THE EFFECTS OF L-ARGININE SUPPLEMENTATION ON PRE AND POST-MAXIMAL EXERCISE IMMUNE RESPONSE

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THE EFFECTS OF L-ARGININE SUPPLEMENTATION ON PRE AND POST-MAXIMAL EXERCISE IMMUNE RESPONSE

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CHAPTER I
INTRODUCTION

Optimal immune function is crucial for the best possible performance in elite athletes and the weekend warrior alike. Research shows that sedentary people do not have as vigorous an immune system as those who habitually exercise. However, moderate exercise (for example, a moderate walking program undertaken by previously sedentary people) has been shown to drastically improve immune response. With regular exercise, the amount of immune cells circulating in the blood increases and the effectiveness of those cells improves (Nieman et al., 1999). There is also evidence that overdoing exercise may suppress the immune system (Nieman, 1994). High-intensity, or prolonged endurance exercise, steps up the output of many stress hormones, two of which are adrenaline and cortisol. These hormones are crucial to performance in that they elevate the heart rate, increase blood pressure, and enhance cellular metabolism. However, both can suppress various components of the immune system and have a negative effect on the athlete. Overtraining is when an athlete is training vigorously beyond the body’s ability to recover and performance deteriorates (Nimmo et al., 2009). The overtraining phenomena affects many athletes but can possibly be prevented with proper training, nutrition and dietary supplementation.
Tri-athletes, marathon runners, and other highly trained athletes alike often report that after intense competition and training, they are more susceptible to common illnesses. Such responses to extreme endurance competition and training can hinder the recovery of the athlete and his/her ability to continue training and to compete, otherwise known as “immunosuppression”. Exercise-related immunosuppression can be related to trauma suffered by bodily tissue during intense exercise bouts (Nimmo, 2009). It is important for anyone, regardless of athletic capabilities, not to allow such a phenomena to deter them from competing or exercisers from exercising. During stress (good or bad), the thymus gland typically shrinks, and the potential for sickness is great. However, L-arginine facilitates the maintenance of the gland's proper size and normal production of lymphocytes (Visek, 1986).

Cardiovascular endurance (CVE) is best measured by maximal oxygen uptake (VO₂max) (Malek et al., 2005). Maximal oxygen uptake is the greatest amount of oxygen (O₂) inspired, transported, and utilized during extreme physical effort. The VO₂max is measured during a graded exercise test (Bruce Protocol) while the subject is connected to a metabolic cart. The metabolic cart measures the amount of oxygen inspired from the ambient air versus the amount of carbon dioxide exhaled from the subject. The result is a measurement of how much oxygen can be transported to and utilized by the working muscles during physical activity shown in the percentage of oxygen compared to carbon dioxide present during exhalation.

L-arginine is a non-essential amino acid, which means it can be produced in our bodies from other amino acids provided from the diet. However, it is also considered a
conditionally essential amino acid in stressful situations (i.e. severe burns, injuries, infections, etc.). L-arginine is a key factor in many roles in the human body, including protein synthesis and detoxification of ammonia waste products, derived from nitrogen catabolism (Visek et al., 1986). L-arginine is also a precursor to the formation of nitric oxide (NO), creatine, and l-glutamine (McConnell et al., 2007).

It has been shown that l-arginine raises nitric oxide (NO) levels with supplementation (McConnell et al., 2007). Nitric oxide is a gas produced by a variety of cells in the body and is involved in many processes, both physiological and pathophysiological. Nitric oxide is generated from l-arginine by a reaction with the enzyme nitric oxide synthase, thus causing a vasodilatory effect. This vasodilation is a sought-after reaction, as it allows for increased blood flow to working muscles and tissue, thus supplying an abundance of oxygen and nutrient rich blood. Moderate doses of 5-20 grams are consumed orally to create such physiological responses (McConnell et al., 2007).

The purpose of our research is to investigate the use of L-arginine as an oral supplementation to aid in exercise recovery and immune system health. Past research has only been done on unhealthy or diseased populations, therefore not giving any opportunity to research the effect on a healthy population. The current study will provide a research base for l-arginine, not only as an ergogenic aid or training supplement but for further medical research. Furthermore, this study will add to the body of knowledge and increase awareness of possible l-arginine supplementation pre-exercise to increase immune function post-exercise.
The immune response we are looking at is the white blood cell count (WBC) and platelets that are in the blood stream in response to a stressor. Also known as leukocytes, white blood cells are the cells of the immune system defending the body against both infectious disease and other foreign materials. White blood cells are found throughout the body primarily in the blood and lymphatic system (Visek et al., 1986).

This study strives to fill in areas where previous research has lacked. This includes the reaction of WBC and platelets to both exercise and l-arginine supplementation in healthy male subjects. Results will be analyzed pre-and post-exercise and treatment will encompass the effects of all factors on the participants.
CHAPTER II
LITERATURE REVIEW

This chapter compiles some of the previous research and how it is relevant to the current study. Research was found in the realm of l-arginine and its ergogenic effects on the body as a dietary supplement. L-arginine is a common ingredient present in many of the popular dietary supplements on the market today. This literature review was performed to clarify the relationship between L-arginine and immune function following VO₂ max testing. To date there is no resolute theory that explains excessive exercise and altered immune response from overtraining.

McConnell et al. (2007) investigated the effects of l-arginine supplementation on exercise metabolism by the increased production of nitric oxide (NO). It is shown that l-arginine supplementation improves aerobic exercise capacity in various cardiovascular disease states, which are associated with endothelial dysfunction. The improvement in exercise capacity is most likely due to l-arginine increasing the production of NO in these individuals with reduced basal NO production. For healthy individuals with normal NO production, it appears that l-arginine administration has little impact on aerobic exercise capacity. However, little research has been conducted to examine the effect of l-arginine supplementation on exercise metabolism. There is some proof that l-arginine infusion
will increase glucose uptake during prolonged exercise and reduce lipolysis (McConnell et al., 2007). It is possible that these effects are due to increases in NO production. However, more research is required to confirm this. Further studies are required to research the potential ergogenic and therapeutic potential of L-arginine in conjunction with long-term, short-term, and high intensity bouts of exercise.

There have been several studies involving the relationship of exercise and risk for cardiovascular disease. A study by Singh et al. (2006) investigated the effect of exercise and training on platelet production. The research hypothesis for that study suggests that platelet characteristics of physically active men will be different than sedentary individuals in response to a progressive incremental exercise test on a cycle ergometer. Eight trained and eight sedentary 18-28 year old males volunteered to be in the study. Fasting blood samples were collected pre- and post- 1-hour cycling exercise at 70% of maximal aerobic power (VO$_{2\text{max}}$) before and after consumption of cocoa or placebo (Singh et al., 2006). The results from the study of Singh et al. (2006) found that trained subjects had higher baseline platelet counts than sedentary subjects and platelet count increased significantly in both groups post-exercise. Trained subjects showed significantly lower platelet activation than sedentary both pre- and post-exercise. Mean platelet volume did not change post-exercise and was not effected by training level. Total antioxidant concentration was lower in trained subjects, but the difference was not found to be significant.

The researchers also investigated how platelet levels change during exercise and with various training levels to better generalize the results to a larger section of the
population. They found a necessity for untrained individuals to utilize less strenuous exercise as they begin a program in order to keep less stress on the cardiovascular system.

The investigators displayed the need for more research in this subject, but could have utilized a more detailed design to address the issues of dosage and chronic usage of cocoa polyphenol to see if it would provide any benefits in the long run.

Pentinnen et al. (1998) studied the role that l-arginine plays in the regulation of blood pressure and autonomic nervous function during exercise. The amino acid l-arginine is a precursor for nitric oxide in the body. The creation of nitric oxide is known to help regulate the functional capacity of the circulatory system. Many positives have been found to come out of this relationship in that l-arginine supplementation can aid in preventing the development of atherosclerosis and help to dilate peripheral blood vessels, creating more room for circulation. This research has mainly been done in diseased populations, creating a need for studies to determine the effects in relatively healthy individuals. This article investigated some of the consequences of l-arginine levels on autonomous nervous function, blood pressure, and oxygen consumption. The sample consisted of 15 non-smoking healthy men age 22-38, using an incremental bicycle ergometer test to the limit of tolerance. Blood samples were then taken to determine l-arginine concentration in plasma using high performance liquid chromatography.

The study concluded that plasma l-arginine level decreased during physical exercise and the higher the level of plasma l-arginine at rest, the lower the systolic blood pressure at rest and with exercise. There was no such significant result for diastolic blood pressure. There was no significant relationship between plasma l-arginine levels at rest
and maximal oxygen consumption. Finally, the higher the plasma l-arginine levels at rest, the higher the variability of heart rate at rest.

There are several implications that can be drawn from their study. Plasma l-arginine level could account for the low amount of nitric oxide produced during exercise because of its similar decrease with physical stress. The finding that baseline l-arginine levels at rest relate to autonomic nervous system function is a novel finding in that few studies on it have been performed. There was no association between plasma l-arginine level and maximal oxygen consumption, suggesting that l-arginine may have only a minor role in fuel homeostasis during exercise. Also, the fitness level of subjects was found to have no effect on the blood pressure relation to l-arginine. Therefore, the blood pressure changes that occur with consistent physical activity may have very little to do with nitric oxide stores in trained individuals. L-arginine can help to lower blood pressure during exercise, but blood pressure also decreases itself during activity. Therefore, it may be useful although negligible to supplement l-arginine during fitness activity in order to help lower blood pressure while working out.
CHAPTER III
RESEARCH DESIGN AND METHODS

The current study explored the ergogenic effect of varied amounts of acute l-arginine supplementation on immune function in conjunction with VO$_2$ max. Immune responses to treatment were measured by white blood cell count (WBC) and platelet count pre-treatment, pre-exercise, and post-exercise. The study was reviewed and approved by The University of Akron Institutional Review Board.

Subjects

The current study comprised of twenty-five male subjects of at least 18 years of age, but younger than 30 years of age. 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 was reviewed and approved by The University of Akron Institutional Review Board.

Preliminary Visit

During the initial visit, the testing protocol was thoroughly explained to the prospective subject verbally. Following the explanation, an informed consent (APPENDIX A) was reviewed and signed by the participant, stating any risks involved with the testing. Subjects were also required to fill out a health history questionnaire (APPENDIX B) to filter out any subjects with possible risk factors. The subject’s height
and weight were measured and recorded using a stadiometer and scale. An air displacement plethysmography body composition assessment was administered to the subject (BOD POD® 2000A. Life Measurement Inc. Concord, CA).

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 testing equipment with appropriate equation. As part of the exclusion criteria, the body fat of each subject was not to exceed 30 percent of his total body mass.

Upon conclusion of body composition assessment, resting blood pressure was measured using a manual dial sphygmomanometer and stethoscope. Subjects were required to have a resting blood pressure no greater than 140/90 mm Hg, which 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, which in this study was among the exclusion criteria.

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

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 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, dietary supplements, or caffeinated drink was consumed for twelve hours previous to their arrival.

The three subsequent visits consisted of a pre-treatment blood draw upon their arrival at the testing facility. Treatment was then given after the pre-treatment blood draw in a double blind randomly assigned order. The treatment consisted of a cocktail that contained three ounces of lemon juice, five ounces of water, one packet of lemonade flavoring, and one of the three treatments, placebo, 3g or 6g. 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 VO_2max 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). True VO_2max test was determined by meeting the following criteria. There were three measurable items that needed to be completed for a successful VO_2max test. The first was a respiratory exchange ratio equal to 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 per 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 is a step-by-step representation of the trial. Upon completion of the VO_2max test, a third blood draw was
taken one minute post-test. Blood samples were later sent to LabCare Plus™ for a complete blood count (CBC) analysis to measure WBC and platelet count.

Figure 3.1 Trial Timeline

Statistical analysis of the data includes the use of an ANOVA. A 3 (time {pretreatment, pre-test and post-test}) x 3 (treatment {placebo, three g dose, six g dose}) with repeated measures on treatment and time for those variables that will be observed at presupplementation, pre-testing, and at the end of each maximal exercise. In possible 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-Geyser estimate. The statistical software package SPSS version 16.0
was used to complete all statistical calculations. The level of significance will be set a priori at (P < 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. Tables and graphs utilized in this document were produced using Microsoft Office Excel Mac 2008.
Chapter IV

Results

The primary goal of the current protocol was to examine the effects of variable dosage of l-arginine on WBC and platelet count before and after VO\textsubscript{2}\text{max} testing of healthy males. The amount of l-arginine was also examined to determine the effectiveness of supplementation per dose. The following graphs will outline how data was analyzed to draw conclusions to support or debate the original hypotheses.

As previously stated, 25 male volunteers were used for data collection; however, WBC and platelet counts were only available from 21 of the participants due to unforeseen circumstances.

The physical characteristics of the male subjects are described in Table 4.1. Measurements taken of the subjects included height, weight, and body composition via air displacement plethysmography. The average age for the included subjects was 22.7 (±2.9) years old. The average height and weight for the included subjects measured to be 70 (±3.5) inches tall and 172.15 (±27.5) pounds, respectively. Finally, the subjects’ average body composition measured to be an estimated 13.5 (± 5.8) percent body fat.

The primary research questions were proposed to determine if there was a positive response on WBC and platelet count as an immune response with varying treatment of l-arginine before and following VO\textsubscript{2}\text{max} testing as way to simulate a vigorous exercise.
Table 4.1 Subject Characteristics

<table>
<thead>
<tr>
<th>MEASUREMENT</th>
<th>AVERAGE OF MEASURES (N=24)</th>
<th>STANDARD DEVIATION (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>22.7 years old</td>
<td>2.9</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>70 inches</td>
<td>3.5</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>172.2 pounds</td>
<td>27.5</td>
</tr>
<tr>
<td>BODY COMPOSITION</td>
<td>13.5%</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Table 4.2 shows the mean WBC levels for each blood draw per dose. There was no statistically significant relationship between dose and WBC with (P= 0.142; F=0.158). With this information, neither the dose of 3g or 6g of l-arginine nor the placebo showed a significant alteration to WBC, thus indicating that no matter the dosage of l-arginine, there was no significant effect on WBC among the subjects. The exercise test itself did show a statistically significant difference over time during the testing. As shown in Table 4.2 and figure 4.1 there was a statistically significant increase in WBC regardless of dosage from pre-exercise to post-exercise testing (P=0.0001; F=92.450).

Table 4.2 Mean WBC Changes Over Time Per Dose

<table>
<thead>
<tr>
<th>MEAN WBC (K/uL) OVER TIME</th>
<th>DOSE</th>
<th>Pre-Treatment (+)</th>
<th>Pre-Exercise (+)</th>
<th>Post-Exercise (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>6.51 (1.92)</td>
<td>6.05 (1.73)</td>
<td>10.69 (2.28)</td>
</tr>
<tr>
<td></td>
<td>3g</td>
<td>6.33 (1.88)</td>
<td>5.96 (1.73)</td>
<td>10.60 (2.40)</td>
</tr>
<tr>
<td></td>
<td>6g</td>
<td>6.38 (1.72)</td>
<td>6.02 (1.68)</td>
<td>10.23 (2.53)</td>
</tr>
</tbody>
</table>

Mean platelet count for each blood draw per dose is shown in table 4.3 and figure 4.2. There was no statistically significant relationship between treatment dose and platelet count (P= 0.829;F=0.116) neither the dose of l-arginine nor the placebo showed a
significant alteration to platelet counts. As shown in Table 4.3 there was a statistically significant change of platelet count from pre-exercise to post-exercise testing (P=0.000; F=9.862). Concluding that regardless of dosage, platelet count increased post exercise.

Table 4.3 Mean Platelet Count Changes Over Time Per Dose

<table>
<thead>
<tr>
<th>DOSE</th>
<th>Pre-Treatment (±)</th>
<th>Pre-Exercise (±)</th>
<th>Post-Exercise (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>257 (59.60)</td>
<td>249 (57.14)</td>
<td>295 (66.39)</td>
</tr>
<tr>
<td>3g</td>
<td>253 (61.37)</td>
<td>251 (60.80)</td>
<td>295 (63.66)</td>
</tr>
<tr>
<td>6g</td>
<td>247 (45.05)</td>
<td>242 (46.08)</td>
<td>288 (54.25)</td>
</tr>
</tbody>
</table>

The findings in Figure 4.1 indicate the relationship that time has on the increase in WBC throughout the testing. The WBC decreases slightly from pre-supplementation to post-supplementation/pre-exercise then dramatically increases post-exercise, concluding that exercise alone provides a significant immune response regardless of supplementation.
The information in Figure 4.2 indicates the relationship that time has on the increase in platelet count throughout the testing. As shown the platelet count drops slightly from pre-supplementation to post-supplementation/pre-exercise then dramatically increases post-exercise, concluding that exercise alone provides a significant response in platelet count regardless of supplementation.
The information in Figure 4.3 shows a visual representation of a significant increase in WBC from pre-supplementation to post-exercise. The star on the graph represents that (P > 0.05), which denotes that there is a significant relationship between exercise and WBC. The slopes of the graph indicate the increase of WBC from the beginning (pre-supplementation) to the end of the test (post-exercise) regardless of supplementation.
The information in Figure 4.4 shows a visual representation of a significant increase in platelet count from pre-supplementation to post-exercise. The star on the graph represents that \( P > 0.05 \), which denotes that there is a significant relationship between exercise and platelet count. The slopes of the graph indicate the increase of platelet count from the beginning (pre-supplementation) to the end of the test (post-exercise) regardless of supplementation.
Figure 4.4 Platelet Changes From Pre-Treatment To Post Exercise

PLATELET CHANGES OVER TIME

Mean

(P < 0.05)
CHAPTER V
DISCUSSION

The purpose of this study was to determine the effects of l-arginine supplementation on pre and post-maximal exercise immune response. Using a treatment of l-arginine (3g, 6g, and placebo), the current study examined the effects on WBC and platelets pre-supplementation, post-supplementation, and post-exercise.

It is abundantly clear that regular exercise has multiple health benefits to those who commit to a moderate and high intensity exercise program. Such benefits include: a decrease in body weight, body composition, Body Mass Index (BMI), and lowering the risk of Type II Diabetes, high blood pressure, and many other cardiovascular risk factors. Exercise has also been confirmed to improve ones mood, build and maintain healthy muscles, bones, joints, and as stated, improve immunity, which will in turn protect against illness and disease. (Nieman et al., 1999)

The overtraining phenomenon is when an athlete is training vigorously beyond the body’s ability to recover, and performance subsequently deteriorates. The overtraining phenomena affects many athletes but can possibly be prevented with proper training, nutrition, and dietary supplementation. (Nimmo et al., 2009) This is where the multi-billion dollar dietary supplement industry comes in to play. The use of supplements containing the amino acid 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.

Other studies have examined how performance would possibly be improved with the use of such ergogenic aids. The vasodilatory effect makes this a popular supplement in the weightlifting and bodybuilding world, where muscular strength and endurance is paramount.

The current study found that l-arginine had no significant relationship with WBC and platelet count, regardless of dosage. The study did, however, show a definitive relationship between time and both the WBC and platelet count, thus indicating and proving that exercise does evoke an immune response. A platelet count increase during exercise regardless of l-arginine dosage as in our study and antioxidant dosage as in the study by Singh et al. (2006) provides strong evidence that exercise alone is responsible for the increased platelet count. The current study complements Singh et al. (2006), showing that there is a positive indication that regular exercise can have an immune boosting effect on those who follow a regular moderate intensity exercise program. In addition to platelet count the WBC increased from pre-supplementation to post-exercise in this study, again showing that exercise has a positive effect on immune response.

The findings in the current study show that it is actually the exercise itself that causes the increase WBC and platelet count. Research by McConnell et al. (2007) suggests that l-arginine supplementation can improve aerobic exercise capacity by the increase in nitric oxide production in diseased populations. Such findings could indicate
that it was the combination of the exercise that the subjects were performing in addition to the l-arginine that was responsible for the increase in NO production and aerobic capacity. More research can be done to observe the changes between sedentary individuals who supplement with l-arginine, those who participate in regular exercise and those who do both.

More investigation should be carried out to examine the chronic effects of l-arginine supplementation and the benefits that may be attained with prolonged daily use. The research performed by Petinnen et al. (1998) and McConnell et al. (2007), only examined the effects of acute usage of l-arginine whereas chronic usage may allow a build up of l-arginine in the system providing benefits that may not be attained from acute dosage. Weight specific dosage could also be considered in future studies. Our subjects all differed in body weights and the concentration of L-arginine would have been different in the smaller subjects compared to the large subjects. Such concentration difference per subject could be a reason that the WBC and platelet count was not significant with the dosage.


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. For the blood draw, trained and experienced personnel will perform venipuncture. 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 VO2max 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 VO2max 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

HEALTH HISTORY QUESTIONNAIRE

The University of Akron Code # __________________

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?
___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
___________________________________________________________________________
May 30, 2007

Matthew R. Feeback
408 37th 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, Nitrite and Nitrate 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 members' recommendations for change. Upon receipt of all requested changes, final approval was given on May 25, 2007. The IRB application number assigned to this project is 20070320.

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 protocol.

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 Otters, Advisor