EFFECT OF ORAL L-ARGININE SUPPLEMENTATION ON LACTIC ACID AND
MAXIMAL OXYGEN CONSUMPTION IN HEALTHY MALES

A Thesis
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

Matthew R. Feeback
May, 2009
EFFECT OF ORAL L-ARGININE SUPPLEMENTATION ON LACTIC ACID AND MAXIMAL OXYGEN CONSUMPTION IN HEALTHY MALES

Matthew R. Feeback

Thesis

Approved:    Accepted:

Advisor Dr. Ronald Otterstetter
Interim Dean of the College Dr. Cynthia Capers

Committee Member Mrs. Stacey Buser
Dean of the Graduate School Dr. George R. Newkome

Committee Member Mrs. Rachele Kappler

Date

Department Chair Dr. Victor Pinheiro
ABSTRACT

The purpose of this study is to investigate the effects of varying doses of oral L-arginine supplementation on platelet aggregation, lactic acid, and nitric oxide synthase in normal health male subjects and the consequent results on maximal oxygen consumption.

The hypothesis of this study is that with treatment of oral l-arginine supplementation, there will be a differential response of VO$_{2\text{max}}$ and lactic acid accumulation concentrations with varying doses. In order to test this hypothesis, subjects will be administered varying doses of l-arginine supplement or placebo and then will complete a VO$_{2\text{max}}$ test.

Prior research has focused on the effects of IV or orally administered arginine and its subsequent enzymatic reduction by nitric oxide synthase to nitric oxide (NO) to benefit patients with hypercholesterolemia, hypertension, and heart disease. However, l-arginine supplementation has not been validated in terms of its cardiovascular effect in healthy populations although it has been studied for weight training. The benefits of using this supplement in cardiovascular training may be related to NO formation which has been shown to dilate blood vessels. Lactic acid accumulation may be delayed or reduced because of better oxygen delivery. Should these effects be substantiated by this study, use of l-arginine as a cardiovascular endurance supplement could provide a legal and healthy way for people to condition. Previous research has demonstrated possible
differential responses to l-arginine supplementation and the physiological markers listed above. These effects should cause increases in time to exhaustion and VO$_{2\text{max}}$.

Twenty-five healthy males between the ages of eighteen and thirty were recruited. Subjects who volunteered to participate in the research completed an informed consent and health history questionnaire and were measured for height, weight, body composition, and blood pressure. Based on the answers to the questionnaire and the measurements recorded, the subjects who qualified were allowed to continue in the study. If the potential participant answered yes to any question and/or does not fall within acceptable quantified limits, they were disqualified from the study. The three quantified items on the Health history questionnaire are alcohol consumption (< 3 drinks per day), excessive fatigue (excluded if still feeling consistently fatigued after at least 7 hours of sleep in previous 24 hour period) and if they have had mononucleosis within six months of first trial date. Subjects were also excluded if they are > 30% body fat, due to an increased chance of a cardiac event, have a resting blood pressure > 140 mm/Hg systolic and > 90 mm/Hg, again due to an increased chance of a cardiac event. Females were also excluded because of potential variations on markers from the menstrual cycle.

To provide rationale for any differential responses, the physiological markers of lactic acid was evaluated. This was measured via finger sticks to produce 0.5 ml of blood at three different points of time during the testing process. The first of the finger sticks occurred upon arrival at the testing facility before any supplementation (l-arginine high dose [6 g], low dose [3 g], or placebo) is administered. The second took place 25 minutes post-supplementation, while the subject was still at rest. Testing via a VO$_{2\text{max}}$ test then
occurred, upon completion of the testing procedure, the third and final finger stick was performed (one minute post-test). Testing trials occurred on three separate occasions seven days apart.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>CHAPTER</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
</tr>
<tr>
<td>L-Arginine</td>
</tr>
<tr>
<td>Lactic Acid</td>
</tr>
<tr>
<td>Conclusion</td>
</tr>
<tr>
<td>III. RESEARCH DESIGN AND METHODS</td>
</tr>
<tr>
<td>Subject Inclusion and Exclusion Criteria</td>
</tr>
<tr>
<td>IV. RESULTS AND DISCUSSION</td>
</tr>
<tr>
<td>V. SUMMARY</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
</tr>
<tr>
<td>APPENDICES</td>
</tr>
<tr>
<td>APPENDIX A. HEALTH HISTORY QUESTIONNAIRE</td>
</tr>
<tr>
<td>APPENDIX B. INFORMED CONSENT</td>
</tr>
<tr>
<td>APPENDIX C. SUBJECT INSTRUCTIONS</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure | Description | Page
--- | --- | ---
3.1 | Trial Design | 17
4.1 | Subject Age | 19
4.2 | Subject Weight | 19
4.3 | Subject Height | 20
4.4 | Subject Body Fat Percent | 20
4.5 | Multivariate Tests on Lactic Acid | 21
4.6 | Subjects Pre-treatment Lactic Acid within Treatments | 21
4.7 | Subjects Post-treatment Lactic Acid within Treatments | 22
4.8 | Subjects Post-exercise Lactic Acid within Treatments | 22
4.9 | Subjects Performance on VO2 Max within Treatments | 23
4.10 | Multivariate Tests on VO2 Max | 23
CHAPTER I

INTRODUCTION

Maximal oxygen uptake (VO₂ max) is a term that is often used by exercise physiologists to measure an individual’s cardio respiratory fitness. Simply put it is the maximal amount of oxygen that an individual can consume and then utilize in a working skeletal muscle during their utmost maximal exertion. There are several physiological factors that make up the oxygen kinetic chain, which is the act of the body to bring in and disperse oxygen to where it is needed. Very few individuals have difficulties with this at rest, however during exercise, the demands are intensified. The cardiac output or amount of blood distributed to the system is increased due to a higher heart rate and stroke volume. Blood, during exercise, is dispersed to the working skeletal muscles and away from less active tissue and musculature.

In the oxygen kinetic chain, the first step is the up-take or the respiratory link. Once oxygen is consumed by the pulmonary system, the responsibility is now the cardiovascular systems to distribute the consumed oxygen. In order to deliver the consumed oxygen to the tissues, it must be transported via the blood. Blood becomes oxygenated in the pulmonary system and then brought back to the cardiovascular system by the pulmonary veins and delivered to the left atrium. At this point, the blood can then travel to the left ventricle, where it can be delivered to the system, or in other words, the
oxygenated blood is circulated to the body. As the oxygen rich blood is dispersed, the working muscles can extract the oxygen and utilize it as an energy source. When the body can no longer utilize and/or deliver the adequate amount of oxygen, it is at this point that an individual is said to have reached VO₂ max.

For years, many endurance athletes have been searching for many training methods and/or ergogenic aids to assist in their training to improve performance. Many popular supplements have been well established as showing a positive ergogenic influence on endurance athletes’ performance. Endurance athletes utilize many popular products and techniques such as caffeine, carbohydrate loading, fat loading, blood doping, growth hormone and synthetic erythropoietin. Previous research has been used to prove the positive effects of many of the above methods of increasing cardiovascular performance among healthy athletes.⁶

In recent years, many popular sports nutrition companies have been marketing a popular substance used by cardiac patients as a means to heighten muscle strength and hypertrophy. The substance is the amino acid l-arginine. L-arginine is a substrate to nitric oxide synthase, which has a vasodilatory effect on the systemic vascular beds and can correct abnormal endothelium-dependant vasodilation. The assumption is that there is some correlation between l-arginine (l-a) and oxygen uptake, delivery and utilization as briefly described in the definition of the oxygen kinetic chain. The answer to l-a affects lies in the delivery aspect of the chain.

Previous research on l-arginine has examined the effects that it has on the exercise performance of diseased populations.²,¹⁰,¹¹,¹³ In populations that suffer from
cardiovascular diseases such as hypertension, angina, hypercholesterolemia or even congestive heart failure, l-arginine has been administered and studied as a means to enhance the exercise capacity in these patients. There is evidence that l-a is beneficial to patients with precapillary pulmonary hypertension, as it has been shown to decrease mean pulmonary arterial pressure. This research study also showed that after oral supplementation of l-a patients exhibited a decrease in pulmonary resistance, as well as systemic vascular resistance. One of the three key physiological aspects of the oxygen-kinetic chain is transporting oxygenated blood to the working musculature. A major deterrent of this mechanism is resistance of the vasculature leading to the skeletal musculature at work caused by vasoconstriction. A one-week supplementation with l-a, patients exhibited a significant increase in peak VO₂ that was also associated with an increase in peak work. Peak work, is higher work intensity; i.e. a heavier resistance or a quicker work rate. This increase in peak VO₂ may be attributed to the hemodynamic and vascular response to l-a.

The hemodynamic and vascular response to l-a is due to vasodilation in addition to an increase in blood flow due to the relaxation of the vasculature. This relates to a decreased vascular resistance. Hypertensive patients can obviously benefit from the supplementation of l-a as it directly relieves the pressure on the blood vessels through relaxation of vessel musculature. A modest hypotensive effect on healthy subjects has also been observed.

Vasodilation in the vasculature of the musculoskeletal system will allow more blood flow to the working musculature. L-arginine infusion in healthy volunteers reduced blood pressure and raised blood flow in the femoral artery. This shows/gives
further evidence that not only does L-a provide positive results for diseased populations that suffer from cardiac illnesses, but may also have a positive effect on healthy subjects.

Other research, although very limited, has examined the relationship between L-arginine supplementation and lactic acid production. In a study, researchers examined the effects of intravenous infusion of L-arginine on lactic acid production. Their findings indicated that L-arginine infusion significantly reduced exercise-induced increases in plasma lactate and ammonia. This may indicate that with a decreased amount of circulating blood lactate, that skeletal muscle may have a higher affinity for oxygen utilization. These results indicate that those who were supplied with an acute supplement of intravenously infused three grams of L-arginine exhibited a lower level of lactate during exercise.

Still other studies have examined chronic supplementation of L-arginine and its relationship and effect on lactate production and ventilatory threshold. Chronic supplementation of L-arginine effected ventilatory threshold in patients with congestive heart failure (CHF). The researchers on this project instructed patients with CHF to supplement with six grams of L-arginine twice daily, and then exercised them to exhaustion on a stationary bike. The researchers examined ventilatory threshold and blood lactate levels. The results indicated that those who supplemented with L-arginine increased their time to ventilatory threshold; whereas lactate levels remained within normal ranges, but did not increase as much when compared to those who ingested a placebo. This may provide evidence that L-arginine may also delay the ventilatory threshold, which in turn would allow an individual to exercise longer in duration and at a higher intensity.
One possible explanation for this response is that l-arginine is a precursor to nitric oxide, which induces a dilatory response of the endothelium of arteries and veins in a subject. Previous research on nitric oxide and its enzyme, nitric oxide synthase, has been performed on the aforementioned cardiac diseased populations. Many studies have researched the effectiveness of l-arginine on endothelium-derived nitric oxide production and how it coincides with aerobic capacity. One such study presented results that l-arginine supplementation may improve exercise capacity in which subjects have a reduced amount of endothelium-derived nitric oxide. This particular study, although it was performed on mice with hypercholesterolemia, provides evidence that l-arginine may increase nitric oxide.

The most prevalent research that has been conducted on l-arginine has been done on subjects with various cardiovascular diseases. The research studies that have been presented above vary from researcher to researcher; in methodologies, in populations, in physiological phenomena studied and in species examined. Therefore, the main objective for this investigation is to observe the influence of varying doses of l-arginine supplementation on healthy human subjects and the physiological effects it has when observing VO\textsubscript{2} max. This study will specifically evaluate lactic acid concentration response before, during and after maximal exercise testing.
CHAPTER II
REVIEW OF LITERATURE

Much of the research that has examined the effects of L-arginine supplementation has focused on the specific population of those afflicted with cardiovascular disease, and their response to exercise. Limited research has been conducted on the experimental effects of L-arginine supplementation on VO2 max in healthy subjects, and even less has attempted to explain the physiological rationale for the results. There is a need to further investigate the ergogenic effects of L-arginine on a healthy population, and to provide some sort of physiological rationale for the results.

This chapter will summarize literature in the areas of interest to the current study. These topics include L-arginine supplementation and its physiological effect, lactic acid and its affect on cardiovascular endurance as it relates to the cardiovascular system.

L-Arginine

L-Arginine (L-a) is an amino acid that is found and consumed in any diet that includes dairy, meat, poultry, fish, or more simply; protein. While it performs its most basic of functions as a building block of muscle in the body, L-arginine also plays a vital role in several other functions of the body. This includes the detoxification of ammonia formed during the nitrogen catabolism of amino acids via the formation of urea.16
L-arginine also plays a role in several other important mechanisms in the body, including cell division, wound healing, immunity to illness, and the secretion of important hormones.\textsuperscript{16}

The majority of the previous research of l-a has explored the effects that it has on the cardiovascular system. However, one study exists in which muscular fatigue is tested after supplementation of l-a. This particular research project investigated oral supplementation by healthy male volunteers.\textsuperscript{12} The subjects performed leg extensions and curls on an isokinetic dynamometer. The aim of the study was to determine muscular fatigue of these two types of exercises at the knee joint. After ingestion of either a placebo or three grams of arginine, subjects then went through two phases. The first phase (control phase) the subjects were tested three times, one as a familiarizing of the testing procedures, then two control tests. After the completion of phase one, phase two ensued in which there were two tests. The results of this study indicate an increase in muscular resistance to fatigue following treatment by the oral administration of arginine.

The predominant theory that supports the previous literature and research on l-a, is that it is a powerful vasodilator. It plays an important role in the cardiovascular system. L-arginine plays a vital role in the “l-arginine-NO” pathway.\textsuperscript{2} This is the mechanism that allows for the vasculature to relax and therefore dilate. Nitric oxide is the substance that physically acts upon the endothelium of vasculature and allows for relaxation. Nitric oxide, itself, is a byproduct of the synthesis of l-a by nitric oxide synthase. Therefore without the presence of l-a, nitric oxide production would be limited, if not completely diminished.
This phenomenon is well supported by other investigations. One such research project explored the short-term administration of l-a and its effect on the hemodynamics in patients with precapillary pulmonary hypertension. Subjects in this study ingested 1.5 grams of l-a per 10 kg of body weight or a placebo for one week. At the conclusion of the week, the subjects underwent cardiopulmonary tests. These tests included peak VO2 and the ventilatory response to carbon dioxide production. The results yielded a positive response in the subject’s mean arterial pressure, indicating a 9% decrease as well as a 16% decrease in pulmonary vascular resistance. The placebo-controlled group showed no hemodynamic or exercise capacity change. The researchers indicated that supplementation with l-a may have beneficial effects on hemodynamics and exercise capacity, due to an increased level of l-citrulline, which indicates nitric oxide production.

In the research provided by Giugliano, l-a supplementation was also examined as a means of investigating the hemodynamic response of the systemic vasculature. Ten healthy young subjects participated in this study. Their involvement included the infusion of l-a in what the researchers coined study I, in study II the subjects were infused with l-a plus ocreotide (a substance that blocks endogenous insulin and glucagons secretion), finally in study III, subjects were infused with l-a plus ocreotide plus basal glucagons and an insulin infusion designed to mimic the insulin response of study I. Blood pressure, heart rate and leg blood flow were measured. The major conclusion found that l-a infusion decreased blood pressure, platelet aggregation and reduced blood viscosity. Other conclusions that were noticed were that of l-a stimulated nitric oxide through the mediation of insulin.
Still other examiners of L-a supplementation have tested the aerobic capacity in conditions where the endothelium derived nitric oxide activity is reduced. One such study examined mice in which endothelium-derived nitric oxide (EDNO) activity was reduced, and whether or not the L-a supplementation would help restore EDNO activity. This study examined two different types of arginine supplements, an L-arginine supplement and a D-arginine supplement. The mice were divided into six groups, four experimental and two control groups. The first two groups each had 16 mice. These two groups were administered an L-a supplement that was mixed with their water supply: one group was EDNO deficient (LE¯), the other group was healthy, (LE+). The second two groups each had eight mice and were administered a D-arginine supplement which is the optical isomer and not considered to be a substrate of nitric oxide. Similar to the first two groups, there was a group that was EDNO deficient (DE¯) and a healthy population (DE+). Again, the supplementation was administered via their water supply. The last two groups were the control groups, one group had 27 mice, and the other had 24 mice. The mice in the control groups were administered no arginine supplementation similar to the arginine supplementing groups, one group was EDNO deficient (NE¯) and the other was a healthy population (NE+). The mice then underwent a VO2 max testing. The mice that were treated with the L-a supplement exhibited an increase in both VO2 max as well as an increase in running distance, when compared to the placebo group. Based on the results the research team working on this project found that the induction of the L-a did in fact help restore the EDNO synthesis.9

Lastly, Kanaya examined the effects of L-a on lower limb vasodilation and the exercise capacity in patients with chronic heart failure. The subjects in this study had an
infusion of l-a for 30 minutes or were given a placebo, the amount infused varied based on weight, as they were given five ml per kg of body weight. Subjects were then exercised on a bicycle ergometer and tested for peak VO$_2$ and blood flow to the calf. At the conclusion of the study the following conclusion was drawn, that acute supplementation of l-a increased lower limb vasodilation but did not improve the exercise capacity of the subjects.$^8$

Based on the above-mentioned research, the overwhelming response to supplemental l-arginine is that it is a potent vasodilator and can be used to help combat cardiac diseases that occlude the vasculature of the body. It still remains that more research is needed on the topic of supplemental l-a in healthy subjects, and to what point it can help the overall effectiveness of their cardiovascular system.

Lactic Acid

Lactic acid or lactate is the metabolic byproduct of excess pyruvate without available oxygen to break it down. When pyruvate is not utilized in the mitochondria as a source of energy production via the Krebs cycle, which requires an aerobic environment, lactate will form from the excess pyruvate.$^1$ This change from an aerobic to an anaerobic energy system is often referred to as the anaerobic threshold, ventilatory threshold or lactate threshold. These terms refer to the point in time during exercise that lactate accumulation will grow at a rapid pace. Studying lactate during exercise can provide important information to physiologists as well as athletes. It can help to determine proper training intensities in order to increase athletic performance.

Research has indicated that supplementation with l-a may increase time to ventilatory threshold. Increasing the time to ventilatory threshold will allow an
individual to time to maximal exercise as well as exhibiting an increase in oxygen uptake. An investigation by Dout releau exemplifies this. The investigators on this particular study examined the chronic affects of l-a on patients with heart failure. Subjects performed four cardiopulmonary tests on a bicycle ergometer. The first test, the subjects were administered no supplementation, giving a pre-supplemental base line for lactate levels and ventilatory threshold. The second test consisted of another bicycle ergometric cardiopulmonary test after a single dose of six grams of l-a. The third test was similar in nature to the second test, except the testing procedures were completed after six weeks of l-a supplementation. The subjects consumed six grams of l-a, twice daily for six weeks and then performed the third cardiopulmonary test. The final test was undertaken at the same intensity as the first test, however it was under the conditions of chronic l-a supplementation and this was done in order to review the level of circulating plasma lactate. After data collection and analysis, the main result that was exhibited was that chronic l-a supplementation delayed ventilatory threshold and reduced the increase in plasma lactate levels in patients with heart failure.  

In a study by Schaefer l-a was examined as a possible means of reducing plasma lactate and ammonia. In this study, the researchers, in a double blind study administered an intravenous supply of l-a or a placebo to eight healthy volunteers. After the ingestion of l-a or placebo subjects were then monitored for VO2 consumption, as well as carbon dioxide production VCO2 along with blood lactate levels. The results rendered a significant decrease in plasma and lactate in those who ingested the l-a supplement. However, there were no significant changes in VO2 or VCO2 in either the placebo or the l-a group. This study provides evidence that l-a infusion can decrease lactate and
ammonia production, which in turn will have an effect on the metabolic byproducts of aerobic and anaerobic exercise.13

These studies suggest that there is a possible mechanism that will inhibit or slow blood lactate production during exercise. This may indicate that a decrease in lactic acid production may also indicate a delay in ventilatory threshold and therefore increase time to exhaustion, as well as oxygen uptake.

Conclusion

L-arginine is an amino acid derived from protein sources that plays a vital role in many physiological mechanisms in the body.16 This is the primary theory that exhibits many of the findings that have lead to many of the positive effects on maximal exercise.

Lactic acid or lactate is the metabolic byproduct of excess pyruvate without available oxygen to break it down and utilize for aerobic fuel.1 Supplemental l-a may lead to a decrease in lactic acid accumulation and an increase in ammonia removal.13 L-arginine supplementation may also be significant in delaying the ventilatory threshold, as well as reducing the amount of increase in lactic acid production.2

Therefore, the purpose of this investigation is to study the influence of the ingestion of varying doses of l-arginine versus a placebo on lactate production during a VO2 max test.
CHAPTER III

RESEARCH DESIGN AND METHODS

This investigation examined the ergogenic effects of varying acute l-arginine supplementation on VO$_2$ max. The research explores varying physiological rationale for any results by collecting and analyzing lactic acid, as well nitrite and nitrate levels. Subjects visited The University of Akron Human Performance Laboratory on four separate occasions.

During the first visit, the testing protocol was explained to the prospective subject and informed consent was obtained prior to enrollment in the protocol. The prospective subjects then completed a health questionnaire and one member of the data collection team took a resting blood pressure. A copy of the informed consent was given to each subject for their further review, as was a copy of Pre-testing instructions, which were also explained orally.

The three subsequent visits consisted of a pre-treatment blood draw upon their arrival at the testing facility. After the pre-treatment blood draw, treatment was given 30 minutes prior to testing. The treatment consisted of a cocktail that contained three ounces of lemon juice, five ounces of water, one packet of Crystal Light “On The Go Lemonade” flavor, and also consisted of one of the three treatments. A digital timer was set for 25
minutes and started after consumption of the treatment cocktail. After the 25 minutes had elapsed, a second blood draw was taken. Testing then occurred, which included a VO₂ max test utilizing a standard Bruce protocol on a treadmill. (Quinton Q-Stress™ TM55. Quinton Cardiology Systems, Inc, Seattle, WA) Gas and ventilation were analyzed utilizing a metabolic measurement system. (Parvo Medics, TrueOne ® 2400, Parvo Medics. Sandy, Utah) upon completion of the VO₂ max test, a third blood draw was taken one minute post-test.

Subject Inclusion and Exclusion Criteria

After consent is given, body composition data was collected for each subject by air displacement plethysmography via a BOD POD. (BOD POD® 2000A. Life Measurement Inc. Concord, CA) All subjects will have a fat mass of total mass not to exceed 30 percent.

Upon conclusion of BOD POD testing, a resting blood pressure was administered using a manual dial sphygmomanometer and stethoscope. Subjects will have a resting blood pressure that will not be greater than (140/90 mm Hg or higher) is considered high (hypertension) as defined by the American Heart Association. These levels are stated as being a systolic blood pressure of 140 mm/Hg and a diastolic blood pressure of 90 mm/Hg.

All subjects were healthy, non-smokers and free of disease (i.e., sickle cell anemia, thyroid disease, diabetes, heart disease) and free of any medication that might alter their metabolic rate at rest or during exercise.
Subjects

25 Male subjects of at least 18 years of age, but younger than 30 years of age, were recruited to serve as subjects in this investigation. Each subject was provided with, and required to read and sign, an informed consent in accordance with the guidelines set forth by The University of Akron Institutional Review Board. The protocol will be reviewed and approved by The University of Akron Institutional Review Board.

Experimental Procedure

Prior to any testing procedures, all subjects were instructed to arrive in proper exercise attire that includes running shoes, athletic shorts, and shirt. The subjects also were instructed to refrain from physical activity, ingestion of alcoholic beverages and over-the-counter medications for a twenty-four hour period previous to arrival. No food or caffeinated drink was consumed for twelve hours previous to their arrival.

Preliminary

During the first visit, each subject completed a Health History Questionnaire and read and completed the approved informed consent form. The subject’s height and weight were measured and recorded using a stadiometer and scale. An air displacement plethysmography body composition was then administered to the subject using a BOD POD. The criteria for the plethysmography required the volume displaced to be two valid measures. Lung volume displacement was compensated by predicted value for the subject by the BOD POD with appropriate equation.

The subject’s blood pressure was then measured after a five-minute period where the subject was resting in a seated position with standard bell stethoscope and manual dial sphygmomanometer.
Trials II, III, IV

The subject received a finger stick to release five ml of blood used for blood lactate analyses using a Lactate Pro LT-1710® (Arkray, Inc. Edina, MN).

The subjects then received one of the three treatments to be ingested. After twenty-five minutes, a second finger stick was administered again to release five ml of blood for lactate analysis. The subjects were then outfitted with the appropriate mouthpiece for the metabolic cart.

From the time of the second blood draw five minutes lapsed before the subjects performed a maximal exercise test on a treadmill according to the standard Bruce protocol. Upon completion of an achieved true VO$_{2\text{max}}$ test, a third finger stick was used to release 5 ml of blood to be analyzed for blood lactate level.

A true VO$_{2\text{max}}$ test was determined as meeting the following criteria. There are three items that needed to be completed. The first was a respiratory exchange ratio of at least or greater than 1.15. The second was a plateau of oxygen consumption, and the third being that the subject achieved a heart rate within 10 beats a minute of age predicted maximal heart rate (220-age). If any of these criteria were not met, the test was deemed invalid and the data collected was not used in the final statistical analyses.
Figure 3.1 Trial Design

*Treatments*

The treatments that were administered to each subject for *Trials II, III, and IV* were randomized order, double-blind method. The first of the treatments is to consist of a treatment of a three-gram L-arginine supplement that will be ingested orally in the form of a liquid cocktail.

The second treatment that each subject ingested was that of a six-gram dose of a L-arginine supplement, again to be taken orally in the form of a liquid cocktail. The third and final treatment that each subject took was that of a placebo, in the form of cornstarch also in the form of the liquid cocktail.

As previously mentioned a secondary investigator randomly assigned the treatments to each subject. The subjects in each treatment and testing procedure were also blind to which treatment they received.
CHAPTER IV

RESULTS AND DISCUSSION

Statistical analysis of the data will include the use of an ANOVA. A 3 (time {pre-treatment, pre-test and post-test}) x 3 (treatment {placebo, three g dose, six g dose}) with repeated measures on time and treatment for those variables that will be observed at pre-supplementation, pre-testing and at the end of maximal exercise. In those situations where the repeated observations may violate the ANOVA assumption of sphericity, the subsequent P values for the within-subjects effects will be adjusted using the Greenhouse-Geysker estimate. The SPSS version 13.0 statistical package will be used to complete all statistical calculations. The level of significance will be set a priori at $p \leq 0.05$. Post-hoc analyses will be performed where applicable, with Bonferroni adjustments (a means of protecting the experimentwise error rate), to determine significant interactions. Graphs utilized in this document were produced utilizing Microsoft Office Excel 2007.

The primary focus of this research was to examine the effects of l-arginine treatment on lactic acid and maximal oxygen consumption in healthy males, the following will outline how data was analyzed to draw conclusions to support or debate the original hypotheses.
The following graphs will give descriptive statistics of the subjects that volunteered to participate in data collection.

Figure 4.1 Subject Age

Figure 4.2 Subject Weight

The above exemplify the average age and weight of the participants that volunteered for data collection in the study.
The graphs below represent the average height and percent body fat percentage of the participants.

As previously described 25 healthy male volunteer subjects were used for data collection.
The primary research questions explored were to see if there was varying response on lactic acid and maximal oxygen consumption with different treatments of l-arginine.

In analyzing lactic acid it was found that it was not statistically significant as demonstrated below.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Pillai's Trace</td>
<td>.002</td>
<td>.013*</td>
<td>2.000</td>
<td>14.000</td>
</tr>
<tr>
<td></td>
<td>Wilks' Lambda</td>
<td>.998</td>
<td>.013*</td>
<td>2.000</td>
<td>14.000</td>
</tr>
<tr>
<td></td>
<td>Hotelling's Trace</td>
<td>.002</td>
<td>.013*</td>
<td>2.000</td>
<td>14.000</td>
</tr>
<tr>
<td></td>
<td>Roy's Largest Root</td>
<td>.002</td>
<td>.013*</td>
<td>2.000</td>
<td>14.000</td>
</tr>
</tbody>
</table>

Within Subjects Design: dose + time + dose * time

Figure 4.5 Multivariate Tests on Lactic Acid

![Figure 4.5 Multivariate Tests on Lactic Acid](image)

Figure 4.6 Subjects Pre-treatment Lactic Acid within Treatments

This graph is a visual representation of the average blood lactic acid level before treatment was given.
The below graphs will provide visual representation of the blood lactic acid levels after treatment and testing.

![Post-treatment Lactate](image)

**Figure 4.7 Subjects Post-treatment Lactic Acid within Treatments**

Although lactic acid accumulation was not determined to be statistically significant, the research question of whether or not there was a varying response on VO₂...
max still remained. The following will assist in illustrating the effects of l-arginine on VO₂ max.

![VO₂ Max Chart]

**Figure 4.9 Subjects Performance on VO₂ Max within Treatments**

As seen there was a positive influence on subjects’ performance on VO₂ max testing within the varying treatments. This data yielded a result that was significant.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>vo2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pillai's Trace</td>
<td>.253</td>
<td>3.888a</td>
<td>2.000</td>
<td>23.000</td>
<td>.035</td>
</tr>
<tr>
<td>Wilks' Lambda</td>
<td>.747</td>
<td>3.888a</td>
<td>2.000</td>
<td>23.000</td>
<td>.035</td>
</tr>
<tr>
<td>Hotelling's Trace</td>
<td>.338</td>
<td>3.888a</td>
<td>2.000</td>
<td>23.000</td>
<td>.035</td>
</tr>
<tr>
<td>Roy's Largest Root</td>
<td>.338</td>
<td>3.888a</td>
<td>2.000</td>
<td>23.000</td>
<td>.035</td>
</tr>
</tbody>
</table>

Within Subjects Design: vo2

**Figure 4.10 Multivariate Tests on VO₂ Max**

Previous research has determined that l-arginine supplementation has had a positive effect on cardiovascular hemodynamics and exercise tolerance in diseased
populations. This study has revealed that the effects of l-arginine treatment have also demonstrated a positive influence on cardiovascular exercise.
CHAPTER V
SUMMARY

In recent history, the desire to become a more dominant athlete, either at the professional or amateur level has never been as prevalent. This unrelenting desire to become bigger, faster, stronger has led to individuals experimenting with different training methods, nutrition habits and ergogenic aids, some even illegal. The use of supplements containing l-arginine has become increasingly popular by individuals attempting to enhance their training. With the popularity of such products, independent research was needed to examine if such products can indeed take athletes to the next level.

As stated before previous research has examined the effects of l-arginine on the cardiovascular system during work of diseased populations. The goal of this research was to set the forward momentum of research of the l-arginine in healthy populations into motion. The findings of this research indicate that l-arginine may enhance the cardiovascular system during aerobic training, more specifically during maximal exercise.
With the apparent positive results found in this study, the need for further research is quite apparent. Ideas for future research in this area can be drawn from the conclusions and design of this study. Future research could include, but is not limited to exploring a chronic treatment, instead of an acute treatment. Dosing individual’s by body weight as compared with a standard dose without regard for body weight. Examining other physiologic processes or byproducts other than lactic acid is another possible research idea. Such processes and byproducts include the enzyme that cause vasodilation of vasculature, nitric oxide synthase. Vascular endothelial growth factor or VEG-F is yet another substance that could lend itself to research in the area of supplementation of l-arginine.

As l-arginine is further explored as a means of positively affecting cardiovascular exercise, VO₂ max is the best marker of cardiovascular fitness, other research areas might include exercise intensity less than that of utmost exertion as required in VO₂ max testing. It is rare that individuals have an all out effort, so a protocol that utilizes sub-maximal trials, may be more appropriate to examine l-arginine as a possible ergogenic aid.

Utilizing a more diverse subject pool is another consideration in moving forward with research in this area. The subject pool in this study was open to all ethnicities, however only Caucasians volunteered for participation. Females were excluded due to possible hormonal changes, however future researchers may consider including them into protocols. Finally, examining varying age groups should also be considered for testing protocols.
As demonstrated, research in the area of l-arginine supplementation on healthy populations is very young and has much room to grow and be explored. It is vital to keep investigating and learning through research in this realm.
BIBLIOGRAPHY


Thank you for volunteering to be a subject for a study to be conducted in the Applied Physiology Research Laboratory. Many of the tests used in our experiments require that you perform very strenuous exercise, sometimes under difficult environmental conditions. Consequently, it is important that we have an accurate assessment of your present health status to assure that you have no medical conditions that would make the tests especially dangerous for you. Please complete the health history as accurately as you can.

THIS MEDICAL HISTORY IS CONFIDENTIAL AND WILL BE SEEN ONLY BE THE PRINCIPLE INVESTIGATORS

Name__________________________________________  Date____/____/____

Present Age_____yrs

Ethnic Group:  ____White
               ____ African American
               ____ Hispanic
               ____ Asian
               ____ Pacific Islands
               ____ American Indian
               ____ Other_____________

HOSPITALIZATIONS AND SURGERIES
In the past six months have you ever been hospitalized for an illness or operation, please complete the chart below.
YEAR_____________

OPERATION OR ILLNESS
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

YEAR______________

OPERATIONS OR ILLNESS
________________________________________________________________________
________________________________________________________________________

In the past six months have you been treated for any chronic illness or chronic condition?  
[  ] Yes   [  ] No  
If Yes, explain:___________________________________________________________
________________________________________________________________________

MEDICATIONS
Please list all medications that you have taken within the past 8 weeks:  (Include
prescriptions, vitamins, over-the-counter drugs, nasal sprays, aspirins, birth control pills,
etc.)  
Check this box [  ] if you have not taken any medication.

MEDICATION________________
REASON FOR TAKING THIS
________________________________________________________________________
________________________________________________________________________

MEDICATION________________
REASON FOR TAKING THIS
________________________________________________________________________
________________________________________________________________________

MEDICATION________________
REASON FOR TAKING THIS
________________________________________________________________________
________________________________________________________________________

MEDICATION________________
REASON FOR TAKING THIS
________________________________________________________________________
________________________________________________________________________

MEDICATION________________
REASON FOR TAKING THIS
________________________________________________________________________
________________________________________________________________________

ALLERGIES
Please list all allergies you have (include pollen, drugs, alcohol, food, animals, etc.)
Check this box [     ] if you have no allergies.

1.______________________________________________________________________
2.______________________________________________________________________
3.______________________________________________________________________
4.______________________________________________________________________

PROBLEMS AND SYMPTOMS
Place an X in the box next to any of the following problems or symptoms that you have had:

General

[   ] Mononucleosis
    If yes, when ______________________________________________________

[   ] Excessive fatigue
[   ] Recent weight loss while not on a diet
[   ] Recent weight gain
[   ] Thyroid disease
[   ] Fever, chills, night sweats
[   ] Diabetes
[   ] Arthritis
[   ] Sickle Cell Anemia
[   ] Heat exhaustion or heat stroke

PROBLEMS AND SYMPTOMS, continued

Heart and Lungs

[   ] Abnormal chest x-ray
[   ] Pain in chest (persistent and/or exercise related)
[   ] Heart attack
[   ] Coronary artery disease
[   ] High blood pressure
[   ] Rheumatic fever
[   ] Peripheral vascular disease
[   ] Blood clots, inflammation of veins (phelebitis)
[   ] Asthma, emphysema, bronchitis
[   ] Shortness of breath
    [   ] At rest
    [   ] On mild exertion
[   ] Discomfort in chest on exertion
[   ] Palpitation of the heart; skipped or extra beats
[   ] Heart murmur, click
[   ] Other heart trouble
Lightheadedness or fainting
Pain in legs when walking
Swelling of the ankles
Need to sleep in an elevated position with several pillows
Hemophilia

G-U SYSTEM
Get up at night to urinate frequently
Frequent thirst
History of kidney stones, kidney disease

G.I. TRACT
Eating disorder (e.g. anorexia, bulimia)
Yellow jaundice
If yes, when
Hepatitis
If yes, when
Poor appetite
Frequent indigestion or heartburn
Tarry (black) stool
Frequent nausea or vomiting
Intolerance of fatty foods
Changes in bowel habits
Persistent constipation
Frequent diarrhea
Rectal bleeding
Unusually foul smelling or floating stools
Pancreatitis

Nervous System
Alcohol problem
Alcohol use
If yes, how many drinks ingested per week?
Frequent or severe headaches
Stroke
Attacks of staggering, loss of balance, dizziness
Persistent or recurrent numbness or tingling of hands or feet
Episode of difficulty in talking
Prolonged periods of feeling depressed or “blue”
Difficulty in concentrating
Suicidal thoughts
Have had psychiatric help
Explain any items checked (when, severity, treatment)

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Have you ever passed out during or after exertion?  YES  NO
Do you have a family history of coronary artery disease  YES  NO
If yes, Who? (Grandparents, parents, siblings, uncles, and aunts)
APPENDIX B

INFORMED CONSENT

Title of Study: “Effect of Oral L-arginine Supplementation on Lactic Acid, Platelet Aggregation and Maximal Oxygen Consumption in Healthy Males”

Introduction: You are invited to participate in a research study designed and conducted by Matt Feeback and Eric Corbett, Masters’ students enrolled in the exercise physiology program at The University of Akron in the Department of Sport Science and Wellness Education under the advisement of Dr. Ronald Otterstetter, faculty member at The University of Akron in the Department of Sport Science and Wellness Education.

Purpose: The main objective for this investigation is to observe the influence of varying doses of l-arginine supplementation on healthy human subjects and the physiological effects it has when observing cardiovascular endurance capacity. This study will specifically evaluate physiological markers found in the blood, before, during and after exercise capacity testing.

Procedure: Thirty subjects will participate in the research. If you volunteer for this study you will be required to take part in a preliminary visit and three testing trials. During the preliminary visit, the testing protocol will be explained and a health questionnaire will be completed by prospective subjects. Baseline measures of blood pressure and body composition will be measured. The three testing trials will be separated by seven days, in which data will be collected. During the testing trials, you will drink a non-nutritive, sugar-free fruit drink containing l-arginine (at three gram and six gram doses) or a placebo, and will be required to complete a cardiovascular endurance capacity test. For the cardiovascular endurance test, you will be equipped with a mouthpiece similar to a scuba-diving snorkel which you will breathe through for the entire exercise session. You will also be equipped with a heart rate monitor and a blood pressure cuff for the entire exercise session. You will perform a cardiovascular endurance capacity test on a treadmill. The treadmill will be set at an incline and slow pace. Both the speed and incline will increase every three minutes until completion. Three blood specimens of 6.5 ml each will be drawn. That is approximately one tablespoon per trial.
The blood specimens will be taken pre-supplementation, 25 minutes post-supplementation and post-exercise testing. Each trial will last approximately one hour, with three total trials.

**Inclusion:** Healthy males between the ages of 18 and 30 years.

**Exclusion:** If you answer yes to any question and/or do not fall within acceptable quantified limits, you will be disqualified from the study. The three quantified items on the Health history questionnaire are alcohol consumption (< 3 drinks per day), excessive fatigue (excluded if still feeling consistently fatigued after at least 7 hours of sleep in previous 24 hour period) and if you have had mononucleosis within six months of first trial date. You will also be excluded if you are > 30% body fat, due to an increased chance of a cardiac event, have a resting blood pressure > 140 mm/Hg systolic and > 90 mm/Hg, again due to an increased chance of a cardiac event.

**Risk and Discomfort:** Risks associated with this investigation are related to withdraw of blood via the venipuncture. Mild discomfort or bruising due to venipuncture is possible. Slight risk of infection similar to any puncture in the skin which will be minimized by using the aseptic technique.

Mild and localized discomfort, not exceeding that incurred during a normal exercise session, associated with delayed onset muscle soreness due to exercise. May experience fatigue and lightheadedness due to exercise.

I understand that the risk of serious injury is no greater than that which I may experience with a very intense physical workout. I also understand that there is an extremely small chance of a serious medical conditions occurring and that according to National statistics, 4 out of every 10,000 people may experience a heart attack and 1 out of every 10,000 people may experience sudden death when engaging in intense physical exercise/exertion. I understand that I should inform the researchers immediately if I start to have pains in my chest, shoulder or legs, feel dizzy or weak, and experience any shortness of breath, difficulty breathing, or other distressing symptom during the testing procedure.

A health history will be taken to screen out anyone for who strenuous exercise may pose a higher than expected risk. It is important that you provide truthful and accurate information so as to not put yourself at unnecessary risk.

We will observe the universal precautions to avoid transmission of blood borne pathogens between testers and subjects. Venipuncture will be performed by trained, experienced personnel. Phlebotomy competency training will be completed and documented for all individuals performing blood draws. In the event of a medical emergency, all researchers are CPR, First Aid, and AED trained and certified. Should an emergency occur, the researchers will activate EMS.

Investigators have completed over 100 VO2 max tests without a subject injury or critical event. An investigator will be monitoring the computer, as to stop the test at any point that the subject requests or if the researcher feels it is unsafe for you to continue based on guidelines set forth by the American College of Sports Medicine. Another
investigator will be standing behind you to catch you in the event you should fall of
treadmill during testing.

**Benefits:** Participating in this study will allow you to learn more about your
cardiovascular endurance via VO_{2max} testing, as well as your personal body composition
via BOD POD testing. Your participation will also help us gain information about l-
arginine supplementation as an ergogenic aid.

**Payments for Participation:** You will be monetarily compensated upon the completion
of your individual participation in the study. Compensation of $10 will be awarded to
you for each trial completed. There will be three trials, so you may be compensated a
total of up to $30. If you are unable to participate in all three trials, your compensation
will be prorated accordingly.

**Right to refuse or withdraw:** Participation in the research is voluntary. You may
withdraw consent and discontinue participation in the study at any time without any
consequence to you.

**Anonymous and Confidential Data Collection:** Any identifying information collected
will be kept in a locked file cabinet, and only the researchers will have access to the data.
As a participant, you will not be individually identified in any publication or presentation
of the research results. Only aggregate data will be used. To insure your privacy, the
information found in this study will be subject to the confidentiality and privacy
regulations of The University of Akron.

**Confidentiality of records:** The project director will store all your information in a
locked research file and will identify you only by a code number. The project director
will keep the code key connecting your name to your number in a separate secure file.

**Who to contact with questions:** If you have any questions at any time, you may contact
either of the researchers at 330-972-7477 or our advisor, Dr. Ronald Otterstetter in the
Department of Sport Science and Wellness Education at 330-972-7738. This project has
been reviewed and approved by The University of Akron Institutional Review Board. If
you have any questions about your rights as a research participant, you may call the IRB
at (330) 972-7666 or 1-888-232-8790.

Thank you for your willingness to participate in this study.

Matt Feeback, B.S.    Eric Corbett, B.S.    Ron Otterstetter, PhD
Researcher            Researcher             Researcher/Advisor
Acceptance & signature: I have read the information provided above and all of my questions have been answered. I voluntarily agree to participate in this study. I will receive a copy of this consent form for my information.

______________________                       Date: __________________
Participant signature

______________________                       Date: ________________
Signature of witness
APPENDIX C

SUBJECT INSTRUCTIONS

“Effect of Oral L-arginine Supplementation on Lactic Acid,, Platelet Aggregation and Maximal Oxygen Consumption in Healthy Males”

Pre-testing instructions (Trials I, II, III) for subjects

24 hours before testing:

➤ Refrain from strenuous physical activity

➤ No ingestion of alcoholic beverages

➤ No over-the-counter medications (OTC) that may alter nervous system or cardiovascular system i.e. Pseudoephedrine, Aspirin.

➤ No illicit drugs or herbal supplements.

12 hours before testing:

➤ Refrain from use of caffeinated beverages i.e. coffee, soda, tea etc…

➤ Perform a fast. This means that no food should be consumed in the 12 hour period before testing trials. Subjects will be allowed to consume water and non-caloric beverages that do not contain caffeine.

Please arrive at testing facility (Human Performance Laboratory in Memorial Hall, Room 64) in clothing conducive for exercise. Athletic shorts, light weight t-shirt, comfortable athletic shoes for running.

If you have any questions regarding the above instructions or any other aspect of the study, feel free to contact either Matt Feeback or Eric Corbett by phone or e-mail.