International Journal of EXERCISE SCIENCE

Original Research

Acute Effects of L-Arginine Supplementation on Oxygen Consumption Kinetics and Muscle Oxyhemoglobin and Deoxyhemoglobin during Treadmill Running in Male Adults

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ABSTRACT

International Journal of Exercise Science 12(2): 444-455, 2019. L-arginine is used as a nitric oxide related supplement intended to improve sports performance, and to enhance muscular recovery during exercise. However, the literature is inconclusive. The aim of this study was to determine the effects of acute oral L-arginine supplementation on O2 consumption kinetics and local muscle blood volume and oxygenation during treadmill running at two different intensities. Using a double-blind, crossover and placebo-controlled design, 11 young healthy male adults were randomly assigned to 6 g of L-arginine (ARG) or placebo (PLA) supplementation that was ingested 60 min before the exercise test. Tests consisted of treadmill run at two different intensities (5 min each; moderate, 90% of ventilatory threshold, VT; and heavy, 50% of the difference between VT and VO2peak) interspersed by 1-min walking. Respiratory gas exchange variables were measured continuously with an automated metabolic cart. Near infrared spectroscopy (NIRS) was used to continuously monitor muscle oxyhemoglobin and deoxyhemoglobin and total hemoglobin. Blood samples were collected before supplementation and 6 min after exercise. Two-way repeated measures ANOVA did not show differences in plasma nitrite concentrations between ARG or PLA conditions during the running tests. No significant differences were observed between ARG and PLA conditions for O2 kinetics as well as for NIRS variables. ARG supplementation does not improve physiological responses associated with oxygen cost and NIRS variables during running treadmill tests. Hence, our results do not support the use of L-arginine as an ergogenic aid for running performance in young healthy males.

KEY WORDS: Muscle oxygenation, pulmonary variables, aerobic exercise, ergogenic aids

INTRODUCTION

Nitric oxide related supplements are amongst the most sold dietary supplements, but their benefits are still debated. Nitric oxide is a small gaseous molecule that has a very short half-life,

which precludes its administration in solid forms by oral route. The rationale beyond its use lies mainly on its known vasodilator properties, which could theoretically increase blood flow to exercising muscles leading to increased delivery of nutrients and oxygen and removal of metabolic waste products (3). Therefore, such products are based on substances that can be converted to nitric oxide, and includes organic nitrates or the amino acids L-arginine and L-citrulline.

L-arginine consists the basis of most nitric oxide related supplements. Despite its widespread use, its ergogenic properties are still debated and controversial findings are reported in the literature. At one side, acute L-arginine administration has shown to increase isokinetic peak torque (24), anaerobic power (11), and cycling time to fatigue (6). However, opposite findings have also been reported, including the absence of ergogenic effects during moderate and severe intensity running or cycling exercise (4, 28).

One important finding was that three days of ARK1 (Arkworld International, USA) supplementation, which contains L-arginine as the major component (6 g per serving), reduced the oxygen (O2) cost of moderate intensity cycling (6). This was the first study demonstrating that oral L-arginine supplementation was able to improve exercise economy, i.e. to reduce O2 consumption (VO2) for the same task. As lactate levels have been shown to be reduced (23) or unaffected (18) following L-arginine supplementation, this finding suggests an important role of this amino acid in the regulation of oxidative metabolism. Actually, nitric oxide related supplements are unique in this aspect, as no other dietary intervention has been shown to do this, and similar findings were shown using dietary nitrate as a supplement (5, 19). In addition to reduced pulmonary VO2, dietary nitrate seems to reduce muscle O2 extraction during moderate intensity cycling (5). These findings can be of relevance not only for exercise performance, but also to clinical populations that show exercise intolerance as a hallmark feature, such as heart failure or peripheral artery disease. Divergent findings, however, have also been reported. Vanhatalo et al. (28) did not observe any significant difference in O2 cost of running or cycling exercise performed at moderate and severe intensities after acute L-arginine administration. The authors attributed the difference in results to the fact that the former study used a commercial product that also contains other components, including L-citrulline, which is also a source for nitric oxide. However, the amount of citrulline per serving (12.5 mg) is well below the amount offered in "pure" L-citrulline (6 g) studies that have shown ergogenic effects of this amino acid (4). Therefore, we do not believe that the divergent findings might be attributed to L-citrulline, and this warrants further research.

L-arginine might exert its beneficial effects via increased nitric oxide production, which occurs via a five-electron oxidation of a guanidino nitrogen of L-arginine in a reaction catalyzed by the family of enzymes nitric oxide synthases (29). Even though it is well known that exogenous L-arginine can be converted into nitric oxide (22), some (4, 6) but not all studies (1, 28) have shown increased systemic nitric oxide levels following acute L-arginine supplementation in healthy subjects. Assuming that L-arginine could lead to increased nitric oxide, blood flow to exercising muscles might be increased, therefore improving muscle performance and recovery. Indeed, we

have previously shown that muscle blood volume was increased during recovery from sets of resistance isokinetic exercise (2).

Considering the inconsistency among the studies that investigated the effects of L-arginine upon aerobic exercise performance, we aimed to assess whether acute oral supplementation of L-arginine would reduce the O2 cost of treadmill running at moderate and severe intensities. In addition, we also investigated the changes in O2 extraction and blood volume in exercising muscle, as well as plasma nitrite levels.

METHODS

Participants

Eleven young adult males, all enrolled at the Brazilian Army Physical Education Undergraduate Program (25 ± 2 years; 75.4 ± 9.5 kg; 177 ± 7 cm) volunteered to participate in the study. As required by the Brazilian Army, all students undergo complete physical and clinical examination assessment prior to the beginning of the undergrad program. Exclusion criteria were as follows: overweight, smoking, vegetarians, current musculoskeletal injuries of lower limbs, and supplements or stimulants intake within the last 30 days. The subjects were advised to maintain their eating and physical activity habits during the study period. All participants received a detailed verbal explanation of the study procedures and risks involved in participating, and signed a written informed consent form. The Ethics Review Board of the Brazilian Air Force Hospital (Galeão) approved the study (Approval #509.212), following the recommendations of the World Medical Association's Declaration of Helsinki.

Sample size was calculated based on a Two-Way ANOVA with repeated measures with interaction intra and inter conditions; Cohen effect size (f) = 0.4; error α = 0.05; power of test = 0.80; number of conditions 2; repeated measures = 2; between repeated measures correlation = 0.80; non sphericity correction = 1. Sample size was estimated to be 8. Due to the characteristics of the study and the increased chance of dropouts, sample size was increased to eleven subjects instead.

Protocol

This study was characterized by a double-blind, cross-over and placebo-controlled design. The entire study consisted of three testing sessions. In the first session, subjects were submitted to an incremental exercise test on a treadmill. The second and third sessions consisted of treadmill running tests under L-arginine (ARG) or placebo (PLA) conditions, in random order, separated by a one-week washout period. In these experimental sessions, blood samples were withdrawn from the antecubital vein of the subject's preferred arm and stored for posterior analysis.

Incremental treadmill test: For determination of describing VO2peak and ventilatory threshold (VT), subjects were submitted to a continuous ramp incremental test protocol on a treadmill (SensorMedics, Yorba Linda, CA, USA), at a 1% slope. The test started with a 3-min warm up walking at 6 km h-1, after which the treadmill speed was increased by 0.7 km.h-1 every minute until the subject was unable to continue. They were connected to an automated metabolic cart

(Ultima CPX, MGC Diagnostics, Minneapolis, USA), and minute ventilation and respiratory gas exchange variables were continuously collected on breath-by-breath mode. Data were registered as the mean value of each three seconds. The metabolic cart was calibrated for volume and gas concentrations prior to each exercise test following the procedures recommended by the manufacturer.

VO2peak was recorded as the highest value attained prior to volitional exhaustion (13). The VT was determined by means of the modified V-slope method (15), using a computer program that utilizes an iterative regression and analysis of the slope of VCO2 vs. VO2 to determine where carbon dioxide production begins increasing disproportionately to the oxygen consumption.

L-Arginine and Placebo Supplementation: Subjects were orally administered 6 g ARG (L-arginine hydrochloride, Labrada Nutrition, CT, USA) or 6 g maltodextrin (PLA). Each dose consisted of twelve non-identifiable capsules, consumed 60 min before the beginning of running tests. Supplementation timing was chosen based on previous studies that have shown an increase in plasma L-arginine levels 60 minutes after oral supplementation (8, 26, 30).

Subjects were asked to refrain from foods and beverages rich in nitrite and nitrate for 24 h before running tests, following a list of prohibited foods according to Pannala et al. (20). Because tests occurred in the afternoon, subjects had breakfast and a morning snack, but remained fasting for 3 hours before each running test.

Experimental running tests: The running tests were performed on a motorized treadmill. The tests initiated 60 min after the ARG or PLA ingestion, with 1-min warm up walking at 6 km.h-1, followed by 5-min running at moderate intensity, 1-min walking at 4 km.h-1, 5-min running at heavy intensity, and 1-min recovery walking at 4 km.h-1 (Figure 1). Moderate intensity running was performed at a speed equivalent to 90 % of the VT, and heavy intensity running, at 50 % of the difference between VT and VO2peak.

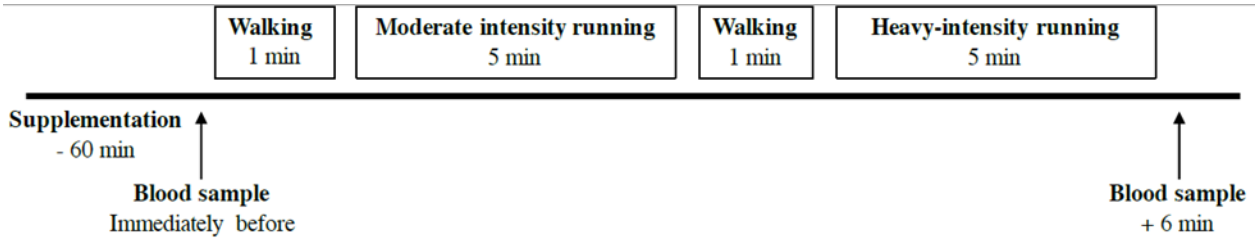

Figure 1. Illustration of an experimental session.

Minute ventilation and respiratory gas exchange variables were collected continuously throughout the running tests using the same automated metabolic cart as indicated previously.

VO2 data for each subject measured breath-by-breath were non-linearly interpolated in order to provide second-by-second values, with minimization of the sum of the squared residuals as the primary goal. A single-exponential model was used to characterize the VO2 responses to

moderate exercise while a bi-exponential model was used for heavy exercise, using the equations described by Bailey et al. (5):

(Eq. 1 – Moderate exercise)

$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + Ap\,[1\text{-}e - (t\text{-}TDp/\tau p)]$
Where
$\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t;
$\dot{V}O_2$ baseline represents the mean $\dot{V}O_2$ in the baseline period;
Ap, TDp, and τp represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline.

(Eq. 2 – Heavy exercise)

$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + Ap\,[1\text{-}e - (t\text{-}TDp/\tau p)] + As\,[1 - e - (t\text{-}TDs/\tau s]$
Where
$\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t;
$\dot{V}O_2$ baseline represents the mean $\dot{V}O_2$ in the baseline period;
Ap, TDp, and τp represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline;
As, TDs and τs represent the amplitude of, time delay before the onset of, and time constant describing the development of the $\dot{V}O_2$ slow component, respectively.

Blood collection and plasma nitrite levels: Samples of 6 mL of blood were withdrawn from the antecubital vein from the subject's preferred arm, using a sterile needle in K2 EDTA containing tubes, immediately before and 6 min after the running tests. Samples were centrifuged at 1000 g for 10 min, and plasma was removed and stored at –70 ^{o}C until analysis.

Plasma nitrite levels were determined as an indicator of NO production according to the Griess reaction. In brief, 50 µL of plasma sample was incubated with 50 µL of Griess reagent (1% sulfanilamide, 0.1% N-1-naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) for 10 minutes at room temperature. A standard curve was performed using sodium nitrite and absorbance was measured at 540 nm (Fluostar Omega, BMG Labtech, Germany).

Muscle blood volume and oxygenation status: The concentration of muscle oxygenated, deoxygenated, and total hemoglobin/myoglobin were collected continuously throughout the exercise protocols using a near-infrared spectroscopy (NIRS) system (PortaMon, Artinis Medical Systems BV, Zetten, Holanda), and later analyzed by a dedicated software (OxySoft versão 2.1.1-2.1.6, Artinis Medical Systems BV, Zetten, Holanda).

The NIRS probe was placed on the skin over the belly of the vastus lateralis muscle of the right thigh. Adipose tissue thickness at this site did not impair light penetration, because in all subjects the skinfold thickness was smaller than the penetration depth of the NIRS light, which is 25 mm (27). To secure the probe on the skin and minimize movement during exercise, an

elastic bandage was wrapped around the subject's thigh. The NIRS device was calibrated according to the manufacturer's recommendation before tests.

NIRS data acquisition consists of an emission probe that irradiates laser beams and a detection probe. Two different wavelength laser diodes provide the light source (760 nm and 850 nm), and a photomultiplier tube in the spectrometer detects the light returning from the tissue. The intensity of incident and transmitted light was recorded continuously at 10 Hz and used to estimate total muscle microvascular concentrations of oxyhemoglobin (O2Hb) and deoxyhemoglobin (HHb). Total hemoglobin (tHb, O2Hb plus HHb) and difference in Hb (HbDiff, O2Hb minus HHb) are considered an indicator of muscle blood volume and oxygenation status, respectively.

Considering the behavior of the NIRS variables during exercise or recovery, the minimum values of tHb, O2Hb and HbDiff, as well as the maximum values of HHb obtained at the end of each running period were reported. During recovery, the maximum values of tHb, O2Hb and HbDiff, as well as the minimum values of HHb were considered for anaylsis.

Statistical Analysis

All data are presented as mean and standard deviation. A dependent t-test was applied to identify differences in all dependent variables between ARG versus PLA conditions for each analyzed period. A two-way analysis of variance with repeated measures on two factors (2 x 2; condition vs. time) was used to test differences in plasma [nitrite] between ARG and PLA. All analyses were performed using SPSS 20.0 for Mac (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at a P value ≤ 0.05.

RESULTS

The ARG and PLA supplements were well tolerated, and no subject reported adverse effects. The mean V̇O2peak was 54.9 ± 3.2 mL.kg-1.min-1, and the VT corresponded to 62.4 ± 6.9% of V̇O2peak. The running speeds corresponding to moderate- and heavy-intensity exercises were 9.7 ± 1.4 km.h-1 and 14.0 ± 0.7 km.h-1, respectively.

The typical behavior of O2 consumption response following placebo or ARG supplementation during moderate- and heavy-intensity running is illustrated in Figure 2. The main finding of the present study was that no significant differences were found for O2 cost of running at neither moderate nor heavy intensity (Table 1). At moderate intensity, there were no statistically significant differences in V̇O2 phase II time constant (τ), amplitude, as well as V̇O2 at steady state (plateau) between ARG and PLA conditions. Similarly, ARG supplementation did not affect any of the aforementioned variables when exercise was performed at heavy intensity.

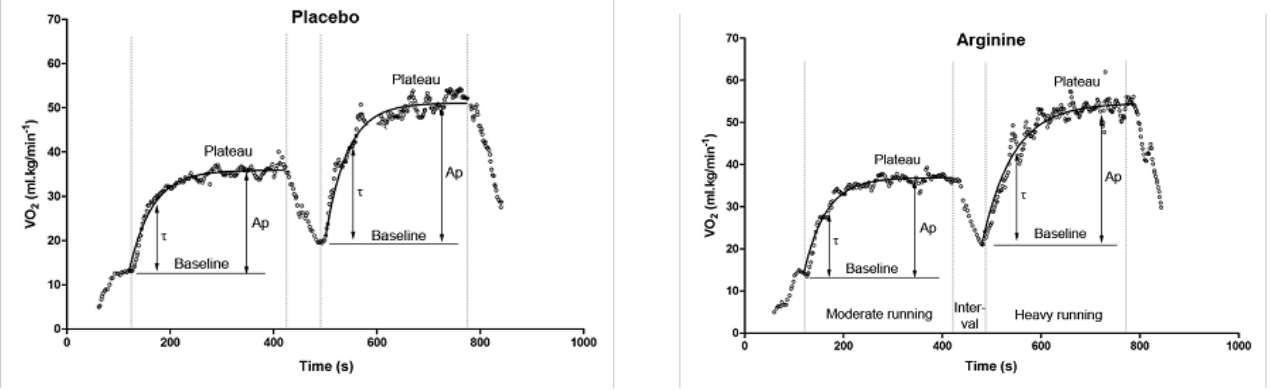

Figure 2. A typical example of oxygen uptake response during the treadmill running test at moderate and heavy intensities for one subject supplemented with placebo (left panel) and arginine (right panel). τ: time constant; Ap: amplitude.

Table 1. Steady-state oxygen kinetics [mean (SD)] during treadmill running tests at moderate- and heavy-intensities under placebo (PLA) and L-arginine (ARG) conditions

	Moderate-intensity		Heavy-intensity	
	PLA	ARG	PLA	ARG
Amplitude (mL.kg^{-1}.min^{-1})	22.1 (6.8)	20.9 (6.7)	30.0 (2.6)	29.3(3.6)
τ (s)	46.6 (10.3)	44.5 (9.4)	50.8 (14.8)	54.2 (12.3)
Plateau (mL.kg^{-1}.min^{-1})	34.8 (5.7)	34.2 (6.0)	49.1 (4.4)	47.3 (5.9)

Figure 3 shows typical NIRS traces for O2Hb, HHb, HbDiff, and tHb in one subject under PLA and ARG conditions. At both intensities, as soon as exercise was initiated, there was a modest increase in muscle blood volume (represented by total hemoglobin), and a sharp decrease in muscle oxygenation (represented by hemoglobin difference), suggesting an increase in O2 extraction by exercising muscle. In recovery periods, hyperemia and re-oxygenation were seen in both conditions and intensities.

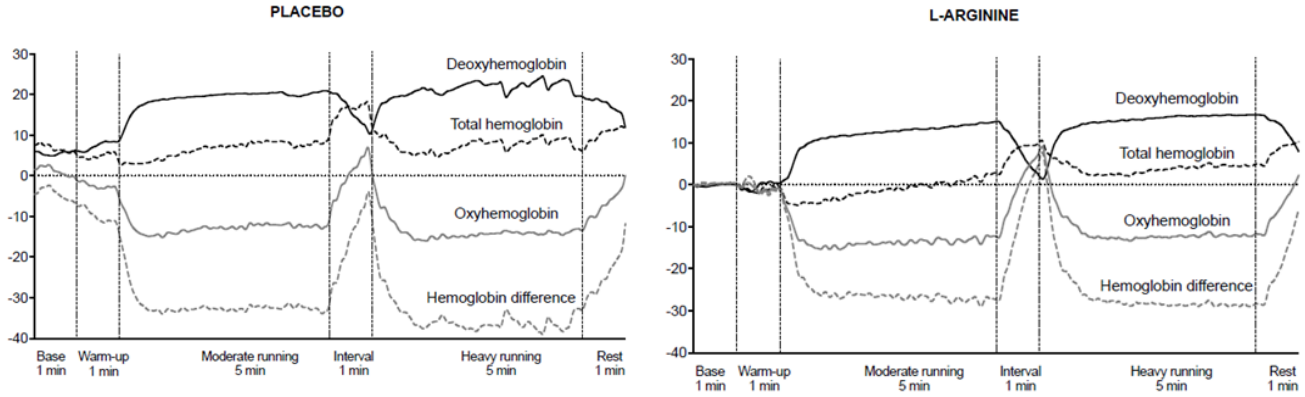

Figure 3. Muscle NIRS variables trends during the treadmill running test at moderate and heavy intensities in placebo (upper panel) and arginine (lower panel) conditions. Data were smoothed, averaging 10 values on each point.

No significant differences were detected in NIRS variables between PLA and ARG conditions during baseline, running or recovery periods (Figure 4).

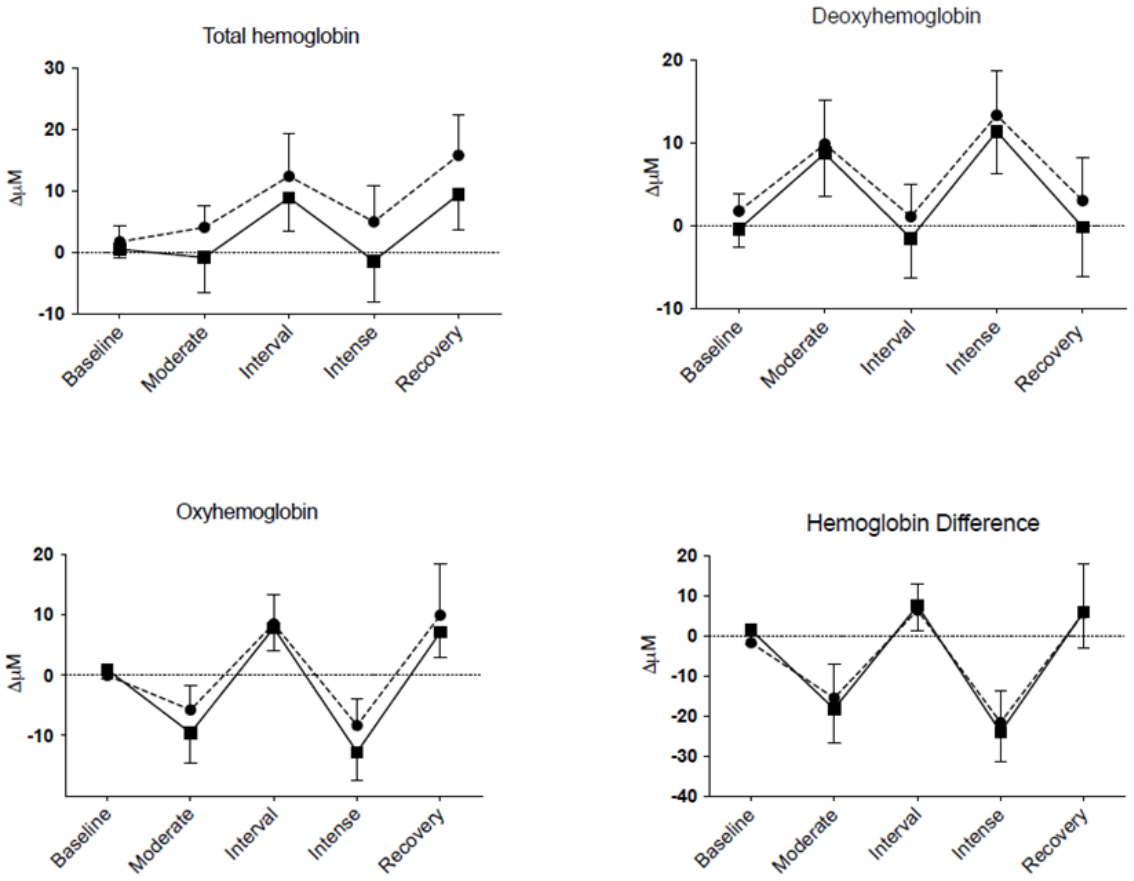

Figure 4. Mean changes in NIRS variables during treadmill running tests in placebo (solid squares and solid lines) and arginine (solid circles and dotted lines) conditions.

A statistically significant main effect for time - before vs after the running tests - was observed in plasma nitrite concentrations, but no interaction was found between PLA (from 0.34 ± 0.16 to 0.51 ± 0.14 mmol/mg protein) and ARG (from 0.40 ± 0.16 to 0.51 ± 0.21 mmol/mg protein).

DISCUSSION

Despite the widespread use of L-arginine based supplements, there is still a lack of consensus about its ergogenic effects. In summary, our data demonstrate that an oral administration of 6 g of L-arginine 60 minutes before aerobic exercise performed at moderate and heavy intensity does not increase NO synthesis, localized blood volume and oxygenation, and does not reduce O2 cost of exercise.

One of the main areas of discussion, related or not to exercise performance, is whether L-arginine supplementation leads to an increase in NO production. Systemic L-arginine levels are well above the Km (concentration of substrate that allows half maximal rate of the enzyme-mediated reaction) of eNOS, but despite that, exogenous L-arginine administration has been demonstrated to lead to increased eNOS activity (22). This phenomenon is termed the "L-arginine paradox" (9). Here, we did not observe increased plasma levels of nitrite, a NO metabolite that is valid to assess its level (22). Similar findings have been reported in the

literature (1, 28), but others have reported increased levels of nitrite after L-arginine supplementation (4, 5). The reason behind different findings is still unknown. It has been argued that Bailey et al. (5) used a commercial supplement (AKR1TM, Arkworld International, USA) that also contained L-citrulline and that was colored with beetroot. However, the amount of L-citrulline given was far below the amount of L-citrulline that has been shown to result in an increase in NO (12.5 mg versus 6 g (4)). The same argument can be used for beetroot as the coloring agent, as the amount of nitrate (5 µmol) given is not expected to exert any physiological effect (28). It is not possible, however, to exclude a synergistic effect between those amino acids to increase NO synthesis.

It is important to highlight that the dose and timing of L-arginine given in the present study does lead to an increase in plasma L-arginine levels in healthy subjects, but that is not always translated into increased NO metabolites levels (4, 14, 25). Therefore, L-arginine bioavailability after oral administration was not a concern. However, its metabolic fate is not so easy to predict. Exogenous L-arginine might be used as a substrate in other metabolic pathways in which this amino acid is involved, or it may be degraded by liver arginases and excreted by the kidneys (26).

In the present investigation, no difference was observed in any of the variables derived from $\dot{V}O_2$ kinetics analysis - phase II time constant, amplitude and $\dot{V}O_2$ at steady state - at both moderate and heavy intensities. The effects of acute or short-term L-arginine supplementation on $\dot{V}O_2$ kinetics are equivocal, and it seems to depend on exercise intensity and mode, as well as on duration of supplementation (4, 6, 7, 18). Theoretical assumptions suggests a possible role of L-arginine in the modulation of $\dot{V}O_2$ during aerobic exercise, which is still one of the major areas of discussion among exercise physiologists, involving two major hypotheses that are not mutually exclusive. Some defend that O_2 supply to exercising muscles might be determinant of $\dot{V}O_2$ kinetics while others state that the limitation is intracellular, related to mitochondrial $\dot{V}O_2$ (21). In this sense, L-arginine supplementation, as a substrate for NO synthesis, might have a dual and opposite role in the modulation of $\dot{V}O_2$ kinetics. Nitric oxide is well known for its vasodilatory properties, which would lead to greater muscle perfusion and O_2 delivery (16), theoretically speeding up $\dot{V}O_2$ kinetics. On the other side, NO is a competitive inhibitor of mitochondrial cytochrome c oxidase, resulting in a slower $\dot{V}O_2$ kinetics (10). It has been shown, inclusive, that L-NAME infusion (a NOS inhibitor) prevents NO inhibitory effect over the mitochondria, leading to a faster $\dot{V}O_2$ kinetics (17).

Regardless all that, no difference was observed in phase II time constant at the onset of moderate or heavy running intensity after acute L-arginine or placebo administration, supporting previous studies (4, 6, 28). This finding suggests that the intracellular metabolic perturbations during the start of exercise were not affected by L-arginine supplementation. Divergent findings, however, have been reported. F days of L-arginine supplementation resulted in a faster $\dot{V}O_2$ kinetics during moderate intensity cycling exercise (18); conversely, three days of supplementation slowed $\dot{V}O_2$ kinetics during cycling at heavy intensity (6). The major difference among these studies was the duration of L-arginine supplementation, but with the available

evidence up to the present moment it is not possible to ascribe the different results found to this variable.

Regarding $\dot{V}O_2$ amplitude, it was demonstrated that L-arginine supplementation causes a reduction during moderate, but not severe intensity cycling (6). This was the only study showing that L-arginine could reduce the O_2 cost of exercise, a result that was also observed when dietary nitrate was administered (5). This was a very impressive finding, as no other dietary supplement has been shown to improve exercise economy. Mechanisms proposed for improved L-arginine metabolic efficiency include increased pool size of the intermediates of tricarboxylic acid cycle, fumarate and α-ketoglutarate (18), and. improved oxidative phosphorylation efficiency by a reduction in mitochondrial proton leak (12). However, this is not a universal finding. Indeed, the vast majority of studies does not support this finding, as demonstrated by our study, in which $\dot{V}O_2$ amplitude at moderate or heavy intensity running did not differ between L-arginine and placebo supplementation, and by others (4, 7, 18, 28).

Contrary to its claims, L-arginine supplementation did not increase blood supply to the vastus lateralis muscle during continuous exercise. There was no difference in the concentration of total hemoglobin following L-arginine administration at baseline, during moderate and intense running or during recovery from these two exercise bouts compared to placebo. Therefore, L-arginine supplementation may not result in an increase in the delivery of O_2 and nutrients to exercising muscle nor in the removal of metabolic waste products. This was probably due to the fact that acute L-arginine supplementation failed to increase NO levels in the present study. In addition, we did not observe an increase in muscle fractional O_2 extraction and oxygenation level. Similar findings were recently reported following pure L-arginine administration, despite an increase in plasma nitrite levels (4).

In conclusion, our findings indicate that acute arginine supplementation is not able to improve physiological responses associated with oxygen cost and hemodynamics during running treadmill tests. Therefore, in the light of our results and the evidence available in the literature, it appears that arginine supplementation is an ineffective ergogenic aid for aerobic training. Further studies are needed to investigate the use of arginine supplementation in aerobic activities in healthy subjects.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Dr. Silvio Rodrigues Marques Neto (Graduate Program in Physical Activity Sciences, Salgado de Oliveira University) for ventilatory data fitting, and Army Captain Jean Soares Amaral (Exercise Physiology Laboratory, Army Physical Training Research Institute - IPCFEx) for helping with the gas exchange data collection during the treadmill tests. No sources of funding were used in the preparation of this article. C Matsuura and PSC Gomes are Universidade do Estado do Rio de Janeiro Prociência Scholarship Recipients. P S C Gomes is a Productivity Research Scholarship Recipient from the Brazilian National Council for Scientific and Technological Development (CNPq)

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