THE ACUTE EFFECTS OF L-ARGININE SUPPLEMENTATION ON FLOW-MEDIATED DILATION AFTER RESISTANCE TRAINING TO FATIGUE

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ABSTRACT

The purpose of this study was to determine the effects of acute L-arginine supplementation on the endothelial health in healthy adults by assessing flow-mediated dilation (FMD) and cardiovascular indicators both before and after resistance exercise to fatigue. Thirty (15 male, 15 female) physically active healthy 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 isokinetic elbow extension/flexion exercise after consumption of either placebo or 3 g L-arginine one hour prior. Baseline brachial artery diameter significantly increased post FMD ($p<0.001$), post-exercise ($p<0.001$), post-exercise FMD ($p<0.001$). There was no significant supplement effect on FMD ($p=0.179$). The increase in brachial diameter due to fatiguing exercise was not enhanced by acute supplementation with L-arginine nor did supplementation alter FMD responses after exercise.

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LIST OF ABBREVIATIONS

1RM ..... One-repetition maximal effort

ANOVA ..... Analysis of variance

ARG ..... L-arginine supplement

BD ..... Brachial artery diameter

BF_{Peak} ..... Peak blood flow

BP ..... Blood pressure

CVD ..... Cardiovascular disease

DC ..... Diameter change

eNOS ..... Endothelial nitric oxide synthase

FBF ..... Forearm blood flow

FMD ..... Flow-mediated dilation

HNES ..... Health, Nutrition, and Exercise Sciences

HR ..... Heart rate

iNOS ..... Inducible nitric oxide synthase

NDSU ..... North Dakota State University

nNOS ..... Neuronal nitric oxide synthase

NO ..... Nitric oxide

NOS ..... Nitric oxide synthase

PAR-Q ..... Physical Activity Readiness Questionnaire

PLA ..... Placebo supplement

PT ..... Peak torque

VO_2 ..... Oxygen consumption

WF ..... Work fatigue

CHAPTER I. INTRODUCTION

In 2010, the American Heart Association updated the heart disease and stroke statistics. Results of this update included: 1 of every 6 deaths in the United States of America in the year 2006 were due to coronary heart disease, 2300 Americans die each day from cardiovascular disease (CVD), and an average of one person every 38 seconds dies from CVD. Cardiovascular disease is the leading cause of death in the USA, accounting for over a third of all deaths. Therefore, prevention and treatment is a high priority (Lloyd-Jones et al., 2010). Vascular endothelium plays a large role in the health of the cardiovascular system, affecting vascular tone, cell growth, platelet and leukocyte interactions and thrombogenicity (Bondonno et al., 2015; Corretti et al., 2002). Vascular endothelium is affected by the body's ability to produce and use nitric oxide (NO), a key molecule in the vasodilation process of the vessels through the production of cyclic guanosine monophosphate. Endothelial health in the vascular system can be measured by a protocol called flow-mediated dilation (FMD). Flow-mediated dilation is a measurement in which the diameter of the brachial artery is measured by ultrasound after being occluded for five minutes by an external pressure cuff. Upon release of the cuff, diameter is measured with the highest diameter being recorded (normally between 45 seconds to 60 seconds after release) (Corretti et al., 2002). About 70% of the change of diameter is the effect of nitric oxide due to the shear stress that is applied (Lieberman et al., 1996).

The relationship between nitric oxide and FMD is partially produced by the oxidation process of the molecule L-arginine, a semi-essential amino acid (Bondonno et al., 2015). Because L-arginine is needed to produce NO and the connection between NO and vasodilation, L-arginine has become a very popular ergogenic aid in healthy adults. Used in many pre-workout

supplements in combination with high doses of caffeine, the vasodilation properties have been proposed to increase blood perfusion to the active muscle (Abel et al., 2005; Rector et al., 1996).

Although there are claims that pre-workout supplements containing L-arginine will increase blood flow to the muscles, studies testing the claims report inconsistent results among healthy participants, which are normally the consumers of such pre-workout supplements (Alvares et al., 2011). In a recent review article on the effects of L-arginine supplementation in healthy participants by Alvares et al., (2011), it cannot be assumed that any positive effects on performance in recent studies are the result of increased nitric oxide via the L-arginine method, due to the many other factors effecting performance (Alvares et al., 2011). Additionally, Bai et. al., (2008) states that short-term oral L-arginine is effective on FMD testing but only when the baseline endothelial function is low, or when the participant is unhealthy. In participants which already had a high endothelial health (>7), the L-arginine supplementation showed no improvement (Bai et al., 2008).

Studies have shown discrepancies in FMD response to acute bouts of exercise, several reporting increases (Harvey et al., 2005; Tinken et al., 2009), others decreases (Dawson et al., 2008; Jones et al., 2010), and some of them showing no change (Rognmo et al., 2008; Varady et al., 2010). With mixed methods such as exercise intensities, modalities, and timing different, it is difficult to compare these studies side-by-side. Although FMD is used in clinical settings to determine endothelial health, vascular response to exercise by measurement of FMD has not been well established.

Fewer studies focus on the effects of L-arginine supplementation combined with resistance exercise on FMD. In a study by Fahs et al., (2009), resistance exercise and moderate L-arginine supplementation are paired to study the hemodynamic and vascular response in the

brachial artery (Fahs et al., 2009). The authors concluded that L-arginine does not have an effect on the hemodynamic and vascular responses to resistance exercise, with data showing no significant increase in blood flow or diameter size (Fahs et al., 2009). However, this is the first study to look at both resistance training and brachial artery paired with L-arginine supplementation. Due to the lack of research on the effects of L-arginine supplementation on flow-mediated response after resistance exercise, more research should be done in order to determine the effects on healthy populations including considerations for gender differences in order to establish if L-arginine has an effect on cardiovascular health.

Purpose of the Study

The purpose of this study is to determine the effects of acute L-arginine supplementation on the endothelial health in healthy populations by assessing the FMD in the brachial artery both before and after resistance exercise to fatigue of the biceps and triceps.

Research Questions

1. Will acute L-arginine supplementation effect endothelial health in healthy participants after resistance exercise to fatigue?
2. Will acute L-arginine supplementation effect endothelial health in males and female participants differently after resistance exercise to fatigue?
3. Will acute L-arginine supplementation effect performance capacity?

Significance of the Study

This research will add to the current information on L-arginine supplementation in healthy adults by determining if the acute supplementation will have a positive effect on the endothelial health of the subjects after they have completed resistance exercise to fatigue.

Limitations of the Study

1. Although FMD is a reliable measure of nitric oxide response, the process is normally done by a trained professional. The research team conducting the study had training for this process, however they were not certified ultrasound technicians.
2. It is not known if menstrual cycle had a large impact on the dilation response to occlusion. Some studies have mentioned the menstrual cycle but not many have studied on females (Corretti et al., 2002).
3. Participants may have had a lack of motivation throughout the exercise protocol and may had have an effect on the results. A participant who did not complete the reps with full strength may cause the exercise protocol fatigue to be underestimated.

Definition of Terms

- Cardiorespiratory System - Made up of the lungs, heart, and blood vessels which deliver oxygen to the cells (Hoeger & Hoeger, 2015).
- Concentric Contraction - A muscle action in which the muscle is able to overcome the resistance, leading to muscle shortening (Coburn & Malek, 2012).
- Dynamometer - An instrument that measures multiple body joints through different types of motions (Feiring, Ellenbecker, & Derscheid, 1990).
- Eccentric Contraction - Action that occurs when a muscle cannot develop sufficient tension and is overcome by an external load, and thus progressively lengthens (Coburn & Malek, 2012).
- Endothelial cells - Regulates vascular tone, platelet activity, leukocyte adhesion, and angiogenesis by producing nitric oxide and other regulatory factors (Corretti et al., 2002; Vita & Keaney, 2002).

- Flow-mediated dilation - A change in size of the diameter of the brachial artery after a five minute occlusion compared to baseline (Corretti et al., 2002).
- Isokinetic - A fixed speed and variable resistance that can be modified (Feiring et al., 1990).
- L-arginine - A semi-essential amino acid used as a precursor to nitric oxide (Alvares et al., 2011).
- Physical Activity Readiness Questionnaire (PAR-Q) - An assessment tool to initially screen apparently healthy clients who want to engage in low-intensity exercise and identify clients who require additional medical screening (Coburn & Malek, 2012).
- Physically active – Participant who participates in 4 ± 1 sessions of moderate intensity physical activity and 2 ± 2 sessions of strenuous intensity physical activity per week (Godin & Shephard, 1985).
- Repetition - One complete movement of an exercise (Fleck & Kraemer, 2014).
- Resistance training - A form of physical activity that is designed to improve muscular fitness by exercising a muscle or a muscle group against external resistance (Feigenbaum & Pollock, 1999).
- Rest intervals - The time interval between two sets (Coburn & Malek, 2012).
- Set - A group of repetitions that are performed consecutively (Coburn & Malek, 2012).

CHAPTER II. LITERATURE REVIEW

Nitric Oxide

Vascular endothelium plays a large role in the health of the cardiovascular system, affecting vascular tone, cell growth, platelet and leukocyte interactions and thrombogenicity (Corretti et al., 2002). Endothelial dysfunction is associated with risk factors such as obesity, smoking, hypertension, hyperglycemia, and a family history of early atherosclerotic disease (Widlansky, Gokce, Keaney, & Vita, 2003). This is due to the impairment of endothelium-dependent relaxation which is partly caused by pro-inflammatory and pro-thrombotic state (Mannheim et al., 2008) All of these factors are associated with arterial stiffness, which is a predictor of cardiovascular risk (Wilkinson et al., 2002).

Arterial stiffness is controlled by vasodilators, specifically an endothelium-derived relaxing factor which in the late 1980's was discovered to be the gas nitric oxide (Moncada & Higgs, 1993). After that discovery, Furchgott (1983) found that endothelial cells reversed the constrictive effects of acetylcholine on vascular smooth muscle through the release of nitric oxide (Furchgott, 1983). Three forms of nitric oxide synthases (NOS) have been since discovered, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), with endothelial NOS having a direct role in the health of the vascular system (Bondonno et al., 2015). This eNOS is reactant upon both a shear stress and pulsatile flow, creating the vasodilation effect on the endothelial tissue (Moncada & Higgs, 1993). Endothelial nitric oxide synthase is combined with other factors, calmodulin, tetrahydrobiopterin, nicotinamide adenosine, dinucleotide phosphate, flavin adenine dinucleotide, nicotinamide adenine dinucleotide, and molecular oxygen, to turn L-arginine into nitric oxide and L-citrulline (Alvares et al., 2011).

Once nitric oxide is produced, there is a number of places that the substrate could travel to and must be used or excreted, based off of it's half-life of only a few milliseconds (Thomas, Liu, Kantrow, & Lancaster, 2001). However, the main action relevant to this study is the direct effect with the endothelial tissue. Nitric oxide binds with haeme moiety on satellite glial cells to increase the cellular second messenger cyclic guanosine monophosphate levels in the cells (Ignarro, 1989). With an increased level of cyclic guanosine monophosphate, the substrate reacts with calcium and calmodulin to create at vasorelaxation effect in the endothelial cells through the potassium channel (Palmer, Ferrige, & Moncada, 1987).

Overall, the vasodilation effect of nitric oxide can provide relief to increased arterial stiffness and increase the overall health of the endothelial cells. The improved health of endothelial cells is dependent upon the production of nitric oxide which is made through the oxidation of L-arginine (Ignarro, 1989; Moncada & Higgs, 1993).

L-arginine

L-arginine is a semi-essential amino acid that is taken in through a person's endogenous diet as well as made by the body (Ernst, 2001). There are many functions of this amino acid. L-arginine is one of the many contributors to protein synthesis and is one of the substrates in the production of nitric oxide. (Ignarro, 1989; Tong & Barbul, 2004). Due to the physiological effect of causing an increased blood perfusion to the muscle, L-arginine has been increasingly used in dietary supplements. L-arginine is an ergogenic aid that is reported to increase the amount of blood available to the muscle; which provides oxygen and other important nutrients to fuel cellular metabolism (Alvares et al., 2011; Camic et al., 2010; Campbell et al., 2006). Supplementing with L-arginine is reported to lead to nitric oxide production, even though the L-arginine stores may already be saturated.

The L-arginine paradox is a theory referring to the saturation of L-arginine in the endothelial cells. Due to L-arginine being one of the only substrates for the production of nitric oxide, the data supports that L-arginine concentrations are high enough to be able to supply eNOS (Alvares et al., 2011). The paradox in this theory is that although the saturation levels are sufficient, supplementation of L-arginine still may have an effect. The greatest effect of L-arginine produced vasodilation has been seen in patients with cardiovascular disease (CVD) (Clarkson et al., 1996; Creager et al., 1992; Pieper, Siebeneich, & Dondlinger, 1996). The area in question and the purpose of this study is to determine the effects of supplementation on healthy individuals.

The effects of L-arginine supplementation on healthy individuals needs to be studied due to L-arginine commonly being combined inside pre-workout supplements as well as to determine if L-arginine supplementation could possibly help increase endothelial health for long term use. Poor endothelial health is a risk factor for cardiovascular disease. If individuals are taking supplements that contain L-arginine, the possible effect on the health of the cardiovascular system needs to be known and more important, is the supplement safe? The next sections will examine L-arginine supplementation combined with exercise to determine the effects on a healthy individual.

L-arginine supplementation research selection. The NDSU Database and Google Scholar were used to identify L-arginine studies. To be included in this review of supplementation, the studies had to fit the following research criteria: the studies had to be randomized, contain an exercise intervention, the subjects had to be healthy individuals, and L-arginine had to have been used in the intervention process. Studies were not included if they: were not randomized, did not recruit a healthy sample, did not apply an exercise intervention,

and did not supplement with the required supplementation (other exclusions may have been applied). The most common exclusion was that the subjects had to be healthy, since multiple studies have been done with clinical subjects. The studies were then split into four categories: chronic supplementation with aerobic exercise (Table 1), chronic supplementation with resistance training (Table 2), acute supplementation with aerobic exercise (Table 3), and acute supplementation with resistance exercise (Table 4). The tables were created by using the tables in Alvares et. al., (2011) as references and guidelines for the table characteristics and any other recent studies found were also included in the table (Alvares et al., 2011).

Supplementation of L-arginine has been applied over various durations, ranging from ten minutes prior to exercise to chronic supplementation (Buford & Koch, 2004) (Fricke, Baecker, Heer, Tutlewski, & Schoenau, 2008). Due to this large range of supplement duration and timing, the exact length to which supplementation is most beneficial is controversial. The dosage also varies between most studies, Little et al., (2008) uses an equation based on body weight (0.1 g/kg/day) while Chen et al., (2010) uses a set amount of 5.2 grams. Even though the different supplementation duration and dosage amount can cause the results to be varied, neither of these variables were used as a limiting criteria.

Chronic effects of L-arginine in healthy subjects. The studies that included chronic supplementation with cardiovascular training had multiple dependent variables (Table 1). Some measured power while others measured the blood lactate levels and other variables (Abel et al., 2005; Chen, Kim, Henning, Carpenter, & Li, 2010; Colombani et al., 1999; Little, Forbes, Candow, Cornish, & Chilibeck, 2008). It is significant to note that none of the studies had dependent variables that had matched the effectiveness of L-arginine supplementation throughout all of the studies.

Long term L-arginine supplementation seemed to have significant effects on peak power, anaerobic threshold, and physical working capacity at fatigue threshold (Camic et al., 2010; Chen et al., 2010; Little et al., 2008) when paired with cardiovascular training. In each study, a cycle ergometer was used, although different exercise protocols were used. Little et al., (2008) had tested male trained subjects (n=35) with 0.1/g/kg/day L-arginine supplement for 10 days in a random, double-blind experiment. An exercise of a bench press test and three sets of 30 second Wingate cycle tests was performed. Increased peak power was the only significant results compared to the two other supplements. Other variables measured were 1RM strength and mean power, both of which showed no significant differences.

Likewise, Chen et al., (2010) had a positive result with the cycle ergometer. Sixteen male cyclists were given either 5.2 grams of L-arginine or a placebo for a 3 week period in a random, double-blind experiment. The exercise protocol consisted of cycling on an ergometer at 60% maximal work rate until exhaustion was performed. Dependent variables included: anaerobic threshold, blood lactate, maximal oxygen consumption (VO_2) and mean power. Similar to Little et al., (2008), mean power was not different between the supplements. The only variable that had a significant increase was the anaerobic threshold response.

Another study that yielded a significant effect from cycle ergometer training was Camic et al., (2010). Fifty untrained males were recruited to supplement with either 1.5 grams of L-arginine, 3.0 grams of L-arginine, or a placebo for 4 weeks, in a random, double-blind fashion. A cycle ergometer test, 80 watts increasing 30 watts every 2 minutes until exhaustion, was applied after supplementation. The main variable studied was the physical working capacity at fatigue threshold, which showed a positive effect in the L-arginine supplementation groups to the placebo groups.

Table 1

Chronic effects of L-arginine supplementation on cardiovascular training.

Study	No. of subjects; sex (sample characteristic); study design	Supplementation	Exercise protocol	Results
(Abel et al., 2005)	30; M (trained); r, db	Asp-Arg (14.4g or 5.0g) or PLA daily; 4 wk	Cycle ergometer (100 W increased every 3 min by 30 W until exhaustion)	FET, VO_2, VCO_2, [La]: Asp-Arg = PLA
(Colombani et al., 1999)	14; M (trained); r, db	Asp-Arg (15g) or PLA daily; 4 wk (1 wk washout)	31 km run	[La], ammonia, TDC: Asp-Arg = PLA Urea: Asp-Arg > PLA
(Little et al., 2008)	35; M (trained); r, db	Cr+AAKG (0.1g/kg/d Cr+0.075g/kg/d AAKG) or Cr or PLA; 10 d	1RM bench-press test; 3 sets 30 s Wingate cycle tests (2 min rest)	1RM strength: Cr+AAKG = Cr > PLA P_{max}: Cr+AAKG > Cr = PLA MP: Cr+AAKG = Cr = PLA
(Chen et al., 2010)	16; M (cyclists); r, db	L-arg (5.2g powder form) or PLA; 3 wk	Cycle ergometer (until exhaustion at 60% MWR)	AT: L-arg > PLA [La], VO_{2max}, MP: L-arg = PLA
(Camic et al., 2010)	50; M (untrained); r, db	L-arg (1.5g or 3.0g) or PLA; 4 wk	Cycle ergometer (80 W increasing 30 W each 2 min until exhaustion)	PWC_{FT}: L-arg > PLA

Note. 1RM = one-repetition maximum; AAKG = arginine alpha-ketoglutarate; Asp-Arg = arginine aspartate; AT = anaerobic threshold; db = double-blind; FET= fatigue exercise time; [La] = lactate concentration; L-arg = L-arginine free form; M = males; MP = mean power; MWR = maximal work rate; PLA = placebo; PWC_{FT} = physical working capacity at the fatigue threshold; r = randomized; reps = repetitions; VCO_2 = carbon dioxide production; VO_2 = oxygen consumption; VO_{2max} = maximal VO_2; > or < indicates significant difference between groups ($p<0.05$); = indicates no significant differences between groups

Studies that have researched the chronic effects of L-arginine supplementation on resistance training in healthy subjects have had mixed outcomes (Table 2). It is important to note that none of the studies had dependent variables that had matched results in favor of L-arginine supplementation throughout all of the studies. From the articles reviewed, chronic supplementation doses of L-arginine paired with resistance training had a positive effect on: one rep max strength (1RM), anaerobic power, fatigue exercise time, peak power, fatigue index, and peak jump force per kilogram (Campbell et al., 2006; Fricke et al., 2008; Little et al., 2008; Santos et al., 2002). Two of those studies include the Wingate test, which was included in the resistance training section due to the strength and anaerobic properties of the test. Campbell et al. (2006) used 35 trained males in the random, double-blind study. Supplementation duration was 8 weeks with 12 grams of an L-arginine alpha-ketoglutarate. Over the course of the 8 weeks, the

subjects did resistance training 4 times a week and did aerobic activity 3 times a week for 30 minutes at 70% of heart rate maximum. There was a significant increase in 1RM strength and anaerobic power in those who had L-arginine supplementation over the course of 8 weeks. This study also saw an increase in time to fatigue suggesting the subjects lasted longer in their final exercise test when receiving L-arginine.

Related to strength, Little et al. (2008) saw an increase in maximum power in the Wingate test with those who had L-arginine supplementation. In a random, double-blind study, 35 trained males were supplemented with either creatine and L-arginine alpha-ketoglutarate, creatine, or a placebo for 10 days. Subjects were tested in 1RM bench press and 3 Wingate tests with 2 minutes of rest in between. Compared to the study by Campbell et al. (2006), Little et al. (2008) also saw an increase in 1RM strength and saw an increase in both the creatine and L-arginine group as well as the creatine group. In addition to 1RM strength, there was also a significant effect on peak power in the creatine and L-arginine group. It is important to note that creatine may have had a large role to play in both of these significant increases.

Santos et al. (2002) used a different exercise method than the two previous mentioned studies. In this random, double-blind, crossover study, 12 untrained males were tested over 15 days with either an L-arginine aspartate supplement or a placebo. An isokinetic dynamometer was used to measure 15 repetitions of concentric knee flexion and extension at 180^{o}/s. Although there was no significant difference in the strength or mean power output, there was a significant decrease in fatigue index, a subjective method that suggests the subjects may have possibly not have had to work as hard to produce the same results. However, this is low amount of repetitions to determine a large amount of fatigue.

In a study by Frick et al. (2008), more objective measures were used, such as strength and power, instead of subjective methods, like the fatigue index. Twenty-three postmenopausal female subjects were tested in a random, double-blind fashion. Over the course of 6 months, the subjects were tested with either 18 grams of L-arginine hydrochloride or a placebo. Strength and power were measured by both a dynamometric grip force and jumping on a force plate. Of the variables tested, there was a significant increase in peak jumping force per kilogram. However, there was not a significant increase in peak jumping force itself. Although the subjects were postmenopausal, the study states that they were healthy as well.

Table 2
Chronic effects of L-arginine supplementation on resistance training.

Study	No. of subjects; sex (sample characteristic); study design	Supplementation	Exercise protocol	Results
(Campbell et al., 2006)	35; M (trained); r, db	AAKG (12g) or PLA daily; 8 wk	RT (4 x/wk 3 sets 8-10 reps, 70-85% 1RM), 1RM bench-press test, Wingate test, Aerobic activity (3 x/wk 30 min at 70% of HR_{max})	1RM strength, AP; AAKG > PLA FET: AAKG>PLA
(Little et al., 2008)	35; M (trained); r, db	Cr+AAKG (0.1g/kg/d Cr+0.075g/kg/d AAKG) or Cr or PLA; 10 d	1RM bench-press test; 3 sets 30 s Wingate cycle tests (2 min rest)	1RM strength: Cr+AAKG = Cr > PLA P_{max}: Cr+AAKG >Cr = PLA MP: Cr+AAKG = Cr+PLA
(Santos et al., 2002)	12; M (untrained); r, db, co	Asp-Arg (3g) or PLA daily; 15 d	Isokinetic dynamometer (15 reps concentric knee flexion/extension 180º/s)	FI: Asp-Arg < PLA FRF (%): Asp-Arg = PLA
(Fricke et al., 2008)	23; F (PM); r, db	L-arg HCI (18g) or PLA; 6 mo	Dynamometric grip force and counter-movement jumping on force plate	MIGF (N), PJP (W) and PJF (N): L-arg = PLA PJF/kg: L-arg > PLA

Note. 1RM = one-repetition maximum; AAKG = arginine alpha-ketoglutarate; AdiP = adiponectin; AP = anaerobic power; Asp-Arg = arginine aspartate; co = crossover; db = double-blind; F = females; FET= fatigue exercise time; FI= fatigue index; FRF = fatigue resistance factor; HR_{max} = maximal heart rate; [La] = lactate concentration; L-arg = L-arginine free form; L-arg HCl = L-arg hydrochloride; M = males; MIGF = maximal isometric grip force; MP = mean power ;PJF = peak jump force; PJP = peak jump peak; PLA = placebo; PM = postmenopausal; P_{max} = peak power; r = randomized; reps = repetitions; RT = resistance training; TC = total cholesterol; TRI = triglycerides; TDC = total distance covered; > or < indicates significant difference between groups ($p<0.05$); = indicates no significant differences between groups

Acute effects of L-arginine in healthy participants. Multiple studies have looked at the acute effects of L-arginine supplementation combined with cardiovascular training in healthy

subjects (Table 3). There seems to be a lack of studies in this discipline, possibly due to less studies finding an effective reaction. It is significant to note that none of the studies had dependent variables that had matched the effectiveness of L-arginine supplementation throughout all of the studies. However, throughout 4 different studies showed that there was no significant effect of L-arginine on blood lactate levels (Bailey et al., 2010; Liu et al., 2009; McConell, Huynh, Lee-Young, Canny, & Wadley, 2005; Yavuz, Turnagol, & Demirel, 2014). It seems the positive effects of supplementation are rather limited. Studies have shown a positive effect on dependents such as: mean power, glucose clearance rate, fatigue exercise time, nitrite, cost of oxygen consumption, slow component of oxygen consumption, and time to exhaustion (Bailey et al., 2010; Buford & Koch, 2004; McConell et al., 2005; Yavuz et al., 2014).

Buford and Koch (2004) studied acute supplementation in 10 resistance trained males in a random, double-blind experiment. Supplementing with 6 grams of L-arginine or a placebo, a cycle ergometer test was set in place to study the supplementation effect on mean power, fatigue index, and max power. The supplementation was given at 45, 30, and 15 minutes prior to exercise and was combined with cranberry juice, same as Stevens et al, (2000). After 5 sets of 10 second sprints, the most significant effect was an increase in mean power throughout the tests. Blood lactate was measured but there was no difference between supplementation groups. The cranberry juice could have had an effect on the supplementation digestion and absorption. Another factor in this study was the L-arginine supplement was in the form of glycine-L-arginine-[alpha]-ketoisocaproic acid, which is 2.0 grams glycine plus 6.0 grams L-arginine monohydrochloride plus 3.2 grams [alpha]-ketoisocaproic acid calcium salt (Buford & Koch, 2004).

Similar to Buford et al., (2004), McConell et al. (2005) used a cycle ergometer to study the effects of L-arginine on blood levels and levels of fatigue. In a random, double-blind crossover study, 9 endurance trained males were intravenously supplemented with 30 grams of L-arginine hydrochloride prior to exercise. The subjects had to complete a cycle ergometer test that was 120 minutes at 70% ± 1% of their VO_2 peak plus an all-out 15 minute cycle test. Although blood lactate, insulin, rating of perceived exhaustion, and fatigue exercise time were all measured, the only significant effect of supplementation was an increase in glucose clearing rate. The lack of insulin reaction helps show that the increased glucose clearance could have been influenced by an increase in nitric oxide production. This was the only study found to study the effects of L-arginine supplementation on glucose clearance rates (McConell et al., 2005).

In a recent study by Bailey et al, (2010), there were many significant effects of L-arginine supplementation. Nine trained males were researched in this random, double-blind crossover study. Supplementation occurred over a span of 3 days, with the last supplement taken 1 hour prior to exercise. The subjects were either taking 6 grams of L-arginine or a placebo supplement. Subjects were tested by completing a cycle ergometer test at 70-90 rpm until exhaustion. Significant effects included: increased fatigue exercise time, increased blood nitrite levels, and reduced oxygen consumption as well as slow oxygen consumption with the L-arginine group. McConell et al, (2005) had no effect on fatigue exercise time, compared to Bailey et al, (2010) showing an increase in time which correlated with the effect displayed by Campbell et al, (2006). Significant effects were given credit to the nitrite producing properties of L-arginine supplementation.

Yavuz et al, (2014) did not focus on how the properties L-arginine supplement affected the subjects in the study, but rather the performance increase. Nine elite male wrestlers were

chosen in a random, double-blind crossover study to focus on the effects of L-arginine supplementation on time to exhaustion. Similar to the effects that Bailey et al, (2010) and Campbell et al, (2006) researched on fatigue exercise time, Yavuz et al, (2014) showed an increase in exercise time to exhaustion. Supplementation consisted of 1.5 grams per kilogram of body weight or a placebo that was taken 1 hour prior to exercise. The wrestler's exercise protocol included a graded cycle ergometer test that started at 90 watts and increased 30 watts every 30 minutes. The only significant difference between supplementation occurred during the exercise time to fatigue, which was (1386.8±69.8 s) with arginine supplementation compared to placebo (1313±90.8 s) ($p < 0.05$). Subjects were on a 12 hour fast and were only allowed to drink water during that time, hydration levels were not measured.

Table 3

Acute effects of L-arginine supplementation on cardiovascular training.

Study	No. of subjects; sex (sample characteristic; study design	Supplementation	Exercise Protocol	Results
(Liu et al., 2009)	10; M (judo athletes); r, db, co	L-arg (6g) or PLA 3d	Cycle ergometer (13 sets at 0.05kp/kg, 1 min rest at 60 rpm after set 9)	[La], ammonia, nitrate, nitrite, L-citr, MP and P_{max} L-arg = PLA
(Buford & Koch, 2004)	10; M (resistance trained); r, db	GAKic or PLA 45, 30, and 10 min before exercise	Cycle ergometer (5 sets of 10 s sprints, 50 s rest intervals)	MP: GAKic > PLA P_{max}, FI, [La]: GAKic = PLA
(McConell et al., 2005)	9; M (endurance trained); r, db, co	L-arg HCI (30g IV) or PLA after 75 min of exercise	Cycle ergometer (120 min at $72^{+/-}$ 1% $VO_{2\ peak}$), 15 min sprint on ergometer	[La], insulin, $VO_{2\ peak}$, RPE, FET: L-arg = PLA GCR: L-arg > PLA
(Bailey et al., 2010)	9; M (trained); r, db, co	L-arg (6g) or PLA 3 d 1 h before exercise	Cycle ergometer (70-90 rpm)	[La]: L-arg = PLA FET, nitrite: L-arg > PLA VO_{2cost}, VO_{2sc}: L-arg < PLA
(Sandbakk et al., 2015)	9; M (endurance trained; r, db	L-arg Nit or Nit or PLA at breakfast and 1 hr before exercise	5 min 10 km/hr run, 5 min 14 km/hr run, 180-m sprint, 5-km run	10km/hr, 14km/hr: L-arg Nit = Nit = PLA MAP: L-arg Nit = Nit = PLA 180-m sprint: Nit > L-arg Nit = PLA
(Yavuz et al., 2014)	9; M (elite wrestlers); r, co	L-arg (1.5g/10kg body weight) or PLA after 12h fast, 1 hr before exercise	Cycle ergometer (60 w warmup, 90 w start, increase 30 w every 30 min) 60-70 rpm	[La], HR_{max}, VO_2: L-arg = PLA $T_{exhaustion}$: L-arg > PLA
(Vanhatalo et al., 2013)	15; M (healthy); r, db, co	L-arg (6g) or PLA 90 min before exercise	2 moderate-intensity 6 min bouts, 1 severe-intensity to exhaustion	MAP, RER, VO_2: L-arg = PLA $Ex_{tolerance}$: L-arg = PLA
(Vanhatalo et al., 2013)	8; M (healthy); r, db, co	L-arg (6g) + CHO (25g) or CHO (25g) 60 min before exercise	2 moderate-intensity 5 min bouts, 1 severe-intensity to exhaustion	MAP, RER, VO_2: L-arg = CHO $Ex_{tolerance}$: L-arg = CHO

Note. CHO = carbohydrates; co = crossover; db = double-blind; FET= fatigue exercise time; FI= fatigue index; GCR = glucose clearance rate; IV = intravenous; [La] = lactate concentration; L-arg = L-arginine free form; L-arg HCl = L-arg hydrochloride; L-arg Nit = L-arginine and nitrate; L-citr = L-citrulline; M = males; MAP = mean arterial pressure; MP = mean power; Nit = nitrate; PLA = placebo; P_{max} = peak power; r = randomized; reps = repetitions; RPE = rating of perceived exertion; rpm = revolutions per minute; = time to exhaustion; VO_2 = oxygen consumption VO_{2cost} = cost of oxygen consumption; VO_{2peak} = peak oxygen consumption; VO_{2sc} = slow component of oxygen consumption; > or < indicates significant difference between groups ($p<0.05$); = indicates no significant differences between groups

Few studies have researched the acute effects of L-arginine supplementation on resistance training in healthy populations (Table 4). It is significant to note that none of the studies had dependent variables that had matched results in favor of L-arginine supplementation throughout all of the studies. In the studies that apply resistance training to healthy subjects, the significant results are: peak torque, total work, fatigue index, capillary density, muscle blood

volume, and plasma L-arginine (Alvares et al., 2012a; Pranskunas, Pranskuniene, Bernatoniene, Vaitkaitiene, & Brazaitis, 2015; Stevens, Godfrey, Kaminski, & Braith, 2000).

Stevens et al, (2000) studied the supplementation effects on an isokinetic dynamometer, focusing on muscle performance. In a random, double-blind crossover study, 13 males were supplemented with 2 grams glycine + 6 grams L-arg HCl + 3.2 grams alpha-ketoisocaproic acid or a placebo 45, 20, and 0 minutes prior to exercise. After an exercise protocol of knee flexion and extension on a dynamometer, there was a significant increase in peak torque, total work, and a lower fatigue index. This result is different from Alvares et al, (2012) who showed no increase in peak torque and total work with supplementation on the elbow joint. However, the lower fatigue index correlates with studies that measure fatigue exercise time and time to exhaustion (Bailey et al., 2010; Santos et al., 2002; Yavuz et al., 2014).

In a related study also using a dynamometer, Alvares et al, (2012) had 15 male subjects participate in a random, double-blind cross-over study that supplemented with either 6 grams of L-arginine or a placebo 80 minutes before exercise. Subjects completed 3 sets of 10 reps on an isokinetic dynamometer of elbow flexion and extension. Significant increases were found when measuring muscle blood volume and blood L-arginine concentrations. Similar to another study, blood L-arginine levels rose due to the consumption of L-arginine (Tang et al., 2011). Muscle blood volume was measured with ultrasound and increased, however it did not result in an increase in strength performance such as peak torque or total work. The study concluded in saying that it is too early to suggest L-arginine supplementation as an ergogenic aid to increase strength dependents.

One study measured a dependent variable that has not been assessed in any other study found. This variable was capillary density, measured by sidestream dark field video microscopy.

Twenty healthy males participated in a random, double-blind study, focusing on the microcirculatory effects of short term L-arginine supplementation (Pranskunas et al, (2015). Participants were supplemented with either 20 grams of liquid L-arginine or a placebo 45 minutes prior to engaging in the BOSCO jumping test, an anaerobic jumping protocol. Capillary density, average power, mean arterial pressure, cardiac output, and post-exercise heart rate were all measured to determine the microcirculatory effects. The significant differences were found in an increased capillary density and decreased post-exercise heart rate. Increased capillary density is attributed to vasodilation caused by nitric oxide that was synthesized by the L-arginine supplement. Pranskunas et al, (2015) discussed that the reduced post heart-rate another factor of nitric oxide production (Pranskunas et al., 2015)

In summary of exercise and L-arginine supplementation, just as Alvares (2009) stated, the results of L-arginine are mixed. The dosage amounts range from 18 grams to 3 grams of L-arginine. When examining the exercise protocols for all of the studies, there is very little independent variables that consistently show positive performance effects, although long term supplementation may seem to have more effects of supplementation (this could be due to more of those studies existing). Many dependent factors that are more performance focused (strength and power) show the least amount of change with supplementation while factors such as increased blood volume and L-arginine plasma concentrations having more positive supplementation effects.

Table 4

Acute effects of L-arginine supplementation on resistance training.

Study	No. of subjects; sex (sample characteristic); study design	Supplementation	Exercise protocol	Results
(Stevens et al., 2000)	13; M (NR); r, db, co	GAKic or PLA 45, 20, and 0 min before exercise	Isokinetic dynamometer (concentric/eccentric knee extension 35 reps at 90º/s (0 min, 5 min, 15 min, and 24 h after GAKic or PLA)	PT, TW: GAKic > PLA FI: GAKic < PLA (except after 24 h)
(Pranskunas et al., 2015)	20; M (healthy); r, db	L-arg (20g liquid) or PLA 45 min before exercise	BOSCO test (anaerobic jumping protocol) 60 s	$D_{capillary}$: L-arg > PLA P_{avg}, MAP, CO: L-arg = PLA Post-HR: L-arg < PLA
(Thiago Silveira Alvares et al., 2012b)	15; M; r, db,	L-arg (6g) or PLA 80 min before exercise	3 sets of 10 maximal voluntary contractions of isokinetic concentric elbow extension (60°·s–1, 2-min rest between sets)	PT, TW, STW, Mox: L-arg = PLA Mbv, Plasma L-arg: L-arg > PLA
(Tang et al., 2011)	8; M (healthy); r, db, c-b	EAA + L-arg (10g) or Cont (EAA) after exercise	10 rep max for seated leg press and knee extension	Plasma L-arg, 30 min EAA: EAA + L-arg > Cont FABF, MPS, PE1, Nitrate, Nitrite: EAA + L-arg = Cont

Note. c-b = counter-balanced; co = crossover; CO = cardiac output; Cont = control db = double-blind; $D_{capillary}$ = capillary density; EAA = 10g essential amino acids; FABF = femoral artery blood flow; GAKic = 2g glycine + 6g L-arg HCl + 3.2g alpha-ketoisocaproic acid; L-arg = L-arginine free form; M = males; Mbv = muscle blood volume; MAP = mean arterial pressure; Mox = muscle oxygenation; NR = not reported; PE1 = Plasma enothelin-1; PLA = placebo; Plasma L-arg = plasma concentration of L-arginine; P_{avg} = average power; Post-HR = post heart rate; PT = peak torque; r = randomized; reps = repetitions; STW = set total work; TW = total work; > or < indicates significant difference between groups ($p<0.05$); = indicates no significant differences between groups

Flow-mediated Dilation

Flow-mediated dilation (FMD) is a process in which the endothelial health of the vessels is assesed. This measurementation is used in clinical settings as a non-invasive way to diagnose endothelial dysfunction (Corretti et al., 2002). An International Brachial Artery Reactivity Task Force was combined in order to create a solid protocol that could be used on special populations with set guidelines and instructions. Due to these guidelines being created by the specialists in this field, their methods will be referenced.

The process of measuring FMD requires the occlusion of either the brachial artery on either the forearm or the mid-upper arm. Before the occlusion, the brachial artery is measured in

the mid-upper arm by a longitudinal ultrasound image acquisition on an ultrasound system that has continuous 2-D imaging and ECG capabilities. The ECG capabilities are used to sync the measurements with the systolic of the cardiac cycle. Anatomical landmarks, such as other vessels or muscle indendations, are used for reproducing the same site of the artery for diameter change. An electric sphygmomanometric cuff is used to occlude the arterial for a total of five minutes. The artery velocity is obtained within 15 seconds post-occlusion release and recorded for 2 minutes. The time frame in which the largest diameter occurs, usually between 45 and 90 seconds post-occlusion relase, is used for the post-occlusion image. Flow-mediated dilation is then calculated by taking the post-occlusion diameter divided by pre-occlusion diameter. A normal FMD measurement is a reponse greater than 7% (Corretti et al., 2002). For more specific instructions, see Corretti et al., (2002).

Flow-mediated dilation produces a nitric oxide response due to the shear stress that is applied to the arteries. Studies have shown that the vasodilation that occurs due to the occlusion shear stress is produced by nitric oxide rather than due to other methods, although the mechanism behind it is not fully understood (Corson et al., 1996; Joannides et al., 1995). By using FMD one can estimate the effectiveness of nitric oxide.

Endogenous nitric oxide can be increased with supplementation of L-arginine, however only if the baseline levels are insufficient. In a meta-analysis by Bai et. al., (2009), thirteen studies were compared when determining FMD response to L-arginine supplementation. The studies included were all acute supplementation studies, with the exception of chronic supplementation. It was discovered that as the baseline FMD response increased, the effect of L-arginine supplementation was lowered. This supports the idea that L-arginine may not have an effect on healthy participants who have a normal FMD response.

Flow-mediated dilation and exercise. Flow-mediated dilation can also be used to determine the effects of exercise on the vascular system. Green et. al., (2004) mentions that although the mechanism behind the increase is unknown, studies have shown that healthy individuals will show an increase in FMD over the course of an exercise regime as long as the stimulus is strong enough (Green, Maiorana, O'Driscoll, & Taylor, 2004). Although this change in long term exercise has been well studied for both the clinical and healthy populations, there is little data about acute FMD change with exercise.

The direct change in FMD as a result of exercise has produced various results, with timing, modalities, and intensity all being a factor, it is hard to say that FMD will increase with exericse. In some cases, FMD may increase after exercise (Harvey et al., 2005; Tinken et al., 2009). However, there are studies in which FMD decreases (Dawson et al., 2008; Jones et al., 2010).

In a study by Johnson et. al., (2012), ten healthy males were studied to determine the effect of different intensities and durations on FMD. Results showed that the moderate intensity, moderate duration and the high intensity, short duration had an increased FMD from pre-exercise to directly post-exericse (Johnson, Padilla, & Wallace, 2012). However, Jones et. al., (2010) had ten males cycle at 70% of VO_2 max with no change in the pre-exercise to post-exercise FMD (Jones et al., 2010).

Comparatively, Oivind et al., (2008) had seventeen athletes and healthy individuals run five sets of five minutess running at 90% of max heart rate. This produced a higher FMD for up to 24 hours post-exercise, however directly post-exercise was not measured (Rognmo et al., 2008). This varies greatly to Dawson et. al., (2008) whom measured fifteen male nonelite

athletes before and after they ran the London marathon. The brachial artery produced a a non-significant change in FMD from pre-run to directly post-run (p=0.96) (Dawson et al., 2008).

As it can be seen, changes in FMD directly after exercise can vary based on the population studied and the intensity of the exercise. From reviewing past studies, in can be noted that less studies focus on FMD response from resistance training and primarily on endurance training. More studies should be directed toward the acute response of FMD after resistance exercise to determine a more accurate predicted reponse.

One study by Fahs et al., (2009), looks at the acute hemodynamic response after exercise paired with L-arginine supplementation, which is similar to FMD by looking at brachial blood flow. Eighteen young healthy males were studied in randomized trial sessions in which the participants received either 7 grams of L-arginine or a placebo. The participants then began an exercise bout, after 30 minutes of ingestion, which consisted of both barbell bench press and dumbell bicep curl, each bout lasting rougly 20 minutes. The L-arginine supplementation did not have an effect on the hemodynamics of the healthy participants, as well as no change in the forearm blood flow (Fahs et al., 2009). However, this study did not look at FMD repsonse but instead looked at hemodynamic measures.

Summary

With cardiovascular health being a large concern for many people, a supplement that effects the nitric oxide production ability of the endothelial cells would be a large benefit. Comparing the reactions of the healthy participants to L-arginine supplementation solidifies little evidence in favor of the supplementation, with most studies having mixed results and different primary focus. The well established data on clinical participants that L-arginine supplementation

may help vascular response and endothelial tone is not applicable to L-arginine supplementaion on healthy individuals who participate in physical activity regularly.
sufficient to prove that supplementation is effective on healthy participants.

Vascular response can be measured accurately by the process of FMD showing that participants with a low FMD response (<7%) means they have or are at a greater risk for endothelial dysfunction. The participants with this low FMD response can improve the response by supplementing with L-arginine, however this has not been the same for healthy participants. The possibility of pairing exercise to deplete some of the stored nitric oxide substrate in the endothelial cells in order to deplete the available substrates may have an effect on FMD. Using the supplementation of L-arginine as an available source to replenish nitric oxide substrates may increase the effects of FMD post-exercise, which has not been tested. If a healthy individual would fatigue on an exercise and use the saturated store of L-arginine, it is possible that an extra supplement of L-arginine could provide further vasodilation.

Alvares (2012) looked at using an isokinetic concentric elbow extension protocol to study the short term effects of 6 grams of L-arginine supplementation. Although there was an increase in muscle blood volume and plasma L-arginine concentration, there was no change in the performance factors that were measured. However, Alvares (2012) only did 3 sets of 10 repetitions for exercise, with a 2 minute rest in between sets. Increasing exercise and using more applicable measures such as FMD and brachial diameter change could show significant results of supplementation. An increase of the exercise protocol to fatigue and short rest periods may produce a greater need for vasodilation, due to muscle's nutrient needs. Causing the vascular system to vasodilate after already expanding for exercise may also increase the need for L-arginine in the blood stream.

Overall, the potential of L-arginine increasing the nitric oxide production in a healthy participant needs further study, especially the link between resistance training and supplementation. Varying results and effects have proved both either effectiveness or no effect of supplementatation, with long term supplementation having more effectiveness. Further studies should focus on determining if acute supplementation of L-arginine have a positive effect on the endothelial system of healthy participants, even when initial FMD response is greater than 7%.

CHAPTER III. METHODOLOGY

The purpose of this study was to determine the effects of acute L-arginine supplementation on the endothelial health on healthy individuals by assessing the FMD response before and after resistance training to a state of fatigue.

Research Questions

1. Will acute L-arginine supplementation effect endothelial health in healthy participants after resistance exercise to fatigue?
2. Will acute L-arginine supplementation effect endothelial health in males and female participants differently after resistance exercise to fatigue?
3. Will acute L-arginine supplementation effect performance capacity?

Participants

Thirty healthy, physically active males and females, fifteen males and fifteen females, 18-25 years of age were recruited, of which all 30 (mean ± SD: age 20.4 ± 1.8 years, height 176.9 ± 10.2 cm, body mass 76.0 ± 12.2) completed the study. A sample size of 30 was prior determined sufficient to be able to determine a difference in FMD responses (Corretti et al., 2002). All were apparently healthy, as determined by a PAR-Q and Health History Questionnaire, and were considered "physically active" as long as they participated in 4 ± 1 sessions of moderate intensity physical activity and 2 ± 2 sessions of strenuous intensity physical activity per week (Godin et al., 1985). Individuals taking medications, supplements, and special circumstances were assessed for eligibility. Individuals had not participated in another clinical trial or ingestion of another investigational product within 30 days of enrollment (Camic et al., 2010). Women who were pregnant or lactating were excluded, due to good practice. If a

supplement containing L-arginine or nitrate was being taken, a three-week washout period was conducted before starting participation.

Recruitment of participants was completed through the North Dakota State University Listserve system, as well as a presentation to a Wellness 100 course and upper level courses. Individuals attended an information session to determine eligibility and learn about the research objectives.

Procedures

All procedures were approved in advance by the North Dakota State University Institutional Review Board and written consent was obtained (Appendix H).

Familiarization session. Familiarization sessions were completed prior to the trial sessions inside the Human Performance Lab of North Dakota State University. Dynamometer settings were recorded and the participant became familiar with the ultrasound and electronic sphygmomanometer. Written, informed consent was received from each participant. The subject's age, height, and weight was also be measured on the data sheet for participant demographics.

Trial sessions. Participants reported to the lab on three different occasions, the trial sessions were separated by a 48-hour washout period, using a double-blind, placebo-controlled, randomized, crossover design. As mentioned, on visit 1 participants became familiar with the protocol. Participants entered the trial sessions (visit 2 and 3) following an 8-hour fast, with the exception of water. Dietary intake was not recorded, although diet may have had an effect on nitric oxide saturation levels even after the fast. The participants were also 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 (Govoni, Jansson, Weitzberg, & Lundberg, 2008).

On the day of the trial, the participant reported to Room 14. Trials were conducted at the same time of day (±2 hrs) to account for circadian variation. Female participants had to take a pregnancy test prior to each trial. Upon entry to the lab, a heart rate monitor was applied across the participant's chest. After being seated for 5 minutes, after which the participant's heart rate, blood pressure, and FMD were be measured, Table 5 includes a further explanation of the sequencing of measurements. Supplementation of either a commercial supplement of 3 grams of L-arginine (NOW Foods, Boomingdale, IL) or 3 gram placebo was administered with 12 oz. of water and the participant remained seated for 55 minutes. Thirty minutes post-supplementation, heart rate and blood pressure was taken.

Exercise protocol. At each trial session, participants completed elbow flexion and extension exercises with their right arm on a Biodex Isokinetic Dynamometer (Biodex Medical Systems, Shirley NY). A 5-minute warm-up on a cycle ergometer was conducted prior to the hour post-supplementation mark. After the warm up, the participants completed an isometric strength test to determine the elbow flexor and extensors peak torque, which was repeated again for the post-fatigue exercise protocol. Participants then completed an exercise protocol to fatigue. The fatiguing exercise protocol was based off the exercise used in Coggan (2014), with modifications being made due to the testing of different muscles (Coggan et al., 2014). Participants had to complete a set of 50 isokinetic flexions and extensions of the elbow joint at 90º/second. Repetitions were completed in sets of 10 with 30 seconds of rest in between sets. Participants were instructed and encouraged to use full-force for all sets. After the fatiguing exercise, isometric peak torque was measured thirty seconds after to determine the fatigue

percentage. Dynamometer settings remained constant for each trial as taken upon familiarization session. The axis of rotation was through the elbow joint, with a support bar under the elbow joint. The participant's grip position was neutral. The participants were be able to view their results and light verbal encouragement was given.

After exercise, heart rate, blood pressure, and FMD was measured (<10 min post-exercise). Upon completion of FMD measurement, the participant received a cereal bar due to the fasting that was required prior to the study and will be released from Room 14 as long as they were feeling well. Equipment was then be cleaned off using antibacterial wipes and paper towels. Equipment was returned to original placement. Data was then recorded in the lab computer and transferred to an encrypted hard drive for storage.

Table 5
Timeline of measurements.

Time	Baseline	30-min post-supplementation	Pre-exercise	During Exercise	Post-exercise
Measurements Taken	HR, BP, FMD	HR and BP	PT	Total work	PT, HR, BP, and FMD

Note. HR= heartrate, BP= blood pressure, FMD= flow-mediated dilation, and PT= peak torque.

Instrumentation

Flow-mediated dilation (FMD). FMD was measured in accordance to Corretti et. al., (2002), a technique report by the International Brachial Artery Reactivity Task Force. For more specific questions, Corretti et. al., (2002) can be referenced. 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 were positioned 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 place. Continuous 2D imaging was used to determine the initial resting artery diameter. Probe placement was outlined with a marker.

A Hokanson E20 electronic sphygmomanometric cuff and rapid cuff inflation system (Bellevue, WA) was placed on the middle of the forearm marked with a sharpie, and inflated to 50 mg above systolic pressure for 5 minutes (Corretti et al., 2002). Upon release of the cuff, the ultrasound probe was placed in the same area in which the first measure was taken from. A continuous 2D imaging was used from cuff release to 120 seconds post-release, due to several studies suggesting the maximal increase is at 60 seconds post-release (Corretti et al., 2002). The largest diameter recorded was the post-measurement used for FMD calculation and was calculated using RadiAnt DICOM software to measure three lines across the diameter and the average was used.

Data were saved on to the Philips HD11xe ultrasound under only the participant number and transferred onto an encrypted hard-drive. The data for this measure and all other measures was then backed up onto a password-secure file on the HNES server.

Blood pressure. Blood pressure was measured using a manual sphygmomanometric cuff (American Diagnostic Corporation, Hauppauge, NY) and stethoscope (American Diagnostic Corporation, Hauppauge, NY) on the left upper arm. Measurements were taken prior to supplementation (after participant had been resting for 5 minutes), thirty minutes post-supplementation, and directly after exercise.

Heart rate. Heart rate was measured using a Polar H7 Bluetooth strap. (Polar Electro, Kempele, FIN). Measurement was recorded prior to supplementation, thirty minutes post-supplementation, and directly after exercise. Measurements were over five minutes of time and the average heart rate was used.

Supplementation. The double-blind design was created by the HNES nutrition faculty. Supplementation was received in a labeled envelope with the participant number and trial session pre-labeled on them. After the baseline measurements were taken, participants ingested either 6 capsules of an L-arginine based supplement or a placebo in identical capsule form. Capsules were consumed with 12 oz. of purified bottled drinking water.

L-arginine. The supplement was L-Arginine (NOW Foods, Bloomingdale, IL). This supplement contains 500 mg of L-arginine per capsule, six capsules per dose for a total of 3.0 grams of L-arginine. Recommendations for timing of the supplement was 30-60 minutes prior to exercise. The latter was used to ensure digestion and availability (Campbell et al., 2006).

Placebo. Corn-starch capsules were used as the placebo for this study (InHealth Specialty Pharmacy, Fargo, ND). Six capsules had a weight of 3.0 grams. Total energy and carbohydrate content are less than 10 calories per dose.

Data Collection and Data Safety

Data were compiled on the North Dakota State University HNES server on a password-secure file type. The participant's name and number was only be recorded on one file, which will be destroyed at the end of the study, eliminating any correlation between the participant and their number. Any transferring of the data occurred through an encrypted hard-drive, which was password protected. After data from physical papers had been transferred and scanned onto the server they were shredded.

Statistical Analysis

A 2 x 4 (group x time) ANOVA with repeated measures was used to identify differences in peak torque (PT), and brachial artery diameter (BD). A 3 x 4 (gender x group x time) ANOVA with repeated measures was used to identify differences in FMD response between males and females. A 2 x 4 (gender x supplement) ANOVA with repeated measures was used to identify differences in BD between males and females. An alpha level of $p < 0.05$ was used to determine differences. If a significant interaction was found, independent and paired t-tests with Bonferroni corrections were used to compare the results. A paired T –test was used to identify differences in FMD, time after exercise (T-Ex), heart rate (HR), blood pressure (BP), diameter change (DC), and work fatigue (WF). All analysis were performed using SPSS (IBM, Armonk, NY). In the event of missing data, the sequence of data in correlation with the missing piece was not be included in statistics. One female subject was dropped from statistical analysis due to technical difficulties with the ultrasound machine (n=29).

CHAPTER IV. ARTICLE

Introduction

With cardiovascular disease becoming one of the number one causes of death for the U.S. population, endothelial health plays a large role in maintaining systemic health (Bondonno et al., 2015; Corretti et al., 2002). The nitric oxide (NO) pathway is critical to cardiovascular health by supporting vasodilation of the arteries and bringing more blood to active working muscles (Bai et al., 2008; Kapadia, Eng, Jiang, Stoyanovsky, & Kibbe, 2009). One of the two key amino acid contributors to the nitric oxide pathway is the semi-essential amino acid L-arginine (Arnal, Dinh-Xuan, Pueyo, Darblade, & Rami, 1999).

L-arginine has been marketed as an ergogenic aid for years in the sports and performance field as a vasodilator to increase blood flow to muscles causing a rise in performance (Abel et al., 2005; Alvares et al., 2012a; Rector et al., 1996). Inconsistent efficacy has been found for L-arginine supplementation on healthy individuals, due to most healthy individuals already having saturated stores of L-arginine available to them (Alvares et al., 2011). To study the effects of supplementation, previous studies have used flow-mediated dilation (FMD) as an assessment tool to cause a vasodilatory response by the body that is measurable and solidified (Corretti et al., 2002).

Combining FMD with resistance exercise, a process that itself creates vasodilation, may allow a possibility for an L-arginine supplement to enhance the vasodilatory response of the FMD tool due to the body needing to use the L-arginine stores that it currently holds. This possible additional vasodilation could provide increased blood flow to working muscles and increase performance. By using FMD to assess endothelial health, which measures the vasodilatory response of the body, we are able to discern if supplementation increases

vasodilation or not. The purpose of this study was to determine the effects of acute L-arginine supplementation on the endothelial health in healthy individuals by assessing the FMD in the brachial artery both before and after resistance exercise to fatigue. With an increased endothelial health, those supplementing with L-arginine may also be able to have an increased performance due to increased blood flow and delivery of nutrients to working muscles.

Methods

Participants. Thirty healthy, physically active males (n=15) and females (n=15), 18-25 years of age were recruited and completed the study (mean ± SD: age 20.4 ± 1.8 years, height 176.9 ± 10.2 cm, body mass 76.0 ± 12.2 kg). All were apparently healthy, as determined by a PAR-Q and Health History Questionnaire, and were considered "physically active" as long as they participated in 4 ± 1 sessions of moderate intensity physical activity and 2 ± 2 sessions of strenuous intensity physical activity per week (Godin & Shephard, 1985). Individuals taking medications, supplements, and special circumstances were assessed for eligibility. Individuals had not participated in another clinical trial or ingestion of another investigational product within 30 days of enrollment (Camic et al., 2010). Women who were pregnant or lactating were excluded, due to good practice. If a supplement containing L-arginine or nitrate was being taken, a three-week washout period was conducted before starting participation.

Procedures. All procedures were approved in advance by the North Dakota State University Institutional Review Board, and written consent was obtained.

Supplementation. The randomized, double-blind design was conducted under the supervision of a board of certified specialist in sports dietetics. Supplementation was administered from a labeled envelope with the participant number and trial session pre-labeled on them. After the baseline measurements were taken, participants ingested either 6 capsules of

an L-arginine based supplement or a placebo in identical capsule form. Capsules were taken with 12 oz. of purified bottled drinking water.

L-arginine and placebo. The supplement was L-Arginine (NOW Foods, Bloomingdale, IL). The supplement contained 500 mg of L-arginine per capsule, six capsules per dose for a total of 3.0 grams of L-arginine. Recommendations for timing of the supplement was 30-60 minutes prior to exercise, the latter was used to ensure digestion and availability (Campbell et al., 2006). Six corn-starch capsules were used as the placebo (InHealth Specialty Pharmacy, Fargo, ND)

Familiarization session. Familiarization sessions were completed prior to the trial sessions. Written informed consent was received from each participant. The participant's age, height (Seca 213, Chino, CA), and weight (Detecto, Webb City, MO) was measured for demographic purposes. Dynamometer (Biodex Medical Systems, Shirley, NY) settings were recorded and the participants became familiar with the ultrasound (Philips Ultrasound, Bothell, WA) and electronic sphygmomanometer procedures.

Trial sessions. Participants reported to the laboratory for two trial sessions, each separated by at least a 48-hour washout period. Trials were conducted at the same time of day (±2 hrs) to account for circadian variation. Participants entered the trial sessions on an 8-hour fast but were encouraged to hydrate. Dietary intake was not recorded. 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 (Govoni et al., 2008). All females were 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. After sitting, heart rate, blood pressure and FMD were measured in the supine position. Heart rate was measured using a Polar H7 Bluetooth strap

(Polar, Kempele, FL) for a 5 minute period and the average heart rate was recorded. After baseline measurements were collected, supplementation of either a commercial supplement of L-arginine (3g) or placebo was administered with 12 oz. of water and the participant rested for 55 minutes. Heat rate and blood pressure were measured 30 minutes post-supplementation.

Exercise protocol. Participants completed elbow flexion and extension exercises with their right arm on an isokinetic dynamometer one hour post-supplementation. Participants warmed up with 5 minutes on a cycle ergometer before the one hour mark. After the warm up, the participants completed an isometric strength test to determine the elbow flexor and extensor peak torque, which was repeated again after the resistance exercise protocol to determine the rate of fatigue. Participants then completed an exercise protocol to fatigue which consisted of 50 maximal isokinetic flexions and extensions of the elbow joint at 90º/second. Repetitions were completed in 5 sets of 10 with 30 seconds of rest in between sets. Participants were instructed and encouraged to use full-force for all sets. After the exercise, isometric peak torque was measured thirty seconds after to determine the fatigue percentage. Dynamometer settings remained constant for each trial as taken upon familiarization session. After exercise, heart rate, blood pressure, and FMD were measured (<10 min post-exercise).

Instrumentation

Flow-mediated dilation. FMD was measured in accordance to Corretti et. al., (2002), a technique report by the International Brachial Artery Reactivity Task Force. 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 place. 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-rest reliability for brachial artery diameter and FMD using intraclass correlation coefficients were 0.953 and 0.488, respectively.

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 mg above systolic blood pressure for five minutes and cuff placement was marked with a marker (Corretti et al., 2002). Upon release of the cuff, the ultrasound probe was placed in the same area in which the first measure was taken from. A continuous 2D imaging was used from cuff release to 120 seconds post-release, due to studies suggesting the maximal vasodilation occurring at 60 seconds post-release (Corretti et al., 2002). The largest diameter recorded was the post-measurement used for FMD calculation and was calculated using RadiAnt DICOM software to measure three lines across the diameter and the average was used.

Statistical Analysis

A 2 x 4 (group x time) ANOVA with repeated measures was used to identify differences in peak torque (PT), and brachial artery diameter (BD). A 3 x 4 (gender x group x time) ANOVA with repeated measures was used to identify differences in FMD response between males and females. A 2 x 4 (gender x supplement) ANOVA with repeated measures was used to identify differences in BD between males and females. An alpha level of $p < 0.05$ was used to determine differences. If a significant interaction was found, independent and paired t-tests with Bonferroni corrections were used to compare the results. A paired t–test was used to identify differences in FMD, time after exercise (T-Ex), heart rate (HR), blood pressure (BP), diameter change (DC), and work fatigue (WF). All analysis were performed using SPSS (IBM, Armonk, NY). In the

event of missing data, the sequence of data in correlation with the missing piece was not be included in statistics. During the trial sessions, one FMD measurement was determined to be incorrect due to technical difficulties, therefore, one female was dropped from statistical analysis (n=29).

Results

A repeated measures ANOVA for PT revealed there was no supplement x exercise interaction. There was, however, an exercise affect suggested a significant decline in post-exercise flexion and extension peak torque ($p<0.001$, $p=0.014$ respectively) (Figure 1). A paired t-test displayed no significant differences in the rate of work fatigue (WF) between set 1 and set 5 between supplement groups (p=0.829, p=0.748). There were no significant differences between time after exercise and FMD measurement time between the supplement groups (4.84 ± 0.86 min, p=0.156). There was no significant interaction between BD x supplement, however, there was a main effect for time. When supplement data was pooled, a pairwise comparison using Bonferroni's adjustment indicated a significant increase in BD ($p<0.001$) (Figure 2). There were no significant differences between FMD and supplement groups after resistance exercise ($p=0.179$). Differences in HR (Figure 3) and BP (Figure 4 and 5) were not significant throughout all time points. There was no significant gender x exercise x supplement interaction (Figure 6). Gender x DC interaction did show a significant decline in DC in females compared to males ($p=0.004$) (Figure 7).

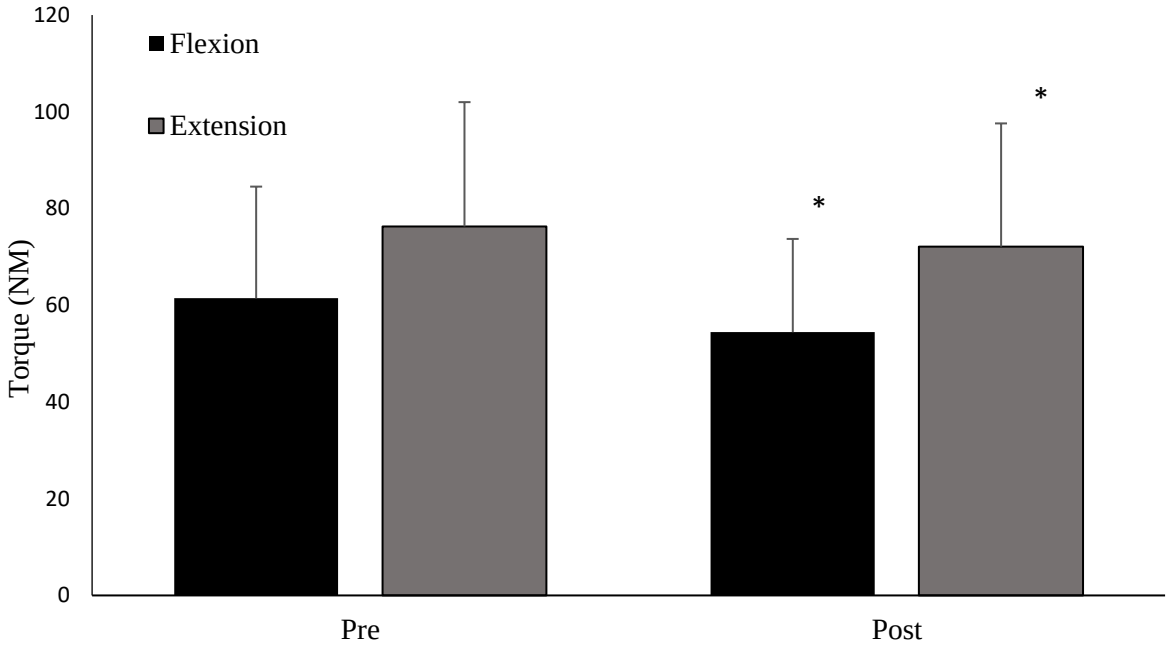

Figure 1. Peak torque (PT) values (mean ± SD) for all participants throughout both trials.
*Significantly lower than Pre-Ex measurement (p<0.05).

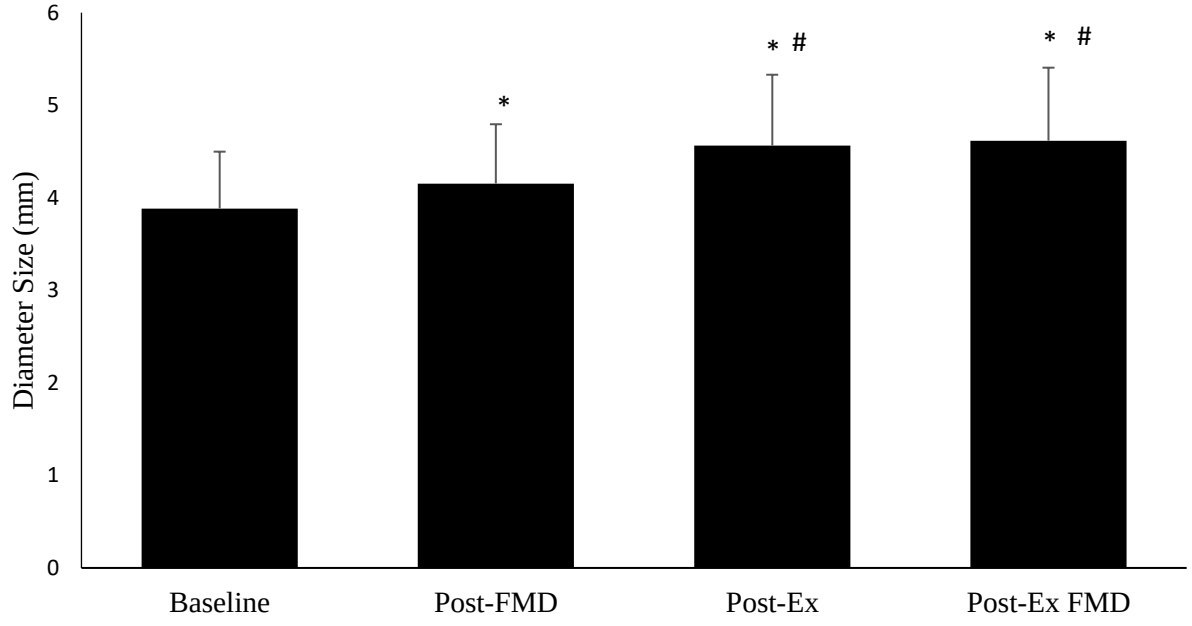

Figure 2. Brachial artery diameter (BD) values (mean ± SD) along time points for all participants.
* Significant from Baseline value (p<0.05).
# Significant from Post-FMD value (p<0.05).

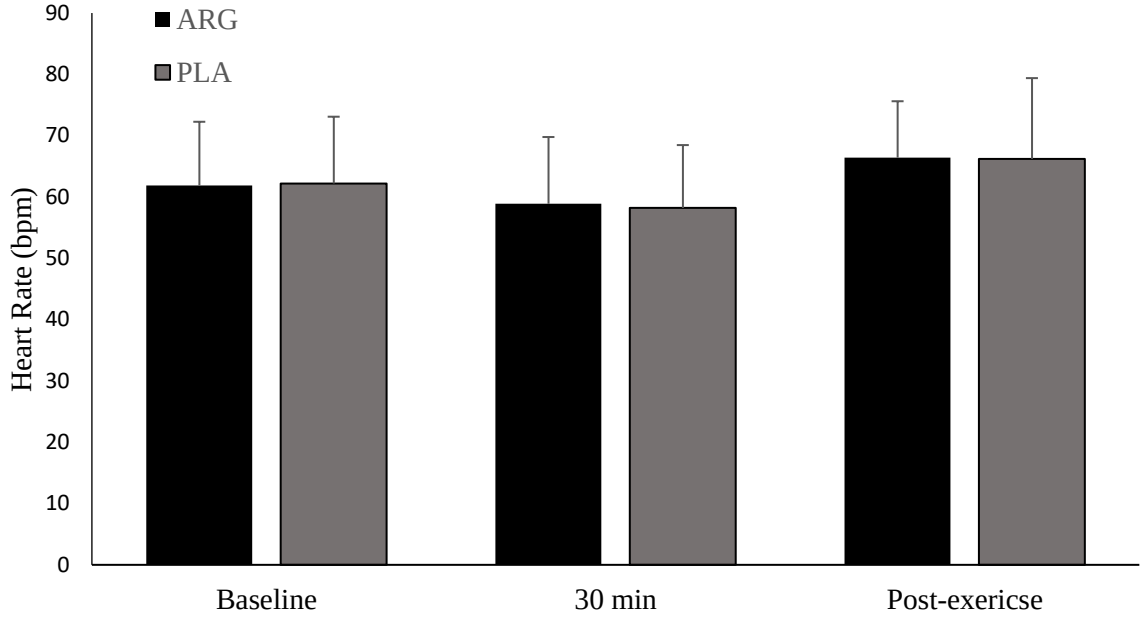

Figure 3. Heart rate (HR) (mean ± SD) changes among time points and supplement trials.
No significant differences between supplements at each time point (p>0.05).

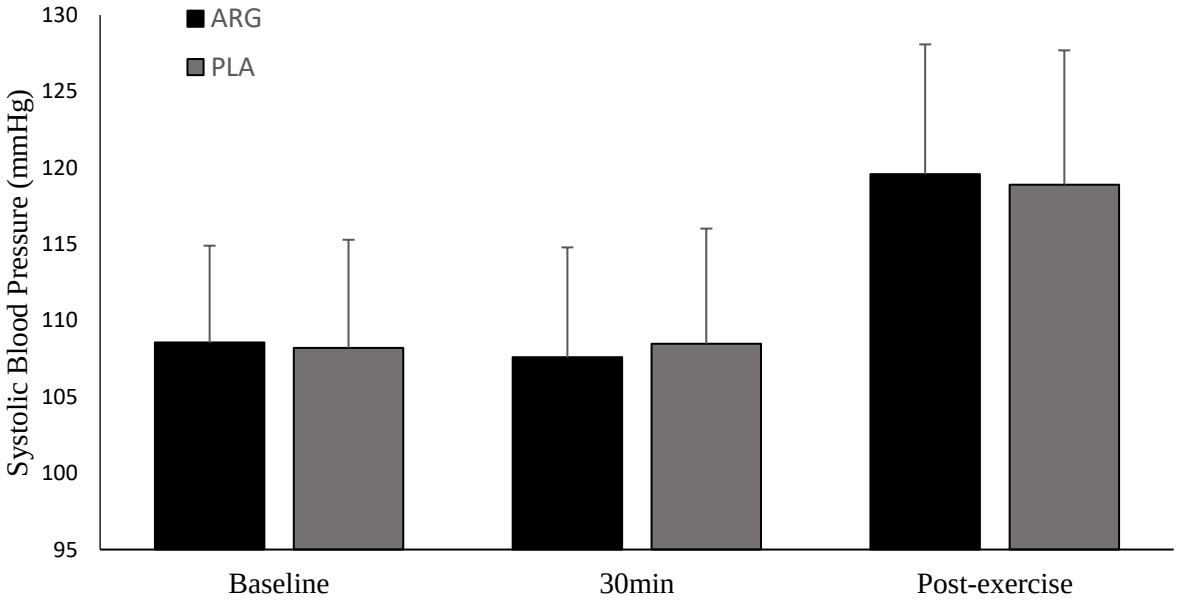

Figure 4. Systolic blood pressure (BP) (mean ± SD) changes among time points and supplement trials.
No significant differences between supplements at each time point (p>0.05).

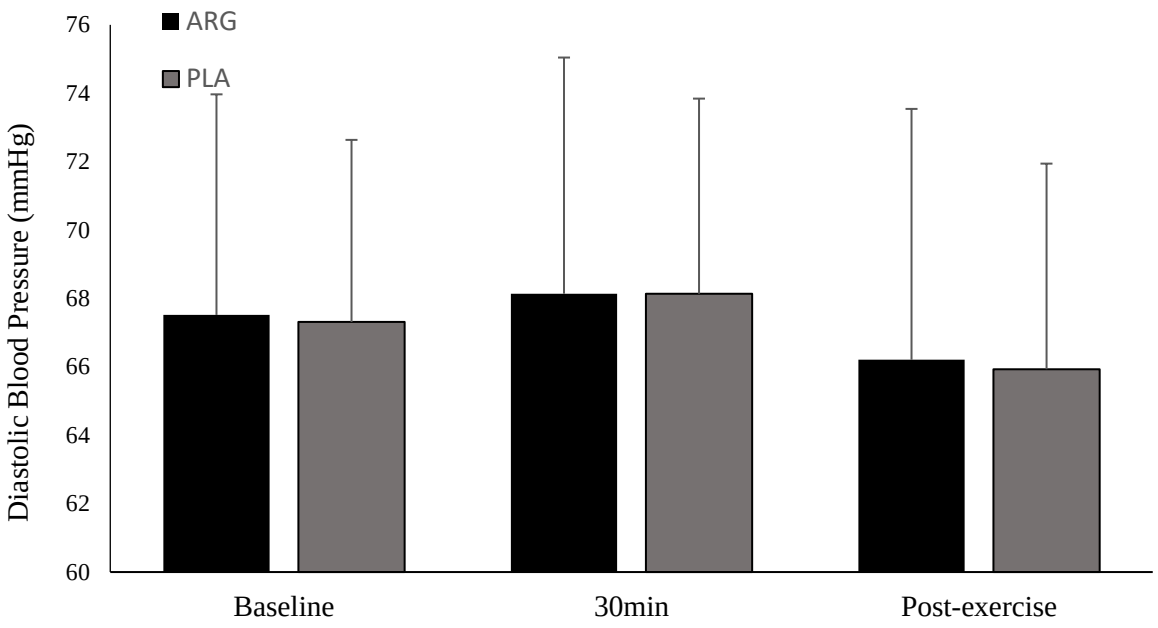

Figure 5. Diastolic blood pressure (BP) (mean ± SD) changes among time points and supplement trials.
No significant differences between supplements at each time point (p>0.05).

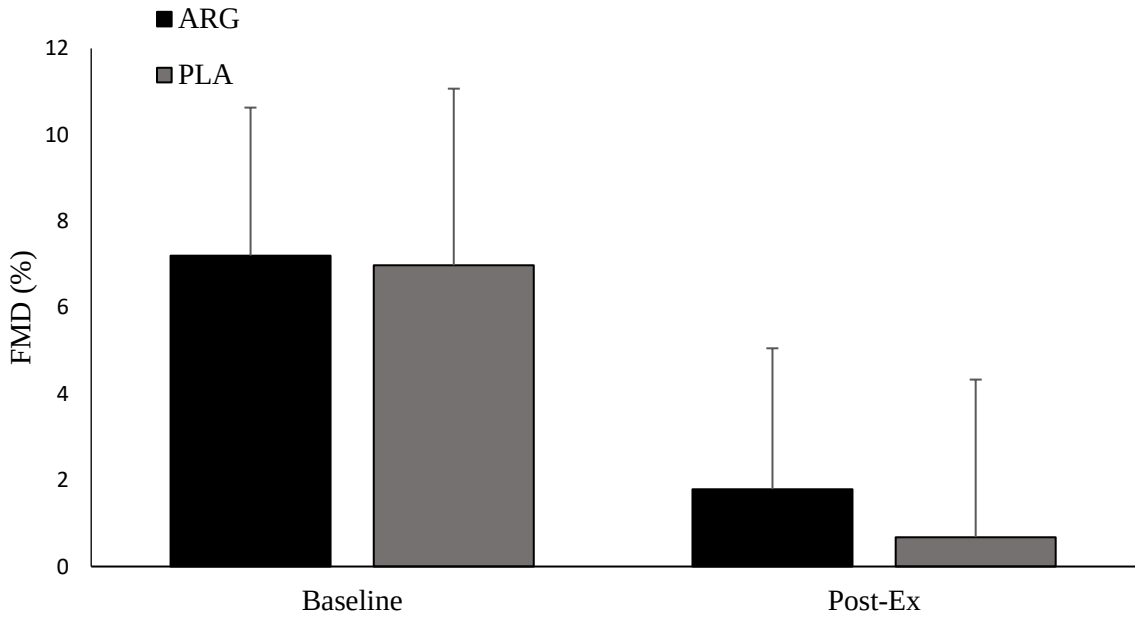

Figure 6. FMD responses (mean ± SD) and supplement for all participants.
No significant difference from other measurement at the same time point (p>0.05).

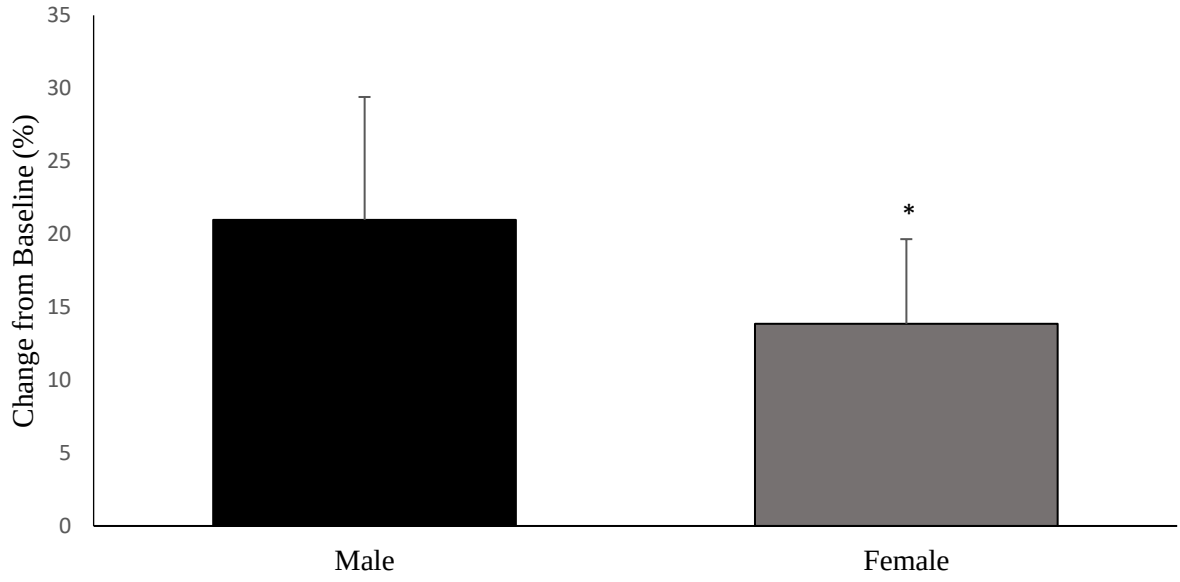

Figure 7. Male vs. female diameter change (DC) (mean ± SD) due to exercise for all participants.
* Significantly lower from other gender (p<0.004).

Discussion

The purpose of this study was to determine the effects of acute L-arginine supplementation on the endothelial health of healthy individuals by assessing the FMD response before and after resistance training to a state of fatigue. The primary research questions for this study were as following: Will acute L-arginine supplementation increase endothelial health in healthy participants after resistance exercise to fatigue? Will acute L-arginine supplementation effect performance capacity? Will acute L-arginine supplementation effect endothelial health in male and participants differently after resistance exercise to fatigue?

L-arginine and arterial response. L-arginine is hypothesized to increase nitric oxide production which will increase the vasodilation response (Alvares et al., 2011). FMD was used to determine the vasodilation responses of the participants' endothelial cells. There was no significant FMD response to the combination L-arginine supplementation and resistance exercise. The lack of effect of L-arginine supplementation could be due to the vasodilation that

was created by resistance exercise itself. The size of the brachial artery was significantly larger post-FMD (6.93%), post-exercise (17.48%), and post-exercise and FMD (18.87%) compared to baseline resting diameter. These data show that vasodilation did occur between the measurements and FMD was measured correctly. Although the post-exercise FMD DC was not different from the post-exercise DC, both measurements were significantly different from post-FMD DC. This could be due to maximal vasodilation occurring during the post-exercise DC measurement, meaning that the post-exercise FMD DC would not be able to increase further.

Only one other study looked at the effect of L-arginine supplementation on hemodynamic and vascular response paired with resistance exercise (Fahs et al., 2009). Eighteen healthy males volunteered to participate in a crossover design study that supplemented with either 7g of L-arginine or a placebo. Participants performed an exercise bout consisting of bench press and bicep curl. Regional arterial stiffness was measured using pulse wave velocity and forearm blood flow (FBF) was measured. Similar to FMD, FBF uses blood-flow occlusion to promote a vasodilation. Fahs et al., (2009) also measured vasodilatory capacity using a blood-flow occlusion technique, occluding the upper arm at 250 mm Hg for 5 minutes. The forearm blood volume was recorded post-occlusion for 3 minutes, the highest reading was observed (BF_{peak}) and the measurements were plotted under the curve. Similar to the current study, Fahs et al., (2009) saw no effect of L-arginine supplementation. This lack of supplementation effect was reinforced from data by Sharmen et al., (2008) that systemic arterial stiffness is not solely nitric oxide dependent and by Mariotti et al., (2007) that a meal with arginine does not affect basal endothelial function (Mariotti et al., 2007; Sharman et al., 2008).

The lack of vasodilatory response that we saw after the post-exercise FMD could be contributed to the shear stress effect on vasodilation (Cooke, Rossitch Jr, Andon, Loscalzo, &

Dzau, 1991; Miura et al., 2001; Olesen, Claphamt, & Davies, 1988). Due to the large amount of blood flow that occurred during and after the resistance exercise, the shear stress of the blood flow caused a large amount of vasodilation. After the application of the occlusion cuff for five minutes, the occlusion was not able to create as much shear stress as the exercise had exhibited causing no change in the diameter of the brachial artery. This may explain why there was a decrease in FMD from the baseline measurement to the post-exercise measurement. However, the lack of increase in BD from baseline to directly post-exercise shows that supplementation of L-arginine will not cause an even greater amount of vasodilation and will not provide a performance enhancement to those using the supplement. Even if the dose was a larger amount, such as the dose used in Fahs et al., (2009) of 7 grams L-arginine, some pre-workout supplements in which L-arginine is used in are not over 10 grams entirely (Table 6). No pre-workout supplements use more than 3.0 grams of L-arginine, thus there is no need to test a larger amount. With other ingredients in pre-workouts combined with L-arginine, it seems unnecessary to include L-arginine as part of the supplement.

Table 6
A display of serving size and L-arginine content in pre-workout supplements.

Pre-Workout Supplement	Total Serving (g)	Total L-arginine (g)
GNC Maxertion N.O. Advanced	4.32	1.5
Cellucor C4	6.5	1.0
Muscle Tech VaporX5	9.0	0.75
RSP Nutrition DyNO	7.5	1.0

L-arginine and performance factors. Due to the vasodilation properties of L-arginine supplementation, it was hypothesized that with increased blood flow there will be an increased performance. In order to perform at maximum capacity, blood flow to muscles must be adequate enough to not be a limiting factor as the exercise occurs. Even though vasodilation is a natural reaction from exercise due to the increased blood flow through the arteries, it is theorized that

even more vasodilation can increase performance due to an increase in oxygen delivery and waste product removal (Alvares et al., 2011).

In the present study, acute L-arginine supplementation did not show any indication of providing a performance benefit to the resistance exercise session. For example, there were no significant differences in PT or WF between placebo and L-arginine. However, PT decreased significantly after exercise throughout all trials. The decrease in PT from pre-exercise to post-exercise is due to the volume of maximal contractions that were required for the exercise protocol which created muscle fatigue. Muscle fatigue can be seen from the average total work fatigue from set 1 compared to set 5 (-19.18% ± 10.14). Our study findings were similar to Alvares et al., (2012) who examined 15 male participants in a random double-blind placebo controlled trial. Six grams of L-arginine or a placebo was supplemented orally 80 minutes prior to exercise which comprised of 3 sets of 10 maximal voluntary contractions of isokinetic concentric elbow extension (60°/s, 2-min rest between sets). Peak torque, total work and set total work were not significantly different between the supplement groups, even though there was an increase in muscle oxygenation (Alvares et al., 2012a).

In contrast to the current study, there have been studies that have shown a significant performance benefits due to L-arginine supplementation (Santos et al., 2002; Stevens et al., 2000). Santos et. al., (2002) studied the effect of chronic L-arginine supplementation (three grams of arginine aspartate) on 12 untrained males for 15 days. Participants performed one set of 15 concentric knee extension/flexion at 180 °/s before and after supplementation. Fatigue index decreased significantly in the L-arginine group compared to the placebo group even though fatigue resistance factor had no difference, which means that the ARG group fatigued at a slower rate. Stevens et al., (2000) focused on the acute effect of L-arginine supplementation (2g glycine

+ 6g L-arginine HCL) on 13 males in a random, double-blinded, placebo controlled study. Participants completed 35 concentric/eccentric knee extensions on an isokinetic dynamometer at 90°/s. Unlike the present study, PT and total work increased significantly between the ARG and PLA groups. There was also a difference in fatigue index between the two groups, with ARG having a better fatigue resistance.

L-arginine and gender. The vasodilation differences between males and females was examined in the current study. There were no differences in FMD between males and females, both genders portrayed a lower FMD response after exercise for both the ARG and PLA trial. Schroeder et al., (2000) showed inversely that there was a larger FMD response by females in a 122 patient study (Schroeder et al., 2000). However, the study concluded that females showed a larger response because FMD response correlated inversely with the size of the brachial artery; the larger the diameter the smaller the FMD response. Another factor that may have played a role in the response of females is the phase of the menstrual cycle. Although the current study did not take into account the menstrual phase of the females, Hashimoto et al. (1995) revealed that FMD response may be highest for females if they are in the follicular and luteal phase compared to the menstrual phase (Hashimoto et al., 1995). This may have been a factor in female FMD measurement.

Vasodilation response to exercise was significantly different between the males and females. Male artery diameter increased an average of 20.98 ± 8.42 percent, while females increased 13.85 ± 5.80 percent. The current study goes against the claim made by Schroeder et al., (2000) because males had a larger increase over females, in which females had the smaller baseline brachial artery (Schroeder et al., 2000). Similarly, Queiroz et al., (2013) had females

respond with a decrease in peripheral resistance as males had an increase in peripheral resistance after resistance exercise (Queiroz et al., 2013).

Conclusion. The increase in brachial diameter due to fatiguing exercise was not enhanced by acute supplementation with L-arginine nor did supplementation alter FMD responses after exercise. This study is the first to combine FMD and resistance training to analyze the effect of L-arginine supplementation. With most pre-workout supplements having the same or similar amounts of L-arginine as an ingredient, it is not necessary to include L-arginine in pre-workout supplements as it has no effect on the vasodilatory capacity of healthy adults. Furthermore, L-arginine does not seem to enhance the endothelial health of healthy adults. However, it needs to be studied in a larger, longer duration in order to determine if L-arginine could help with the current high cardiovascular disease prevalence in the U.S.

Limitations. The dosage amount used for the study may have been low compared to other studies. However, the dosage was selected to be similar to doses that are included in common pre-workout supplements. Larger doses may have been difficult for participants to consume, as they had to consume six capsules to get to 3 grams. Another limitation was the experience of the researchers on the ultrasound equipment. Normal ultrasounds tests are conducted by licensed technicians who have years of experience, the researchers in the study had one year of ultrasound experiences and no licensure. Further limitations include: motivation of participants, reliability of participants, and the effect of menstrual cycle on vasodilatory response in female participants.

CHAPTER V. SUMMARY AND CONCLUSIONS

Summary

The purpose of this study was to determine the effects of acute L-arginine supplementation on the endothelial health in healthy populations by assessing the FMD in the brachial artery both before and after resistance exercise to fatigue of the elbow flexors and extensor muscles. L-arginine is an amino acid commonly used as an ergogenic aid for its documented vasodilation properties. This study used a randomized, cross-over, double-blind, placebo controlled clinical trial, in which thirty physically active, healthy males and females completed two trial sessions each supplemented with either 3g of L-arginine or a placebo. Participants completed an exercise protocol consisting of 50 maximal isokinetic elbow flexions and extension repetitions on an isokinetic dynamometer. Flow-mediated dilation (FMD), heart rate (HR), blood pressure (BP), brachial diameter (BD), peak torque (PT), and work fatigue (WF) were all dependent variables in the study.

Our first and primary research question focused on the possible effect of L-arginine supplementation on FMD after exercise. Statistical analysis revealed that supplementation of L-arginine had no effect on FMD ($p>0.05$). Flow-mediated dilation decreased after exercise and the supplementation did not improve the reaction. Although there was no interaction, brachial diameter did increase significantly from baseline at all other time points ($p<0.05$). This shows us that the resistance exercise selected may have caused maximum vasodilation and thus did not allow FMD to be a factor after exercise.

Our second question addressed whether the L-arginine supplementation would have a different effect on males than it did on females. The overall ANOVA determined that there was no significance between the genders. However, it was revealed that male vasodilation to exercise

(20.98 ± 8.42 percent) was significantly larger than female vasodilation to exercise (13.85 ± 5.80 percent) ($p<0.05$). This has not been documented in findings and goes against the current literature that is available.

Our final question examined the effect of the supplement on performance capacity. Analysis of isokinetic dynamometer measurements showed no increase in peak torque (PT) between supplements, as well as no difference in work fatigue (WF) throughout the trials. Although the exercise protocol effectively decreased the participants' ability for work output, decrease of -19.8 ± 10.14 percent from set 1 total work to set 5 total work, the supplement had no effect on the participants' ability to complete more work. This leaves us to believe that supplementing with L-arginine would be not be beneficial for healthy physically active adults.

Conclusion

The increase in brachial diameter due to fatiguing exercise was not enhanced by acute supplementation with L-arginine nor did supplementation alter FMD responses after exercise. This study is the first to combine FMD and resistance training to analyze the effect of L-arginine supplementation. With most pre-workout supplements having the same or similar amounts of L-arginine as an ingredient, it is not necessary to include L-arginine in pre-workout supplements as it has no effect on the vasodilatory capacity of healthy adults. Furthermore, L-arginine does not seem to enhance the endothelial health of healthy adults and needs to be studied for a longer duration in order to determine if L-arginine could help with the current high cardiovascular disease prevalence in the United States of America.

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APPENDIX A. INFORMED CONSENT

NDSU North Dakota State University

Health, Nutrition, and Exercise Sciences

Department # 2620, PO Box 6050

Fargo, ND 58108-6050

701-231-6706

Title of Research Study: The Acute Effects of L-arginine Supplementation on Flow-mediated Dilation after Resistance Training to Fatigue

This study is being conducted by: Principal Investigator- Kyle Hackney, PhD, CSCS, kyle.hackney@ndsu.edu, 701-231-6706. Co-investigator- Daniel Streeter, BA, EP-C daniel.streeter@ndsu.edu, 612-735-3045

Why am I being asked to take part in this research study?

We are looking to recruit 30 participants for this study.

You are being asked to participate in this study because you:

- Are a male or female between the ages of 18-25.
- Are apparently healthy as identified by two health questionnaires.
- Have participated in 4 hours or more exercise or recreational activity for the past 6 months.
- Are available to devote roughly 4.5 hours to the study.

You should not participate in this study if you:

- Answered “Yes” to any questions on the PAR-Q or Health History Questionnaire.
- Are a current tobacco-user/ e-cigarette user.
- Are currently taking any prescription medications.
- Are currently taking anabolic steroids.
- Have any current or previous cardiovascular, musculoskeletal, or neurological medical problems.
- If you are known to have had violent allergic reactions to drugs, chemicals, or food ingredients including milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybeans.
- Have consumed other nitrite/nitrate/L-arginine containing substances within the past three weeks.

- Are pregnant or nursing.

What is the reason for doing the study?

Supplements containing L-arginine claim to increase blood flow and increase the health of the cardiovascular system. Flow-mediated dilation is a test of the cardiovascular system and is done by having a person lay down and placing an electric blood pressure cuff on their forearm for five minutes. However, in healthy populations there has been confusing data both in favor of the substance and against it. By possibly pairing resistance exercises with flow-mediated dilation, a small positive effect may be seen from L-arginine supplementation.

What will I be asked to do?

We are asking you to complete on familiarization trial and two testing trials. During the testing trials, the order of events will be: resting measurements, consummation of six tablets with water (either the placebo, 2.8 g or L-arginine, 3.0 g), going through an exercise protocol, and then having measurements taken again.

Measurements:

- Heart rate
- Blood pressure
- Flow-mediated dilation- A process in which an electric blood pressure cuff is placed on the forearm and inflated for 5 minutes.

Exercise Protocol: Elbow flexion and extension (bringing right arm up to your shoulder and down to your waist)

- One arm strength test measuring how much force you can apply when your arm is at a 90 degree angle. Then 30 seconds of rest.
- A 50 repetition arm curling exercise, in which we will ask you to perform 5 sets of 10 repetitions with 30 seconds rest between each set.
- One arm strength test measuring how much force you can apply when your arm is at a 90 degree angle.

Where is the study going to take place, and how long will it take?

This study will take place in room 14 of Benson-Bunker Fieldhouse. The estimated time for each session is as follows:

Visit #1 = 30 minutes

Visit #2 = 120 minutes

Visit #3 = 120 minutes

It is estimated that the total time for this study will be ~270 minutes (4.5 hours).

What are the risks and discomforts?

It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known risks to the participant. If new findings develop during the course of this research which may change your willingness to participate, we will tell you about these findings. Below are examples of known risks for this study.

Muscle Strength Testing and Resistance Exercise

1. Muscle soreness following testing- Exercising with higher than accustomed resistance, performing new exercises, maximal exercises, or performing eccentric (muscle lengthening) movements (risk- moderate).
2. Muscle cramping- Inadequate warm-up or stretch may cause cramping (risk-low).
3. Musculoskeletal injury during testing- Muscle overload or improper performance of a test can cause muscle, ligament, tendon, or bone injury (risk- low).
4. Adverse cardiovascular responses- Abnormal blood pressure responses from holding ones breathe to help generate force or from the standard exercise (risk-low).
5. Lightheadedness- Quickly standing following exercise or the strain of standard exercise (risk-low).
6. General personal injury- Inadvertently walking into test stations during operations, having contact with sharp edges, pinch points, or hardware/software failure could cause injury (risk-low).

Dietary Supplement, Caffeine, or Placebo Intake

1. Nausea- Upset stomach from known or unknown ingredient in dietary supplement or placebo (risk- moderate).
2. Allergic reaction- to known or unknown ingredient in dietary supplement or placebo (risk- low).
3. Adverse cardiovascular responses- Abnormal heart rate or blood pressure responses from dietary supplement or placebo (risk-low).

Measurements

1. During the forearm occlusion period, light tingling or numbing sensation may be felt in the right arm (risk-low).
2. Rash or skin irritation -At the site of application of the ultrasound gel or heart rate monitor (risk-low).

Risk Minimization

The study team has minimized the known risks by studying healthy, participants that have exercise experience. By being healthy and used to exercise all exercise testing risks are lowered. We will also adopt previously tested protocols and all of the members of the research team have had training in proper resistance exercise techniques. We will also monitor heart rate and stop the session if heart rate is raised above age predicted heart rate max (220-age) for more than 30 seconds. If you have any history of violent allergic reactions to drugs, chemicals, or food ingredients; you should not take part in this study. We will use non-allergenic ultrasound gel to reduce the risk of rash or skin irritation. If any adverse side-effects occur, please contact Kyle Hackney, NDSU: 701-231-6706 or cell: 616-886-0226 or call Student Health Services directly at 701-231-7331. If you feel it is an emergency please call 911.

What are the benefits to me?

You are not expected to get any benefit from being in this research study.

What are the benefits to other people?

Information from this study could add to the data of L-arginine's effect on the cardiovascular system. Information that supports a correlation between supplementation and an improved cardiovascular system may cause an increase in research of this substance. Due to cardiovascular health being a major problem in the United States, discovering a way to improve it would be beneficial to many people.

Do I have to take part in the study? Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What will it cost me to participate? There are no direct costs for participation in the study.

What are the alternatives to being in this research study? Instead of being in this research study, you can choose not to participate.

Who will see the information that I give?

We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places and password protected.

If you withdraw before the research is over, your information will be retained in the research record and we will not collect additional information about you.

Can my taking part in the study end early?

You can choose to not be in the study at any time, however, we ask that you please contact the researchers if you choose to do so. If you fail to show up to sessions or fail to comply with the study guidelines you may be removed from the study.

Will I receive any compensation for taking part in this study? A compensation of $28.00 will be given upon completion of this study.

What happens if I am injured because of this research?

If you receive an injury in the course of taking part in the research, you should contact Kyle Hackney at the following phone number NDSU 701-231-6706 or cell 616-886-0226. Treatment for the injury will be available including first aid, emergency treatment and follow-up care as needed. Payment for this treatment must be provided by you and your third party payer (such as health insurance). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

What if I have questions?

Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact a researcher, Daniel Streeter at 612-735-3045 and daniel.streeter@ndsu.edu or Dr. Kyle Hackney at 701-231-6706 (office) or 616-886-0226 (cell) and kyle.hackney@ndsu.edu.

What are my rights as a research participant?

You have rights as a participant in research. If you have questions about your rights, or complaints about this research, you may talk to the researcher or contact the NDSU Human Research Protection Program by:

- Telephone: 701.231.8995 or toll-free 1-855-800-6717
- Email: ndsu.irb@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

The role of the Human Research Protection Program is to see that your rights are protected in this research; more information about your rights can be found at: www.ndsu.edu/irb .

Documentation of Informed Consent:

You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

_____ _____

Your signature Date

_____

Your printed name

_____ _____

Signature of researcher explaining study Date

_____

Printed name of researcher explaining study

Documentation of release of video or images:

You also have the choice to allow all images and/or video obtained during this study to be used by the research team in publications, manuscripts, poster presentations, Powerpoint presentations, and University websites. Images will only be used in a professional context when describing the study. You name will never be associated with the image/video.

Yes _____

No _____

_____ _____

Your signature Date

_____

Your printed name

APPENDIX B. PARTICIPANT RECRUITMENT FORM

The Acute Effects of L-arginine Supplementation on Flow-mediated Dilation after Resistance Exercise to Fatigue

Principal Investigator- Kyle Hackney, PhD, kyle.hackney@ndsu.edu, 701-231-6706.

Co-investigator- Daniel Streeter, BA, daniel.streeter@ndsu.edu, 612-735-3045.

Supplements containing L-arginine claim to increase blood flow and increase the health of the cardiovascular system, measured by endothelial health. Flow-mediated dilation tests this endothelial health. However, in healthy populations there has been confusing data both in favor of and against the supplementation effects. By possibly pairing resistance exercises with flow-mediated dilation, a positive effect may be seen from L-arginine supplementation on the endothelial health of participants. This study will determine if L-arginine will have an effect on the cardiovascular system by measuring the flow-mediated dilation after resistance exercise to fatigue.

Who are we looking for?

To participate, you must:

- Be a male or female
- Between the ages of 18-25
- Generally healthy
- Participate in 4 or more hours of exercise or recreational activity per week (for the past 6 months)

We are asking you to complete one laboratory familiarization session and two testing trials where to will randomly be assigned to consume either placebo or L-arginine tablets. During each trial, we will measure your heart rate, blood pressure, and flow-mediated dilation (using ultrasound). The exercise protocol that is a part of the study is a bicep/triceps arm curl on a machine. We will ask you to complete 5 sets of 10 at full force, with a strength test both before and after. The trials will last approximately 2 hours.

Is there compensation?

Yes. Compensation of $40 will be awarded for full completion of the study.

Interested?

Contact Daniel Streeter at: daniel.streeter@ndsu.edu or 612-735-3045.

This research has been approved by the North Dakota State University Institutional Review Board (Protocol # HE16235)

APPENDIX C. DATA AND SAFETY MONITORING PLAN

Brief description of the protocol

Participants will be recruited through the campus wide ListServ email system as well as through the HNES 100 class. Once eligibility is confirmed, participants will be scheduled for a familiarization session and two trial sessions.

At the familiarization session, informed consent and a health questionnaires will be given to guarantee the health of the participants and the knowledge of the study. Participants will be shown equipment and procedures will be described thoroughly.

After successful completion of the familiarization session, two trial sessions will be scheduled 48 hours apart. At the trial sessions, participants will arrive at the same time (±2hr) on the two separate occasions after fasting for 8 hours prior (water intake is recommended). Participants will have their baseline heart rate, blood pressure, and flow-mediated dilation recorded upon settling. Once baseline measurements are completed, participants will consume either the placebo or L-arginine supplement along with 12 oz. of water. At the 30 minute post-consumption mark, heart rate and blood pressure will be recorded again. Warmup will begin 5 minutes prior to exercise start time. The exercise protocol will begin at 60 minutes post-consumption and will consist of using a Biodex dynamometer to conduct right elbow flexion and extension. Participants will complete 5 isometric repetitions, along with 50 isokinetic repetitions (5 sets of 10 repetitions, and then another 5 isometric repetitions, all to the best of their ability. Upon completion of exercises (<10min), heart rate, blood pressure, and flow-mediated dilation will be measured. Once measurements have been recorded, participants will receive a cereal bar (due to the fasting that was required) and will then be able to leave as long as they are feeling well.

Flow-mediated dilation (FMD) process: Flow-mediated dilation is the process of measuring the body's reaction to a sheer force. With the participant lying down on a table, the right brachial artery will be measured longitudinally with a 3D ultrasound. Then an electric blood sphygmomanometer will be placed on the subject's forearm and inflated to 50 mmHG above the subject's systolic blood pressure for 5 minutes. Upon release of the cuff, the brachial artery diameter will be recorded again to determine the response. For more information, please reference:

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., & Vallance, P. (2002). *Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology, 39(2), 257-265.*

Primary and Secondary outcome measures

Primary Outcome Measures: The primary measurement of this study the FMD response of the cardiovascular system with L-arginine supplementation compared to a placebo.

Secondary Outcome Measures: Exercise fatigue rate will be recorded as a secondary outcome measures. This is to ensure that the participants became fatigued throughout the protocol.

Inclusion/exclusion criteria

Inclusion:

- Recreationally active males and females between the ages of 18-25.
- Generally healthy as evidenced by Physical Activity Readiness Questionnaire and Health History Forms (Appendix C and D).
- Available for two trial sessions, each lasting roughly two hours.
- Individuals are to not have had participation in another clinical trial or ingestion of another investigational product within 30 days of enrollment.

Exclusion:

- Answered "Yes" to any questions on the Physical Activity Readiness Questionnaire.
- Are a current smoker.
- Are currently taking any prescription medications.
- Are currently taking anabolic steroids.
- Have any current of previous cardiovascular, musculoskeletal, or neurological medical problems.
- If you are known to have had allergic reactions to drugs, chemicals, or food ingredients including milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybeans.
- Have consumed other nitrite/nitrate/L-arginine containing substances within the past three weeks.
- Are pregnant or nursing.

Sample size

A samples size of 30 is required to determine relevant changes in FMD response.

List of participating enrolling clinics or data collection centers

The only participating data collection center is within the North Dakota State campus, Bentson-Bunker Room 14, the Human Performance Lab.

Target population distribution (e.g., women, minorities, etc)

15 males and 15 females who are recreationally active and healthy (as determined by PAR-Q and Health History Questionnaire).

Data acquisition and transmission

Data will be recorded both manually on trial data sheets and recorded on the Biodex computer. All paper-and-pencil trial data will be transferred onto the password protected Excel spreadsheet on the NDSU secure server and then shredded upon entry. Data that is transferred from the Biodex computer to the server will be transferred using an encrypted hard-drive. Data will also be erased from hard-drive once transferred onto server.

The only data that will have identifying information will be the informed consent and the relation to participant in their number on a link Excel sheet. The link sheet will be destroyed upon completion data collection, ensuring there will be no identifying information to lead back to the participants.

Data entry methods

Data will be both paper-and-pencil and electronic data in which both will be transferred onto electronic storage. All data will be stored in a password protected Excel spreadsheet on the NDSU secure server. Any paper-and-pencil forms that need to be stored before entry will be locked in a file cabinet in a secure office. Once documents have been transferred onto the secure server, the paper will be shredded.

Data analysis plan

A 2 x 4 (group x time) ANOVA with repeated measures will be used to identify differences in heart rate and blood pressure. A 2 x 2 (group x time) ANOVA with repeated measures will be used to identify differences in isometric strength and set work fatigue. Statistical significance will be set at the 0.05 level of confidence. When a significant F is found, addition tests with Bonferroni adjustment will be performed. A paired T-test will be used to identify differences in FMD response. All analysis will be performed using SPSS (IBM, Armonk, NY).

Quality assurance plan

Data quality will be monitored by weekly compliance rates for pre- and post- intervention assessments in the EMA data as well as compliance and accuracy rates during the intervention phase. Monitoring occurs at each weekly check-in, and participant compliance/accuracy are discussed on a weekly basis.

Reporting mechanisms of AEs/SAEs to the IRB, FDA.

In this study we use the FDA definition of serious adverse events (SAEs). SAEs will be systematically assessed each week during the weekly check-ins. Any SAE, whether or not related to study intervention, will be reported to the IRB and NDSU using the SAE Entry Form. The initial SAE report will be filed within 72 hours of the incident report. This will be followed by submission of a completed SAE report to both institutions. In the event that a participant either withdraws from the study or the investigator decides to discontinue a patient due to SAE, the patient will be monitored by the investigator via ongoing status assessment until (1) a resolution is reached, (2) the SAE is determined to be clearly unrelated to the study intervention,

or (3) the SAE results in death. Outcome of SAEs will be reported to the IRB and NDSU as soon as possible following the resolution of the SAE.

Reporting mechanisms of IRB actions to NDSU

Any IRB actions that directly affect the viability or feasibility of the study will be reported to the NDSU Program Officer within 48 hours of such action. A detailed report examining how the actions affect study protocols will be submitted to NDSU as soon as such information is available.

Report of changes or amendments to the protocol

Any changes or amendments to the study protocol will be addressed with NDSU prior to submitting official requests through the Institutional IRB. Once the amendment is approved by NDSU, it will be forwarded to the IRB for consideration. Upon official approval of the change/amendment, the revised protocol and official IRB approval of the change will be forwarded to the NDSU program officer. Any changes/amendments will be included in the annual report submitted to NDSU.

Trial stopping rules

Any SAE or AE will result in the cessation of the trial until (1) it is determined that the SAE/AE was unrelated to the study protocol or (2) the SAE/AE is resolved. SAE/AE will only result in trial stopping if it is determined that the SAE/AE is a direct result of study protocol. If the results of the blind interim analysis show statistically overwhelming significant differences between groups, the blind will be broken and the study stopped.

Conflict of interest

There are no potential conflicts of interest. Any identified conflicts of interest will be reported to NDSU and to the institutional IRB using the conflicts of interest report form. All conflicts of interest will be included in the annual report to NDSU.

Potential risks and benefits for participants

Muscle Strength Testing and Resistance Exercise Risks

1. Muscle soreness following testing- Exercising with higher than accustomed resistance, performing new exercises, maximal exercises, or performing eccentric (muscle lengthening) movements (risk- moderate).

2. Muscle cramping- Inadequate warm-up or stretch may cause cramping (risk- low).

3. Musculoskeletal injury during testing- Muscle overload or improper performance of a test can cause muscle, ligament, tendon, or bone injury (risk- low).

4. Adverse cardiovascular responses- Abnormal heart rate or blood pressure responses from holding ones breath to help generate force or from the standard exercise (risk-low).

5. Lightheadedness- Quickly standing following exercise or the strain of standard exercise (risk-low).

6. General personal injury- Inadvertently walking into test stations during operations, having contact with sharp edges, pinch points, or hardware/software failure could cause injury (risk-low)

Dietary Supplement or Placebo Intake

1. Nausea- Upset stomach from known or unknown ingredient in dietary supplement or placebo (risk- moderate).

2. Allergic reaction- to known or unknown ingredient in dietary supplement or placebo (risk- low).

3. Adverse cardiovascular responses- Abnormal heart rate or blood pressure responses from dietary supplement or placebo (risk-low).

Measurements

1. During the forearm occlusion period, light tingling or numbing sensation may be felt in the right arm (risk-low).

2. Rash or skin irritation -At the site of application of the ultrasound gel or from wearing the heart rate monitor (risk-low).

Risk Minimization

The study team has minimized the known risks by studying healthy, participants that have exercise experience. By being healthy and used to exercise all exercise testing risks are lowered. We will also adopt previously tested protocols and all of the members of the research team have had training in proper resistance exercise techniques. We will also monitor heart rate and stop the session if heart rate is raised above age predicted heart rate max (220-age) for more than 30 seconds. We will alert the participant to report any history of violent allergic reactions to drugs, chemicals, or food ingredients; these participants should not take part in this study. We will use non-allergenic ultrasound gel to reduce the risk of rash or skin irritation. The flow-mediated procedure is a safe procedure even used on clinical populations and has been shown not to have any adverse side effects. We have taken care to ensure that all responses remain confidential. It is not possible to identify all potential risks in research procedures, but the PI has taken reasonable safeguards to minimize any known risks to participants. There are no expected benefits for the participants.

Participants will be asked to report any adverse events that may occur after trial sessions to the PI.

Collection and reporting of AEs and SAEs

SAE/AE evaluated on a weekly basis with each participant. The collection of SAE/AE will be conducted by Lab research assistants with follow-up by the PI. All SAE/AE will result in the filing of an SAE report to the university IRB and NDSU using the SAE reporting form. The initial SAE report will be filed within 72 hours of the incident report. This will be followed by submission of a completed SAE report to both institutions. In the event that a participant either withdraws from the study or the investigator decides to discontinue a patient due to SAE, the patient will be monitored by the investigator via ongoing status assessment until (1) a resolution is reached, (2) the SAE is determined to be clearly unrelated to the study intervention, or (3) the SAE results in death. Outcome of SAEs will be reported to the IRB and NDSU as soon as possible following the resolution of the SAE. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NDSU.

Management of SAEs or other study risks

The collection of SAE/AE will be conducted by lab personnel during weekly check-ins. Any participant with an SAE/AE will be followed up by the PI until the resolution of the SAE/AE. All SAE/AE will result in the filing of an SAE report to the university IRB and NDSU using the SAE reporting form within 72 hours. The SAE reporting form will also be submitted once the SAE has been resolved. All SAEs will be tracked and submitted to NDSU during the annual report.

Plans for Interim Analysis of efficacy data

Blind interim analyses of the data will be conducted when 50 of the sample has been accrued. If the results show statistically overwhelming significant differences between groups, the blind will be broken and the study stopped.

Responsibility for data and safety monitoring

The Principal Investigator will be responsible for monitoring the safety and efficacy of this trial, executing the Data and Safety Monitoring (DSM) plan, and complying with the reporting requirements. The PI will provide a summary of the DSM report to NDSU after the completion of the study. The DSM report to NDSU will also include, when available, the results of any efficacy data analysis conducted.

Content of DSM report

The DSM report will include any SAE/AE, resolutions/outcomes of any SAE/AE, protocol adherence, record adherence (i.e., maintenance of signed consent), confidentiality adherence, data storage and coding adherence, and appropriate training of all lab personnel to include certificates of human subjects protection training.

APPENDIX D. FAMILIARIZATION SESSION DATA FORM

Participant (#) _____ **Date**_____

Age: _____ Gender: _____

Height (cm): _____

Weight (kg): _____

Biodex Measurements:

Seat Monorail:	Dyna Monorail:
Seat Height:	Dyna Height:
Seat Rotation:	Dyna Rotation:
Seat Back Tilt:	Arm Attach Length:
Seat Fore/Aft:	Arm Rest Height:

APPENDIX E. TRIAL PROTOCOL FORM

Participant (#) _____ Date _____ Trial (#) _____ Researchers Present (initials)

Before Subject Arrives:

- O Ultrasound on
- O Biodex on
- O Biodex attachment in place
- O Participant ID brought up on Ultrasound and Biodex
- O Take out heart rate monitor
- O Take out blood pressure equipment
- O Occlusion station set up
- O Supplement and water ready
- O Bike ergometer moved into Room 14

Subject Arrives:

- O Heart rate monitor fitted and tested
- O Participant sits in chair Time:_____
- O Baseline measures after 5 minutes Time:_____
- O Heartrate measure- BASELINE- record on data sheet
- O Blood pressure measure- BASELINE- record on data sheet
- O FMD measure- BASELINE- record in computer
- O Supplementation Time:_____

Pre-Exercise:

- O 30 minute post-consumption Time:_____
- O Record systolic pressure for electric cuff
- O 55 minute post-consumption Time:_____
- O 4 minute light warm up on bike ergometer
- O Light arm stretches
- O Set-up in Biodex

Exercise:

- O Set range of motion
- O Begin exercise protocol Time:_____

Post-Exercise (>10 minutes of finish):

- O Heart rate measure- POST-EX- record on data sheet
- O Blood pressure measure- POST-EX- record on data sheet
- O FMD measure- POST-EX- record in computer
- O Give discharge instructions and ensure participant is well
- O Subject data collection complete Time:_____

APPENDIX F. TRIAL DATA FORM

Participant (#) _____ **Date**_____ **Trial** (#) _____

Trial Start Time:		Product Consumption Time:	
Exercise Start Time:		Participant Discharge Time:	

Biodex Measurements:

Seat Monorail:	Dyna Monorail:
Seat Height:	Dyna Height:
Seat Rotation:	Dyna Rotation:
Seat Back Tilt:	Arm Attach Length:
Seat Fore/Aft:	Arm Rest Height:

Anatomical Reference: 90 degrees ROM: Toward: _____ Away:_____

Blood Pressure and Heart Rate Measurements

	Heart Rate	**Blood Pressure**
Pre-Supplementation		
30min Post-Supplementation		
Post-Exercise		

Ultrasound:

	Baseline Pre-Cuff	**Baseline Post-Cuff**	**Post Pre-Cuff**	**Post Post-Cuff**
Comments				

Electric Sphygnamometer Measurements

	Baseline	**Post-Ex**
Pressure		

APPENDIX G. PARTICIPANT DISCHARGE INSTRUCTIONS

Date_____

Dear Study Participant:

Thank you for participating in the L-arginine and resistance exercise study. With any supplement or ingested compound please let us know if you experience any side effects. We are not expecting any specific issues given L-arginine is a commonly consumed amino acid that is available over the counter. However, if you experience any nausea, rapid heart rate, chest pain, dizziness, or other bothersome side effects that may be associated with an allergic reaction (hives, swelling of the throat, itching), please seek medical attention (your personal provider) and contact the research team.

If you have any questions, do not hesitate to call or email.

Thank you,

Dan Streeter, BA, EP-C
Daniel.streeter@ndsu.edu
612-735-3045 (cell)

Dr. Kyle Hackney, PhD, CSCS
Kyle.hackney@ndsu.edu
701-231-6706 (office)
616-886-0226 (cell)

Dr. Sherri Stastny, PhD, RD
Sherri.stastny@Ndsu.edu
701 231 7479

APPENDIX H. IRB APPROVAL FORM

NDSU NORTH DAKOTA STATE UNIVERSITY

July 15, 2016

Dr. Kyle J. Hackney
Department of HNES

IRB Approval of Protocol #HE16235, "The Acute Effects of L-arginine Supplementation on Flow-mediated Dilation after Resistance Training to Fatigue"
Co-investigator(s) and research team: Dan Streeter, Sherri Stastny, Kara STone, Tylor Bennett, Lauren Mcintosh, Dan Ewert, James Grier

Approval period: 7/15/2016 to 7/14/2017 Continuing Review Report Due: 6/1/17

Research site(s): NDSU Funding agency: Northland American College of Sports Medicine
Review Type: Full Board, meeting date – 5/13/2016
Risk Level: A minor increase over minimal risk
IRB approval is based on original submission, with revised: protocol materials (received 7/14/2016).

Additional approval is required:
o prior to implementation of any proposed changes to the protocol (Protocol Amendment Request Form).
o for continuation of the project beyond the approval period (Continuing Review/Completion Report Form). A reminder is typically sent two months prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:
o any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (Report of Unanticipated Problem or Serious Adverse Event Form).
o any significant new findings that may affect risks to participants.
o closure of the project (Continuing Review/Completion Report Form).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,
Kristy Shirley
Digitally signed by Kristy Shirley
DN: cn=Kristy Shirley, o=NDSU, ou=Institutional Review Board, email=kristy.shirley@ndsu.edu, c=US
Date: 2016.07.15 16:08:26 -05'00'
Kristy Shirley, CIP
Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult www.ndsu.edu/irb. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8995 | Fax 701.231.8098 | ndsu.edu/irb
Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102