Endothelial, Cardiovascular, and Performance Responses to L-Arginine Intake and Resistance Exercise

DANIEL M. STREETER†1, KARA A. TRAUTMAN†1, TYLOR W. BENNETT*1, LAUREN E. MCINTOSH*1, JAMES W. GRIER‡2, SHERRI N. STASTNY‡1, and KYLE J. HACKNEY‡1

1Muscle, Metabolism, and Ergogenics Workgroup, Department of Health, Nutrition, and Exercise Sciences, North Dakota State University, Fargo, North Dakota, USA; 2Department of Biological Sciences, North Dakota State University, Fargo, ND, USA

*Denotes undergraduate student author, †Denotes graduate student author, ‡Denotes professional author

ABSTRACT

International Journal of Exercise Science 12(2): 701-713, 2019. The purpose of this study was to examine the acute endothelial, cardiovascular, and performance responses to L-arginine intake by assessing flow-mediated dilation (FMD) and various indicators (e.g., heart rate, heart rate variability (HRV), blood pressure, torque) both before and after resistance exercise. Thirty (15 male, 15 female) physically active participants (mean ± SD: age 20.4 ± 1.8 years, height 176.9 ± 10.2 cm, body mass 76.0 ± 12.2 kg) volunteered for a randomized, cross-over, double-blind, placebo-controlled clinical trial. Participants completed five sets of elbow extension-flexion exercise after consumption of either 3 g L-arginine or 3 g of placebo. There was a significant decline in post-exercise elbow extension (p = 0.014) and flexion peak torque (p < 0.001). FMD response after exercise was ~5.8% less than before resistance exercise (L-arginine and placebo data pooled, p < 0.001). Baseline brachial artery diameter significantly increased post-FMD (p < 0.001), post-resistance exercise (p < 0.001), and post-resistance exercise FMD (p < 0.001). There were significant time effects for HRV when expressed as the square root of the mean of the sum of squares of differences between adjacent RR intervals (RMSSD) or the proportion of differences between adjacent normal (NN) RR intervals that exceed 50 ms (pNN50) (all p-values < 0.05), but there were no treatment or interaction effects (all p-values > 0.05). We conclude the increased vasodilation due to acute resistance exercise was not enhanced by acute supplementation with L-arginine nor was exercise performance augmented. Further, the relative contribution of sympathetic nervous system input increased with resistance exercise but was not influenced by the addition of L-arginine.

KEY WORDS: Vasodilation, flow mediated dilation, heart rate variability, performance

INTRODUCTION

The vascular endothelium plays an important role in maintaining systemic cardiovascular health (9). In particular, the nitric oxide pathway is a critical regulator of vasodilation (4, 28) via the conversion of the semi-essential amino acid L-arginine to L-citrulline and nitric oxide via nitric oxide synthase within the endothelium (3). Supplementation with L-arginine has increased blood flow and physical function in patients with heart failure (35). However, L-
arginine has also been marketed for athletes as a vasodilator that is hypothesized to increase oxygen and nutrient delivery to muscles causing a subsequent rise in performance. To date, the vast majority of studies are related to endurance performance with both acute and chronic L-arginine intake data (2). However, reported findings have been inconsistent (2). For example, it has been previously reported that L-arginine concentrations in plasma in adults range from 40 to 100 µmol/L (8), which is a high enough concentration to fully saturate endothelial nitric oxide synthase activity (10, 32). This suggests that adults receive sufficient L-arginine from the standard diet to produce the required amount of nitric oxide synthase without the need for additional supplementation. However, higher circulation levels of nitric oxide inhibitors (such as asymmetric dimethylarginine) or increased production of reactive oxygen species may interfere with nitric oxide bioavailability (25) and could explain why benefits of supplemental L-arginine are observed when circulating levels are within the physiological range (e.g. L-arginine paradox) (2).

Acute resistance exercise is a stress stimulus that triggers reactive oxygen species which could reduce the bioavailability of nitric oxide leading to potential acute benefits of L-arginine supplementation. Both strength and hypertrophy resistance exercise protocols have increased plasma concentrations of protein carbonyl, which is an indicator of oxidative stress (27). However, research into the acute influence of L-arginine on resistance exercise performance is limited. Stevens et al. (39) reported 28% and 10% increases in fatigue resistance and total work, respectively with glycine and L-arginine salt of alpha-ketoisocaproic acid calcium compared to isocaloric sucrose control. Further, Alveras et al. (1) reported increased muscle blood volume but did not observe a corresponding increase in performance. Similarly, Fahs et al. (22) did not find significant differences in blood flow or limb circumference following resistance exercise with either L-arginine or placebo, which suggests little peripheral benefit. In addition, the researchers did not observe differential hemodynamics (systolic and diastolic blood pressure) in response to the acute dietary intervention and resistance exercise (22). Previous studies exploring L-arginine infusion (without exercise) have shown reduced systolic blood pressure response with a corresponding increase in indexes of vagal (parasympathetic) cardiac control (15, 31). Currently, there is no information on the changes in autonomic function with the combination of L-arginine intake and resistance exercise. Furthermore, any potential physiological and performance changes with L-arginine intake requires clarification. Therefore, the purposes of this study were to examine the acute endothelial, cardiovascular, and performance responses to acute L-arginine and resistance exercise. We hypothesized that L-arginine supplementation would enhance the vasodilatory response and lead to a corresponding increase in exercise performance. We further hypothesized that the stress of resistance exercise would increase the relative role of the sympathetic nervous system and L-arginine intake could mitigate that response.

METHODS

Participants
Thirty healthy, physically active males (n = 15) and females (n = 15), 18-25 years of age completed the study. Physical activity status was established from a questionnaire where subjects self-
reported that they participated in 4 ± 1 sessions of moderate intensity physical activity and 2 ± 2 sessions of vigorous intensity physical activity per week (24). Further, subjects had not participated in another clinical trial or consumed another study related investigational product within 30 days of enrollment (11). If a supplement containing L-arginine, or other nitrate precursors, was being taken, a three-week washout period was conducted before starting participation. Additionally, women who were pregnant or lactating were excluded. All procedures were approved in advance by the North Dakota State University Institutional Review Board, and written consent was obtained.

Protocol
A randomized, cross-over, double-blind, placebo-controlled research design was implemented for this investigation. Prior to the randomized testing sessions, participants completed an anthropometric measurement and familiarization session which included: collection of participant age, height (Seca 213, Chino, CA), and body mass (Detecto, Webb City, MO), and familiarization with the Biodex Dynamometer (Biodex Medical Systems, Shirley, NY), ultrasound (Philips Ultrasound, Bothell, WA), and electronic sphygmomanometer (Hokanson Rapid Cuff Inflation System, Bellevue, WA) procedures. Participants then reported to the laboratory for two testing sessions, each separated by at least a 48-hour washout period. Testing sessions were conducted at the same time of day (± 2 hours) to account for circadian variation. Participants entered the trial sessions on an 8-hr fast but were encouraged to arrive at the session well-hydrated. The participants were instructed not to exercise or consume caffeine 24 hours prior to trial time or use toothpaste, chewing gum, or mouthwash the morning of the trials, due to the possible effects on nitric oxide absorption (26). All females tested negative for pregnancy based on a urinary pregnancy test (Clinical Guard, Atlanta, GA) prior to each trial session. At the start of the trial, participants rested for 5 minutes while sitting.

Endothelial responses were measured by a technique termed flow mediated vasodilation (FMD) which assessed the maximal vasodilatory response to shear stress (17). FMD was measured in accordance with recommendations from the International Brachial Artery Reactivity Task Force (17). A Philips HD11XE ultrasound system (Philips, Amsterdam, NL) equipped with 2D imaging, color and spectral Doppler, and a high-frequency vascular transducer was used for the protocol. The participants laid supine with the right arm inside a pillow for a stabilizer. Probe placement was on the brachial artery above the antecubital fossa in the longitudinal plane. Continuous 2D imaging was used to take an initial brachial artery diameter for 10 seconds to determine the resting artery diameter (to cover the full cardiac cycle). Probe placement was outlined with a marker. The test-retest reliability for brachial artery diameter intraclass correlation coefficient was 0.953. A Hokanson E20 electronic sphygmomanometric cuff and rapid cuff inflation system (Bellevue, WA) was placed on the middle of the forearm and inflated to 50 mmHg above systolic blood pressure for 5 minutes and cuff placement was marked with a marker. Upon release of the cuff, the ultrasound probe was placed in the same area in which the first measure was taken. A continuous 2D imaging was used from cuff release to 120 seconds post-release, due to studies suggesting the maximal vasodilation occurs at 60 seconds post-release (17). The average of three diameters measured using RadiAnt DICOM software was used...
for determination of FMD (%), which was expressed as the change in post-stimulus diameter as a percentage of baseline diameter (13).

Measures of the cardiovascular system included blood pressure (BP), heart rate (HR), and heart rate variability (HRV). Blood pressure was measured manually using a sphygmomanometer and stethoscope. HR and HRV were recorded using a Polar H7 Bluetooth strap (Polar, Bethpage, NY) combined with a Personal Pro Elite HRV smartphone application (elitehrv.com) over 5 minute intervals. Data were then further analyzed with Kubios software (31, 39, kubios.com) using the “very low” artifact correction setting. HRV explored the differences in beat-to-beat intervals based on the time (milliseconds) between adjacent R to R (RR) peaks within the PQRST waveform (18, 20, 21). HRV provides a dynamic, sensitive meter of the balance or tone between the two branches of the autonomic nervous system, sympathetic (SNS) and parasympathetic (PNS), and, thereby, the effects of various stressors. Stress, from any source, increases the relative role of SNS input which results in increased average HR, decreased HRV, and increased BP. Recovery from stress involves increased PNS input followed by a return to a more equal balance between the SNS and PNS (37, 40). There are numerous variables that have been used for measuring HRV in two general categories referred to as “time domain” and “frequency domain”. A complete list of definitions is published elsewhere (21). In our laboratory, time domain calculations for the square root of the mean of the sum of squares of differences between adjacent ECG RR intervals, the “root mean square successive differences,” (RMSSD) and the proportion of differences between adjacent normal (NN) RR intervals that exceed 50 milliseconds (pNN50) showed the most internal consistency (Cronbach’s alpha > 0.70 and intraclass correlations > 0.75) (30). RMSSD and pNN50 are also the most commonly used and recommended metrics for studies such as this (7, 16, 19, 20, 23, 29, 33, 34, 38). Baseline HR, HRV, BP, and FMD were all measured in the supine position.

After baseline measurements were collected, each participant consumed either 6 capsules (3 g) of L-arginine (NOW Foods, Bloomingdale, IL) or 6 capsules (3 g) of corn starch placebo (InHealth Specialty Pharmacy, Fargo, ND), which was identical in capsule size and appearance. Each supplement was consumed with 12 oz. of water. The participant then rested for 55 minutes to ensure enough time for digestion, absorption, and availability (12). HR, HRV, and BP were re-measured 30 minutes post-supplementation. At 55 minutes post-supplementation, participants completed a 5-minute warm up using a cycle ergometer (Monark 828E, Vansbro, SV). Sixty minutes post-supplementation, participants completed elbow flexion and extension exercise with their right arm on an isokinetic dynamometer to determine the elbow flexor and extensor peak torque. This was repeated after resistance exercise to determine the rate of fatigue. The exercise protocol, designed to induce fatigue, consisted of five sets of 10 maximal isokinetic extension repetitions of the elbow joint at 90º per second with 30 seconds of rest in between sets. Participants were instructed and encouraged to use full-force for all sets. Isometric peak torque was measured prior to and thirty seconds after exercise to determine the fatigue percentage. Dynamometer settings remained constant for each testing session as recorded during the anthropometric and familiarization session. At 10 minutes post-exercise, HR, HRV, BP, and FMD were measured for the final time.
In summary of the protocol, subjects participated in three sessions: an anthropometric and familiarization session followed by two testing sessions in which they received either (at random) the L-arginine or the placebo supplement. The sessions consisted of the following time sequence:

1. Baseline measurements and supplement consumption. Participants arrived, rested 5 minutes, then were measured for HR, HRV, BP, and FMD, then given the supplement.
2. 30 minutes post-supplement intake. At 30 minutes after consuming supplement participants were re-measured for HR, HRV, and BP.
3. Resistance exercise with pre- and post-exercise isometric peak torque measurements. At 55 minutes after being given the supplement, participants warmed up with leg (cycle ergometer) exercise and measured for peak torque at 60 minutes after supplement intake. Five sets of 10 repetitions of elbow extension-flexion resistance exercise were completed. At 30 seconds following the exercise, the peak torque was re-measured as an indicator of fatigue. Work fatigue during the 5 sets was also examined to determine exercise performance.
4. Post-exercise measurements. At 10 minutes after exercise the final set of HR, HRV, BP, and FMD measurements were taken.

Statistical Analysis
A 2 x 2 (treatment by time) analysis of variance (ANOVA) with repeated measures was used to identify differences in peak torque pre and post-resistance exercise. At each of the three main measurement periods (baseline, 30 minutes post-supplement intake, and post-exercise) the other measurements were taken, including HR, HRV, BP, and analyzed with a 2 x 3 (treatment by time) ANOVA. Because the measurement of FMD (%) requires arterial diameter measures before and after cuff inflation, this was further examined at baseline and post-exercise and analyzed with 2 x 2 (treatment by time) ANOVA. Given that brachial diameter was collected on 4 occasions (baseline, post-FMD, post-resistance exercise, post-exercise FMD) to calculate FMD (%), it was also analyzed separately in a 2 x 4 (treatment by time) ANOVA. A significance level of α < 0.05 was used to determine significance. If a significant interaction (treatment by time) or main effect (treatment or time) was found, paired t-tests with Bonferroni corrections were used for further interpretation. Paired t-tests were used to identify differences in the time after exercise FMD was obtained and the work fatigue initiated from the resistance exercise session. All analyses were performed using SPSS (IBM, Armonk, NY). During the treatment sessions, one brachial artery diameter and FMD measurement was determined to be incorrect due to technical difficulties, therefore, one female was dropped from statistical analysis for that variable only (n = 29). All other analyses were performed with n = 30.

RESULTS
Thirty participants (age: 20.4 ± 1.8 years, height: 176.9 ± 10.2 cm, body mass: 76.0 ± 12.2 kg) were included in the analysis (with the exception of brachial artery diameter and FMD, n = 29). There were no significant treatment by time interactions for elbow extension (p = 0.839) or flexion (p = 0.455) There were, however, significant time effects resulting in a significant decline in post-
exercise elbow extension (-5.5%, \( p = 0.014 \)) and flexion (-11.4%, \( p < 0.001 \)) peak torque (Figure 1).

![Figure 1](image)

**Figure 1.** Elbow extensor and flexor peak torque (mean ± SD) for all participants (\( n = 30 \)) pre- and post-acute bout of resistance exercise (L-arginine and placebo data pooled). *Significantly different from the pre-exercise measurement (\( p = 0.014 \) and \( p < 0.001 \), respectively).

Given the complexity of measuring brachial artery diameter using ultrasound from the same location on the arm at each time point, we recorded the time it took to obtain the post-exercise FMD measurement to make sure there were no differences between L-arginine and placebo trials. There were no significant differences in time after exercise when the FMD measurement was obtained (mean: 4.84 ± 0.86 minutes, \( p = 0.156 \)). Brachial artery diameter showed no significant treatment by time interaction (\( p = 0.979 \)), however, there was a main effect for time (\( p < 0.001 \)). When data were pooled, pairwise comparisons using Bonferroni’s adjustment indicated significant increase in brachial artery diameter as a result of FMD measurement, acute resistance exercise, and the measurement of FMD following resistance exercise (Figure 2).

There was no significant treatment by time interaction (\( p = 0.467 \)) or treatment effect (\( p = 0.163 \)) for FMD. There was, however, a significant time effect (\( p < 0.001 \)). Follow-up Bonferroni corrected t-tests determined the FMD response after exercise was ~5.85% less than before resistance exercise (\( p < 0.001 \), Figure 3) suggesting a reduced ability to vasodilate further after resistance exercise vasodilation was stimulated. Significant time effects for HR (\( p < 0.001 \)), systolic blood pressure (\( p < 0.001 \)) and diastolic blood pressure (\( p < 0.001 \)) are shown in Table 1.
Figure 2. Brachial artery diameter values (mean ± SD) along time points (n = 29) (L-arginine and placebo data pooled). *Significantly different from the baseline value (p<0.05). #Significantly different from post-FMD value (p < 0.05).

Table 1. HR and BP responses between L-arginine and placebo treatments.

<table>
<thead>
<tr>
<th></th>
<th>L-arginine</th>
<th>Placebo</th>
<th>Pooled</th>
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<tbody>
<tr>
<td><strong>HR (beats · minute⁻¹)</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>61.89 ± 10.38</td>
<td>62.19 ± 10.89</td>
<td>62.04 ± 10.63</td>
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<tr>
<td>30 minutes</td>
<td>58.89 ± 9.19</td>
<td>58.20 ± 10.26</td>
<td>58.53 ± 9.72*</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>66.41 ± 12.38</td>
<td>66.20 ± 13.18</td>
<td>66.31 ± 12.78* #</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>108.55 ± 6.34</td>
<td>108.21 ± 7.08</td>
<td>108.38 ± 6.71</td>
</tr>
<tr>
<td>30 minutes</td>
<td>107.59 ± 7.20</td>
<td>108.48 ± 7.53</td>
<td>108.03 ± 7.36</td>
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<tr>
<td>Post-exercise</td>
<td>119.59 ± 8.49</td>
<td>118.90 ± 8.79</td>
<td>119.24 ± 8.64* #</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>67.51 ± 6.42</td>
<td>67.31 ± 5.40</td>
<td>67.41 ± 5.89</td>
</tr>
<tr>
<td>30 minutes</td>
<td>68.14 ± 6.90</td>
<td>68.14 ± 5.71</td>
<td>68.14 ± 6.30*</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>66.20 ± 7.33</td>
<td>65.93 ± 6.01</td>
<td>66.07 ± 6.67* #</td>
</tr>
</tbody>
</table>

*Significantly different than baseline, †significantly different from 30 minutes.
A paired t-test displayed no significant differences in the rate of work fatigue between set 1 and set 5 between supplement groups ($p = 0.829, p = 0.748$). There were significant time effects for HRV when expressed as RMSSD and pNN50 (all $p$-values < 0.05, Figure 4). However, there were no significant difference between the L-arginine and placebo trials ($p > 0.05$).
DISCUSSION

Contrary to our hypothesis, our study was unable to detect any significant differences in vasodilation or resistance exercise performance with acute supplementation of L-arginine compared to placebo. Our vasodilation response data appear consistent with Fahs et al. (22). In that study, eighteen healthy males performed upper body resistance exercise after supplementing with either 7 g of L-arginine or a placebo. Similar to the current study, Fahs et al. (22) reported no interaction effect of L-arginine supplementation and resistance exercise. Further, in the present study, acute L-arginine supplementation did not show any indication of providing a performance benefit to the resistance exercise session. Our study is consistent with Alvares et al. (1) who evaluated the effect of 6 grams of L-arginine prior to maximal isokinetic elbow extension. Peak torque, total work, and set total work were not significantly different between L-arginine and placebo, even though there was an increase in muscle oxygenation (1).
The only study showing potential acute benefits of L-arginine intake and resistance exercise was Stevens et al. (39) who examined 2 g glycine + 6 g L-arginine salt of alpha ketoisocaproic acid. Peak torque and total work increased significantly in the group provided the cocktail supplement versus a placebo. There was also a difference in fatigue index between the two groups, with 2 g glycine + 6 g L-arginine salt of alpha ketoisocaproic acid having greater fatigue resistance (39). An upregulation of metabolic pathways associated with L-arginine, glycine, and alpha ketoisocaproic acid may have worked synergistically in that investigation to improve performance. However, our data do not support the recommendation of any ergogenic benefit from isolated intake of L-arginine when combined with acute resistance exercise. Several currently marketed pre-workout supplements that contain L-arginine have a lower dose than that used in either the Fahs et al. (22) (7 g) or the current study (3 g). However, L-arginine was recently shown to activate mTORC1, a major pathway for muscle protein synthesis, through CASTOR1 (14). Thus, it is plausible L-arginine may have benefits pertaining to muscle growth.

A strength of our study was the addition of cardiovascular measures to complement our vasodilatory and performance indicators. Basic HR and BP responses do not show significant differences between L-arginine and placebo treatments and are similar to what has been previously reported following resistance exercise (5). Similarly, our HRV metrics (RMSSD and pNN50) showed no differences between L-arginine versus placebo treatments but are otherwise as predicted following an acute bout of resistance exercise (i.e., increased sympathetic nervous system activation) (36). Our hypothesis of a potential reduction in the sympathetic response through increased vagal tone from L-arginine was not supported by these data. It should be noted that HR and HRV in general tend to be negatively correlated (6, 20) partially due to a mathematical consequence from curvilinear heart rate aspects and partially due to physiological consequences involving the two. Billman et al. (6) recommended that HRV metrics be corrected for HR (6). However, the correlation between raw HRV and corrected HRV (all metrics) is 1.0 except with major, autonomic interventions as discussed by Billman et al. (6). Thus, either can be used for most purposes. The nearly universal convention for studies such as ours that do not directly intervene in the autonimcs nervous system is to use the raw HRV metrics. L-arginine versus placebo treatment did not produce a significant effect in our raw HRV metrics and it is unlikely that correcting for HR would make any difference.

In summary, this study is one of the few in the literature to examine the acute influence of L-arginine when combined with acute resistance exercise. Acute resistance exercise induced muscle fatigue, increased brachial artery diameter, decreased FMD. Contrary to our hypothesis, we could not detect differences in vasodilation, performance, or any autonomic or cardiovascular indicators between L-arginine and placebo at any specific time point, particularly post-resistance exercise. We conclude that the increased vasodilation due to fatiguing exercise was not enhanced by acute supplementation with L-arginine nor was exercise performance augmented. Further, the relative contribution of sympathetic nervous system input increased with resistance exercise but was not reduced by the addition of L-arginine.
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