Acute L-arginine supplementation increases muscle blood volume but not strength performance

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Abstract: L-Arginine (L-arg) is an amino acid precursor to nitric oxide (NO). Dietary supplements containing L-arg have been marketed with the purpose of increasing vasodilation, thereby elevating blood flow to the exercising muscle and enhancing the metabolic response to exercise. Our goal was to identify the acute effect of L-arg supplementation on biceps strength performance, indicators of NO production (nitrite and nitrate – NOx), and muscle blood volume (Mbv) and oxygenation (Mox) during recovery from 3 sets of resistance exercise. Fifteen males participated in a randomized, double-blind, placebo-controlled study. After withdrawing resting blood samples, the subjects were supplemented with 6 g of L-arg (ARG) or placebo (PLA). Monitoring of Mbv and Mox with near-infrared spectroscopy began 30 min after supplementation and lasted for 60 min. The exercise protocol (3 sets of 10 maximal voluntary contractions of isokinetic concentric elbow extension at $60°{\cdot}s^{-1}$, 2-min rest between sets) was initiated 80 min after supplementation. Blood samples were drawn at 30, 60, 90, and 120 min after supplementation. Repeated measures ANOVA showed that Mbv significantly ($p \leq 0.05$) increased in ARG compared with the PLA during the recovery period of each set of resistance exercise. NOx, Mox, peak torque, total work, and set total work were not significantly different between groups. We found that acute L-arg supplementation increases Mbv during recovery from sets of resistance exercise with no increase in strength performance. It is still premature to recommend nutritional supplements containing L-arg as an ergogenic aid to increase muscle strength during resistance training in healthy subjects.

Key words: nutritional supplements, amino acids, vasodilation, nitric oxide, resistance exercise.

Résumé : La L-arginine (L-arg) est un acide aminé précurseur de l'oxyde nitrique (NO). Les suppléments alimentaires contenant de la L-arg ont été commercialisées comme étant des substances susceptibles d'augmenter la vasodilatation et le débit sanguin dans les muscles sollicités ainsi qu'augmenter la réponse métabolique à l'effort. Cette étude se propose d'analyser les effets immédiats de la supplémentation en L-arg sur la force musculaire du biceps brachial, les indicateurs de la production de NO (nitrite et nitrate - NOx), le volume sanguin dans le muscle (Mbv) et l'oxygénation musculaire (Mox) durant la période de récupération consécutive à trois séries d'exercices contre résistance. Quinze hommes participent à une étude expérimentale à double insu incluant un groupe de contrôle (placebo). Après le prélèvement d'échantillons de sang au repos, on donne aux sujets un supplément contenant 6 g de L-arg (ARG) ou un placebo (PLA). Le suivi de Mbv et de Mox par spectroscopie dans le proche infrarouge commence 30 min après la supplémentation et dure 60 min. Quatre-vingts minutes après la supplémentation, on demande aux sujets de réaliser les 3 séries de 10 contractions maximales volontaires des fléchisseurs / extenseurs du coude en mode miométrique et isokinétique ($60°{\cdot}s^{-1}$), chaque série étant intercalée d'une période de repos de 2 min. On prélève des échantillons de sang à la 30^e, 60^e, 90^e et 120^e min après la supplémentation. Résultats. L'analyse de variance avec mesures répétées révèle une augmentation significative du Mbv ($p \leq 0{,}05$) au cours de la période de récupération consécutive à chaque série d'exercices contre résistance chez le groupe ARG, et ce, comparativement au groupe PLA. On n'observe pas de différence significative de NOx, de Mox, de moment de force de pointe, de travail total et de travail au cours d'une série, d'un groupe à l'autre. Nous avons constaté que la supplémentation en L-arg augmente le Mbv durant la période de récupération à la suite de séries d'exercices contre résistance sans augmenter la performance

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musculaire. Il est hâtif de recommander une supplémentation en L-arg à titre d'agent ergogène pour augmenter la force musculaire au cours d'un programme d'entraînement contre résistance chez des sujets en bonne santé.

Mots-clés : suppléments alimentaires, acides aminés, vasodilatation, oxyde nitrique, exercice contre résistance.

[Traduit par la Rédaction]

Introduction

Many nutritional supplements have been introduced into the market with the purpose of optimizing gains in muscle strength and hypertrophy induced by resistance training (Kreider et al. 2010). Recently, supplements based on L-arginine (L-arg) have been advocated to promote acute vasodilatation because of an increased production of nitric oxide (NO) in the exercising muscle (Álvares et al. 2011; Tang et al. 2011). The resulting vasodilation would elevate blood perfusion and theoretically induce a higher nutrient and oxygen delivery to the active tissues during exercise, enhance protein synthesis, and facilitate muscle recovery. L-arg is considered a semi-essential amino acid because the body normally produces it in sufficient amounts. However, supplementation may be needed in special conditions such as malnutrition, excessive ammonia production, burns, infections, peritoneal dialysis, rapid growth, urea synthesis disorders, and (or) sepsis (Appleton 2002).

The physiological concentrations of L-arg (ranging between 40 to 100 $\mu mol \cdot L^{-1}$) in healthy individuals are enough to saturate endothelial nitric oxide synthase, which is ~2–20 $\mu mol \cdot L^{-1}$ (Durante et al. 2007). Therefore, theoretically, supplementary L-arg should not promote increased enzyme activity, and consequently, no further NO production should occur — hence the condition known as the "L-arginine paradox" (Bode-Böger et al. 2007). Nevertheless, early evidence suggests that L-arg supplementation may help treat individuals with atherosclerosis risk factors, such as hypercholesterolemia, hypertension, diabetes mellitus, kidney failure, hyperhomocysteinemia, smoking, and aging — all of which are conditions that are associated with reduced NO biosynthesis (Clarkson et al. 1996; Creager et al. 1992; Pieper et al. 1996).

Despite the theory regarding L-arg supplementation improving vasodilation from increased NO production, a recent review (Álvares et al. 2011) about the ergogenic effect of L-arg supplementation in healthy subjects shows that there were only 5 acute studies that evaluated exercise performance after L-arg supplementation, 3 of which reported significant improvements. Stevens et al. (2000) supplemented 13 subjects orally with a product that comprised L-arg (6 g) plus glycine (2 g) plus α-ketoisocaproic acid (3.2 g) or 9.46 g sucrose isocaloric control in 3 equal aliquots at 45-, 30-, and 10-min periods before exercise, and observed significant increases in peak torque, total work, and fatigue index using an isokinetic dynamometer. Employing a similar supplement protocol, Buford and Koch (2004) observed a significant improvement of average power during repeated sets of supramaximal exercise during cycle ergometry. Bailey et al. (2010) trialled 9 healthy recreationally active men with a supplement that contained 6 g of L-arg (dissolved in 500 mL of water) or placebo 1 h before a series of moderate- and severe-intensity exercise bouts performed on an electronically braked cycle ergometer for 3 days. A significant increase was observed in the time to task failure, with concomitant reductions in the O_2 cost of moderate-intensity cycle exercise and slow oxygen uptake component amplitude between groups. Unfortunately, only one (Bailey et al. 2010) of these studies measured any variable that could explain the underlying mechanism for the increase in NO production and its relationship with performance. Furthermore, there have only been 3 scientific reports with evidence of blood perfusion changes after an acute supplementation of L-arg in healthy subjects during resistance exercise (Fahs et al. 2009; Robinson et al. 2003; Tang et al. 2011). However, none of these studies evaluated muscle oxygenation or muscle performance during exercise, particularly strength exercise.

Oxidation of NO via several metabolic reactions results in the formation of nitrite (NO_2^-) and nitrate (NO_3^-) as the 2 major end products (Tsikas 2005). NO_2^- is the principal oxidation product of NO synthesis in aqueous solutions (in the absence of biological constituents, such as hemoproteins). The further oxidation to NO_3^- requires the presence of additional oxidizing species, such as oxyhemoproteins (Ignarro 1990). For example, NO is quickly oxidized to NO_2^- via autoxidation in aqueous solutions, such as biological fluids, and may react with superoxide anion to produce $ONOO^-$. In the presence of heme groups in proteins, such as hemoglobin and myoglobin, NO reacts with oxyhemoglobin to produce metahemoglobin and NO_3^-. Therefore, measurement of NO_2^- plus NO_3^- (NOx) in various biological fluids has been recognized as the most suitable, practical, and reliable noninvasive method to assess systemic NO synthesis in vivo (Tsikas 2005).

One of the possible mechanisms behind the effects of L-arg, namely vasodilation caused by increased NO production, may be evaluated using near-infrared spectroscopy (NIRS). NIRS is a noninvasive optical technique that has been used successfully to monitor muscle oxygenation and blood volume levels in humans (Bhambhani 2004). The validity of NIRS in measuring venous oxygen saturation during forearm exercise has been demonstrated and the contributions of oxymyoglobin and skin blood flow to the overall absorbency changes are quite low (Mancini et al. 1994). As well, the validity of NIRS against regional blood flow measured by the direct Fick and plethysmography during forearm exercise (Van Beekvelt et al. 2001*b*) and using indocyanine green in combination with magnetic resonance imaging during plantar flexion has been established (Boushel et al. 1998).

To test the claim that L-arg supplementation enhances muscle performance during resistance exercise in healthy individuals by improving vasodilation, the present study was conducted to identify the acute effects of L-arg supplementa-

tion regarding changes in (*i*) strength performance; (*ii*) muscle blood volume (Mbv) and muscle oxygenation (Mox) measured noninvasively by NIRS; and (*iii*) markers of NO production – NOx in healthy male subjects. Based on the L-arginine paradox, it was hypothesized that there would be no significant differences in strength performance, or higher levels of Mbv, Mox, and NOx as a result of L-arg supplementation when compared with the placebo condition.

Materials and methods

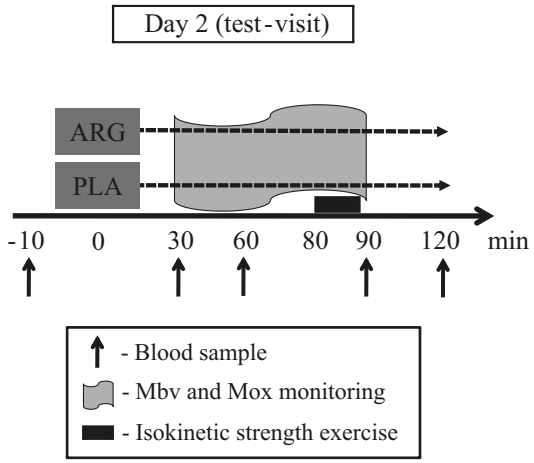
Subjects

Fifteen healthy male volunteers with previous resistance training experience (at least 3 months; mean, 5.9 years; SD, 3.9 years) and who were not familiar with isokinetic exercise were recruited to participate in the study. The height and weight of the subjects were measured on a calibrated analogue anthropometric scale (model 31; Filizola, São Paulo, Brazil). Body density was predicted from the Jackson and Pollock (1978) equation, using skinfold measurements at 3 sites (chest, abdomen, and thigh) taken with a Lange caliper (Beta Technology Inc., Cambridge, Md., USA) from which body fat percentage was calculated using the Siri (1956) equation. In addition, biceps skinfold thickness was measured at the same site where the NIRS probe was placed. All subjects were fully informed of the nature and purpose of the investigation and gave their written consent to participate. They were instructed not to deviate from their current training regimen during the course of the study, except for refraining from exercise for the 24 h prior to each testing day. The exclusion criteria for participation in the study were any known cardiovascular, pulmonary or metabolic diseases (asthma, diabetes, hypertension, dyslipidemia, smoking, etc.), upper limb injury, and (or) the use of either nutritional ergogenics or anabolic steroids 6 months prior to the beginning of the study. All experimental procedures were performed in accordance with the ethical standards of the Declaration of Helsinki and were approved by the Institutional Ethics Committee of the Universidade Gama Filho, Rio de Janeiro (protocol no. 4936.0.000.312-10).

Experimental design

The study was conducted in a randomized, double-blind, and placebo-controlled fashion. All subjects reported to the laboratory on 2 occasions, with at least a 1-week interval between visits. The first visit was used to explain the experimental procedures, collect anthropometric data, and familiarize the subjects with the exercise protocol. In the second visit, blood samples were drawn from an antecubital forearm vein at baseline after a 10-min period of quiet rest in the supine position. Thereafter, subjects were randomly divided into either a placebo (PLA) or an L-arg group (ARG). Monitoring of Mbv and Mox began 30 min after the supplementation and lasted for 60 min. The exercise protocol was initiated 80 min after the supplementation, and lasted approximately 10 min. Blood samples were drawn again at 30, 60, 90, and 120 min after supplementation (Fig. 1). The NIRS monitoring began 30 min after supplementation, based on evidence (Tang et al. 2011) that the plasma L-arg concentration reaches a peak approximately 30 min after oral supplementation, and NO synthesis may be expected only after this time period.

Fig. 1. Experimental design.



Dietary control

One day before conducting the study, the subjects were oriented as to the NO_2^- and NO_3^- content of foods and were requested to restrict their diets from foods rich in these compounds. A list describing foods and groups of food to be avoided and to be preferred was distributed to the subjects to simplify their dietary choices for low NO_2^- and NO_3^- foods for the 24-h period prior to the study. This dietary orientation was based on a list developed to estimate dietary NO_2^- and NO_3^- (Griesenbeck et al. 2009). Adherence to the diet was monitored by asking the subjects to provide information pertaining to their dietary intake for the preceding 24-h period.

Supplementation

All subjects were orally administered either 6 g of encapsulated L-arg hydrochloride or a placebo (as cornstarch) in identical forms with 400 mL of water in a double-blind and randomized manner. We chose to provide 6 g of L-arginine because such a dose would be well tolerated when consumed orally and has been reported to increase vasodilation (Bode-Böger et al. 1998).

Exercise protocol

The subjects performed dominant elbow flexion and extension exercise with an isokinetic dynamometer (Cybex Norm, Cybex International Inc., Ronkonkoma, N.Y., USA) in the concentric–concentric mode. Each subject lay down in a supine position, with the legs flexed and supported by a footplate. The body was stabilized in the bed and strapped with Velcro to minimize movements other than the elbow flexion and extension. The elbow flexion–extension adapter was adjusted to the semi-prone position, according to the body dimensions of each subject. These adjustments were recorded so they could be repeated accurately in each subsequent visit. The exercise movement was performed with a joint range of motion from 0° to 100°, beginning with concentric elbow flexion, followed by passive elbow extension. Prior to the exercise test, the subjects performed a warm-up that consisted of a 5-repetition set at a velocity of $60°\cdot s^{-1}$, during which the subjects were advised not to perform at maximal effort. After 2 min of rest, 3 sets of 10 maximal voluntary contractions were performed at a velocity of $60°\cdot s^{-1}$ in both the extension (passive movement) and flexion (active movement) phases,

with a recovery period of 2 min between sets. The strength variables analyzed were peak torque (Nm), total work (Joules), and set total work (Joules). The maximal exerted force sustained under fatiguing conditions as a fraction of the maximal work exerted by each individual over the 3 sets was quantified by using the endurance ratio (which indicates the rate at which a person fatigues). The Cybex Norm (Cybex International Inc.) system calculates the endurance ratio by dividing the work done in the last 50% of the repetitions in the set by the work performed in the first 50% of the repetitions in the set.

Mbv and Mox

Mbv and Mox were continually monitored for 60 min, including the 2-min recovery period between the 3 sets of exercise. A continuous dual-wave near-infrared spectroscopy device (MicroRunman, NIM Inc., Philadelphia, Pa., USA) was used to record these measurements. The NIRS unit consists of 3 components: (*i*) a probe with 2 tungsten lamps, which had a light penetration depth of approximately 2.5 cm; (*ii*) 2 silicone diodes that absorbed light at 760 and 850 nm; and (*iii*) 1 unit that amplified the absorbance signal. The probe was placed on the skin, over the dominant biceps muscle, exactly 2 cm below the middle point between the acromiale and radiale anatomical landmarks. Since the penetration depth of the NIRS signal is influenced by skinfold thickness (Van Beekvelt et al. 2001*a*), this value was recorded on the biceps at the same site where the NIRS probe was placed. For each subject, the half skinfold thickness (ARG = 3.8 mm and PLA = 3.4 mm) was less than the penetration depth (approximately 25 mm) of the NIRS probe, ensuring that measurements were recorded from the muscle tissue. To secure the probe on the skin and minimize movement during exercise, an elastic bandage was wrapped around the subject's arm. The wrap also helped to minimize the possibility that extraneous light could influence the signal. The NIRS device was calibrated according to the manufacturer's recommendation before each test. A deep penetration and a light intensity of 110 mV were used. All data were collected online at a frequency of 1 Hz, using specific software (Nircom, NIM Inc.). Mox and Mbv were calculated using standard algorithms provided by NIRCOM software (version 1.5) developed for this instrument. The modified Beer–Lambert law was applied to calculate optical density (OD) from the milliVolt measurements at both wavelengths, according to the following equations:

$$OD_{760} = \log_{10}(I_{760cal}/I_{760meas})$$

$$OD_{850} = \log_{10}(I_{850cal}/I_{850meas})$$

where OD_{760} and OD_{850} are optical densities for the respective wavelengths; and $I_{760cal}/I_{760meas}$ and $I_{850cal}/I_{850meas}$ are calibration and measured light intensities at the 2 wavelengths, respectively. The difference in OD between 850 and 760 nm was used as an index of Mox, and the sum of the ODs at these 2 wavelengths was used as an index of Mbv.

The variables used to determine the levels of Mbv and Mox were min, which is the minimum absolute value measured during the exercise phase; max, which is maximum absolute value measured during the exercise recovery phase; and range, which is the difference between max and min.

Blood sample and analysis

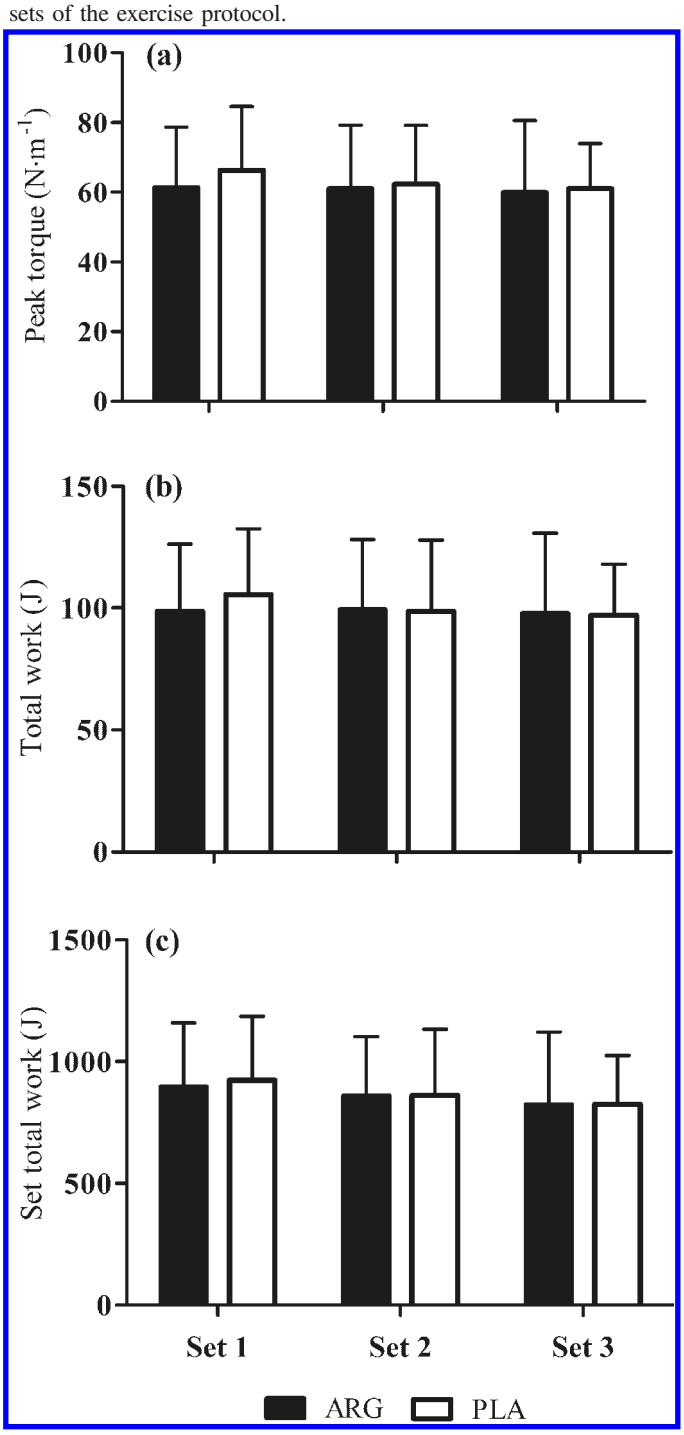
Blood was drawn from the antecubital vein and collected in EDTA-containing tubes and immediately centrifuged at 3000*g* for 10 min at 4 °C to separate the plasma before storage at –80 °C for subsequent analysis. NO production was assayed by measuring plasma NOx as described by Li et al. (2000). In brief, plasma was diluted in H_2O in a proportion of 1:10 and 1:100 to analyze NO_2^- and NO_3^-, respectively. After dilution, the samples were filtered using a 10-kDa cutoff ultrafilter membrane (Vivaspin 2, GE Healthcare, Björkgatan, Uppsala, Sweden) at 14 000*g* for 15 min to remove high-molecular weight substances. NO_3^- was enzymatically converted to NO_2^- by nitrate reductase from *Aspergillus* species EC 1.6.6.2 (Roche Diagnostics, Mannheim, Germany). The solution was incubated at room temperature for 1 h. Following the conversion of NO_3^- to NO_2^-, the sample was incubated at 24 °C with 316 mmol·L^{-1} 2,3-diaminonaphthalene to convert NO_2^- into the highly fluorescent 2,3-naphthotriazole, followed by the addition of 2.8 mol·L^{-1} NaOH, and immediately analyzed by high-performance liquid chromatography (HPLC) coupled to a fluorescence detector model RF-10AXL (Shimadzu, Tokyo, Japan) monitoring excitation and emission wavelengths at 375 and 415 nm, respectively.

The amino acids L-arg, L-citrulline, and L-ornithine were analyzed as previously described by Wu and Meininger (2008). In brief, plasma was mixed with 1.5 mol·L^{-1} perchloric acid (*v*/*v*) to remove proteins. After 2 min, H_2O and potassium carbonate were added to the mixture and then centrifuged at 10 000*g* for 2 min. The sample was mixed with the *o*-phthaldialdehyde reagent solution (*v*/*v*) for 1 min. The solution derivatized was immediately analyzed using an HPLC device with a fluorescence detector that monitored excitation and emission wavelengths at 340 and 455 nm, respectively. All chromatographic procedures were performed at room temperature. These chromatographic methods are highly sensitive, specific, and accurate, and provide a useful tool to study the L-arginine–NO pathway.

Statistical analyses

A 2-way ANOVA with repeated measures on 2 factors (2 × 3; group × set) was used to identify differences in strength variables (peak torque, total work, and set total work), endurance ratio, and NIRS variables (max, min, and range values of Mbv and Mox during the recovery period of 3 sets of resistance exercise) between ARG vs. PLA groups. Two-way ANOVA with repeated measures on 2 factors (2 × 5; group × time) was utilized to identify differences in NOx and plasma amino acids at each time point. When a significant *F* was found, additional post hoc tests with Bonferroni adjustment were performed. Pearson's correlation coefficient was calculated to examine the relationship between total work in 3 sets (TW3S, the sum of total work of each set) and the range values of Mbv and Mox in both ARG and PLA groups. The correlation coefficients between NOx (measured immediately postexercise or 90 min postsupplementation) and Mbv and Mox were also calculated. Statistical significance was set at the 0.05 level of confidence. All anal-

Fig. 2. Changes in peak torque (*a*), total work (*b*) and set total work (*c*) on ARG (L-arginine group) and PLA (placebo group) over the 3 sets of the exercise protocol.

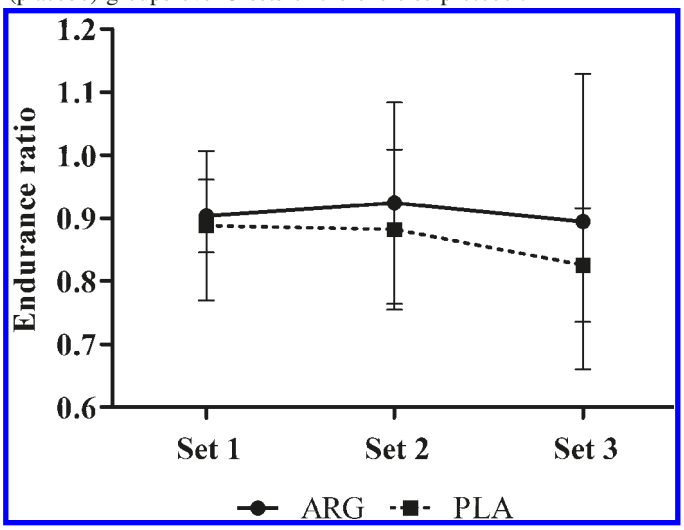


yses were performed using a commercially available statistical package (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, Calif., USA).

Results

The amount of L-arg offered in the present study (6 g) was well tolerated and no side effects were related by the subjects.

Fig. 3. Changes in endurance ratio on ARG (L-arginine) and PLA (placebo) groups over 3 sets of the exercise protocol.



Subject characteristics

At the study onset, there were no significant differences ($p > 0.05$) between the randomly assigned PLA vs. ARG groups with respect to age (ARG, 26.3 ± 4.9 years vs. PLA, 24.7 ± 1.8 years), height (ARG, 175.4 ± 8.3 cm vs. PLA, 177.7 ± 8.0 cm), body weight (ARG, 79.2 ± 13.4 kg vs. PLA, 78.3 ± 9.1 kg), body mass index (ARG, 25.6 ± 2.6 kg·m^{-2} vs. PLA, 24.8 ± 2.4 kg·m^{-2}) and body fat (ARG, 14.1% ± 6.0% vs. PLA, 16.2% ± 2.6%).

Strength performance

Strength performance changes during L-arg and placebo supplementation over the 3 sets are presented in Fig. 2. There were no significant differences between ARG vs. PLA groups after the supplementation period with regard to peak torque, total work, and set total work over the 3 sets of the exercise protocol. The endurance exercise ratio values are presented in Fig. 3. There was no statistical significance in ARG (0.90 ± 0.1; 0.92 ± 0.2; 0.89 ± 0.1 for sets 1, 2, and 3, respectively) when compared with PLA group (0.89 ± 0.1; 0.88 ± 0.1; 0.83 ± 0.1 for sets 1, 2, and 3, respectively).

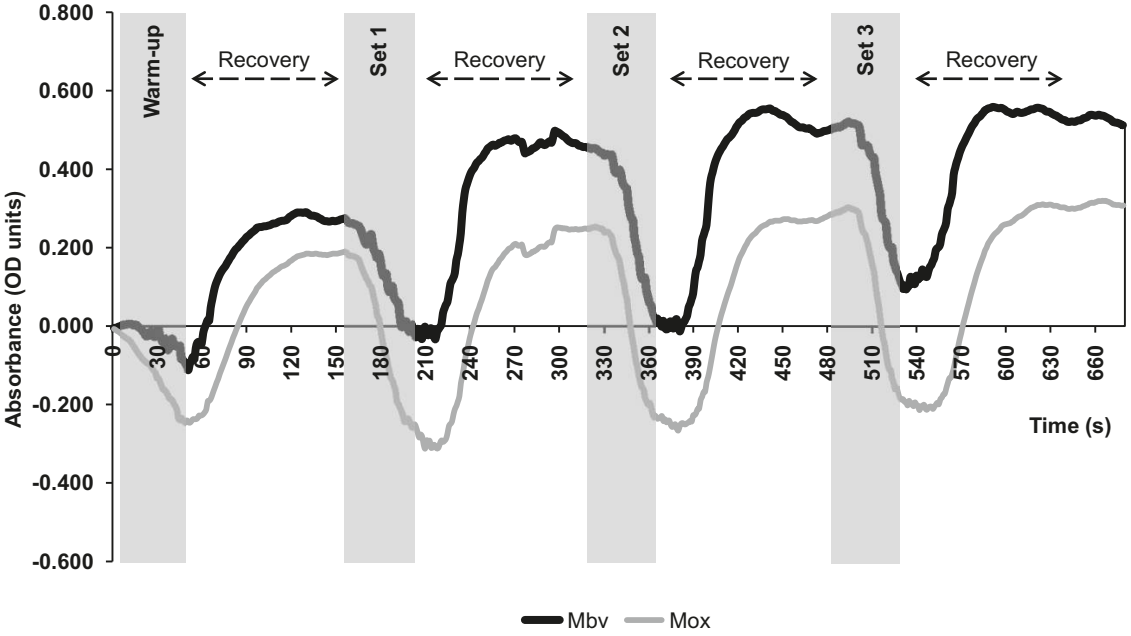
Muscle blood volume and oxygenation changes

The Mbv and Mox trends of the biceps brachii during the exercise protocol in a representative subject are illustrated in Fig. 4. The mean Mbv and Mox values measured during recovery from each set of exercise are shown in Table 1. No significant difference was observed in Mox between ARG vs. PLA groups. However, a significant increase in Mbv during the recovery period of the 3 sets of exercise was observed in the ARG compared with the PLA group ($p < 0.05$). The correlation coefficients revealed that there were no significant relationships between TW3S performed and Mbv for ARG ($r = 0.30$; $p = 0.469$) and PLA ($r = 0.12$; $p = 0.781$) groups, and TW3S and Mox for ARG ($r = –0.02$; $p = 0.950$) and PLA ($r = –0.11$; $p = 0.811$) groups.

NO production

Plasma NOx concentrations at each time point are depicted in Fig. 5. There were no significant differences found be-

Fig. 4. Graphic trends of muscle oxygenation (Mox) and muscle blood volume (Mbv) in a representative subject during submaximal warm-up and maximal 3 sets of dominant elbow flexion–extension at 60°·s^{-1} with a 2-min rest interval between sets. The vertical gray columns indicate the moment of muscle contraction. The white spaces indicate the recovery phase. OD, optical density.



tween groups at any of the 5 time points in which measurements were taken. However, the correlation coefficients revealed that there was a significant relationship between NOx and Mbv for ARG ($r = 0.74$; $p = 0.03$), but not for the PLA ($r = 0.10$; $p = 0.816$) group. There were no significant correlations between NOx and Mox for ARG ($r = –0.15$; $p = 0.721$) and PLA ($r = –0.41$; $p = 0.358$) groups.

Plasma amino acids

The plasma concentrations of L-arg, L-citrulline, and L-ornithine at each time point are summarized in Table 2. Plasma L-arg increased significantly at 30 min after supplementation in the ARG group when compared with the PLA group ($p < 0.05$). A significant increase in plasma L-ornithine was observed at 30, 60, and 90 min after supplementation in the ARG group when compared with the PLA group ($p < 0.05$). No significant difference was observed between groups in plasma L-citrulline at any time point.

Discussion

The present study was designed to test our hypothesis that oral L-arg supplementation does not influence (*i*) strength performance, (*ii*) Mbv and Mox, and (*iii*) markers of NO production – NOx in healthy male subjects.

Overall, the major and unexpected finding of the present study was that oral supplementation with a single dose (6 g) of L-arg was able to increase Mbv during the recovery period between successive sets of resistance exercise with no significant change in Mox as measured by NIRS. However, no significant differences in NOx and elbow flexion strength performance (peak torque, total work, and set total work) were observed between the groups.

L-arg supplementation and NIRS parameters during exercise recovery

It appears that L-arg supplementation can rapidly induce vasodilation in skeletal muscle via vascular smooth muscle NO biosynthesis, as observed by the significant increase of Mbv in the ARG group during the recovery period of resistance exercise sets when compared with the PLA group. However, other studies that have measured blood flow did not support this finding. Robinson et al. (2003) submitted 6 healthy males to oral L-arg supplementation (10 g) 30 min after repeated bouts of squatting exercise and measured forearm blood flow during a 3-h period using venous occlusion plethysmography. The authors did not observe any significant change in peripheral blood flow. Fahs et al. (2009) also did not observe any significant change in forearm blood flow (using straingauge plethysmography) after supplementing 18 young men with 7 g of L-arg before each resistance exercise bout, which consisted of 1-repetition maximum (1RM) tests for the bench press and the biceps curl. Tang et al. (2011) did not observe any significant increase in muscle blood flow (using a linear array pulse Doppler ultrasound probe) in 8 healthy young men after supplementing them with 10 g of oral L-arg after a bout of unilateral leg resistance exercise.

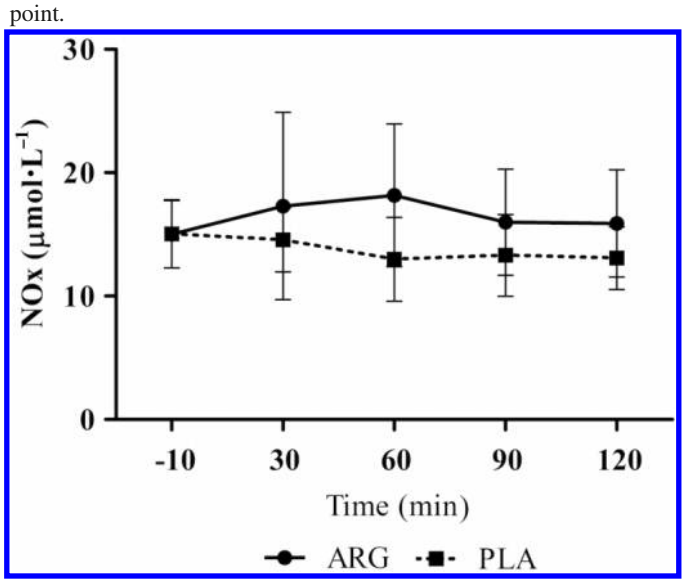
A possible explanation for the lack of significant differences between placebo and supplemented groups in the results of the above mentioned studies may be due to the association between the time subjects were supplemented and the time that blood flow measurements were taken. In the present study, the subjects were continuously measured with NIRS during all exercise bouts with data retrieved immediately following each exercise set. Furthermore, the exercise bouts were initiated 80 min after supplementation, which is enough time for absorption (the plasma L-arg concentration signifi-

Table 1. Mean ± standard deviation optical density values for muscle blood volume (Mbv) and oxygenation (Mox) during the recovery period of each set of exercise.

	Set 1			Set 2			Set 3		
Group	Max	Min	Range	Max	Min	Range	Max	Min	Range
Mbv									
ARG	0.87±0.45*	0.07±0.38	0.81±0.53*	1.01±0.43*	0.36±0.34	0.65±0.24	1.07±0.43*	0.45±0.33	0.62±0.26*
PLA	0.42±0.22	0.04±0.24	0.38±0.15	0.46±0.29	0.03±0.40	0.43±0.21	0.40±0.29	0.14±0.25	0.26±0.14
Mox									
ARG	0.07±0.43	–0.41±0.35	0.49±0.3	0.09±0.43	–0.48±0.36	0.57±0.24	0.09±0.46	–0.48±0.37	0.58±0.29
PLA	0.15±0.34	–0.21±0.27	0.36±0.17	0.09±0.34	–0.31±0.29	0.40±0.16	0.02±0.38	–0.31±0.36	0.33±0.12

Note: Max, maximum recorded value; Min, minimum recorded value; Range, difference between the Max and Min recorded values; ARG, L-arginine group; PLA, placebo group.
*Significantly different from PLA ($p < 0.05$).

Fig. 5. Plasma NO_2^- plus NO_3^- (NOx) concentrations (μmol·L^{-1}) for ARG (L-arginine group) and PLA (placebo group) at each time point.



cantly increased 30 min after supplementation). In the 3 aforementioned studies, however, the measurement of blood flow had been taken several minutes after exercise bouts: the Robinson et al. (2003) study measured 30 min after exercise bout cessation; the Fahs et al. (2009) study, 25 min afterwards; and in the Tang et al. (2011) study, subjects were supplemented after exercise and measurements were taken subsequently.

Interestingly, no difference was observed in Mox for either of the groups. A possible explanation for this phenomenon could be due to the high affinity of NO for molecular oxygen (O_2). After synthesis, NO quickly reacts with the content of O_2 in Oxy-Hb/Mb, producing metahemoglobin and NO_3^- (Ignarro 1990; Ignarro et al. 1993). For example, NO released from endothelial cells may reach the erythrocytes to react with Oxy-Hb/Mb to form NO_3^-, or with hemoglobin to form nitrosylhemoglobin, or with the 93-cysteine residue of the L-subunit to form (*S*)-nitrosohemoglobin (Eich et al. 1996). The combination of NO and Oxy-Hb/Mb is almost instantaneous. The rate of this reaction has been determined with a second-order rate constant of 3.4×10^7 mol·L^{-1}·s^{-1} (Carlsen and Comroe 1958). The rate constant for NO uptake by human red blood cells was reported to be 167 mmol·L^{-1}·s^{-1} (Carlsen and Comroe 1958). Furthermore, the ratio of these reactions is dependent on partial pressure of oxygen. In the plasma, NO may react with O_2 to form NO_2^-, or with superoxide anion to form $ONOO^-$. The subsequent decomposition of ONO_2^-, and thus the ratio of formed NO_2^- and NO_3^-, depends on the surrounding conditions (Ghosh and Salerno 2003). Since NIRS measures Oxy-Hb/Mb and deoxy-Hb/Mb content, the high affinity of NO for O_2 present in Oxy-Hb/Mb may buffer NIRS measurements, which may explain why no significant change was found in Mox after L-arg supplementation. There is evidence showing that performance during multiple sets of resistance exercise is not significantly affected by Mox levels, even when these returned to pre-exercise levels before the next set was initiated (Matsuura et al. 2011).

Table 2. Values of plasma amino acids (μmol·L^{-1}) at –10, 30, 60, 90, and 120 min postsupplementation.

	ARG					PLA				
Amino acid	–10	30	60	90	120	–10	30	60	90	120
Citrulline	130.7±24.3	158.5±48.7	182.8±51.8	178.1±73.3	163.2±57.9	128.5±43.8	114.5±21.2	123.0±38.0	140.4±18.7	149.9±38.5
Arginine	75.4±54.7	100.6±73.2*	62.2±24.8	57.1±38.8	65.0±72.4	47.0±13.8	37.8±14.2	55.5±25.3	49.3±29.4	59.9±37.4
Ornithine	99.2±19.2	140.4±28.0*,[a]	148.4±33.4**,[a]	154.6±44.1**,[a]	127.4±49.8	98.7±38.5	82.6±32.6	81.2±32.7	87.9±29.9	94.2±42.0

Note: The values are means ± standard deviation. The symbols **($p < 0.01$) and *($p < 0.05$) denote significantly different from placebo at same time point. ARG, L-arginine group; PLA, placebo group.
[a]Significantly different from time – 10.

L-arg supplementation and strength performance

For optimal muscular function, it has been speculated that an adequate blood supply is needed to deliver essential substrates (e.g., glucose, amino acids, and oxygen) to the muscles and to remove metabolites (e.g., lactate and ammonia). Since L-arg is the only substrate for NO synthesis, and it is a potent vasodilator, L-arg supplementation would thus be expected to enhance muscle function by increasing the blood supply. However, there is no evidence to support the claim that increased nitric oxide production from L-arg supplementation leads to better muscle vasodilation and (or) improved exercise performance in healthy subjects.

In the present study, L-arg supplementation increased Mbv during recovery period of sets of resistance exercise. This condition may theoretically favor muscle performance, according to the aforementioned hypothesis that increased blood supply may optimize muscular function. However, no significant difference was observed on muscle strength variables (peak torque, total work, and set total work) as well as endurance ratio between the groups. Furthermore, no significant correlations were observed between TW3S and Mbv and Mox in both ARG and PLA groups. This lack of significant correlation is in accordance with a previous study from our group (Pereira et al. 2007), which observed that Mox recovery (considered as the moment when Mox measured by NIRS stabilizes to begin the next set of resistance exercise protocol) was not a determinant factor for activity performance. Recently, Matsuura et al. (2011) did not observe any significant correlations between TW and changes in Mbv and Mox, when subjects exercised at 50% or 75% of 1RM to voluntary fatigue.

On the other hand, other studies have shown the positive effect of L-arg supplementation on muscle performance (Santos et al. 2002; Stevens et al. 2000). For example, Stevens et al. (2000) supplemented 13 subjects orally with a product that comprised L-arg (6 g) plus glycine (2 g) plus α-ketoisocaproic acid (3.2 g) or 9.46 g sucrose isocaloric control in 3 equal aliquots at 45-, 30-, and 10-min periods before exercise, which consisted of 35 continuous isokinetic concentric–eccentric knee extension repetitions at 90°·s^{-1}. They observed a significant increase in peak torque and total work as well as a significant decrease in the fatigue index. In another study, Santos et al. (2002) observed a significant decrease in the work fatigue indexes after 15 days of L-arg supplementation (3 g) in healthy volunteers who completed an exercise test protocol, which consisted of isokinetic dynamometry at an angular velocity of 180°·s^{-1} using 15 repetitions of knee extension and flexion. Campbell et al. (2006) observed significant increases in Wingate peak power and 1RM bench press after supplementing 35 resistance-trained healthy males during 3 weeks with 12 g of oral AAKG.

The present study has shown no significant difference in resistance to fatigue to the exercise protocol between ARG and PLA groups. However, there are some possible limitations that may be addressed: it is important to note that the number of repetitions used in the exercise protocol of the present study was smaller when compared with the study of Stevens et al. (2000) and Santos et al. (2002), which had found a significant tolerance to fatigue. Furthermore, both Stevens et al. (2000) and Santos et al. (2002) submitted the subjects to a fatigue-inducing exercise protocol, whereas

Campbell et al. (2006) submitted the subjects to whole-body resistance training. Moreover, Santos et al. (2002) and Campbell et al. (2006) evaluated the effects of chronic supplementation, which was contrary to the protocol of the present study.

The apparent controversy between the results of these studies could be explained by differences in the exercise protocols. L-arg supplementation may only be effective for exercises with a large number of repetitions (>30 repetitions), or for exercises that induce complete muscle fatigue (the point at which an individual can no longer withstand the load) or for applied multiple-joint exercises. However, it cannot be assumed that the positive results for exercise performance (Santos et al. 2002; Stevens et al. 2000; Campbell et al. 2006) were due to increased NO production via L-arg supplementation since none of these reports investigated the underlying mechanisms.

L-arg supplementation and NO production

Several papers have described techniques to detect NO production, both directly and indirectly, to quantify NO synthesis in biological models (Li et al. 2000; Xia and Zweier 1997). Since NO is produced in small amounts (picomolar to nanomolar ranges) in vivo and is rapidly oxidized (short half-life) to NO_2^- and NO_3^-, direct measurement of its production is difficult. Therefore, the analysis of NO_2^- and NO_3^- — stable products of NO oxidation — is often performed to indirectly measure NO synthesis in biological fluids (Jobgen et al. 2007; Rhodes et al. 1995; Tsikas 2005). There is compelling experimental evidence that NO_2^- and NO_3^- may indicate NO synthesis in the endothelium (Rhodes et al. 1995). Jungersten et al. (1997) observed significant increases in plasma markers of NO in humans after an acute and 2- to 3-month protocol of incremental cycle ergometer exercise, respectively. Bode-Böger et al. (1994) observed increases in urinary NO_2^-, NO_3^-, and cGMP only during incremental cycle ergometer exercise, when compared with 1 h after exercise.

In the current study, no significant difference in plasma NO_2^- and NO_3^- concentrations after L-arg supplementation was observed at any point. This finding supports the results of other studies where no significant changes were observed after an acute treadmill test (Poveda et al. 1997) and cycle ergometer exercise (Yamamoto et al. 2007). On the other hand, Bailey et al. (2010) observed significant increases in plasma NO_2^- and time to task failure in 9 healthy recreationally active men supplemented with 6 g of L-arginine (dissolved in 500 mL of water) or placebo 1 h before a series of moderate- and severe-intensity exercise bouts performed on an electronically braked cycle ergometer for 3 days. There was also a significantly reduced O_2 cost of moderate-intensity cycle exercise and reduced oxygen uptake slow component amplitude observed between groups. It is important to note that the study cited included other amino acids, such as L-citrulline (quantities not expressed), besides L-arginine, which have been shown to increase NO production as measured by plasma concentrations of NO_2^- (Sureda et al. 2009) and urinary excretion of NO_3^- and cGMP (Schwedhelm et al. 2008). Interestingly, these investigators did not measure plasma nitrite at baseline but undertook measurements 1 h after supplementation. This is a major methodological limitation since it is not known whether there were any differences between the samples prior to supplementation. Furthermore, taking into consideration that diet can influence nitrite plasma concentrations, no dietary control to limit the consumption of foods rich in nitrite and nitrate was conducted. The authors concluded that the precise mechanisms responsible for improving exercise efficiency and exercise tolerance remained to be elucidated.

In the present investigation, there was a significant correlation between NOx and Mbv in ARG group compared with PLA group. It may be speculated that L-arg supplementation promoted local increases in NO synthesis. However, the method for analyzing plasma NO_2^- and NO_3^- in the present study indirectly measures systemic NO production, as opposed to local production, which may explain the lack of significant increase in NOx after L-arg supplementation. It may be that the intensity of this exercise protocol was not sufficient to promote significant changes in systemic NO production, since the exercise protocol of the present study was performed in a small muscle group (unilateral elbow flexion–extension).

L-arg is oxidized to NO and L-citrulline in equimolar concentrations, a reaction referred to as the L-arginine–NO pathway. This reaction is mediated by nitric oxide synthase (NOS), which is dependent upon the availability of substrates and cofactors (nicotinamide adenine dinucleotide phosphate, flavin mononucleotide, tetrahydrobiopterin, flavin adenine dinucleotide), as well as the rate of electron transfer (Ghosh and Salerno 2003). In the presence of sufficient cofactors, NOS activity is dependent on L-arg and oxygen availability. It is important to point out that one of the main factors that may influence NOS activity is its competition with arginase for the same substrate, which is L-arg. Arginase is the enzyme that catalyzes L-arg into L-ornithine and urea. This pathway is called the urea cycle, and it is vital to the elimination of nonessential nitrogen-containing substances from the body. Although the affinity of L-arg is much higher for purified NOS (Km ~2–20 $\mu mol \cdot L^{-1}$) than for arginase (Km ~1–5 $mmol \cdot L^{-1}$), the maximum activity of arginase is more than 1000 times that of NOS (Durante et al. 2007).

There was no significant difference between the 2 groups in the plasma L-citruline concentrations in the present study, which may indicate that the supplementary L-arg was not utilized by the L-arginine–NO pathway. On the other hand, there was a significant increase in the plasma L-ornithine in the ARG group when compared with the PLA group. This finding may indicate that L-arg was possibly diverted to the urea cycle, which may explain why NO increased plasma L-ornithine but did not increase plasma L-citruline after L-arg supplementation.

According to our hypothesis, L-arg supplementation should not enhance any NO production in healthy subjects. However, there is evidence showing that increasing plasma L-arg levels by supplementation improves endothelial function in nonhealthy individuals (Clarkson et al. 1996; Creager et al. 1992; Pieper et al. 1996). Thus, the term L-arginine paradox has been used to describe the phenomenon, whereby L-arg supplementation, at least in vivo, improves NO function despite the known high concentration of L-arg compared with the low Km values for NOS. Thus, it has been postulated that, at least in vivo, the lack of L-arg is caused

by a substrate delivery system that may possibly be rate-limiting in terms of NO biosynthesis, despite NOS saturation (Ghosh and Salerno 2003). Not surprisingly, given the complexity of L-arg metabolism, its bioavailability is regulated by various factors, including the exogenous supply through diet–supplements, endogenous release through protein degradation, endogenous L-arg resynthesis, L-arg catabolism, and L-arg transport. Therefore, because of the multiple roles of L-arg, its supplementation is likely to result in increased metabolism via pathways other than NO synthesis, such as those that increase L-ornithine, polyamines, creatine, and proline (Durante et al. 2007). It is therefore important to note that although L-arg supplementation can produce effects via NO-dependent mechanisms, other effects that are independent of NO may well play a functional role.

There is evidence demonstrating that insulin has vasoactive action on skeletal muscle vasculature in humans. Giugliano et al. (1997) demonstrated that systemic infusion of L-arg (1 g·min^{-1} during 30 min) in healthy subjects increases leg blood flow and inhibits platelet aggregation. Furthermore, the authors conclude that these effects are partially mediated by endogenously released insulin (mediated by L-arg). The effect of insulin release by L-arg purportedly involves the stimulation of electrical activity of the pancreatic β-cells, which leads to membrane depolarization (Henquin and Meissner 1981). Depolarization of the plasma membrane could result in activation of voltage-dependent calcium channels, an increase in cytosolic Ca^{2+}, and subsequent stimulation of insulin secretion (Newsholme et al. 2006). However, no human studies have yet explored the L-arg metabolism in detail in the pancreatic β-cells; thus, any conclusion about the potential effect of L-arg on insulin-mediated vasodilation must be made with caution.

Conclusion

L-arg supplementation did not stimulate an increase in NO synthesis or Mox in healthy men in response to exercise, despite an increase in Mbv. In addition, L-arg supplementation did not enhance isokinetic concentric elbow extension strength performance. These results bring into question the ergogenic potential of L-arg for acutely increasing strength and NO production in healthy men, and thereby suggest that other mechanisms may be involved in the improved blood perfusion in response to L-arg supplementation. Long-term studies investigating the effects of L-arg supplementation on different populations (healthy and nonhealthy subjects, using a large number of subjects), and exercise protocols involving whole-body muscle groups and muscle fatigue are needed to better understand the underlying mechanism(s) of this supplement before making any recommendation about its utilization as an ergogenic aid.

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