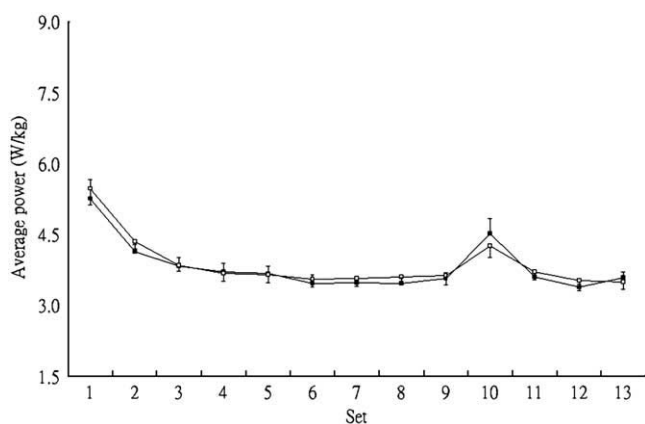


Fig. 8. Average power in each set of intermittent anaerobic exercise in ARG (■) and CON (□) trials.

tation ($P=.013$) and time ($P<.001$) effects were significant. Plasma arginine concentrations were significantly increased from the baseline before, during and after exercise in ARG trial, while it remained unchanged throughout the sampling period in CON trial. As the result, ARG trial had significantly higher arginine concentrations than CON trial at the same time point before, during and after exercise.

Plasma NO_x concentrations at each time point in both trials are depicted in Fig. 3. No significant difference was found between the 2 trials at any time point. In both trials, NO_x concentration was significantly higher during and 6 min after exercise comparing to the basal concentration. The increase in NO_x concentration during exercise in both trials was parallel to the increase in plasma citrulline concentrations (Fig. 4). There was no significant difference in citrulline concentration between the 2 trials at any sampling time point.

Plasma lactate concentration showed significant time effect ($P<.001$). Plasma lactate increased sharply during and immediately after the exercise, then returned to the baseline 60 min after the exercise in both trials (Fig. 5). No significant difference was found between the 2 trials at any time point. Similarly, time effect was significant in plasma ammonia concentration ($P<.001$). Exercise induced significant increase in plasma ammonia concentrations in both trials (Fig. 6). There was no significant difference in ammonia concentrations between the two trials at any time point. There was no significant difference in area under the curve between the two trials in NO_x , citrulline, lactate and ammonia concentrations.

ARG and CON trials showed similar peak and average power in each set of the intermittent anaerobic exercise (Figs. 7 and 8, respectively). In addition, the total power in the entire exercise test was not significantly different between the 2 trials (ARG: 1011.32 ± 0.08 J/kg, CON: 1008.22 ± 0.11 J/kg). Peak and average power declined after the second set then remained stable until the ninth set. After 1 min rest, peak and average power increased in the 10th set then dropped to the similar level of the ninth set.

4. Discussion

The results of this study suggested that short-term arginine supplementation had no effect on NO production, lactate and ammonia metabolism and performance in intermittent anaerobic exercise in well-trained male judo athletes.

Our results showed that the performance in each set of the intermittent anaerobic exercise test were similar in ARG and CON trials. The intermittent exercise protocol used in this study was similar to the regular training and competition pattern of these well-trained judo athletes. The exercise protocol could minimize the potential learning effect. Similarly, it has been shown that supplementation of arginine aspartate for 14 days prior to a marathon run did not affect the subsequent performance in trained runners [27]. On the contrary, it has been reported that supplementation of arginine in combination with glycine and ketoisocaproic acid could reduce the power drop in repeated supramaximal 10-s cycling sprints with 1-min rest intervals in healthy subjects [24]. However, the effect may result from the combination of the compounds, rather than arginine alone.

Both ARG and CON trials showed exercise-induced NO production, as NO_x and citrulline concentrations were significantly elevated during exercise. The mechanism for the increase in NO_x at 6 min after exercise was not clear, as plasma citrulline concentration did not change. However, arginine supplementation had no effect on exercise-induced NO production in our well-trained subjects. This result was in contrast to previous studies that suggested arginine supplementation could improve exercise-induced NO production and vasodilation in subjects with various cardiovascular diseases [9–11,13–16]. In addition, it has been reported that regular exercise training and arginine supplementation may have additive effects on improving endothelium-dependent vasodilation in chronic heart failure patients [32]. It is possible that our athletes already had higher basal concentration of NO than general population and these patients [33]. Regular exercise training has been shown to increase basal NO production [9,34] by stimulating endothelial NO synthase expression and phosphorylation [35]. Therefore, arginine supplementation did not provide any additional effect on NO production in our subjects.

In agreement to our results, Colombani et al. [27] suggested that supplementation of arginine aspartate 15 g/d for 14 days before a marathon run had no effect on postexercise plasma ammonia concentrations in trained runners. Four weeks of arginine aspartate supplementation also had no effect on plasma lactate concentration after graded maximal test in endurance athletes [36]. It has been hypothesized that enhanced muscle perfusion resulted from elevated NO production could lead to improved muscular aerobic metabolism and less lactate accumulation [21]. In addition, NO may increase glucose uptake by working muscles in healthy men, possibly by facilitating the

contraction-induced translocation of GLUT 4 [37]. However, the lack of difference in NO production indicated similar level of blood supply and glucose uptake in working muscles in the two trials. Therefore, glucose metabolism during the intermittent anaerobic exercise in our study may not be altered.

Several studies have shown that short-term oral supplementation or intravenous infusion of arginine could reduce exercise-induced lactate and ammonia accumulation [21,22]. However, these studies used general population with less exercise capacity than our well-trained athletes. The postexercise lactate (ARG: 15.0 ± 1.4 mmol/L, CON: 14.3 ± 1.2 mmol/L) and ammonia (ARG: 162.6 ± 10.2 μ mol/L, CON: 161.8 ± 15.4 μ mol/L) concentrations were similar to the highest values reported in the literature after strenuous anaerobic exercise [38,39]. On the other hand, Schaefer et al. [21] and Denis et al. [22] reported much lower postexercise lactate and ammonia concentrations comparing to our study. In addition, the exercise protocol used in the present study lasted approximately 9 min, while others used graded maximal or submaximal exercise protocols that lasted 15–45 min. It is possible that the large amounts of lactate and ammonia generated during the relatively short period time by athletes may exceed the potential effect of arginine in removing these two metabolites.

The present study used a 6 g/d, 3-day arginine supplementation protocol because it has been shown that a single oral supplementation of 6 g arginine could result in a significant improvement of flow-mediated dilatation in hypertensive patients [3]. It has also been suggested that 4–5 g of oral arginine doses may produce similar results in lactate and ammonia metabolism, comparing to 10–30-g doses, at least in healthy subjects [21]. Thus, the 3-day short-term supplementation protocol was selected instead of those lasting for weeks used by other studies [32,40]. In a preliminary study, we found that plasma arginine concentrations peaked at 60 min after oral consumption (data not shown). In agreement, a pharmacokinetic study showed that plasma arginine concentration reached the highest level at 60 min after consumption of arginine and α -ketoglutarate [26]. It has also been suggested that the vasodilation effect of arginine became significant approximately 60 min after the consumption, when mean pulmonary arterial pressure and pulmonary vascular resistance began to fall [13]. As the result, the exercise test was performed 60 min after the supplementation in the present study. In our subjects, plasma arginine concentration increased by 31.3% (78.9 ± 6.5 μ mol/L) at 60 min and by 58.1% (95.0 ± 6.8 μ mol/L) during exercise comparing to the baseline (60.1 ± 3.0 μ mol/L) in ARG trial. Previous studies have revealed that intravenous infusion of arginine could significantly increase plasma arginine concentration while effectively increasing endothelial-dependent vasodilation [41,42] and decrease post-exercise lactate and ammonia accumulation [21]. Therefore, it is reasonable to assume that arginine concentrations in our subjects after supplementation

were similar to those in previous studies that showed beneficial effect of arginine.

This study suggested that the short-term supplementation of arginine had no effect on plasma NO, lactate and ammonia concentrations and performance in intermittent anaerobic tests in well-trained male athletes. The use of arginine supplementation to increase NO production, reduce metabolites accumulation and improve exercise performance in athletes should be critically reevaluated even though it may have beneficial effect in certain patients and general population. Further investigations with higher dosages, extended supplementation periods or in combination with other compounds are warranted.

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