The Effects of Chronic Arginine Supplementation on Muscle Strength and Hypertrophy Following Resistance Training

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The Effects of Chronic Arginine Supplementation on Muscle Strength and Hypertrophy Following Resistance Training

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**Abstract**

Arginine is known to be the only precursor in the human body to stimulate nitric oxide (NO) production. NO induces vasodilation via smooth muscle relaxation allowing for increased blood flow and greater waste removal at the tissue. **PURPOSE:** The purpose of this study was to determine whether or not arginine supplementation increased muscle strength and hypertrophy when compared to a placebo group. **METHODS:** Nine male subjects were designated into one of three groups: 1) placebo, 2) Arginine supplementation 20 minutes prior to exercise, and 3) Arginine supplementation two hours post exercise. The experimental groups consumed 6g of arginine before or after each workout. The placebo group consumed 6g of capsulated powdered sugar 20 minutes prior to exercise. Three compound lifts (chest press, leg press, shoulder press) were performed three times per week for four total weeks. A 1 repetition maximum (1RM) was performed for all three lifts on the first day of training and the last day of training. Skin fold analysis was performed on the same days as the 1RM to account for body composition changes. **RESULTS:** Strength performance and muscle hypertrophy of participants in both experimental groups showed no significant increase compared to the placebo group (p>0.05). **CONCLUSION:** The results of this study showed all groups to increase in strength performance following a 4-week resistance training regime but it was found that the experimental groups showed no significant difference compared to the placebo group. Arginine administration failed to significantly increase strength performance and muscle hypertrophy.

**Introduction**

Resistance exercise, when performed for a sufficient duration and intensity, will stimulate adaptations to the working muscle causing changes in the underlying skeletal muscle morphology allowing for increased force output and mass (19). The adaptations that result due to chronic resistance training are diverse and not yet fully understood, but there is a preference for increased type II fiber cross-sectional area (19). Additionally, hormonal concentrations play an increasing role in skeletal muscle
adaptations necessary to promote increased strength performance (3, 14). Outside stimuli, i.e. ergogenic supplementation can potentially be used to help evoke these hypertrophic muscular mechanisms (5, 19).

Arginine is an amino acid that directly produces the signaling molecule nitric oxide (NO). NO is a molecule that causes vasodilation, increasing blood flow, potentially delivering more nutrients and metabolic substrates while removing more waste products. ARG is taken up by endothelial cells through a family of cationic amino acid transporter proteins where nitric oxide synthase enzymes then use the newly introduced ARG as its substrate. The reaction creates an intermediate complex known as Nω-hydroxy-L-arginine (L-NOHA) followed by the release of NO and the formation of L-citrulline. NO is then released into vascular smooth muscle cells, causing relaxation and consequently vasodilation (16). This concept is particularly appealing to athletes because it potentially allows for greater nutrient delivery and waste removal due to the increased diameter following vascular dilation. It is hoped that greater oxygen availability will increase aerobic and anaerobic performance and decrease fatigue in skeletal muscle.

In mice, ARG supplementation has demonstrated positive effects on aerobic exercise performance and skeletal muscle adaptations (1, 9, 13). Hypercholesterolemic mice were used in two studies because the increased cholesterol levels exhibit a reduced endothelial vasodilator function and reduced maximal oxygen consumption during maximal exercise (aerobic capacity). ARG supplementation appeared to restore this exercise-induced endothelium-derived nitric oxide (EDNO) synthesis and prevent the decline of aerobic capacity associated with hypercholesterolemia (1). Conditions
that reduce EDNO production disrupt exercise induced hyperemia. A reduced hyperemic response results in less blood flow to the working muscle, effectively reducing overall exercise capacity (13). From this, the importance of NO is evident to achieve optimal exercise performance (1, 13).

Given that ARG is a precursor for nitric oxide, ARG supplementation could potentially enhance the hyperemic response to exercise in other species as well. Thus, humans could potentially benefit from increasing ARG concentrations due to its ability to be converted to NO which causes vascular smooth muscle relaxation and subsequently an increased hyperemic response allowing for augmented nutrient delivery and waste removal. Acute oral ARG supplementation has been reported to increase muscle blood volume and hyperemia during resistance exercise (5). Moreover, the physical working capacity at the fatigue threshold (PWCFT), or the ability to sustain exercise while at the fatigue threshold, was analyzed to see if there is a relationship between arginine-based supplements and performance during exercise. Supplemented groups were shown to increase in PWCFT by 22.4% and 18.8% respectively for the 1.5g and 3.0g supplement groups, but no change for the placebo group (10).

Furthermore, exercise is known to evoke significant increases in growth hormone (GH) concentrations (20). It is believed by many that GH concentrations play a significant role in promoting the muscular adaptations necessary to achieve increased strength performance during resistance exercise (3). Oral ARG ingestion has also been shown to induce increased basal GH secretion through an inhibition of somatostatin (21). For reasons unknown though, ARG supplementation has been found in multiple
studies to attenuate the release of exercise-induced GH secretion when taken pre-exercise (3, 14, 15).

Given the findings described above, it is possible the NO substrate L-arginine might reduce metabolic waste accumulation as well as increase nutrient delivery and hormonal concentrations (growth hormone) to significantly increase protein synthesis and thus muscle hypertrophy. This study is designed to investigate the present discrepancies concerning the possible ergogenic benefits of ARG by approaching ARG supplementation from a different methodology commonly seen. ARG will not only be supplemented to a group pre-exercise but also to a group post-exercise which is not commonly performed. Additionally, resistance training will be the focus of this study as opposed to endurance training which is the common form of exercise performed in many studies dealing with both human and rodent subjects.

The aim of this study was to determine whether the oral ingestion of ARG would promote the necessary skeletal muscle adaptations to increase strength performance and hypertrophy. We hypothesized that chronic L-arginine supplementation will result in a greater strength performance for experimental group 2 (ingestion of ARG 2 hrs post exercise) compared to the placebo group but that experimental group 1 (ingestion of ARG 20 min prior to exercise) would show no significant difference. ARG is well studied but questions remain. It is fairly common to pick up a pre-workout supplement and find ARG on the ingredients label. But it is still unclear if ARG should be included at all, or if ARG is truly optimal for resistance training following pre-exercise ingestion.
Methods

Subjects

Nine healthy, collegiate athlete men (mean ± SD, age 19 ± 3) volunteered to participate after hearing about the study through flyer advertisements located on the Ohio Dominican University campus in Columbus, Ohio. All subjects were fully aware of all laboratory procedures, associated risks, potential benefits and necessary responsibilities disclosed to them in a consent form which was signed by all participants. None of the subjects were current tobacco users or taking any other dietary supplements. All procedures were approved by the Ohio Dominican University Institutional Review Board.

Procedures

A double-blind study was conducted using nine trained subjects. Three groups (n=3 per group) took part in the four week study. The control group consumed a placebo supplement 20 minutes prior to resistance training. The first experimental group (EG1) orally consumed 6 grams of arginine powder 20 minutes prior to resistance training. The second experimental group (EG2) consumed 6 grams of arginine powder 2 hours following resistance training. Subjects were asked not to consume anything, except water if they wished, 90 minutes before the resistance training sessions.

The researcher supervised the subjects for each of their first resistance training sessions. These sessions included the following exercises: leg press, smith-machine bench press, and smith-machine shoulder press. The resistance training sessions took place twice a week, one on Monday and one on Thursday. The one rep max was
determined for each exercise and then 80% of this weight was used for the first days training session. Subjects were required to train with this weight until they were able to complete the required training exercise program of eight repetitions for all three sets. Once the subject could complete this requirement, they were told to increase the weight by 5%. Subjects were permitted to progressively increase the weight as long as they were able to complete three sets of eight repetitions at the previous weight. Following each training session, the amount of weight used for each exercise and the number of repetitions performed in each set was recorded. For every workout following the first training session, the subjects then performed each exercise on their own and were responsible for recording their own repetition ranges. Each subject recorded every weight used in pounds for each repetition in each set. For the subject’s last workout after the four week period, the researcher accompanied the subjects to determine the subject’s final one rep max.

Prior to resistance training, the girth of the muscle groups being tested was recorded in centimeters, these included: chest width, shoulder width, and thigh width. Additionally, a standard fat caliper was used to determine the subcutaneous fat of each subject. Skinfold thickness was found in three separate regions all residing on the right side of the body: the chest, thigh, and subscapular. Skinfold thickness was measured to the nearest mm, except in cases of low subcutaneous fat (5mm or less) where thickness was measured to the nearest 0.5 mm. Body fat percentage was not calculated. Skinfold thickness was determined in order to account for potential subcutaneous fat increases or decreases that would affect the girth measurement.
Statistical Analysis

Three separate ANOVAs were used to find the statistical significance of the data obtained. An ANOVA was performed for 1) strength performance 2) girth measurements and 3) skinfold thickness.

Results

Resistance Training

Baseline one repetition maximum (1RM) values pre- and post-training for the three exercises used in this study are reported in Table 1. Briefly, there were no statistical differences between groups for baseline values before or after the 4-week training period (p>0.05). Figure 1 and 2 provide a visual of the 1RM values found for all three experimental groups prior to and following the 4-week training regime. Additionally, Figures 3, 4, and 5 provide a comparison between groups for each respective resistance training exercise. In summary, four weeks of resistance training improved 1RM for each exercise in all groups tested. However, despite the increase within each group, there were no statistical differences between groups (p>0.05) that consumed differing amounts of ARG or placebo.

Girth & Fat Measurements

Baseline values of girth measurements for the three girth sites used in this study are reported in Table 1. Briefly, there were no statistical differences between groups for baseline values (p>0.05). The average skinfold thickness at the three sites used in this study is also reported in Table 1. In summary, four weeks of resistance training showed no significant effect for both girth measurements and skinfold thickness (p>0.05). There
were no statistical differences between groups that consumed differing amounts of ARG or placebo (p>0.05).

**Discussion**

The purpose of this study was to determine the effect of chronic L-arginine supplementation on muscle hypertrophy and strength increases. It was hypothesized only chronic L-arginine supplementation would show a significant increase in strength performance and hypertrophy compared to the other groups. In contrast to our hypothesis, chronic L-arginine supplementation had no significant effect on muscle hypertrophy or strength increases following a resistance training routine for any experimental group (p>0.05).

Numerous studies have been performed showing varying results regarding the possible ergogenic effects of ARG supplementation; however, relatively few have been conducted to investigate the effects of oral ARG supplementation on resistance training (5, 9, 11, 26, 27). Alvares et. al. (5) reported acute increases in localized blood volume following a resistance training session for ARG supplementation compared to a placebo. Although there was a significant increase in blood volume, there was no significant increase in strength performance following acute ARG supplementation which is consistent with our findings. Based on previous research, we postulated that an acute bolus of ARG may not provide the body with enough time to make the skeletal adaptations necessary to increase strength performance. For this reason, ARG was administered over a 4-week time frame in the current study. The increased length of
time was to allow for possible increases in strength performance, however no significant differences existed between the supplemented groups and the placebo.

Figure 6 displays the metabolic pathway of L-Arginine as interpreted by Racké & Warnken (16). This illustration helps to establish ARG as the only significant physiological precursor to NO production, an idea well established and supported in multiple studies (1, 8, 12, 17). Although ARG is known to increase NO production, this may not have a significant effect on overall exercise performance. ARG is present in baseline blood concentrations at levels as high as 0.1-1.0 mM, which significantly exceeds the $K_m$ of endothelial NOS (eNOS) for ARG (2). There is only so much ARG that can be utilized due to NOS saturation with its substrate ARG already present in baseline blood concentrations. The fact that administered exogenous L-arginine causes NO-mediated physiological effects regardless of this supposed saturation is known as the arginine paradox (18). eNOS is necessary for the formation of ARG to L-citrulline and subsequently NO release. At baseline concentrations though, asymmetric dimethylarginine (ADMA) is known to be the endogenous eNOS inhibitor. A proposed mechanism is that ARG antagonizes ADMA, resulting in eNOS activity and NO production (22). As a result, NO production is able to cause endothelial vascular vasodilation.

Resistance training alone has been shown to induce rapid vasodilation in humans, resulting in immediate exercise-induced hyperemia (23, 24). Endothelial vasodilation is restricted to the maximal dilation capacity in that endothelial tissue (25). From this, it is possible that additional vasodilator stressors and stimulators will have no effect on vascular endothelial dilation. Thus, the exercise-induced hyperemic response
would be unaffected by NO stimulators. This concept is consistent with a study reporting no significant increases in hemodynamics or brachial-artery hyperemia following supplementation of arginine-alpha-ketoglutarate (NO stimulator) to physically active men during resistance exercise (11). They concluded that the increase in hemodynamics and brachial-artery hyperemia reported in the experimental group and placebo group can only be attributed to metabolic by-products released from the exercising muscle. Therefore, trained athletes during resistance training sessions are potentially unable to increase their hyperemic response past a certain point and therefore would not benefit from the supposed increase in nutrient delivery/waste removal proposed with vasodilation. Thus, as with the current study, trained subjects would fail to see any significant increase in strength performance.

Additionally, the subjects in the above study were trained subjects, a factor that could have implications when administering NO stimulating supplements, i.e. ARG. Untrained individuals have shown increased tolerance to aerobic and anaerobic exercise following acute ARG supplementation (13). However, highly trained athletes in related studies showed no increase in anaerobic or aerobic performance (13). These findings can possibly be attributed to evidence that indicates exhaustive exercise leads to increased arginase activity in trained athletes (13). Arginase enzymes participate in the final step of the urea cycle, competing with NOS for ARG, as can be seen in Figure 6. If there is a decrease in arginase activity for untrained individuals, there will be more available ARG for NOS and thus they may see significant increases in anaerobic performance due to an enhanced hyperemic response compared to trained subjects. These findings are consistent with reports from Sureda et. al. (4) indicating that
untrained or moderately healthy subjects can improve their aerobic and anaerobic exercise tolerance while trained subjects show no positive effect on performance following chronic/acute ARG supplementation. Similarly, the current study used healthy trained subjects and found no significant increase in strength performance following a resistance training regime as can be seen in Figure 1 and 2. Thus, trained subjects appear to have skeletal muscle and vascular adaptations that negate the possible ergogenic effects of ARG supplementation as seen with untrained subjects.

**Limitations**

One limitation of this study was sample size. Only nine healthy trained males were tested during the 4-week training program. A larger sample size would have decreased the variation of the data. More subjects should be obtained for future studies. Additionally, the time frame of 4 weeks used in the current study may have been insufficient to see significant increases in muscular performance. An increased length of study should be used in future studies to decrease chances of variability.

**Conclusion**

Our results showed there was no significant increase in muscle hypertrophy and strength performance following administration of arginine during a 4-week resistance training program (p>0.05). This could be the result of many factors such as the training status of the subjects, enzyme activity, sample size, and the length of the study. Although there is significant research regarding ARG administration, questions still remain concerning its ergogenic effects. In untrained subjects and sedentary
individuals, ARG supplementation seems to have beneficial components (4). However, for trained athletes, the results are more controversial. Our results are consistent with the majority of resistance training investigations that find no significant increase in strength performance following oral ARG administration (4, 5, 7, 11, 14).
References


Tables and Figures

<table>
<thead>
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<th>Variable</th>
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Table 1: Measured variables for the 4-week training study. Variables include: Chest Press, Shoulder Press, Leg Press, Chest Girth, Shoulder Girth, Thigh Girth, Chest Skinfold, Thigh Skinfold, Subscapular Skinfold.
Figure 1: Total weight obtained for all experimental groups for the three resistance exercises pre-4-week training.
Figure 2: Total weight obtained for all experimental groups for the three resistance exercises post-4-week training.
Figure 3: Chest press pre- and post-4-week training comparison for all three experimental groups.
Figure 4: Leg press pre- and post-4-week training comparison for all three experimental groups.
Figure 5: Shoulder press pre- and post-4-week training comparison for all three experimental groups.
Figure 6: Arginine Metabolic Pathway. Compounds included: ARG, arginase, ASL, argininosuccinat lyase, argininosuccinat synthetase 1, OTC, ornithine, carbamoyltransferase; CPS1, carbamoyl-phosphate synthetase 1, mitochondrial; ADC, arginine decarboxylase; GATM, glycine aminotransferase; RARS arginyl-tRNA-synthetase; NOS, nitric oxide synthase; ODC1, ornithine decarboxylase; OAT, ornithine aminotransferase; PYCR1, pyrroline-5-carboxylate, reductase 1; P5CDh = ALDH4A1, aldehyde dehydrogenase family 4, member A1; PSCS, pyrroline-5-carboxylate synthetase; PRODH, proline dehydrogenase (oxidase) 1; SRM, spermidine synthase; SMS, spermine synthase. (41)