The Influence of L-arginine on Vascular Endothelial Function at Rest and During Sympathetic Activation

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Andrew Wells

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DEDICATION PAGE

I dedicate this thesis to my family, mentors, peers and students for all their support in making this work a reality.

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ABSTRACT

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Endothelial dysfunction, arterial stiffness and hypertension increase the risk of cardiovascular disease. Stiff arteries are a consequence of chronic systolic hypertension and atherosclerosis. The resulting increase in peripheral resistance due to a narrow arterial lumen increases the rate pressure product and demand on the heart. One way to reduce the demand on the heart is to lower peripheral resistance by vasodilating the peripheral vessels. L-arginine is an endothelial substrate used to produce nitric oxide, a potent vasodilator. Vasodilation increases arterial lumen diameter through smooth muscle cell relaxation, improving arterial elasticity, blood flow and lowering blood pressure. Slower pulse wave transit times in the arterial tree indicate arterial elasticity decreasing demand on the heart and decreasing risk for cardiovascular disease. While L-arginine may stimulate nitric oxide production and reduce peripheral resistance, no known studies have investigated L-arginine supplementation on arterial stiffness in healthy individuals. In order to establish the effects oral L-arginine supplementation on pulse wave transit

times, this study hypothesizes (1) L-arginine will decrease peripheral arterial stiffness in young healthy subjects, (2) L-arginine will decrease peripheral arterial stiffness in active tissue, and (3) L-arginine will decrease peripheral arterial stiffness in inactive tissue. The hypotheses tested acute L-arginine supplementation during three different conditions: rest, reactive hyperemia and ischemic handgrip exercise. Fifteen subjects were recruited (N=15), and visited the laboratory on two separate days: the first day without L-arginine supplementation and the second day, a dosage of 70 mg per kg lean body mass was given as a water L-arginine solution. No differences were found comparing the three conditions with L-arginine supplementation. This study concludes L-arginine does not decrease peripheral arterial stiffness in young healthy subjects and does not decrease peripheral arterial stiffness with active and inactive tissue.

CHAPTER I

INTRODUCTION

Background

Cardiovascular disease kills 17.3 million per year, and hypertension is a leading risk factor of the disease. Last year, 970 million cases of hypertension were diagnosed worldwide. Hypertension is linked to endothelial dysfunction, atherosclerosis, heart attack, cognitive impairment, impotence and renal failure (14, 21, 54). In an aging population endothelial function is an important factor in managing vascular peripheral resistance that influences blood pressure at rest and during exercise. If the vascular endothelium does not respond properly to stimuli such as shear stress or metabolites, the resulting impairment of vascular function provided hypertension is observed increases aortic impedance and afterload on the heart (10, 12, 38, 53). This increased afterload can lead to left ventricular hypertrophy, coronary heart disease, and congestive heart failure (10, 25, 37). Numerous drugs can treat hypertension by inhibiting vasoconstriction and activating pathways for vasodilation, with the intention to improve aortic elasticity and decrease afterload on the heart. Improving arterial stiffness indicates greater arterial compliance, arterial recoil and circulation that reduce the work load of the heart.

However, changes in cardiac dimensions such as increased left ventricular mass also occur in athletic populations (4, 5). As a result, changes in blood pressure from either exercise or hypertension facilitates adaptation to cardiac muscle. This provides a

contradiction between physiologic and pathologic cardiac adaptation as adaptations appear to be similar creating difficulty in diagnosis (29).

A possible explanation, prompts the use of a cardiac adaption model that can express cardiac hypertrophy through concentric or eccentric activity (32). The concentric model examines resistance training, but is relatable to hypertensive individuals at rest provided with increased peripheral resistance (4, 32). Where the eccentric model examines active individuals during exercise with increased venous return to the heart through a muscle pump effect.

In concentric hypertrophy the heart overcomes the increase in peripheral resistance by increasing contractility of cardiac muscle with no change in venous return. Conversely, in eccentric hypertrophy the stimulus is provided by an increase in venous return to the heart from exercise.

It may be argued that hypertensives at rest have difficulty maintaining circulation, while normotensives during exercise facilitate circulation because of increased venous return. However, in either case the assumption that regulation of peripheral resistance is the stimulus for cardiac adaptation. Evidence for this argument is when cardiac output is controlled, modulation of peripheral resistance maintains blood pressure and circulation as the body is placed in different positions (48).

Furthermore, arterial smooth muscle cell tone at rest determines peripheral resistance by local tissue, hormone and neural control. These pathways influence endothelial nitric oxide production, a power vasodilator, providing a decrease in

peripheral resistance. The semi-essential amino acid L-arginine, a known substrate for Nitric Oxide (NO) production may provide a decrease in peripheral resistance through arterial smooth muscle cell relaxation, increasing arterial elasticity. L-arginine has shown promise in diseased animal and human populations to help reduce peripheral resistance and reduce the risk of a cardiovascular disease (51). However, in moderately active normotensive individuals that don't fit the concentric cardiac adaptation model, L-arginine supplementation may and provide the benefit of improved blood flow at rest, without cardiac adaptations provided by hypertension.

Improved blood flow provided by arterial elasticity can be assessed by measuring arterial stiffness quantified by the velocity of the pulse waves generated from ventricular contraction. If peripheral resistance is high provided in hypertensives, arterial smooth muscle cell tension will provides a medium for pulse waves to travel faster, where elastic arteries a reduction in smooth muscle cell tension will provide a medium for pulse waves to travel slower throughout the arterial tree (24, 35, 38, 45) In a pathophysiological model an increase in pulse wave velocities (PWV) are reported to be independent predictors of cardiovascular mortality (21, 40, 44). If L-arginine reduces PWV at rest in a moderately active population, it may provide evidence for improved modulation of peripheral resistance and improved blood pressure control lowering the risk of cardiovascular disease.

Statement of the Problem

Hypertension is linked to cardiovascular disease in an aging population. Healthy cardiovascular aging starts in young adulthood, where the risk of hypertension may be reduced by supplementing L-arginine. If arterial stiffness is reduced in a young healthy population with acute supplementation, chronic supplementation may reduce the risk of hypertension in an aging population.

Statement of Purpose

The purpose of this study was to establish the effects of oral L-arginine supplementation on arterial stiffness at rest and in active and inactive tissues during sympathetic activation. This study hypothesized that (1) Acute L-arginine supplementation will decrease peripheral arterial stiffness in young healthy subjects, (2) Acute L-arginine supplementation will decrease peripheral arterial stiffness in active tissue, and (3) Acute L-arginine supplementation will decrease peripheral arterial stiffness in inactive tissue.

Theoretical bases and organization

The amino acid L-arginine promotes vasorelaxation. Acute L-arginine supplementation has shown promise as an endothelial-dependent vasodilator, which produces nitric oxide catalyzed by the enzyme endothelial nitric oxide synthase (eNOS). Since L-arginine is an endothelial-dependent vasodilator, it requires hormonal, neural or mechanical action for nitric oxide production via eNOS. At rest, L-arginine may enhance

arterial compliance, which reduces the workload on the heart and improves circulation. Vasorelaxation reduces afterload on the heart and lowers the risk of vascular disease (39, 51).

Limitations

1. The diets were not controlled prior to measurements.
2. Physical activity history was self-reported.
3. The study used an indirect measure of endothelial function
4. Age
5. Sex; only male subjects
6. Sample size, N=15
7. Relative dosage 70mg/kg lean body mass
8. Oral administration of L-arginine, and no blood plasma levels of L-arginine were not measured

Delimitations

1. The results are delimited to healthy, men with no known cardiovascular disease.
2. The results are delimited to young adults less than 40 years of age.
3. The results are delimited to L-arginine-naïve subjects, to avoid possible effects of adaptation to the supplement.

Definition of Terms

Arterial stiffness - is a general term for the elasticity (or compliance) of the arteries. The stiffness of arteries influences cardiac afterload.

Carotid-radial pulse wave velocity (CRPWV)- a measure of arterial stiffness between the carotid and radial artery.

L-arginine - a semi-essential amino acid involved in protein synthesis and a substrate for endothelial nitric oxide synthase.

Nitric oxide (NO) - Nitric oxide is a potent vasodilator produced mainly in the vascular endothelium.

Endothelial nitric oxide synthase (eNOS)- are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule. It helps modulate vascular tone, insulin secretion, airway tone, and peristalsis, and is involved in angiogenesis and neural development.

Pulse wave velocity (PWV) - a measure of arterial stiffness

CHAPTER II

REVIEW OF LITERATURE

Background

Cardiovascular disease kills 17.3 million people per year. Each year, more than 970 million cases of hypertension are diagnosed worldwide, which is linked to endothelial dysfunction, atherosclerosis, myocardial infarction, cognitive impairment, impotence and renal failure (14, 21, 22, 25, 54). Endothelial dysfunction contributes to hypertension. If the vascular endothelium does not respond properly to stimuli such as shear stress or metabolites, the resulting impairment of vasomotion is associated with increases in aortic impedance and afterload on the heart (12, 53). This increased afterload can lead to left ventricular hypertrophy, coronary heart disease, and congestive heart failure (10, 19, 25, 37). Numerous drugs treat chronic hypertension by inhibiting vasoconstriction and activating pathways for vasodilation that improves aortic elasticity and decreases cardiac afterload. In treating chronic hypertension, reducing cardiac afterload lowers cardiovascular risk.

Arterial Compliance

The inhibition of vasoconstriction and the activation of vasodilation improves arterial compliance through smooth muscle cell relaxation, which increases the volume of blood in the arteries.

$$\text{Arterial compliance} = \Delta\text{Volume}/\Delta\text{Pressure}$$

Non-compliant arteries or stiff arteries reflect a decrease in blood volume and an increase in pressure exerted on the arterial wall. If pressure exerted on the arterial wall did not increase this would alter arterial blood flow as illustrated by Poiseuille's law.

$$\text{Flow rate} = [\pi*(\text{Pressure})*(\text{Radius})^4]/[8(\text{Viscosity})*(\text{length of tubing})]$$

Following this principle, a decrease in blood volume requires a decrease in arterial diameter in order to maintain blood flow to metabolic tissues at rest. The decrease in arterial diameter increases intra-arterial blood pressure to maintain perfusion (40). As arterial blood pressure (BP) is a function of cardiac output (CO) and total peripheral resistance (TPR).

$$BP = CO*TPR$$

If arterial blood pressure is to increase at rest, cardiac output as a function of heart rate and stroke volume remains constant. The increase in peripheral resistance provides a decrease in arterial diameter, which is attributed to smooth muscle cell contraction, placing an additional force on the heart. This additional force is overcome by increasing the force of ventricular contractions resulting in hypertension. Chronic hypertension at rest results in ventricular hypertrophy, which eventually reduces stroke volume as the ventricular volume is reduced by concentric cardiac hypertrophy and leads to congestive heart failure.

Sympathetic neural control, hormones, and local tissues regulate blood pressure. In arteries producing high peripheral resistance, moderators such as norepinephrine and

angiotensin actively signal smooth muscle cell contraction to produce high intravascular pressures. Smooth muscle cells also contract when stretched rapidly on their own, termed myogenic autoregulation. As previously indicated by Poiseuille's law, a small change in radius requires a large change in pressure to maintain adequate circulation. Conversely, this produces high wall tension in people with hypertension, as illustrated by the Law of Laplace.

$$\text{Wall Tension} = (\text{Pressure} * \text{Radius})/2$$

High wall tension produces stiff arteries. An augmented pulse wave is sent through the arterial tree with each cardiac cycle. Pulse wave velocity (PWV) can indicate arterial stiffness (21, 24, 46).

$$\text{Pulse Wave Velocity} = \sqrt{Tension/(\frac{mass}{length})}$$

PWV is a measure of intrinsic arterial wall elasticity dependent upon pressure exerted on the arterial wall. In hypertensives, arterial remodeling such as atherosclerosis increases mass and pressure due to loss of arterial diameter. The increase in wall tension and PWV is the result of organic physical property change in the arterial wall. Another example of organic change is the loss of elastin, which is replaced with less compliant collagen fibers, increasing arterial stiffness. A loss in the ability to control vascular tone that involves a deficiency in sympathetic and parasympathetic neural stimulation, hormonal dysregulation and myogenic autoregulation is labeled as a loss of physiological function (45).

The loss of the ability to control vascular tone can be observed not only in hypertensives but also in those with orthostatic hypotension. In treating deficient vascular control, numerous drugs can inhibit and produce vasoconstriction or vasodilation. In hypertensives, vasoconstriction can be produced by overproduction of norepinephrine or angiotensin.

Myogenic autoregulation is dependent upon smooth muscle cell stretch accelerations that are produced with high intra-arterial pressures. This mechanism maintains high vascular tone and “locks” smooth muscle cell contraction to prevent rapid dilation accompanied by possible tissue damage. A moderator for this mechanism is nitric oxide (NO), a potent vasodilator. NO production with high shear stress placed on the endothelium, lowers intracellular calcium levels, producing smooth muscle cell relaxation and allowing an increase in arterial diameter.

L-arginine is a substrate utilized by endothelial nitric oxide synthase (eNOS) to produce NO, which decreases the vasoconstrictive properties at the local tissue level. Acute supplementation of L-arginine has shown promise as an endothelial-dependent vasodilator in both human and animals (8, 28, 39). In the deficient regulation of vascular control in hypertensives, L-arginine may help alleviate high blood pressure and arterial stiffness. The reduction in high blood pressure would enhance perfusion to ischemic tissues when increased pressure can no longer compensate for the loss of arterial diameter. However, this implies that hypertensives have compromised circulation.

Under hypoxic conditions vasodilation occurs via metabolites producing a chemoreflex, which inhibits sympathetic nerve pathways to attenuate vasoconstriction (20, 27, 40). If blood flow did not return in excess post-ischemia, the reduction in perfusion during recovery may be a measure of vascular control and poor endothelial health.

This is tested, comparing normotensives to hypertensives, the physiological response to post-ischemic conditions provided by the removal of an artificial occlusion. The removal of the artificial occlusion produces an exaggerated reperfusion response called reactive hyperemia. In reactive hyperemia trials, hypertensives demonstrated no immediate change in arterial stiffness, whereas normotensives demonstrated arterial elasticity (12, 20). Under these circumstances, hypertensives exhibit poor vascular control, as perfusion remains compromised due to no change in arterial diameter. It may be that supplementation of L-arginine, as an endothelial-dependent vasodilator contributes to improvement in vascular tone control in both hypertensive and normotensive individuals.

CHAPTER III

METHODOLOGY

Sample Population

The study was submitted to and approved by the Institutional Review Board, California State University, Chico. A total of 15 subjects were studied. Inclusion criteria were: 1) males under 40 years of age, and 2) moderately physically active as classified by the Paffenberger physical activity questionnaire (36). All subjects were normotensive, did not smoke within the previous 6 months, non-diabetic, and had no history of heart disease, blood clotting or recent infection or illness.

Control Day

Subjects were asked to fast 8-hours overnight before testing. Testing began at 0700 AM. Subjects were asked to rest for 5 minutes in a seated position. Resting heart rate was obtained, followed by resting blood pressure using manual sphygmomanometery of the brachial artery. Body composition was then assessed by air plethysmography (COSMED Concord, CA). Subjects then rested for 20 minutes in the supine position and resting arterial stiffness (Millar SPT-301) was obtained, followed by reactive hyperemia. Finally, after another 20-minute rest, the subject performed an ischemic handgrip exercise.

Treatment Day

Subjects were asked to fast 8-hours overnight before testing. Testing began at 0700 AM. Subjects were asked to rest for 5 minutes in a seated position. Resting heart rate was obtained, followed by resting blood pressure using manual sphygmomanometery of the brachial artery. Subjects ingested 70 mg/kg of lean body mass of L-arginine 40 minutes prior to assessment of resting arterial stiffness. Subjects rested in a supine position for 20 minutes prior to taking a resting arterial stiffness measurement, followed by reactive hyperemia and finally ischemic handgrip exercise.

Pulse wave velocity measurements

All Arterial stiffness measurements were taken on the non-dominant arm, from carotid and radial pulse sites. Arterial stiffness data was collected for 1 minute immediately after the subject completed each condition. Probe displacement was calculated by the difference from the suprasternal notch to the radial and carotid sites (24, 46).

Reactive hyperemia protocol

A blood pressure cuff was placed on the subjects non-dominate arm and inflated to supra-systolic levels (220 mmHg) for five minutes (24). Immediately after the pressure in the cuff was released, arterial stiffness was assessed by probe measurements at the carotid and radial sites.

Ischemic handgrip exercise

Prior to ischemic handgrip exercise, a max voluntary handgrip test was performed. After a blood pressure cuff was placed on the dominant arm and inflated to supra-systolic levels (220 mmHg) for two minutes. During that time the subject engaged in 40% max voluntary muscle contractions every two seconds for 2 minutes to establish sympathetic activation (13). A metronome was provided to assist the subject in timing muscle contractions and visual feedback provided by either a computer digital display or numerical digital display. Immediately after the pressure in the cuff was released, arterial stiffness was assessed by probe measurements at the carotid and radial sites.

Data Analysis

SPSS version 22 was used to calculate a two-way repeated measures ANOVA was used to test for main and interactions effects and a one-way repeated measures ANOVA was used to establish differences within testing days. SPSS version 22 was used for Paired-T tests and Vassarstats was used for Wilcoxon rank sign tests, used to establish differences within conditions comparing control with L-arginine. Paired-T tests were used to establish baseline characteristics between testing days.

CHAPTER IV

ABSTRACT

Endothelial dysfunction, arterial stiffness and hypertension increase the risk of cardiovascular disease. Stiff arteries, particularly in the face of coronary artery disease, characterize systolic hypertension. This increases peripheral resistance due to a narrow arterial lumen, which increases the rate pressure product, the energy demand on the heart, and myocardial oxygen consumption. One way to alleviate increased demand on the heart is to lower peripheral resistance by vasodilating the peripheral vessels. L-arginine is an endothelial substrate used to produce nitric oxide, a potent vasodilator. Vasodilation increases arterial lumen diameter through smooth muscle cell relaxation, improving arterial elasticity, blood flow and lowering blood pressure. Reducing pulse wave transit times in the arterial tree suggests increased arterial elasticity, decreased demand on the heart, and a reduced risk for cardiovascular disease. L-arginine may be indicated as a treatment to lower blood pressure in hypertensive to reduce risk of a cardiovascular event, but no known studies have investigated L-arginine supplementation on arterial stiffness in healthy individuals. In order to establish the effects oral L-arginine supplementation on pulse wave transit times, this study hypothesized that 1) The effect of L-arginine will decrease peripheral arterial stiffness in young healthy subjects at rest, 2) the effect of L-arginine will decrease peripheral arterial stiffness in active tissue, and 3) the effect of L-arginine will decrease peripheral arterial stiffness in inactive tissue. The

hypotheses tested acute L-arginine supplementation during three different conditions: rest, reactive hyperemia and ischemic handgrip exercise. Fifteen subjects were recruited (N=15), and visited the laboratory on two separate days. The first day the subjects did not take L-arginine supplementation. The second day, the subjects were given an L-arginine solution in a dosage of 70mg per kg lean body mass. There were no differences between the three conditions in arterial stiffness. This study concluded that L-arginine does not decrease peripheral arterial stiffness in young healthy subjects, and does not decrease peripheral arterial stiffness with active and inactive tissue.

Introduction

Cardiovascular disease kills 17.3 million per year with the presence of 970 million diagnoses of hypertension worldwide, which is linked to endothelial dysfunction that promotes atherosclerosis, heart attack, cognitive impairment, impotence and renal failure (14, 21-23, 47). One mechanism that has been identified to contribute to hypertension is endothelial function. If the vascular endothelium does not respond properly to stimuli such as shear stress or metabolites, the resulting impairment of vasomotion is associated with increases in aortic impedance and afterload on the heart (12, 53). This increased afterload can lead to left ventricular hypertrophy, coronary heart disease and congestive heart failure (10, 25). Numerous drugs can be employed to treat hypertension by inhibiting vasoconstriction and activating pathways for vasodilation, with the intention to improve aortic elasticity and decrease afterload on the heart. Improving arterial stiffness allows for greater arterial compliance, arterial recoil and

circulation reduces the work of the heart. Arterial stiffness can be quantified by measuring the velocity of the pulse waves generated from ventricular contractions. These pulse wave velocities (PWV) are independent predictors of cardiovascular mortality (40, 44).

One substance that has been identified as a substance that promotes vasorelaxation is the amino acid L-arginine. Acute L-arginine supplementation has shown promise as an endothelial-dependent vasodilator, which produces nitric oxide using the enzyme endothelial nitric oxide synthase (eNOS). Since L-arginine is an endothelial-dependent vasodilator, it requires hormonal, neural or mechanical action for nitric oxide production via eNOS. At rest, L-arginine may enhance arterial compliance, which reduces the workload on the heart and improves circulation. This vasorelaxation reduces afterload on the heart and lowers the risk of vascular disease (3, 17, 34, 39, 51).

However contradictions still exist in the literature regarding L-arginine supplementation as potentially either beneficial or harmful. Studies have demonstrated that L-arginine can act as an ergogenic aid, improving time to exhaustion and blood flow (2, 3, 52). In contrast a study has shown harmful effects of L-arginine supplementation, increasing intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecules (VCAM-1), upregulating the enzyme arginase contributing to arterial stiffness or no effect in improving prognosis post myocardial infraction or peripheral arterial disease (41, 50, 51). This is to include that other studies have also shown no change in NO production with L-arginine supplementation, or improved muscle performance (1, 49). It

may be argued that as a semi-essential amino acid received from food, that there may exist in context a physiological response, trained versus untrained healthy adults with l-arginine supplementation and a pathological response to l-arginine supplementation by a mechanism that may be currently unknown.

Another item of interest regarding vascular disease involves the physiological response to post-ischemic conditions. During an ischemic event, with a blockage present an effort is made by the vascular system to shunt blood to the hypoxic area. After the removal of the blockage, blood flow to the area temporarily increases. Under hypoxic conditions vasodilation occurs via metabolites producing a chemoreflex inhibiting sympathetic nerve pathways to attenuate vasoconstriction (20, 27, 40). This phenomenon is called reactive hyperemia. If blood flow did not return in excess post-ischemia, the reduction in perfusion during recovery may be the result of poor endothelial health. During reactive hyperemia, hypertensives experience no change in arterial elasticity, while it increases in people with normal blood pressure (24, 40). L-arginine may further improve arterial elasticity in normotensive individuals, as an endothelial-dependent vasodilator.

During exercise, L-arginine is popularly used as an ergogenic aid to increase blood flow to active muscles. At the onset of exercise, a resetting of the baroreflex increases sympathetic activity to defend a higher blood pressure (40, 42). Locally, active tissues override this signal via functional sympatholysis, which inhibits adrenergic mediated vasoconstriction in active limbs. Functional sympatholysis inhibits sympathetic

adrenergic mediated vasoconstriction as active tissues have lower pH, increased temperature, potassium, and adenosine concentrations. Increased potassium levels effectively block nerve transmission by changing the electrochemical gradient of the nerve during depolarization. A change in pH and temperature in active tissues could change binding affinity of alpha-adrenergic receptor sites, where increased adenosine concentrations compete with norepinephrine, inhibiting vasoconstriction. An alternative circumstance involve observations in human skin circulatory capacitance with varied environmental temperatures (40). L-arginine could potentiate this inhibition of myogenic vasoconstriction by acting as a substrate that produces nitric oxide, which enhances vasodilation in active tissues. In turn, this increased blood flow could improve the response to metabolic demand of active tissues and improve aerobic exercise performance.

Delivery of blood flow to working muscles during exercise is a careful balance of vasodilation of active tissue and vasoconstriction of inactive tissue. A systemic response to exercise includes both baroreflex resetting and generation of metabolites that facilitates vasodilation via functional sympatholysis. Conversely, vasoconstriction is vital in inactive tissues to shunt blood toward metabolically active tissues. If L-arginine vasodilates inactive tissues during exercise, then circulation could fail to meet oxygen demand in active tissues. This failure limits oxygen uptake, and maximal capacity for aerobic performance (6, 15, 26).

Therefore the purpose of this study is to establish the effects of oral L-arginine supplementation on arterial stiffness at rest and in active and inactive tissues during sympathetic activation. This study hypothesizes 1) Acute L-arginine supplementation will decrease peripheral arterial stiffness in young healthy subjects at rest, 2) Acute L-arginine supplementation will decrease peripheral arterial stiffness in active tissue, and 3) Acute L-arginine supplementation will decrease peripheral arterial stiffness in inactive tissue.

Methods

The study was submitted to and approved by the Institutional Review Board, California State University, Chico. Participants: a total of 15 subjects were studied. Inclusion criteria were: 1) males under 40 years of age, and 2) moderately physically active as classified by Paffenberger physical activity questionnaire (36). All subjects were normotensive, did not smoke within the previous 6 months, non-diabetic, and did not have any history of heart disease, blood clotting or recent infection or illness. Subjects were asked to perform an 8-hour overnight fast before testing on each day. All testing began at 0700 AM. Day one: Subjects were asked to rest for 5 minutes in a seated position. Resting heart rate was obtained, followed by resting blood pressure using manual sphygmomanometery of the brachial artery. Body composition was then assessed by air plethysmography (COSMED Concord, CA). Subjects then rested for 20 minutes in the supine position and resting arterial stiffness (Millar SPT-301) was obtained, followed by reactive hyperemia. Finally after another 20 minute rest the subject performed an ischemic handgrip exercise. All arterial stiffness measurements were taken on the non-

dominant arm, from carotid and radial pulse sites. Arterial stiffness data was collected for 1 minute immediately after the subject completed each condition. Day two: Subjects ingested 70 mg/kg of lean body mass of L-arginine. During the 40 minutes prior to assessment of resting arterial stiffness, 20 minutes after ingestion the subjects, rested in a supine position for 20 minutes prior to taking a resting arterial stiffness measurement, followed by reactive hyperemia and finally ischemic handgrip exercise. All arterial stiffness measurements were taken on the non-dominant arm, from carotid and radial pulse sites. Arterial stiffness data was collected for 1 minute immediately after the subject completed each condition. Probe displacement was calculated by the difference from the suprasternal notch to the radial and carotid sites (23, 24, 46). Reactive Hyperemia protocol: A blood pressure cuff was placed on the subjects non-dominate arm and inflated to suprasystolic levels (220 mmHg) for five minutes. Immediately after the pressure in the cuff was released, arterial stiffness was assessed by probe measurements at the carotid and radial sites. Ischemic Handgrip Exercise: Prior to ischemic handgrip exercise, a max voluntary handgrip test was performed. After a blood pressure cuff was placed on the dominant arm and inflated to suprasystolic levels for two minutes. During that time the subject engaged in 40% max voluntary muscle contractions every two seconds. A metronome was provided to assist the subject in timing muscle contractions and visual feedback provided by either a computer digital display, or numerical digital display. Immediately after the pressure in the cuff was released, arterial stiffness was assessed by probe measurements at the carotid and radial sites. Data Analysis: A two-way

repeated measures ANOVA, was used to test for main and interactions effects. A one-way repeated measures ANOVA was used to establish differences within testing days. Paired-T tests were used to establish baseline characteristics between testing days. Effect sizes were calculated to establish magnitude of difference to infer impact of L-arginine had on different conditions.

Results

All subjects in the study were male that were attending university courses during the time of the study. The subjects' age ranges approximately from 18 to 30 years of age. Respectively, weight ranges from 53.86 to 108.7 kg, height from 1.63 to 1.95 meters, and body fast percentage 5.5% to 26.5%.

Subject Characteristics in Table 1.

Table 1.	Subject Characteristics
Subjects	N= 15
Age (Years)	24.07±3.28
Weight (Kg)	81.28±13.71
Height (m)	1.79±0.08
Body Fat %	16.00±5.25

Mean±*SD*

Baseline characteristics during the first day (control) demonstrates ranges in heart rate from 57.59 to 69.35 beats per minute, systolic pressures from 108.06 to 122.74 mmHg, and diastolic pressures from 69.34 to 78.66 mmHg. During the second day (L-arginine), heart rate ranges from 55.47 to 68.27 beats per minute, systolic pressure from 111.59 to 119.35 mmHg, and diastolic pressures from 67.57 to 75.09 mmHg. No statistical

difference was found for resting heart rate, systolic pressure and diastolic pressure between testing days ($p>0.05$) (Table 2.), demonstrating comparable testing days.

No statistical difference was found for resting heart rate, systolic pressure and diastolic pressure between testing days ($p>0.05$) (Table 2.).

Table 2. Baseline Characteristics

	Control	*L-arginine*	*p*
Heart Rate (bpm)	63.47±2.94	61.87±3.20	p=0.543
Systolic Blood Pressure (mmHg)	115.40±3.17	115.47±1.94	p=0.338
Diastolic Blood Pressure (mmHg)	74±2.33	71.33±1.88	p=0.979

Mean±SE

No statistical differences were found between control and L-arginine supplementation, at rest, during reactive hyperemia and ischemic handgrip exercise (Figure 1), demonstrating neither a benefit nor detriment to blood pressure regulation. Statistical significance was found between the marginal means of control and L-arginine supplementation (Figure 2). The difference across days in comparing marginal means, demonstrates the inclusion of all conditions offering a perspective of no controlled variables (Figure 2).

CHAPTER V

DISCUSSION AND CONCLUSION

Research Hypotheses

The study hypothesized that 1) Acute L-arginine supplementation will decrease peripheral arterial stiffness in young healthy subjects at rest, 2) Acute L-arginine supplementation will decrease peripheral arterial stiffness during reactive hyperemia, and 3) Acute L-arginine supplementation will decrease peripheral arterial stiffness in inactive tissue after an ischemic handgrip exercise.

First Hypothesis

A two-way repeated measures analysis shows no statistical difference in peripheral arterial stiffness with supplementing L-arginine in young healthy subjects at rest (Figure 1). This provides evidence opposing our first hypothesis, that L-arginine supplementation at a dosage of 70 mg per kg lean body mass does not reduce peripheral arterial stiffness in a sample with presumably little to no cardiovascular disease. Further statistical analyses show no difference in arterial stiffness during reactive hyperemia and in inactive tissues after an ischemic handgrip test, offering no support for the latter two hypotheses. This demonstrates that L-arginine supplementation at a dosage of 70 mg per

kg lean body mass did not reduce peripheral arterial stiffness in any specific testing condition (Figure 1).

The support for the first hypothesis found in this study, is supported with current standing literature involving healthy individuals. No difference was found in peripheral arterial stiffness at rest with L-arginine supplementation. A study done on animals demonstrate an increase in vascular flow in the ear artery when provided L-arginine and L-citrulline, but not with L-arginine alone (33). In young healthy adult human males, there is evidence to support that L-arginine does not increase nitric oxide levels even though L-arginine is shown to be a precursor for nitric oxide production (1, 39). The support for the first hypothesis of this study shows no reduction in peripheral arterial stiffness at rest.

Second Hypothesis

The second hypothesis of the study regarding reactive hyperemia is referred to current standing literature involving healthy individuals that L-arginine, as an ergogenic aid shows increased time to exhaustion in male wrestlers, significant differences in Wingate, and 1-RM bench press (9, 52). Another study demonstrated improved muscle blood flow during recovery between sets, but no improvements in muscular strength (2). Even though this study did not measure performance on handgrip exercise in terms of exhaustion or power output, it did mimic hypoxia provided in the activities listed. If improved muscle blood flow is present between sets, this study should have found a decrease in arterial stiffness with L-arginine supplementation when comparing reactive

hyperemia tests. A change in arterial diameter increases arterial compliance, blood volume in the hypoxic area and would reduce pulse wave transit time (16).

Third Hypothesis

Current standing literature involving healthy individuals, examining inactive tissues during an ischemic handgrip exercise, supports the last hypothesis of the study. This study shows no difference when comparing peripheral arterial stiffness with inactive tissues during an ischemic handgrip exercise with L-arginine at the dosage provided. It may be interpreted, that inactive tissue would respond to the same way as tissues at rest. However in the presence of ischemic handgrip exercise, inactive tissues, would be regulated by a sympathetic neural response, to shunt blood away from inactive tissues toward hypoxic tissues (6, 40).

Additional findings of note

On a final note, a statistical difference was found between the marginal means with L-arginine in comparison to control (Figure 2). In one interpretation this demonstrates L-arginine supplementation at the dose provided, reduced pulse wave transit times averaged from all three conditions. Even though this study found no difference between heart rates and blood pressure with L-arginine supplementation, it is difficult to tell if this was not due to the influence of reactive hyperemia and ischemic handgrip exercise. Another interpretation questions if a number and/or duration of different conditions are required to utilize L-arginine as a vasodilator, if L-arginine is

used at all for nitric oxide production as demonstrated in the literature on healthy individuals.

Justifications in study design

Protocols developed are in reference to pulse wave velocity studies in hypertensive and COPD populations. It was necessary to understand resting steady state conditions established by 10 or more minutes of supine rest prior to resting pulse wave measurements, to include morning testing (11, 24, 43). On the control day, 20 minutes of rest was implemented based on VO2 resting steady state data (11). This is to include using the suprasternal notch as a reference point to measure displacement of the waveform to establish a velocity (24). Reactive hyperemia protocols were followed as a 5 minute occlusion followed by a 1 minute pulse wave velocity recording of the brachial artery that have been establish in the literature (24, 30, 43). Ischemic handgrip exercise was adapted from an incremental power output ergometer test with occluded blood flow to the arm (7). A constant power output was used at 40% max voluntary contraction, to establish an assumed constant sympathetic output (13).

During the intervention day protocol the literature was referenced for oral L-arginine supplementation absorption times and dosage (18).

Limitations

There are limitations to this study identified as age, sex, relative dosage of L-arginine, oral administration of L-arginine and sample size. A sign of vascular aging, shows the replacement of elastin with collagen, increasing arterial stiffness and faster pulse wave velocities (45). Age was controlled for to presume the effect of L-arginine on vascular stiffness, without limited viscoelasticity. Females were excluded from the study due to a change in hormone regulation and blood volume loss during the menstrual cycle, making the effects of L-arginine difficult to measure (31). The relative dosage of L-arginine may change arterial stiffness. This study used a 40 minute ingestion period prior to testing, with the intent to test arterial stiffness as L-arginine reached peak levels in blood plasma at 60 minutes (18). This allowed for a 20 minute testing window. Following, the assumption was made that oral L-arginine, was absorbed, and that through the process of digestion L-arginine remained intact. Due to the small sample size in the study a type II error may have been present in the parametric paired t-tests, so a non-parametric Wilcoxon rank sign test was used help confirm statistical measures of the data.

Other study designs of note include that the longest time spent between testing days for any one subject was five days and diet logs provided were dropped from the study due to a poor return rate. This is also to include that subjects may have adjusted to the stress of the protocol that may provide a shift in the data.

Conclusion

No differences were found with L-arginine supplementation in resting, reactive hyperemia and ischemic handgrip protocols regarding pulse wave velocities. This seems to fit with current standing literature. Although when the data was pooled together a significant difference was found between the control day and the L-arginine supplementation day. This difference is difficult to determine as pooling the data together allows for a more general perspective, since those who supplement with L-arginine have some level of physical activity during the day. However the counter point to this argument would be how that physical activity relates to conditions set by reactive hyperemia and ischemic hand grip exercise, to include half-life of L-arginine levels in the plasma. The need for more frequent doses throughout the day may be required and activity dependent. Studies have found L-arginine acting as a ergogenic aid by increasing time to exhaustion and improved blood flow between weightlifting sets (2, 3, 52).

Further investigation is necessary to better understand the influence of L-arginine on vascular function. Multiple pathways, influence endothelial nitric oxide production leading to vasodilation. However nitric oxide may play roles, other than acting as a regulator for vasodilation in healthy individuals. Other roles of nitric oxide include lowering blood plasma viscosity by reducing platelet aggregation. This change in blood plasma viscosity may influence endothelial shear stress, a known stimulus for vasodilation. Another venture would be identifying variables leading toward a phenotypic

expression and profile of vascular aging, related to the various roles nitric oxide regulates. An epidemiological study describing heart disease in a population consuming foods higher in L-arginine content. In conclusion, this study does not provide any evidence to support, that acute L-arginine supplementation in young healthy subjects, decreases peripheral vascular stiffness at rest, during reactive hyperemia or in inactive tissues after ischemic handgrip exercise.

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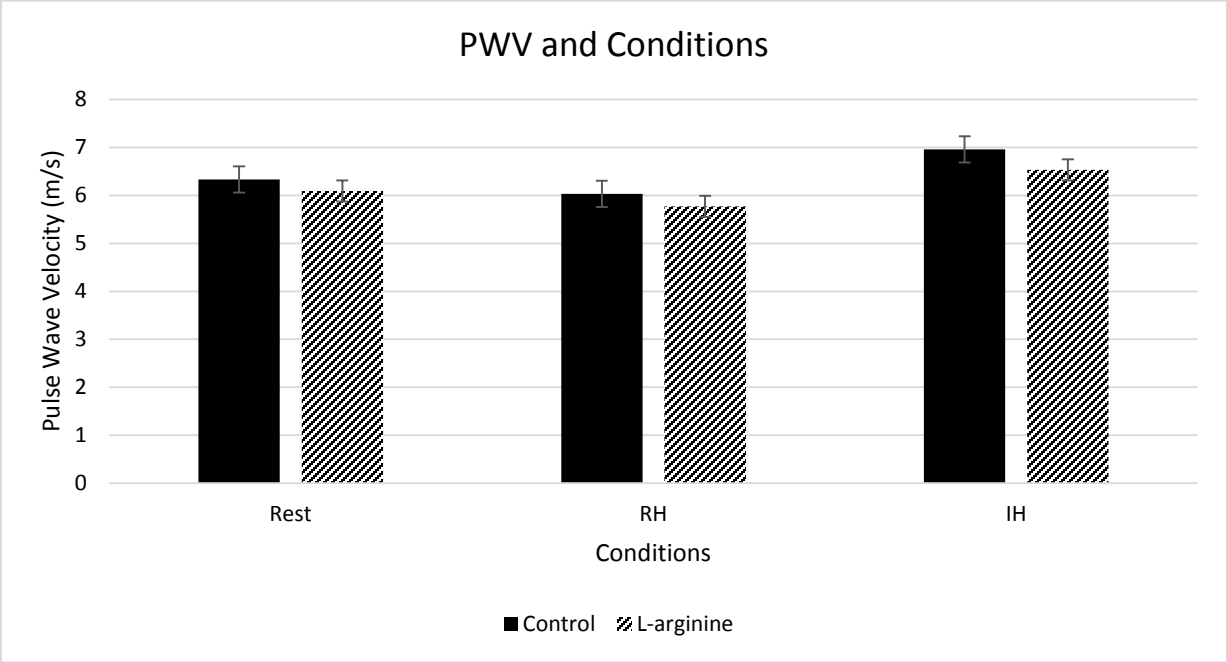
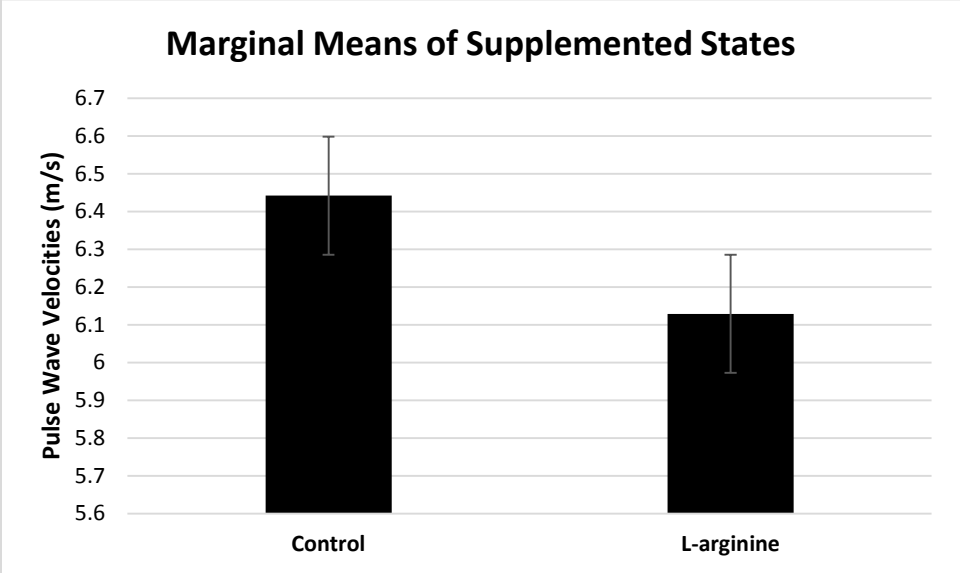
Figure 1.



Mean±SE, RH-Reactive Hyperemia, IH-Ischemic Handgrip Exercise

Figure 2.



Mean±SE

APPENDIX A

Protocol Diagram

APPENDIX B

Raw Data

	Day 1 PWV (m/s)			Day 2 PWV (m/s)		
Subject #	Rest	RH	IH	Rest	RH	IH
Subject 1	6.02	4.95	7.06	6.43	4.96	7.25
Subject 2	5.87	5.65	7.33	4.47	5.61	5.95
Subject 3	6.23	5.46	7.48	4.97	4.49	5.43
Subject 4	6.79	5.44	5.7	5.41	5.43	6.16
Subject 5	5.01	5.17	6.03	4.9	5.75	5.93
Subject 6	8.78	8.31	8.15	7.27	7.66	8.35
Subject 7	6.49	8.02	7.68	7.72	6.97	8.44
Subject 8	5.61	5.61	6.51	6.41	5.24	6.38
Subject 9	6.1	5.54	5.86	5.44	5.41	6.05
Subject 10	6.07	5.66	6.49	5.37	5.27	6.8
Subject 11	6.37	6.59	7.3	5.85	6.27	6.46
Subject 12	6.64	6.81	7.77	6.87	6.45	6.94
Subject 13	6.08	5.88	6.45	6.61	4.94	5.71
Subject 14	6.5	5.83	7.1	6.98	6.07	5.04
Subject 15	6.38	5.59	7.55	6.66	6.05	7
Average	**6.33**	**6.03**	**6.96**	**6.09**	**5.77**	**6.53**
SD	**0.81**	**0.99**	**0.75**	**0.96**	**0.83**	**0.97**
SE	**0.21**	**0.25**	**0.19**	**0.25**	**0.21**	**0.25**

	Day 1 SBP	Day 1 DBP	Day 1 HR	Day 2 SBP	Day 2 DBP	Day 2 HR
Subject #	Rest	Rest	Rest	Rest	Rest	Rest
Subject 1	114	60	44	118	78	46
Subject 2	110	80	78	118	80	78
Subject 3	118	78	70	124	74	60
Subject 4	120	70	56	118	72	64
Subject 5	118	78	72	110	72	60
Subject 6	126	78	56	118	70	54
Subject 7	118	78	72	130	74	52
Subject 8	118	60	48	110	52	56
Subject 9	140	90	50	120	62	54
Subject 10	88	66	78	100	70	74
Subject 11	120	88	72	122	78	60
Subject 12	120	72	54	116	68	48
Subject 13	94	68	60	110	80	56
Subject 14	108	66	70	108	68	90
Subject 15	119	78	72	110	72	76
Average	**115.40**	**74.00**	**63.47**	**115.47**	**71.33**	**61.87**
SD	**12.29**	**9.04**	**11.38**	**7.50**	**7.28**	**12.39**
SE	**3.17**	**2.33**	**2.94**	**1.94**	**1.88**	**3.20**

APPENDIX C

	Sample Characteristics			
	Age (Years)	Weight (Kg)	Height (m)	BF%
	22	76.14	1.75	18.9
	22	74.3	1.73	18.1
	22	73.99	1.77	19.8
	33	108.9	1.92	28.1
	22	80.95	1.69	17
	27	92.4	1.91	20.9
	22	81.6	1.82	14.7
	25	67.73	1.76	11.2
	24	93.5	1.78	15.5
	24	73.7	1.88	5.2
	23	97.1	1.88	13
	22	99.05	1.84	17.1
	21	64.6	1.67	15.2
	23	65.5	1.66	15.1
	29	69.73	1.74	10.2
Average	**24.07**	**81.28**	**1.79**	**16.00**
SD	**3.28**	**13.71**	**0.08**	**5.25**
SE	**0.85**	**3.54**	**0.02**	**1.36**

Pulse Wave Velocities			
	Control	**L-arginine**	
Subject #	**Rest Day 1**	**Rest Day 2**	**Rest Difference**
Subject 1	6.02	6.43	0.41
Subject 2	5.87	4.47	-1.4
Subject 3	6.23	4.97	-1.26
Subject 4	6.79	5.41	-1.38
Subject 5	5.01	4.9	-0.11
Subject 6	8.78	7.27	-1.51
Subject 7	6.49	7.72	1.23
Subject 8	5.61	6.41	0.8
Subject 9	6.1	5.44	-0.66
Subject 10	6.07	5.37	-0.7
Subject 11	6.37	5.85	-0.52
Subject 12	6.64	6.87	0.23
Subject 13	6.08	6.61	0.53
Subject 14	6.5	6.98	0.48
Subject 15	6.38	6.66	0.28
Average	6.329333333	6.090666667	-0.238666667
SD	0.807566598	0.964324091	0.888392341
SE	0.208512799	0.24898741	0.229381916

APPENDIX D

Difference Data

Pulse Wave Velocities			
	Control	**L-arginine**	
Subject #	**RH Day 1**	**RH Day 2**	**RH Difference**
Subject 1	4.95	4.96	0.01
Subject 2	5.65	5.61	-0.04
Subject 3	5.46	4.49	-0.97
Subject 4	5.44	5.43	-0.01
Subject 5	5.17	5.75	0.58
Subject 6	8.31	7.66	-0.65
Subject 7	8.02	6.97	-1.05
Subject 8	5.61	5.24	-0.37
Subject 9	5.54	5.41	-0.13
Subject 10	5.66	5.27	-0.39
Subject 11	6.59	6.27	-0.32
Subject 12	6.81	6.45	-0.36
Subject 13	5.88	4.94	-0.94
Subject 14	5.83	6.07	0.24
Subject 15	5.59	6.05	0.46
Average	6.034	5.771333333	-0.262666667
SD	0.986087507	0.831451972	0.497126984
SE	0.254606699	0.214679976	0.128357635

Pulse Wave Velocities			
	Control	**L-arginine**	
Subject #	**IH Day 1**	**IH Day 2**	**IH Difference**
Subject 1	7.06	7.25	0.19
Subject 2	7.33	5.95	-1.38
Subject 3	7.48	5.43	-2.05
Subject 4	5.7	6.16	0.46
Subject 5	6.03	5.93	-0.1
Subject 6	8.15	8.35	0.2
Subject 7	7.68	8.44	0.76
Subject 8	6.51	6.38	-0.13
Subject 9	5.86	6.05	0.19
Subject 10	6.49	6.8	0.31
Subject 11	7.3	6.46	-0.84
Subject 12	7.77	6.94	-0.83
Subject 13	6.45	5.71	-0.74
Subject 14	7.1	5.04	-2.06
Subject 15	7.55	7	-0.55
Average	6.964	6.526	-0.438
SD	0.748186379	0.968583649	0.875941942
SE	0.193180892	0.250087223	0.226167237

APPENDIX E

Resting Condition

Data Entry

Pairs	Data Cells X_a	Data Cells X_b	S/R of $\lvert X_a - X_b \rvert$	Import/Export Box X_a and X_b	S/R ="signed rank"
1	6.02	6.43	-4	8.78 7.27 6.49 7.72 5.61 6.41 6.1 5.44 6.07 5.37 6.37 5.85 6.64 6.87 6.08 6.61 6.5 6.98 6.38 6.66	Import data to data cells
2	5.87	4.47	+14		
3	6.23	4.97	+12		
4	6.79	5.41	+13		
5	5.01	4.9	+1		
6	8.78	7.27	+15		
7	6.49	7.72	-11		
8	5.61	6.41	-10		
9	6.1	5.44	+8		
10	6.07	5.37	+9		
11	6.37	5.85	+6		
12	6.64	6.87	-2		
13	6.08	6.61	-7		
14	6.5	6.98	-5		
15	6.38	6.66	-3		

Clear this box

Reload

Reset

Calculate

W=	36		
$n_{s/r}$=	15	P(1-tail)	P(2-tail)
z=	1.01	0.1562	0.3125

Reactive Hyperemia Conditions

Data Entry

Pairs	Data Cells X_a	Data Cells X_b	S/R of $\lvert X_a - X_b \rvert$
1	4.95	4.96	-1.5
2	5.65	5.61	+3
3	5.46	4.49	+14
4	5.44	5.43	+1.5
5	5.17	5.75	-11
6	8.31	7.66	+12
7	8.02	6.97	+15
8	5.61	5.24	+8
9	5.54	5.41	+4
10	5.66	5.27	+9
11	6.59	6.27	+6
12	6.81	6.45	+7
13	5.88	4.94	+13
14	5.83	6.07	-5
15	5.59	6.05	-10

Import/Export Box

X_a and X_b

8.31 7.66 +12
8.02 6.97 +15
5.61 5.24 +8
5.54 5.41 +4
5.66 5.27 +9
6.59 6.27 +6
6.81 6.45 +7
5.88 4.94 +13
5.83 6.07 -5
5.59 6.05 -10

Clear this box

S/R ="signed rank"

Import data to data cells

Reload

Reset

Calculate

		P(1-tail)	P(2-tail)
W=	65		
$n_{s/r}$=	15		
z=	1.83	0.0336	0.0673

Ischemic Hand Grip Exercise Condition

Data Entry

Pairs	Data Cells X_a	Data Cells X_b	S/R of $\lvert X_a - X_b \rvert$
1	7.06	7.25	-3.5
2	7.33	5.95	+13
3	7.48	5.43	+14
4	5.7	6.16	-7
5	6.03	5.93	+1
6	8.15	8.35	-5
7	7.68	8.44	-10
8	6.51	6.38	+2
9	5.86	6.05	-3.5
10	6.49	6.8	-6
11	7.3	6.46	+12
12	7.77	6.94	+11
13	6.45	5.71	+9
14	7.1	5.04	+15
15	7.55	7	+8

Import/Export Box X_a and X_b

```
8.15  8.35  -5
7.68  8.44  -10
6.51  6.38  +2
5.86  6.05  -3.5
6.49  6.8   -6
7.3   6.46  +12
7.77  6.94  +11
6.45  5.71  +9
7.1   5.04  +15
7.55  7     +8
```

Clear this box

S/R ="signed rank"

Import data to data cells

Reload

Reset

Calculate

		P(1-tail)	P(2-tail)
W=	50		
$n_{s/r}$=	15		
z=	1.41	0.0793	0.1585