


University of Chester

# *Department of Clinical Sciences*

## *MSc In Exercise and Nutrition Science*

Module Title: Research Project

Module Code: XN7523

September 2012

# Literature Review

## The Effects of L-Arginine Supplementation on Exercise Performance

**Word count: 4757**

***Contents***

## 1.1 - ***Abstract***

L-arginine is one of the most metabolically versatile amino acids in the human body. In its most extensively reviewed function, L-arginine serves as the pre-cursor for the biosynthesis of nitric oxide (NO), an essential substance in regulating vascular tone, triggering vasodilation and increasing blood flow. Despite conflicting literature, supplemental L-arginine may theoretically support general health and exercise capacity, through enhanced blood flow to tissues. While L-arginine supplementation has been compelling reported to improve exercise tolerance in patient populations, the findings in healthy and athletic populations are less consistent and often conflicting. Ergogenic effect has been demonstrated in previous literature, yet the supportive evidence-base is limited. Equally, a range of recent studies have reported no effect on exercise performance. The reported physiological effects of L-arginine have served as the rationale behind the development and marketing of a number of NO stimulating dietary supplements. Such products profess to augment NO production, therefore improving blood flow to muscle during training, and in turn, leading to greater training adaptations. Consequently, supplementation with L-arginine and similar “NO boosters” has soared in popularity over the last decade, despite the fact that there is an overall lack of supportive data in healthy human cohorts, as ergogenic potential remains controversial and inconclusive.

## 1.2 – ***Introduction***

L-arginine is regarded as a semi-essential or conditionally essential amino acid (Morris, 2006). It is one of the most metabolically versatile amino acids in the human body, demonstrated by its numerous important roles in a number of crucial physiological and biochemical processes (Morris, 2006).

Indeed, L-arginine plays a number of significant functions within mammalian cells. For example, it plays a critical role in protein and creatine syntheses and in the biosynthesis of other amino acids (Campbell et al. 2006). Literature has also demonstrated L-arginine to play important roles in the secretion of growth hormone (HGH), augmenting the production of nitric oxide (NO) and in the regulation of the urea cycle. In the latter essential biochemical pathway, urea is synthesized from L-arginine to enable the body to remove excess ammonia, which is toxic to cells. (Campbell et al. 2006).

A typical Western diet contains approximately 3–6 g of arginine per day, most of which is derived from plant proteins such as soy (Visek, as cited by Paddon-Jones, Borsheim & Wolfe, 2004), yet the rate of de novo synthesis remains unaffected by several days of an arginine free diet (Castillo *et al.*, 1995). In humans, most endogenous L-arginine is derived from another amino acid, L-citrulline, which is a bi-product of glutamine metabolism (Appleton, 2006). As such, L-arginine can be synthesized within the human body as part of glutamine, glutamate, and proline metabolism (Appleton, 2006). The amino acid L-citrulline is recycled into L-arginine, after being produced during processes such as NO synthesis or glutamine metabolism, via a pathway termed 'the Arginine-citrulline pathway' (Figure 1) by Hecker et al. (as cited by Wu & Morris, 1998).

1.3 **- *L-Arginine and Nitric Oxide***

In one of its most important and extensively reviewed roles, L-arginine serves as the precursor for the biosynthesis of NO, an essential substance in regulating vascular tone, triggering vasodilation and increasing blood flow (Wu & Meininger, 2000; Campbell *et al*. 2006). Nitric Oxide has several anti-atherogenic actions; reducing oxidative stress, monocyte adhesion and platelet aggregation (Walker et al. 2001). NO synthesis occurs via the L-arginine-NO pathway (Lerman et al., 1998) by the enzyme endothelial nitric oxide synthase (eNOS). In this reaction, endothelial nitric oxide synthase (eNOS), one of a family of enzymes known as the NO synthases, catalyses the oxidation of L-arginine to NO, with L-citrulline produced as a bi-product (Castillo et al. 1995). NADPH and $O_2$ serve as co-substrates for the oxidation of L-arginine (Castillo et al. 1995), demonstrated in Figure 1.

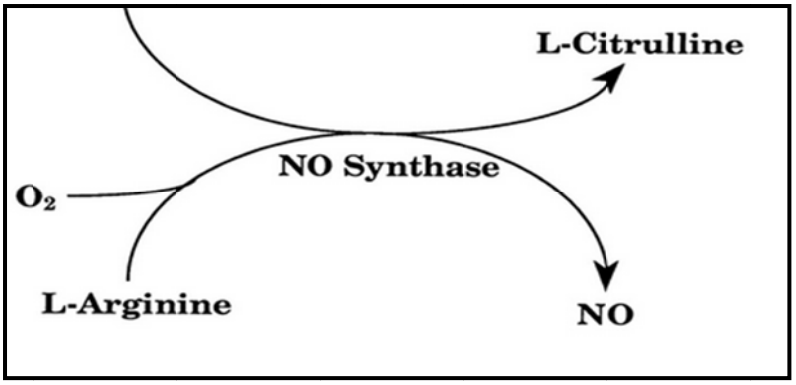


**Figure 1**: Synthesis of Nitric Oxide – The Arginine-Citrulline pathway. *Adapted from; Appleton (2002).*

## 1.4 - ***L-Arginine Supplementation***

L-arginine is not considered an “essential” amino acid because humans can synthesize it “*de novo”* from glutamine, glutamate, and proline (Appleton, 2006). This means the body produces sufficient functional amounts on its own to meet most normal physiological demands, yet during times of stress or increased demand, for example, in exercise or disease, additional L-arginine is required from external dietary sources (Appleton, 2006).

As such, despite the fact that L-arginine is already stored in significant intracellular amounts (Hambrecht et al. 2000), previous literature has indicated that L-arginine supplementation is associated with improvements in endothelial function, for example, increasing blood flow by increasing arterial diameter via NO-mediated vasodilation (Lerman et al. 1998). Hambrecht et al. (2000) stated that L-arginine supplementation increases endothelial substrate availability, in turn augmenting the synthesis of NO, as a result of the increased levels of L-arginine as the substrate for NO synthesis.

Support for this notion comes from Bailey et al. (2010) who observe that exogenous L-arginine administration has previously been reported to increase urinary NO concentration ($p<0.05$, Maxwell et al., as cited by Bailey et al. 2010), plasma NO concentration ($p<0.05$, Xiao et al., as cited by Bailey et al. 2010), and L-citrulline concentration ($p<0.05$, Schaefer et al., as cited by Bailey et al. 2010), whilst reducing resting systolic blood pressure. Furthermore, an L-arginine-free diet has been demonstrated to reduce plasma NO concentration and synthesis, whilst also reducing L-arginine flux (Bailey et al. 2010).

Conversely, however, it has equally been suggested that L-arginine is ineffective in augmenting NO-mediated vasodilation (Tang et al. 2011). Tang et al. (2011) investigated the effects of an acute bolus of L-arginine on muscle blood flow and muscle protein synthesis in eight healthy young men (22.1 ± 2.6 y). Participants were recruited to two trials where they performed a single bout of resistance exercise (seated leg press) after consuming either a drink containing either 10 g essential amino acids (EAAs) with 10 g L-arginine, or a control beverage. Femoral artery blood flow was assessed using Doppler ultrasound, with results indicating that blood flow increased 270% above basal in the exercised leg but not in the non-exercised leg (Table 1) and intriguingly there was no differences between the L-arginine and control trials ($p<0.001$). As expected, L-arginine administration increased ($p<0.001$) plasma L-arginine levels; however, levels of plasma nitrate, nitrite, and endothelin-1, all markers of NO synthesis, did not change during either the ARG or CON trial, nor was there significant difference between the trials ($p<0.001$). Tang et al. (2011) concluded therefore that an oral, acute bolus of L-arginine does not increase NO synthesis, or consequently, muscle blood flow.

Despite conflicting literature, according to Tong et al. (as cited in Campbell et al. 2006), supplemental L-arginine may, theoretically, support general health and capacity, through enhanced blood flow to tissues. This would potentially be beneficial to an athlete undergoing exercise training.

**Table 1:** Femoral artery blood flow in the non-exercised and resistance-exercise legs of young men during ARG and CON trials. *(Redrawn from Tang et al. 2011).*

## 1.5 - ***L-Arginine and Pre-existing CVD***

The therapeutic, general health effects of L-arginine supplementation discussed by Tong et al. (as cited in Campbell et al. 2006), may also rationally apply to those populations whose health and ability to exercise was compromised by underlying pathology, such as in individuals with pre-existing cardiovascular disease including angina and atherosclerosis (Paddon-Jones et al. 2004). For example, Hambrecht et al. (2000) found beneficial effects on endothelial function and blood flow in people with chronic heart failure. It was reported that endothelial dysfunction, characterised by attenuated vasodilation and the presence of ischaemia (Hambrecht et al. 2000), was markedly improved following L-arginine supplementation, as a result of the aforementioned benefits on NO production. Forty patients with severe chronic heart failure were randomised into an L-arginine group (8g/day), a training group with daily forearm exercise training, or an inactive control group. It was observed that L-arginine supplementation had increased radial artery diameter from baseline (280 ± 33 vs 77 ± 5 μm) and therefore blood flow ($p<0.001$), demonstrating that

supplemental L-arginine is therefore also related to improved endothelial function in humans. Endothelial dysfunction is a marker of cardiovascular risk, and as such, L-arginine supplementation could potentially aid in reducing CV risk (Hambrecht et al. 2000; Wu & Meininger, 2000).

Furthermore, clinical studies with L-arginine have also suggested beneficial counteraction to clinical ischemia (Ceremuzynski, Chamiec & Herbaczynska-Cedro, 1997). In a double-blind trial in 22 patients with healed myocardial infarction and stable angina, three-day oral supplementation of L-arginine (6g/day) was associated with enhanced exercise capacity (Ceremuzynski et al., 1997). Following three days L-arginine treatment, it was observed that maximum MET workload (ARG 7.4 ± 3 vs CON 6.4 ± 2, $p<0.006$) and time to maximal ST-segment depression ($p<0.04$ were both significantly enhanced when compared to baseline data. This data suggests beneficial effects on ischaemia. Thus, according to Paddon-Jones et al. (2004), the anti-atherogenic and vasodilatory properties of L-arginine action appear to be indicative of improved exercise tolerance in patients with pre-existing CVD.

Appleton (2006) reviewed and concluded that L-arginine supplementation appears to be a safe and effective therapy for many cardiovascular diseases which are responsive to modulation of nitric oxide, with the therapeutic effect thought to be as a result of improved NO-mediated vasodilation.

## 1.6 - ***Application to Exercise Performance***

The aforementioned biochemical and physiological effects of L-arginine would reasonably suggest that supplemental L-arginine could improve exercise performance (Campbell, La Bounty & Roberts, 2004), yet the available literature evidence is limited and conflicting (Paddon-Jones et al. 2004). An extensive summary of relevant literature related to L-arginine supplementation and exercise performance can be observed in Appendix 1.

L-arginine supplementation has been associated with improved exercise capacity, in both healthy subjects (Campbell et al., 2006) and also in people with stable angina (Ceremuzynski et al*.,* 1997). According to Lerman et al. (1998) improving endothelial function (i.e. NO-mediated vasodilator capacity via L-arginine supplementation) will increase blood flow. In theory, increasing blood flow will then in turn improve exercise performance via an increased rate of oxygen and substrate delivery (Tang et al. 2011), glucose uptake, and removal of waste exercise bi-products.

This assumed relationship between blood flow and exercise performance was investigated in a recent study by Liu et al*.* (2010). Liu and colleagues observed the effects of acute L-arginine supplementation on vasodilation and subsequent exercise performance in elite judo. Ten elite male college judo athletes completed two trials, with subjects receiving 6g/day L-arginine or placebo for three days before undertaking an intermittent anaerobic cycling test. Pre-test, Liu et al. (2010) hypothesised that L-arginine supplementation would improve exercise performance as a result of enhanced NO-mediated vasodilation, concurrent with the aforementioned work of Lerman et al. (1998). Vasodilation was measured using photoplethysmography in pre-supplementation, pre-exercise, and at regular intervals post-

exercise. Both conditions demonstrated exercise-induced vasodilation. However, L-arginine supplementation had no additional effect on exercise-induced vasodilation (ARG 32.8 ± 4.3 vs CON 30.8 ± 4.0 % reflection index) in these well-trained subjects ($p<0.05$). This study therefore suggested that the short-term arginine supplementation had no effect on exercise-induced vasodilation and performance in intermittent anaerobic tests in well-trained male judo athletes. Liu et al. (2010) concluded that the lack of effect on performance was potentially as a consequence of the lack of L-arginine effect on NO-mediated vasodilation, thus highlighting the relationship between blood flow and exercise capacity.

While L-arginine supplementation has been compellingly established to improve exercise tolerance in patient populations (Hambrecht et al. 2000; Ceremuzynski et al. 1997), the evidence in healthy humans is less consistent and often conflicting. Specifically, improvements in sprint performance (Buford & Koch, as cited in Bailey et al. 2010) and muscular power and fatigue resistance (Campbell et al. 2006) have been observed in studies following L-arginine supplementation, yet the supportive evidence-base is limited. Furthermore, an equal range of studies have indeed reported no effect on exercise performance (Liu et al. 2010).

One study purporting the ergogenic potential of L-arginine supplementation was conducted in Brazil in 2002. Santos et al. (2002) aimed to evaluate the effect of oral supplemental L-arginine on muscular fatigue during isokinetic exercise. Twelve healthy, untrained male volunteers consumed 3g of L-arginine daily over a 15 day period, before undergoing testing evaluating resistance to muscular fatigue via quadriceps isokinetic dynamometry. Data demonstrated that there was significant resistance to muscular fatigue following oral administration of L-arginine (10% and 7.6% respectively for left and right leg Fatigue Indexes), when compared to control baseline data ($p<0.05$). Santos et al. (2002) speculated

that these results may have been due to increased availability of nitric oxide and therefore the endothelium-dependent dilation, subsequently resulting in an increased muscular resistance to fatigue. Yet, although initially this study appears to provide substantial evidence for the beneficial effects of L-arginine supplementation, when evaluated by Campbell et al. (2004), the study was not deemed to be high quality due to the fact that the investigators had failed to utilize a double-blinded protocol or placebo group.

An important, recent study conducted by Bailey et al. (2010) attempted to explore the hypothesis that acute L-arginine supplementation would elevate plasma NO levels, thus reducing systolic blood pressure and improving exercise capacity, as a result of enhanced NO bioavailability. In a double-blind study, nine healthy, recreationally active males (19-38 years) took part in a supplementation and exercise testing program. They consumed a 500 ml beverage containing 6g of L-arginine or a placebo beverage, before completing a series of moderate and severe intensity exercise bouts, 1 hour after ingestion of the beverage. This was repeated on seven occasions over a 4 to 5 week period. It was reported that plasma NO levels were significantly greater in the L-arginine group than in the placebo group (331 ± 198 vs 159 ± 102 nM, $p<0.05$), whilst, as hypothesised, systolic blood pressure readings were significantly reduced (123 ± 3 vs 131 ± 5 mmHg, $p<0.01$). In terms of exercise performance, it was observed that dietary L-arginine supplementation reduced the oxygen cost of moderate intensity exercise by 7% ($p<0.05$) and extended the time to exhaustion (707 ± 232 vs 562 ± 145 seconds, $p<0.05$) during severe-intensity exercise. Bailey et al. (2010) speculated that this is as a consequence of L-arginine supplementation elevating NO bioavailability, and the associated, discussed benefits of NO.

The work of Bailey et al. (2010) provides rationale for the acute administration of L-arginine. As such, it is feasible to speculate that the acute oral ingestion of L-arginine may result in an

increase in NO synthesis and bioavailability, with corresponding physiological outcomes and effects on exercise capacity. However, as demonstrated, support for this concept is scarce, with the concept of acute supplementation providing a sizeable research gap.

Supplemental L-arginine is often given in the form of arginine alpha keto-glutarate (AAKG), as this is seen to improve the bioavailability of L-arginine and therefore enhance any potential effects. Campbell et al. (2006) used this supplement format to assess the effects of AAKG on body composition, training adaptations and exercise performance in adult men. Thirty-five resistance trained men (30-50 years) were randomly assigned to ingest supplements, containing either a placebo or AAKG (12g/day) for an eight-week period of controlled, standardised training, during which, body composition and a series of exercise tests were performed at weeks 0, 4 and 8. Although, no significant differences were observed between the placebo and AAKG groups in terms of body composition; several training adaptations were observed in the AAKG group. Change in bench press 1RM over the eight weeks was significantly greater in the AAKG group (8.82 ± 7.33 vs 2.67 ± 9.11kg, $p=0.03$), whilst sprint peak power, time to peak power, and rate to fatigue were each significantly improved in the AAKG group (all, $p<0.05$). This formed the basis of the researchers' conclusion that "AAKG supplementation may augment one-rep max strength, sprint power and rate to fatigue in response to training".

In contrast, AAKG has also been observed to have no effects on muscular endurance and blood pressure in trained college-aged men (Greer & Jones, 2011). Three sets each of chin-ups, reverse chin-ups, and push-ups were performed to exhaustion with three minutes of rest between each set. AAKG supplementation did not improve muscle endurance or significantly affect the BP response to this anaerobic work (Greer & Jones, 2011). In fact, subjects performed fewer total chin-ups (AAKG 23.75 ± 6.38 vs. PLA 25.58 ± 7.18) and total

trial repetitions (AAKG 137.92 ± 28.18 vs. PLA 141.08 ± 28.57) in the supplement trial ($p<0.05$).

As aforementioned, the work of Campbell et al. (2006) at Baylor University, Texas, was one of the early studies in healthy subjects to really push the ergogenic potential of L-arginine supplementation and formed the basis for the rationale that L-arginine supplementation could indeed be a worthwhile ergogenic aid for athletes. Intriguingly, however, five years later, as part of the same research group at Baylor University, Willoughby et al. (2011) provided evidence in direct contradiction to the earlier work of Campbell et al. (2006).

Twenty-four healthy, resistance-trained men were recruited to ingest 12g/day for seven days in a double-blind design. The exact same dosage and ingestion protocols were used as earlier set out by Campbell et al. (2006). Prior to supplementation, subjects underwent baseline muscle strength testing, provided venous blood samples (pre-test, day 1 and day 7), whilst brachial artery blood flow was assessed throughout the testing sessions via Doppler ultrasound (Willoughby et al. 2011). The testing sessions in questions consisted of standardised resistance-exercise in which subjects completed three sets of 15 repetitions at 75% 1RM bicep-flexion exercise.

In terms of hemodynamics, analysis revealed that although heart rate, systolic and diastolic blood pressure and MAP each increased significantly with the exercise protocol, there was no significant difference between the groups ($p<0.05$). Likewise, there was no difference ($p=0.014$) between ARG and PLA in terms of brachial-artery blood flow responses, which brings into question the very rationale of L-arginine ergogenics. Indeed, although plasma-L-arginine significantly increased ($p=0.001$) in the ARG group (179.33 ± 75.79 vs 91.01 ±

16.27μmol/L), there was no significant difference between groups (p=0.073) in levels of serum NOx (nitrites/nitrates).

There is a recent selection of literature in support of the findings of Willoughby et al. (2011). For example, it has been shown that single-doses of three separate alleged nitric-oxide-inducing supplements (each containing L-arginine) were ineffective at increasing circulating nitric oxide levels and blood flow in response to resistance exercise (Bloomer et al. 2010). Similarly, work by Fahs, Heffernan & Fernhall (2009) observed that there was no significant change ($p<0.05$) in blood flow (Figure 2) or hemodynamic and vascular responses when 7g of L-arginine was given acutely, immediately before resistance exercise.

**Figure 2:** Forearm blood flow responses pre and post resistance exercise. No significant difference ($p<0.05$) was observed between 7g ARG and PLA conditions (*Fahs, Heffernan & Fernhall, 2009).*

Along these lines, work from Iran by Imanipour et al. (2011) also found L-arginine to be ineffective for increasing NO during intermittent anaerobic tests in elite male bodybuilders. 30 matched weight bodybuilder consumed either 12g/day L-arginine or a placebo for six

weeks, providing blood samples at baseline, after three weeks and again after six weeks. Analysis revealed, however, that there was no significant difference between the ARG and PLA conditions ($p<0.05$), allowing the researchers to conclude that as long-term supplementation of L-arginine had no effect on plasma NO, the rationale for L-arginine supplementation requires critical re-evaluation (Imanipour et al. 2011).

A recent study by Wax et al. (2012) also provided evidence against the ergogenic potential of L-arginine in a simple study investigating muscular performance. Sixteen healthy males (19.8 ± 1.9 years), eight of whom were engaged in regular resistance training (with the remaining eight classified as 'untrained'), were recruited to ingest 3g of AAKG or a placebo (PLA) 45 minutes prior to a resistance exercise protocol in a double-blind design. Following the determination of one repetition maximum (1RM), subjects completed repetitions to failure at 60% 1RM on both barbell bench press and leg press. Analysis revealed that there was no significant difference ($P<0.05$) in bench press performance between AAKG or PLA conditions for either 1RM of total load volume (number of repetitions before failure) regardless of the training status of the subjects. Likewise, there was no significant difference ($p<0.05$) in leg press 1RM or total load volume regardless of the supplementation condition or training status (Figure 3).

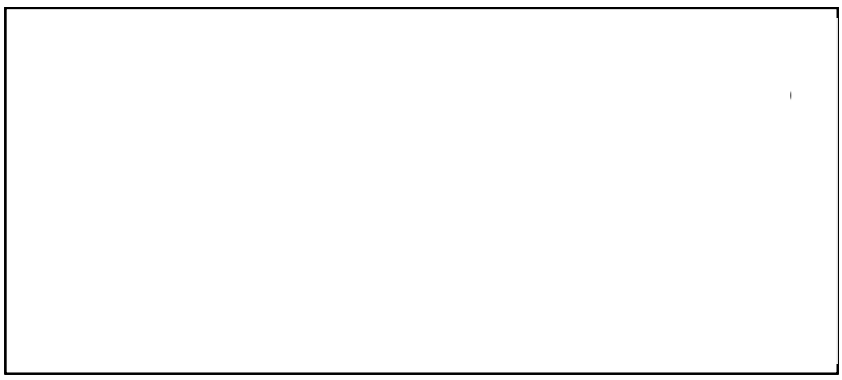

Figure 3: Total load volume (60% 1RM repetitions to failure): bench press and leg press.

*Adapted from Wax et al. (2012).*

A recent study did observe improved exercise performance in elderly cyclists following three-weeks of L-arginine and antioxidant supplementation (Chen et al. 2010). Youthful, healthy, athletic individuals generally have a more efficient NO system, compared with aged individuals, with NO quantity and availability diminishing as we age (Taddel et al., as cited by Chen et al. 2010). Furthermore, exercise capacity declines with advancing age. The study subjects had an average age >55 years, suggesting lower baselines in vasodilatory capacity and exercise tolerance, which may explain the significant improvement on exercise performance (Chen et al. 2010).

However, a particularly relevant recent study did concur that L-arginine could indeed provide a useful ergogenic aid in healthy subjects. Ranchordas & Whitehead (2011) investigated the effect of acute L-arginine on 20km time-trial performance in competitive male cyclists (n = 6, 23 ± 5 years), in a double-blind, cross-over study. Subjects consumed either 500ml placebo (PLA) or L-arginine (ARG) beverage 6g L-arginine over a 3-day period. Following a 3-day supplementation, participants completed an incremental test to exhaustion, followed by a 20km time trial. It was observed that time-trial completion time was significantly reduced ($p<0.05$) by 34 seconds (ARG 32:04 ± 1:38 vs PLA 32:38 ± 1:50 min), whilst $O_2$ consumption and both systolic and diastolic blood pressure were also significantly reduced following ARG supplementation (all $p<0.05$). While these results seem to suggest ergogenic potential of acute L-arginine supplementation, unfortunately, the sample size (n = 6) was very small, and all cyclists were very well-trained, making it difficult to draw strong conclusions from this data as a consequence.

## 1.7 - ***Safety, Tolerance & Side Effects***

A recent review by Alvares et al. (2011) suggested that low oral doses are well tolerated and clinical side effects are rare in healthy subjects. Indeed, the majority of research within healthy subjects report tolerance to the consumption of L-arginine containing supplements. For example, in the work of Willoughby et al. (2011), participants reported by questionnaire that ingestion had been tolerated with no side effects. Campbell et al. (2006) concur that a daily dose as high as 12g appears safe and well tolerated.

In contrast, however, consumption of L-arginine containing supplements has been associated with adverse effects on health (Prosser et al. 2009). Prosser et al. (2009) reported the cases of three patients, all of whom were admitted to hospital following the self-reported use of L-arginine containing supplements. A 33-year-old man presented with palpitations, dizziness, vomiting, and syncope, after the use of *$NO_2$ Platinum*. In addition, a 21-year-old man with palpitations and near syncope had used a "nitric oxide" supplement, whilst a 24-year-old man presented after taking *NO-Xplode* with palpitations and a headache. According to Prosser et al. (2009), the purported active ingredient in each of these products is L-arginine, and therefore consumption of L-arginine containing supplements may be associated with adverse effects on health, adding a further element of doubt regarding the necessity of L-arginine supplementation in healthy subjects.

## 1.8 – ***Concluding Remarks***

The reported physiological and biochemical effects of L-arginine have served as the rationale behind the development and marketing of a number of NO stimulating dietary supplements to elite athletes (Campbell et al. 2006). Such supplements profess to augment NO production, therefore improving blood flow to muscle during training, and in turn, leading to greater training adaptations. Consequently, in recent years, professional sports individuals have begun to use L-arginine supplementation prior to competition as an ergogenic aid. Indeed, supplementation with L-arginine and similar "NO boosters" has soared in popularity over the last decade (Campbell et al. 2006), despite the fact that there is an overall lack of supportive data in healthy human cohorts, as ergogenic potential remains controversial and inconclusive (Bailey et al. 2010; Liu et al. 2010).

As demonstrated, there is a wealth of evidence exploring the ergogenic potential of L-arginine supplementation for improved exercise performance. The findings in healthy humans seem inconsistent and often conflicting. Specifically, improvements in sprint performance (Buford & Koch, as cited in Bailey et al. 2010), time to exhaustion (Bailey et al. 2010), cycling time trial performance (Ranchordas & Whitehead, 2011), muscular power and fatigue resistance (Campbell et al. 2006) have each been observed in studies following L-arginine supplementation, yet the supportive evidence-base is limited and conflicted. Equally, supplementation with L-arginine has been shown to be ineffective in improving muscular endurance and resistance to fatigue (Wax et al. 2012; Greer & Jones, 2011; Willoughby et al. 2011).

The possible adverse health effects (Prosser et al. (2009) should not be neglected or minimalised, however, in an industry where the margins between success and failure can be incredibly small, it is feasible to suggest that any potential benefit to athletes provided by L-arginine should be considered worthwhile.

## 1.9 – ***Rationale for Further Research***

As demonstrated, the ergogenic potential of L-arginine-based supplementation remains equivocal in the literature, and the need for further research is therefore well established. Experimentation with higher dosages, perhaps as high as 30g as hypothesised by Bode-Boger et al. (1998) may be justified. However, concerns with tolerance and aforementioned side effects may arise.

In addition, research into different supplementation protocols may be warranted. As many L-arginine containing sports products are marketed as 'pre-trainers' and 'NO-boosters' (Campbell et al. 2006), it is problematic that the rationale for acute supplementation should provide such a sizeable research gap. Indeed, it does appear that industry instructions to ingest L-arginine before exercise are supported by little evidence (Greer & Jones, 2011). Only a handful of studies have adopted this ingestion protocol, and the data is inconsistent in terms of ergogenic effect (Ranchordas & Whitehead, 2011) and no effect (Greer & Jones, 2011; Wax et al. 2012). Further research into the ergogenic effects of acute doses of L-arginine prior to an exercise bout is therefore justified.

***Appendix 1***

***Literature Review Summary Table: The effects of L-arginine supplementation on exercise performance***

| Researchers | Subjects | Supplementation Protocol | Methodology | Ergogenic Effects? | Results | Concluding Remarks | Critique |
|---|---|---|---|---|---|---|---|
| **Bailey *et al*. (2010)** *University of Exeter* | n = 9 Recreationally active young men (26 ±6yr) | 6g Arg once daily (1hour pre-test) for 3 days of testing (no caffeine or alcohol for 24h)<br><br>10 day washout period | Moderate intensity cycling & high intensity cycling to fatigue (over 3 consecutive days) | ✓ | **Plasma $NO_2$ was increased** (ARG 331±198 vs PL 159±102nM, p<0.05). **Systolic BP was reduced** (ARG 123±3 vs PL 131±5mmHg, p<0.05). **Time to exhaustion was extended** (ARG 707±232 vs PL 562±145s, p<0.05). **$O_2$ consumption was reduced** (ARG 1.48±0.12 vs PL 1.59±0.14l/min, p<0.05). | "Arg significantly increased plasma $NO_2$, reduced systolic BP and resulted in reduced $O_2$ cost and improved time to fatigue in moderate/high intensity exercise" | Supplement composition (ARK – containing vitamins, other amino acids and fructose) |
| **Campbell *et al*. (2006)** *Baylor University, USA* | n = 20 ARG n = 15 PLA Resistance trained adult men (38.9±5.9yr) | Four caplets AAKG, three times per day (12 caplets = 6g per day) for eight weeks<br><br>IOC certified supplement | 4 days/week resistance training programme and 70% max aerobic work 3 days/week for a total of 8 weeks.<br><br>Recorded reps & weights on training cards | ✓ | **Significant difference in 1RM bench press performance** (AAKG 8.82±7.33 vs PLA 2.67±9.11, p=0.03) Significant positive effects on Wingate peak power performance. **No significant effects on aerobic capacity variables (time to exhaustion, $O_2$ consumption), BP or body composition.** | "AAKG supplementation appeared safe and well tolerated, and positively influenced 1RM bench press and Wingate peak power performance. AAKG did not influence body composition or aerobic capacity" | Sponsored by relevant nutrition facility (Medical Research Institute, San Francisco) who provided supplement<br><br>Older subjects |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Chen *et al.* (2010)**<br>*UCLA, USA* | n = 8 ARG<br>n = 8 PLA<br>Sixteen 'elderly' male cyclists (50-73yr) | 5.2g ARG once daily before bed over a 3 week testing period<br>Each serving contained 5.2 g L-arginine with L-citrulline, ascorbic acid, vitamin E, folic acid, L-taurine) | Incremental cycle ergometer test to fatigue<br><br>Constant work rate test at 60% max | ✓ | **Anaerobic threshold significantly increased in Arg group: by** 16.7% at week 1, and the effect was sustained by week 3 with a 14.2%. In the control group, there was no change in anaerobic threshold at weeks 1 and 3 compared to baseline. The anaerobic threshold for the supplement groups was significantly higher than that of placebo group. | "An arginine containing supplement increased the anaerobic threshold in elderly cyclists. This study indicates a potential role of L-arginine and antioxidant supplementation in improving exercise performance in elderly" | Low applicability to younger subjects (impaired NO production & exercise capacity)<br><br>Supplement composition |
| **Ranchordas & Whitehead (2011)**<br>*Sheffield Hallam University* | n = 6<br>Male competitive cyclists (23±5yr) | 6g Arg once daily for 3 days preceding testing<br><br>10 day washout period | Incremental cycle test to fatigue followed by 1 hour rest and 20km time trial. | ✓ | **Time trial completion time was reduced by 34s** (PLA 32.38±1.50 vs ARG 32.04±1.38m, $p<0.05$).<br>**$O_2$ consumption reduced** (PLA 51.6±8.2 vs ARG 47.5±6.1ml/kg/min, $p<0.05$).<br>**Both systolic & diastolic BP reduced** (PLA 132±7 vs ARG 127±7mmHg, $p<0.05$ and PLA 79±5 vs ARG 74±5mm Hg, $p<0.05$). | "L-arg supplementation at a dose of 6g/day increases 20km time trial performance, reduces $O_2$ consumption and reduces systolic and diastolic BP" | Small sample size |
| **Tang *et al*. (2011)**<br>*McMaster University, Canada* | n = 8<br>Recreationally active males (22.1±2.6 yr) | 10g Arg as part of a **post-exercise beverage** | Seated leg press and knee extension resistance exercise – Single leg only | **X** | Plasma endothlein-1, nitrate, and nitrite concentrations were unchanged, with **no difference between ARG and CON.**<br><br>Following the bout of exercise, femoral artery blood flow increased ~270% above basal in the exercised leg but not in the non-exercised leg, with **no difference between ARG & CON.**<br><br>Muscle protein synthesis greater in the exercised leg than non-exercised leg, with **no difference between ARG & CON**. | "Despite a 4-fold increase in plasma levels, arginine supplementation does not stimulate an increase in NO synthesis or muscle blood flow in young men at rest or after resistance exercise, questioning the ergogenic potential of arginine in healthy young men" | Novel approach for use of Arg as a post-exercise supplement |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Liu *et al*. (2010)** *National College of Physical Education, Taiwan* | n = 10<br>Elite male judo athletes (20.2±0.6 yr) | 6g Arg/day for two days pre-testing with final dose of 6g 1 hour pre-test<br><br>4 day washout period | Intermittent anaerobic exercise test. Alternated 20-sec all-out exercise and 15-sec rest periods for 13 sets cycle ergometer. Designed to mimic the actual judo competition. | X | No significant difference in peak power (ARG: 29.0±4.9%; CON: 35.1±4.5%) and average power (ARG: 28.0±4.3%; CON: 28.6±3.4%).<br><br>No significant difference was found between the ARG (85.0 ± 3.6%) and CON (83.6 ± 2.9 %) trials before exercise for Vascular Reflection Index (a measure of vasodilation). | "The results of this study suggested that short-term arginine supplementation had no effect on exercise-induced vasodilation and performance in intermittent anaerobic exercise in well-trained male judo athletes" | Potential low applicable to Western athletic diet |
| **Greer & Jones (2011)** *Sacred Heart University, USA* | n = 12<br>Trained college male athletes (22.6 ±3.8yr) | 3700mg AAKG 4 hours and again 30 minutes before exercise (no caffeine on day of testing)<br><br>7 day washout period | Three sets each of chin-ups, reverse chin ups and push ups to exhaustion with 3 mins rest between sets | X | **No effect on chin-up repetitions** (AAKG 23.75±6.38 vs PL 25.58±7.18).<br>**No effects on total repetitions** (AAKG 137.92±28.18 vs PL 141.08±28.57).<br>**No effects on BP** | "Arg had no positive ergogenic benefit and may acutely be counterproductive for muscular endurance"<br>"Because AAKG may hinder muscular endurance, the use before resistance training should be questioned" | Supplement quality control – Ingredients not tested (cited as a potential confounder by authors) |
| **Santos *et al*. (2002)** *University of Paraiba Valley, Brazil* | n = 12<br>Healthy male volunteers (23.8±3.5yr | 3g Arg (1.5g twice a day) daily for 15 days during testing period. | Five minute, cardiovascular warm-up and stretching followed by 15 repetitions of isokinetic knee extension & flexion. | ✓ | Significant difference between control (no beverage) and ARG condition for various fatigue indices and fatigue resistance factor (FRF). | "Treatment by oral arginine leads to an increase in the capacity of resistance to muscular fatigue" | Single-blind design |
| **Wax et al. (2012)** *Missisippi State University, USA* | n = 16<br>Eight resistance-trained, eight untrained (19.8 ± 1.9 yr) | 3000mg AAKG or placebo: 45 minutes prior to a resistance exercise protocol. | Following the determination of one repetition maximum (1RM), subjects completed repetitions to failure at 60% 1RM on both barbell bench press and leg press. | X | **No significant difference (P<0.05)** in bench press or leg press performance between AAKG or PLA conditions for either 1RM of total load volume, regardless of the training status of the subjects. | "Acute AAKG supplementation provided no ergogenic benefit, regardless of the subject's training status..... and AAKG is therefore not recommended for healthy individuals" | Supplement quality control – Ingredients not tested (cited as a potential confounder by authors)<br><br>Groups determined by self-reported training status. Significant differences between the groups at baseline. |

References

Álvares, T. S., Meirelles, C. M., Bhambhani, Y. N., Paschoalin, V. M. F. & Gomes, P. S. C. (2011). L-Arginine as a Potential Ergogenic Aid in Healthy Subjects. *Sports Medicine*. 41: 3, 233-248

Appleton, J. (2002). Arginine: Clinical Potential of a Semi-Essential Amino Acid. *Alternative Medical Review*; 7(6): 512-522

Bailey, S. J., Winyard, P. G., Vanhatalo, A., Blackwell, J. R., DiMenna, F. J., Wilkerson, D. P. & Jones, A. M. (2010). Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *Journal of Applied Physiology.* 109:1394-1403

Bloomer, R. J., Farney, T. M., Trepanowski, J. F., McCarthy, C. G., Canale, R. E., & Schilling, B. K. (2010). Comparison of pre-workout nitric oxide stimulating dietary supplements on skeletal muscle oxygen saturation, blood nitrate/nitrite, lipid peroxidation, and upper body exercise performance in resistance training men. *Journal of International Society of Sports Nutrition.* 7, 16-30

Bode-Boger, S. M., Boger, R. H., Galland, A., Tsikas, D. & Frolich, J. C. (1998). L-arginine induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. *British Journal of Clinical Pharmacology.* 46, 489-497

Campbell, B. I., La Bounty, P. M. & Roberts, M. (2004). The Ergogenic Potential of Arginine. *Journal of the International Society of Sports Nutrition.* 1(2):35-38

Campbell, B. I., Roberts, M., Kerksick, C., Wilborn, C., Marcello, B., Taylor, L., Nassar, E., Leutholtz, B., Bowden, R., Rasmussen, C., Greenwood, M. & Kreider, R. (2006).

Pharmacokinetics, safety, and effects on exercise performance of L-arginine a-ketoglutarate in trained adult men. *Nutrition. 22*. 872-881

Castillo, L., Sánchez, M., Vogt, J., Chapman, T. E., DeRojas-Walker, T. C., Tannenbaum, S. R., Ajami, A. M. & Young, V. R. (1995). Plasma arginine, citrulline, and ornithine kinetics in adults, with observations on nitric oxide synthesis. *American Journal of Physiology, Endocrinology and Metabolism.* 268: E360–E367

Ceremuzynski, L., Chamiec, T. & Herbaczynska-Cedro, K. (1997). Effect of Supplemental Oral L-Arginine on Exercise Capacity in Patients with Stable Angina Pectoris. *American Journal of Cardiology*. 80: 331-333

Chen, S., Kim, W., Henning, S. W., Carpenter, C. L. & Li, Z. (2010). Arginine and antioxidant supplement on performance in elderly male cyclists: a randomized controlled trial. *Journal of the International Society of Sports Nutrition.* 7:13, 111-117

Fahs, C. A., Heffernan, K. S. & Fernhall, B. (2009). Hemodynamic and vascular response to resistance exercise with L-arginine. *Medicine & Science in Sports & Exercise.* 41: 4, 773-779

Greer, B. K. & Jones, B. T. (2011). Acute Arginine Supplementation Fails To Improve Muscle Endurance Or Affect Blood Pressure Responses To Resistance Training. *Journal of Strength & Conditioning Research.* 25(7), 1789-1794

Hambrecht, R., Hilbrich, L., Erbs, S., Gielen, S., Fiehn, E., Schoene, N. & Schuler, G. (2000). Correction of Endothelial Dysfunction in Chronic Heart Failure: Additional Effects of Exercise Training and Oral L-Arginine Supplementation. *Journal of the American College of Cardiology.* 35: 706-713

Imanipour, V., Naderi, A., Mahdi, F. & Shahedi, V. (2011). The effects of supplementary L-arginine on serum nitric oxide concentration in male bodybuilders. *International Conference on Bioscience, Biochemistry and Bioinformatics.* IPCBEE, Singapore

Lerman, A., Burnett, J. C., Higano, S. T., McKinley, L. J. & Holmes, D. J. (1997). Long-term L-Arginine Supplementation Improves Small-Vessel Coronary Endothelial Function in Humans. *Circulation.* 97: 2123-2128

Liu, T. H., Hung, W., Jang, T. R. & Chang, C. K. (2010). Effect of Short-term Arginine Supplementation on Vasodilation and Performance in Intermittent Exercise in Judo Athletes. *International Journal of Sport and Exercise Science,* 1(2): 7-12

Morris, S. M. (2006). Arginine: beyond protein. *American Journal Clinical Nutrition*. 83, 508S-512S

Paddon-Jones, D., Børsheim, E. & Wolfe, R. R. (2004). Potential Ergogenic Effects of Arginine and Creatine Supplementation. *Journal of Nutrition.* 134: 2888S–2894S

Prosser, J. M., Majlesi, N., Chan, G. M., Olsen, D., Hoffman, R. S. & Nelson, L. S. (2009). Adverse effects associated with arginine alpha-ketoglutarate containing supplements. *Human & Experimental Toxicology*. 28(5), 259-262

Ranchordas, M. K. & Whitehead, T. (2011). Effect of acute L-arginine supplementation on 20km time trial performance in competitive male cyclists. *British Journal of Sports Medicine.* doi: 10.1136/bjsports-2011-090606.35

Santos, R. S., Pacheco, M. T. T., Martins, R. A. B. L., Villaverde, A. B., Giana, H. E. & Baptista, F. & Zangaro, R. A. (2002). Study of the effect of oral administration of L-arginine

on muscular performance in healthy volunteers: An isokinetic study. *Isokinetics and Exercise Science.* 10, 153–158

Tang, J. E., Lysecki, P. J., Manolakos, J. J., MacDonald, M. J., Tarnopolsky, M. A. & Phillips, S. M. (2011). Bolus Arginine Supplementation Affects neither Muscle Blood Flow nor Muscle Protein Synthesis in Young Men at Rest or After Resistance Exercise. *The Journal of Nutrition.* 141(2), 195-200

Walker, H. A., McGing, E., Fisher, I., Boger, R. H., Bode-Boger, S. M., Jackson, G., Ritter, J. M. & Chowiencyzk, P. J. (2001). Endothelium-dependent vasodilatation is independent of the plasma L-Arginine/ADMA ratio in men with stable angina. *Journal of the American College of Cardiology.* 38: 499-505

Wax, B., Kavazis, A. N., Webb, H. E. & Brown, S. P. (2012). Acute L-arginine alpha ketoglutarate supplementation fails to improve muscular performance in resistance trained and untrained men. *Journal of the International Society of Sports Nutrition.* doi: 10.1186/1550-2783-9-17

Willoughby, D. S., Boucher, T., Reid, J., Skelton, G. & Clark, M. (2011). Effects of 7 days of arginine-alpha-ketoglutarate supplementation on blood flow, plasma L-arginine, nitric oxide metabolites and asymmetric dimethyl arginine after resistance exercise. *International Journal of Sports Nutrition and Exercise Metabolism.* 21: 291-299

Wu, G. & Meininger, C. J. (2000). Arginine Nutrition and Cardiovascular Function. *Journal of Nutrition.* 130: 2626-2629

Wu, G. & Morris, S. M. (1998). Arginine metabolism: nitric oxide and beyond. *Biochemical Journal. 336*, 1-17

# Research Paper

## The effects of acute arginine supplementation on 10 mile cycling time trial performance in young adult males

**Word count: 3670**

***Contents***

With special thanks to Mike Morris, University of Chester

*Department of Clinical Sciences*

*****

**Rationale for Journal Publication**

A potential target journal for publication of this work would be *The Journal of Strength & Conditioning Research (JSCR).* The JSCR pledges to "advance the knowledge and understanding of conditioning and sport through applied exercise science" and, according to the 2011 ISI Citation Report, is ranked in the top 30 journals in the field of Sport & Exercise Science. A unique aspect of this journal is that it includes recommendations for the practical applications of research findings, allowing the front-line sports practitioner to immediately benefit from contemporary findings. With a relatively successful Impact Factor of 1.831, and a proven track record in the subject area (a literature search shows that four relevant studies regarding arginine ingestion and exercise performance have been published in the journal since 2010), the JSCR appears a feasible choice for potential publication.

*****

**Abstract**

**Introduction:** L-arginine is one of the most metabolically versatile amino acids in the human body, most notably serving as the pre-cursor for the biosynthesis of nitric oxide (NO). The reported physiological effects of L-arginine have served as the rationale behind the development and marketing of a number of NO stimulating dietary supplements, which profess to augment NO production and improve blood flow to muscle during exercise. Supplementation with L-arginine and similar "NO boosters" has soared in popularity over the last decade, despite the fact that there is an overall lack of supportive data in healthy humans, as ergogenic potential remains inconclusive. The aim of this study was therefore to determine the effects of acute supplementation of commercially available L-arginine on exercise performance.

**Methods:** Twelve recreationally trained, young adult males (22.3 ± 4.1 yr, 79.3 ± 7.9 kg, 180.9 ± 2.3 cm) consumed either: a placebo (PLA), an L-arginine beverage containing 8g L-arginine (ARG) or no beverage (CON) in a double-blind, repeated-measures design. 45 minutes following consumption, participants completed a 10 mile time trial on a cycle ergometer.

**Results:** There was no significant difference (p=0.643) in time-trial performance between the conditions (CON 29:49 ± 2:19 vs ARG 29:49 ± 3:18 vs PLA 29:30 ± 2:42 minutes). There was no significant difference (p=0.276) between conditions in volitional power output (W) (CON 119.3 ± 8.7 vs ARG 120.1 ± 7.7 vs PLA 121.2 ± 6.2 W), or in heart rate responses (p=0.129) (CON 169.2 ± 11.3 vs ARG 167.2 ± 10.8 vs PLA 166.3 ± 7.8 bpm). Significant differences (p=0.033) were observed between conditions (CON 15.6 ± 1.6 vs ARG 15.2 ± 2.0 vs PLA 15.0 ± 1.7) in RPE responses.

**Conclusions:** With no ergogenic benefits observed in this study, the rationale for pre-exercise supplementation with arginine may be further brought into question.

## Introduction

L-arginine is one of the most metabolically versatile amino acids in the human body, featuring in a number of crucial physiological and biochemical processes (Morris, 2006). In its most extensively reviewed role, L-arginine serves as the precursor for the biosynthesis of nitric oxide (NO), an essential substance in regulating vascular tone, triggering vasodilation and increasing blood flow (Wu & Meininger, 2000).

Despite the fact that L-arginine is already stored in significant intracellular amounts (Hambrecht *et al.* 2000), previous literature has indicated that L-arginine supplementation is associated with improvements in endothelial function, for example, increasing blood flow by increasing arterial diameter via NO-mediated vasodilation (Bailey *et al*. 2000; Lerman *et al.* 1998). In theory, increasing blood flow will then in turn improve exercise performance via an increased rate of oxygen and substrate delivery (Tang *et al.* 2011), glucose uptake, and removal of waste exercise bi-products. The aforementioned physiological pathways of L-arginine would therefore reasonably suggest that supplemental L-arginine could improve exercise performance (Campbell, La Bounty & Roberts, 2004), yet the available literature evidence is limited and conflicting.

The reported physiological and biochemical effects of L-arginine have served as the rationale behind the development and marketing of a number of NO stimulating dietary supplements to elite athletes (Campbell *et al.* 2006). Such supplements profess to augment NO production, therefore improving blood flow to muscle during training, and in turn, leading to greater training adaptations. Consequently, in recent years, professional sports individuals have begun to use L-arginine supplementation prior to competition as an ergogenic aid

(Campbell *et al.* 2006) Indeed, supplementation with L-arginine and similar “NO boosters” has soared in popularity over the last decade (Campbell *et al*. 2006), despite the fact that there is an overall lack of supportive data in healthy human cohorts, as ergogenic potential remains controversial and inconclusive (Bailey *et al*. 2010; Liu, Hung, Jang & Chang. 2010).

While L-arginine supplementation has been compellingly established to improve exercise tolerance in patient populations (Hambrecht *et al*. 2000; Ceremuzynski, Chamiec & Herbaczynska-Cedro, 1997), the evidence in healthy humans, and athletic populations, is less consistent and often conflicting. Specifically, improvements in repeated sprint performance (ARG -1 ± 9 vs PLA -47 ± 18 W, power output decrease between sprints, Buford & Koch, 2004); time to exhaustion (ARG 707 ± 232 vs PLA 562 ± 145 seconds, Bailey *et al.* 2010); cycling time trial performance (ARG 32:04 ± 1:38 vs PLA 32:38 ± 1:50 min, Ranchordas & Whitehead, 2011); muscular power and fatigue resistance (ARG 8.82 ± 7.33 vs PLA 2.67 ± 9.11 kg change in 1RM, Campbell *et al*. 2006) have each been observed in studies following L-arginine supplementation; yet the supportive evidence-base is limited and conflicted. Equally, supplementation with L-arginine has been shown to be ineffective in improving muscular endurance and resistance to fatigue (Wax, Kavazis, Webb & Brown, 2012; Greer & Jones, 2011; Willoughby *et al*. 2011).

## Aims & Hypotheses

In the absence of fully substantiated claims from supplement manufacturers supporting the notion that arginine-containing supplements indeed provide ergogenic benefit; the aim of this study was therefore to determine the effects of acute supplementation of commercially available L-arginine on cycling time trial performance, heart rate (HR) response, volitionally selected power output (PO) and rating of perceived exertion (RPE). The following hypotheses were made prior to the commencement of the study. Acute consumption of arginine pre-training will significantly: 1) improve cycling time trial performance, 2) enhance power output, 3) reduce perceived exertion, and 4) reduce heart rate.

## Methods

### *Participants:*

Twelve recreationally trained, young adult males volunteered to participate in three trials in a double-blind, repeated-measures design. The sample size was justified through the use of a sample size power calculation to achieve a power of 0.8 (Appendix 1). The definition of 'recreationally trained' was adopted from Gearhart (2008) and, as such, inclusion criteria was anyone that was not training for a specific event or activity, but was training ''recreationally'', on a regular, planned basis, for health, cosmetic or other benefits. Exclusion criteria for participants was any pre-existing illness, ailment or medical condition, screened before the commencment of the study via a health screening questionaire (Appendix 2). All participants provided written, informed consent and ethical approval was obtained from the Faculty of Applied and Health Sciences ethics committee at the University of Chester.

***Procedures:***

This study utilised a repeated measures design in which each participant was required to attend the gym facility on three separate occasions. Upon the first visit to the gym, participants were provided a thorough familiarisation of procedures, anthropometric measurements of height and weight were taken, and subsequently a 10-mile cycling time trial was performed on a cycle ergometer (Corival Ergometer, Lode, Netherlands) to establish baseline characteristics. No pre-test beverage was provided in this control (CON) trial.

The use of time-trial as a measure of exercise performance was justified by the work of Jeukendrup, Saris, Brouns and Kester (1996) who validated that tests to exhaustion have a much lower reproducibility, and therefore reliability, than time-trial protocols, which are therefore a better solution for performance evaluation.

Throughout the time trial, participants volitionally selected a level of PO, using the bike's keypad, pressing 'up' to increase the load, and 'down' to reduce it. Cycling time trial completion time, PO, HR and RPE (via Borg scale) were recorded throughout, the latter three measures being taken by the researcher at pre-determined time intervals of 5 minutes until test completion. All measures were concealed from participants at all times.

During the next two testing sessions, participants consumed either a pre-prepared 500ml placebo (PLA) or an L-arginine beverage containing 8g L-arginine (ARG). In PLA, the drink consisted of 400ml water and 100ml fruit juice concentrate (Robinson's "Apple and Blackcurrent - No Added Sugar", Britvic PLC, UK). In the ARG condition, the drink was the same but also contained 8g of commercially available arginine in powdered form (Primary

Arginine ®, Maximuscle Ltd, Hemel Hempstead, UK). This dose had previously been shown to be 'safe and well tolerated' (Campbell *et al*. 2006) and a seven-day washout period seperated the trials, in line with previous work (Greer & Jones, 2011).

Supplements were ingested immediately upon arrival at the gym and 45 minutes prior to exercise, in line with previous protocols (Wax *et al.* 2012; Buford & Koch, 2004). They were the same colour, texture and taste, and were administered in a double-blind fashion, with beverages taken in a randomised counter-balanced manner to prevent a learning effect taking place. Following ingestion of the supplement, participants rested during a full briefing and equipment set-up and then prepared for testing, prior to a short warm-up and subsequently a 10-mile cycling time trial.

Participants were instructed to refrain from caffeine and alcohol in the 24 hours preceding each test as these have been previously demonstrated to effect exercise capacity and performance variables (Cox *et al*. 2002). All tests were performed at the same time of day (± 2 hours) for each participant to minimise the effects of biological variation on physiological responses and exercise performance (Atkinson & Reilly, 1996).

***Data Analysis:***

Statistical analysis was performed using commercially available software, SPSS 19 (SPSS Inc., Chicago, IL, USA). Statistical significance was accepted when $p < 0.05$ and data are presented as mean ± SD. Following a check on data distribution normality via the Shapiro-Wilk statistic, time-trial performance, HR, PO and RPE, were each assessed by use of separate One-way (Repeated Measures) ANOVAs. Paired t-tests were used Post Hoc to identify where significant differences lay, followed by Bonferroni multiple comparison

adjustment as appropriate to reduce the potential for a type 1 statistical error. Mauchly's test of sphericity was used to confirm homogeneity.

## Results

### *Participant Characteristics:*

All twelve volunteers met the inclusion criteria and completed all testing procedures. Participants had a mean age, weight and height of 22.3 ± 4.1 years, 79.3 ± 7.9 kg, 180.9 ± 2.3 cm, respectively. Participant compliance with supplement ingestion was 100%.

### *Time Trial Performance:*

In terms of 10-mile cycling time-trial performance, there was no significant difference ($p = 0.643$) between the conditions (CON 29:49 ± 2:19 vs ARG 29:49 ± 3:18 vs PLA 29:30 ± 2:39 minutes). There was a mean 19 second reduction in time-trial completion time in the PLA condition, but this was deemed insignificant when compared to the CON ($p = 0.276$) and ARG ($p = 0.394$) trials (Fig. 1).

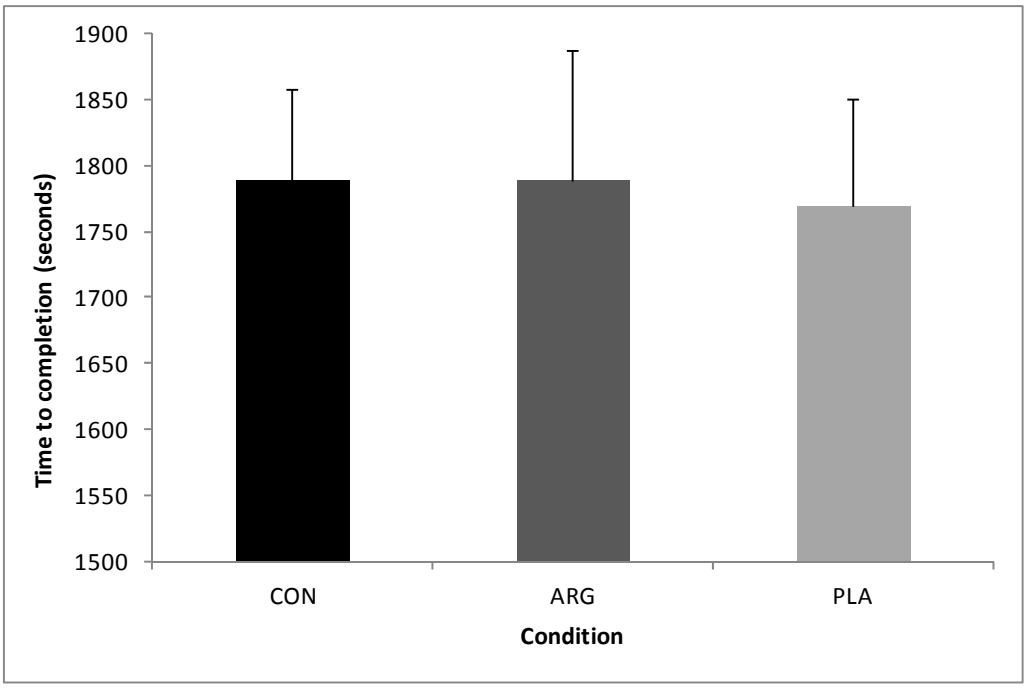


**Figure 1: Time to completions (seconds) for 10-mile cycling time trial on Corival Ergometer. Data are displayed as mean ± SD. There was no significant different difference between the conditions (p = 0.643).**

***Heart Rate:***

HR increases linearly as exercise intensity increases. While the present findings reflect this relationship across all conditions (Fig. 2), HR responses were not significantly different (p = 0.129) between conditions (CON 169.2 ± 11.3 vs ARG 167.2 ± 10.8 vs PLA 166.3 ± 7.8 bpm).

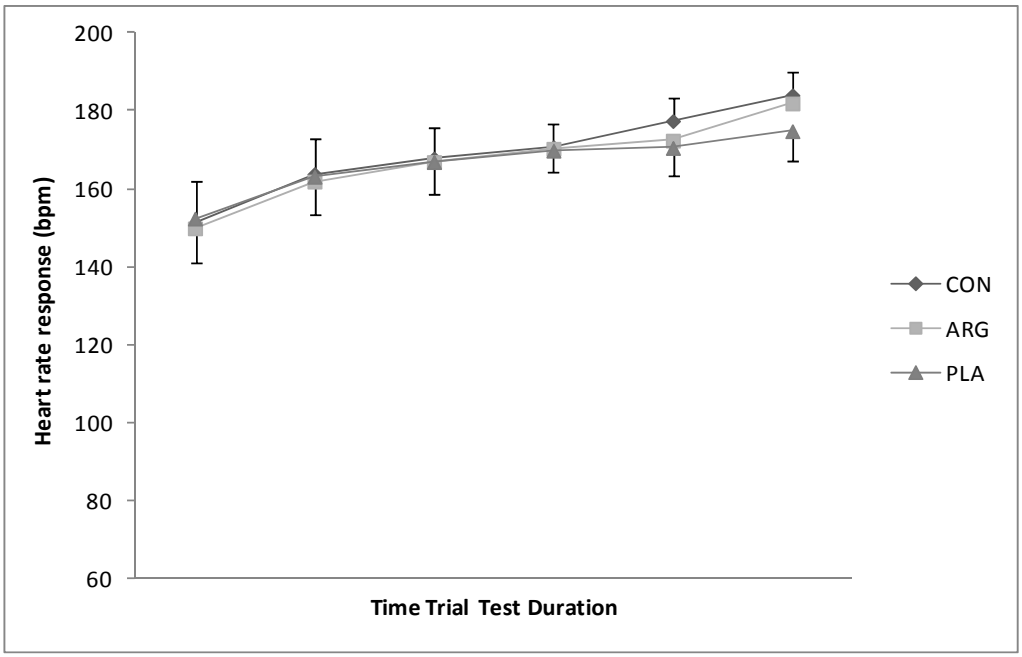


**Figure 2: Heart rate (bpm) responses throughout duration of time-trial protocol. Data are presented as mean ± SD. There was no significant difference between the conditions (p = 0.129).**

***Power Output:***

No significant difference (p = 0.276) was observed between conditions (CON 119.3 ± 8.7 vs ARG 120.1 ± 7.7 vs PLA 121.2 ± 6.2 W) in terms of self-selected PO (Fig. 3).

***Rating of Perceived Exertion:***

Significant difference (p = 0.033) was observed between conditions (Fig. 4) (CON 15.6 ± 1.6 vs ARG 15.2 ± 2.0 vs PLA 15.0 ± 1.7). Post Hoc analysis via multiple paired t-tests revealed that this difference lay between CON & PLA (p = 0.0005), while no significant difference was observed between CON & ARG (p = 0.148) or ARG & PLA (p = 0.149). After a Bonferroni adjustment for multiple comparisons, statistical significance was accepted as $p < 0.017$.

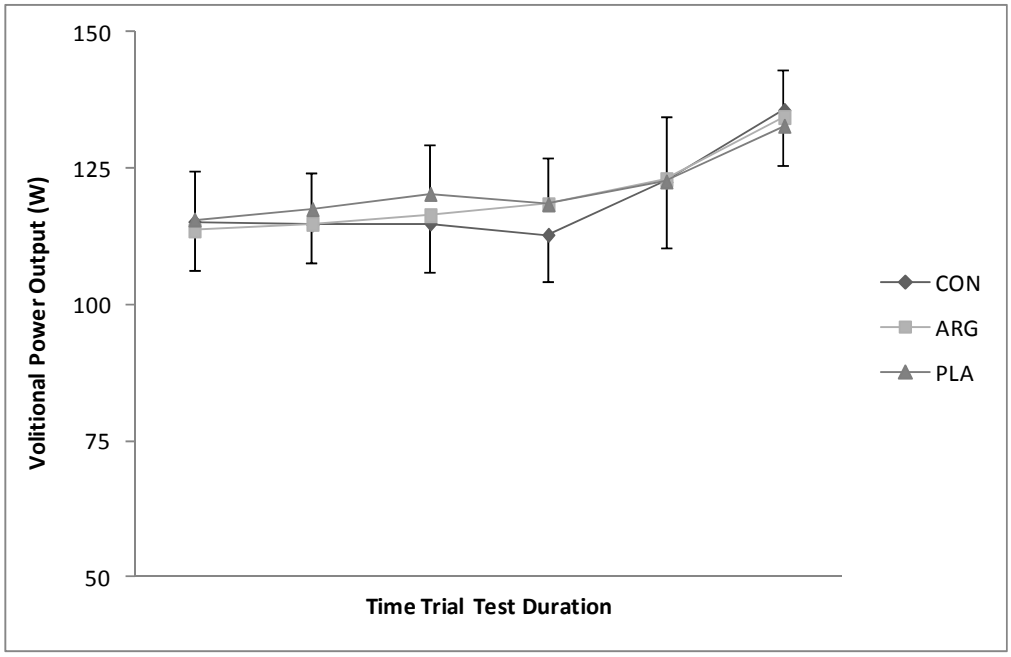


**Figure 3: Self-selected power output (W) values throughout duration of time-trial protocol. Values were hidden from participants throughout by taping across the bike's output display. Data are presented as mean ± SD. There was no significant difference between the conditions (p = 0.276).**

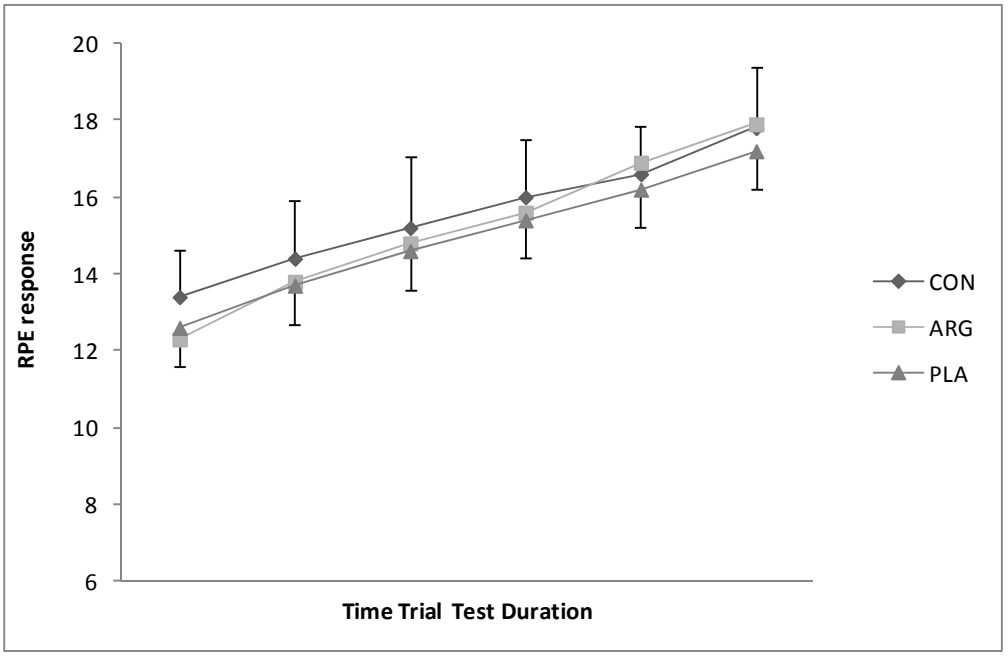


**Figure 4: RPE responses throughout the duration of the time-trial protocol. Data are presented as mean ± SD. There was significant difference between CON & PLA (p = 0.0005) but no significant difference was observed between the experimental trials ARG & PLA (p = 0.149) or between CON & ARG (p = 0.148).**

## Discussion

The main finding of this study was that acute supplementation with L-arginine, 45 minutes prior to exercise, provided no ergogenic benefit. These results bring into question the ergogenic potential of arginine in healthy young men. The ergogenic potential of arginine-based supplementation remains equivocal in the literature.

Previously, improvements in sprint performance (Buford & Koch, 2004), time to exhaustion during high intensity cycling to fatigue (Bailey *et al.* 2010), muscular power and fatigue resistance (Campbell *et al.* 2006) have each been observed in studies following L-arginine supplementation. However, concurrent with the findings of the present study, Greer & Jones (2011) did not observe any ergogenic effect on muscular endurance following acute supplementation with AAKG; neither did Wax *et al*. (2012) find any improvement in muscular performance following an acute dose of AAKG in either trained and untrained individuals.

A very relevant, recent study (Ranchordas & Whitehead, 2011) did observe improvements (ARG 32:04 ± 1:38 vs PLA 32:38 ± 1:50 min) in 20km cycling time trial performance in highly trained, competitive male cyclists, following a 3 day supplementation with L-arginine, however, the small sample size (n=6) makes it difficult to draw conclusions.

Although arginine-containing 'NO stimulating' supplements are marketed to increase exercise performance, this study does not support this contention with acute ingestion. It is true that the major advertised ergogenic benefit of arginine-based supplements rests on the assumption of augmented production of NO. However, it should be noted that this is not a foregone conclusion (Greer & Jones, 2011). Indeed, Tang *et al*. (2010) reported that, despite

a 300% increase in plasma arginine levels, arginine supplementation did not stimulate an increase in NO synthesis or muscle blood flow in young men, at rest or after resistance exercise ($p > 0.05$).

Likewise, Willoughby *et al.* (2011) observed that 7-days L-arginine supplementation provided no benefit in terms of hemodynamics or blood flow. Analysis revealed that although heart rate, systolic and diastolic blood pressure and MAP each increased significantly ($p = 0.001$) with a resistance exercise protocol, there was no significant difference ($p > 0.05$) between ARG and PLA groups. Furthermore, although plasma-L-arginine significantly increased in the ARG group, was no significant difference between groups ($p = 0.73$) in levels of serum NOx (nitrites/nitrates). Likewise, there was no significant difference between ARG and PLA in terms of brachial-artery blood flow responses ($p = 0.14$), which brings into question the very rationale of L-arginine ergogenics.

There is a recent selection of literature in support of the findings of Willoughby *et al*. (2011). For example, it has been shown that single-doses of three separate alleged nitric-oxide-inducing supplements (each containing L-arginine) were each ineffective at increasing circulating nitric oxide levels and blood flow in response to resistance exercise (Bloomer *et al*. 2010). Similarly, work by Fahs, Heffernan & Fernhall (2009) observed that there was no significant change in blood flow or hemodynamic and vascular responses when 7g of L-arginine was given acutely, immediately before resistance exercise.

The lack of ergogenic effect in the present study may speculatively be as a result of the amount of L-arginine ingested. According to Willoughby *et al.* (2011), there is no empirical evidence demonstrating any acute vasodilatory effects with doses less than 15g/day,

Moreover, the issue of L-arginine's bioavailability must be taken into consideration. It has previously been suggested that as much as 50% of L-arginine consumed orally is metabolised in the liver (Castillo *et al*. 1995). Indeed, L-arginine bioavailability following oral administration has been found to be approximately 68% (Bode-Boger *et al.* 1998). As a result, it is conceivable to conclude that lower doses of L-arginine may lack the bioavailability required to augment nitric oxide production. Therefore it is plausible to suggest that the lack of ergogenic effect observed in the present study may be as a consequence of an inadequate amount of bioavailable L-arginine.

The lack of effect demonstrated by the present study, and previously by Greer & Jones (2011) and Wax *et al*. (2012) suggest that small, acute doses of L-arginine may be insufficient in harnessing ergogenic benefits. However, larger doses and extended loading protocols have also proved unsuccessful (Willoughby *et al.* 2011). This brings into question the rationale for pre-exercise supplementation with arginine-based products.

It is conceivable to speculate that the lack of effect observed on PO and HR in the present study is consequential of a lack of effect on nitric oxide stimulation, as aforementioned. However, no assessment on nitric oxide levels, or blood flow was conducted in the present study, a potential weakness to the study design.

One surprising finding in the present study was the significant difference ($p = 0.0005$) in participant RPE responses observed between CON and PLA trials. No significant difference was observed between CON & ARG ($p = 0.148$) or ARG & PLA ($p = 0.149$). It is speculated, however, that this difference could be accounted for merely by placebo effect, and emphasises a further potential limitation within the study design. The possibility of placebo

effect was not completely alleviated, owing to the complete absence of a beverage in the initial CON condition.

As demonstrated, the ergogenic potential of L-arginine-based supplementation remains equivocal in the literature, and the need for further research is therefore well established. Experimentation with higher dosages, perhaps as high as 30g as hypothesised by Bode-Boger *et al.* (1998) may be justified. However, concerns with tolerance and aforementioned side effects may arise.

Indeed, arginine doses of 10g have been associated with gastrointestinal pain and diarrhoea (Robinson, Sewell & Greenhaff, 2003). Meanwhile, Prosser *et al.* (2009) have reported the adverse side effects associated with cases of hospital admittance following the consumption of L-arginine containing supplements. The possible adverse health effects should not be neglected or minimalised, and with ergogenic benefit equivocal, Industry directives to consume doses of arginine before exercise may therefore be unwarranted (Greer & Jones, 2011).

In summary, the present study provides data herein that somewhat refute the claim that arginine-based supplements are effective 'pre-trainers' for enhancing exercise performance. No significant differences were observed between CON, ARG or PLA conditions in terms of cycling time-trial performance or performance variables of heart rate, power output or RPE.

## Practical Applications

Arginine-based 'NO boosting' supplements are marketed to increase muscular endurance and benefit exercise capacity. However, this study is in agreement with previous work (Greer & Jones, 2011; Wax *et al.* 2012; Willoughby *et al*. 2011) in not supporting this contention with acute ingestion.

A review of the literature suggests that L-arginine can indeed provide an alternative therapeutic approach in cases of endothelial dysfunction (Hambrecht *et al*. 2000). Yet, while there is compelling evidence for the potential benefits of supplemental L-arginine administration in these patient populations, the evidence in healthy humans is less consistent. Evidence such as that provided by the present study brings into question the ergogenic potential of L-arginine supplementation and therefore the efficacy of L-arginine products in healthy humans.

With concerns regarding possible adverse health effects and ingestion discomforts previously reported (Prosser *et al*. 2009; Robinson *et al.* 2003), and ergogenic potential remaining unclear, industry instructions to consume large doses of arginine before exercise may therefore be unwarranted (Greer & Jones, 2011), and the rationale for supplementation should be questioned.

**References**

Atkinson, G. & Reilly, T. (1996). Circadian variation in sports performance. *Sports Medicine*. 21(4): 292-312

Bailey, S. J., Winyard, P. G., Vanhatalo, A., Blackwell, J. R., DiMenna, F. J., Wilkerson, D. P. & Jones, A. M. (2010). Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *Journal of Applied Physiology.* 109:1394-1403

Bloomer, R. J., Farney, T. M., Trepanowski, J. F., McCarthy, C. G., Canale, R. E., & Schilling, B. K. (2010). Comparison of pre-workout nitric oxide stimulating dietary supplements on skeletal muscle oxygen saturation, blood nitrate/nitrite, lipid peroxidation, and upper body exercise performance in resistance training men. *Journal of International Society of Sports Nutrition.* 7, 16-30

Bode-Boger, S. M., Boger, R. H., Galland, A., Tsikas, D. & Frolich, J. C. (1998). L-arginine induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. *British Journal of Clinical Pharmacology.* 46, 489-497

Buford, B. N. & Koch, A.J. (2004). Glycine-arginine-α-ketoisocaproic acid improves performance of repeated cycling sprints. *Medicine & Science in Sports & Exercise.* 36: 583-587

Campbell, B. I., La Bounty, P. M. & Roberts, M. (2004). The Ergogenic Potential of Arginine. *Journal of the International Society of Sports Nutrition.* 1(2):35-38

Campbell, B. I., Roberts, M., Kerksick, C., Wilborn, C., Marcello, B., Taylor, L., Nassar, E., Leutholtz, B., Bowden, R., Rasmussen, C., Greenwood, M. & Kreider, R. (2006).

Pharmacokinetics, safety, and effects on exercise performance of L-arginine a-ketoglutarate in trained adult men. *Nutrition. 22*. 872-881

Castillo, L., Sánchez, M., Vogt, J., Chapman, T. E., DeRojas-Walker, T. C., Tannenbaum, S. R., Ajami, A. M. & Young, V. R. (1995). Plasma arginine, citrulline, and ornithine kinetics in adults, with observations on nitric oxide synthesis. *American Journal of Physiology, Endocrinology and Metabolism.* 268: E360–E367

Ceremuzynski, L., Chamiec, T. & Herbaczynska-Cedro, K. (1997). Effect of Supplemental Oral L-Arginine on Exercise Capacity in Patients with Stable Angina Pectoris. *American Journal of Cardiology*. 80: 331-333

Cox, G. R., Desbrow, B., Montgomery, P. G., Anderson, M. E., Bruce, C. R., Macrides, T. A., Martin, D. T., Moquin, A., Roberts, A., Hawley, J. A. & Burke, L. M. (2002). Effect of different protocols of caffeine intake on metabolism and endurance performance. *Journal of Applied Physiology*. 93: 990-999

Fahs, C. A., Heffernan, K. S. & Fernhall, B. (2009). Hemodynamic and vascular response to resistance exercise with L-arginine. *Medicine & Science in Sports & Exercise.* 41: 4, 773-779

Gearhart, R. F. (2008). Ratings of perceived exertion and oxygen consumption during maximal, graded, treadmill exercise following different anchoring procedures. *European Journal of Sport Science*. 8:1, 35-40

Greer, B. K. & Jones, B. T. (2011). Acute Arginine Supplementation Fails To Improve Muscle Endurance Or Affect Blood Pressure Responses To Resistance Training. *Journal of Strength & Conditioning Research.* 25(7), 1789-1794

Hambrecht, R., Hilbrich, L., Erbs, S., Gielen, S., Fiehn, E., Schoene, N. & Schuler, G. (2000). Correction of Endothelial Dysfunction in Chronic Heart Failure: Additional Effects of Exercise Training and Oral L-Arginine Supplementation. *Journal of the American College of Cardiology.* 35: 706-713

Jeukendrup, A., Saris, W. H. M., Brouns, F. & Kester, A. D. M. (1996). A new validated endurance performance test. *Medicine & Science in Sports & Exercise*. 28(2), p266-270

Lerman, A., Burnett, J. C., Higano, S. T., McKinley, L. J. & Holmes, D. J. (1997). Long-term L-Arginine Supplementation Improves Small-Vessel Coronary Endothelial Function in Humans. *Circulation.* 97: 2123-2128

Liu, T. H., Hung, W., Jang, T. R. & Chang, C. K. (2010). Effect of Short-term Arginine Supplementation on Vasodilation and Performance in Intermittent Exercise in Judo Athletes. *International Journal of Sport and Exercise Science,* 1(2): 7-12

Morris, S. M. (2006). Arginine: beyond protein. *American Journal Clinical Nutrition*. 83, 508S-512S

Prosser, J. M., Majlesi, N., Chan, G. M., Olsen, D., Hoffman, R. S. & Nelson, L. S. (2009). Adverse effects associated with arginine alpha-ketoglutarate containing supplements. *Human & Experimental Toxicology*. 28(5), 259-262

Ranchordas, M. K. & Whitehead, T. (2011). Effect of acute L-arginine supplementation on 20km time trial performance in competitive male cyclists. *British Journal of Sports Medicine.* doi: 10.1136/bjsports-2011-090606.35

Robinson, T. M., Sewell, D. A. & Greenhaff, P. L. (2003). L-arginine ingestion after rest and exercise: Effects on Glucose Disposal. *Medicine & Science in Sports & Exercise.* 35: 8, 1309-1315

Tang, J. E., Lysecki, P. J., Manolakos, J. J., MacDonald, M. J., Tarnopolsky, M. A. & Phillips, S. M. (2011). Bolus Arginine Supplementation Affects neither Muscle Blood Flow nor Muscle Protein Synthesis in Young Men at Rest or After Resistance Exercise. *The Journal of Nutrition.* 141(2), 195-200

Wax, B., Kavazis, A. N., Webb, H. E. & Brown, S. P. (2012). Acute L-arginine alpha ketoglutarate supplementation fails to improve muscular performance in resistance trained and untrained men. *Journal of the International Society of Sports Nutrition.* doi: 10.1186/1550-2783-9-17

Willoughby, D. S., Boucher, T., Reid, J., Skelton, G. & Clark, M. (2011). Effects of 7 days of arginine-alpha-ketoglutarate supplementation on blood flow, plasma L-arginine, nitric oxide metabolites and asymmetric dimethyl arginine after resistance exercise. *International Journal of Sports Nutrition and Exercise Metabolism.* 21: 291-299

Wu, G. & Meininger, C. J. (2000). Arginine Nutrition and Cardiovascular Function. *Journal of Nutrition.* 130: 2626-2629

Appendix 1 – Sample Size Power Calculation

[1] -- *Tuesday, April 10, 2012 -- 22:47:31*
**F tests** – ANOVA: Repeated measures, within factors

| | | | |
|---|---|---|---|
| **Analysis**: | A priori: Compute required sample size | | |
| **Input**: | Effect size f | = | 0.4 |
| | α err prob | = | 0.05 |
| | Power (1–β err prob) | = | 0.8 |
| | Number of groups | = | 1 |
| | Number of measurements | = | 3 |
| | Corr among rep measures | = | 0.5 |
| | Nonsphericity correction ε | = | 1 |
| **Output**: | Noncentrality parameter λ | = | 11.5200000 |
| | Critical F | = | 3.4433568 |
| | Numerator df | = | 2.0000000 |
| | Denominator df | = | 22.0000000 |
| | Total sample size | = | 12 |
| | Actual power | = | 0.8162038 |

Appendix 2 – Pre-test Health Screening Questionnaire


**Pre-test Questionnaire**

**The effects of acute arginine supplementation on 10 mile cycling time-trial performance in young adult males**

**Researcher***:* *Sam Gill*

Name:_____ Test date:_____

Contact number:_____ Date of birth:_____

In order to ensure that this study is as safe and accurate as possible, it is important that each potential participant is screened for any factors that may influence the study. Please circle your answer to the following questions:

1. Has your doctor ever said that you have a heart condition *and* that you should only perform physical activity recommended by a doctor? YES/NO

2. Do you feel pain in the chest when you perform physical activity? YES/NO

3. In the past month, have you had chest pain when you were not performing physical activity? YES/NO

YES/NO

4. Do you lose your balance because of dizziness *or* do you ever lose consciousness?

YES/NO

5. Do you have bone or joint problems (e.g. back, knee or hip) that could be made worse by a change in your physical activity?

6. Is your doctor currently prescribing drugs for your blood pressure or heart condition? YES/NO

7. Have you injured your hip, knee or ankle joint in the last six months? YES/NO

8. Do you know of any other reason why you should not participate in physical activity? YES/NO

Thank you for taking your time to fill in this form. If you have answered 'yes' to any of the above questions, unfortunately you will not be able to participate in this study.