International Journal of EXERCISE SCIENCE

Original Research

No Effect of L-Arginine on Vascular Stiffness at Rest and During Reactive Hyperemia in Young Healthy Subjects

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ABSTRACT

International Journal of Exercise Science 12(2): 556-566, 2019. The efficacy of L-arginine in augmenting blood flow via nitric oxide (NO) production is controversial, with several studies in the literature providing equivocal findings. The purpose of this study was to examine the effect of L-arginine supplementation on vascular stiffness at rest and during induced reactive hyperemia. Young healthy males (n = 15) were studied on two separate study days. On day one, a resting pulse wave velocity (PWV) was measured from the carotid and radial sites at rest and immediately following an induced reactive hyperemia (RH). On day two, subjects rested for 40 minutes following a dose of 70 mg of L-arginine per kg of lean mass and PWV was again measured from the same two sites at rest and immediately following a RH. There was no difference in PWV between control and supplemented states at rest (6.33 ± 0.81 m/s and 6.09 ± 0.96 m/s; p = 0.32) or immediately following RH (6.03 ± 0.99 m/s and 5.77 ± 0.83 m/s; p = 0.06). In conclusion, L-arginine supplementation did not reduce PWV in healthy subjects at rest or during induced RH.

KEY WORDS: Vasorelaxation, arterial, supplementation, PWV, arterial stiffness, exercise, vasodilation, ergogenic aid, endothelial function

INTRODUCTION

In 1886, L-arginine was first extracted from a lupine seedling. L-arginine, the biologically active variation of arginine, is a semi-essential amino acid used in the biosynthesis of proteins, nitric oxide, urea, creatine, polyamines, proline, and glutamate (33). *In vivo*, arginine is synthesized mainly from citrulline. Dietary sources include fish, red meat, chicken, dairy products, seeds, and nuts. L-arginine supplementation has been shown to improve mitogen-stimulated lymphocyte proliferation and collagen disposition, suggesting roles in wound healing (7, 28). Also, several meta-analyses report lower infection and hospital length of stay outcomes (8, 20, 21, 32, 51) Further, studies have shown improvements to pulmonary hypertension, blood flow, nitrogen balance, and myofibrillar protein catabolism outcomes (9, 13, 26, 30) Thus, severe metabolic stress, such as burns, infections, hemodynamic compromise or intense physical

activity may necessitate supplementing L-arginine. L-arginine is also the primary substrate for the synthesis of nitric oxide (NO) using the enzyme nitric oxide synthase (22).

Nitric oxide (NO), once termed "endothelial-derived relaxing factor", is an ephemeral but powerful vasodilator (36, 37). An elaborate molecular pathway relying on the enzyme nitric oxide synthase leads to the formation of NO using the substrate L-arginine in the presence of oxygen (42). L-arginine supplementation in both smokers and patients with chronic kidney disease has shown decreases in arterial stiffness, which improves blood flow (5, 43, 44). Conversely, athletes and physically active people often take L-arginine supplements to increase blood flow, improve endurance, and promote muscle hypertrophy (12, 18).

L-arginine supplementation augments antioxidant enzyme expression and attenuates inflammation (23); both functions of NO. This connection has led many to believe L-arginine could also augment blood flow and thus oxygen delivery, ultimately increasing exercise performance. Published data exploring the connection between L-arginine supplementation, blood flow, and exercise performance is equivocal. For example, Aguiar et al. (3) found that a single 8 gram dose of L-arginine did not increase leg blood flow during basal or exercise conditions in healthy physically active older women; and Olek et al. (35) found no increase in power output, oxygen consumption, or plasma nitrate levels following a 2 gram L-arginine dose. Conversely, Yavuz et al. (33) found increases in time to exhaustion with no changes in oxygen consumption, lactate and heart rate in wrestlers after a 1.5-gram dose per kilogram body weight. However, Fricke et al. (18) found that 14.2 g/day of L-arginine increased power scaled to body weight in older postmenopausal women following 6 months of supplementation; and Camic, et al. (12) found that supplementing 3.0 grams L-arginine per day for four weeks increased physical work capacity at fatigue threshold in untrained college-aged men.

Previously published data using L-arginine and exercise performance have focused on broad outcome variables such as oxygen consumption kinetics and muscular power (3, 12, 18, 23, 33, 35). The lack of specific data on vascular function with L-arginine use is notable, as positive findings may have resulted from confounders such as L-arginine's role as a secretagogue; L-arginine can augment the release of hormones germane to exercise performance such as catecholamines and growth hormone (29). Thus, a potential exists for L-arginine supplementation to enhance performance in untrained or physically inactive healthy populations taking part in exercise.

A more specific measure of vasomotion, such as arterial stiffness, could better elucidate the effects of L-arginine on vascular function in humans at rest and during aerobic exercise performance. Recent literature shows an inverse relationship between nitric oxide bioavailability and pulse wave velocity (19), a measurement of the speed of the pressure wave propagated down the vascular tree by ventricular contraction. This measurement could be an appropriate tool for investigating the local and direct effects of L-arginine on arterial stiffness. Indeed, pulse wave velocity (PWV) has been established as the gold standard for determination of vascular stiffness (1). To date, no published studies have used PWV measurements to quantify acute changes in vascular tone whilst exercising in an L-arginine supplemented state.

Therefore, the purpose of this study was to establish the effects of oral L-arginine supplementation on vascular stiffness at rest and following induced reactive hyperemia (RH) in healthy young men. To complete this aim, we measured peripheral PWV in young healthy males at rest and immediately following RH in a control and L-arginine supplemented state. We hypothesized that L-arginine supplementation would decrease peripheral arterial stiffness in both conditions.

METHODS

Participants

A total of 15 subjects were studied. All subjects in the study were male university students between 18 and 30 years of age (Table 1). To determine sample size, pilot data was collected from a singular subject to determine approximate mean and standard deviation difference for the experimental and control conditions. Both effect size (ES = 0.95) and sample size (n = 14) were calculated with alpha set to 0.05 and power to 0.90 through G*Power software (version 3.1.2.9.2) for matched pairs (16). All subjects were normotensive, reported abstinence from smoking tobacco during the previous 6 months, were non-diabetic and without any history of heart disease, blood clotting, or recent infection or illness. The research protocol was explained thoroughly, and subjects provided written informed consent. The study was approved in advance by the Institutional Review Board at California State University, Chico. Inclusion criteria were: 1) males under 40 years of age, and 2) moderately physically active as classified by the Paffenberger physical activity questionnaire (34).

Protocol

Subjects reported to the lab on two separate days, both days beginning at 0700 AM following an 8-hour overnight fast.

Day one: Subjects arrived at the California State University, Chico human performance laboratory and rested for 5 minutes in a seated position. A resting heart rate followed by resting blood pressure using manual sphygmomanometery of the brachial artery was obtained with the subject in a seated position at the end of the 5-minute rest period.

Percentage body fat was measured using the Bod Pod air-displacement plethysmography system (Life Measurements Instruments, Concord, CA) (10). Prior to measurement, a system volume calibration using a cylinder of a known volume (49.794 L) and calibration of the scale using two 10 kg weights was performed. Fasting-state body weight was measured to the nearest 0.1 kg on a calibrated electronic scale and subjects entered the Bod Pod chamber wearing only a tight fitting swimsuit and swim cap. Body volume measurements were taken in duplicate and repeated if measures were not within 150 mL of each other (15). Body density was calculated as mass/body volume, and body fat percentage was calculated by using Siri's formula (46). Body mass index (BMI) was calculated as kg body mass divided by height in meters squared. A measure of lean mass was obtained for determining dosage of L-arginine for consumption on day two.

Subjects then rested for 20 minutes in the supine position and a peripheral pulse wave velocity (PWV) measurement was obtained from the carotid and radial sites using arterial tonometry. Briefly, two pressure sensitive probes (Millar SPT-301) were placed over the arteries of the non-dominant arm to obtain arterial pressure wave tracings. The distance between each probe was measured as the difference from the suprasternal notch to the radial and carotid sites (24, 25, 47). Pressure wave tracings were recorded at 250 Hz for 1 full minute of rest. Recordings were saved for later analysis using Windows Data Acquisition Software (DATAQ, Akron, OH). The time difference between the foot of each waveform was measured, and this time component was divided into the measured distance between the probes to calculate PWV. All PWV data was collected and analyzed by one trained investigator.

To measure the effects of L-arginine during a shear stress stimulus, a blood pressure cuff was placed on the subject's non-dominant arm and inflated to suprasystolic pressure (220 mmHg) for five minutes. The pressure in the cuff was then released to create a RH and another peripheral PWV measurement was immediately taken from the carotid and radial arteries for one minute.

Day two: Subjects again arrived at the human performance laboratory at California State University, Chico and then ingested an L-arginine dose dissolved in tap water equivalent to 70 mg/kg of lean body mass (Piping Rock Health Products, Ronkonkoma, NY). A resting heart rate and blood pressure measurement was taken with subjects in a seated position, after 5 minutes of quiet rest. Subjects rested quietly for a total of 40 minutes, with at least 20 minutes of rest in a supine position prior to obtaining a resting PWV measurement from the non-dominant arm. Again, a blood pressure cuff was used to occlude blood flow for 5 minutes, followed by the cuff release and RH. Another one-minute recording of PWV was made immediately following the cuff release.

Table 1. Subject characteristics.

Subjects	*n* = 15
Age (Years)	24 ± 3
Weight (Kg)	81.28 ± 13.71
Height (m)	1.79 ± 0.08
Body Fat (%)	16.00 ± 5.25

Mean ± standard deviation.

Statistical Analysis

Data analysis was performed using SPSS version 22. A two-way repeated measures ANOVA was used to test for differences between the control and L-arginine supplemented states for resting and RH conditions. Paired-T tests were used to establish baseline characteristics between testing days. Effect sizes were calculated using Cohen's d. Statistical significance was set at $p < 0.05$. Coefficient of variation (CV) for our PWV measurements was calculated as the standard deviation of each condition divided by the mean multiplied by 100 (CV = (SD / Mean) * 100).

RESULTS

All subjects completed the protocol. At rest, there were no significant differences between control days and L-arginine supplementation for heart rate, systolic or diastolic blood pressure (Table 2). The average coefficient of variation for all PWV measurements was 14.4%. On day one, subjects had an average resting PWV of 6.33 ± 0.81 m/s and 6.09 ± 0.96 m/s 40 minutes following L-arginine supplementation on day two (Table 3). The *p*-value comparing the resting condition of the control vs. L-arginine supplemented state was not statistically significant (p = 0.32). Also, no statistical difference was found for RH comparing control to L-arginine (6.03 ± 0.99 m/s vs. 5.77 ± 0.83 m/s; p = 0.06). In both conditions, a decrease in each mean is observed but large variance and small sample size prevent the rejection of the null hypothesis and no statistical significance was found (Table 3). The effect size as expressed as Cohen's *d* was small at -0.27 (95% confidence interval: -0.73, 0.25). The results should be reported in a logical sequence, giving the main findings first. The use of descriptive text, tables, and figures should be unique and not repeat information. Tables and figures should be restricted to those needed to explain the argument of the paper. Graphs should be used as an alternative to tables with many entries.

Table 2. Baseline characteristics.

Variable	Control	L-arginine	*p*-value
Heart Rate (bpm)	63 ± 3	62 ± 3	$p > 0.05$
Systolic Blood Pressure (mmHg)	115 ± 3	115 ± 2	$p > 0.05$
Diastolic Blood Pressure (mmHg)	74 ± 2	71 ± 2	$p > 0.05$

Mean ± standard error.

Table 3. Pulse wave velocities at rest and during reactive hyperemia.

Variable	Control	L-arginine	p-value
Resting PWV (m/s)	6.33 ± 0.81	6.09 ± 0.96	p>0.05
Reactive hyperemia PWV (m/s)	6.03 ± 0.99	5.77 ± 0.83	p>0.05

Mean ± standard deviation, PWV- Pulse wave velocity

DISCUSSION

The objective of this study was to investigate the efficacy of L-arginine in modifying arterial stiffness during rest and RH. We hypothesized L-arginine supplementation would result in a reduction of arterial stiffness in peripheral arteries as measured by a decrease in PWV in both conditions. Since both p-values were greater than our a priori p-value of significance set to $p <$ 0.05, neither of our experimental hypotheses were supported. This may have been a result of our study population (young, healthy, active males with a presumably healthy, functioning endothelium) or variation of the tool we used to measure arterial stiffness (average coefficient of variation for all PWV measurements was 14.4% in comparison to 9.3% in another study (48). While the effect size confidence interval was mostly negative, favoring a decrease of arterial stiffness following L-arginine supplementation, it was still considered a small at -0.27. We cannot support the popular notion that L-arginine supplementation alone provides a hemodynamic advantage at rest or during times of shear stress in a healthy, young, physically

active male population. Although, the findings of the current study agree with Adams et al. (2), concluding oral L-arginine supplementation (7 grams three times per day for three days) found no difference in brachial artery dilation with ultrasound measurements.

While L-arginine has many physiological functions, the function germane to this investigation is the conversion of L-arginine to NO via NO synthases. As NO is produced by the vascular endothelium, it binds to guanylate cyclase to create the second messenger cGMP, which ultimately causes the relaxation of vascular smooth muscle and the subsequent vasodilation. This vasodilation reduces resistance to blood flow and if cardiac output is held constant, the tissue in question will see an increase in oxygen perfusion. During exercise, the increase in vascular shear stress during hyperemia augments vasodilation in working tissue through an NO dependent mechanism (14). Traditional thinking holds that augmenting serum L-arginine concentration in a healthy human should also increase blood flow at rest and during exercise. This hypothesis is the basis for many popular ergogenic aids claiming to improve cardiometabolic risk profile and exercise performance.

To establish the efficacy of supplemental L-arginine in increasing blood flow, an ingestion of L-arginine must at least lead to a rise of serum L-arginine or other reservoir for NO production, like nitrite (NO_2^-). Some studies found an increase in serum L-arginine following consumption of L-arginine (4, 52), however, other studies report no change to plasma NO_2^- (4, 17, 31, 41), again leaving doubt as to the efficacy of supplemental L-arginine. While we did not measure plasma NO in our study, our use of arterial tonometry would have detected changes in peripheral resistance presumably mediated through its pathway. Our lack of statistical significance and small effect size suggests that L-arginine was not an effective agent for production of supplemental endogenous NO in our young, active population. This finding was true at rest and immediately following an induced RH to simulate an increase of endothelial shear stress found during physical activity.

Despite our lack of supportive data for role of exogenous L-arginine in vasorelaxation, some studies found a positive role for L-arginine as an ergogenic aid. For example, in healthy rats, Silva, et al. (45) found that L-arginine supplementation decreased oxidative stress and improved exercise performance. In rats with myocardial infarction, L-arginine plus exercise increases indices of cardiac systolic function to a greater degree than exercise alone (40). There are also positive findings in humans. For example, in postmenopausal women, L-arginine plus aerobic exercise was effective at decreasing diastolic blood pressure, whereas aerobic exercise only was not (39). Curiously, this observation was not supported by a concomitant change in nitric oxide pathway or redox status changes. This indicates there may be another yet undiscovered effect of L-arginine on blood flow regulation during exercise.

People with circulatory limitations might benefit from the L-arginine supplementation. Research by Brown et al. studied 12 women with pulmonary arterial hypertension with both a walking regimen and 6 g/day of L-arginine. While there was no control group for L-arginine consumption and thus no statistical comparison, an improvement of 6 minute walk time test coupled with an increase in VO_2max was found (11). They also found gains in measures of

quality of life and no side effects of L-arginine supplementation were noted, indicating L-arginine is likely safe and effective for use in stable clinical populations using exercise training as treatment.

In contrast to older or clinical populations, use of L-arginine as an ergogenic aid in young, healthy humans appears to be less effective. In elite male wrestlers, time to exhaustion on an incremental cycle ergometer test increased following a dose of L-arginine (1.5 g per 10 kg of body weight), but metabolic markers such as lactate and oxygen consumption remained similar between supplemented and controlled states (52). In healthy subjects, 6 g of L-arginine did not influence plasma NO_2^- (31), oxygen consumption, strength (31) or exercise tolerance (50).
The same dosage in a group of highly trained cross-country skiers (VO_2max: 69.3 ± 5.8 ml · min^{-1} · kg^{-1}) also did not increase NO_2^-concentration or exercise economy (41). In trained cyclists, 0.075 g/kg body weight of L-arginine had no effect on lactate, glucose, oxygen consumption, carbon dioxide production, respiratory exchange ratio, and blood nitrates during exercise (17). Even after 4 weeks of regular L-arginine supplementation (6 g daily) and a significant increase of L-arginine concentration in the blood of trained runners, no increases of nitrate, NO_2^-, insulin, growth hormone, insulin growth factor-1 or decreases in lactate or ammonia during exercise were found (4). However, in contrast, an acute 6 g of L-arginine supplementation in a group of healthy men reduced oxygen cost of moderate-intensity exercise and improved exercise tolerance (6)

Recent research may offer insight as to differences in efficacy of L-arginine across populations. Podgorska et al. (38) used acetylsalicyclic acid and 16.0 g of direct L-arginine infusion to measure flow mediated dilation in both athletes and otherwise healthy normotensive inactive individuals. They concluded that a decrease in endothelial function in inactive men reflects increased degradation of nitric oxide rather than decreased production (38). In contrast, Tsukiyama et al. (49) found that increased fitness yields a decrease in serum L-arginine with an increase in arginase activity and nitric oxide production. The concept of an increased production of NO is supported by data from Lefer et al. (27), who found that exercise trained individuals also have elevated levels of NO_2^-, which they concluded was likely a storage reservoir for NO. These studies highlight the lack of agreement on the question of supplementing L-arginine as a method for increasing blood flow in active young adults. Future research should explore factors governing the preservation and degradation of NO in this population.

In conclusion, L-arginine did not change PWV at rest or during reactive hyperemia in healthy, active subjects. This finding does not support the role of L-arginine supplementation in modifying arterial stiffness for improvement of blood flow in healthy, physically active young men. While L-arginine has documented and consistent benefits in aging, sedentary, and clinical populations, we were not able to confirm these same benefits are extended to an already healthy and active population.

ACKNOWLEDGEMENTS

I would like to thank my committee Dr. Michael Smith, Dr. Thomas Fahey, and Dr. George Swanson, without their guidance, work, and expertise this study would not have been possible. Additionally, I would like to thank the undergraduate assistants whom provided support for this project. We would like to thank our subjects for their time and efforts.

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