EFFECTS OF ORAL L-ARGININE SUPPLEMENTATION ON PLATELET COUNT
AND MAXIMAL OXYGEN CONSUMPTION IN HEALTHY MALES

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Eric Corbett
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EFFECTS OF ORAL L-ARGININE SUPPLEMENTATION ON PLATELET COUNT AND MAXIMAL OXYGEN CONSUMPTION IN HEALTHY MALES

Eric Corbett

Thesis

Approved:

Advisor
Dr. Ronald Otterstetter

Committee Member
Mrs. Stacey Buser

Committee Member
Mrs. Rachele Kappler

Department Chair
Dr. Victor Pinheiro

Accepted:

Interim Dean of the College
Dr. Cynthia Capers

Dean of the Graduate School
Dr. George Newkome

Date
DEDICATION

This work, and the completion of this work, is dedicated to my parents who encouraged and motivated me to finish it. Thank you.
ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

Page

LIST OF TABLES............................................................................................................ vii

LIST OF FIGURES ......................................................................................................... viii

CHAPTER

I. INTRODUCTION ............................................................................................................1

II. LITERATURE REVIEW ................................................................................................7

   L-Arginine ................................................................................................................7

   L-Arginine Effects ...................................................................................................8

   L-Arginine – Platelet Aggregation Relationship ...................................................11

   Blood Viscosity – Cardiovascular System Relationship ......................................14

III. METHODOLOGY ......................................................................................................15

   Subject Inclusion and Exclusion Criteria ...............................................................16

   Subjects ..................................................................................................................16

   Experimental Procedure .........................................................................................17

      Preliminary Visit ........................................................................................17

      Trials I, II, and III ......................................................................................17

   Blood Draws ..........................................................................................................19

   Treatments ...............................................................................................................19

   Blood Analysis .......................................................................................................20
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Subjects’ characteristics</td>
<td>21</td>
</tr>
<tr>
<td>4.2 Mean platelet count per dose and time</td>
<td>22</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Trial timeline</td>
<td>18</td>
</tr>
<tr>
<td>4.1 Platelet count over time</td>
<td>23</td>
</tr>
<tr>
<td>4.2 Maximal oxygen consumption over dose</td>
<td>24</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION

Cardiovascular endurance (CVE) is one of the most important measures of overall health. A person’s level of cardiovascular endurance helps predict probability of disease, quality of life, and ability to react to acute physical and mental stress. For healthy individuals, higher cardiovascular endurance also indicates an elevated level of physical fitness.

The fitness industry spends an overabundant amount of money each year attempting to develop ways to increase and improve cardiovascular endurance. Training regimens and training environments have been studied to find the most advantageous combination. However, improvements in cardiovascular endurance are not solely exercise-based. Now, training supplements of all different modes are being tested and designed to make athletes bigger, faster, stronger, and more durable. The studies and discoveries that benefit elite athletes trickle down to aid moderate athletes and eventually the general population.

Maximal oxygen uptake (VO$_{2\text{max}}$) is a recognized standard for measuring cardiovascular endurance (Malek et al. 2005). Maximal oxygen uptake is the utmost amount of oxygen (O$_2$) inhaled, absorbed, and utilized in working muscle during exhaustive physical exertion. It is measured during a graded exercise test using open-circuit spirometry with a metabolic cart analyzes the air breathed by a person. The
measurement is simply a drawn comparison between the ambient air, which is air in the surrounding environment, and the exhaled air. The comparison involves percentage changes in oxygen and carbon dioxide (CO$_2$). When a person’s cardiovascular system is more efficient, more oxygen is delivered to and used by the working muscle. The changes in percentage of gases are different with less oxygen and more carbon dioxide being exhaled.

The best indicator for the athlete’s cardiovascular performance is the maximal oxygen consumption assessment, according to Brooks, Fahey and Baldwin$^4$. A greater amount of oxygen consumed by the body relative to body mass indicates a higher cardiovascular efficiency. Higher cardiovascular efficiency allows muscle to work at a higher intensity for a longer time period without tiring. This is possible because the body can only exercise as long as oxygen is delivered to muscle and waste, such as carbon dioxide, is removed and eliminated. A longer time to exhaustion benefits exercisers by allowing them to physically stress their bodies to a fuller extent. When the body is overloaded with more stress it will create a larger adaptation to that stress for the future.

Better cardiovascular endurance benefits exercisers in rest and recovery as well. A stronger and more efficient cardiovascular system will supply tissues damaged by exercise with the materials to heal more rapidly. This reduces total rest time in between exercise sessions.

For oxygen consumption and carbon dioxide elimination to occur, blood must travel past muscle. Two factors affect the rate at which oxygen and carbon dioxide are exchanged and delivered in the blood. The first factor is the effectiveness of working muscle to extract oxygen from the blood and through the blood vessel. The second factor,
which is more important for this study, is the rate at which oxygenated blood is delivered to the working muscle.

Blood flow rate depends on many factors. Included in these factors is blood viscosity. Blood viscosity is simply the friction of blood within itself. High viscosity blood travels more slowly because it is less mobile in the blood vessel. Low mobility of blood is congestive. Lowering the viscosity of blood should decrease the resistance to blood flow which would decrease the afterload on the heart. Afterload is defined as the ventricular pressure at the end of ventricular contraction, systole.

Blood viscosity naturally increases during exercise due to a change in the concentration of blood components. The amount of hematocrit, the element of total blood volume made up of blood cells, remains the same. However, the plasma volume, the component in blood without any cells, decreases during exercise because of exchange of fluid between intracellular and extracellular spaces. The increased concentration of hematocrit will cause an increase in blood viscosity.

Platelet aggregation, the self-adhesiveness of blood, also determines the viscosity of blood. When the aggregation of platelets is lowered, blood adheres less readily to itself and to the blood vessel through which it is flowing. The adhesion of blood is a factor in its viscosity. By lowering the adhesiveness of blood, the viscosity lowers and the resistance to flow also lowers. The flow rate of blood then increases.

Measuring for platelet aggregation requires compensating for the change in concentration of hematocrit. The plasma volume change formula developed by Dill and Costill\(^5\) conveniently converts the post-exercise concentration to resemble the pre-exercise concentration. After the value conversion, all changes in blood viscosity due to
plasma volume changes are compensated. All other blood viscosity changes are accredited decreases in blood platelet aggregation.

Blood can only flow out of the heart as long as the pressure created by the ventricle is greater than the pressure in the artery. As the artery’s pressure decreases from the lowered viscosity, more blood is ejected with each stroke. This allows more oxygenated blood to reach the working muscle with each heart beat. The increase in amount of blood results in an increase of the amount of oxygen delivered.

The increase in delivery rate of oxygenated blood allows the muscle to work more efficiently consuming less energy and delaying fatigue. Delaying fatigue increases the length and intensity of aerobic physical activity capacity which is itself an increase in cardiovascular endurance.

L-Arginine is a basic natural amino acid found in many foods of the normal diet, and has been marketed as a body-building supplement. The increase in body creatine, an amino acid that aids in the supply of energy, levels due to L-arginine aids in muscular hypertrophy and muscle recovery from exercise. L-arginine has also been shown to aid cardiac patients. The current belief is that the increase in exercise capacity is due to the conversion of L-arginine into nitric oxide (NO), a vasodilator. Vasodilation caused by NO allows blood to flow to the coronary blood vessels more readily. This decreases the likelihood of a myocardial infarction for cardiac patients or at least lowers the amount of cardiac cell damage should an infarction occur. The vasodilation will occur in the systemic blood vessels, as well, increasing the diameter of all arteries and letting more blood flow through wider channels.
During the metabolism of L-arginine into NO, endothelium-derived relaxing factor (EDRF) is developed. While EDRF acts itself as a nitrovasodilator, it also inhibits platelet aggregation and adhesion. This inhibition occurs because EDRF stimulates intracellular guanylate cyclase which stimulates intracellular levels of cyclic guanosine monophosphate (cGMP) in platelet cytosol.

The administration of L-arginine to patients with heart failure has shown an increase in exercise capacity\textsuperscript{12}. Blood pressure and other risk factors of heart disease have improved as a consequence of L-arginine supplementation. This is due to the decreased work load on the heart. Any decrease in cardiac work load whether in a diseased or healthy heart returns benefits. Therefore, the increased exercise capacity found in cardiac patients due to L-arginine supplementation could also implicate improvement in cardiovascular endurance in healthy subjects.

A study performed by Rector \textit{et al.}.\textsuperscript{12} investigated the effect of L-arginine on NO concentration. Regardless of the findings concerning the effects of NO, the study did not investigate or allow for the possibility that a lowered platelet aggregation caused the increased exercise capacity.

Much research, such as the study performed by Giugliano \textit{et al.}\textsuperscript{7} entitled “The Vascular Effects of L-Arginine in Humans”, has explained that increased cardiovascular endurance lowers blood platelet aggregation. However, there has been no attempt to discover if the reciprocal is also true: Will lowering blood platelet aggregation through a means such as L-arginine supplementation affect cardiovascular endurance?

When blood platelet aggregation is decreased the blood platelets themselves become less likely to adhere or aggregate to other platelets. When evaluating platelets,
tests will show that platelets that have adhered to each other during a platelet count will be counted as one. Effectively, a higher blood platelet aggregation will show a lower platelet count because all the adhered platelets seem to be one platelet, and a lower platelet aggregation will be viewed as a higher platelet count.

L-arginine should maintain a higher blood platelet count which would correspond with a lower platelet aggregation and lower blood viscosity. The end result should be that L-arginine supplementation, through these mechanisms, increases total oxygenated blood delivery to working muscle and therefore increases in maximal oxygen consumption and cardiovascular endurance.

The purpose of this study was to discover and determine the effect of L-arginine supplementation on cardiovascular endurance, and whether or not these effects were due to a higher blood platelet count. If there proved to be a corollary effect between the dose of L-arginine and blood platelet count, then cardiovascular effects would result. If this study found significant advantage in cardiovascular endurance due to changes in blood platelet count through L-arginine supplementation, then exercisers in all levels would have access to advantages to the extent that are currently undiscovered.
CHAPTER II
LITERATURE REVIEW

This chapter organizes the previous related research and imposes a structure to clarify the relationship. Research was performed on the areas of L-arginine, how L-arginine affects blood platelet aggregation, how blood platelet count relates with blood platelet aggregation and how the level of cardiovascular system is affected blood viscosity.

L-Arginine

L-arginine is a semi-essential amino acid. It is found in natural food sources such as walnuts, filberts, pecans, Brazil nuts, sesame and sunflower seeds, brown rice, raisins, coconut, gelatin, buckwheat, almonds, barley, cashews, cereals, chicken, chocolate, corn, dairy products, meats, oats, and peanuts. L-arginine has been found necessary in the production of urea and synthesis of creatine in the body.

L-arginine breaks down in the body into nitric oxide and L-citrulline. Nitric oxide causes vasodilation, the relaxation and expansion of blood vessels. Because of the vasodilation, research studies have been performed to determine the benefits for populations with coronary heart disease. Some of the research has credited the L-arginine - nitric oxide pathway for benefits in peak VO$_{2\text{max}}$.
L-Arginine Effects

Nagaya et al.\textsuperscript{11} performed a study entitled “Short Term Oral Administration of L-Arginine Improves Hemodynamics and Exercise Capacity in Patients with Pre-capillary Pulmonary Hypertension” in 2001. That study examined acute hemodynamic responses to oral L-arginine or placebo in 19 patients with primary or pre-capillary secondary pulmonary hypertension. The researchers measured peak VO\textsubscript{2} and ventilatory response to CO\textsubscript{2} production during an exercise test.

After one week supplementation of either placebo or L-arginine, the patients performed the same test and were measured again. Included in these measures were: heart rate, mean systemic arterial pressure, mean pulmonary arterial pressure, mean right atrial pressure, and pulmonary capillary wedge pressure. The patients’ exercise tests were performed on cycle ergometer at 55 rpm without gradual increases in work load.

The researchers found increases in peak VO\textsubscript{2}, and found decreases in mean pulmonary arterial pressure and pulmonary vascular resistance. The researchers considered the cause for the improvement and lowered pressure to be pulmonary vasodilation due to NO mechanism. Nagaya et al.\textsuperscript{11} did not investigate the possibility that the changes in performance and measured values were due to L-arginine via a decrease in blood viscosity from lowered platelet aggregation. The lowered resistance could be related to lowered platelet aggregation and thus increase platelet count, but that was not the focus of the Nagaya et al.\textsuperscript{11} research. Further research was needed on this as a possible cause.

An investigation performed by Giugliano et al.\textsuperscript{7} studied the possibility that increased vascular tone during acute hyperglycemia related to decreased NO. The study
included twenty non-obese men with no family history of diabetes or hypertension. The researchers investigated the idea by inducing hyperglycemic events. When steady-state hyperglycemia had been achieved, the subjects were infused with solutions to either increase L-arginine or inhibit the L-arginine synthesis process. A control was also performed by simply inducing the hyperglycemic event without augmenting any L-arginine reaction. In all cases, blood pressure and heart rate were taken non-invasively, and femoral artery flow information was obtained with image directed ultrasonography.

The hyperglycemic events increased blood pressure and heart rate and decreased leg blood flow. The researcher found that increased availability of L-arginine normalized blood pressure and femoral blood flow during the hyperglycemic event.

Böger et al.\textsuperscript{3} found that dietary L-arginine stopped progression of atherosclerosis and atherosclerotic plaque in hypercholesterolemic rabbits. The rabbits were chronically fed a hypercholesterolemic diet and treated with L-arginine. Another group of similarly cholesterol-fed rabbits were treated with lovastatin, a cholesterol-lowering therapy. A third group of rabbits were used as a control and fee normal rabbit chow. The rabbits were studied for aortic and carotid, intimal plaque area. The L-arginine treatment stopped the plaque progression more effectively than the lovastatin treatment.

A study by Engelman et al.\textsuperscript{6} investigated the effects of L-arginine on endothelial inflammation. The study explored twelve control group and twelve experimental group Yorkshire pigs. The pigs were sedated and measured for left ventricular pressure, left ventricular dimensions, and LAD regional segment length by performing a median sternotomy and inserting a catheter into the left ventricle and the coronary sinus.
All the subjects were tested for baseline measures prior to any control or experimental protocol. The subjects were then intravenously given either a lactated Ringer’s solution for the control group, or an L-arginine for the experimental group. After the intravenous solution was administered, the left anterior descending coronary artery was ligated for thirty minutes and reperfused for 90 minutes. The subjects were analyzed during the ischemia, during the reperfusion and after the reperfusion. The researchers’ results showed that the L-arginine infusion reduced the excessive endothelial inflammation response subsequent to injury.

A study was conducted to ascertain whether L-arginine affected wound healing by Wittman et al.\textsuperscript{15} in 2005. The researchers investigated male mice. The researchers induced a soft tissue trauma to the mice and gave the mice either L-arginine or saline solution. The researchers measured hydroxyproline, a metabolite of collagen synthesis, in the wound fluid via high-performance liquid chromatography and wound breaking strength at seven and ten days post-trauma. In the control group, the hydroxyproline was significantly decreased, while the L-arginine group showed significant restoration of the metabolite in the wound. The L-arginine group also showed an increase in wound breaking strength. The researchers showed, with their investigation into this study, that L-arginine increased wound healing.

Research on the subject of L-arginine has provided a wealth of information suggesting that supplementation with L-arginine can improve peak oxygen consumption, blood pressure, leg blood flow, plaque progression risk, inflammation and wound healing. L-arginine has been studied and has already been proven to be an amazing and significantly important supplement. The research in this study attempted to investigate
the possibility that L-arginine supplementation could have an even greater impact on the health and wellness of society; an impact on increases in maximal oxygen consumption via increases in platelet count.

**L-Arginine – Platelet Aggregation Relationship**

Decreases in blood platelet aggregation lower blood viscosity. Conversely, increases in blood platelet aggregation will affectively increase blood viscosity. Less adhesive liquid flows faster due to the lowered resistance, an idea that is supported by Poiseuille’s law. Poiseuille’s law includes four factors in the determination of flow rate: pressure gradient, length, radius, and viscosity. The viscosity is inversely proportional to flow rate\(^4\).

In a study by Adams *et al.*\(^1\), twelve healthy men between the ages of twenty-seven and thirty-seven were given either placebo or three times daily (t.i.d.) supplementation of L-arginine (7g). The trial was performed as a crossover study with washout periods of 7 to 14 days. The researchers investigated platelet physiology and found, in humans, marked impairment of platelet aggregation that correlated with the level of plasma L-arginine after supplementation.

A study performed with male hypercholesterolemic rabbits\(^13\) showed that ten weeks chronic supplementation of oral L-arginine significantly lowered platelet aggregation. The control group in this study, having been simply fed a controlled diet without L-arginine supplementation, showed no change in aggregation levels. There was no difference between control and experimental groups in regards to basal production of
platelets. Therefore, the number of platelets present for aggregation had no effect on the study.

Rector et al.\textsuperscript{12}, though not evaluating the effects of L-arginine on platelet aggregation in the study they performed, still found that aggregation was lowered. Fifteen subjects were involved in the crossover study and were given six weeks of L-arginine supplementation and another six weeks with placebo crossover. This was done in random sequence. The subjects were tested on distance walked in six-minutes and a questionnaire concerning “Living With Heart Failure.”

Their study found that peripheral blood flow, in patients with heart failure, was increased and responses to the questionnaire became more positive. The researchers did not attempt to relate the lowered aggregation with the L-arginine supplementation or the peripheral blood flow. The purpose of their study was to investigate the effects of supplemental oral L-arginine on peripheral blood flow in patients with heart failure. The tangential data, however, alluded to the possibility that the L-arginine supplementation lowers platelet aggregation and thus could cause the increases in blood flow.

Giugliano et al.\textsuperscript{8} completed another investigation in 1997, entitled “The Vascular Effects of L-arginine in Humans”. In Study I, the researchers investigated constant rate infusions of L-arginine into five males and five females that were resting for thirty minutes. The findings of their research implicated the connection between L-arginine supplementation and lowered blood platelet aggregation. Lowered blood viscosity was also correlated with the supplementation. This Giugliano et al.\textsuperscript{8} study showed a connection with infusion of L-arginine into normal subjects at rest and decreased platelet
aggregation. However, the results can not be inferred to exercising subjects and oral supplementation.

The research studies collected in this section all indicate that L-arginine relate with blood platelet aggregation, and blood viscosity. Certain studies also explain the beneficial effects of lower blood platelet aggregation and viscosity towards coronary heart disease. Increased blood flow was an apparent advantage of the L-arginine supplementation in the Rector et al.\textsuperscript{12} study. The other studies also found important links between L-arginine and blood platelet aggregation, but increased blood flow due to decreased platelet aggregation was never linked with increases in cardiovascular endurance. That link was an important part of this study.

Research in the area surrounding L-arginine supplementation and its effects on cardiovascular endurance has previously focused on unhealthy populations and their responses. The research involving normal subjects excluded some topics of key interest to this study. Effects of oral supplementation of L-arginine, effects to exercise capacity, and the possible relationship of platelet aggregation to exercise improvements all are areas that needed to be collectively investigated. This study investigated the incorporated effects of oral supplementation of L-arginine on platelet aggregation in normal subjects and the consequent results on maximal oxygen consumption.

In order to fully benefit the general population, including recreational exercisers and amateur athletes, this study investigated an area lacking in research: the completed connection between L-arginine-induced increases in platelet count and increased maximal oxygen consumption. Any marked increases in VO\textsubscript{2max} that relate with changes
in platelet count found in this study would open new avenues of that lead to increases in cardiovascular endurance for everyone.

**Blood Viscosity – Cardiovascular System Relationship**

Junker et al.\(^9\) prepared a collective in which they analyzed the blood viscosity of 1142 male myocardial infarct patients and correlated it with the severity of the infarct. Blood was drawn four to six weeks post infarct and was analyzed for viscosity, fibrinogen, and cholesterol. The researchers found a positive correlation between the viscosity and the severity of coronary heart disease events even after adjusting for age, fibrinogen, and use of diuretics. The researchers decided that the results supported their hypothesis that plasma viscosity related positively to coronary heart disease. However, their study concerned only coronary heart disease patients, and no effort was made to link those results to the healthy population or to cardiovascular endurance.
CHAPTER III

METHODOLOGY

This investigation examined the ergogenic effects of varying acute l-arginine supplementation on VO$_2$ max. The research explored varying physiological rationale for any results by collecting blood platelets. 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. A health questionnaire was completed by prospective subjects, as well as a measurement of resting blood pressure using a stethoscope and manual sphygmomanometer.

The three subsequent visits consisted of a pre-treatment blood draw (PRE-TRE) upon arrival at the testing facility. After the pre-treatment blood draw, treatment was given 30 minutes prior to testing. Five minutes prior to testing, a second blood draw (PRE-EX) was then collected. Testing then occurred, which included a VO$_2$ max test that utilized a standard Bruce protocol on a treadmill (Quinton Q-Stress™ TM55, Quinton Cardiology Systems, Inc. Seattle, WA). Gas and ventilation analysis utilized a metabolic measurement system (Parvo Medics’ TrueOne® 2400, Parvo Medics. Sandy, UT.). Upon completion of the VO$_2$ max test, a third blood draw (POST-EX) was then taken one minute post test.
Subject Inclusion and Exclusion Criteria

After consent was 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 subject had to have a fat percentage of total mass not to exceed 30 percent.

Upon conclusion of BOD POD testing, a resting blood pressure was measured with a manual dial sphygmomanometer and stethoscope. Subjects had to have a resting blood pressure that was not greater than Stage One hypertensive levels as defined by the American College of Sports Medicine\(^2\). 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 that were free of disease (i.e., sickle cell anemia, thyroid disease, diabetes, heart disease). All subjects were free of any medication that might have altered their metabolic rate at rest or during exercise.

Subjects

Twenty-six male subjects between the ages of 18 and 25 years old were recruited to serve as subjects in this investigation. Each subject was provided with, and was required to read and sign an informed consent (APPENDIX A) in accordance with the guidelines set forth by The University of Akron Institutional Review Board. The protocol was 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 included running shoes, athletic shorts, and shirt. The subjects were also 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 arrival.

Preliminary Visit

During the first visit, each subject completed the Health History Questionnaire (APPENDIX D) and read and completed the approved informed consent form. The subject’s height and weight were 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 measured after a five minute period where the subject was resting in a seated position with standard bell stethoscope and manual dial sphygmomanometer.

Trials I, II, and III

When the subject arrived at The University of Akron Human Performance Laboratory, the first blood specimen (PRE-TRE) was taken according to standard blood draw procedures. The blood specimen was packaged for shipment to be analyzed for
complete blood count (CBC). The subject then received and ingested one of the three treatments.

After twenty-five minutes, a second blood specimen (PRE-EX) was taken according to standard blood draw procedures. The blood specimen was packaged for shipment to be analyzed for CBC. The subject was fitted with the appropriate mouthpiece for the metabolic cart.

Five minutes after the second blood draw, each subject performed a maximal exercise test on the treadmill according to the standard Bruce protocol. Upon completion of an achieved true VO\(_{2\text{max}}\) test, a third blood specimen (POST-EX) was attained and packaged for shipment to be analyzed for CBC. Figure 3.1 below shows a visual representation of the trial timeline.

![Figure 3.1 Trial timeline](image)

The following criteria needed to be met in order for the testing to be considered a “true” VO\(_{2\text{max}}\) test\(^2\). There were three items that needed to be completed. The first was a respiratory exchange ratio of at least 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 were not met, the test was deemed invalid and the data collected was not used in the final statistical analyses.

Blood Draws

For each blood draw, subjects were venipunctured with a vacuum-cannulus placed in the cephalic vein at the antecubital fossa according to standard blood draw procedures. A blood specimen was drawn by trained personnel. The specimen was packaged for shipment to be analyzed for CBC according to the instructions given by the blood testing facility.

Treatments

The treatments were administered to each subject for Trials I, II, and III, in a random-order, double-blind fashion. One of the treatments consisted of a three-gram (low) L-arginine supplement (LD-LA) dissolved in a solution of lemon juice and Crystal Light Lemonade ® and ingested completely.

Another treatment that each subject ingested at a different trial was that of a six-gram (high) dose of an L-arginine supplement (HD-LA), again to be ingested in the lemon juice and Crystal Light ® solution. Another treatment that each subject took was that of a placebo (PLA-D), in the form of corn starch dissolved the lemon juice and Crystal Light ® solution.
As previously mentioned the treatments were randomly assigned by a secondary investigator to each subject. The subject in each treatment and testing procedure was also blind to which treatment they received.

**Blood Analysis**

Blood analysis was done by a private blood testing facility (LabCare Plus ®). The blood specimens that were drawn at each stage were packaged in a biohazard-safe shipping bag and stored with ice. Upon pick-up by the lab each day, the blood was analyzed for CBC. The CBC allowed the determination of the blood platelet count during the time of each blood draw and therefore the changes in the count from one time to the next.

**Statistical Analysis**

Statistical analysis was conducted using SPSS 16.0 and included the use of a 3 \((treatment \ [placebo, \ three-gram \ L-arginine \ supplement, \ six-gram \ L-arginine \ supplement]) \times \ 3 \ (time)\) ANOVA, with repeated measures on time and treatment for those variables that were observed at pre-supplement, pre-exercise, and post-exercise. The level of statistical significance was fixed a priori at \(P \leq 0.05\).
CHAPTER IV
RESULTS

The purpose of this study was to evaluate the effects of oral L-arginine supplementation on blood platelet count and maximal oxygen consumption. Twenty-six subjects were recruited and all signed the Institutional Review Board approved Informed Consent form (APPENDIX A) and a Health History Questionnaire (APPENDIX D). Out of those twenty-six subjects, twenty-four completed all three trials (i.e. PLA-D, LD-LA, and HD-LA). The information compiled in this chapter included data from the twenty-four subjects that completed their trials.

The subjects’ characteristics are described in Table 4.1 below. Subjects were measured for height, weight, body composition via body plethysmography. The average measure for the subjects’ ages was 22.76 years old. The average measure for subjects’ heights and weight was 70 inches tall and 172.15 pounds, respectively. Also, the subjects’ average measure for body composition was 13.5 percent body fat.

Table 4.1 Subjects’ characteristics

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>AVERAGE OF MEASURES (N = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>22.76 years old</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>70 inches</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>172.15 pounds</td>
</tr>
<tr>
<td>BODY COMPOSITION</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

The information on Table 4.2 shows the mean platelet levels for each blood draw per dose. There was no significant relationship with P = .261 being greater than 0.05,
with $F = 1.429$. With no statistically significant relationship between dose and platelet count, neither the doses nor the placebo showed significant change to platelet levels compared to the other doses. This indicated that individual L-arginine doses had no significant effect on the platelet count of the subjects above what was already caused by the exercise itself.

Table 4.2 Mean platelet count per dose and time

<table>
<thead>
<tr>
<th>(Dose/Time)</th>
<th>Mean (Platelets)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA-D/PRE-TRE</td>
<td>248.33</td>
<td>42.269</td>
</tr>
<tr>
<td>PLA-D/PRE-EX</td>
<td>244.33</td>
<td>44.053</td>
</tr>
<tr>
<td>PLA-D/POST-EX</td>
<td>290.42</td>
<td>51.618</td>
</tr>
<tr>
<td>LD-LA/PRE-TRE</td>
<td>257.83</td>
<td>55.871</td>
</tr>
<tr>
<td>LD-LA/PRE-EX</td>
<td>250.79</td>
<td>54.500</td>
</tr>
<tr>
<td>LD-LA/POST-EX</td>
<td>296.46</td>
<td>64.245</td>
</tr>
<tr>
<td>HD-LA/PRE-TRE</td>
<td>253.38</td>
<td>57.901</td>
</tr>
<tr>
<td>HD-LA/PRE-EX</td>
<td>250.71</td>
<td>57.219</td>
</tr>
<tr>
<td>HD-LA/POST-EX</td>
<td>295.75</td>
<td>58.708</td>
</tr>
</tbody>
</table>

The information in Figure 4.1 shows the visual representation of the examination of the possible relationship of the dose of L-arginine with an effect in platelet count. The slopes of the graphed lines indicate any changes in the platelet count between times.
Figure 4.1 does, however, display a significant (p = .000, p < 0.05, with F = 1.066) relationship of time’s effect on platelet count. Changes in platelet count are found to be statistically significant with time but not with dose.

Figure 4.2 shows a statistically significant increase in maximal oxygen consumption when compared across doses supplemented. The investigation showed that to be significant at p = .035 which is less than 0.05, with F = 3.888. The differences between the values show the three-gram dose (LD-LA) to have significantly higher maximal oxygen consumption than the placebo dose (PLA-D). Also, the differences between the values show the six-gram dose (HD-LA) to have significantly higher oxygen consumption than the three-gram dose (LD-LA).
Figure 4.2 Maximal oxygen consumption over dose
CHAPTER V
DISCUSSION

Multiple factors affect maximal oxygen consumption. Oral L-arginine supplementation is one of those factors. This study, along with others before it, has proven a relationship between oral L-arginine supplementation and increased maximal oxygen consumption. However, L-arginine supplementation’s pathway to cause an increase in maximal oxygen consumption does not include an increase in platelet count. While there is a change in platelet count during exercise, the decrease that occurs does not show any relationship with oral L-arginine supplementation.

Platelet count increases were found in the subjects during this study. The increases were universal throughout the trials. These universal decreases depended only on the time at which the samples were taken, and had no significant relationship with the presence of L-arginine supplementation.

The research performed previously by Rector et al., Adams et al., and Giugliano et al. on humans, and the Tsao et al. research performed on animal subjects suggest that the platelet count should have increased at a greater level than it did simply from exercise in the placebo\textsuperscript{1,7,8,12,13}. However, the research in this area has focused on the resting and diseased populations with few actual studies investigating L-arginine’s effects on platelet physiology during exercise.
This study found that platelet count changes had no significant relationship with dosage of L-arginine, but platelet count is only an indirect measure for platelet aggregation. Further research should focus on measuring platelet aggregation directly with a platelet aggregometer. With a direct method of measuring platelet aggregation, the researchers could measure, more precisely, the effects and relationships between oral L-arginine supplementation and platelet aggregation. A more precise view into the relationship may show significance that platelet count alone could not.

This study did show an encouraging relationship between L-arginine, based on dosage, and increased maximal oxygen consumption. The values show that the six-gram dose caused significantly higher maximal oxygen consumption than the three-gram dose which had caused significantly higher maximal oxygen consumption than the placebo dose.

Nagaya et al.’s\textsuperscript{11} research did suggest that the L-arginine supplementation would improve maximal oxygen consumption. The previous research was performed, as is most of the research on L-arginine supplementation, on a diseased population, but those findings match the findings in this study.

L-arginine supplementation did not prove to significantly affect platelet aggregation, but the investigation into relationships between blood viscosity, platelet aggregation and maximal oxygen consumption should continue to be an important avenue of research for this field. Other areas of research should examine the other steps in the blood platelet aggregation cascade. There are many steps involved in the cascade and affecting only one could bring about the change in blood viscosity that shows significant improvement in maximal oxygen consumption. Similarly, there are other
things that affect blood viscosity, and investigating possible changes in those factors could prove successful in increasing maximal oxygen consumption.

The effects of L-arginine supplementation on maximal oxygen consumption do prove to be promising for athletes and recreational exercisers alike. Increases seen in maximal oxygen consumption can be attributed to oral supplementation of L-arginine. The nitric oxide pathway is a known cause for that increase. However, much more research is needed to investigate other possible reasons for the increases. The majority of research has focused on the increased delivery of oxygenated blood to working muscle tissue, but that is only a part of the full process determining the efficiency of oxygen usage in the body.

Research investigating changes in oxygen absorption rate into the blood stream may not show much promise considering the relatively short time blood becomes oxygenated on its route through the pulmonary blood vessels. Research into L-arginine supplementation’s effect on the ability of working muscle to absorb oxygen from the blood may be a more promising study. No research has been performed to investigate that possibility and therefore cannot be discounted.

More investigation should also focus on the possible chronic effects that have not been seen in such short examinations. None of the current research extends into long-term exploration. Short-term maximal oxygen consumption increases are very positive results that allow increased performance. L-arginine’s half-life is so short that it would not have a chronic effect on a person’s body, but repeated and continuous supplementation may affect a person’s vascular system. If there are chronic results due to extended L-arginine supplementation, the results may be good or bad, and so they should
be investigated. Positively, a person’s response time to exercise may be more efficient, and negatively that person’s blood vessels could remain chronically dilated resulting in blood pressure difficulties.

Future research could also investigate weight-specific dosage. This study only created static three- and six-gram doses. However, our subjects differed in body weights and the concentration of L-arginine would have been different in the small subjects compared to the large subjects. That concentration difference could be a reason that the platelet count was not significant. A set milligram dose of L-arginine per kilogram body weight would standardize the L-arginine concentration in blood and could more closely precisely impact platelet count.

Other areas of research may be inspired by the lack of effect oral L-arginine supplementation has on platelet aggregation. Since L-arginine supplementation does increase maximal oxygen consumption without significantly affecting platelet aggregation during exercise, people with blood-clotting disorders may be able to supplement with L-arginine safely. They could see positive results in exercise without worrying about the effect the supplementation may have on their blood’s ability to form clots. More research concentrating on L-arginine supplementation’s effect on blood needs to be done before that could become a reality.
REFERENCES


APPENDICES
APPENDIX A

INFORMED CONSENT

Title of Study: “Effect of Oral L-arginine Supplementation on Lactic Acid, Nitrate and Nitrite Levels, 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$_{2\text{max}}$ 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 B

HUMAN SUBJECTS APPROVAL

May 30, 2007

Matthew R. Feeback
408 3rd St. N.W.
Canton, Ohio 44709

Mr. Feeback:

The University of Akron’s Institutional Review Board for the Protection of Human Subjects (IRB) processed your application for the research project entitled: “Effect of Oral L-arginine Supplementation on Lactic Acid, Nitrate and Nitrite Levels, Platelet aggregation and Maximal Oxygen Consumption in Healthy Males”. At the May 9, 2007 convened meeting of the IRB, this protocol was approved contingent upon your compliance with IRB member’s recommendations for changes. Upon receipt of all requested changes, final approval was given on May 25, 2007. The IRB application number assigned to this project is 10070320.

Your research is now approved without further qualifications until May 9, 2008. Per federal guidelines, if you wish to continue the project beyond one year, you must submit a request for continuing review to the IRB. In addition, any changes in the original research protocol must be approved by the IRB prior to implementation.

Enclosed is a copy of the informed consent document that the IRB has approved for your use in this research. A copy of this document must be submitted with any application for the continuation of this project.

Please note that within one month of the expiration date of this approval, the IRB will forward an annual review reminder notice to you by email as a courtesy. Nevertheless, please note that it is your responsibility as principal investigator to remember the renewal date of your protocol’s review.

If your project terminates prior to the annual renewal date, please complete the Final Report form in order to complete your IRB file.

Please retain this letter for your files. If this research is being conducted for a master’s thesis or doctoral dissertation, you must file a copy of this letter with the thesis or dissertation. If you should have any questions, please do not hesitate to contact me.

Good luck with your research!

Sincerely,

Rosalie Hall
Ph.D.
Chair, Institutional Review Board

cc: Eric Corbett, Co PI
    Ronald Otterstatter, Advisor
APPENDIX C
CONTINUING REVIEW OF RESEARCH INVOLVING HUMAN SUBJECTS
APPROVAL

NOTICE OF APPROVAL

Date: April 22, 2008
To: Matthew Feeback
408 37th St NW
Canton, Ohio 44709

From: Sharon McWhorter, IRB Administrator

Re: IRB Number 20070326-2
"Effect of Oral L-Arginine Supplementation on Leptin, Insulin and Metabolic Rates,
Platelet Aggregation and Maximal Oxygen Consumption in Healthy Adults"

Thank you for submitting your Application for Continuing Review of Research Involving Human Subjects for the referenced project. Your protocol represents minimal risk to subjects and has been approved under Expedited Category #7.

Approval Date: April 22, 2008
Expiration Date: May 9, 2009
Continuation Application Due: April 24, 2009

In addition, the following is/are approved:
- Waiver of documentation of consent
- Waiver or alteration of consent
- Research involving children
- Research involving prisoners

Please adhere to the following IRB policies:
- IRB approval is given for not more than 12 months. If your project will be active for longer than one year, it is your responsibility to submit a continuation application prior to the expiration date. We request submission two weeks prior to expiration to insure sufficient time for review.
- A copy of the approved consent form must be submitted with any continuation application.
- If you plan to make any changes to the approved protocol you must submit a continuation application for change and it must be approved by the IRB before being implemented.
- Any adverse reactions/incidents must be reported immediately to the IRB.
- If this research is being conducted for a master's thesis or doctoral dissertation, you must file a copy of this letter with the thesis or dissertation.
- When your project terminates you must submit a Final Report Form in order to close your IRB file.

Additional information and all IRB forms can be accessed on the IRB web site at:
http://www.uakron.edu/research/oripap/compliance/IRBHome.php

☐ Approved consent form/s enclosed

Cc: Ronald Osterstetter - Advisor
Cc: Eric J. Gerbett - Co PI
Cc: Rosalie Hall - IRB Chair

Office of Research Services and Sponsored Programs
Akron, OH 44325-0101
330-972-7600 • 330-972-4281 Fax

The University of Akron is an Equal Education and Employment Institution.
HEALTH HISTORY QUESTIONNAIRE

The University of Akron

__________________

Human Performance Laboratory

HEALTH HISTORY
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
If you have been hospitalized in the past six months for an illness or operation, please complete the chart below. Do not include childhood tonsillectomy, or broken bones.

OPERATIONS OR ILLNESS
In the past six months have you been treated for any disease or chronic condition, even if presently not taking medication?  [  ] 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, etc.)
Check this box [  ] if you have not taken any medication.

MEDICATION
__________________________________________
__________________________________________
__________________________________________
__________________________________________
__________________________________________
__________________________________________

REASON
__________________________________________
__________________________________________
__________________________________________
__________________________________________
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

PROBLEMS AND SYMPTOMS, continued

Heart and Lungs
[     ] Heart attack
[     ] Coronary artery disease
[     ] High blood pressure
[     ] Rheumatic fever
[     ] Peripheral vascular disease
[     ] Blood clots, inflammation of veins (phlebitis)
[     ] 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
[ ] Hemophilia

G.I. TRACT

[ ] Eating disorder (e.g. anorexia, bulimia)
[ ] Hepatitis

Nervous System

[ ] Alcohol use
  If yes, how many drinks ingested per week? ________________
[ ] Stroke

Please list any weight-training products you have taken in the past week?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________