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Can L-arginine Influence the Acute Hormonal, Metabolic, and Physiological Responses at Rest and Prior to Exercise?

by

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ABSTRACT

L-arginine is a conditionally essential amino acid and has recently been purported as ergogenic for both strength and aerobic athletes; however, the value of oral L-arginine supplementation in physically active participants is controversial. The purpose of this dissertation was threefold: first to examine the hormonal and metabolic responses of low vs. high relative doses of oral L-arginine at rest; second to determine the GH response when L-arginine was ingested prior to a bout of whole-body resistance exercise in strength trained men; and third to evaluate the hormonal and metabolic responses when L-arginine was consumed prior to a bout of submaximal aerobic exercise in trained cyclists. Study 1: fourteen physically active men (age: 25 ± 5 y; body mass: 78.0 ± 8.5 kg; height: 179.4 ± 4.7 cm) volunteered to be in a randomized, double-blind, repeated measures design. Each subject was provided three treatment conditions (placebo: flour, low dose: 0.075 $g \cdot kg^{-1}$ or high dose: 0.150 $g \cdot kg^{-1}$ body mass of L-arginine). L-arginine plasma concentrations significantly increased to a similar concentration at any time point in both the low and high dose conditions, while there was no change over time in the placebo condition. There was no significant difference between conditions for plasma growth hormone (GH), nitrate+nitrite (NOx), insulin-like growth factor-1 (IGF-1), or insulin. Study 2: fourteen strength trained men (age: 25 ± 4 y; body mass: 81.4 ± 9.0 kg; height: 179.4 ± 6.9 cm) participated in a randomized crossover design. L-arginine (0.075 $g \cdot kg^{-1}$ body mass) or a placebo was ingested 60 minutes prior to performing an acute bout of resistance exercise (3 sets of 8 exercises, 10 repetitions at ~75% 1RM). There were no differences between conditions for GH, GH-releasing hormone (GHRH), ghrelin, or IGF-1 at any time point. GH-inhibiting hormone (GHIH) was significantly lower in the L-arginine condition. However; integrated area under the curve (iAUC) for GH was significantly blunted in the L-arginine condition. Study 3: fifteen

aerobically trained men (age: 28 ± 5 y; body mass: 77.4 ± 9.5 kg; height: 180.9 ± 7.9 cm; training experience: 5.9 ± 3.4 y, VO_2 max: 59.6 ± 5.9 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in a randomized crossover design. L-arginine (0.075 $g \cdot kg^{-1}$ body mass) or a placebo was ingested prior to performing an acute bout of submaximal aerobic exercise. There were no difference between conditions for GH, non esterified fatty acids (NEFA), lactate, glucose, VO_2, VCO_2, RER, and NOx. However, there was a small but significant elevation in plasma glycerol at the 45 minute time point after L-arginine consumption. In summary, L-arginine consumed orally at rest was effective at increasing plasma L-arginine. L-arginine prior to resistance exercise attenuated plasma GH compared to resistance exercise alone. Lastly, L-arginine before aerobic exercise did not enhance GH, glucose, lactate, NOx, or any cardio-respiratory parameters; however there was a small but significant increase in glycerol during exercise. In conclusion, L-arginine is effective at increasing L-arginine plasma concentrations; however the hormonal and metabolic responses are small and further research is required to examine the ergogenic potential.

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GH	Growth hormone
IGF-1	Insulin-like growth factor 1
GHRH	Growth hormone releasing hormone
GHIH	Growth hormone inhibiting hormone
NO	Nitric oxide
NOS	Nitric oxide synthase
NOx	Nitrate+Nitrite
HR	Heart rate
RER	Respiratory exchange ratio
$\dot{V}O_2$ max	Maximal Oxygen Uptake
RM	Repetition Maximum
RR	Respiratory Rate
ADMA	Assymetrical Dimethylarginine
iAUC	Integrated Area Under the Curve
kg	Kilogram
y	Years
cm	Centimeters
m	Meters

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Athletic competitions continue to push the limits of human performance and athletes often seek ergogenic aids to gain an edge. A nutritional ergogenic aid is defined as any nutrient capable of enhancing energy utilization, including energy production, control, and efficiency (1). One nutritional ergogenic aid sought commonly by athletes is protein (or amino acid) supplementation (2-6). L-arginine is an amino acid that has been purported to be ergogenic and, as such, has become very popular in the food supplement industry (2, 3, 5-8). Malinauskas et al. (9) observed that 27 of the 145 National Collegiate Athletic Association (NCAA) male and female athletes at a south-eastern state university in the United States of America were interested in taking supplements. Among these athletes, 13% were taking L-arginine.

L-arginine (2-amino-5-guanidinovaleric acid) is one of the 20 most common amino acids; it has been shown to be in relatively high (e.g. as much as 16% of the protein content) concentrations in foods such as watermelon juice, nuts, seeds, algae, meats, seafood, rice protein concentrate, and soy protein isolate (10-12). A typical North American diet contains approximately 4.4 $g \cdot day^{-1}$ of L-arginine (11). L-arginine can also be synthesized endogenously in the kidney and liver (13) and therefore has traditionally been termed non-essential; however, during periods of rapid growth, in response to a traumatic incident, pathologic insult, or some other type of physiological stressor (14-18), the demand for L-arginine may not be fully met by de novo synthesis and normal dietary intake alone. Since exercise can be considered a physiological stressor, athletes under heavy physical training regimes (catabolic stress) may benefit from exogenous L-arginine supplementation (3, 5). In older adults with cardiovascular

diseases, such as heart failure, myocardial infarction, stable angina, and pulmonary hypertension, L-arginine has been shown to be ergogenic (e.g. improved 6 minute walk; 8, 16, 19-21). However, currently the literature examining L-arginine as an ergogenic aid in physically active healthy young humans is sparse and conflicting.

1.2 L-Arginine Supplementation and Performance

L-arginine supplementation has been effective in improving exercise performance (e.g., aerobic capacity or 6 minute walk) in older adults with cardiovascular diseases such as stable angina, congestive heart failure, healed myocardial infarction, and pulmonary hypertension (16, 19-22). However; L-arginine supplementation on performance in physically active healthy young subjects is limited and can be considered to be conflicting (3, 5), as shown in table A-2 and A-4 in the appendix. For example, Santos and colleagues (23) supplemented untrained men with 3 g of L-arginine for 15 days and underwent a test-retest protocol evaluating the resistance to muscular fatigue in the knee extensors using isokinetic dynamometry. This latter study was able to demonstrate a significant increase in the resistance to muscular fatigue but they did not utilize a double-blinded protocol nor was there a placebo or control group. Conversely, Walberg-Rankin et al. (24) supplemented male weight lifters on a hypo-caloric diet with 8 g of L-arginine daily and found no positive influences on muscle function (bicep/quadriceps isokinetic assessment) or body composition compared to a placebo condition. Recently, Alvares et al. (2) provided 6 g of L-arginine 60 minutes prior to an elbow extension protocol consisting of 3 sets of 10 repetitions and found no effect on peak torque, total work, and set total work. Liu and colleagues (25) also demonstrated no effect of short term L-arginine supplementation (6 $g \cdot day^{-1}$ for 3 days) on indirect measures of nitric oxide (NO) production [nitrate+nitrite (NOx), L-citrulline], metabolic markers and repeated sprint performance in well-trained judo athletes.

Buchman et al. (26) examined the effect of 10 g of L-arginine three times per day for 14 days on marathon performance and found a detrimental effect compared to a predicted time (+23 ± 21 minutes, $p<0.049$). Interestingly, the temperature at the start of the marathon was -2ºC, with a wind chill of -12ºC and freezing rain; however, Buchman and colleagues (26) suggested the weather only played a minor role. Thus, the ergogenic potential of L-arginine is difficult to evaluate because much of the literature is conflicting and sometimes lacks scientific rigor.

1.3 Potential Ergogenic Mechanisms

The mechanisms by which L-arginine supplementation may play a role in enhancing performance are not fully understood. Intravenous administration of L-arginine stimulates GH secretion from the anterior pituitary in humans (27, 28) primarily due to an inhibition of endogenous GHIH (29). In addition, L-arginine is a necessary substrate involved in the detoxification of ammonia, produced during the catabolism of amino acids via the formation of urea (13). L-arginine is also gluconeogenic because it has the potential to be converted to glucose in the liver. In addition L-arginine may be catabolized to produce energy because it can be converted to alpha-ketoglutarate and enter the citric acid cycle (13). Furthermore, L-arginine is utilized by a number of metabolic pathways that produce a variety of biologically active compounds such as NO and creatine (13). Nitric oxide is an endogenously produced, cellular signalling molecule that is involved in a variety of endothelium-mediated effects in the vasculature (30). Nitric oxide serves as a second messenger to trigger blood vessel dilation and increase blood flow (31). L-arginine is the only endogenous nitrogen-containing amino acid substrate of nitric oxide synthase (NOS) and thus is an important governor of the production of NO.

Potential ergogenic effects of L-arginine are unique and intriguing due to the fact that L-

arginine may be beneficial for both strength and aerobic trained athletes. Strength trained athletes ingest L-arginine in an attempt to stimulate GH secretion, believing that this practice will promote greater gains in muscle mass and strength compared to resistance training alone (4). Growth hormone also stimulates the production of hepatic IGF-1 through the JAK-STAT signalling pathway which is known to enhance muscle protein synthesis (32). From an aerobic exercise perspective, enhanced L-arginine induced endogenous GH may influence endurance exercise performance by increasing lipolysis (33) and fat oxidation (34). During submaximal exercise, GH administration increases plasma glycerol and free fatty acids (FFA) in healthy (35, 36) and well-trained endurance athletes (35, 37). These altered metabolic effects may in turn increase time to exhaustion during exercise by sparing skeletal muscle and/or liver glycogen (38, 39). Secondly, L-arginine may enhance endurance performance through a NO induced vasodilation (38). There is increasing evidence that interventions that influence NO bioavailability can alter the O_2 cost of exercise (40-42), influence blood flow (43, 44), nutrient delivery (45-47) and aid in metabolic waste product removal (48). However, research regarding the potential ergogenic responses, both hormonally and metabolically, to L-arginine supplementation at rest or when combined with either resistance or aerobic exercise in trained participants is limited.

1.4 L-Arginine Supplementation at Rest

Although it is well known that intravenous infusion of L-arginine at rest stimulates a GH response in clinical and some healthy populations (49, 50), oral supplementation is much more controversial. Oral L-arginine seems to result in a blunted response compared to intravenous infusion, which may be due to the low bioavailability of ingested L-arginine. Infusion with a high dose (e.g. 30 g) has been shown to be a potent secretagogue of both GH and insulin (27). In

fact, intravenous infusion has been used clinically to determine the responsiveness of the GH axis when GH deficiency is suspected (8). Oral L-arginine supplementation has also demonstrated increases in resting GH at rest in healthy individuals (51) while others have shown no effect (24, 52). One difficulty in interpreting the effectiveness of L-arginine taken orally has been due to the various dosages utilized and it would be important to establish an effective dose of L-arginine to elicit a physiological effect (8). Collier et al. (51) attempted to establish an effective dose for ingestion of oral L-arginine on the GH response. They used a randomized placebo controlled repeated measures design in which all the subjects received either a placebo, 5, 9, or 13 g of L-arginine in a double-blind fashion. An increase in the peak GH and area under the curve (AUC) was observed with increasing doses of L-arginine up to 9 g. Furthermore, they demonstrated a peak GH response 30 minutes post-ingestion. However, the limitations of this latter study included a small sample size (n=8), the removal of non-responders from the sample without adequate justification, and they only measured GH. It seems prudent that future studies are needed to examine how much L-arginine can be absorbed using different doses and these doses should be based on a relative load (e.g. using a dose relative to body mass). In support of this contention, Collier et al. (51) suggested that the highest absolute dose (13 g) used was not absorbed due to an osmotic imbalance that subsequently caused gastro-intestinal distress. Previous research in clinical populations suggests that a plasma concentration of 1000 μmol/L must be achieved to stimulate any hormonal or metabolic response (30). This provides strong rationale for using a relative dose based on body mass that may be more appropriate and effective at achieving a high blood plasma concentration of L-arginine while limiting any negative side effects. A recent review (6) examining the upper tolerable limit of L-arginine in healthy young humans suggested that 20 g was the maximum dosage when consumed orally.

However, these limits were based off studies that utilized unhealthy participants (i.e. cystic fibrosis), that is known to alter the absorption at the gut.

Research examining NO production and blood flow at rest as a result of L-arginine supplementation is equivocal. Intravenous infusion of L-arginine enhanced blood flow in healthy young rats (53), improved endothelial function in healthy older humans (54) and hypercholesterolemic patients (55). Further, Vallance et al. (50) demonstrated that infusion of NG monomethyl-L-arginine, a specific inhibitor of NO synthesis, to healthy participants significantly impaired blood flow. In contrast, Kubota et al. (56) did not show any significant effect on blood flow in healthy young participants, when L-arginine (6 g) was ingested. In summary, although L-arginine may promote a hormonal and metabolic effect at rest in some studies, the research is inconclusive; these contradictory findings may be related to relative dosing and possibly different effects in physically active individuals.

1.5 Acute Effects of L-arginine Supplementation and Strength Exercise

The potential mechanism(s) of L-arginine when combined with resistance exercise has been conflicting (52, 57, 58). Fahs and colleagues (58) examined the effects of acute L-arginine supplementation and resistance exercise on arterial function and found no significant difference between L-arginine supplementation and placebo for any hemodynamic or vascular response after a resistance training session. Collier and colleagues (57) examined the effects of 7 g of L-arginine ingestion combined with a whole body resistance exercise session, previously shown to stimulate GH secretion (59). L-arginine alone resulted in a significant increase (2-fold) in GH compared to the placebo, while exercise alone stimulated a 5-fold increase; however, an attenuated response (3 fold increase) was observed when L-arginine and resistance exercise were combined compared to resistance exercise alone. Marcell et al. (52) examined the effects of 5 g

of L-arginine consumed orally that did not significantly change basal GH concentrations significantly nor did it enhance the GH response compared to resistance exercise alone. Contrary to Collier and colleagues (57) they found no significant difference between the resistance exercise alone trial or when resistance exercise was combined with L-arginine on GH. These studies suggest that L-arginine supplementation may in fact be detrimental to the positive GH response consequent to acute resistance exercise. Collier et al. (57) suggest two potential possibilities for the attenuation of GH. First, there may be a down regulation of GHRH induced GH release and secondly there may be an auto-negative feedback induced by elevated IGF-1 prior to the resistance exercise bout suppressing subsequent stimulation of GH. However, these mechanisms have not been explored and future studies are needed to identify the underlying mechanisms for the attenuation in GH secretion when combining L-arginine and resistance exercise. Furthermore, it would be of special interest to examine these responses in strength trained individuals.

1.6 Acute Effects of L-arginine Supplementation and Aerobic Exercise

Although it has been shown that infusion of L-arginine influences blood pressure, heart rate and blood flow at rest (50, 60), several other studies have shown that L-arginine has little effect on haemodynamics during exercise in humans (56, 61, 62). Schaefer et al. (48) found that L-arginine administered intravenously reduced both lactate and ammonia and increased L-citrulline plasma concentrations during aerobic exercise, perhaps due to an enhanced NO production. In addition, Koppo et al. (56) demonstrated that oral L-arginine enhanced the speed of phase II pulmonary (slow component) VO_2 kinetics by 12% at the onset of moderate-intensity aerobic exercise. Others have shown no effect on NO production during aerobic exercise following L-arginine ingestion (25, 63, 64). Recently, Wideman et al. (49) found an enhanced

post exercise GH secretion when L-arginine (30 g) was infused intravenously 30 minutes prior to a submaximal cycling protocol. Enhancing GH may increase gluconeogenesis and enhance lipolysis thereby sparing muscle glycogen (65). Future studies are needed to assess the acute effects of an effective oral dose of L-arginine on GH and the subsequent metabolic changes during an aerobic bout of exercise, especially in athletes.

1.7 Summary

It is clear that interpreting the effectiveness of L-arginine as an ergogenic aid is difficult due to the different dosages utilized, method of delivery and population investigated. Wideman et al. (49) have demonstrated elevated GH levels when L-arginine is infused but since oral ingestion will be the predominate method of delivery for the potential ergogenic benefits in non clinical populations such as athletes, establishing an effective oral dose of L-arginine is important (8). Collier and colleagues (51) examined the dose response effect of L-arginine on GH and observed increased GH levels in response to an absolute dose of L-arginine up to 9 g but a reduced GH response was noted with a higher absolute dose (13 g). It seems intuitive that a relative dose of L-arginine based on the size of the individual (i.e. body mass) may promote a more effective absorption of L-arginine and elicit higher plasma L-arginine while reducing any gastro-intestinal distress. Furthermore, the ergogenic potential of L-arginine may only be realized when the individual is experiencing a particular stress (3). L-arginine combined with resistance exercise seems to blunt the GH response, suggesting a potential detrimental effect of L-arginine supplementation (57). With respect to aerobic exercise, Wideman et al. (49) found an enhanced post-exercise GH secretion when L-arginine was infused prior to a submaximal cycling protocol. Schaefer et al. (48) infused L-arginine intravenously and demonstrated a reduced lactate and ammonia plasma concentration during an incremental cycling protocol. The

effectiveness of L-arginine as an ergogenic aid to either acute resistance or aerobic exercise is controversial and not well understood especially in physically active individuals; further research is warranted.

1.8 Purposes and Hypotheses:

Study 1: The acute effects of a low and high dose of oral L-arginine supplementation in young active males at rest.

The purpose of this study was to examine the effectiveness of two relative doses of L-arginine on the primary variable growth hormone and other secondary markers including metabolic (nitrate/nitrite and glucose), hormonal (IGF-1, insulin), and plasma L-arginine concentrations at rest in young physically active men.

It was hypothesized that the high relative dose of L-arginine would result in the greatest growth hormone response in addition to elevated L-arginine plasma concentration and subsequently higher concentrations of other metabolites (NO) and hormones (GF-1, insulin).

Study 2: The effects of acute L-arginine combined with resistance exercise on growth hormone, growth hormone secretagogues, and insulin-like growth factor-1 in strength trained men.

The purpose of this study was to investigate GH response when L-arginine is ingested prior to resistance exercise in strength trained participants. As well as to examine the GH secretagogues (GHRH, GHIH, and ghrelin) associated with the GH response.

It was hypothesized that the ingestion of L-arginine prior to an acute bout of resistance exercise will attenuate the GH response compared to resistance exercise alone and will be accompanied by a decrease in plasma GHRH. A secondary hypothesis was to determine if an

elevation of GH and IGF-1 occurred prior to exercise, because GH and IGF-1 are known to suppress subsequent stimuli for GH, GHRH, ghrelin, and the release of GHIH

Study 3: The effects of acute L-arginine prior to submaximal aerobic exercise on growth hormone and metabolic responses in trained cyclists.

The purpose of this study was to evaluate whether L-arginine consumed orally prior to a bout of aerobic exercise would stimulate plasma GH above exercise alone, and to examine the subsequent metabolic (NEFA, glucose, glycerol, lactate, VO_2, VCO_2, and RER) responses in well-trained individuals.

It was hypothesized that the ingestion of L-arginine prior to submaximal aerobic exercise will increase circulating concentrations of GH which will promote an increase in circulating concentrations of glycerol and NEFA while lowering lactate concentration during exercise compared to aerobic exercise alone. A secondary hypothesis was to explore the possibility that L-arginine consumption prior to an aerobic bout of exercise will increase markers of nitric oxide (nitrate+nitrite; NOx) production.

1.9 Significance

The commercial market for L-arginine is large and the advertised claims of its effectiveness seem exaggerated. The ergogenic effects of L-arginine supplementation remains an area of controversy and debate based on the contradictory findings of a number of investigations (5). While some investigators have reported enhanced performance from L-arginine supplementations (23, 40, 41) others have not (24-26, 66, 67). Furthermore, the underlying hormonal and metabolic effects of exogenous oral L-arginine supplementation in physically active or trained individuals remain unclear (5). The studies in this thesis will investigate a relative oral dose response (based on body mass and its effects on several hormones and

metabolites), investigate why growth hormone may be attenuated when L-arginine is consumed prior to resistance exercise, and examine the acute hormonal and metabolic effects of oral L-arginine prior to submaximal aerobic exercise.

1.10 Delimitations

Men between the ages of 18 and 35 years in good health without any medical conditions that may confound the hormonal or metabolic responses were recruited in all three studies. Study 2 used strength athletes and study 3 used aerobic athletes. A group with such characteristics limits the influence of age (68), gender (69), and health status (4) on physiological or hormonal responses. Men were selected for this study because they are the prominent users of nutritional supplements and have a different hormonal and metabolic response at rest and during exercise (69). Prior to each testing condition in all three experiments, pre-experiment nutrition ($\geq$24 hours) and physical activity were controlled. In the first study the subjects were involved in all three nutritional conditions in a double-blinded randomized within subject design during which a placebo, low, or high dose of L-arginine was consumed based on the subject's body mass. The second and third study used a computer randomized double blinded repeated measures placebo controlled design in which participants completed both conditions. Consistency was maintained, with respect to both the investigators conducting the tests and with the equipment and protocols used in order to maximize reliability.

1.11 Limitations

Inherent limitations within the studies include the reliance on subjects' volitional adherence to pre-study nutrition and physical activity controls. Furthermore, only plasma samples were used to measure hormonal and metabolic responses, therefore it is difficult to make

clear interpretations on absorption, synthesis, and degradation of these types of variables. In addition, these studies only examined the acute responses; chronic supplementation may provide differing results. Another limitation for all three studies includes the lack of direct comparison between trained and untrained subjects.

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CHAPTER 2

A low and high dose of oral L-arginine elevates plasma L-arginine to a similar concentration at rest in young active men.

2.1 Introduction

The popularity of amino acids as supplemental ingredients in various dietary supplements and functional foods has increased tremendously with their prevalence being highest in sport nutrition products (1). L-arginine (2-amino-5-guanidinovaleric acid) is considered to be a conditionally essential amino acid and a typical western diet contains ~3-6 $g \cdot d^{-1}$ (3). L-arginine is involved in several metabolic pathways and in the production of a number of biologically active compounds. Two of the most widely explored metabolic fates of L-arginine has been its conversion to NO via NOS and its stimulation of GH (4). First, the ergogenic potential of NO has received attention and has clinical applicability due to the vasodilatory response thereby increasing blood flow (5-7). Further benefits of this latter response include enhanced nutrient delivery (e.g. creatine, glucose) and metabolic waste-product removal from peripheral tissues such as skeletal muscle (8). Chronic exposure to NO also increases mitochondrial biogenesis, activation of satellite cells, and reduces a number of the processes associated with the onset of atherosclerosis (9, 10). Secondly, L-arginine has also been shown to enhance the maximal pituitary somatotroph responsiveness to GHRH, and increase GH release by suppression

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1 A version of this chapter has been published:
Forbes SC and Bell GJ. 2011. The acute effects of a low and high dose of oral L-arginine in young active males at rest. *Appl Physiol Nutr Metab* 36(3):405-411.

of endogenous GHIH (11). The metabolic effects of GH are diverse and involve increased lipolysis, hyperinsulinemia, and elevated blood concentration of IGF-1 (12, 13). However, research examining the metabolic and hormonal effects of L-arginine in physically active subjects is relatively small and conflicting (10, 14) that may be at least partially due to the dose and/or mode of delivery.

Research examining the effects of L-arginine provided intravenously (IV) consistently demonstrates an enhanced NO and GH response in a dose-dependent manner from 6 to 30 g (7, 15, 16). However, IV delivery of L-arginine is only used in a clinical setting while oral intake is the most common non-clinical option. Recently, Bescos et al. (17) examined the effects of either a control diet (L-arginine = 5.5 $g \cdot d^{-1}$), a diet enriched with L-arginine containing foods (L-arginine = 9.0 $g \cdot d^{-1}$), or a control diet supplemented with 15 g of L-arginine (L-arginine = 20.5 $g \cdot d^{-1}$) for 3 days in trained subjects. They found no significant effect on plasma nitrate. However, Bailey et al. (18) found that 6 g provided orally to healthy young participants was effective at increasing plasma nitrite, suggesting that a single large bolus is a more effective method at increasing NO. With regards to GH response, Collier et al. (19) examined different absolute oral doses of L-arginine on GH response in healthy young subjects and this research found a dose-response effect up to a consumption of 9 g and a reduced GH response with 13 g (placebo < 5g < 9g > 13 g). Collier et al. (19) suggested that L-arginine plasma concentrations may have been reduced with the 13 g dose due to an osmotic imbalance leading to gastro-intestinal distress; an undesirable side effect of high oral doses of L-arginine (20). However, circulating levels of L-arginine in the blood were not measured in this latter research and the amount of L-arginine provided to the participants was the same regardless of body size. Body size is known to influence various physiological responses to ingested supplements (21).

Potentially a relative dose of L-arginine would provide a standard delivery and enhance the various metabolic and hormonal responses while avoiding the negative side effects of high absolute doses in individuals that vary in body size. Therefore, the purpose of this study was to examine a low and high dose of L-arginine provided as a single bolus orally relative to body mass in physically active subjects using a within subject, randomized, double-blind placebo controlled trial. It was hypothesized that the high relative dose of L-arginine would result in the greatest L-arginine plasma concentration and subsequently elicits higher concentrations of metabolites (NO, glucose) and hormones (GH, IGF-1, insulin).

2.2 Materials and methods

Subjects

Fourteen physically active male participants (age: 25 ± 5 y; weight: 78.0 ± 8.5 kg; height: 179.4 ± 4.7 cm; average protein intake: 1.2 ± 0.2 $g \cdot kg \cdot d^{-1}$) were recruited from a University population. Participants were all non-smokers and were screened for food allergies, vegetarianism and any medical condition that would prevent participation in this study. They were participating in 4 ± 1 sessions of strenuous physical activity and 2 ± 2 sessions of moderate physical activity per week (22). Seven participants were strength training, 3 were endurance training, and 4 were performing concurrent strength and endurance training. Participants had been free from other nutritional supplements (e.g. creatine or L-arginine) for at least 12 weeks before initial testing to eliminate any effects from other supplementation. A University Research Ethics Board for human subject research approved the study and all participants provided both verbal and written consent.

Experimental Protocol

The study used a double blind, repeated measures design in which every subject participated in all three testing conditions in a randomized order, each condition was separated by 7 days. Randomization was accomplished by a computerized random number generator where all participants had an equal chance of being assigned to the three groups. Prior to the first visit the participant's height and body mass were recorded and a 1-day dietary record was completed by each participant to determine total daily energy, carbohydrate, fat, protein intake using a computer software program (The Food Processor II for Window, Nutritional Analysis Nutritional Software Version 6.11, Salem, OR). Following the analysis, the nutritional intake of each subject was modified to obtain a 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ of mixed food protein at the same time as maintaining the total calories reported on the original diet record and all participants were required to consume this exact diet one day prior to each experimental condition, since protein and energy intake are known to influence metabolic and hormonal responses (21). In addition, participants refrained from vigorous physical activity the day prior to each experimental condition. Each subject came to the laboratory in the morning after a 10-hour overnight fast to obtain resting blood samples. Following this, the participants were provided with one of three treatment conditions in capsule form: placebo (flour), 0.075 or 0.150 $g \cdot kg^{-1}$ of body mass of L-arginine (purchased from General Nutrition Centre) and 500 ml of water in a triple blind fashion (0.5g per capsule and the equivalent number of capsules were provided in both conditions, that varied in number based on body mass). These relative doses were utilized based off of upper tolerable limits in healthy participants (Collier et al. 2005). Blood samples (~10 ml per sample) were obtained using an intravenous cathelon inserted into a forearm vein kept patent with sterile saline (0.5 ml of 0.9% NaCl). Note that 2 ml's of blood was drawn and discarded prior to

obtaining each sample to clear saline from the line. Blood was taken at 30, 60, 90, 120, and 180 minutes after each condition. The blood samples were immediately put on ice and centrifuged for 10 minutes at 1500 ×g. The plasma was aliquotted, frozen and stored at -80°C until analyzed.

Biochemical Analysis

All samples from the same subjects were run in the same assay and in duplicate. L-arginine concentrations were determined spectrophotometrically (23) by the oxidation of NADH using octopine dehydrogenase. The intra-assay coefficient of variation (CV) for the duplicate samples was 3.4%. Nitrate and Nitrite (NOx), the major NO metabolites, were analyzed in plasma using a commercially available colorimetric assay kit (Cayman #780001, Caymen Chemical, Ann Arbor, MI) according to the procedures provided by the manufacturer. Briefly, following the addition of nitrate reductase co-factor to each diluted sample, nitrate reductase was added and the mixture was incubated for 3 hours to allow for the full conversion of nitrate to nitrite. Greiss reagent was then added, which converts nitrite into a deep purple azo compound. The absorbance was measured at 540 nm and quantification of each sample was performed with a calibration curve. The intra-assay CV for the duplicate samples was 2.3%.

Growth hormone was analyzed in plasma using a commercially available enzyme-linked immunoassay kit (Diagnostics Biochem., Canada Inc., London, Ontario) according to the procedures provided by the manufacturer. This assay follows a typical sandwich type procedure in which a monoclonal antibody specific for GH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of GH is conjugated to horse radish peroxidase. The absorbance was measured at 415 nm and quantification of the samples was performed with a calibration curve. The intra-assay CV for the duplicates was 10.3%

Glucose was analyzed in plasma using the glucose oxidase method (Sigma Aldrich 2004). Briefly, glucose oxidase was added to an unknown sample that results in the oxidation of any serum glucose to gluconic acids and hydrogen peroxide. Hydrogen peroxide then reacted with o-dianisidine in the presence of peroxidase to form a coloured product, while oxidized o-dianisidine reacted with sulphuric acid to form a more stable coloured product. Measured at 540 nm, the intensity of the pink colour was proportional to the original glucose concentration and quantification of all samples was performed with a calibration curve. The intra-assay CV for these duplicate samples was 7.5%.

Total IGF-1 was analyzed in plasma using a commercially available enzyme linked, immunoassay kit (Diagonostics Biochem, London; Immunodiagnosticsystem, Fountain Hills; Cayman Chemical Company, Michigan) according to the procedures of the manufacturer. Samples were incubated with a reagent to inactivate binding proteins and then diluted. A purified sheep polyclonal anti-IGF-1 was coated onto the inner surface of polystyrene microtitre wells. The pre-treated, diluted samples were then incubated, together with horseradish peroxidase labelled monoclonal anti-IGF-1, in antibody-coated wells for 2 hours at room temperature. The wells are washed and a single component chromogenic substrate was added to develop colour. Measured at 450 nm, the intensity of the yellow colour is proportional to the original IGF-1 concentration (ImmunoDiagnostics 2008) and quantification of the samples was performed with a calibration curve. The intra-assay CV for these duplicate samples was 7.5%.

Insulin was analyzed in plasma using a commercially available radioimmuno-assay (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles) according to the procedures of the manufacturer. Briefly, ^{125}I-labelled insulin competed for a fixed time with insulin in the patient sample for sites on insulin-specific antibody. The radioactivity in the tubes

was counted for 1 minute in a gamma counter and a standard curve of known values was used to quantify the insulin present in the sample. The intra-assay CV for the duplicate samples was 18.9%.

Statistical Analysis

A 3 dose (placebo, low, high) × 6 blood samples (6 time points) repeated measures ANOVA was used to determine whether there were any differences between the different dosages and the placebo condition over time for the dependent variables (plasma L-arginine, NOx, GH, insulin, IGF-1, and glucose). Integrated area under the curve (iAUC) for the 3 h of sampling was determined for each variable using Prism software (Graphpad 4, San Diego, CA) and a 1-way repeated measures ANOVA was used to determine if the iAUC was different between conditions. iAUC was used to assess the complete bioavailability of each hormone and metabolite over time. Significant F ratios were further analyzed with a Tukey's multiple comparison procedure. All results are expressed as means ± standard deviation (unless otherwise noted). Statistical significance was set at $p \leq 0.05$.

2.3 Results

Resting values for all the dependent variables (L-arginine, NOx, GH, insulin, IGF-1, and glucose) were not significantly different between conditions ($p > 0.05$). Two subjects reported mild GI distress after consuming the highest relative dose of L-arginine, while no side effects were noted during the low dose or the placebo condition.

L-arginine plasma concentrations significantly increased ($p < 0.0001$) to a similar concentration at all time points in both the low and high dose conditions, while there was no change in the placebo condition over time (Figure 1A). There was a significant main effect for insulin ($p = 0.003$) that showed the concentration at 30 and 90 minutes was increased from 180

minutes (Fig 5A). NOx concentration was different between rest, 30 and 60 minutes compared to 180 minutes (main effect, p=0.004; Figure 3A). There was no significant difference over time or between conditions for GH (Figure 2A; time: p=0.166, group: p=0.892), IGF-1 (Figure 4A; time: p=0.584, group: p=0.995), and glucose (Figure 6A; time: p=0.623, group: p=0.982).

The iAUC analysis for plasma L-arginine concentrations revealed a significant difference between the L-arginine conditions compared to the placebo and between the high and low relative doses (Figure 1B; p<0.0001). The iAUC for NOx with the high relative dose was significantly lower than the low dose of L-arginine (Figure 3B; p=0.023). There were no significant differences between conditions for iAUC for GH (Figure 2B; p=0.551), IGF-1 (Figure 4B; p=0.621), insulin (Figure 5B; p=0.314), and glucose (Figure 6B; p=0.418).

2.4 Discussion

This study was the first to compare the effect of two different doses of L-arginine (relative to body mass) on plasma L-arginine, NOx, GH, insulin, IGF-1, and glucose concentrations in physically active subjects. The main findings of the present study were that both a low and high dose of L-arginine delivered orally increased plasma L-arginine concentrations significantly (178% and 204% above resting values, respectively) that earlier research has not documented (19, 24, 25). As well, the two doses were not significantly different at any single time point; however, the high dose of L-arginine did provide a significantly greater iAUC compared to the low dose (e.g. GH). Previous research has shown that a substantial amount of orally administered L-arginine does not enter the systemic circulation because approximately 40% of orally administered dietary L-arginine was degraded by the small intestine (26, 27). Furthermore, high doses of L-arginine can cause GI distress due to the osmotic movement of water into the stomach and intestine (19, 28) potentially leading to an increase in

the excretion of L-arginine (19, 28, 29). In the present study two of the fourteen subjects reported gastro-intestinal distress with the high dose of L-arginine (0.15 $g \cdot kg^{-1}$ or ~12 g of L-arginine), whereas Collier et al. (19) reported seven out of eight subjects having gastro-intestinal distress with an absolute oral dose of 13 g. Therefore, the relative dosing method used in this study was able to reduce this side effect. However, despite the increase in L-arginine and absence of side effects, the doses used in the present study had little effect on certain metabolites or hormonal concentrations at rest.

The signalling molecule, NO, is produced by the NOS group of enzymes which catalyze the oxidation of L-arginine yielding NO and L-citruline (30). It is well known that NO production can elevate cyclic guanosine monophosphate (cGMP), resulting in the relaxation of smooth muscle and vasodilation, and there is increasing evidence that interventions which influence NO bioavailability and perhaps through enhanced blood flow can also alter the O_2 cost of exercise in humans (18, 31, 32) and may enhance blood flow (5) and nutrient delivery (8). Further, exogenous L-arginine administration has been reported to increase urinary (nitrate) (33) and plasma [nitrite]+[nitrate] (NOx; (34)) in mice. However, in healthy humans, markers of NO bioavailability were not increased (17, 32, 35) which supports our findings. Recently, Bailey et al. (18) found an increased plasma nitrite concentration following 6 g of L-arginine supplementation. A potential difference is the marker used for NO bioavailability. Thus, a potential limitation of the present study and recent studies (17, 32, 35-37) may be that nitrate+nitrite which are conventionally used to assess NOS activity may not be as sensitive as nitrite alone (38). Bode-Boger et al. (5) demonstrated that intravenous infusion of 30 g of L-arginine significantly increased arterial blood flow in the femoral artery of healthy subjects by a mean of 44%. However, in a subsequent study, a lower dose of L-arginine (6 g), administered

either intravenously or orally, failed to produce acute vasodilation. Furthermore, Schellong et al. (8) found that a single systemic infusion of 30 g of L-arginine increased nutritive muscle blood flow by a mean 43%, whereas a lower dose of 8 g of L-arginine had no significant effect. This latter research suggests that to achieve a metabolic effect at rest in healthy young humans it seems that a high dose, beyond which can be consumed orally may be necessary. Conversely, iAUC for NOx was higher for the low L-arginine dose compared to the high L-arginine dose. The reason for this is unclear but it is possible that the high dose of L-arginine consumption may resulted in an increase production of assymetrical dimethylarginine (ADMA), a naturally occurring endogenous L-arginine metabolite than can inhibit all NOS isoforms, thereby reducing NOx (4).

L-arginine is also known to enhance the maximal pituitary somatotroph responsiveness to GHRH, and increase GH release on the hypothalamic level by suppression of endogenous growth hormone inhibiting hormone release (11). Previous research using IV administration of 1/12, 1/6, and 1/4 g of L-arginine per pound of body mass demonstrated an increase in GH response with each level of dosing (39). However, the lowest dose (1/12 $g \cdot pound^{-1}$ of body mass) did not significantly increase GH. Previous research has demonstrated that orally administering absolute amounts of 5 and 9 g of L-arginine resulted in a differential effect on circulating concentrations of GH (19), while other research has not shown a dose effect (25, 40-42). The present study supports this latter research because no differences were observed for circulating concentrations of GH when different doses of L-arginine relative to body mass were ingested. It is also important to note that Collier et al. (19) removed 2 participants deemed to be non-responders from their data set prior to their final data analyses that may have influenced

their findings. The occurrence of responders and non-responders in healthy participants ingesting L-arginine at rest is not currently known.

The metabolic effects of GH are diverse and involve increased lipolysis, hyperinsulinemia, and elevated blood concentrations of IGF-1 (12, 13). In fact, many athletes use L-arginine to stimulate the GH-IGF-1 axis, believing that this will promote gains in strength and muscle mass (43). The complex anabolic effects leading to tissue growth, repair, and re-modeling processes are also regulated by changes in insulin (10, 14). However, since we did not observe a significant GH response, there was no expectation that IGF-1 or insulin concentrations would be increased. The present findings support other research showing minimal physiological effects of different absolute doses of oral L-arginine supplementation (25, 37, 40, 42). Because L-arginine can be synthesized endogenously in the kidney and liver (44) as well as being present in a variety of food sources, the stimulatory effect of supplementing with L-arginine would logically be diminished in a healthy individual at rest and fasted despite some previous research to the contrary (19). A limitation of the present study was that protein was adjusted to 0.8 g/kg/d in all subjects the day prior. It is not clear that this time frame to the adjusted diet would allow for accommodation and may have influenced the present findings. However, this strategy was used to normalize all participants to the same protein intake. Regardless, it has been shown that during periods of rapid growth, a traumatic incident, a pathologic insult, or some other type of physiological stressor such as exercise (14, 45-47), the demand for L-arginine may not be fully met by de novo synthesis and normal dietary intake alone and this needs to be further explored.

2.5 Conclusion

The present study was able to establish that two different relative doses of orally administered L-arginine elevated plasma L-arginine concentrations significantly at rest.

However, neither dose was sufficient to increase any metabolite or hormone measured at any time point at rest. Future research is needed to assess the hormonal and metabolic ergogenic pathway stimulated by L-arginine consumption when combined with acute exercise stimuli.

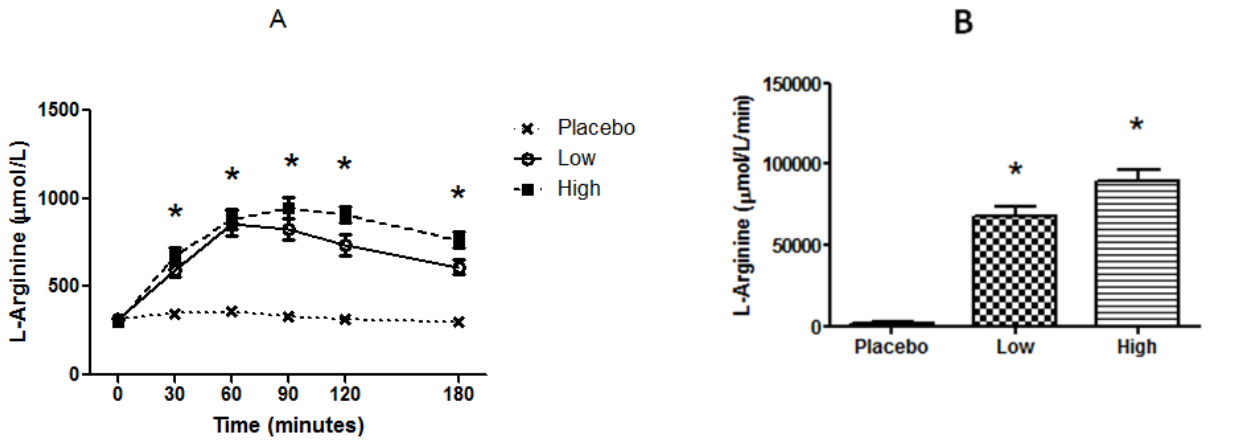

Figure 1 - Mean ± SEM. Plasma L-arginine concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine. * = a significant difference between the L-arginine condition and the placebo condition.

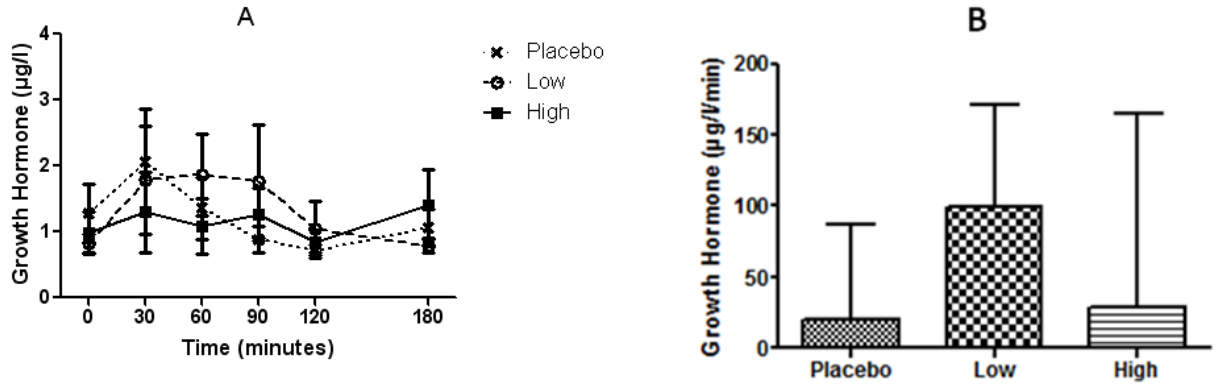

Figure 2 - Mean ± SEM. Plasma growth hormone concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine.

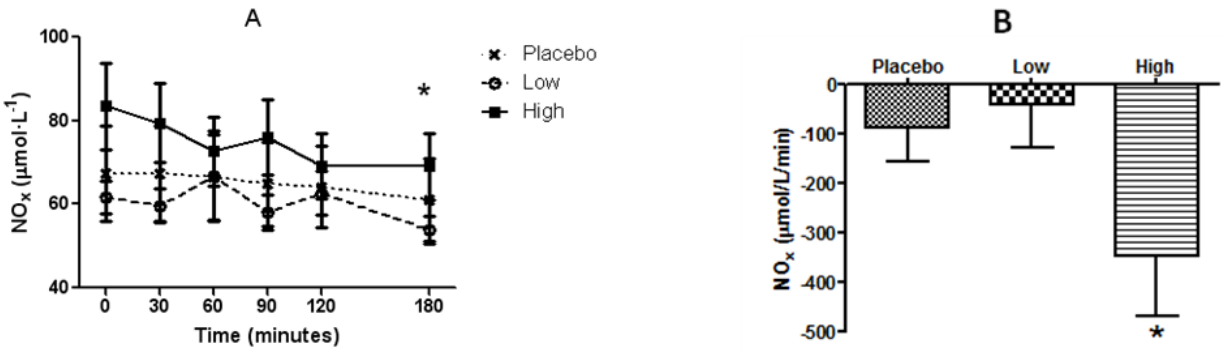

Figure 3 - Mean ± SEM. Plasma nitrate+nitrite concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine. * = indicates a significant main effect.

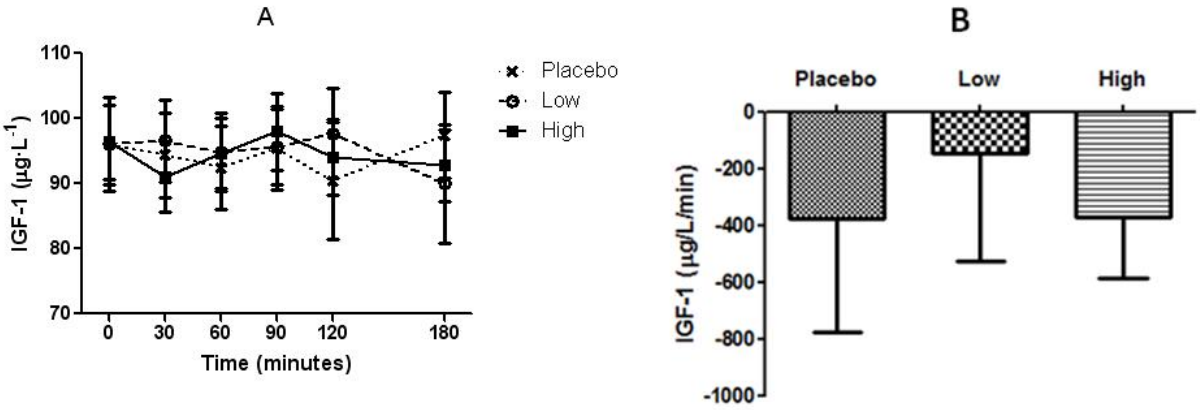

Figure 4 - Mean ± SEM. Plasma insulin like growth factor-1 concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine.

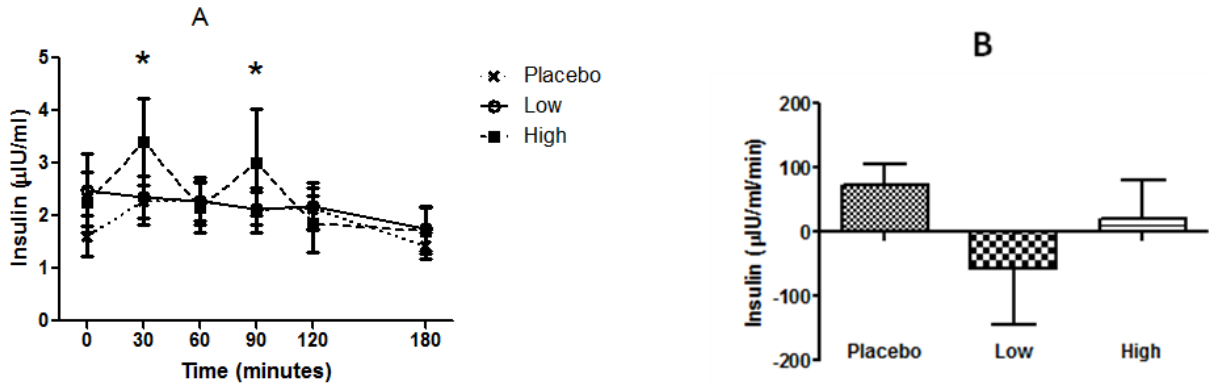

Figure 5 - Mean ± SEM. Plasma insulin concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine. * = a significant main effect.

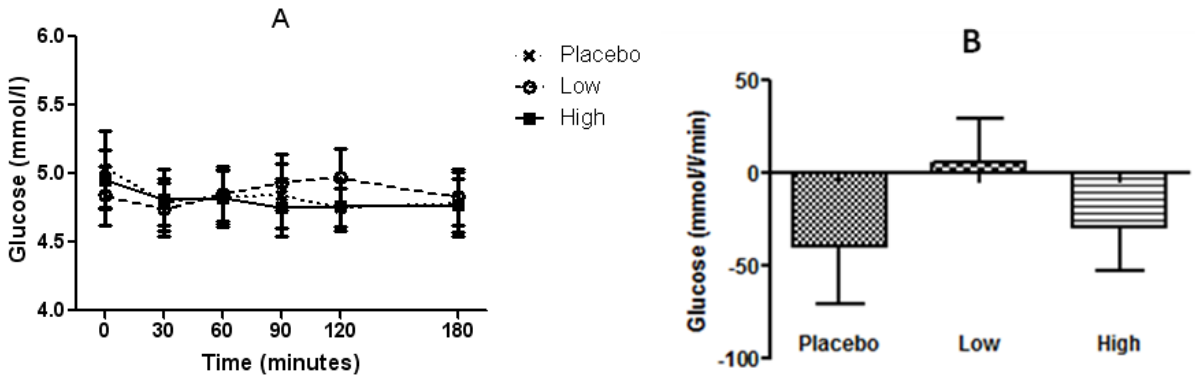

Figure 6 - Mean ± SEM. Plasma glucose concentrations (A) over time and (B) integrated area under the curve after ingesting a placebo, low (0.075g/kg) or high (0.150g/kg) of L-arginine.

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CHAPTER 3

The effects of acute L-arginine combined with heavy resistance exercise on growth hormone, growth hormone secretagogues, and insulin-like growth factor-1 in strength trained males[2]

3.1 Introduction

The popularity of amino acids as supplemental ingredients in various diets and functional foods has increased tremendously with their prevalence being highest in sport nutrition products (1, 2). Strength trained athletes ingest select amino acids in an attempt to stimulate growth hormone (GH) secretion, believing that this practice will promote greater gains in muscle mass and strength compared to resistance training alone (1). An amino acid which has recently gained popularity and has been purported as ergogenic is L-arginine (3, 4). L-arginine (2-amino-5-guanidinovaleric acid) is considered a conditionally essential amino acid. A typical western diet contains ~3-6 $g \cdot d^{-1}$ (5) and in healthy adults, L-arginine can be synthesized in sufficient quantities required for most physiological demands (6, 7). However, during periods of rapid growth, in response to a traumatic or pathologic insult, or due to the physiological demands of exercise, the requirements for L-arginine may not be met by de novo synthesis and normal dietary intake alone (4, 6, 8-10). In such circumstances L-arginine supplementation may be beneficial (9).

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2 A version of this chapter has been submitted:
Forbes SC, Harber V, and Bell GJ. Oral L-arginine prior to resistance exercise blunts growth hormone in strength trained males. *J. Strength Cond. Res.* Submitted Mar 31, 2012.

Intravenous infusion and orally ingested L-arginine have both been shown to increase GH in men (11-13) despite some research showing little change with oral ingestion at rest (14, 15). It has also been established that resistance exercise elevates circulating concentrations of GH (11, 16-19) and this may contribute to gains in muscle mass and strength (17, 20), however these gains in muscle mass may also be attributed to GH stimulatory effects on insulin-like growth factor-I (IGF-I) which has been shown to stimulates the PKB/Akt mTOR muscle protein synthetic pathway (21) and increase satellite cell proliferation and differentiation (22). The way in which L-arginine ingestion may increase GH secretion has been suggested to be due primarily to an inhibition of endogenous GH-inhibiting hormone (GHIH) release (23); while resistance exercise without L-arginine supplementation may enhance GH secretion through a variety of mechanisms such as an augmented hypothalamic secretion of GH-releasing hormone (GHRH) and ghrelin in addition to suppression of hypothalamic GHIH (13, 24).

The literature is equivocal concerning the GH response to resistance exercise when ingesting L-arginine. Marcell et al. (19) found no significant difference with the ingestion of 5 g of L-arginine prior to a bout of resistance exercise compared to resistance exercise alone in untrained participants. Conversely, Collier et al. (12) demonstrated that 7 g of ingested L-arginine attenuates the GH response following a resistance exercise bout in recreationally physical active participants, suggesting that L-arginine supplementation may actually impede the anabolic response. These results may vary based on strength training status, which is known to influence GH responses to resistance exercise (17, 25). Ahtiainen and colleagues (25) demonstrated a significantly higher GH response to a relative bout of resistance exercise in participants that were strength trained. This blunted growth hormone response when combing L-arginine and resistance exercise is similar to studies examining repeated bouts of exercise that

have shown an attenuated GH response to the second exercise bout (26). The mechanism by which GH may be attenuated is unclear (12, 19, 26). Previous research has shown that infusion of IGF-1 suppresses the pulsatile GHRH-stimulated GH secretion in male subjects (27). In addition, GH can directly inhibit its own release, possibly at the pituitary gland (28) or an auto-negative feedback at the level of the hypothalamus mediated by an increase in GHIH and/or a decrease in the release of GHRH. Lanzi and Tannenbaum (29) demonstrated that the immuno-neutralization of GHIH prevented the attenuation of spontaneous GH release after GH pre-treatment in rats. Furthermore, Lanzi and Tannenbaum (29, 30) demonstrated a potential role of GHIH in the attenuation of exogenous GHRH-induced GH release. Therefore, there is controversy as to whether or not L-arginine supplementation prior to a bout of resistance exercises interferes with GH secretion and if so, what mechanism underlies this attenuation and whether this attenuation occurs in strength trained individuals.

The purpose of this study was to investigate GH and GH sercretagues (GHRH, GHIH, and ghrelin) when L-arginine ingestion is combined with resistance exercise in strength trained participants. It was hypothesized that the ingestion of L-arginine combined with an acute bout of resistance exercise will attenuate the GH response compared to resistance exercise alone and will be accompanied by a decrease in plasma GHRH. A secondary hypothesis was to explore the possibility that a GH and IGF-1 are elevated prior to the exercise, because GH and IGF-1 may suppress subsequent stimuli (e.g. exercise) for GH, GHRH, ghrelin, and the release of GHIH (27, 31).

3.2 Methods

Subjects

Eighteen subjects began the study however only fourteen were able to complete the study. Therefore fourteen subjects (age: 25 ± 4 y; body mass: 81.4 ± 9.0 kg; height: 179.4 ± 6.9 cm; body fat: 11.5 ± 3.8%: experience: 6.3 ± 3.4 y; one repetition maximum (1RM) bench press relative to body mass ratio: 1.3 ± 0.2; average protein intake: 1.7 $g \cdot kg^{-1} \cdot d^{-1}$; mean ± SD) participated in this study. Participants were actively strength training at least 3 times per week for at least 1 y. Participants were screened for food allergies, vegetarianism or any medical or musculoskeletal condition that would prevent participation in this study. Participants were not consuming any other nutritional supplement (e.g. creatine or L-arginine) for at least 12 weeks before initial testing. In addition, all subjects were required to complete a Physical Activity Readiness Questionnaire (PAR-Q; Thomas et al. 1992) and provide written informed consent. A University Research Ethics Board for human research approved the study.

Experimental Procedures

This study used a randomized double blind, cross over design and the two conditions were separated by ~7 days. Randomization was accomplished by a computerized random number generator where all participants had an equal chance of being assigned to the two groups. At the first visit subjects completed a medical history and physical activity questionnaire followed by anthropometric (height and body mass) and body composition measurements. Body composition was assessed with hydrostatic weighing according to the procedures previously published for our laboratory (32); body density was calculated and subsequently % body fat was determined using the prediction formula of Siri (33). On the same day each subject completed a 1 repetition maximum (1RM) test for bench press and incline leg press and a multiple RM for 6

other strength exercises (lat pull-down, leg extension, leg flexion, shoulder press, tricep extension, bicep curls, and calf raises) according to the protocols outlined in Bell et al. (42). The multiple RM test results for these latter 6 exercises were used to calculate a predicted 1RM using a computer software program (Power 5.1, Florida, USA), according to the procedures previously published for our laboratory (34). In addition a 2-day food record was completed on the days leading up to visit 2 and the participants were required to consume this exact diet prior to visit 3 (15). This dietary record was analyzed to determine the total daily energy, carbohydrate, fat, protein intake using a computer software program (Food Processor II for Windows, version 6.11; Salem, Ore., USA).

At least 72 hrs following the initial assessment participants returned for the 2 experimental conditions (visit 2 and 3). During visit 2 and 3 (separated by ~7 days) subjects entered the laboratory at 08:00 after a 10-hour overnight fast and no prior exercise the previous 24 hours to obtain a resting blood sample. Following this, the participants were required to consume either L-arginine (NOW Foods, Bloomingdale, Il) in an amount equivalent to 0.075 $g \cdot kg^{-1}$ of body mass (15) or a placebo containing flour in capsule form (0.5g per capsule and the equivalent number of capsules were provided in both conditions, that varied in number based on body mass) with 500 mL of water in a double blind fashion. The L-arginine and placebo capsules were identical in size and shape and a certificate of authenticity was provided (NOW Foods, Bloomingdale, Il). Resting blood samples (~10 mL per sample) were obtained via venipuncture and the multiple blood samples taken during recovery from exercise were obtained using an intravenous catheter inserted into a forearm vein kept patent with sterile saline (0.5 mL of 0.9% NaCl) in a seated position. The intravenous catheter was inserted immediately following the exercise and the first rest recovery blood sample was taken within 2 min. Prior to obtaining each

sample, 2 mL of blood was drawn and discarded to clear the line of saline. Blood samples were taken at rest prior to supplementation, immediately before exercise (60 min following ingestion), and 0, 15, 30, 60 min during rest-recovery. For each sample, two aliquots of blood (~40 uL) were drawn into 50 uL heparinized tubes for the duplicate measurement of hematocrit by micro-centrifugation (International Micro Capillary Centrifuge – MB). The remaining blood samples were immediately put on ice prior to being centrifuged for 10 minutes at 1500 xg. The plasma aliquots were immediately frozen at -20ºC and subsequently stored at -80ºC until analysed.

During visits 2 and 3, resistance exercise was also performed; 3 sets of 10 repetitions (1st set at 65% of 1-RM and the next two sets were set at 75% of 1-RM) for the 8 different strength exercises described previously were performed. A 1.5 minute rest was given between each set (23) and each exercise (total time to completion ~55 min). To maintain hydration status, subjects drank 500 mL of water during the exercise session and 250 mL during recovery for each experimental condition. Heart rate (HR) was recorded from a telemetric monitor (Polar Electro, Finland) at rest, immediately before exercise, and 0, 15, 30, 60 minutes post exercise. A rating of perceived exertion (RPE) borg scale was used to estimate effort immediately upon completion of the exercise session.

Biochemical Analysis

All samples from the same subjects were run in the same order for each assay and in duplicate. L-arginine concentrations were determined spectrophotometrically (35) by the oxidation of NADH using octopine dehydrogenase as previously described for our lab (15). GH, GHRH, GHIH, unacylated ghrelin, and IGF-1 were measured using commercially available enzyme-linked immunoassay kits using the manufacturers recommended procedures. Briefly, GH and unacylated ghrelin were analyzed using a double- antibody sandwich technique that

elicits a colorimetric response. Unknown samples were measured spectrophotometically and compared against known standards (GH: Diagnostics Biochem., Canada Inc., London, Ontario; Unacylated ghrelin: SPI-BIO Bertin Pharma, Montigny Le Bretonneux, France). GHRH, GHIH and total IGF-1 involved a monoclonal antibody procedure and were measured spectrophotometrically (GHRH and GHIH: Uscn Life Science Inc. Wuhan, China;; IGF-1: Diagnostics Biochem Canada Inc.; Immunodiagnostic Systems Inc., Fountain Hills, Ariz., USA; Cayman Chemical Company). The intra-assay coefficient of variation (CV) for the duplicate samples for hematocrit, L-arginine, GH, GHRH, GHIH, ghrelin, and IGF-1 was 1.0%, 3.3%, 14.5%, 7.3%, 8.9%, 6.2%, 7.2%, respectively.

Statistical Analysis

A 2 (placebo vs. L-arginine) x 6 (time points) repeated measures ANOVA was used to examine differences between the two experimental conditions (ARG and PLA) and over time for each dependent variable. Paired t-tests were used to analyze differences between peak heart rate and RPE between the two conditions. The integrated area under the curve (iAUC) for total time was determined for each variable using Prism software (Graphpad 4, San Diego, CA) and a one-way, repeated measure ANOVA was used to assess the iAUC between conditions. iAUC was used to assess the complete bioavailability of each hormone and metabolite over time. Significant F ratios were further analyzed with a Tukey's pair-wise comparison. Statistical analyses were carried out using Statistica, version 8.0 (StatsSoft Inc., Tulsa, OK). All results are expressed as means ± standard deviation, except for the figures where means ± SEM are presented to enhance visual appeal. Statistical significance was set at $p \leq 0.05$.

3.3 Results

Of the original 18 participants that volunteered, 14 completed the study. Three participants experienced emesis and 1 subject became light-headed and could not complete the resistance training session when L-arginine was ingested. Results from these participants were excluded. Fasting values at rest were not significantly different between conditions for all dependent variables (L-arginine, GH, GHRH, GHIH, ghrelin, IGF-1, HR; $p>0.05$).

There was no significant difference over time or between conditions for hematocrit. There was no difference in physical effort between conditions as indicated by peak HR (ARG: 148 ± 18 bpm; PLA: 147 ± 23 bpm, $p>0.05$) and RPE (ARG: 14±2; PLA: 15±2, $p=0.150$). L-arginine plasma concentrations significantly increased after resistance exercise in the ARG condition (120%) while there was no change over time for the PLA condition (Figure 1A; $p<0.001$). There was a significant main effect for time for GH (increased: $p=0.0001$), GHRH (increased: $p<0.0001$), and ghrelin (decreased: $p<0.0001$); however, there were no differences between conditions (Figure 2A,3A,4A; $p>0.05$). IGF-1 was elevated immediately following exercise compared to 30 and 60 min of rest-recovery (Figure 5A; $p=0.001$). GHIH was significantly lower in the L-arginine condition (Figure 6A; $p=0.009$).

The analysis of the iAUC for plasma L-arginine ($p<0.0001$) and GH ($p=0.0492$) revealed a significant difference between conditions, as shown in Figure 1B and 2B, respectively. There were no significant differences in iAUC for GHRH, ghrelin, IGF-1, or GHIH (Figure 3B, 4B, 5B, 6B; $p>0.05$).

3.4 Discussion

Strength-trained athletes consume select amino acids in the belief that they will increase anabolic hormone responses such as GH beyond resistance exercise alone and lead to greater

increases in strength and muscle mass (1). Intravenous administration of L-arginine has been shown to be effective for inducing increases in GH in both clinical and non-clinical settings (8, 13). Research has previously shown that different doses of orally administered L-arginine (0.075 and 0.150 $g \cdot kg^{-1}$ of body mass) at rest significantly elevate circulating concentrations of L-arginine in the blood (15); however the effect of L-arginine consumed orally on GH responses associated with resistance exercises is unclear (12, 19). The main finding of the present study is that there was an attenuated GH response, which supports one of the present hypotheses; but this occurred despite a suppression of GHIH and no difference was observed between conditions for GHRH, ghrelin, and IGF-1, when L-arginine was combined with resistance exercise vs resistance exercise alone. This blunted GH response occurred even though there was no significant difference between conditions for GHRH, ghrelin, and IGF-1.

Previous literature examining L-arginine ingestion prior to an acute bout of resistance exercise has demonstrated either no difference (19) or an attenuated GH response (12). Marcell et al. (19) examined the effects of ingesting 5 g of L-arginine prior to a bout of resistance exercise (3 sets of 8-10 repetitions at 85% 1RM) on GH in untrained males and females. Although this latter research found no statistical difference, the authors reported that the AUC was ~20% lower in the L-arginine condition compared to the placebo condition. More recently, Collier et al. (12) examined the effects of ingesting 7 g of L-arginine prior to a resistance training session (3 sets of 10 reps at 80% 1RM) in recreationally active males. They observed a significantly reduced GH response as indicated by the AUC in the L-arginine and exercise condition compared to exercise alone. These varying results may be due to differences in participant sex, training status, dosing, timing, and lack of statistical power due to small sample sizes and high individual variability. Neither study measured plasma L-arginine concentrations,

GH secretagogues, nor did they examine strength trained participants (12, 19). Strength training status is known to influence GH responses to resistance exercise (16, 17, 25) and Ahtiainen et al. (25) demonstrated a significantly higher GH response to a relative bout of resistance exercise in participants that were strength-trained. In the present investigation, strength trained participants were used and it was shown that resistance exercise alone significantly elevated circulating concentrations of plasma GH above resting values supporting Collier and colleagues (12). However, when L-arginine was consumed prior to resistance exercise, the GH response was attenuated (-192%) in the strength-trained participants of the present study which is consistent with previous research (12).

The attenuation in GH as a result of consuming L-arginine prior to resistance exercise has been postulated to be due to differences in the mechanism of GH release (12). The regulatory augmentation mechanism of L-arginine, when 30 g was administered intravenously, on GH release has been shown to be through a suppression of GHIH at rest in healthy men (23, 36). L-arginine has no known direct effects on GHRH release or the ghrelin effector pathway (13). The regulatory control of GH release during exercise is less clear. Some evidence suggests that exercise stimulates GH release by similar mechanisms as L-arginine via suppression of GHIH. More recently, deVries et al. (24) exposed healthy men to an incremental exercise bout on a cycle ergometer and demonstrated that the exercise-induced GH response is not due to complete inhibition of hypothalamic GHIH release but that exercise also stimulates other GH secretagogues such as GHRH. However, other factors such as adrenergic and cholinergic stimulation, metabolic acidosis and the metabo-reflex have been implicated in controlling GH release (37-40).

Therefore, it could be postulated that combining L-arginine and resistance exercise should induce a larger GH release compared to exercise alone. However, the present study and others (12, 19) have been unable to support this hypothesis. Collier et al. (12) postulated two possible reasons for the attenuated GH response when L-arginine was ingested prior to an acute bout of resistance exercise. First they noted that the attenuated GH response was similar to research that has shown a reduction of the GH after repeated intravenous injections of GHRH at rest (36). This latter response was thought to be due to a down regulation of GHRH-induced GH release. Secondly, it has also been shown that acute resistance exercise (16, 17) and potentially L-arginine supplementation (9) can stimulate the release of IGF-1 which is involved in an auto-negative feedback loop that reduces GH release (12, 27). In addition, GH can directly inhibit its own release, possibly at the pituitary gland (28) or via an auto-negative feedback at the level of the hypothalamus mediated by an increase in GHIH release and/or a decrease in the release of GHRH. Lanzi and Tannenbaum (29) demonstrated that the immuno-neutralization of GHIH prevented the attenuation of spontaneous GH release after GH pre-treatment in rats. In addition, Lanzi and Tannenbaum (29, 30) demonstrated a potential role of GHIH in the attenuation of exogenous GHRH-induced GH release. Furthermore, ghrelin is known to be a potent GH secretagogue so along with GHRH it may be a synergistic stimulator of GH release during exercise (24). However, there were no differences between conditions and, if anything, a decrease in ghrelin following resistance exercise was observed, which supports previous research (41). These results may be associated with hunger (41) and suggest that ghrelin is not a stimulant for the resistance exercise-induced GH release.

The present study found a significant elevation in GHRH during recovery in both conditions; however, there was no change over time for GHIH. This suggests that the primary

mechanism to enhance plasma GH concentrations during resistance exercise is through the stimulation and elevation of GHRH. Although there was no significant difference between conditions for GHRH; the placebo condition did demonstrate a 28% higher peak GHRH compared to the L-arginine condition, indicating a possible down regulation of GHRH release with L-arginine ingestion. However, caution is advised with this interpretation due to the lack of statistical significance and no direct measures of receptor activation were assessed. Furthermore the present results do not support the auto-negative feedback loop via enhanced IGF-1 or GH because IGF-1 was not elevated from rest and GH did not rise prior to the exercise bout nor was there any significant difference between conditions.

It remains possible that part of the blunted GH response may have been due to the timing of the exercise following the ingestion of L-arginine. Previously, Marcell et al. (19) and Collier et al. (12) found either no difference in GH response or a blunted response when L-arginine was ingested 30 minutes prior to exercise while the present study consumed the supplement 60 minutes prior to exercise. Perhaps, consuming the supplement immediately before, during or after the exercise session may eliminate any potential auto-negative feedback or down regulation.

3.5 Conclusion

Acute L-arginine supplementation when combined with resistance exercise blunted the resistance exercise-induced rise in plasma GH concentration. The reason for this was not clear in the present study; however, the attenuation in GH after resistance exercise did not appear to be due to changes in circulating concentrations of GHRH, ghrelin, or IGF-1. This information is important for strength trained athletes who purchase this supplement in the belief that it will

unequivocally enhance GH concentrations after resistance training and enhance the training stimulus. Consequently, the use of L-arginine ingestion by strength athletes seems unwarranted.

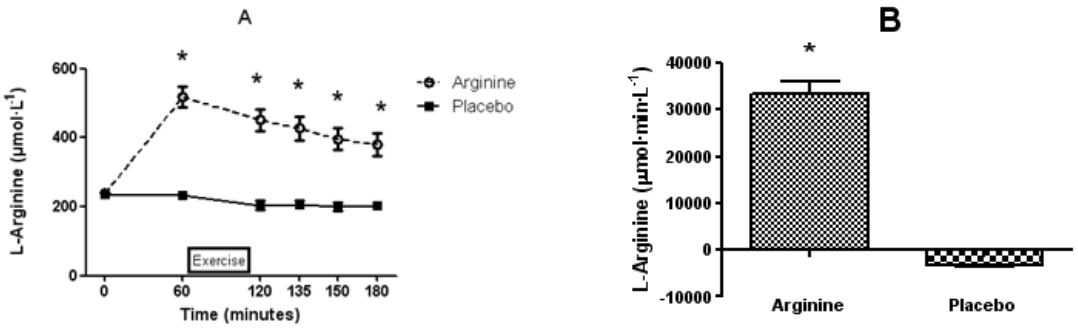

Figure 1 - Mean ± SEM. Plasma L-arginine concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. * = a significant difference between the L-arginine condition and the placebo condition ($p<0.0001$).

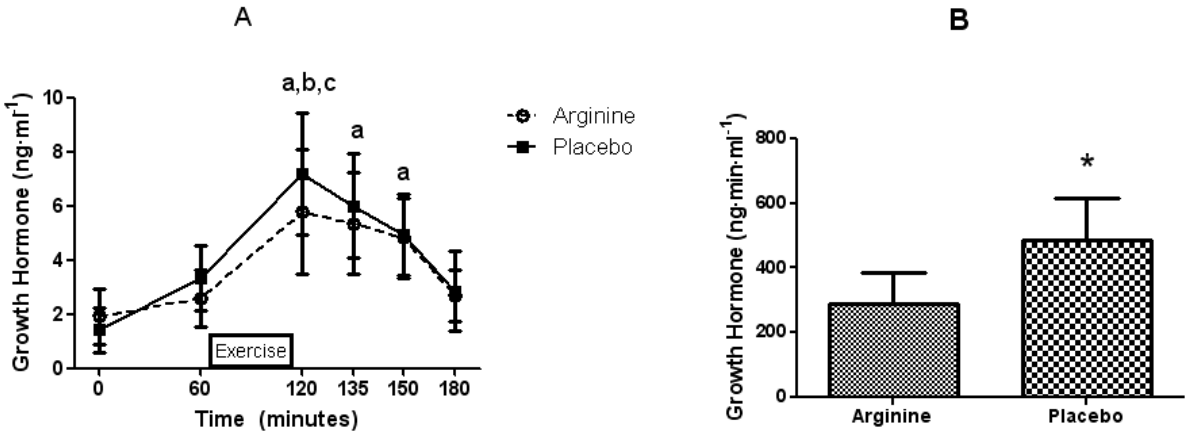

Figure 2 - Mean ± SEM. Plasma growth hormone (GH) concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. * = a significant difference between the L-arginine condition and the placebo condition. a = significant difference from 0 min time point. b = significant difference from 60 min time point. c = significant difference from 180 min time point.

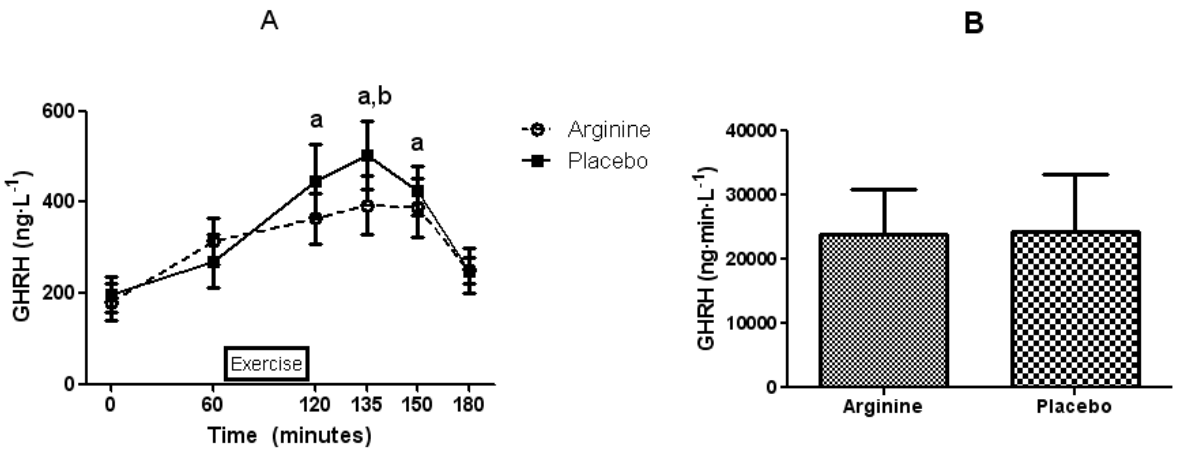

Figure 3 - Mean ± SEM. Plasma growth hormone releasing hormone (GHRH) concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. a = significant difference from 0 min time point. b = significant difference from 180 min time point.

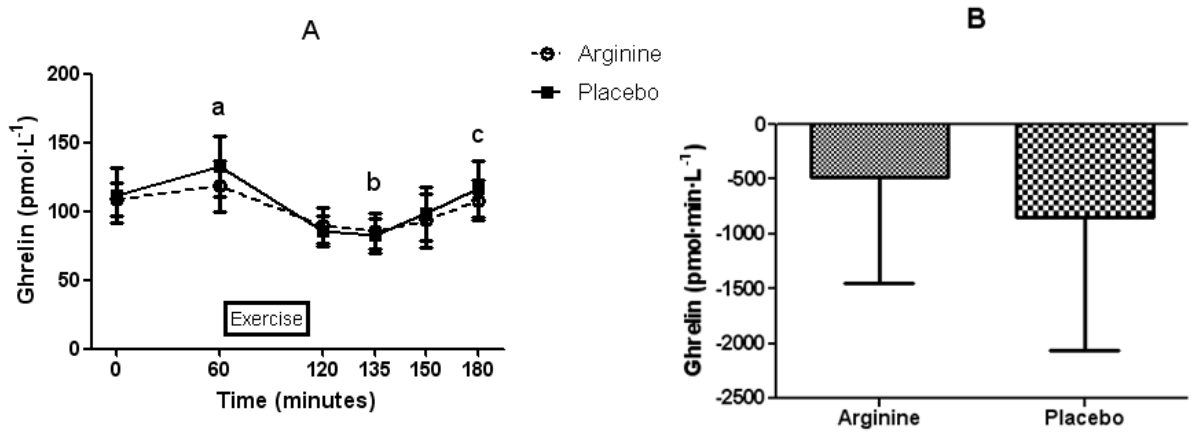

Figure 4 - Mean ± SEM. Plasma ghrelin concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. a = significant difference from 135, 150, 180 min time points. b = significant difference from 0, 60, 180 min time points.

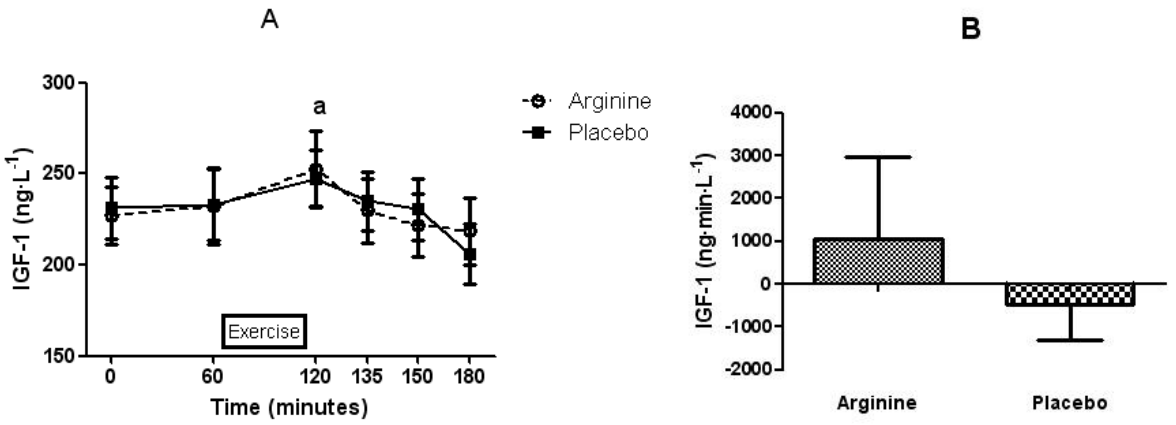

Figure 5 - Mean ± SEM. Plasma insulin-like growth factor-1 concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. a = significant difference from 150, 180 min time points.

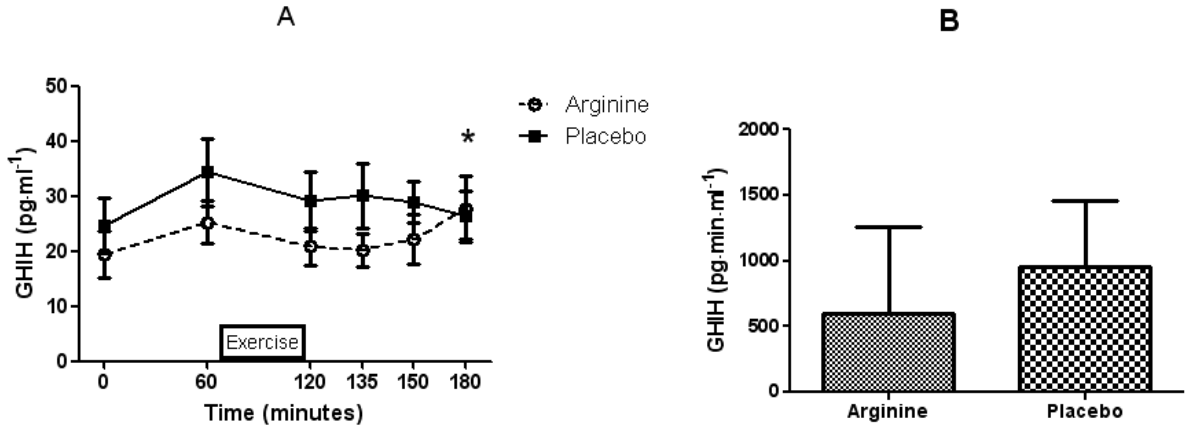

Figure 6 - Mean ± SEM. Plasma growth hormone inhibiting hormone concentrations (A) over time and (B) integrated area under the curve after ingesting L-arginine or a placebo and completing a resistance exercise session. * = a significant difference (group effect) between the L-arginine condition and the placebo condition.

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CHAPTER 4

The acute effects of L-arginine on hormonal and metabolic responses during submaximal exercise in trained cyclists

4.1 Introduction

L-arginine (2-amino-5-guanidinovaleric acid) is a conditionally essential amino acid and has been purported to be ergogenic (1-4). It has been suggested that L-arginine can enhance endurance exercise performance by two primary mechanisms including an enhanced secretion of endogenous GH and NO. First, GH may influence endurance exercise performance by enhancing lipolysis (5) and fat oxidation (6). During submaximal exercise, GH administration has shown to increase plasma glycerol and NEFA in healthy (7, 8) and well-trained endurance athletes (7, 9). These effects may in turn increase time to exhaustion during exercise by sparing skeletal muscle and liver glycogen (1, 10). Secondly L-arginine may enhance endurance performance through a NO induced vasodilation response (1, 11). Nitric oxide is a signalling molecule produced by the NOS group of enzymes, catalyzed by the oxidation of L-arginine as precursor (1, 12). Nitric oxide production can elevate cyclic guanosine monophosphate (cGMP), resulting in the relaxation of smooth muscle and inducing vasodilation, and there is increasing evidence that interventions that influence NO bioavailability can also alter the O_2 cost of exercise (11, 13, 14), influence blood flow (15, 16), nutrient delivery (17-19) and aid in metabolic waste product removal (20). However, there is controversy as to the effectiveness of L-arginine ingested orally for increasing GH and NO bioavailability in humans at rest and during endurance exercise (11, 14, 21-26).

In animals and in some diseased populations, such as with congestive heart failure, stable angina, and pulmonary hypertension, L-arginine ingestion has demonstrated positive effects on

aerobic exercise performance and skeletal muscle adaptations (27-31); however in healthy physically active or trained athletes the research has shown a positive effect (20, 21), no effect (19, 32), or a detrimental effect (33) on performance. Schaefer et al. (20) examined L-arginine (3 g) administered intravenously and found a lower lactate response during submaximal exercise. Others (19, 32) found no effect on graded exercise tests and submaximal aerobic exercise in physically active participants.

Currently there is little research that has examined the GH response to L-arginine consumed orally when combined with aerobic exercise. Therefore, the purpose of this study was to evaluate whether L-arginine consumed orally prior to a bout of aerobic exercise would stimulate plasma GH above exercise alone, and to examine the subsequent metabolic (NEFA, glucose, glycerol, lactate, VO_2, VCO_2, and RER) responses in endurance-trained individuals. It was hypothesized that the ingestion of L-arginine prior to submaximal aerobic exercise will increase circulating concentrations of GH which will promote an increase in circulating concentrations of glycerol and NEFA while lowering lactate concentration during exercise compared to aerobic exercise alone. A secondary hypothesis was to explore the possibility that L-arginine consumption prior to an aerobic bout of exercise will increase markers of nitric oxide (nitrate+nitrite; NOx) production.

4.2 Methods

Subjects

Fifteen aerobically trained men (age: 28 ± 5 y; body mass: 77.4 ± 9.5 kg; height: 180.9 ± 7.9 cm; body fat: 11.5 ± 3.5%; experience: 5.9 ± 3.4 y; VO_2 max: 59.6 ± 5.9 $ml \cdot kg^{-1} \cdot min^{-1}$, average protein intake: 1.5 ± 0.3 $g \cdot kg^{-1} \cdot d^{-1}$: mean ± standard deviation) participated in this study. Participants were actively training at least 3 times per week during the 12-month period prior to

the start of this study. Participants were verbally screened for food allergies, vegetarianism or any medical condition that would prevent participation in this study and an investigator recorded this information. Participants were required to abstain from consuming any other type of nutritional supplement for at least 12 weeks before the start of the study to eliminate effects from other supplementation. In addition, all subjects were required to complete a Physical Activity Readiness Questionnaire (PAR-Q; Thomas et al. 1992) and provide written informed consent. A University Research Ethics Board for human subject research approved the study.

Experimental Procedures

This study used a randomized double blind, cross over design and the two conditions were separated by ~7 days. Randomization was accomplished by a computerized random number generator where all participants had an equal chance of being assigned to the two groups. At the first visit subjects completed a medical history and physical activity questionnaire followed by anthropometric (height and body mass) and body composition measurements. Body composition was assessed with hydrostatic weighing according to the procedures previously published (34); body density was calculated and subsequently % body fat was determined using the prediction formula of Siri (35). In addition a 2-day food record was completed, analysed and returned to the participants so that they could consume this exact diet 2 days prior to each experimental condition. This dietary record was analyzed to determine the total daily energy, carbohydrate, fat, protein intake using a computer software program (Food Processor II for Windows, version 6.11; Salem, Ore., USA). During this same visit each participant completed an exercise test to determine maximal oxygen consumption ($\dot{V}O_{2\,max}$). The $\dot{V}O_{2\,max}$ was performed on a cycle ergometer (Monark, 828E, Sweden) and involve a graded, incremental exercise to volitional exhaustion. Subjects began cycling at 80 watts (W) and maintained a

pedaling frequency of 80 revolutions per minute (rpm). Every 2 minutes the power output was increased by 40 W; after ventilatory threshold (VT) was reached, power output was increased by 40 W every minute until $\dot{V}O_{2\,max}$ was attained. Ventilatory threshold during the tests were determined by a decrease and plateau in the minute ventilation to carbon dioxide production ratio (VE/VCO_2) before a systematic increase with increased power output (36) and a respiratory exchange ratio greater than 1.0. Following the test, the ventilatory threshold was determined as the point at which CO_2 production and O_2 consumption deviated from linearity using the V-slope method (37). Attainment of VO_2 max was indicated by a least two of the following: levelling (<0.100 L·min^{-1}) or decrease in VO_2 with increasing workload; a plateau in heart rate (<5bpm) and (or) attainment of age predicted maximum heart rate; a respiratory exchange ratio >1.1; and volitional fatigue (38). During the test, expired air was collected and analyzed for O_2 and CO_2 using a calibrated metabolic system (ParvoMed True Max 2400, Utah). Heart rates were recorded every minute from the receiver of a telemetric heart rate monitor (Polar Electro, Finland).

At least 72 h and a maximum of 1 week following the initial assessment participants returned for the two experimental conditions (visit 2 and 3). During visit 2 and 3 (separated by ~7 days) subjects returned to the laboratory at 08:00 after a 10-hour overnight fast and no prior exercise to obtain a resting blood sample. Following this, the participants were provided either L-arginine (NOW FOODS; Bloomingdale, IL); 0.075 g·kg^{-1} of body mass (25) or a placebo containing flour in capsule form (0.5g per capsule and the equivalent number of capsules were provided in both, that varied in number based on body mass) with 500 ml of water in a double blind fashion. Resting blood samples (~10 ml per sample) were obtained via venipuncture while exercise and post exercise blood samples were obtained using an intravenous catheter inserted

into a forearm vein kept patent with sterile saline (0.5 ml of 0.9% NaCl) in a seated position. Note that 2 ml of blood was drawn and discarded prior to obtaining each sample to ensure no saline was present in the sample used for analysis. Blood samples (~10 mls) were taken at rest, immediately before exercise, every 15 min during exercise, and 15, 30, 60 min post exercise. Two aliquots of blood were removed for the measurement of hematocrit by microcentrifugation (International Micro Capillary Centrifuge – MB). The remaining blood samples were immediately put on ice and centrifuged for 10 minutes at 1500 xg at 1°C. The plasma was aliquotted, immediately frozen at -20ºC and subsequently stored at -80ºC until analysed.

The exercise protocol consisted of a standardized warm up at 40 W for 5 minutes followed by 60 minutes at a power output equivalent to 80% of the power output achieved at VT, as determined during the $\dot{V}O_{2\,max}$ protocol. To maintain hydration status, subjects drank ad libitum during and following the exercise bout and were required to consume this identical volume of water during the second experimental condition. Heart rate (HR) was recorded from a HR monitor throughout the test. Blood pressure from an automated device (Automatic Plus Blood Pressure Monitor, Life Brand, Toronto, Ontario) at rest, immediately before exercise, and during recovery and manual blood pressure was obtained during exercise. The ratings of perceived exertion (RPE) scale (Borg, 1970) was used to estimate effort during exercise at 15, 30, 45 and 60 min. Expired-gas samples for determination of VO_2, carbon dioxide output (VCO_2), and the respiratory exchange ratio (the ratio between VCO_2 and VO_2) were collected for 6-minute periods at the beginning of exercise (1-6 minutes), middle (27-33 minutes), and end (54-60 minutes).

Biochemical Analysis

All samples from the same subjects were assayed in the same order and in duplicate. L-arginine concentrations were determined spectrophotometrically (40) by the oxidation of NADH using octopine dehydrogenase. GH, glycerol, NOx were measured using commercially available enzyme-linked immunoassay kits (GH: Diagnostics Biochem., Canada Inc., London, Ontario; glycerol and NOx: Cayman Chemical Company). Plasma glucose and lactate were determined spectrophotometrically (Sigma Chemical Inc. USA). Plasma NEFA was determined by an enzymatic colorimetric procedure (NEFA-C test; Wako, USA). The intra-assay coefficient of variation (CV) for the duplicate samples for hematocrit, L-arginine, GH, NEFA, glycerol, glucose, lactate, and NOx was 1.2%, 3.1%, 9.8%, 7.8%, 4.2%, 5.0%, 8.2%, 4.3%, respectively.

Statistical Analysis

A 2 (placebo vs. L-arginine) x 6 (time points) repeated measures ANOVA was used to examine differences between the two experimental conditions (ARG and PLA) and over time for each dependent variable. Paired t-tests were used to determine whether there were any differences between peak heart rate and RPE between the two conditions. Significant F ratios were further analyzed with a Tukey's paired-wise comparison. Statistical analyses were completed using Statistica, version 8.0 (StatsSoft Inc., Tulsa, OK). All results are expressed as means ± standard deviation. Statistical significance was set at $p \leq 0.05$.

4.3 Results

All of the participants completed the study with no reported or observed side effects. Resting values for all the dependent variables (L-arginine, GH, NEFA, glycerol, lactate, glucose, glycerol, NOx, blood pressure, HR) were not significantly different between conditions ($p > 0.05$).

There was no significant difference over time or between conditions for hematocrit. L-arginine plasma concentrations significantly increased in the ARG condition (146%) while there was no change over time for the PLA condition (Figure 1; $p<0.0001$). There were significant increases over time in plasma GH ($p<0.0001$), NEFA ($p<0.0001$), lactate ($p<0.0001$), and a significant decrease in glucose ($p<0.0001$); however, there were no differences between conditions. There were no significant differences over time or between conditions for NOx, $p=0.465$. There was a significant difference ($p=0.049$) between ARG and PLA 45 minutes into the bout of exercise for glycerol, as shown in figure 2.

Diastolic ($p=0.08$) and systolic ($p<0.0001$) blood pressure changed over time; however there were no significant difference between conditions ($p>0.05$). Cardio-respiratory data are shown in Table 4.1. Mean VO_2 was ~60% VO_2 max during the exercise protocol, with no differences between conditions ($p>0.05$). There were no difference between conditions for VCO_2 ($L \cdot min^{-1}$), VE ($L \cdot min^{-1}$), respiratory rate (RR), VE/VCO_2, VE/VO_2**,** and RER (VCO_2/VO_2). There was a significant time main effect ($p<0.001$) for RPE indicating an increase throughout the exercise, while no difference between conditions was observed ($p=0.4211$).

4.4 Discussion

This study examined the hypothesis that L-arginine may serve as an ergogenic aid during prolonged submaximal exercise for endurance-trained athletes. Theoretical rationale was based on research indicating that L-arginine administered intravenously (30 g) increased GH (26) that is known to increase lipolysis, NEFA release, and enhance fat oxidation. In addition, L-arginine may increase NO production (11), which has been shown to enhance vasodilation thereby increasing blood flow (41), nutrient delivery (19), and waste product removal (20). This is the first study to examine the effects of L-arginine ingested orally prior to a bout of submaximal

aerobic exercise on GH and the subsequent metabolic responses in trained cyclists. The findings of the present study demonstrated that circulating concentration of L-arginine was increased by 143% in blood and an increased glycerol (15% higher) concentration at the 45 min time point, which partially supports one of the present hypotheses. However, there were no differences between conditions for GH, NEFA, glucose, and lactate that was contrary to the hypothesis. In addition, we found no change in NOx between the two conditions suggesting that L-arginine had no effect on nitric oxide synthase activity.

Intravenous infusion and orally ingested L-arginine at rest have both been shown to increase GH (26, 42, 43) despite some research showing little change with oral ingestion (25, 44). Enhancing GH is known to increase lipolysis and fat oxidation and spare muscle and liver glycogen. Recently, Wideman et al. (26) found an enhanced effect of L-arginine administered intravenously (30 g) prior to a bout of aerobic exercise on plasma GH. The present study found no effect of L-arginine consumed orally prior to a submaximal exercise bout compared to exercise alone in trained subjects. These differences are most likely due to the method of administration; intravenous compared to oral ingestion (45). Previous research has shown that a substantial amount of orally administered L-arginine does not enter the systemic circulation because approximately 40% of orally administered dietary L-arginine was degraded by the small intestine (46, 47). High doses of L-arginine can cause GI distress due to the osmotic movement of water into the stomach and intestine (25, 42, 48) potentially leading to an increase in the excretion of L-arginine (42). The present study showed a significant increase in plasma L-arginine and research has previously shown no difference in L-arginine plasma concentrations when consuming 0.075 $g \cdot kg^{-1}$ compared to 0.150 $g \cdot kg^{-1}$ of body mass of L-arginine, suggesting

that the dose used in the present investigation was appropriate and represented a high oral dose while limiting side effects.

Although there was no statistical difference in GH between conditions, there was a 26% lower GH in the ARG condition, which is similar to previous research examining L-arginine consumed orally prior to a bout of resistance exercise (43). Because this occurred with no changes in plasma NEFA concentration, it is possible that L-arginine ingestion may have led to a reduction in intramyocellular lipid oxidation. However, this is speculative and there was no influence on RER. Further research would be necessary to determine the influence of the L-arginine on intramyocelular lipid metabolism during exercise. It is interesting to note that McConnell et al. (19) infused 30 g of L-arginine prior to a bout of aerobic exercise and found a significant elevation in skeletal muscle glucose clearance. McConell et al. (19) postulated that the elevated glucose clearance may be associated with a greater NO production because plasma insulin concentration was unaffected; however, they did not measure any markers of NO. Interestingly, in the present study, there was a significant increase in glycerol after 45 minutes of exercise, which occurred without a change in GH and NEFA.

Secondly, L-arginine is thought to enhance endurance performance as a precursor for NO. Nitric oxide is a signalling molecule produced by the nitric oxide synthase group of enzymes, catalyzed by the oxidation of L-arginine (12). Nitric oxide production can elevate cyclic guanosine monophosphate, resulting in the relaxation of smooth muscle and vasodilation, and there is evidence that interventions that influence NO bioavailability can also alter the O_2 cost of exercise in humans (11, 13, 14); improve blood flow (15) and nutrient delivery [e.g. O_2, glucose, free fatty acids; (18, 19)]; and, enhance the recovery processes of the activated tissues (e.g. lactate, CO_2; Schafer et al. 2002). Exogenous L-arginine administration has been reported

to increase urinary (nitrate) (27) and plasma [nitrite]+[nitrate] [NOx; (49)] in mice. However, in healthy humans markers of NO bioavailability were not increased (14, 21, 50) which supports the present findings. Recently, Bailey et al. (11) found an increased plasma nitrite concentration following 6 g of L-arginine supplementation. A potential difference is the marker used for NO bioavailability. Bode-Boger et al. (15) demonstrated that intravenous infusion of 30 g of L-arginine significantly increased arterial blood flow in the femoral artery of healthy subjects by a mean of 44%. However, in a subsequent study, a lower dose of L-arginine (6 g), administered by either intravenously or orally, failed to produce acute vasodilation. Furthermore, Schellong et al. (18) found that a single systemic infusion of 30 g of L-arginine increased nutritive muscle blood flow by a mean 43%, whereas a lower dose of 8 g of L-arginine had no significant effect. This latter research suggests that to achieve a metabolic effect in healthy young humans it seems that a high dose, beyond which can be consumed orally may be necessary. In addition, the present study found no difference in any cardio-respiratory parameters, such as VO_2, HR, VE, systolic or diastolic blood pressure.

4.5 Conclusion

L-arginine ingested at a dose of 0.075 $g \cdot kg^{-1}$ prior to aerobic exercise significantly elevates plasma L-arginine concentration in endurance trained cyclists. The acute ingestion of L-arginine before aerobic exercise did not enhance any hormonal, metabolic, or cardio-respiratory parameter except for a small but significant increase in glycerol during submaximal cycling in endurance trained cyclists.

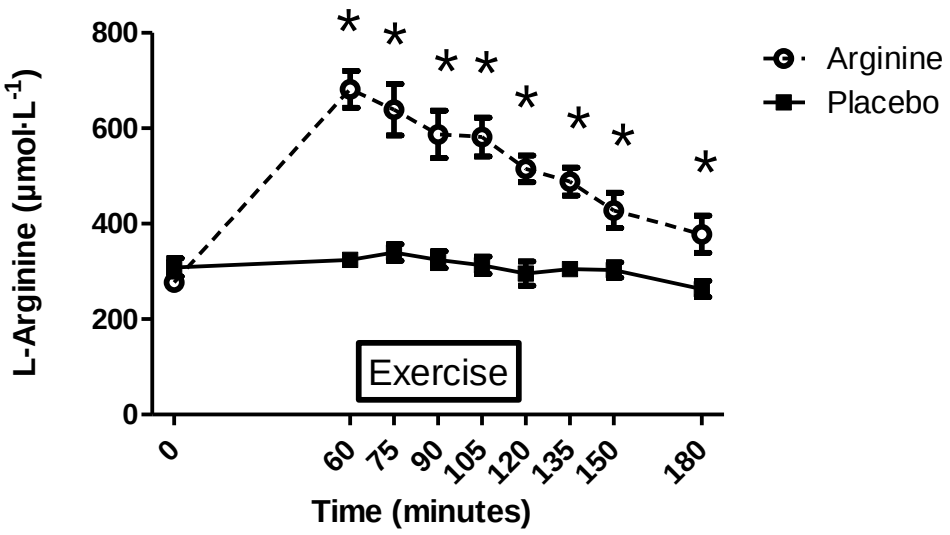

Figure 1 - Mean ± SEM. Plasma L-arginine concentrations over time after ingesting L-arginine or a placebo and completing an aerobic exercise session. n=9. * = indicates a significant difference between the L-arginine condition and the placebo condition.

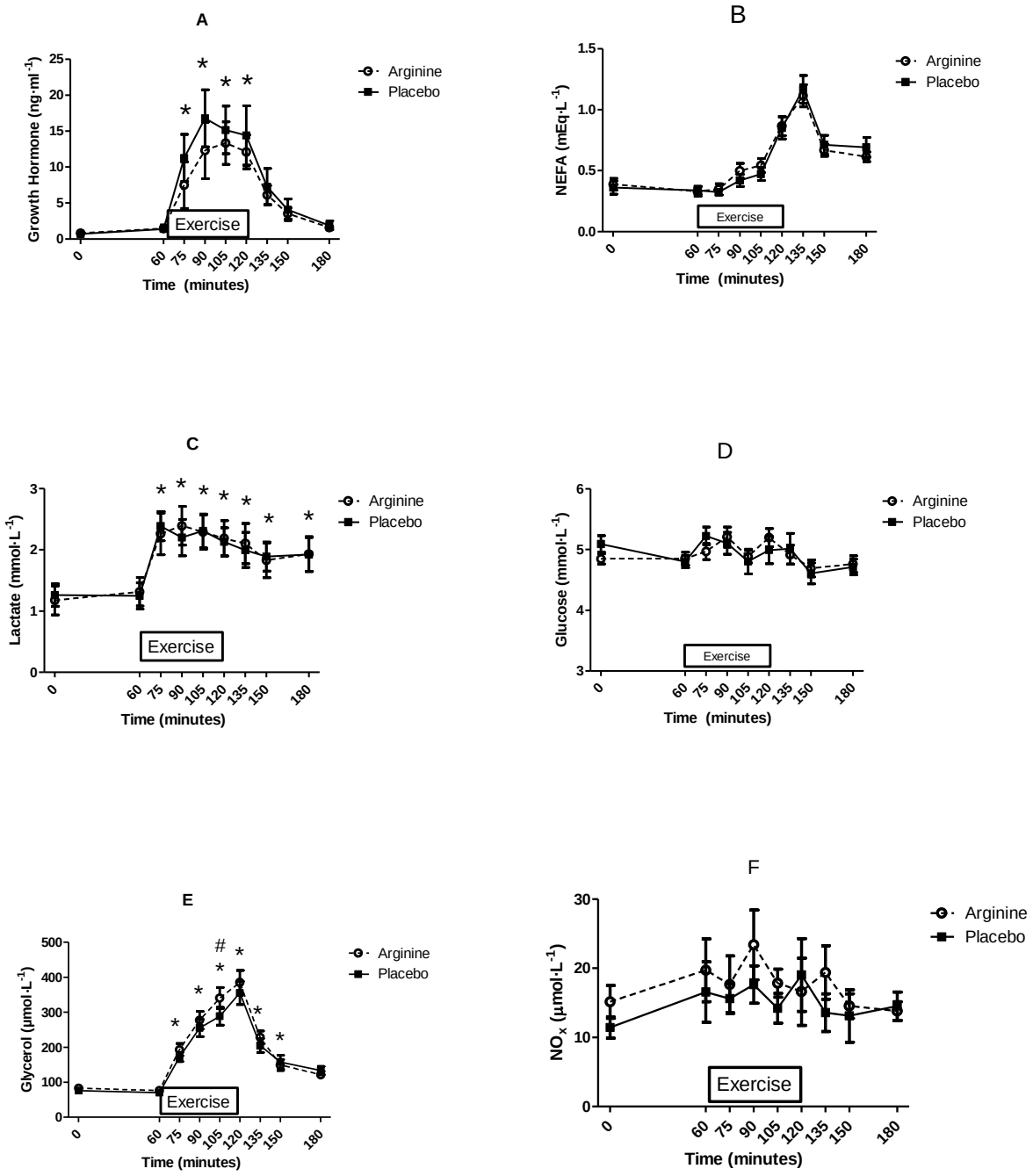

Figure 2 - Mean ± SEM. Plasma (A) growth hormone (GH) (B) NEFA (C) Lactate (D) glucose (E) Glycerol (F) NOx over time after ingesting L-arginine or a placebo and completing an aerobic exercise session. * = significantly different between the arginine and placebo condition.

Table 4.1: Mean ± SD. Cardio-respiratory responses between the L-arginine and placebo condition during the submaximal exercise at the start (0-6 min), middle (27-33 min), and end (54-60 min).

	L-arginine			Placebo		
	Start	**Middle**	**End**	**Start**	**Middle**	**End**
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	35.2±6.5	36.9±6.8	37.0±6.1	34.9±6.2	36.2±5.9	36.5±5.9
VO_2 ($L \cdot min^{-1}$)	2.71±0.54	2.85±0.55	2.86±0.52	2.70±0.52	2.79±0.50	2.80±0.49
VCO_2 ($L \cdot min^{-1}$)	2.49±0.46	2.55±0.45	2.55±0.44	2.44±0.44	2.49±0.43	2.50±0.43
RER (VCO_2/VO_2)	0.92±0.05	0.90±0.04	0.89±0.04	0.91±0.4	0.90±0.3	0.89±0.3
VE ($L \cdot min^{-1}$)	64.3±11.5	69.7±12.2	72.3±12.4	62.2±10.6	68.7±11.5	71.1±12.2
VE/VO_2	24.2±2.2	25.1±2.5	26.0±2.4	23.5±2.0	25.2±2.5	25.8±2.5
VE/VCO_2	25.5±1.8	27.0±2.2	27.9±2.2	25.3±2.1	27.3±2.6	28.2±2.7
RR ($b \cdot min^{-1}$)	24.7±4.3	27.6±5.0	29.6±5.0	23.7±3.7	27.9±5.7	29.45.7

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CHAPTER 5

General Discussion

5.1 Introduction

Despite the popularity of L-arginine as a nutritional supplement in physically active individuals (1) and the purported ergogenic benefits (2), the underlying physiological mechanisms remain to be elucidated. Recently, Maughan, Greenhaff, and Hespel (3) noted L-arginine ingestion as an emerging and growing trend among athletes. The ergogenic benefits of L-arginine have been observed in certain clinical populations, such as individuals with congestive heart failure, stable angina, and pulmonary hypertension (4-8); however in young physically active or athletic populations the research is limited, controversial in some cases and the mechanism(s) underlying the purported benefits remain to be elucidated (2, 9). The theoretical rationale for L-arginine as an ergogenic aid has come primarily from literature using intravenous administration (9-11). Intravenous administration of L-arginine has been shown to produce a more direct hormonal and metabolic response (10, 12, 13); however, intravenous infusion is not a practical method of delivery for athletes. Thus, ingestion of L-arginine in capsule or powder mixed in various solutions is the primary method of supplementation practised by individuals and the form sold in nutrition and supplement stores. Despite this, it is important to note that a high dose of L-arginine consumed can cause gastro-intestinal distress due to the osmotic movement of water into the stomach and intestine (14-16) potentially leading to an increase in the excretion of the ingested L-arginine (14, 15, 17). Therefore, the purpose of the present dissertation was to determine a oral dose of L-arginine that was able to elevate plasma L-arginine concentrations while minimizing gastro-intestinal distress at rest; and, secondly, to examine the physiological responses associated with L-arginine ingestion prior to

both a bout of whole body resistance and aerobic exercise in strength and endurance trained athletes. These two modes of exercise were selected because L-arginine may be beneficial for both resistance and aerobic exercise (2, 18) and there is some controversy as to the effectiveness of L-arginine supplementation when combined with exercise (19, 20).

5.2 Dose-Response Characteristics of L-Arginine

The dose-response study (Chapter 2) demonstrated that L-arginine plasma concentrations significantly increased to a similar concentration at any time point in both the low (0.075 $g \cdot kg^{-1}$ or ~5.9 g) and high (0.150 $g \cdot kg^{-1}$ or ~11.7 g) dose conditions, while there was no change over time when the placebo was consumed. In addition, there were no reported side effects in the low dose and only two of the fourteen participants experienced minor gastro-intestinal distress following the high dose condition. Previous research using an absolute dosing method reported seven out of eight subjects having gastro-intestinal distress with an absolute oral dose of 13 g (14). Therefore, standardizing the amount consumed based on a dosing method that is relative to body mass was effective at reducing negative side effects. However; despite a reduction in side effects and achieving a significant plasma elevation in L-arginine, there were no significant changes in plasma GH, insulin, IGF-1, glucose, and NOx concentrations at rest. These findings were somewhat unexpected, but although there were no effects at rest, it remains feasible that ingesting L-arginine may influence certain physiological responses known to occur as a result of an acute stressor such as exercise. In a clinical setting, L-arginine has been shown to be effective during periods of rapid growth and following a traumatic incident and a pathologic insult (21-23), therefore athletes under a physiological stress (e.g. exercise) may respond differently than at rest (study 2 and 3).

5.3 L-arginine and Acute Exercise

L-arginine and resistance exercise

L-arginine and resistance exercise have both been shown to independently increase GH (14, 19, 24), despite some research showing little change in GH with oral L-arginine ingestion at rest (25, 26). If L-arginine can stimulate GH concentration, then it is plausible that over time and combined with resistance training, this could lead to gains in muscle mass and strength (24, 27). However; some of these gains may also be attributed to GH stimulatory effects on IGF-I. It was important to establish whether L-arginine can have any effect in strength-trained individuals because these are the primary users of L-arginine and these individuals have largely been ignored in the literature. Interestingly, an examination of L-arginine provided before an acute whole body resistance exercise session (Chapter 3) blunted the integrated area under the curve for GH significantly. This result suggests that L-arginine may actually be detrimental to the post-exercise anabolic hormonal response (i.e. GH). Furthermore, this blunted GH response was not associated with changes in GHRH or ghrelin, because no differences between conditions were observed. In addition, L-arginine had no effect on IGF-1 compared to the placebo condition. These findings agree with Marcell et al. (20) and Collier et al. (19) who demonstrated that L-arginine ingested prior to resistance exercise had either no effect or a blunted response on GH. Based on the present dissertation, the effectiveness of L-arginine as a nutritional aid to resistance exercise cannot be supported and the blunted GH response requires further study.

L-arginine and aerobic exercise

Athletes also consume L-arginine as a potential ergogenic aid to endurance performance. However, there is much less literature investigating the role that L-arginine may have in influencing endurance performance. Wideman et al. (10) found that GH was elevated

significantly following 30 g of L-arginine administered intravenously combined with submaximal aerobic exercise. However, the effects of oral L-arginine combined with submaximal aerobic exercise, the additional potential physiological benefits, and whether these potential effects can be observed in trained athletes have not been well examined. Thus, it was also of interest and important to examine the various factors known to be influenced by L-arginine during submaximal aerobic exercise in endurance-trained individuals. In Chapter 4, L-arginine was ingested prior to submaximal aerobic exercise in trained cyclists and it was hypothesized that GH would be elevated and this would lead to increases in free fatty acids while lactate would be reduced and glucose maintained. However, there was no effect on circulating concentrations of GH compared to the placebo condition. There was a small but significant elevation in plasma glycerol concentrations near the end of exercise. There were no further differences observed between conditions for plasma glucose, lactate, and NOx. Based on these findings, the use of L-arginine as an ergogenic aid in endurance exercise performance is not warranted. However, whether L-arginine could provide some additional benefits during long term, endurance training is not known.

5.4 Study design

Although all three studies used a randomized, double blind, repeated measures, placebo-controlled design, considered to be one of the gold standard experimental designs when attempting to formulate a causal claim, there were several inherent limitations. The present protocols were chosen to minimize the effects of confounding variables known to influence hormonal responses such as dietary intake prior to experimentation (28); however such a protocol may lack external validity because it is also known that athletes consume a mixed diet containing a variety of fats, carbohydrates, and proteins. There have been several studies that

have utilized L-arginine combined with a multitude of ingredients (e.g., ornithine, creatine, glucose, etc.). For example, Little et al. (29) examined an L-arginine-creatine based supplement on anaerobic power and muscular endurance. This latter research found a significant increase in peak anaerobic power attained during repeated Wingate sprint tests in the L-arginine-creatine condition compared to creatine alone while there were no differences in the muscular endurance condition. Furthermore, Camic et al. (30) found a significant increase in the ventilatory threshold after 4 weeks utilizing an arginine-based supplement compared to a placebo, however, formulating a causal claim regarding a single nutrient warrants caution.

5.5 Side effects

Another important issue when selecting a sport supplement surround the notion of risk versus benefits (3). Following the ingestion of L-arginine and upon completion of the resistance exercise protocol four of the 18 participants (22%) vomited despite utilizing a "safe" dose (0.075 $g \cdot kg^{-1}$ or ~5.9 g) (16). These results suggest that this L-arginine dose was near the tolerable limit at least when ingested prior to whole-body resistance exercise. Interestingly, there were no observed or reported side effects with this same relative dose at rest or following submaximal aerobic exercise. These differences may be associated with mode of exercise (resistance versus aerobic exercise), intensity, and aerobic fitness status. The resistance exercises were completed to failure or near failure while the aerobic exercise was below ventilatory threshold; interestingly the peak heart and RPE were similar between the two modes of exercise (resistance: 148 ± 18 bpm vs. aerobic 145 ± 14 bpm; resistance: 14 ± 2 vs. aerobic 14 ± 1). A systematic risk assessment of L-arginine has been previously conducted (16) and concluded that 20 $g \cdot day^{-1}$ of L-arginine achieved an observed safe concentration for normal healthy adults. Although they noted that much higher concentrations of L-arginine have been tested, as high as 42 $g \cdot day^{-1}$ (31) in

cystic fibrosis patients, without adverse or reported side effects. The present results do not support oral doses above 0.075 $g \cdot kg^{-1}$ of body mass.

5.6 Timing of supplementation

An emerging trend and current academic debate in protein and amino acid supplementation is the importance of the timing of ingestion (32-36). Collier et al. (19) postulated that L-arginine may stimulate GH and GH is known to inhibit subsequent stimuli via a negative feedback loop. Previously, Marcell et al. (20) and Collier et al. (19) found either no difference in GH or a blunted response when L-arginine was ingested 30 minutes prior to exercise while the present study consumed the supplement 60 minutes prior to exercise, suggesting that these two times points may be detrimental. Certainly, in Chapter 2, it was established that circulating concentrations of L-arginine were elevated 30 minutes following ingestion, peaked at 60 minutes, and remain above baseline values for 180 minutes. Future research is required to elucidate a potential timing effect of L-arginine.

5.7 Responders versus non-responders

Lastly, there is a need to examine responders and non-responders with respect to nutritional supplements (37-39). Hormonal responses to acute exercise show large inter-individual variability. In order to uncover mechanisms mediating exercise supplement related responses and to develop new strategies, an understanding of the hypothalamic, pituitary, adrenal, and hepatic response is essential. Such research depends on substantial knowledge of moderating and intervening variables that affect hormonal responses to different stressors and stimuli. It is known that several modulating factors such as age and gender, as well as dietary energy supply and habitual protein intake (e.g. chronic versus acute L-arginine supplementation) may alter hormonal responses to acute stressor and nutritional interventions (28). Furthermore,

there is a role of genetic factors and methodological issues in terms of habituation to repeated stress exposures (i.e., training). These studies are warranted to examine why the metabolic and hormonal response to L-arginine differs across participants.

5.8 Limitations

Inherent limitation of the present study is that the insertion of a catheter or venipuncture in close proximity to the resting blood sample. The catheter is known to cause a hormonal response (Tremblay et al. 1990). However, both conditions were similarly controlled (i.e. that is the same person took the bloods, bloods were taking at the the same time of day, participants were all seated, and all studies utilized a within-subject design). In addition, there were no differences between resting concentrations nor was an order effect found.

Another limitation to the current dissertation is that no markers of performance were assessed. Therefore, it is difficult to interpret the ergogenic value of L-arginine supplementation. Future research is required to examine both the metabolic, hormonal, physiological and performance aspects of L-arginine supplementation.

Furthermore, it is important to note that the current dissertation only used a single bolus of L-arginine. Future studies are required to examine the chronic effects of L-arginine supplementation in athletic populations. It is important to note that although the resting dose response study showed no difference between the low and high dosages, that this may not represent the optimal dose response characteristics when L-arginine is consumed prior to either resistance or aerobic exercise. Future research is required to examine the possibility of a dose response relationship when L-arginine is combined with exercise.

The sample size used in all three studies was calculated from previous research using a power of 0.80 and an alpha of 0.05, however, the sample sizes utilized in the present studies

were small. This increases the likelihood of a type II error, that is, the chance of accepting the null hypothesis when the research hypothesis is actually true ("false negative"). To test for the likelihood of a type II error, a power calculation was done to assess the predicted subject number needed to demonstrate a significant difference. A power of 80% with an alpha level of 0.05 was used. The results show for study 1: GH n = 94, glucose n = 204, insulin n = 186, IGF-1 n = 686, NOx n = 385; study 2: GHRH n = 53, GHIH n = 47, IGF-1 n = 851, GH n = 279, ghrelin n = 775; study 3: GH n = 339, NEFA n = 562, lactate n = 283, glucose n = 139, and NOx n = 88, demonstrating a weak chance of a type II error.

5.9 Practical Application and Future Directions

L-arginine is a popular nutritional supplement utilized by a diverse group of athletes. L-arginine is involved in several metabolic and hormonal pathways and is intriguing in that it may be beneficial for both strength and aerobic athletes. This dissertation examined the acute hormonal, metabolic, and physiological responses to L-arginine supplementation in physically active participants at rest and when combined with resistance and aerobic exercise. These results suggest that oral L-arginine ingestion is effective at elevating plasma concentrations of L-arginine, but has limited metabolic and hormonal potential and may in fact be detrimental to the anabolic response in strength athletes. Further research is warranted to examine the influence of timing, responders versus non-responders, and the chronic effects of L-arginine supplementation with a variety of exercise training interventions.

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APPENDIX A: Literature Review

Biochemistry of L-Arginine

L-arginine (2-amino-5-guanidinovaleric acid) is one of the 20 most common natural amino acids. Its occurrence in mammalian protein was discovered by Hedin in 1895 (1). L-arginine has since been shown to be in relatively high concentrations in seafood, watermelon juice, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate (2-4), but low in the milk of most mammals (5, 6). Visek (7) suggests that approximately 5.4 g of L-arginine is absorbed each day in adults who ingest an average North American diet (7). Thus each gram of dietary protein supplies about 54 mg of L-arginine. Walser (8) estimated that a 70-kg adult person consuming 50 g of protein per day consumes about 0.2 mmol (34.8 mg) of L-arginine· kg^{-1} of body mass per day or a total of 2.4 g of L-arginine·day^{-1}. Recently results of the third National Health and Nutrition Examination Survey indicated that mean L-arginine intake for the U.S. adult population is 4.4 g·day^{-1}, with 25, 20 and 10% of people consuming <2.6 (suboptimal), 5-7.5, and >7.5 g·day^{-1}, respectively (3).

Homeostasis of plasma L-arginine concentrations is regulated by dietary L-arginine consumption, protein turnover, L-arginine synthesis, and metabolism. The main tissue in which endogenous L-arginine synthesis occurs is the kidney, where L-arginine is formed from L-citrulline, which is released mainly by the small intestine (9). Cell types containing nitric oxide synthase (NOS) have been demonstrated to be able to reutilize L-citrulline, the by-product of NO synthesis, to L-arginine via the so-called L-arginine-citrulline cycle (10, 11). In healthy adults the concentration of endogenous synthesis is sufficient; however in cases of catabolic stress (e.g. burns, infections, and physiological stress) concentration of endogenous synthesis may not meet the metabolic demands (12). Therefore, L-arginine is considered a conditionally-essential amino

acid (13) and Campbell and colleagues (14) have postulated that L-arginine may be beneficial during bouts of physiological stress such as strength or aerobic exercise.

L-Arginine Metabolism

The current knowledge of L-arginine is the outcome of discoveries that were made during the 20th century. One such discovery was made by Krebs and Henseleit who demonstrated that L-arginine is an essential component of the urea cycle (15). This is the only pathway in mammals that allows elimination of continuously generated toxic ammonia from the body. In 1939, Foster and colleagues (16) found that L-arginine is also required for the synthesis of creatine. Creatine phosphate is an essential energy source for rapid muscle contraction. In the 1980s it was discovered that L-arginine is a precursor for nitric oxide (NO) (17-19). In the 1990s the enzyme arginine decarboxylase was discovered in mammalian cells (20). This enzyme converts L-arginine into agmatine, a molecule whose physiological functions are still under investigation. Agmatine has been shown to bind to alpha-2 adrenoceptors and imidazoline receptors, potentially evoking clonidine-like effects on blood pressure (20).

Hormonal Responses to L-Arginine at Rest

L-arginine is also a potent hormone secretagogue. L-arginine infusion at rest increases plasma insulin, glucagon, growth hormone, epinephrine, and norepinephrine blood concentrations (21-23). Intravenous infusion of several different amino acids stimulates insulin secretion, with L-arginine being the most potent (24). Furthermore, it is important to note that oral L-arginine provides a lower bioavailability compared to intravenous infusion. Bode-Boger and colleagues (25) examined the effects of L-arginine induced vasodilation in healthy humans when either consuming an oral dose (6 g) or when receiving an intravenous infusion (6 g or 30 g). They found that the bioavailability of L-arginine was 68 ± 9% when consuming 6 g of L-

arginine compared to 6 g infused. Furthermore, they suggest that the effects of L-arginine are closely related the plasma concentration. Table A.1 shows the difference between oral consumption and intravenous infusion on the growth hormone response.

Table A.1: Oral versus intravenous infusion on growth hormone response.

Reference	Subject age (y)	Sex	Fitness/training status	Dosages	Oral/IV	Growth Hormone Response
Suminski et al. (26)	**22.4±0.8**	**Males and Females**	**Resistance training, 2-3d/wk**	**1.5 g arg + 1.5 g lys**	**Oral**	**↑ 2.7 fold at 60 min**
Isodori et al. (27)	**15-20**	**Males**	**"Healthy"**	**1.2 g arg + 1.2 g lys**	**Oral**	**↑ 8 fold at 90 min**
Lambert et al. (28)	**22.6±1.0**	**Males**	**Bodybuilders**	**2.4 g arg + 2.4 g lys**	**Oral**	**no effect**
Merimee et al. (29)	**17-35**	**Males and Females**	**"Healthy"**	**183 mg arg/kg**	**IV**	**Females ↑; Males no change**
				367 mg arg/kg	**IV**	**Females ↑; Males ↑**
Tanaka et al. (30)	**17.2±1.0**	**Males and Females**	**BMI = 34.7**	**0.5 g arg/kg**	**IV**	**↑ 13 fold**
	25.3±0.9	**Males and Females**	**BMI = 35.6**	**0.5 g arg/kg**	**IV**	**↑ 7 fold**
	50.4±3.4	**Males and Females**	**BMI = 35.5**	**0.5 g arg/kg**	**IV**	**↑ 6 fold**
Collier et al. (31)	**24.8±1.2**	**Male**	**"Healthy"**	**5 g arg**	**Oral**	**↑**
				9 g arg	**Oral**	**↑**
				13 g arg	**Oral**	**no change**

In trained individuals at rest, there appears to be diminished L-arginine-stimulated insulin secretion, however, in trained individuals the L-arginine-stimulated increases in plasma glucagon and growth hormone was not affected (32). Therefore, the primary ergogenic effect of L-arginine in strength trained individuals is thought to be by enhanced growth hormone secretion (33). This increase in growth hormone secretion from L-arginine has been attributed to the

suppression of endogenous growth hormone inhibiting hormone secretion (34). Growth hormone inhibiting hormone inhibits growth hormone secretion, therefore, removing an inhibitory hormone will increase growth hormone secretion.

Physiological Effects of L-Arginine via Nitric Oxide Production at Rest

One of the primary purported effects of L-arginine supplementation for endurance athletes is through the L-arginine nitric oxide link. L-arginine is the precursor for the endogenous synthesis of NO due to the activity of NOS (35). Nitric oxide generated from L-arginine is a highly reactive radical gas and an important messenger molecule that is involved in vasodilation (36). At low concentrations like those produced by constitutive endothelial NOS in the vasculature in vivo, NO acts as a paracrine-signalling molecule, mediating vasodilation (18). After beneficial effects of L-arginine on endothelial function in animal experiments were demonstrated, it was shown that local intracoronary infusion of L-arginine normalized coronary vasomotor responses to acetylcholine in hypercholesterolemic humans (37). A similar observation was made upon systemic infusion of L-arginine in hypercholesterolemic subjects, in whom endothelium-dependent forearm vasodilation was improved (38). Recently, Ohta and colleagues (39) examined L-arginine in normotensive rats and demonstrated increased muscle blood flow. However, others have shown no effect on NO production or blood flow at rest (40-42). These differences may be attributable to dosage and population.

Potential Ergogenic effects of L-Arginine

A nutritional supplement that enhances exercise capacity is said to have an ergogenic effect. L-arginine as a nutritional ergogenic aid is intriguing for two reasons. First L-arginine has been shown to be a potent stimulus for growth hormone secretion. Increasing growth hormone stimulates insulin-like growth factor-1 which has been shown to increase muscle mass

and strength (43). Secondly, it has the potential to enhance ones cardiovascular system (increased blood flow via nitric oxide). This may enhance nutrient delivery (e.g. oxygen, glucose, free fatty acids, amino acids) and assist in the removal of metabolic wastes (e.g. CO_2, lactate). Therefore, L-arginine may be beneficial for both strength and aerobically trained athletes. However, the majority of the literature examining these potential mechanism(s) have used either a cardiovascular disease population or animal models and little is known about the hormonal or metabolic responses to L-arginine supplementation at rest or when combined with either resistance or aerobic exercise in healthy or exercise trained subjects.

Supplementation with L-Arginine on Performance

L-arginine has been shown to be ergogenic for older adults with cardiovascular diseases, such as heart failure, myocardial infarction, stable angina, and pulmonary hypertension (44-48). For example, subjects with congestive heart failure ingested 9 g of L-arginine daily for 7 days, which enhanced exercise duration as compared to a placebo (44). In patients with healed myocardial infarction, Ceremuzynski et al. (45) supplemented participants with 6 g of L-arginine for 3 days demonstrated a beneficial effect on exercise capacity in patients with stable angina. Nagaya et al. (47) examined the effects of oral supplementation of L-arginine on exercise capacity in patients with pulmonary hypertension. Cardio-respiratory exercise tests were performed to measure peak oxygen consumption. Participants consumed 1.5 g·10 kg^{-1} of body mass·day^{-1} for 7 days. A significant increase in peak oxygen consumption was observed. The literature on the ergogenic effects of L-arginine in young healthy adults or athletes is limited and controversial, as shown in table A.2-4. Santos and colleagues (49) supplemented untrained males with 3 g of L-arginine for 15 days and underwent a test-retest protocol evaluating the resistance to muscular fatigue in the knee extensors using isokinetic dynamometry. This study

was able to demonstrate a significant increase in the resistance to muscular fatigue but they did not utilize a double-blinded protocol nor was there a placebo or control group. Conversely, Walberg-Rankin and colleagues (50) examined male weight lifters on a hypocaloric diet. They supplemented these athletes with 8 g of L-arginine daily and found no positive influences on muscle function (bicep/quadriceps isokinetic assessment) or body composition compared to a placebo condition. Similarly, Alvares et al. (51) found no effect of 6 g prior to 3 sets of 10 reps on total work or peak torque. Liu and colleagues (52) and Olek et al. (53) demonstrated no effect of short term or acute effects on indirect measures of nitric oxide production (nitrate+nitrite), metabolic markers and repeated sprint performance in physically active or well trained judo athletes. Buchman and colleagues (54) examined the effect of 10 g 3 times per day of L-arginine on marathon performance and found a detrimental effect compared to a predicted time (+23±21 minutes, $p<0.049$). Interestingly, the temperature at the start of the marathon was -2ºC, with a wind chill of -12ºC and freezing rain; however, Buchman and colleagues (54) suggested the weather only played a minor role.

Table A.2: L-arginine and performance

Study	No. of Subjects: Sex (subject characteristics): study design	Supplementation	Exercise Protocol	Results
Liu et al. (52)	10; M (judo athletes), r, db, co	L-Arg (6 g) or Pla, 3 d	Cycle ergometer (13 sets at 0.05kp/kg, 1 min rest at 60 rpm after set 9)	[La], ammonia, nitrate+nitrite, MP and Pmax: L-Arg = Pla
Olek et al. (53)	6; M (physically active); r, db, co	L-Arg (2 g) or Pla	Repeated Wingate (3 times) w/ 4 min rest	[La], nitrate+nitrite; PP, MP: L-Arg = Pla

Buchman et al. (54)	23; M (runners); r, db	10 g * 3 times /d, 14 d or Pla (glycine)	Marathon	Time: L-Arg > (slower) Pla
Ranchordas & Whitehead, (Abstract)	6; M (trained cyclists); r, db, co	L-Arg (6 g) or Pla, 3 d	20 km time trial	Performance time, VO2, BP: L-Arg > Pla
Alvares et al. (51)	15 M; r, db, p, co	6 g (L-arg) or Pla	3 sets of 10 conc. Elbow extensions @ 60°/S, 2 min rest	Mbv: L-Arg > Pla; Nox, Mox, peak torque, total work, and set total work: L-Arg = Pla
Walberg-Rankin et al. (50)	18 Weight trainers, r, db	5 g (L-arg), Pla, Control, 10 days	hypocaloric diet for 10 days (cutting weight)	FFM, FM, Ptorque, GH, IGF-1, NBAL: L-arg = Pla
Santos et al. (49)	12 healthy M, pre-post	3 g (L-arg), 15 days	isokinetic dynamometer, 15 reps	Fatigue Index: L-Arg < Pla

Supplementation with L-Arginine containing compounds on performance

One of the difficulties and potential misinterpretation of L-arginine research is due to compounds that contain L-arginine as one of the potential ergogenic ingredients, as shown in table A.3. Such as, Elam et al. (55) combined 1 g of L-arginine with 1 g of ornithine over a 5 week strength training program. They found a significant increase in bench press 1-repetition maximum (1RM) and lean body mass. Campbell and colleagues (56) combined 6 g of L-arginine with 6 g of alpha-ketoglutarate over an 8 week strength training program. They demonstrated a significant increase in bench press 1RM and peak power on a Wingate protocol; however they found no effect on body composition, total body water, isokinetic quadriceps muscle endurance, or aerobic capacity. Furthermore, Buford and Koch (57) and Stevens et al. (58) have shown positive effects of an L-arginine containing supplement (glycine-arginine-α-ketoisocaproic acid mixture: GAKIC). Buford and Koch (57) demonstrated an enhanced fatigue resistance while Stevens et al. (58) found an increase in muscle torque and work in the GAKIC condition

compared to a placebo. Little and colleagues (59) examined the effects of an arginine-creatine compound compared to creatine alone and an isocaloric placebo. They found a significant increase in peak power during a Wingate in the arginine-creatine compound compared to either creatine or placebo alone. However, other L-arginine containing compounds have demonstrated no effects (60, 61). Colombani et al. (60) investigated the effects of 15 $g \cdot day^{-1}$ of arginine-aspartate compound compared to a carbohydrate-based placebo, in a randomized double blind cross over design, for 14 days before a marathon run. The plasma concentration of carbohydrate and fat metabolites, cortisol, insulin, ammonia, lactate dehydrogenase, and creatine kinase as well as respiratory exchange ratio and marathon time were not significantly different between conditions. However, plasma concentration of somatotropic hormone, glucagon, urea, and L-arginine were significant increased. Abel et al. (61) found similar results, they examined whether daily intake of two different dosages of L-arginine and aspartate during a four weeks aerobic training program influenced parameters of overtraining syndrome, such as, performance, and metabolic and endocrine parameters. Thirty male endurance trained athletes were included in a randomized, double-blind, placebo-controlled study and divided into three groups; high (5.7 g L-arginine and 8.7 g aspartate), low (2.8 g L-arginine and 2.2 g aspartate), or placebo. Independent of condition, they found no effect on performance, or selected metabolic or endocrine parameters. These studies utilizing L-arginine containing supplements provide some indirect support for athletes (both strength and aerobic) to supplement with L-arginine; however, they need to be interpreted with caution.

Table A.3: L-arginine containing compounds on performance.

Reference	Subject number and age (y)	Fitness / training status	Dosages	Oral/IV	Performance	Metabolic	Hormonal

Campbell et al. (56)	**n=35, 38.9±5.8**	**Resistance trained, ≥1yr**	**6 g L-arginine + 6 g AAKG/d, 8 wks**	**Oral**	**↑ 1RM bench press, Wingate peak power. No effects on body composition, muscular endurance, and aerobic capacity**	**No effect on lipid profiles, liver enzymes, renal function, electrolytes, calcium, total protein, nitrate/nitrite, agmatine, hematocrit.**	**N/A**
Elam et al. (55)	**n=22**	**Healthy**	**1 g L-arginine + 1 g L-ornithine, 5 wks**	**Oral**	**↑ total strength, lean body mass.**	**↓ urinary hydoxyproline**	**N/A**
Colombani et al. (60)	**n=14, 37±2.5**	**Endurance Trained**	**15 g L-arginine-L-aspartate, 14 days**	**Oral**	**No effect on marathon time.**	**No effect on CHO and fat metabolites, ammonia, LDH, creatine kinase, RER. ↑ urea, arginine.**	**No effect on cortisol, insulin. ↑GH, glucagon**
Abel et al. (61)	**n=30, 38.6±10.0**	**Endurance Trained**	**High: 5.7 g L-arg + 8.74 g aspartate. Low: 2.85 g L-arg + 2.15 g aspartate, 4 wks**	**Oral**	**No effect on time to exhaustion**	**No effect on VO_2, lacate, Urea.**	**No effect on GH, glucagon, cortisol, testosterone.**
Buford & Koch, (57)	**n=10, 21±1**	**Phsically active, ≥3 d/wk**	**11.2 g GAKIC**	**Oral**	**Less drop in mean power between sprint 1 and 2 compared to placebo**	**No effect on lactate**	**N/A**
Stevens et al. (58)	**n=13, 20.9±1.9**	**Healthy**	**11.2 g GAKIC**	**Oral**	**↑ muscle torque and work**	**N/A**	**N/A**

Little et al. (59)	**n=35, 22.8±2.8**	**Physically active**	**0.075 g/kg AAKG + 0.1 g/kg Creatine**	**Oral**	**↑Muscular endurance, Peak Power. No effect on body composition.**	**No effect on lactate**

L-Arginine combined with Strength Training

The research examining the potential mechanisms of L-arginine supplementation when combined with resistance training has been counter-intuitive (62-64). Fahs et al. (64) examined the effects of acute L-arginine supplementation and resistance exercise on arterial function and found no significant difference between L-arginine supplementation and a placebo condition for any hemodynamic or vascular response after a resistance training session. Collier and colleagues (62) examined 7 g of L-arginine combined with a whole body resistance training session, previously shown to stimulate growth hormone secretion (65). L-arginine alone resulted in a significant increase in growth hormone response (2-fold increase) compared to the placebo, while exercise alone stimulated a 5-fold increase; however an attenuated response was observed when L-arginine and strength exercise were combined compared to strength exercise alone, demonstrating only a 3-fold increase. Marcell et al. (63) examined 5 g of L-arginine orally which did not significantly change basal growth hormone concentrations nor did it enhance the growth hormone response to exercise. Similar to Collier et al. (62) they found a tendency for a blunted growth hormone response when combined with resistance exercise compared to exercise alone however it was not statistically significant. These studies provide evidence that L-arginine supplementation may in fact be detrimental to the positive growth hormone response consequent to acute strength exercise. Collier et al. (62) suggest two potential possibilities for the attenuated growth hormone response. First, there may be a down regulation of growth hormone releasing hormone (GHRH) induced growth hormone release (66) and secondly there may be an auto-

negative feedback induced by an enhance IGF-1 prior to the resistance exercise bout suppressing subsequent stimulation of growth hormone release (67). Future studies are needed to explore the mechanisms controlling growth hormone secretion when combining L-arginine and resistance exercise.

L-Arginine combined with Aerobic Exercise

Although it is well known that infusion of L-arginine influences blood pressure, heart rate and blood flow at rest (68, 69), several studies have shown that L-arginine has little effect on haemodynamics during exercise in humans (40, 41, 70). Schaefer and colleagues (71) infused 3 g of L-arginine or a placebo 90 minutes prior to an incremental maximal protocol and found reduced lactate and ammonia and increased L-citrulline plasma concentrations, suggesting an enhanced nitric oxide production. In addition, Koppo et al. (72) demonstrated that exogenous L-arginine administration enhanced the speed of phase II pulmonary VO_2 response by 12% at the onset of moderate-intensity aerobic exercise. Furthermore, Wideman et al. (73) found an enhanced post exercise growth hormone secretion when L-arginine was infused 30 minutes prior to a submaximal cycling protocol. Enhancing growth hormone may increase gluconeogenesis and enhance lipolysis thereby sparing muscle glycogen (74). Future studies are needed to assess the acute effects of an appropriate oral dose of L-arginine on these metabolic and hormonal changes during an aerobic bout of exercise in athletes.

Table A.4: L-arginine combined with aerobic exercise.

Study	No. of Subjects: Sex (subject characteristics): study design	Supplementation	Exercise Protocol	Results
Buchman et al. (54)	23; M (runners); r, db	10 g * 3 times /d, 14 d or Pla (glycine)	Marathon	Time: L-Arg > Pla
Ranchordas & Whitehead, (Abstract)	6; M (trained cyclists); r, db, co	L-Arg (6 g) or Pla, 3 d	20 km time trial	Performance time, VO_2, BP: L-Arg > Pla

Bescos et al. (75)	9; M (Tennis Players); r, db, co	Control diet (5.5 g/d) or L-Arg rich diet (9.0 g/d or L-Arg supplement (20.5 g/d), 3 d	Treadmill submaximal test 10-11 km/hr, increase 1 km/hr every 4 min until 85-90% VO2max.	[la]: L-Arg < Pla; VO2, VCO2, nitrate: L-Arg = Pla
McConell et al. (76)	9; M (endurance trained); r, db, co	L-Arg HCl (30g IV) or Pla after 75 min of exercise	Cycle ergometer (120 min at 72 ± 1% VO2 peak)	[La], VO2peak, RPE, FET: L-arg = Pla; GCR: L-arg > Pla; glycerol, NEFA: L-Arg < Pla
Bailey et al. (77)	9; M (trained); r, db, co	L-Arg (6 g) or Pla, 3 d	Cycle ergometer (70–90 rpm)	[La]: L-arg = PLA FET, nitrite: L-arg > PLA VO2cost, VO2sc: L-arg < PLA
Sunderland et al. (78)	18; M (trained cyclists); r, db	L-Arg (2 X 6 g/d) or Pla, 28 d	Clycle ergometer GXT	VO2 max, VT: L-Arg = Pla
Schaefer et al. (71)	8; M (healthy); r, db, co	L-Arg (3g IV) or Pla	Cycle ergometer (Incremental protocol)	HR, VO2, VCO2: L-arg = Pla; [La]: L-arg < Pla; L-citrulline: L-Arg > Pla
Koppo etl al. (72)	7; M (physically active); r, db, co	L-Arg HCL (7.2 g/d) or Pla; 14 d	Cylce ergometer (2 bouts of 6 min @ 80% VT on two days)	[La]: L-Arg = Pla; O2 kinetics time constant: L-Arg < Pla
Linden et al. (79)	7; M (healthy); r, db, cb	L-Arg (30g IV) or Pla (saline)	Cycle ergometer (120 min at 64 ± 1% VO2 peak)	GCR: L-Aar > Pla; NOS: L-Arg = Pla

Summary

The evidence provided is limited and controversial for the use of L-arginine supplementation from an ergogenic perspective in physically active humans. L-arginine is involved in several metabolic and physiological processes, such as GH and NO production; however, the majority of this research has been conducted on cardiovascular disease patients. The mechanism(s) underlying the potential benefits in healthy physically active humans needs to be elucidated. The differences between studies may be due to the dose or the stressor (strength versus aerobic).

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APPENDIX B: Completed Assays

Glucose Assay: General Principle: Glucose oxidase is added to an unknown sample which results in the oxidation of any serum glucose to gluconic acids and hydrogen peroxide. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a coloured product, while oxidized o-dianisidine reacts with sulphuric acid to form a more stable coloured product. Measured at 540 nm, the intensity of the pink colour is proportional to the original glucose concentration (Sigma-Aldrich, 2004). Glucose concentrations can be determined mathematically using the slope of a standard curve of known concentrations of glucose.

IGF-1 (Insulin-like Growth Factor 1) assay 1: General Principle: Samples are incubated briefly with a reagent to inactivate binding proteins and then diluted for the assay. A purified sheep polyclonal anti-IGF-1 is coated onto the inner surface of polystyrene microtitre wells. The pre-treated, diluted samples are then incubated, together with horseradish peroxidase labelled monoclonal anti-IGF-1, in antibody-coated wells for 2 hours at room temperature. The wells are washed and a single component chromogenic substrate is added to develop colour. Measured at 450 nm, the intensity of the yellow colour is proportional to the original IGF-1 concentration (Enzo, Life Sciences). IGF-1 concentrations can be determined mathematically using the slope of a standard curve of known concentrations of IGF-1 samples. This procedure measures free IGF-1.

IGF-1 (Insulin-like Growth Factor 1) assay 2: General Principle: (IGF-1 was analyzed in plasma using a commercially available enzyme-linked immunoassay kit (Diagnostics Biochem Canada Inc.; Immunodiagnostic Systems Inc., Fountain Hills, Ariz., USA; Cayman Chemical Company), according to the procedures of the manufacturer. Samples were incubated with a

reagent to inactivate binding proteins, and then diluted. A purified sheep polyclonal anti-IGF-1 was coated onto the inner surface of polystyrene microtitre wells. The pretreated, diluted samples were then incubated, together with horseradish-peroxidase-labelled monoclonal anti-IGF-1, in antibody-coated wells for 2 h at room temperature. The wells were washed, and a single component chromogenic substrate was added to develop colour. Measured at 450 nm, the intensity of the yellow colour is proportional to the original IGF-1 concentration (Immunodiagnostic Systems Inc. 2008), and quantification of the samples was performed with a calibration curve. This procedure measures total IGF-1.

Insulin: General Principle: The Coat-A-Count Insulin procedure is a solid-phase radioimmunoassay, wherein ^{125}I-labelled insulin competes for a fixed time with insulin in the patient sample for sites on insulin-specific antibody. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radio-labelled insulin. Counting the tube in a gamma counter then yields a number, which converts by way of calibration curve to a measure of the insulin present in the sample.

L-arginine: General Principle: Arginine can be estimated spectrophotometrically (Bergmeyer, 1977) by the oxidation of NADH using octopine dehydrogenase. This method is highly specific for this amino acid and is applicable to the estimation of arginine in the presence of mono- and disubstituted guanidino compounds.

hGH: Growth hormone: General Principle: The principle of this enzyme immunoassay (Diagnostics Biochem. Canada Inc., London, Ontario) test follows a typical sandwich type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody

specific for hGH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of hGH is conjugated to horse radish peroxidase (HRP). hGH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured at 415 nm. The intensity of the colour is proportional to the concentration of hGH in the sample. hGH concentrations can be determined mathematically using the slope of a standard curve of known concentrations of hGH samples.

NOx: Nitrate/Nitrite: General Principle: Nitrate/Nitrite determination is a colorimetric assay (Cayman #780001). The first step of this assay is to convert nitrate to nitrite utilizing nitrate reductase. The second step is the addition of the Griess Reagents which converts nitrite into a deep purple axo compound. The absorbance is measured at 540 nm. The intensity of the colour is proportional to the concentration of NOx in the sample. NOx concentrations can be determined mathematically using the slope of a standard curve of known concentrations of NOx samples.

GHRH (Growth hormone releasing hormone): General Princple: GHRH was measured using an enzyme-linked immunoassay kit (Uscn Life Science Inc. Wuhan, China) according to the procedures provided by the manufacturer. The microtiter plate provided with this ELISA kit was pre-coated with an antibody specific to GHRH. Standards and samples were pipetted into the appropriate plate wells, with a biotin -conjugated polyclonal antibody preparation specific to GHRH. Avidin was added to each well to bind to the biotin and incubated. Only those wells that contain GHRH, biotin-conjugated antibody and enzyme-conjugated avidin exhibited a change in

color. The enzyme-substrate reaction was then terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of GHRH in the samples was then determined by comparing the optical densities of the samples to the standard curve.

GHIH (Growth Hormone Inhibiting Hormone): General Principle: GHIH was measured using an enzyme-linked immunoassay kit (Uscn Life Science Inc. Wuhan, China) according to the procedures provided by the manufacturer. The microtiter plate provided with this ELISA kit was pre-coated with an antibody specific to human GHIH. Standards and samples were pipetted into the appropriate plate wells, with a biotin -conjugated polyclonal antibody preparation specific to GHIH. Avidin conjugate to horseradish peroxidise was added to each well to bind to the biotin and incubated. The amount of bound horseradish peroxidise conjugate is reverse proportional to the concentration of GHIH in the sample. The enzyme-substrate reaction was then and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of GHIH in the samples was then determined by comparing the optical densities of the samples to the standard curve.

Ghrelin: General Principle: Ghrelin was analyzed in plasma using a commercially available enzyme-linked immunoassay kit (SPI-BIO Bertin Pharma,Montigny Le Bretonneux, France), according to the procedures of the manufacturer. Samples were incubated in a plate coated with a monoclonal antibody specific to the C-terminal aspect of ghrelin. Acetylcholinesterase FAB conjugate recognizes the N-terminal part of unacylated ghrelin. This allows the two antibodies to form a sandwich by binding on different parts of the human unacylated ghrelin. Measured at 415 nm, the intensity of the yellow colour is proportional to the original ghrelin concentration, and quantification of the samples was performed with a calibration curve.

Lactate: Lactate were determined spectrophotometrically (Sigma Chemical Inc. USA).

NEFA (Non-esterified fatty acids): Plasma NEFA was determined by an enzymatic colorimetric procedure (NEFA-C test; Wako, USA).

Glycerol: General Principle: Cayman Chemical Company. The glycerol assay measures glycerol by a coupled enzymatic reaction system. Glycerol is phosphorylated by glycerol kinase to produce glycerol-3-phosphate and adenosine-5'-diphosphate. The G3P is oxidized by glycerol diphosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase catalyzes the redox-couple reaction of H_2O_2 with 4-aminoantipyrine and N-ethyl-N-(3-sulfopropyl)-m-anisidine, producing a purple product with an absorbance of 540 nm. The intensity of the purple colour is proportional to the original glycerol concentration, and quantification of the samples was performed with a calibration curve.

Appendix C
Individual Responses

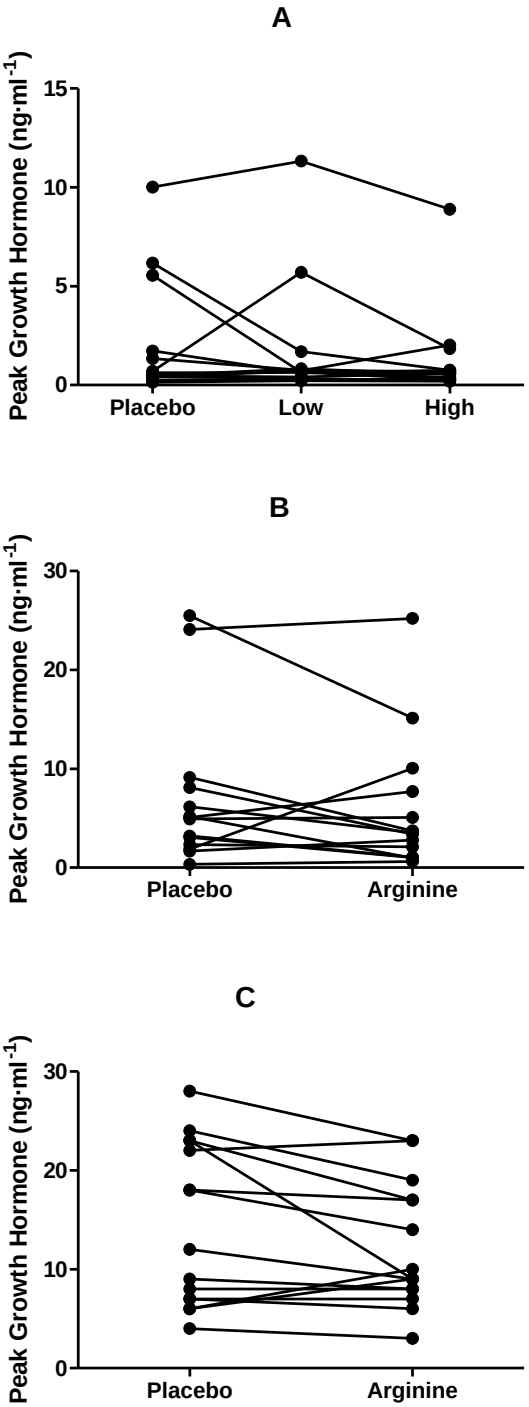

Figure A.C.1: Individual peak growth hormone responses at rest (A), following resistance exercise (B), and following aerobic exercise (C).