Research Article

L-Arginine does not Blunt Sympathetic Vasoconstriction in Metabolically Inactive Regions During Ischemic Handgrip Exercise in Young Healthy Males

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Abstract

Blood flow is carefully distributed during exercise to support oxygen delivery to working muscles. This is accomplished by sympathetic vasoconstriction of metabolically inactive tissue, with a blunting of this vasoconstriction in metabolically active tissue, often accomplished through nitric oxide production. The supplement L-arginine has been reported to elevated nitric oxide levels and may impair the careful distribution of blood flow during exercise. The purpose of this study was to determine the impact of L-arginine supplementation on vascular stiffness of metabolically inactive regions following sympathetic activation. 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 ischemic Hand Grip Protocol (IHG). 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 an IHG. 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 IHG (6.96±0.75 m/s and 6.53±0.97 m/s; p = 0.07). In conclusion, L-arginine supplementation did not enhance impair vasoconstriction of inactive regions during sympathetic activation.

Introduction

At the onset of exercise, muscular oxygen demand rises sharply. To meet this demand, the baroreflex operating point rises with exercise intensity [1], which results in augmented blood pressure and flow to working muscles. This elevated blood flow and pressure is accomplished by an outflow of muscle Sympathetic Nerve Activity (SNA) is directed at all vascular beds [2,3], which causes vasoconstriction via α1 adrenoreceptor activation [4]. However, metabolically active tissue releases metabolites that oppose this sympathetic stimulation, which opposes this signal and results in vasodilation. This process results in an efficient distribution of oxygen to working muscles while avoiding a drop in blood pressure and is termed "functional sympatholysis".

The circulatory system can simplistically be divided into compliant (metabolically active muscle) and non-compliant (visceral, renal, and skin) regions during exercise. Compliant muscle regions have been referred to as the "sleeping giant"; if a significant amount of vascular tissue vasodilates fully, a syncopal episode may occur [5]. Indeed, there is evidence that during whole body exercise, vasodilation to muscle is restrained [6,7], which is an important adaptation to upright exercise in a temperate environment. Maintaining arterial blood pressure to these compliant regions requires a vasoconstriction of inactive areas with a graded vasodilation in metabolically active tissue. This vasodilation is appropriate only in metabolically active muscles.

One of the mechanisms used to blunt sympathetically mediated vasoconstriction to active muscles is release of the substance Nitric Oxide (NO). NO is an ephemeral but powerful vasodilator substance, which relies on the enzyme nitric oxide synthase [8] to form of NO using the substrate L-arginine in the presence of oxygen [9]. Athletes and physically active people

often take L-arginine supplements to increase blood flow, improve endurance, prevent osteoporosis, and promote muscle hypertrophy [10,11,12]. While increasing blood flow to active muscle beds could certainly be advantageous to aerobic exercise performance, a supplement that non-specifically blunts vasoconstriction in metabolically inactive tissue would be deleterious to oxygen delivery to working muscles. While it is clear that L-arginine can support endothelium dependent vasomotion in select populations, it is not currently known if L-arginine could blunt the vasoconstrictor effect in metabolically inactive tissue beds during exercise in a young active population.

Therefore, the purpose of this study was to establish the effects of oral L-arginine supplementation on vascular stiffness of inactive tissue during rest and sympathetic activation in young healthy men. To complete this aim, we measured the peripheral PWV of inactive tissue in young healthy males at rest and immediately following ischemic Hand Grip Exercise (IHG) in a control and L-arginine supplemented state. We hypothesized L-arginine would decrease arterial stiffness of inactive vascular beds in both conditions.

Methods

Participants

A total of 15 subjects were studied. 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 [13].

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 human performance laboratory and rested for 5 minutes in a seated position. A resting heart rate, followed by resting blood pressure using manual sphygmomanometer 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) [14]. 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 [16]. 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 [17-19]. 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.

To measure the effects of L-arginine during sympathetic activation, an IHG protocol was used. Prior to IHG exercise, a maximum voluntary handgrip test was performed and a target of 40% of this maximum was calculated. Then, a blood pressure cuff was placed on the dominant arm and inflated to supra-systolic levels (220 mmHg) for two minutes. During that time the subject engaged in 40% max voluntary hand grip contractions every two seconds for 2 minutes to stimulate activation of the sympathetic nervous system [20]. A metronome was provided to assist the subject in timing muscle contractions and visual feedback provided by either a computer digital graph displays or digital numerical display. Immediately after the pressure in the cuff was released, arterial stiffness was assessed by probe measurements at the carotid and radial sites on the non-dominant arm.

Day two: Subjects again arrived at the human performance laboratory at Chico state and then ingested an L-arginine dose equivalent to 70 mg/kg of lean body mass (Piping Rock Health Products, Ronkonkoma, NY). A resting heart rate and blood pressure measurement was obtained 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 PWV measurement during sympathetic activation. Again, subjects completed another IHG test. A blood pressure cuff was used to occlude blood flow for two minutes while subjects rhythmically contracted at 40% of max every two seconds. Another one-minute recording of PWV was made immediately following the cuff release.

Statistical Analysis

Data Analysis: A two-way repeated measures ANOVA was used to test for differences between the control and L-arginine supplemented states for resting and IHG conditions. Paired-T tests were used to establish baseline characteristics between testing days. 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 in the study were male university students between 18 and 30 years of age (Table 1). 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. During IHG exercise, there was a slight but statistically insignificant drop of PWV from 6.96±0.75 in the control state to 6.53±0.87 m/s while supplemented with L-arginine (Table 3). While the p value comparing the resting conditions of the control vs L-arginine supplemented state were not statistically significant (p=0.32), the p value comparing the IHG conditions of control vs L-arginine trended towards statistical significance (p=0.07). 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 (Figure 1).

Subjects	N= 15
Age (Years)	24.07±3.28
Weight (Kg)	81.28±13.71
Height (m)	1.79±0.08
Body Fat %	16.00±5.25
Mean±SD	

Table 1: Subject Characteristics.

	Control	L-arginine	p
Heart Rate (bpm)	63.47±2.94	61.87±3.20	0.54
Systolic Blood Pressure (mmHg)	115.40±3.17	115.47±1.94	0.34
Diastolic Blood Pressure (mmHg)	74±2.33	71.33±1.88	0.98
Mean±SE			

Table 2: Baseline Characteristics.

	Control	L-arginine	P value
Resting PWV (m/s)	6.33±0.81	6.09±0.96	0.32
Ischemic handgrip PWV (m/s)	6.96±0.75	6.53±0.97	0.07
Mean±SD			

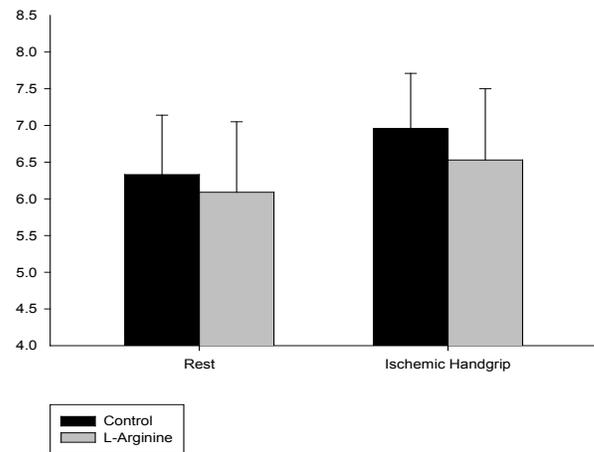**Table 3:** PWV at Rest and During Ischemic Handgrip.

Figure 1: Pulse Wave Velocity (PWV) at rest and immediately following ischemic handgrip exercise in un-supplemented (control) and L-arginine supplemented subjects. No statistical differences were found between control and L-arginine at rest or immediately following reactive hyperemia. Expressed as means±SD.

Discussion

The objective of this study was to investigate the role supplemental L-arginine may have in blunting sympathetically mediated vasoconstriction of metabolically inactive vascular beds. We hypothesized L-arginine supplementation would result in an augmented vasodilation of peripheral arteries as measured by a decrease in PWV at rest and during IHG exercise. 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 resulted from our subject population, as L-arginine seems to have little effect in healthy active humans with a presumably healthy endothelium. To the best of our knowledge, this is the first study to examine the effects of L-arginine supplementation on functional sympatholysis. L-arginine has many physiological functions, including the conversion of L-arginine to NO via NO synthases. In the case of exercise, an increase in shear stress across the vascular endothelium activates NO synthase enzymes, and in the presence of oxygen and L-arginine, NO is produced [21]. It then binds to guanylate cyclase and creates the second messenger cGMP, which ultimately causes the relaxation of vascular smooth muscle and

the subsequent vasodilation found in vessels feeding metabolically active tissue. NO production also blunts vasoconstriction in active muscles [22-24] by decreasing α1 adrenoreceptor responsiveness [25], which ultimately causes vasodilation in this tissue. 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. Other exercise induced factors that may blunt α1 adrenergic receptor mediated vasoconstriction include low pH [26], hypoxia [27] and ischemia [28].

Constricting inactive circulatory beds during exercise is essential to maintain venous return to the heart. Releasing vasoconstriction would redirect blood and oxygen away from metabolically active tissues at best, and could cause syncope at worst. In trained subjects, muscle blood flow can reach as high as 4 L kg^{-1}min^{-1} in the quadriceps muscle [29]. If this same hyperemia were elicited in the majority of muscle groups, venous return would be compromised and the pumping capacity of the heart would not be able to meet blood flow demand. For example, when leg cycling is added to arm ergometry exercise, blood flow to arm muscles is reduced by 10% [30].

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 NO$_2^-$. Some studies found an increase in serum L-arginine following consumption of L-arginine [31,32], however, other studies report no change to plasma nitrite [20,31,33,34], 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 the augmented production of NO at rest or during sympathetic activation. This finding is positive from the perspective that L-arginine does not appear to unfavorably impact functional sympatholysis in young healthy males during exercise.

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, L-arginine has a favorable effect on cultured human osteoblasts [35], and NO can inhibit osteoclastic bone resorption [36]. L-arginine supplementation in rats decreased oxidative stress and improved exercise performance [37]. In rats with myocardial infarction, exercise coupled with L-arginine increases indices of cardiac systolic function to a greater degree than exercise alone [38]. There are also positive findings in humans. For example, in postmenopausal women, L-arginine plus aerobic exercise decreased diastolic blood pressure, whereas aerobic exercise without supplementation did 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.

Studies supporting the use of L-arginine as an ergogenic aid in young, healthy human populations during physical activity are not favorable. In elite male wrestlers, time to exhaustion on an incremental cycle ergometer test increased following a dose of L-arginine (1.5g per 10 kg of bodyweight), but metabolic markers such as lactate and oxygen consumption remained similar between supplemented and controlled states [32]. In healthy subjects, 6 g of L-arginine did not influence plasma nitrite [33], oxygen consumption, strength [33] or exercise tolerance [40]. The same dosage in a group of highly trained cross-country skiers (.VO$_2$max: 69.3±5.8 ml · min^{-1}. kg^{-1}) also did not increase nitrite concentration or exercise economy [34]. 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 [20]. 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, nitrite, insulin, growth hormone, insulin growth factor-1 or decreases in lactate or ammonia during exercise were found [31]. Our findings fit with these observations, as we did not find a role for L-arginine in vasomotion in either active or inactive circulatory beds.

In conclusion, L-arginine did not enhance vasodilation at rest or oppose vasoconstriction in active muscle beds during sympathetic activation in healthy young males. Our findings do not support the concept of L-arginine supplementation as a substrate for NO production. Furthermore, L-arginine did not demonstrate any attenuation of vasoconstriction in metabolically inactive tissue, indicating it does not impair functional sympatholysis and oxygen delivery. While L-arginine has documented and consistent benefits in aging, bone health, and in sedentary clinical populations, we were not able to confirm these same benefits are extended to an already healthy and active population.

(Figure 1) Pulse wave velocity (PWV) at rest and immediately following ischemic handgrip exercise in un-supplemented (control) and L-arginine supplemented subjects. No statistical differences were found between control and L-arginine at rest or immediately following reactive hyperemia. Expressed as means±SD.

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