

**Effects of Chronic Ingestion of L-arginine
alpha ketoglutarate Combined With Creatine
monohydrate on Anaerobic Performance in
Strength-Trained Athletes**

By

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Abstract

There has been a vast growth in the use of 'pre' workout nutritional aids for sports performance over the past decade, particularly those containing single use amino acids such as L-arginine. There are many purported physiological benefits to suggest combination of L-arginine with creatine will expedite a greater performance, however exact mechanisms are equivocal and evidence suggesting an actual performance benefit are lacking. **PURPOSE:** To determine the effect of L-arginine alpha ketoglutarate (AAKG) on total body mass, fat free mass, peripheral blood flow ($\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$), isometric bench press and half squat, countermovement jump and peak power output in a single anaerobic Wingate. Further investigation into additive effects of creatine monohydrate (Cr) is undertaken where warranted. **METHOD:** Strength trained individuals ($n = 24$, Age: 24.5 ± 4.9 years; Height: 179.2 ± 8.1 cm; Weight: 77.1 ± 10.3 kg) were randomly assigned in a double blind manner either: (1) AAKG combined with Cr; (2) AAKG combined with a matched cornflour placebo (PLA); (3) Matched cornflour PLA alone, or (4) Control (CONT) across an 8 week intervention. Participants' anthropometric measures, peripheral blood flow, isometric strength and power were tested. To minimise dietary covariance, subjects completed weekly nutritional assessments to maintain dietary consistency. **RESULTS:** Total body mass (TBM) was not significantly different at any point during the trial, overall TBM from baseline to week 8 was not significantly altered between any group (AAKG + Cr: 1.0 ± 0.7 kg, AAKG + PLA: 0.4 ± 0.5 kg, PLA: -0.1 ± 0.5 kg and CONT: 0.2 ± 0.1 kg; $P > 0.05$). Similarly, fat free mass (FFM) showed no significant weekly change and no significant overall difference from baseline to week 8 (AAKG + Cr: 1.3 ± 0.8 kg, AAKG + PLA: 0.3 ± 0.6 kg, PLA: -0.4 ± 0.5 kg and CONT: -0.5 ± 0.4 kg; $P > 0.05$). Blood flow PRE (AAKG + Cr: 0.35 ± 0.29 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, AAKG + PLA: -0.07 ± 0.21 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, PLA: 0.08 ± 0.12 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ and CONT: 0.27 ± 0.17 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$) INTER (AAKG + Cr: 0.10 ± 0.29 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, AAKG + PLA: -0.03 ± 0.25 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, PLA: 0.04 ± 0.10 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ and CONT: 0.30 ± 0.22 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$) and POST (AAKG + Cr: 0.19 ± 0.32 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, AAKG + PLA: -0.11 ± 0.22 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$, PLA: 0.12 ± 0.09 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ and CONT: 0.32 ± 0.14 $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$) showed no significant difference at any point ($P > 0.05$). Isometric bench press demonstrated significant differences between AAKG + Cr and CONT only (83.28 ± 20.68 N and 15.51 ± 6.35 N respectively, $P = 0.026$) no significant differences between any other groups (AAKG + PLA: 48.33 ± 15.76 N and PLA: 26.63 ± 15.24 N, $P > 0.05$). In contrast isometric squat showed no significant differences at any point (AAKG + Cr: 117.50 ± 59.72 N, AAKG + PLA: 95.33 ± 27.27 N, PLA: 33.39 ± 47.88 N and CONT: -0.34 ± 9.48 N, $P > 0.05$). In addition there was no significant difference between any of the power variables at any stage throughout the trial, countermovement jump (AAKG + Cr: 1.43 ± 0.72 cm, AAKG + PLA: 1.47 ± 0.97 cm, PLA: -0.53 ± 2.23 cm and CONT: -2.41 ± 1.84 cm, $P > 0.05$) and peak wingate trial (AAKG + Cr: 23.92 ± 9.40 W, AAKG + PLA: 16.92 ± 16.80 W, PLA: -0.58 ± 8.53 W and CONT: 5.25 ± 2.38 W, $P > 0.05$). **CONCLUSION:** There was no significant difference between the majority of the variables at any stage throughout the trial, the only significant difference was in isometric bench press between AAKG + Cr and CONT. This would suggest that the combination of L-arginine alpha ketoglutarate and Creatine monohydrate might only lead to limited improvements in strength gains over those gained without supplementation.

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Abbreviations and Terminology

AA – Amino Acids

Cr – Creatine Monohydrate

NO – Nitric Oxide

EAA – Essential Amino Acids

NOS – Nitric Oxide Synthase

 eNOS – Endothelial

 nNOS – Neuronal

 iNOS – Isoform

PCr – Phosphocreatine

ATP – Adenosine Triphosphate

AAKG – L-arginine Alpha Ketoglutarate

ELISA – Enzyme-Linked Immunosorbent Assay

PL – Placebo

CONT – Control

ADMA – Asymmetric Dimethyl Arginine

OAT – Ornithine Aminotransferase

ASS – Argininosuccinate Synthase

AGAT – Arginine:Glycine Amidino-transferase

ADP – Adenosine Diphosphate

SAM – S-adenosyl methionine

SAH – S-adenosyl homocysteine

TBM – Total body mass

FFM – Fat free mass

FM – Fat mass

hGH – Human growth hormone

1RM – One repetition maximum

HPLC – High performance liquid chromatography

MS – Mass spectrometry

P:W – Power to weight ratio

1.0

Introduction

Nutritional interventions for the healthy individual have increased over the past decade, particularly those containing single use of amino acids (AA) such as L-arginine or more popular supplementation such as creatine monohydrate (Cr) to enhance resistance exercise, strength and power efforts, as well as body composition (Campbell, Bounty & Roberts, 2004; Wax, Kavazis, Webb & Brown, 2012). Research suggests that L-arginine can increase nutrient delivery to the working muscles due to its activity as a precursor to nitric oxide (NO) production, a cell-signalling molecule that is known to facilitate the vasodilation of blood vessels and reduce vascular resistance (Little et al., 2008). Studies infer that in combination L-arginine may increase creatine synthesis in addition to increasing the rate of delivery of creatine to the muscle increasing muscle creatine concentration (Campbell, Bounty & Roberts, 2004), and hence may provide additional benefits in sports performance.

In the majority of individuals L-arginine is sufficiently manufactured within the human body and can be obtained through the diet in a variety of animal and plant sources. Meat such as beef, pork and poultry, along with seafood's such as lobster and salmon, contain abundant amounts of arginine (Adams, Forsyth, Jessup, Robinson & Celermajar, 1995). Typically individuals who undertake a resistance-training programme have a greater need for exogenous arginine consumption, this is often provided in regular dietary protein intake but supplementation may be required where sufficient amounts are not supplied. Along with other metabolic fates evidence suggests that L-arginine may increase protein synthesis, nitric oxide production and creatine synthesis (Figure 1.1) (Campbell, Bounty & Roberts, 2004).

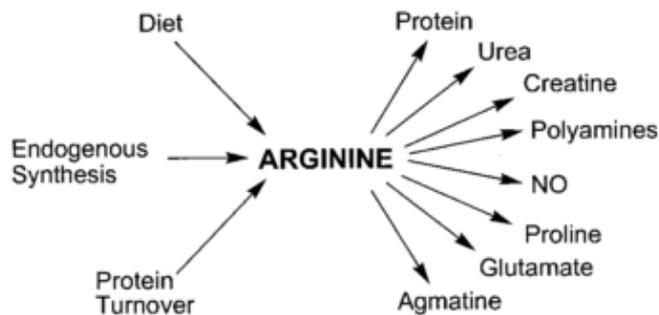


Figure 1.1 Sources and metabolic fate of arginine (Morris, 2006).

The effect of L-arginine on protein synthesis is disputed in research, consumption of essential amino acids (EAA) is acknowledged as having the same effect on muscle protein synthesis with or without the addition of L-arginine (Paddon-Jones, Borsheim & Wolfe, 2004). Therefore if protein synthesis is to occur it may be as a result of NO production (Paddon-Jones, Borsheim & Wolfe, 2004), nitric oxide is synthesised from arginine via the enzymatic activity of nitric oxide synthases (NOSs). Along with glycine and methionine, arginine is key in the synthesis of creatine, this occurs at a rate of approximately $1 - 2\text{g}\cdot\text{d}^{-1}$ (Figure 1.2) (Paddon-Jones, Borsheim & Wolfe, 2004).

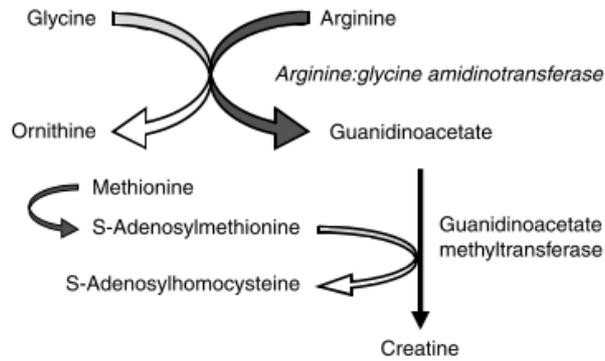


Figure 1.2 Pathway of creatine synthesis from arginine (Persky, Brazeau & Hochhaus, 2003).

Creatine is also a non-essential dietary component that may provide an ergogenic benefit when supplemented to regular food intake. Similarly to arginine, dietary sources of creatine include meat and fish, in particular red meats such as beef (Paddon-Jones, Borsheim & Wolfe, 2004). Creatine is found in abundance in skeletal muscle, more than 95% of total body stores are found here. The major rationale for the utilisation of creatine supplementation is that of increasing phosphocreatine (PCr). Phosphocreatine is used to create energy for short periods of time, 2 – 7 seconds on average. A fundamental role of PCr is maintaining the homeostasis of adenine nucleotides, in part the production/regeneration of adenosine triphosphate (ATP) (Persky, Brazeau & Hochhaus, 2003). The first system recruited during exercise for energy production is the ATP-PCr system, this is known to produce energy under oxygen deprived conditions at the fastest rate, however its relative small capacity culminates in rapid depletion during high intensity exercise. Creatine supplementation has been shown to increase the creatine and PCr levels in muscle and nervous tissue, ensuing greater ATP-production capacity allowing cells to better handle energetic challenges and reducing cell damage or death (Hultman, Soderlund & Timmons, 1996).

Very few studies have investigated the ergogenic benefits of combining both arginine and creatine into a single dose, many of the reported benefits are derived from the physiological mechanisms as opposed to evidential research. A study by Little et al. (2008) initially initiated the research with the following rationale behind a combination supplement opposed to a single supplement.

“Both Creatine and L-arginine supplementation appear to improve muscle performance yet work by different proposed mechanisms, their effects could be additive. Second, because L-arginine might enhance muscle blood flow, combining L-arginine with Cr could increase the delivery of Cr to skeletal muscle and increase the effectiveness of Cr supplementation” (Little et al., 2008).

Completion of the study concluded that the combination of Cr and AAKG had a beneficial effect over Cr alone reporting significant increases in anaerobic sprint performance, however this was not attributed to increased Cr uptake/retention as previous eluded to. A possible reason for this may be due to the beneficial factors occurring through different mechanisms; McGuire, Gross, Van Pilsum & Towle (1984) previously deduced that the effect of adding Cr to AAKG may support performance as a result of decreasing the conversion of arginine to creatine thus increasing the

availability of L-arginine for NO synthesis, decreasing homocysteine levels and reducing methylation demand (Taes et al., 2003).

Current research into the area is limited; the few studies that have investigated the matter have focussed on physiological effects as opposed to the relative performance improvements, even though many users of the supplement do so expecting performance improvements. Physiological testing has led to recommendations for consumption being as late as 30 minutes prior to exercise when in fact a performance scenario may not yield peak results until much later. Due to this nature there is very little to determine recommended dosage timings for the participants, in order to counter this the current study has been divided into two stages. The first stage, pre-experimental study; will investigate the most appropriate time to administer the supplement in order to best ensure that L-arginine reaches peak bioavailability for the training/testing session. The second stage, main experimental study; will aim to determine the potential influence of L-arginine and creatine on performance.

1.1 Aims and Objectives: Pre-Experimental Study

Aims

Firstly this study will establish the bioavailability of oral consumption, L-arginine alpha ketoglutarate (AAKG) combined with Cr when taken as a mixture with 250 ml diluted fruit juice. The secondary aim is to determine whether capillary blood samples of 500 µl are an adequate and viable method of collection.

Objectives

The aims for this study will be achieved through recruitment of 10 healthy, active participants to the laboratory. Participants will be supplied with a ready mixed formula containing 6g.d⁻¹ AAKG and 10g.d⁻¹ Cr, capillarised blood samples being collected and assessed via enzyme-linked immunosorbent assay (ELISA) testing at 0, 1, 2 and 4 hours post consumption.

1.2 Hypotheses: Pre-Experimental Study

H₁ – L-arginine plasma concentration will peak between zero and one hour post consumption.

H₀ – L-arginine plasma concentration will not peak between zero and one hour post consumption.

H₂ – Capillary blood sampling will provide indubitable results relative to other collection methods.

H₀ – Capillary blood sampling will not provide indubitable results relative to other collection methods.

1.3 Aims and Objectives: Main-Experimental Study

Aims

The initial aim will be to assess the influence of AAKG and Cr in combination on anthropometric measures. The secondary aim is the assessment of physiological measures, such as blood flow, heart rate and blood pressure after ingestion of AAKG and Cr supplementation. The third aim will be to assess the influence of AAKG and Cr in combination on performance variables for strength and power. The final aim will be to determine whether combining AAKG with or without Cr positively affects power to weight ratio.

Objectives

The first aim will be resolved through tracking measurements of weight, body fat percentage and fat free mass using scales and skinfold calliper's. To determine the effect to a physiological degree heart rate, blood pressure and blood flow will be tracked and analysed. The third aim will be achieved via strength testing of an isometric bench press and isometric half squat with the power testing utilising a countermovement jump and anaerobic wingate measurements. The conclusive aim will be met using the strength and power variables aforementioned and calculated against the participants' body weight to determine power to weight ratio.

1.4 Hypotheses: Main-Experimental Study

H₁ – A formula containing AAKG and Cr will increase total body mass and decrease fat free mass greater than all other groups when compared.

H₀ – A formula containing AAKG and Cr will not increase total body mass and not decrease fat free mass greater than all other groups when compared.

H₂ – A formula containing AAKG and Cr will improve peripheral blood flow greater than all other groups when compared.

H₀ – A formula containing AAKG and Cr will not improve peripheral blood flow greater than all other groups when compared.

H₃ – A formula containing AAKG and Cr will increase isometric strength variables greater than all other groups when compared.

H₀ – A formula containing AAKG and Cr will not increase isometric strength variables greater than all other groups when compared.

H₄ – A formula containing AAKG and Cr will increase power variables greater than all other groups when compared.

H_0 – A formula containing AAKG and Cr will not increase power variables greater than all other groups when compared.

H_5 – A formula containing AAKG and Cr will improve power to weight ratio greater than all other groups when compared.

H_0 – A formula containing AAKG and Cr will not improve power to weight ratio greater than all other groups when compared.

2.0

Literature Review

2.1 L-Arginine Alpha Ketoglutarate: Mechanisms of Action

Proteins consist of linear polymers built from a varying series of up to twenty amino acids (AA), twelve non-essential and eight essential. L-arginine (2-amino-5-guanidinovaleric acid) is one of these non-essential amino acids; non-essential stipulates that 'sufficient amounts for activities of daily living are produced naturally by the body' (Adams, Forsyth, Jessup, Robinson & Celermajar, 1995). However, it has been classified as a conditionally essential or 'semi-essential' amino acid when demand exceeds supply (Moncada et al., 1991; Adams et al., 1997; Wu & Morris, 1998). L-arginine serves as a precursor for the synthesis of urea's, polyamines, proline, glutamate, creatine and agmatine (Moncada & Higgs, 1995; Williams, 1997); its most important role being the biosynthesis of nitric oxide (NO); an endogenously produced, cellular signalling molecule, involved in a variety of endothelium-mediated effects in the vasculature (Moncada & Higgs, 1993; Boger & Bode-Boger, 2001). Arginine is the only substance found within the body to contain the nitrogen substrate of NOS and therefore governs the production of NO; as a direct consequence of this the plasma concentration of L-arginine may be rate limiting for NO production (Nakaki & Hishikawa, 2002). L-arginine is also classified as a glucogenic amino acid due to it being metabolized into alpha-ketoglutarate (AKG) and entering the citric acid cycle, otherwise known as Krebs cycle (Campbell et al., 2006).

A key intermediate in Krebs cycle, α -ketoglutarate is produced from the oxidative decarboxylation of isocitrate; before subsequently being converted to succinyl coenzyme A, a reaction catalysed by the AKG dehydrogenase complex (Hammarqvist, Wernerman, von der Decken & Vinnars, 1991). Wiren, Permert & Larsson (2002) concluded that oral supplementation of AKG alone had no significant benefits on protein metabolism or catabolism therefore decided that combining it with another supplement such as arginine would be more beneficial. In order to obtain the final product of L-arginine α -ketoglutarate (AAKG), L-arginine and α -ketoglutaric acid must be bound together to form the salt that is sold as a commercial product. NO potentiating nutritional aids, such as AAKG, have been developed and marketed towards resistance-trained athletes based purely on their reported physiological effects (Campbell et al., 2006). Purported benefits propose that AAKG stimulates NO production, improve blood flow to muscle during resistance training, increase protein synthesis and reduce catabolism, therefore leading to greater training adaptations (Campbell et al., 2006; Wu & Meininger, 2000). Bode-Boger (2006) determined that both acute and chronic administration evoked these benefits in endothelial function.

Substantial evidence exists that reinforce the statements regarding NO production; it appears that the major influence of L-arginine is production of NO and L-citrulline via NO synthases (NOSs) conversion (McConnell, 2007). NO acts as a messenger for vasodilation (Nisoli et al., 2003); by binding to the guanylate cyclase in order to increase production of one of the cyclic nucleotides, second messenger cGMP (McConnell, 2007). Vasodilation will occur when endothelial NOS (eNOS), the primary isoform of NOS found in the endothelial cells, plays a role in blood vessel dilation; in skeletal muscle both eNOS and neuronal NOS (nNOS) are expressed within muscle fibres themselves (Lau et al., 2000; McConnell & Kingwell, 2006). It appeared that human skeletal muscle nNOS expressed much higher than eNOS. Furthermore asymmetric dimethyl arginine (ADMA) can interact with eNOS and inhibit its activity (McConnell & Kingwell, 2006). Metabolic diseases such as diabetes could increase the inducible isoform of NOS (iNOS) in skeletal muscle by inflammatory

processes thus slowing the rate of vasodilation further (Torres, De Sanctis & de L Briceno, as cited in Campbell et al., 2006). There are currently sparse amounts of literature on the mechanisms of AAKG and blood flow, it has however been shown in theory that blood flow should increase due to the vasodilation of the blood vessels (Sander, Chavoshan & Victor, as cited in Campbell et al., 2006). There is little substantiating evidence to suggest that AAKG will heighten this effect anymore than exercise alone would.

Endogenous production of L-arginine follows a multitude of pathways (Figure 2.1), in mammalian cells this process occurs via P5C synthetase and proline oxidase (Wu, Davis, Flynn, Knabe & Davidson, 1997). A selection of enzymes shown are present in a variety of different cells, though expression of other enzymes is highly restricted; phosphate-dependant glutaminase, ornithine aminotransferase (OAT), argininosuccinate synthase (ASS), argininosuccinate lyase (ASL) and aspartate aminotransferase are widely found in tissue (Wu et al., 1997). Whereas, carbamoyl-phosphate synthase I (CPS I), ornithine carbamoyltransferase (OCT) and N-acetylglutamate synthase are restricted solely to the liver and intestinal mucosa (Morris, 1992).

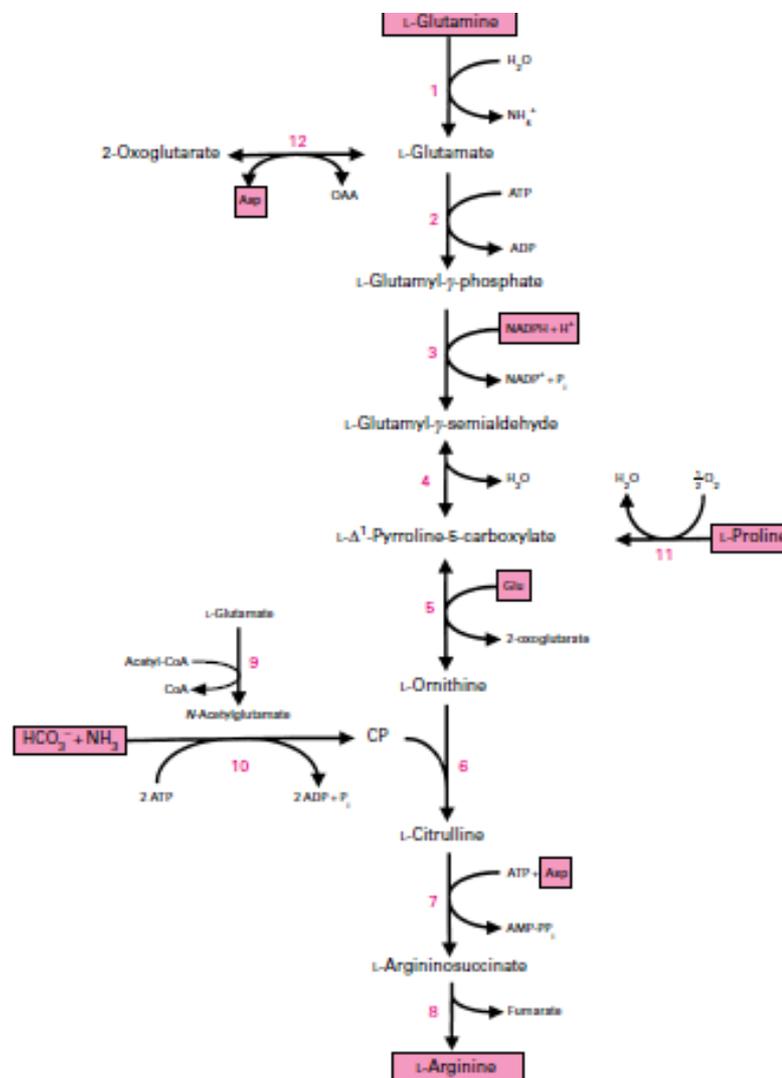


Figure 2.1 Pathways of L-arginine synthesis (Wu & Morris, 1998).

As stated by Campbell et al. (2006) supplementation of AAKG has been touted as a reducer of protein catabolism, research has indicated however that this may actually be detrimental to performance rather than beneficial. A reduction in muscle catabolism due to greater synthesis would in fact be a more valid statement. The products of arginine catabolism have proved a great attraction for researchers in the field of performance improvements, the cell signalling molecules produced in this process are: NO, glutamate and agmatine (Figure 2.2) (Wu & Morris, 1998). Glutamate can also aid the production of other cell signalling molecules, γ -aminobutyric acid ('GABA') (Williams, 1997). Furthermore, arginine has been reported to stimulate hormones such as insulin, human growth hormone (hGH), glucagon and prolactin (Reyes, Karl & Klahr, 1994).

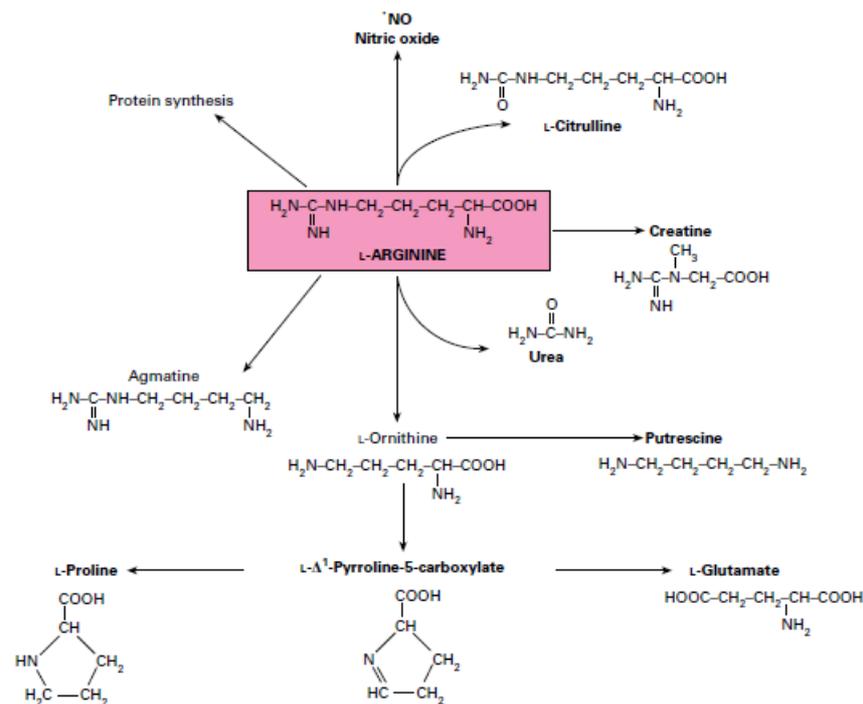


Figure 2.2 Processes of L-arginine catabolism (Wu & Morris, 1998).

2.2 AAKG: Bioavailability

L-arginine bioavailability investigation unveils a different response curve dependent on the dosage bolus, the larger the dosage the more rapid the response. There also appears to be a difference depending on method of administration, intravenously there is a much faster response opposed to oral ingestion of the same dose. Intravenous administration is usually chosen as the preferred method for dosages above that of comfortable oral consumption, studies to knowledge have used 30 g.d^{-1} for intravenous and $5 - 10 \text{ g.d}^{-1}$ for oral consumption. Bode-Boger et al. (1994), and Bode-Boger et al. (1998) reported that maximal plasma concentrations were observed at 20 – 40 minutes, Bode-Boger et al. (1998) went on to further show the differences between dosages reporting a peak of $6223 \pm 407 \mu\text{mol.L}^{-1}$ in 30 minutes for those infused with 30 g.d^{-1} , a peak of $822 \pm 59 \mu\text{mol.L}^{-1}$ at 22 minutes for those receiving intravenous infusion of 6 g.d^{-1} and a peak of $310 \pm 152 \mu\text{mol.L}^{-1}$ at 90 minutes for oral administration of 6 g.d^{-1} , this would suggest that intravenous infusion is approximately 4 times faster compared with oral consumption.

Comparing this with data investigating oral consumption alone it can be seen that dosage quantity and frequency has the same effect on appearance rates in the blood. Oral administration appears to delay the onset of peak concentration values over intravenous infusion but additionally maintains these values for a longer period of time. Tang et al. (2011) found there to be a peak concentration value at 60 minutes post ingestion, this then was documented as remaining elevated for a further 120 minutes, these results are concurrent with those recorded by Bode-Boger et al. (1998).

2.3 Creatine monohydrate: Mechanisms of action

Creatine (α -methyl guanidino-acetic acid) is produced naturally by the body within the liver, kidneys and pancreas, and derived from the synthesis of three AA, arginine, glycine and methionine. Production occurs at a rate of approximately 1 - 2 g.d⁻¹ (Walker, 1979) with a further consumption of around 1 g.d⁻¹ being ingested through regular dietary intake of meat and fish, in particular red meats (Paddon-Jones, Borsheim & Wolfe, 2004). Degradation of creatine (Cr) forms creatinine, this enters circulation by diffusion and once eliminated through glomerular filtration is excreted in the urine at a rate of around 2 g.d⁻¹ (Walker, 1979). Most creatine is stored in skeletal muscle, around 90 - 95% of total bodily stores are found here; of this roughly one-third is in the form of free creatine and two-thirds exists as phosphocreatine (PCr), the remaining pool is found in the brain, liver, kidney and testes (Walker, 1979).

Derivatization of Cr from glycine and arginine occurs from the formation of guanidinoacetate and ornithine in a reaction that is catalysed by arginine:glycine amidino-transferase (AGAT) (Wyss & Kaddurah-Daouk, 2000). Guanidinoacetate is formed in the kidney and then transported to the liver via the blood stream; in the liver the S-adenosylmethionine donates a methyl group to guanidinoacetate by S-adenosylmethionine:guanidinoacetate N-methyltransferase (GAMT). Cr synthesis is limited by the formation of guanidinoacetate by AGAT (Walker 1979, Wyss & Kaddurah-Daouk, 2000). Factors that have also been shown to regulate Cr synthesis include testosterone, ornithine, thyroid hormone, growth hormone and dietary deficiencies (e.g. vitamin E).

Research shows that there is little Cr found at the site of production; therefore it is critical that it is transported from the area of synthesis to areas of utilization and storage. Phosphorylation of Cr to PCr occurs in the presence of creatine kinase. Characteristically organs that express the greatest levels of AGAT and/or GAMT have the lowest levels of creatine kinase (Walkers, 1979). Due to Cr being utilized in organs other than those of production it is critical that it enters the blood stream to reach skeletal muscle tissue, failure of adequate cellular uptake causes potential for down-regulation of these systems under chronic Cr exposure (Guerrero-Ontiveros & Wallimann, 1998). Transportation of Cr into tissues where it is required against a concentration gradient is facilitated through a sodium and chloride-dependant transporter (CreaT). CreaT shares similarities with the transporters for taurine, dopamine and guanidino γ -aminobutyric acid (Guerrero-Ontiveros & Wallimann, 1998). The location of creatine kinase expression matches that of CreaT as its mRNA has been found in skeletal muscle, kidney, brain, heart and colon.

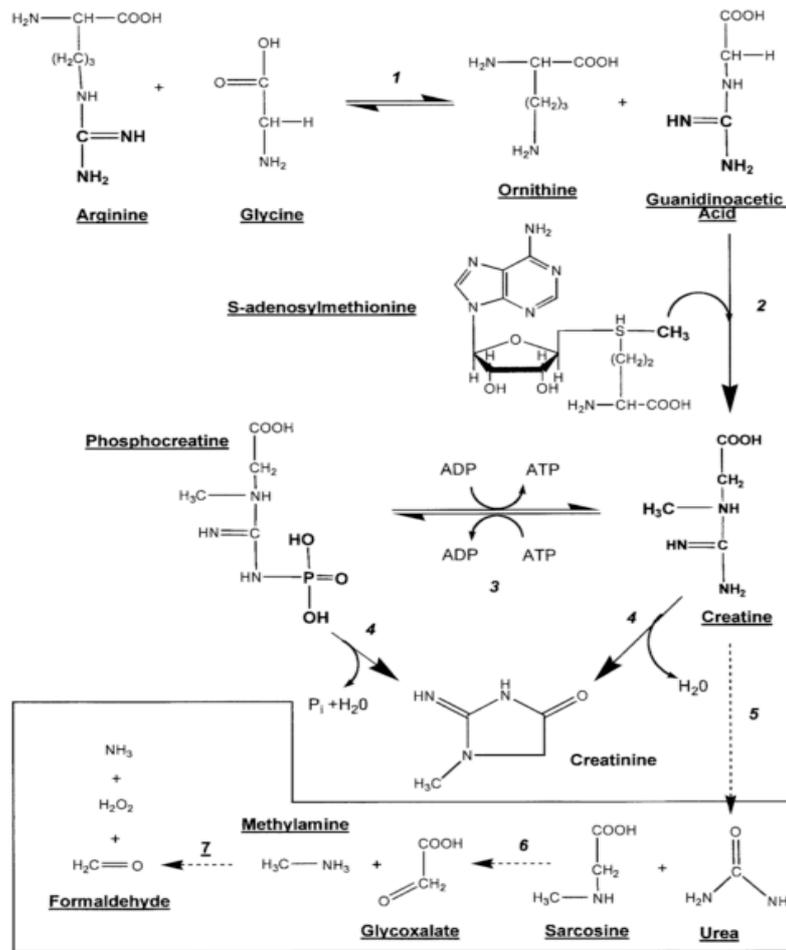


Figure 2.3 Pathway of creatine synthesis and degradation (Persky & Brazeau, 2001).

Net uptake of Cr in skeletal muscle can be influenced by external factors; catecholamines, insulin, insulin-like growth factor 1 (IGF-1) and exercise have all been shown to have an effect on uptake. Odoom, Kemp and Radda (1996) suggested that thyroid hormone (T₃) increased total Cr content three fold, while IGF-1 increased it by 40 – 60% compared to control. Other studies have shown that both insulin and carbohydrate intake can increase total Cr accumulation in humans (Haugland & Chang, 1975). Work on nonspecific agonists such as β -agonist isoproterenol have shown increases in total Cr accumulation while others, such as α_1 -agonist methoxamine showed a decrease in total Cr levels (Odoom, Kemp & Radda, 1996).

Muscle fibre type plays a role in total Cr content; type 2 fibres will have a much higher levels of Cr and PCr (Meyer, Brown and Kushmerick, 1985). Type 2a and 2b fibres in rodent samples contained approximately 32 mM PCr and 7 mM Cr, while on showing 16 mM PCr and 7 mM in type 1 fibres (Willot et al., 1999). PCr is a limiting factor when trying to maintain ATP resynthesis during short-term exercise at maximal intensity; theoretically an increase in PCr concentration will lead to an exaggerated energy reserve for such exercise (Kreider, 2003). Hence, it is suggested that the process of creatine loading as a performance enhancer for maximal short-term exercise is analogous to the method of carbohydrate loading often advantageous in endurance exercise (Kreider, 2003; Lemon, 2002). Average concentration values of skeletal muscle, total creatine; is around 120 mmol.kg⁻¹ (dry mass), with the upper limit typically reaching saturation at around 150 – 160 mmol.kg⁻¹ (Casey & Greenhaff, 2000; Hultman et al., 1996). Repeated studies have shown that oral

consumption of creatine supplementation can indeed increase muscle concentration, most protocols recommending a loading phase of approximately 20 g.d⁻¹ creatine monohydrate for 4 – 7 days, immediately followed by a daily maintenance dose of about 5 g.d⁻¹ for 2 – 4 weeks (Burke, 2003; Harris, Solderlund and Hultman, 1992; Hultman et al., 1996). Dosages such as these are advocated as this theoretically increases creatine concentration in the blood to an optimal level for muscle uptake, levels above this will most likely lead to an excess secretion in the urine. Additionally lower doses have not been discounted, the use of 2 – 3 g.d⁻¹ daily will also increase total muscle creatine values but the process takes far longer, usually peaking over a period of 3 – 7 weeks.

There have been several proposed mechanisms by which exercise performance can be improved with creatine supplementation in both acute and chronic ingestion. The major mechanism behind exercise performance improvements lies with Cr role in energy metabolism; as already stated Cr is phosphorylated into PCr that in turn plays a role in adenosine triphosphate (ATP) production. ATP concentrations maintain physiological processes and provide protection from hypoxia-induced tissue damage (Persky & Brazeau, 2001). The PCr system acts as a temporal and spatial energy buffer, as well as providing a pH buffer. As a spatial energy buffer it is the involvement of Cr and PCr in ATP shuttling from the inner mitochondria to the cytosol that promotes performance adaptations (Bessman & Carpenter, 1985). The processes that operate as a temporal energy and pH buffer are seen in the production of PCr and adenosine diphosphate (ADP) from Cr and ATP; catalyzed by creatine kinase (Figure 2.4).

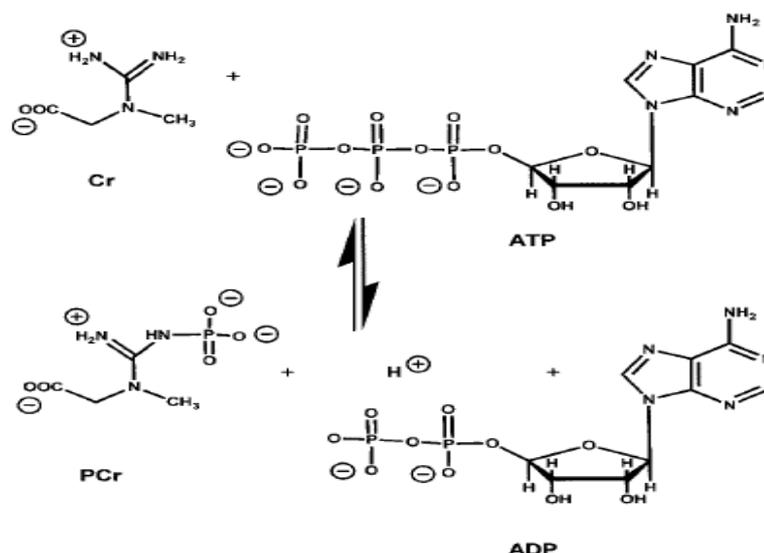


Figure 2.4 Phosphorylation of Cr by ATP to form PCr and ADP (Greenhaff, 1997).

Due to opposing charges, Cr is “locked” in the muscle by the polar PCr maintaining retention of Cr and preventing partitioning through biological membranes (Greenhaff, 1997). At times of low pH levels this reaction will favour the production of ATP, conversely however during recovery periods where production of ATP will occur aerobically, levels of PCr will increase (Greenhaff, 1997). The energy and pH buffer is one of the mechanisms by which exercise performance can be increased with Cr. A further mechanism by which exercise performance may be increased is the regulation of glycolysis. It is thought that certain proteins when human tissue Cr is depleted; will adapt by increasing oxidative enzymes found in aerobic metabolism that can offset the lack of anaerobic

energy supplied by the PCr system. Depletion will increase enzymes such as succinate dehydrogenase, citrate synthase, mitochondrial creatine kinase and GLUT-4 glucose transporters (Ren, Semenkovich & Holloszy, 1993). Little research is available to determine whether increasing intracellular Cr stores will affect enzyme activity, those that have investigated this have been discredited due to control of PCr showing traces of impurities like inorganic pyrophosphate (Wyss & Kaddurah-Daouk, 2000).

Another process by which Cr may help improve exercise performance over a longer period of time is its potential effect on increasing protein synthesis and decreasing protein catabolism. Many studies have found that Cr supplementation in young, healthy males has increased muscle fibre size and increased fat-free mass when consumed for 4 to 28 days (Vandenbergh et al., as cited in Little et al., 2008). It is theorised that Cr may perform as a stimulus of protein synthesis and muscular hypertrophy; however, more recent studies have suggested that there is in fact no increase in protein synthesis but actually a decrease in protein catabolism (Parise, Mihic, MacLellan, Yarasheski & Tarnopolsky, 2000). Protein metabolism regulation is further supported in studies researching the effect of cell swelling on protein synthesis during use of osmotic agents such as Cr. It has been shown that Cr retention in cells can increase hydration levels; hyper hydration can act as an anabolic signalling stimulating protein synthesis (Haussinger, Lang & Gerok, 1994). The reverse of this, hypo-osmolality, has been shown to increase protein sparing thus reducing protein degradation (Berneis, Ninnis, Haussinger & Keller, 1999).

2.4 Potential mechanisms of AAKG and Cr combined

Exploration into the use of AAKG and Cr alone is very limited, many researchers, guided by a commercial market; have studied the use of these products with a varied blend of branched chain AA, medium chained triglycerides and carbohydrate sources. Despite this, theoretically the use of AAKG and Cr alone could produce improved performance down to differing physiological mechanisms creating a proposed additive effect, there could be an increase in free L-arginine destined for different metabolic fates, improved waste product removal or improved rate of ATP production through increased PCr stores (Little, Forbes, Candow, Cornish & Chilibeck, 2008). Manipulation of Cr uptake has been shown possible in studies stimulating insulin secretion through carbohydrate co-ingestion (Green, Hultman, MacDonald, Sewell & Greenhaff, 1996) and alpha-lipoic acid (Burke, Chilibeck, Parise, Tarnopolsky & Candow, 2003) suggesting that co-ingestion with AAKG, a potential vasodilator; could increase effectiveness of Cr through increased skeletal muscle delivery (Little et al., 2008).

A large proportion of L-arginine use is attributed to synthesis of Cr by the enzyme AGAT (Persky & Brazeau, 2001); it is estimated that around 70% of available methyl groups are consumed during the generation of Cr, with S-adenosyl methionine (SAM) serving as the primary methyl donor (Wyss & Kadurrah-Daouk, 2000). Figure 2.5 shows a basic representation of this process. This demand for free methyl groups may reduce the availability of those required in other biological reactions, consequently having adverse effects on endothelial function and growth due to hypomethylation and aberrant methylation (Jamaluddin, Yang & Wang, as cited in Little et al., 2008). The process of donating methyl groups to guanidinoacetate results in SAM being metabolised to S-adenosyl homocysteine (SAH) and eventually homocysteine. Homocysteine is known to have

adverse effects on endothelial function; consequently the production of Cr from L-arginine may impair endothelial function.

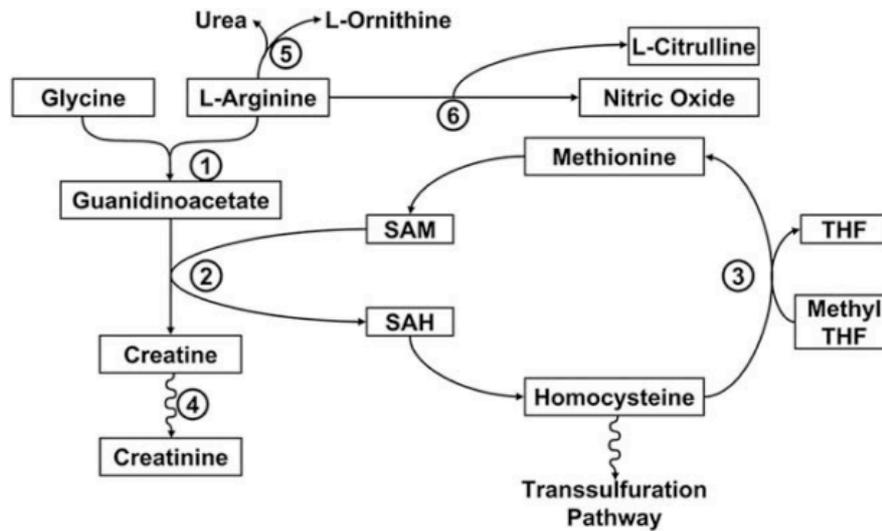


Figure 2.5 Relationship between L-arginine, creatine and homocysteine metabolism (Jahangir et al., 2009).

Activity of the enzyme AGAT is reduced by Cr due to feedback inhibition, augmenting Cr concentrations through supplementation has the potential to decrease conversion rate of L-arginine to Cr (McGuire, Gross, Van Pilsum & Towle, 1984). It is predicted that this effect could increase the availability of L-arginine for NO synthesis, decrease demand for free methyl groups thus decrease homocysteine levels (Taes et al., 2003).

2.5 Effect of AAKG and Cr on Body Composition

Documentation on body composition changes when supplementing with both Cr and AAKG separately is in vast supply, however effects of the two in combination as already stated are not with much of the current research purely based on the physiological benefits of a combination as opposed to the actual physical benefits. Similarities can still be drawn in those studies that investigated each separately when compared with those that have combined the two.

Arciero et al. (2001) investigated the effect of Cr consumption along both with or without resistance training. Results showed a significant increase in all groups ($P < 0.05$) with Cr alone increasing total body mass (TBM) from 73.9 ± 11.5 kg to 75.6 ± 12.5 kg, fat free mass (FFM) from 58.1 ± 8.1 kg to 59.0 ± 8.8 kg and fat mass (FM) 12.6 ± 3.8 kg to 13.1 ± 3.9 kg. And a significant difference in Cr with resistance training ($P < 0.050$) increasing TBM from 78.9 ± 6.7 kg to 80.8 ± 6.8 kg, FFM 63.0 ± 2.8 kg to 64.7 ± 3.6 kg and finally FM change from 12.8 ± 4.7 kg to 13.1 ± 4.4 kg, this shows that TBM gain was roughly 50 % FFM and 50 % FM. This coincides with a study by Little et al., (2008) who found that with Cr alone TBM increased by on average 1.2 kg of which it appears that 0.5 kg is FFM and 0.9 kg is FM, significance was found between pre and post in the Cr only group ($P < 0.01$) (Table 2.1). It can also be seen in the table that a combination of AAKG and Cr has already been used, resulting in non-significant differences in TBM, FFM and FM pre to post despite the apparent reduction in FM and percentage body fat.

Table 2.1 Body composition measures (mean \pm standard deviation) before and after 10 days of supplementation (Little et al., 2008).

	Cr + A-AKG		Cr		Placebo	
	Pre	Post	Pre	Post	Pre	Post
Body mass (kg)	81.1 \pm 9.3	81.5 \pm 9.9	79.9 \pm 13.0	81.1 \pm 13.8*	78.9 \pm 11.3	78.8 \pm 13.7
Lean-tissue mass (kg)	68.4 \pm 4.4	69.5 \pm 4.6	66.6 \pm 7.6	67.1 \pm 8.2	65.1 \pm 6.2	64.8 \pm 5.8
Fat mass (kg)	12.5 \pm 6.1	12.0 \pm 6.6	13.2 \pm 6.4	14.1 \pm 6.4	13.8 \pm 8.7	13.9 \pm 9.0
% body fat	14.9 \pm 5.7	14.1 \pm 6.2	15.8 \pm 5.8	16.8 \pm 5.4	16.5 \pm 7.4	16.5 \pm 8.3

*Significant increase in body mass from pre- to postsupplementation.

The effect of L-arginine alone on body composition is much less apparent with much of the research concluding little to no differences, and none showing any significance in body composition changes (Campbell et al., 2006; Liu et al., 2009; Tang et al., 2011). Campbell et al., (2006) reported data as seen in Table 2.2, it can be seen that there is a decrease in body fat percentage and an increase in TBM in both the AAKG and placebo groups leading towards the conclusion that supplementation of AAKG does not accentuate the body composition changes and it is in fact the exercise itself that causes the adaptations. Many older studies looking to discover how L-arginine can alter body composition have looked at the production of growth hormone (GH) and how that may affect TBM, FFM and FM. One study considered the comparisons between L-arginine production of GH and exogenous GH, concluding that L-arginine produced no significant values of GH compared to exogenous GH with post testing significance only being seen in body composition of the exogenous GH group ($P < 0.001$).

Table 2.2 Body composition variables (Campbell et al., 2006).

	AAKG	Placebo	P (group \times time)
Lean mass (kg)			
T1	59.8 \pm 8.0	63.6 \pm 9.4	
T2	60.0 \pm 8.0	64.3 \pm 9.3	
T3	60.6 \pm 7.9	64.5 \pm 8.9	0.90
Fat mass (kg)			
T1	13.9 \pm 6.6	15.7 \pm 4.7	
T2	14.8 \pm 6.2	15.4 \pm 4.9	
T3	14.6 \pm 6.3	15.1 \pm 4.9	0.25
Total body mass (kg)			
T1	77.0 \pm 12.7	81.9 \pm 12.7	
T2	77.1 \pm 12.6	82.2 \pm 13.0	
T3	77.6 \pm 13.0	82.1 \pm 12.5	0.53
Body fat (%)			
T1	18.7 \pm 5.8	19.1 \pm 3.8	
T2	18.7 \pm 5.6	18.6 \pm 3.8	
T3	18.2 \pm 5.5	18.1 \pm 3.9	0.31

AAKG, L-arginine/ α -ketoglutarate; T1 to T3, time 1 to time 3

Physiologically it is thought that Cr would increase TBM and FFM through an increase in cellular water retention and increased protein synthesis and decreased protein catabolism. There is

potential for this to happen in both trained and untrained individuals as proven by Arciero et al. (2001) but that said there will be a quicker response; possibly as soon as 7 days, in those who undertake resistance training also. Research on this remains conflicting however as the exact reason behind increases TBM is yet to be fully discovered in human subjects (Earnest, Snell, Rodriguez, Almada & Mitchell, 1995). Considering the results found in these studies it would seem to suggest that the additive effect of AAKG to Cr results in a desired increase in TBM and FFM but when compared to Cr alone may decrease FM.

2.6 Effect of AAKG and Cr on Blood Flow

The benefit of Cr on the aforementioned parameter is known to have very little influence regardless of increasing consumption levels through supplementation. Nevertheless this does not disregard the potential benefit of increasing total tissue Cr levels in order to allow a greater availability of L-arginine (Taes et al., 2003). It is well known that L-arginine is a precursor to NO, which in turn is known to vasodilate the blood vessels; a greater available pool of L-arginine due to a decrease in losses through Cr synthesis may prove beneficial to increasing blood flow (Taes et al., 2003; Campbell et al., 2006). It may prove beneficial as the more available L-arginine within the body the more there is potential for greater NO production, due to the many metabolic fates of L-arginine it is impossible to say for certain that it will increase NO production but should this be the case there will be a greater vasodilatory effect on the vascular system.

Regardless of this apparent lack of benefit one study is found investigating the effects of Cr and resistance training on energy expenditure and limb blood flow. Arciero et al. (2001) sought to determine whether Cr ingestion with no resistance training had any difference in effect when compared to Cr coupled with resistance training and placebo with resistance training. Table 2.3 shows the results that were obtained, it can be seen that significant increases were noted in blood flow for both the Cr and resistance group and the placebo and resistance group (30% and 38% respectively, $P < 0.001$).

Table 2.3 Interaction means for body composition, energy expenditure and blood flow parameters (Arciero et al., 2001).

	Cr (n = 10)			Cr-RT (n = 10)			P-RT (n = 10)		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
TBM (kg)	73.9 ± 11.5 ^a	74.9 ± 11.8 ^a	75.6 ± 12.5 ^b	78.9 ± 6.7 ^a	81.7 ± 5.5 ^b	80.8 ± 6.8 ^b	78.8 ± 11.3 ^a	78.7 ± 10.9 ^a	79.0 ± 11.7 ^a
FFM (kg)	58.1 ± 8.1 ^a	59.0 ± 8.8 ^a	59.0 ± 8.8 ^a	63.0 ± 2.8 ^a	64.5 ± 3.4 ^b	64.7 ± 3.6 ^b	62.3 ± 6.5 ^a	62.2 ± 6.6 ^a	62.5 ± 6.5 ^a
FM (kg)	12.6 ± 3.8 ^a	12.5 ± 3.8 ^a	13.1 ± 3.9 ^a	12.8 ± 4.7 ^a	12.7 ± 4.4 ^a	13.1 ± 4.4 ^a	12.9 ± 7.0 ^a	12.6 ± 8.0 ^a	13.1 ± 7.6 ^a
% Fat	16.9 ± 3.5 ^a	16.6 ± 3.7 ^a	17.2 ± 3.5 ^a	16.4 ± 4.9 ^a	16.0 ± 4.6 ^a	16.4 ± 4.6 ^a	16.3 ± 6.4 ^a	15.8 ± 7.4 ^a	16.4 ± 6.8 ^a
TBW (L)	40.4 ± 6.9 ^a	40.0 ± 7.3 ^a	42.6 ± 7.3 ^b	40.6 ± 2.4 ^a	41.7 ± 3.8 ^{a,b}	42.3 ± 2.2 ^b	40.8 ± 5.2 ^a	39.9 ± 6.7 ^a	40.9 ± 5.3 ^a
CBF (mL · 100 mL ⁻¹ · min ⁻¹)	2.98 ± 1.0 ^a	2.58 ± 1.0 ^a	2.65 ± 1.2 ^a	2.22 ± 0.3 ^a	2.91 ± 0.9 ^b	2.87 ± 0.8 ^b	2.91 ± 0.9 ^a	2.95 ± 0.6 ^a	3.04 ± 0.90 ^a
FBF (mL · 100 mL ⁻¹ · min ⁻¹)	2.98 ± 1.3 ^a	3.24 ± 1.2 ^a	2.75 ± 0.7 ^a	2.90 ± 1.1 ^a	4.1 ± 1.5 ^b	4.0 ± 1.6 ^b	3.5 ± 2.0 ^a	3.4 ± 1.5 ^a	3.7 ± 1.6 ^a
RMR	1,860 ± 165 ^a	1,740 ± 477 ^a	1,907 ± 173 ^b	1,971 ± 172 ^a	2,029 ± 175 ^a	2,086 ± 184 ^b	2,043 ± 211 ^a	2,070 ± 180 ^a	2,050 ± 192 ^a

FM = Fat mass, TBW = Total body water, CBF = Calf blood flow, FBF = Forearm blood flow.

Studies have reported beneficial effects after oral administration of L-arginine, such as improved blood flow, reductions in blood pressure and improved immune function (Preli, Klein & Herrington, 2002). On the other hand however, it has also been shown that AAKG supplementation does not increase muscle blood flow after resistance exercise (Tang, Lysecki, Manolacos,

Tamopolsky & Philips, 2011). The inconsistency between these two studies is found in the methodology; whereas Preli, Klein & Herrington (2002) have looked at aerobic exercise where an increased blood flow would be expected due to oxygen demand of the muscles being matched by supply (Bode-Boger, Boger, Galland, Tsikas & Frolich, 1998; Liu et al., 2009). Tang et al. (2011) study involved an anaerobic discipline on the cycle ergometer. Both studies recorded no significant difference ($P > 0.05$) between supplement groups and controlling variables, suggesting that an increased blood flow is not directly linked to L-arginine consumption as physiological mechanisms would speculate.

Contrasting with the lack of research that has currently been undertaken regarding benefits of AAKG and strength, there are vast resources available in respect to AAKG and blood flow. A selection of studies that looked at these effects all determined blood flow using high resolution, real-time pulsed-wave Doppler ultrasound (SonoSite M-Turbo, SonoSite Inc, Bothell, WA) providing very comparable data between studies (Thomson, Thomson, Woods, Lanos & Sage, 2001; Matthiessen, Zeitz, Richard & Klemm, 2004; Billinger & Kluding, 2009; Hotoleanu, Fodor & Suciu, 2010; Willoughby et al., 2011). Brachial artery was located by palpitation while subjects were relaxed in the supine position, 30 second sampling was acquired at a depth of no more than 6 cm dependant on circumference of thickest part of the arm. Earlier studies carried out by Thomson et al. (2001); Matthiessen, Zeitz, Richard & Klemm (2004); Billinger & Kluding, 2009; Hotoleanu, Fodor & Suciu (2010) were only determining blood in the forearm immediately pre and post exercise, finding no significance ($P > 0.05$).

Willoughby et al. (2011) differentiated their study on brachial blood flow with the inclusion of an additional time point; this gives rise to study potential lasting affects of supplementation as opposed to the acute effects often studied (Table 2.6).

Table 2.6 Brachial artery blood flow ($\text{cm}\cdot\text{s}^{-1}$) pre, post and 30 minutes post exercise (Willoughby et al., 2011).

	Placebo T1	Placebo T2	AAKG T1	AAKG T2
Pre	85.69 ± 20.34	90.55 ± 18.20	85.85 ± 17.76	82.15 ± 15.24
Post	116.91 ± 8.09	119.88 ± 10.21	115.51 ± 11.18	113.31 ± 11.18
30 min Post	90.74 ± 20.34	94.50 ± 21.43	85.01 ± 10.74	91.52 ± 24.56

Although no significant differences were found ($P = 0.094$) similar to other studies on the area, it is interesting to note that the group supplemented with AAKG ($n = 12$) actually exhibited a decrease in blood flow pre and post exercise as opposed to an increase. Perhaps signalling that L-arginine may be having an inhibitive effect on blood flow during exercise as opposed to the beneficial effects that are theorised. Where the placebo group increased by $5.14 \text{ cm}\cdot\text{s}^{-1}$ the AAKG group actually decreases by $3.70 \text{ cm}\cdot\text{s}^{-1}$, this may have inhibited blood flow to the muscles that needed it impeding performance. Increases are only noted 30 minutes post exercise whereas the placebo group ($n = 12$) exhibited a total increase over each exercise test. This gives rise to the theory that

there may be a strong “placebo effect” (Smilios, Piliandis, Karamouzis & Tokmakidis, as cited in Willoughby et al., 2011). However, it is much more likely that NO production has become inhibited by the up-regulation of ADMA due to inflammatory cytokines (Stuhlinger et al., 2001). Inflammatory cytokines have been shown to increase in response to resistance exercise (Izquierdo et al., 2009). Inhibition of NO has been proven to be concentration-dependent, this means that when ADMA levels increase and L-arginine levels do not NO will decrease, the same can be said it reverse (Izquierdo et al., 2009). Resistance training is proven to increase inflammatory cytokines, should L-arginine levels not be sufficient a resultant increase in ADMA may become prevalent. A greater concentration of ADMA coupled with an inadequate concentration of L-arginine may result in NO inhibition thus leading to minimal vasodilation.

Deeper investigation into the dose response of L-arginine and blood flow improvements uncovers a skewed view of its benefits. The study that has been most often cited as evidence of L-arginine improving blood flow is that by Bode-Boger et al. (1998); this study concluded that vascular effects are closely correlated with plasma L-arginine concentrations. It has long been taken as a given that increasing oral consumption of L-arginine will increase plasma concentration thus leading to a conclusion of potential blood flow improvements. However, this study originally determined this via intravenous sampling of 30 g.d⁻¹ AAKG as opposed to the more regularly seen 5 – 10 g.d⁻¹ oral consumption, without further investigation it would appear that only intravenous inclusion of these levels will promote blood flow hence a possible reason why no significant data was obtained in this study. The same study did nonetheless research the intravenous effects of 6 g.d⁻¹ and found the response to be much slower than 30 g.d⁻¹ intravenous in terms of time taken to reach peak concentration values, but still well below what is seen with oral consumption.

2.7 Effect of AAKG and Cr on Strength and Power

Cr is well researched in terms of the effect on strength and power exercise, it is known that PCr energy system is used in exercise lasting up to 15 seconds; therefore resistance training that is anaerobic in nature would benefit greatly from increased Cr stores. It would appear that the training status of the subjects play an important role in the benefits that are noted in this area. Pearson, Hamby, Russel and Harris (1999) reported that both upper and lower body strength and full body power was increased when supplemented with Cr (Table 2.5).

Table 2.5. Pre and post study measurements (Pearson, Hamby, Russel & Harris, 1999).

	Measure	Before	After
Bench press (kg)	Creatine monohydrate	149.12 ± 12.64	154.22 ± 12.54*
	Placebo	130.24 ± 26.73	128.63 ± 22.09
Squats (kg)	Creatine monohydrate	241 ± 12.64	268.59 ± 56.18*
	Placebo	221.88 ± 67.95	232.47 ± 73.43
Power clean (kg)	Creatine monohydrate	123.32 ± 21.65	130.97 ± 20.57*
	Placebo	111.58 ± 15.42	109.32 ± 29.32

This differs to a study by Izquierdo, Ibanez, Gonzalez-Badillo & Gorostiaga (2001) who found there to be no significant difference ($P > 0.05$) in upper body strength, compared with a 14.7 ± 2.2 kg

increase against a slight decrease in the placebo group in lower body strength ($P < 0.01$) when supplemented with Cr (Figure 2.6).

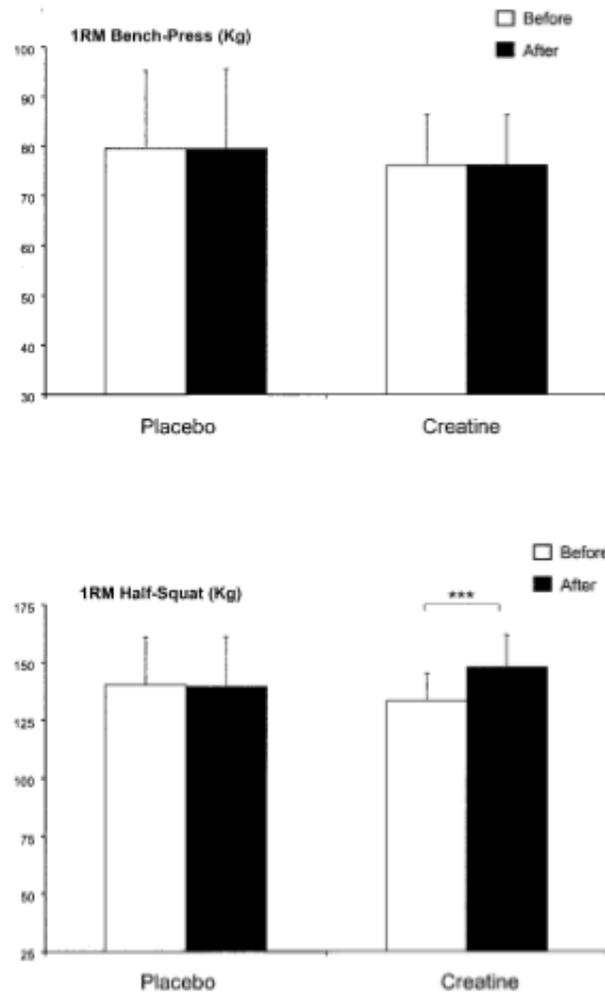
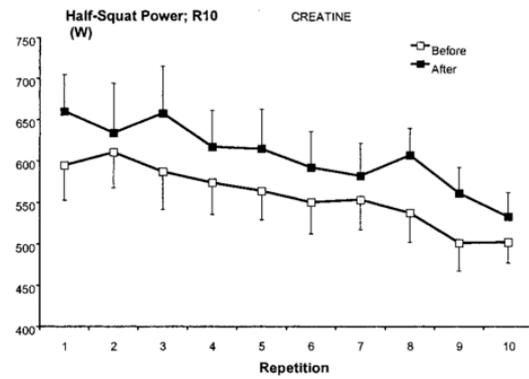
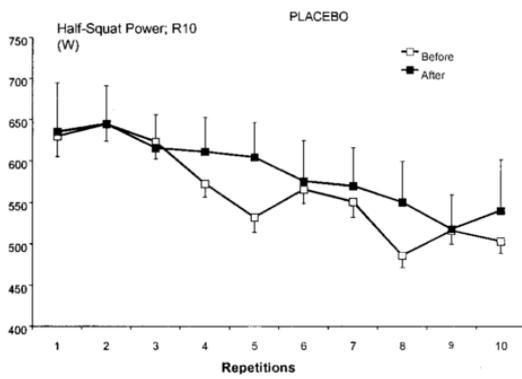


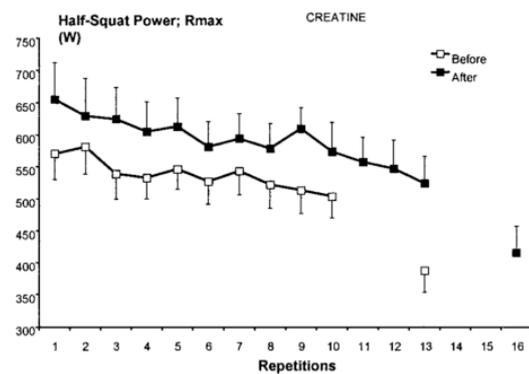
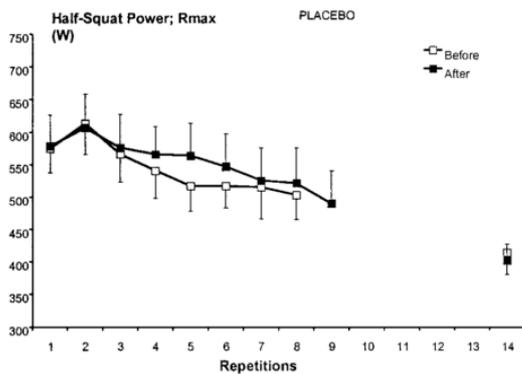
Figure 2.6 One repetition maximum (1RM) bench press and half squat pre and post supplementation (Izquierdo et al., 2001).

Both studies in this case utilized a $20 \text{ g}\cdot\text{d}^{-1}$ loading phase for 7 days and then continued with $5 \text{ g}\cdot\text{d}^{-1}$ for a further 3 weeks. The variance in upper body strength gains could be attributed to the training programme or the sport that the subjects played. The study by Pearson et al. (1999) investigated the effect on collegiate American football players in comparison to Izquierdo et al (2001) who used collegiate handball players; all of which followed a structured training programme for upper and lower body strength. Lower body strength was also attributed to lower body power adaptations when supplementing with Cr (Greenhaff et al., 1996; Pearson et al., 1999). Figure 2.7 demonstrates the average power increments over each repetition, pre and post placebo or Cr consumption. It would suggest that there is no benefit in taking Cr supplementation prior to resistance exercise in the hope of getting performance benefits; both placebo and Cr groups have performed better in the second trial, albeit the Cr group would appear to have performed beyond that of the placebo but to significant difference.



(A)

(B)



(C)

(D)

Figure 2.7 Average muscle power pre and post supplementation for half squat (Izquierdo et al., 2001).

Similar to Cr supplementation evidence supporting AAKG supplementation tends to support its use over long periods. The AAKG-enhanced vasodilation and blood flow to working muscles during resistance exercise is alleged to provide and even greater impetus for increasing muscle strength and hypertrophy than exercise alone (Willoughby, Boucher, Reid, Skelton & Clark, 2011). Despite research claiming that NO is shown to stimulate mitochondrial synthesis in most body muscles, specifically skeletal muscle (Kobzik, Reid, Bredt & Stamler, 1994), the current campaigns marketing AAKG as a body building supplement and the theoretical research into the potential mechanisms of action there is still sparse research in the area of AAKG and improvements in strength. Currently there are only very few known studies that have determined AAKG does in fact increase strength parameters as is claimed; with no data to knowledge determining these effects during co-ingestion of AAKG and Cr.

Studies by Santos et al. (2002) and Campbell et al. (2006) looked into the ergogenic effects of AAKG on muscle function; using the variables one repetition maximum (1RM) bench press, isokinetic knee extension fatigue index and Wingate anaerobic power indices. Santos et al. (2002) reported no significant changes in 1RM bench press or Wingate power indices, but did show an 8.5% improvement in isokinetic knee extension fatigue index when supplemented with AAKG. This is in direct opposition to results found by Campbell et al. (2006) who stated that no significance was prevalent in fatigue indexes, but significance showed in both 1RM strength improvements and anaerobic power indices.

Analysis of the 1RM bench press (Figure 2.8) showed significant improvements between groups ($P=0.03$), AAKG showing improvements of 8.82 ± 7.33 kg versus Placebo 2.67 ± 9.11 kg, over an 8-week intervention period (Campbell et al., 2006).

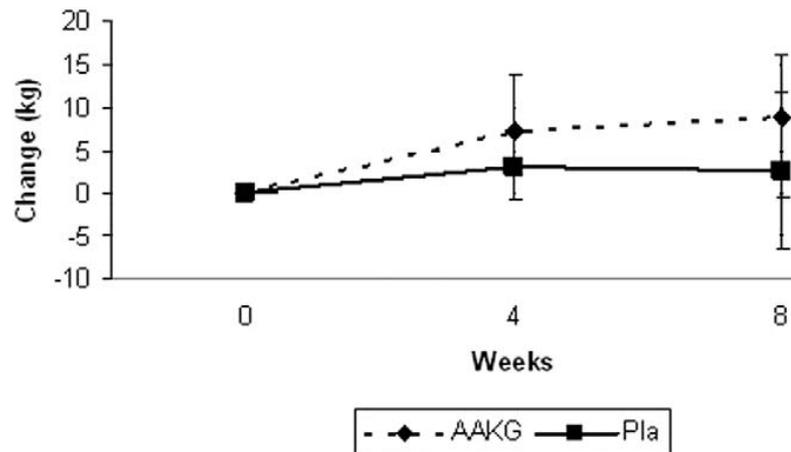


Figure 2.8 1RM bench press change (kg) in resistance-trained individuals (Campbell et al., 2006).

The tabulated data from the three stages (Table 2.6) of the anaerobic power test as carried out by Campbell et al. (2006), data shows that significance only occurred during the final stage of the test, peak power ($P = 0.005$), time to peak power ($P = 0.05$) and rate to fatigue ($P = 0.005$). In contrast, no significance was found at any stage during research by Santos et al. (2002) who reported ($T1 - P = 0.125$, $T2 - P = 0.343$ and $T3 - P = 0.063$).

Table 2.6 Wingate anaerobic power indices (Campbell et al., 2006).

	AAKG	Placebo
Peak Power (W)		
T1	1251 ± 236	1271 ± 257
T2	1291 ± 254	1282 ± 219
T3	1331 ± 242*	1202 ± 241
Time to Peak Power (s)		
T1	3.77 ± 0.55	3.83 ± 1.02
T2	3.80 ± 0.80	4.12 ± 0.84
T3	3.88 ± 0.48*	3.32 ± 1.25
Rate to Fatigue (W/s)		
T1	34.9 ± 8.90	35.6 ± 8.60
T2	36.4 ± 10.00	35.6 ± 9.10
T3	37.6 ± 8.80*	31.9 ± 9.50

Other studies; also using untrained individuals, determined that there were no significant improvements in any of the parameters for 1RM strength. Bednarz, Jaxa-Chamiec, Gebalska, Herbaczynska-Cedro & Ceremuzyński (2004); Paddon-Jones, Borsheim & Wolfe (2004) both used 1RM bench press as a determinant of strength but found no significant results ($P = 0.125$ and $P = 0.267$, respectively). Similarly, no significance was found in 1RM deadlift performance (AAKG: 4.57 ± 0.37 kg and Placebo: 3.72 ± 0.89 kg, $P = 0.546$; AAKG: 6.79 ± 1.24 kg and Placebo: 6.02 ± 1.46 kg, $P = 0.734$) during oral administration (Siani et al., 2000; Nagaya et al., 2001).

In summary, there are many potential benefits for ingesting a combined supplementation. Unfortunately the majority of the research simply looks at physiological mechanisms or laboratory based testing protocol. There is a gap in the research to suggest there is a sporting benefit despite the many purported similarities and connections L-arginine and creatine have.

3.0

Methodology

3.1 Pre-Experimental Methodology

3.1.1 Participants

Ten healthy, active male volunteers (Table 3.1) were recruited through contacts within the University of Hertfordshire. Inclusion in the study required all participants to be aged 18 – 40 years, actively participate in resistance training and have a normotensive blood pressure of less than 140/90 mmHg.

Table 3.1. Overview of subject details (n = 10), all values are mean \pm SD.

Subject Details	
Age	19.6 \pm 1.3 years
Height	177.4 \pm 6.3 cm
Weight	83.5 \pm 3.5 kg

Ethical approval was obtained prior to testing via the University of Hertfordshire, Life Sciences ethics committee, protocol number LS5/12/12P; in addition to informed consent and health monitoring screens being completed by the participants (Appendix 1).

3.1.2 Collection Protocol

Participants were required to attend the Human Performance Laboratory at the University of Hertfordshire on a single occasion; the session will last a minimum of four hours excluding time taken to fill out appropriate forms. Prior to entering the laboratory participants were instructed to forgo strenuous exercise 24 hours previous in addition to a 4-hour fast period immediately preceding the testing date. Upon arrival participants receive a full verbal briefing in addition to that already signed (Appendix 2), and encouraged to ask any questions that they may have about the study.

Preliminary anthropometric data was collected, this involved measurement of height (m) using a Seca 220 Stadiometer (Seca United Kingdom, Birmingham, United Kingdom), weight (kg) with the Seca 780/783 Weight Scales (Seca United Kingdom, Birmingham, United Kingdom) and using the Omron MX3 plus digital blood pressure monitor, HEM-742-E (Omron Health Care, Kyoto, Japan) heart rate (bpm) and blood pressure (mmHg) were recorded. To provide safety subjects were seated in a phlebotomy chair and the tips of three fingers on the chosen hand were sterilised using a steret isopropyl alcohol wipe (Medlock Medical Ltd, Oldham, United Kingdom). After ensuring that the participant is prepared for the blood collection process a Accucheck Safe-T-Pro Plus Lancet (Roche Diagnostics GmbH, Mannheim, Germany) is used to pierce the skin and subsequent blood collected via a 600 μ l Multivette® 600 EDTA-K (Sarstedt Ltd, Leicester, United Kingdom), this is then labelled hour zero. Once collected participants remain seated and are provided with a pre-made intervention drink consisting of 6 g.d⁻¹ AAKG combined with 10 g.d⁻¹ Cr mixed into 250 ml diluted fruit juice; this is to be consumed within 15 minutes. Further 600 μ l samples are collected at one, two and four hours; participants were required to remain in the laboratory throughout the investigation, all the while keeping movement and activity to a minimum, although food was prohibited they were encouraged to drink water *ad libitum* throughout the study.

Capillary sampling was chosen as the preferred method of collection over multiple venepunctures to minimise participant discomfort. Once each blood sample had been collected, vials

were centrifuged immediately at 11,000 rpm for 5 minutes in room temperature using a Hettich Zentrifugen EBA 12 (Andreas Hettich GmbH & Co. Kg, Tuttlingen, Germany). Following this plasma was aspirated using a Pipetman Classic P1000 (Gilson Inc. Middleton, USA) into the relevant labelled CRY-260-030A cryotube (Fisher Scientific UK Ltd, Leicestershire, United Kingdom), placed into a cryobox before being stored at -80 degrees Celsius in an MDF-U5V3 VIP-86 freezer (SANYO Biomedical Solutions, Illinois, USA).

Participants were kept under constant observation by both researcher and additional qualified first aider for any signs of adverse effects, such as fainting nausea and/or vomiting due to supplement or blood taking procedures. In addition to this blood pressure and heart rate were monitored throughout to ensure no undue changes. Before being allowed to vacate the premises all participants were checked by a present first aider and given clearance.

3.1.3 Analysis Protocol

Analysis of blood plasma for L-arginine concentration was undertaken at the University of Hertfordshire, Biomedical Science Laboratory by the researcher, with the aid of the laboratory technician. An L-arginine Enzyme-linked Immunosorbent Assay (ELISA) kit (BioSupply UK Ltd, Yorkshire, United Kingdom) was chosen as the method for blood evaluation, overleaf is a schematic illustrating the process by which this was obtained (Figure 3.1); the full guideline document can be found in Appendix 5. Once ELISA testing had been concluded a calibration curve was documented using the standards and controls that are provided, this is then used to determine the L-arginine concentration for all blood samples collected.

3.1.4 Data Analysis: Pre-Experimental

Quantitative, continuous ratio data was collected and measurements input to SPSS 21, PASW Statistics 21.0 (IBM, New York, United States of America) for analysis. Normality tests confirmed that the data was normally distributed and therefore required the use of a one-way within samples ANOVA test and a Bonferonni post-hoc test selection to ascertain any differences in variables. Upon completion of these significance tests acceptance or rejection of the null hypothesis was determined ($P < 0.05$).

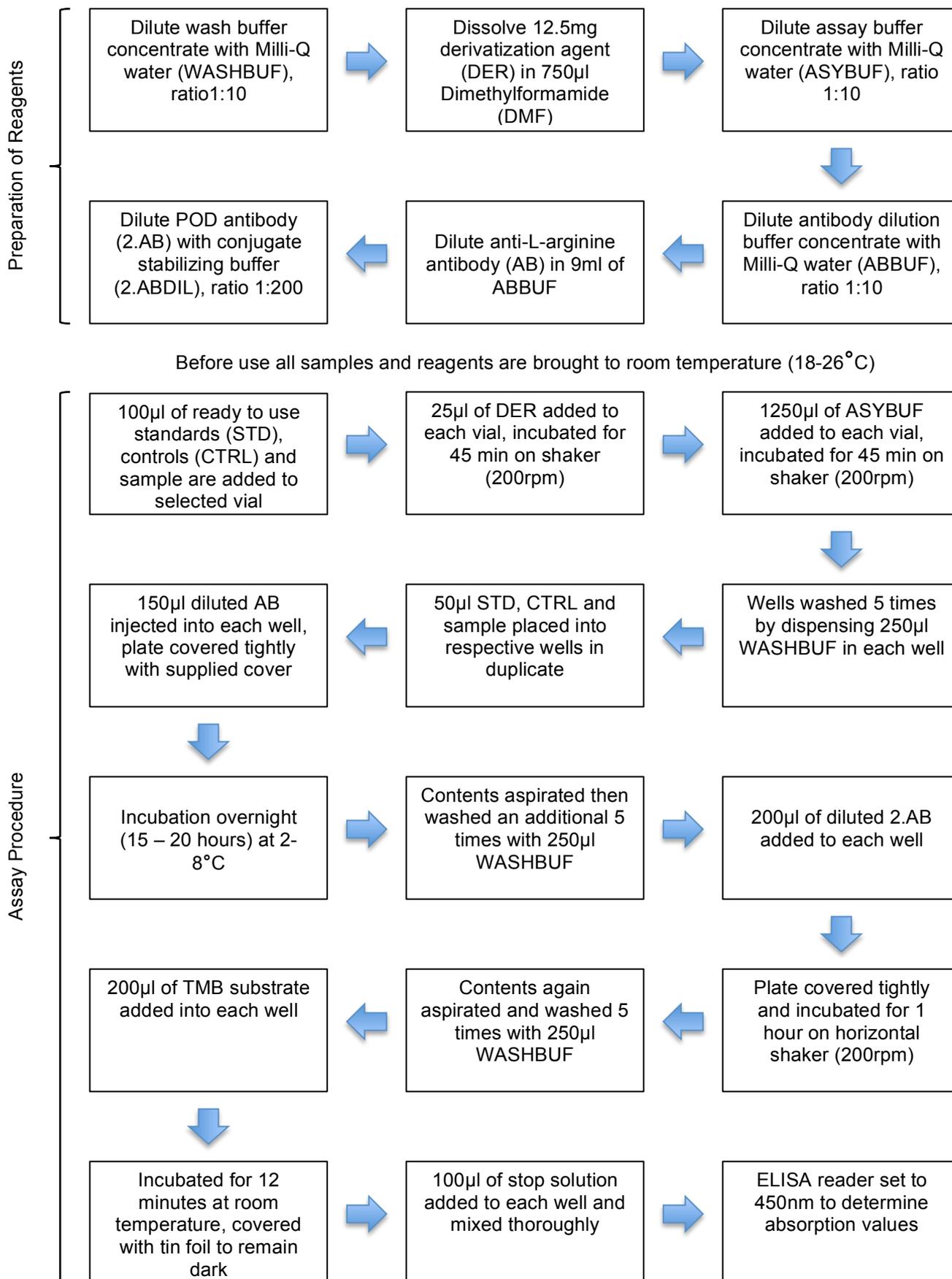


Figure 3.1 Flow diagram illustrating the steps taken when analysing L-arginine absorbance using ELISA testing (Immun Diagnostik Ltd, Bensheim, Germany).

3.2 Main-Experimental Methodology

3.2.1 Participants

Twenty-four habitually trained male participants (Table 3.2) participated in this study, vital inclusion criterion consisted of; aged between 18 – 40 years, at least one years strength training experience and currently participating in aforementioned exercise type, twice a week or more. These along with further inclusion and exclusion criteria can be seen in (Appendix 4), all of which were stringently adhered to.

Table 3.2 Overview of subject details at baseline (n = 24), all values are mean \pm SD.

Subject Details	
Age	24.5 \pm 4.9 years
Training Age*	2.5 \pm 0.9 years
Height	179.2 \pm 8.1 cm
Weight	77.1 \pm 10.3 kg
Fat Free Mass	68.59 \pm 8.71 kg

*Training age is defined as the number of consecutive years of participation in resistance training.

Ethical approval was obtained prior to testing via the University of Hertfordshire, Life Sciences ethics committee, protocol number LS6/12/12P; in addition to informed consent and regular health monitoring screens being completed by the participants (Appendix 1). Recruitment was accomplished through word of mouth at the place of work, local gym and sports facilities.

3.2.2 Calculation of Participant Numbers

A priori analysis was conducted to compute required sample size to find significance between treatments for AAKG and Cr. The alpha level was set 0.05, the power level was set at 80% and the effect size was estimated at 0.78 and 0.88 for AAKG and Cr, respectively, based on previous research. The total sample size was estimated at 8 and 6 participants for AAKG and Cr, respectively. A sensitivity analysis was completed to compute the required effect size to find significance with the seven participants that completed the study. It was calculated that an effect size of 0.80 would be required.

3.2.3 Study Design

The most appropriate design to be utilised for this study was a double blind, placebo and controlled intervention. This ensures that neither the researcher nor the subject can influence the results due to knowledge of what is being consumed, reducing the probability of bias.

One permutation that was discussed during planning was the inclusion of a creatine only group as a replacement for the inactive control group. However, after deliberation it was shown that the inclusion of this group would take the research costs above the upper limits of the planned project. An application for further funding to cover these costs was placed but not granted, therefore it was decided to exclude a creatine only group, as the study would benefit from keeping costs down to allow funds to be better spent elsewhere.

Furthermore, it is uncommon to see the use of both a placebo and inactive control group, however this study employed the use of both. The active placebo group would help with understanding whether there is a learning effect of the training or the mere perception that taking a 'pre-workout' formula may improve performance, whilst the inactive control group acted as a means to assess potential differences between those who were active (taking placebo) and inactivity, aiding understanding of the response to exercise as opposed to L-arginine.

3.2.4 Control of Data

Where possible all external variables that may influence results have been considered and every effort is made to reduce the impact these have. Temperature was monitored daily, where possible extraneous temperatures were avoided, average temperatures recorded (21.94 ± 0.44 °C) and humidity (38.33 ± 6.20 %). In addition to this 3-day food diaries were collected every two weeks (Table 3.3) to ensure there was no dietary co-variance throughout the study, no significant differences were noted ($P > 0.05$).

Table 3.3 Actual dietary intakes, values shown are mean \pm SD

	AAKG + Cr	AAKG + PLA	PL	CONT
PRE (Week 0)				
Carbohydrates (g.d ⁻¹)	152.32 \pm 8.39	157.93 \pm 9.82	150.36 \pm 11.22	155.51 \pm 8.91
Fats (g.d ⁻¹)	50.40 \pm 7.24	41.20 \pm 8.34	38.41 \pm 6.24	30.71 \pm 5.60
Proteins (g.d ⁻¹)	65.69 \pm 6.30	58.28 \pm 9.56	55.33 \pm 7.48	62.71 \pm 7.50
INTER (Week 4)				
Carbohydrates (g.d ⁻¹)	153.49 \pm 9.57	158.03 \pm 6.45	152.76 \pm 10.34	156.23 \pm 7.50
Fats (g.d ⁻¹)	50.98 \pm 6.46	42.53 \pm 6.19	38.94 \pm 7.23	31.76 \pm 6.07
Proteins (g.d ⁻¹)	67.21 \pm 6.49	60.05 \pm 8.39	57.18 \pm 8.29	61.64 \pm 8.03
POST (Week 8)				
Carbohydrates (g.d ⁻¹)	151.62 \pm 12.33	156.83 \pm 8.37	153.60 \pm 11.10	155.83 \pm 8.65
Fats (g.d ⁻¹)	51.22 \pm 6.80	43.59 \pm 7.12	39.42 \pm 6.35	29.87 \pm 5.50
Proteins (g.d ⁻¹)	64.38 \pm 6.45	61.57 \pm 9.32	56.21 \pm 7.39	63.45 \pm 7.10

Co-efficient of variation (CoV) was conducted within pilot work on the following apparatus to determine the validity of use. The Omron MX3 plus digital blood pressure monitor (CoV = 1.8), HEM-742-E (Omron Health Care, Kyoto, Japan), Harpenden skinfold calliper (CoV = 2.3), HSK-BI (Baty International, West Sussex, United Kingdom), Probotics jump mat (CoV = 1.0) (Probotics Inc, Alabama, USA) and MIE myometer strain gauge (CoV = 1.8) (MIE Research Ltd, Leeds, United Kingdom). Manufacturer recommended calibration was conducted on those apparatus that required it prior to each participant starting the test.

3.2.5 Collection Protocol

Participants were required to attend the University of Hertfordshire Human Performance Laboratory once every two weeks, over the course of an eight-week period. Each session would last approximately one hour, the initial assessment lasting slightly longer due to familiarisation of testing procedures. Prior to testing all participants were required to refrain from exercise for 24 hours previous, and enter the laboratory fasted for 8 hours, to ensure that habitual daily habits were

affected as little as possible, every effort was made to book each participant in to the same time of day for all further tests. Upon entering the laboratory all appropriate consent forms and health screens were completed by the participant and signed by the researcher, a 10-minute rest period is then implemented where communication between researcher and participant was kept to a minimum.

Once this was complete, participants would be fitted with Omron MX3 plus digital blood pressure monitor, HEM-742-E (Omron Health Care, Kyoto, Japan), to record heart rate (HR) and blood pressure (BP). Along with this, anthropometric data was collected using the SECA 220 Stadiometer (SECA United Kingdom, Birmingham, United Kingdom) for height (cm), weight (kg) using the SECA 780/783 Weight Scales (SECA United Kingdom, Birmingham, United Kingdom) and forearm length, wrist circumference and forearm circumference using the SECA 201 (SECA United Kingdom, Birmingham, United Kingdom). Forearm length was measured from the humeral medial epicondyle to the styloid process, wrist circumference was measured 1 inch proximal from the styloid process and forearm circumference was measured 1 inch distal to the humeral medial epicondyle. To determine forearm volume the following equation was used:

$$V = \frac{\pi L}{3} (r_1^2 + r_1 r_2 + r_2^2)$$

This simply translates to, volume equals, pie times length of the forearm divided by three, times by the sum of the radius of the wrist and the radius of the upper forearm. To determine radius the circumference measurements were halved. The formula will only however calculate the forearm as if it were a truncated cone so would only be used to show total forearm blood flow to all tissue, as opposed to the actual flow of skeletal muscle.

Body fat percentage was calculated using a four site skinfold equation adapted and tested by Jackson & Pollock (1985), they concluded that the standard errors associated with skinfold assessment and the below equation were comparable with that when measured using bioelectric impedance analysis and hydrostatic weighing. Sites selected for measurement are a vertical fold on the triceps measured to the mid point of the upper arm between the acromiale and radiale, diagonal fold on the suprailiac measured immediately superior to the iliac crest following its natural angle, vertical fold on the abdominal measured 1 inch to the right hand side of the umbilicus and a vertical fold on the thigh measured as the midway point from the proximal border of the patella to the inguinal groove. The equation below demonstrates the process by which body fat is obtained:

$$(0.29288 \times \text{sum of skinfolds}) - (0.0005 \times \text{square of the sum of skinfolds}) + (0.15845 \times \text{age}) - 5.76377$$

(Jackson & Pollock, 1985)

After all anthropometric data has been collected participants are seated at the Hokanson EC6 plethysmograph strain gauge (P.M.S. Instruments Ltd, Maidenhead, England) this is then placed around the arm at the same point as the measurement for forearm circumference. The size of the band used is to be 1 cm smaller than forearm circumference measurements. A Hokanson E20 rapid cuff inflator and Hokanson AG101 cuff inflator source (P.M.S. Instruments Ltd, Maidenhead, England) is placed around the upper arm, just above the bend of the elbow, to be

restricted to 80mmHg, the pressure is adjusted prior to each subject, using the dial on the front to ensure this always reads the same. Another cuff is placed around the wrist and inflated to 180mmHg to restrict arterial influx towards the hand; this is done manually using an Omron HEM-18 Manual Sphygmamometer (Omron Health Care, Kyoto, Japan). Chart 5 software (ADInstruments, Spechbach, Germany) is opened on the computer and calibrated. To calibrate, the EC6 Plethysmograph was set to 0 % using the 'position' dial on the front and then adjusted 1 % in both a positive and negative manner using the calibration switch. The 'set up' tab then 'display settings' option on Chart 5 provided a display of this change and allowed the units to be altered. From previous tests it was decided that each unit of the display would represent a 0.5% change of the derived signal from the strain gauge otherwise experience suggested that the readings would plateau. The readings on the dial should correlate to the readings on the computer screen before the equipment is used for testing. Chart 5 recorded rate of forearm expansion (%.s⁻¹) for 45 seconds. Using Chart 5 an average expansion rate over 30 seconds (%.s⁻¹) was calculated and then multiplied by sixty to get percentage change per minute (%.min⁻¹).

The calculation of total forearm blood flow (ml.100ml⁻¹.min⁻¹) was a multi stage process; firstly forearm volume is calculated using the aforementioned equation for measuring a truncated cone. Forearm volume prior to occlusion was used to calculate volume change over the time period using the formula below and then added together to provide a post occlusion measurement.

$$\text{Volume change (ml)} = \text{initial forearm volume (ml)} \times (\text{volume change (\%.min}^{-1}\text{)} / 100)$$

Once a post occlusion measurement had been obtained it was then possible to determine the blood flow to the forearm during the minute that was recorded.

$$\text{Forearm flow (ml.min}^{-1}\text{)} = \frac{\text{post occlusion volume (ml)} - \text{pre occlusion volume (ml)}}{\text{time (min)}}$$

Current research into blood flow has reported results as ml.100ml⁻¹.min⁻¹; this is a more accurate measurement of blood flow than ml.min⁻¹. As volume change has been calculated during the research process the formula below is the final standardised formula that was used to calculate forearm blood flow.

$$\text{Total forearm blood flow (ml.100ml}^{-1}\text{.min}^{-1}\text{)} = \frac{\text{volume change (ml)}}{\text{pre occlusion volume (ml)} / 100} \times \text{time (min)}$$

The strength element is determined by an isometric bench press, measured in Newtons, and an isometric half squat, also measured in Newtons. All subjects have the bench set to flat, the bar is set so that when the MIE myometer restraining cables are placed around there is 2 inches of slack and the upper arms are parallel with the floor. To determine the correct height for the half squat all participants were required to stand to maximum height un-weighted before lowering the head of the femur until it went parallel with the patella; displacement of the shoulder joint is then measured and halved to determine bar height for the half squat. The same procedure with the restraining straps and

2 inches of slack is repeated for this determined height. Each participant is asked to complete 3 repetitions at maximal force production on each exercise before an average is taken, when completed HR, BP and blood flow is measured once more.

The power element is determined through a countermovement jump and single 20-second anaerobic wingate test; these were chosen as exercises that closely mimicked the demands on a sporting field compared with the static tests already conducted. During the instruction for the countermovement jump test participants are told that they may use their arms for assistance but must ensure that their legs remain straight throughout the jump and can only bend upon landing. Using a Probotics jump mat (Probotics Inc, Alabama, USA) height is measured and again completed 3 times to provide an average result. To conclude the physical tests is a 20-second anaerobic wingate, this is designed to mimic the energy demands of a sprint in sport and thus give indication as to whether the intervention may help in both a controlled and uncontrolled environment. All participants are provided with a 3-minute warm up with only the cradle as resistance (1kg); weight equalling 7.5% of their body weight placed on the cradle at the end of the warm up period and all resistance was momentarily removed. With a countdown from three to one in which participants are to build up speed the cradle was released and the full resistance was returned to the bike. Once 20-seconds has been completed participants will have one further HR, BP and blood flow measurement recorded.

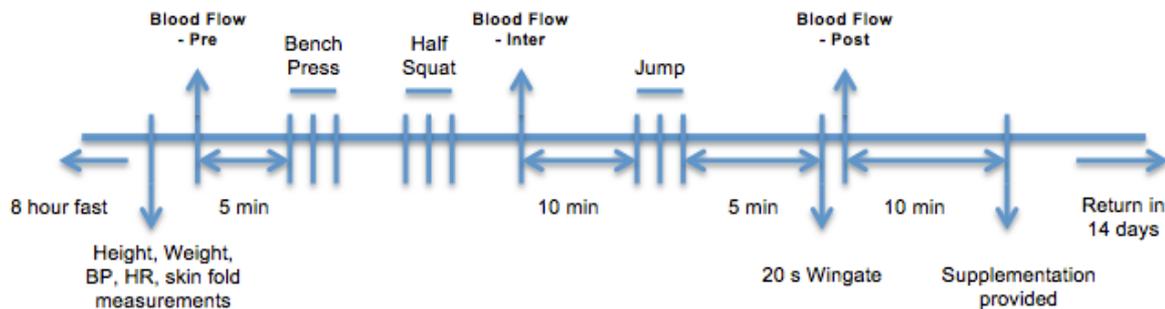


Figure 3.2 Main experimental schematic of events.

3.2.6 Intervention and Nutritional Supplementation

Before leaving the laboratory the participants are checked by both the researcher and a further first aid trained individual to make sure that they are fit to leave. Participants are randomly assigned one of four groups as labelled in table 3.4 and provided with a supplement as necessary, this procedure remains unchanged for future tests at week 2, 4, 6 and 8. All supplements were provided by Myprotein (Myprotein Inc, Northwich, United Kingdom) and mixed with 250 ml of a fruit juice of the participants' choice.

Table 3.4 Breakdown of the intervention groups utilised in this study.

	Group			
	A	B	C	D
Loading Dose (Day 0 - 14)	6g.d ⁻¹ AAKG + 10g.d ⁻¹ CR	6g.d ⁻¹ AAKG + 10g.d ⁻¹ PL	16g.d ⁻¹ PL	N/A
Maintenance Dose (Day 15 - 56)	3g.d ⁻¹ AAKG + 5g.d ⁻¹ CR	3g.d ⁻¹ AAKG + 5g.d ⁻¹ PL	8g.d ⁻¹ PL	N/A

AAKG = L-arginine alpha ketoglutarate, CR = Creatine, PL = Placebo.

3.2.7 Data Analysis

Quantitative, continuous ratio data was collected and measurements input to SPSS 21, PASW Statistics 21.0 (IBM, New York, United States of America) for analysis. Normality tests on, total body mass, fat free mass, peripheral blood flow, isometric bench press and half squat, countermovement jump and anaerobic sprint performance, will confirm distribution, for normally distributed data a One-Way ANOVA with Tukeys post hoc test will be conducted, not normally distributed tests will use the Kruskal-Wallis ANOVA method with a Mann Whitney-U post hoc test. Additional t-tests will be used where only two data sets are being analysed ($P < 0.05$).

4.0

Results

4.1 Pre-Experimental Results

4.1.1 Bioavailability of L-arginine

Analysing L-arginine concentration in ELISA testing requires the use of a calibration curve; this is drawn from standard values as provided. The use of an exponential trendline and linear equation establishes the arginine concentration of each individual blood sample.

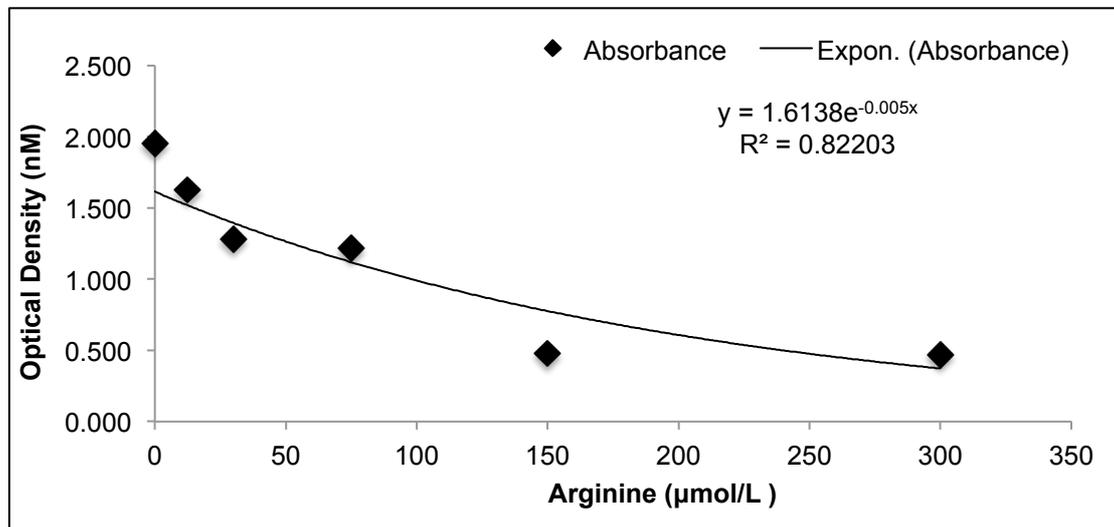


Figure 4.1 Calibration curve showing ELISA optical density and arginine standards.

To determine the most appropriate time to initiate supplement intake, participants were required to consume 6 g.d⁻¹ AAKG mixed with 10 g.d⁻¹ Cr immediately after the collection of a baseline capillary blood sample. Below is a graphical representation of L-arginine concentration over a 4-hour period (Figure 4.2).

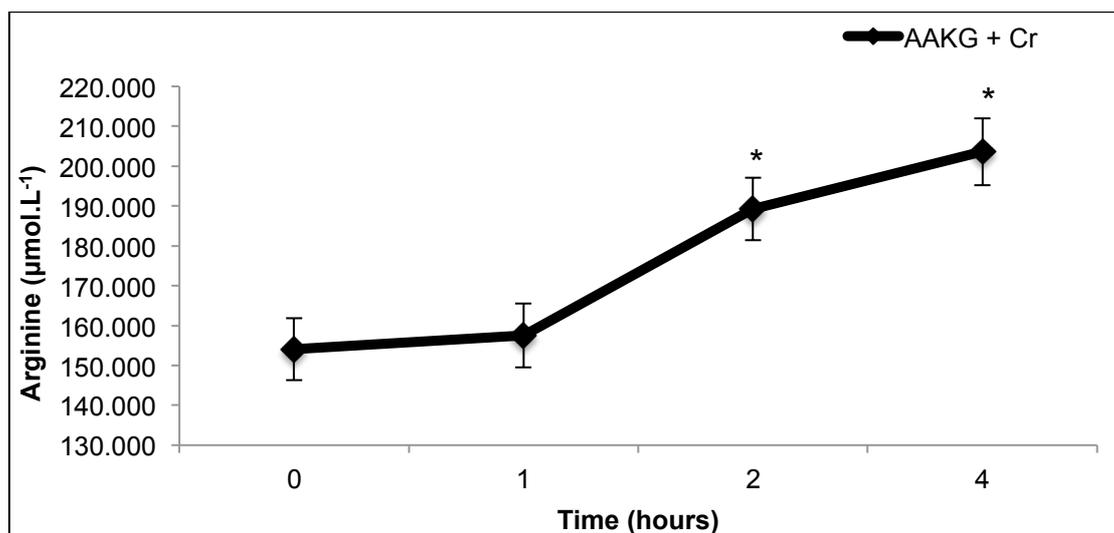


Figure 4.2 L-arginine concentrations in capillary sampled blood plasma; all values are mean \pm standard error of the mean (SEM). Significant differences were seen between hours two and four compared to zero (*P = 0.012 and P = 0.037 respectively).

Baseline plasma concentration was $154.075 \pm 7.826 \mu\text{mol.L}^{-1}$, this increased significantly to $189.273 \pm 7.849 \mu\text{mol.L}^{-1}$ at 2 hours post consumption and further increase was seen up until the final sample at 4 hours post consumption, $203.617 \pm 8.403 \mu\text{mol.L}^{-1}$.

4.2 Main-Experimental Study Results

4.2.1 Effect of intervention on body weight and fat free mass

Establishment of body fat percentage and weight allowed for the calculation of fat free mass (FFM). Table 4.1 and 4.2 show the weekly differences from baseline of total body mass (TBM) and FFM respectively.

Table 4.1 TBM (kg) change over 8 weeks between individual groups; values are mean \pm SEM, significance was only found between baseline values (*P = 0.001 between AAKG + Cr and AAKG + PL; ^P = 0.009 between AAKG + PL and PL).

	Baseline	Week 2	Week 4	Week 6	Week 8
AAKG + Cr	$68.0 \pm 2.5^*$	0.3 ± 0.1	-0.2 ± 0.4	0.6 ± 0.5	1.0 ± 0.7
AAKG + PL	$88.3 \pm 3.1^{\wedge}$	0.0 ± 0.5	-0.1 ± 0.5	0.3 ± 0.4	0.4 ± 0.5
PL	73.0 ± 2.7	-0.1 ± 0.5	-0.1 ± 0.4	-0.3 ± 0.6	-0.1 ± 0.5
CONT	78.7 ± 3.4	0.0 ± 0.2	-0.4 ± 0.1	0.0 ± 0.2	0.2 ± 0.1

Table 4.2 FFM (kg) change over 8 weeks between individual groups; values are mean \pm SEM, significance was only found between baseline values (*P = 0.004 between AAKG + Cr and AAKG + PL; ^P = 0.049 between AAKG + Cr and CONT; ^P = 0.033 between AAKG + PL and PL).

	Baseline	Week 2	Week 4	Week 6	Week 8
AAKG + Cr	$61.2 \pm 2.9^{* \wedge}$	0.5 ± 0.1	0.4 ± 0.5	0.8 ± 0.6	1.3 ± 0.8
AAKG + PL	$76.7 \pm 3.1^{\wedge}$	0.0 ± 0.5	-0.1 ± 0.6	0.3 ± 0.5	0.3 ± 0.6
PL	64.8 ± 2.0	0.2 ± 0.4	-0.2 ± 0.3	-0.5 ± 0.4	-0.4 ± 0.5
CONT	71.7 ± 2.6	-0.3 ± 0.3	-0.6 ± 0.3	-0.4 ± 0.5	-0.5 ± 0.4

4.2.2 Effect of intervention on blood flow

Table 4.3 signifies the average blood flow for all groups at baseline while Figure 4.3 signifies the overall weekly differences between all groups.

Table 4.3 Baseline values for forearm blood flow at rest, inter exercise and post exercise, values shown are mean \pm SEM, no significant differences were found (P > 0.05).

	Flow ($\text{ml.100ml}^{-1}.\text{min}^{-1}$)			
	AAKG + Cr	AAKG + PL	PL	CONT
Pre	9.89 ± 0.71	12.14 ± 0.72	10.36 ± 0.20	11.65 ± 0.68
Inter	10.16 ± 0.78	12.21 ± 0.76	10.44 ± 0.26	11.77 ± 0.72
Post	10.30 ± 0.73	12.46 ± 0.80	10.62 ± 0.19	12.00 ± 0.74

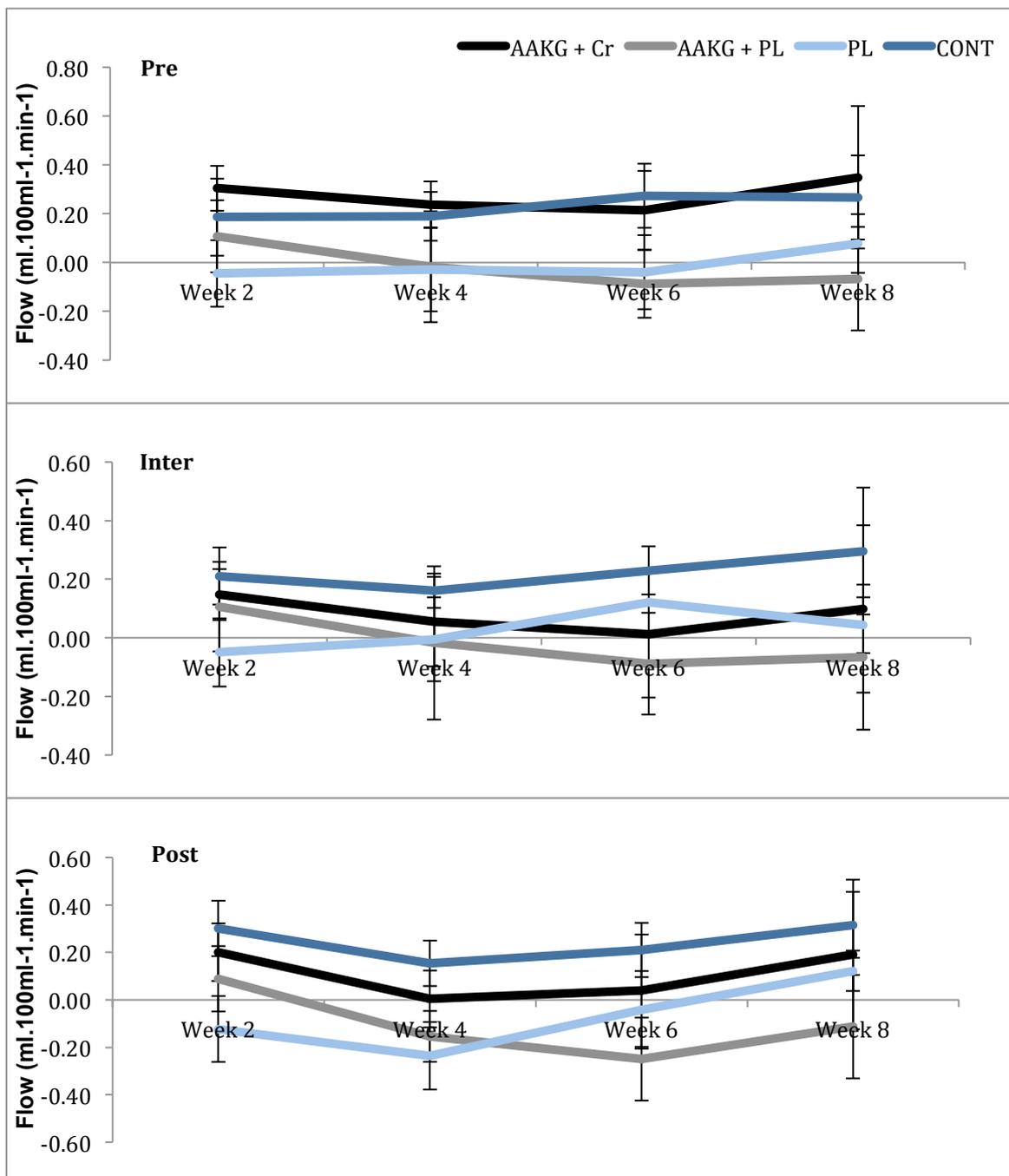


Figure 4.3 Change over 8 weeks from baseline in pre, inter and post forearm blood flow, values are mean \pm SEM, no significance was found ($P > 0.05$).

Pre blood flow increased by 0.14 ± 0.18 ml.100ml⁻¹.min⁻¹, less than 0.17 ± 0.10 ml.100ml⁻¹.min⁻¹ that was observant in the group without AAKG. Results at mid way show a modest increase in blood flow, 0.03 ± 0.18 ml.100ml⁻¹.min⁻¹ compared with an increase of 0.17 ± 0.12 ml.100ml⁻¹.min⁻¹ in the PL group. The final marker exhibited an increase in those taking AAKG, 0.04 ± 0.19 ml.100ml⁻¹.min⁻¹ compared to those without 0.22 ± 0.08 ml.100ml⁻¹.min⁻¹.

4.2.3 Effect of intervention on strength variables

Baseline values for the bench press and squat can be found in Table 4.4, units used to measure bench press and squat were newtons (N).

Table 4.4 Bench press and Squat variable force at baseline across all groups, values are mean \pm SEM, no significant differences were found ($P > 0.05$).

Baseline Scores (N)				
	AAKG + Cr	AAKG + PL	PL	CONT
Bench	200.9 \pm 25.8	256.8 \pm 20.6	239.8 \pm 10.4	265.8 \pm 36.6
Squat	445.3 \pm 73.1	507.3 \pm 71.2	473.6 \pm 99.5	666.6 \pm 63.7

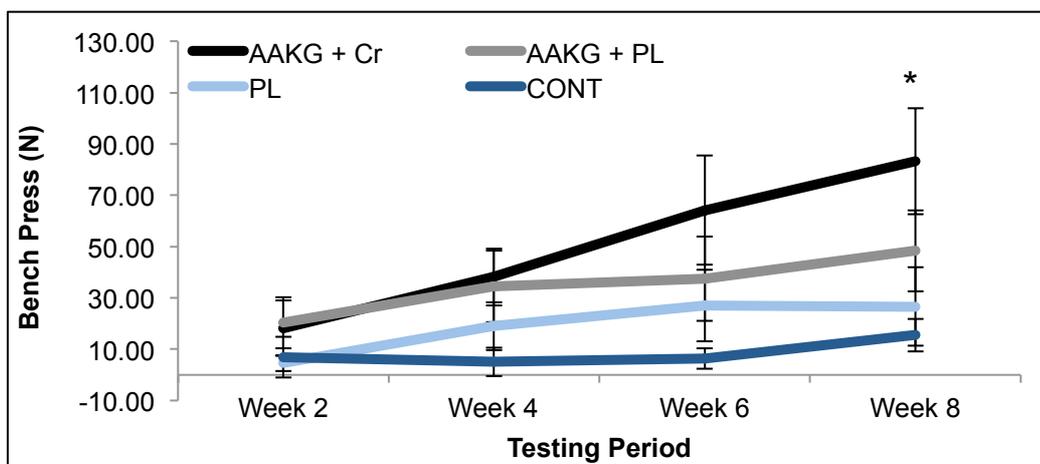


Figure 4.5 Weekly changes in bench press from baseline in all groups, values shown are mean \pm SEM. Significant differences were seen between AAKG + Cr and CONT (* $P = 0.026$) at week 8 only.

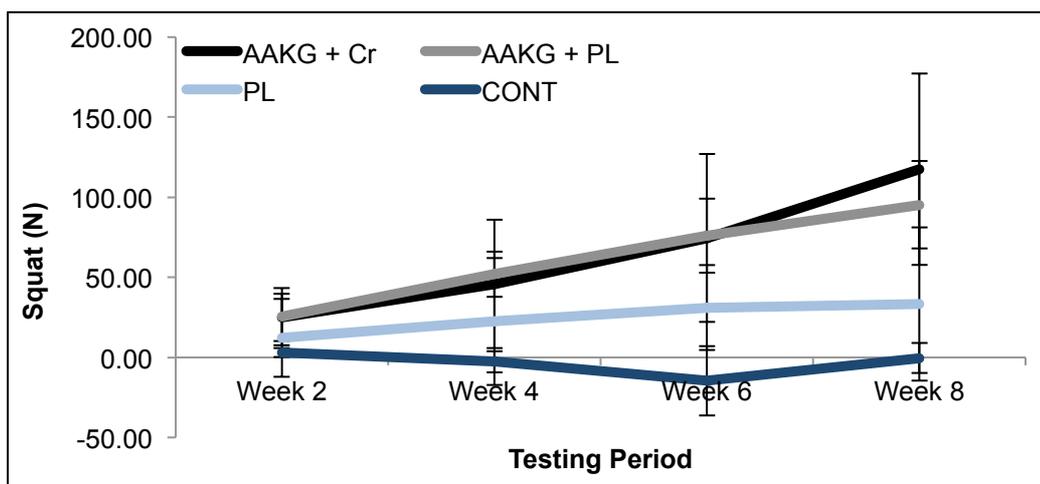


Figure 4.7 Weekly changes in isometric half squat from baseline in all groups; values shown are mean \pm SEM. No significant differences were seen ($P > 0.05$).

Over the 8-week period the only significant differences observed were on the final week of testing for the bench press variable, this showed significant changes between AAKG and Cr when compared with CONT. The former showed a change of 83.28 ± 20.68 kg which was far greater than the change observed in the latter which was 15.51 ± 6.35 kg. There were no significant differences seen at any stage during the isometric squat.

4.2.4 Effect of intervention on power variables

To better understand the effect that this type of supplementation will have on sporting performance two comparable variables were chosen. A countermovement jump, with the use of the arms to gain momentum, was selected to mimic the jump in sport. Baseline variables have been tabulated to aid a better understanding of performance change in each of the variables of power.

Table 4.5 Countermovement jump variable heights at baseline across all groups, values are mean \pm SEM, no significant differences were found ($P > 0.05$).

Countermovement Jump (cm)				
	AAKG + Cr	AAKG + PL	PL	CONT
Baseline	50.4 ± 3.4	46.9 ± 4.1	50.2 ± 3.0	57.8 ± 3.6

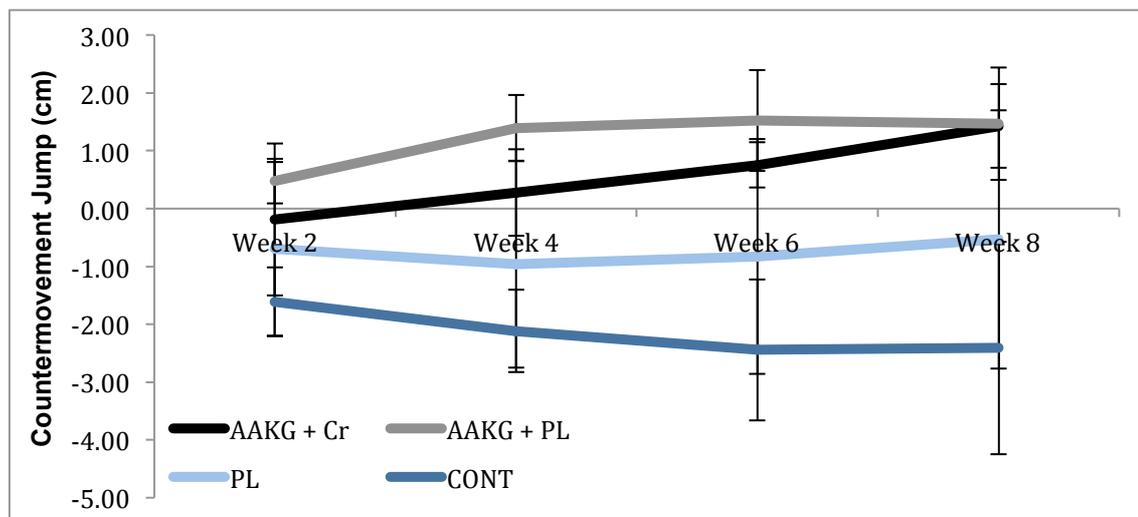


Figure 4.8 Countermovement jump performance between all groups over the research period, values shown are mean \pm SEM. No significant differences were found ($P > 0.05$).

No significant differences have been shown at any stage of the testing period, this is despite changes from baseline to week 8 being positive in both AAKG + Cr (1.43 ± 0.72 cm) and AAKG + PL (1.47 ± 0.97 cm) whereas they are negative in PL (-0.53 ± 2.23 cm) and CONT (-2.41 ± 1.84 cm); this is probably due to the relatively large standard error.

An anaerobic wingate has been used to mimic the usual demands of intermittent sprinting in most sports. As before baseline variables have been tabulated to aid a better understanding of performance change.

Table 4.6 Peak power at baseline across all groups, values are mean \pm SEM, significant differences were found between AAKG + Cr, AAKG + PL and CONT (*P = 0.006 and ^P = 0.015 respectively).

Peak Power (W)				
	AAKG + Cr	AAKG + PL	PL	CONT
Baseline	512.6 \pm 28.9 * ^	714.3 \pm 48.1 *	613.8 \pm 33.6	691.8 \pm 36.9 ^

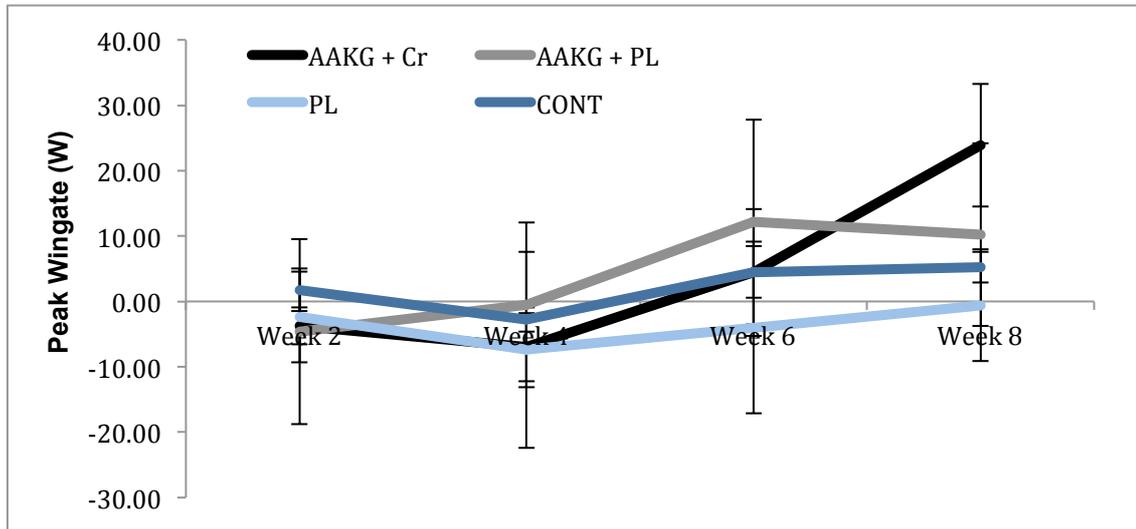


Figure 4.9 Peak power between all groups over the 8-week period, values shown are mean \pm SEM. No significant differences were found (P > 0.05).

Peak power changed throughout all four groups over the 8-week period, AAKG + Cr observed the greatest change with increases of 23.92 ± 9.40 W, AAKG + PL and CONT followed a similar trend with overall increases being 10.25 ± 13.99 W and 5.25 ± 2.38 W respectively. There was no change from baseline to week 8 in PL despite a drop in weeks 4 and 6.

4.2.5 Effect on power to weight ratio

Power to weight ratio (P:W) was determined so that changes in strength and power could be correlated with changes in TBM. The only significant change was noted in the AAKG + Cr group compared with control only, the differences between the two were 1.20 ± 0.31 N.kg⁻¹ for the former and 0.18 ± 0.08 N.kg⁻¹ for the latter. No other groups exhibited a significant difference during the power to weight ratio analysis.

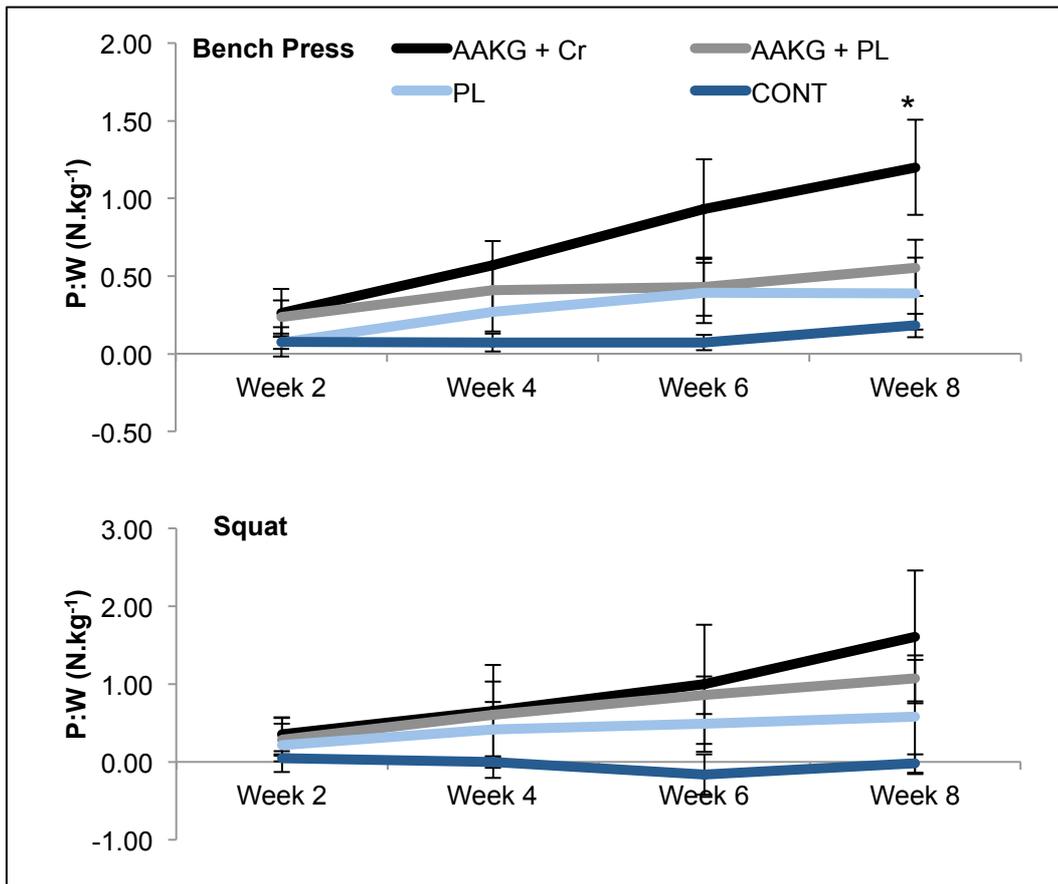


Figure 4.11 Weekly bench and squat changes from baseline in all groups, values shown are mean \pm SEM. Significant differences were seen between AAKG + Cr and CONT (* $P = 0.016$) at week 8 for bench press only, no significant differences were seen at any point between groups for the squat ($P > 0.05$).

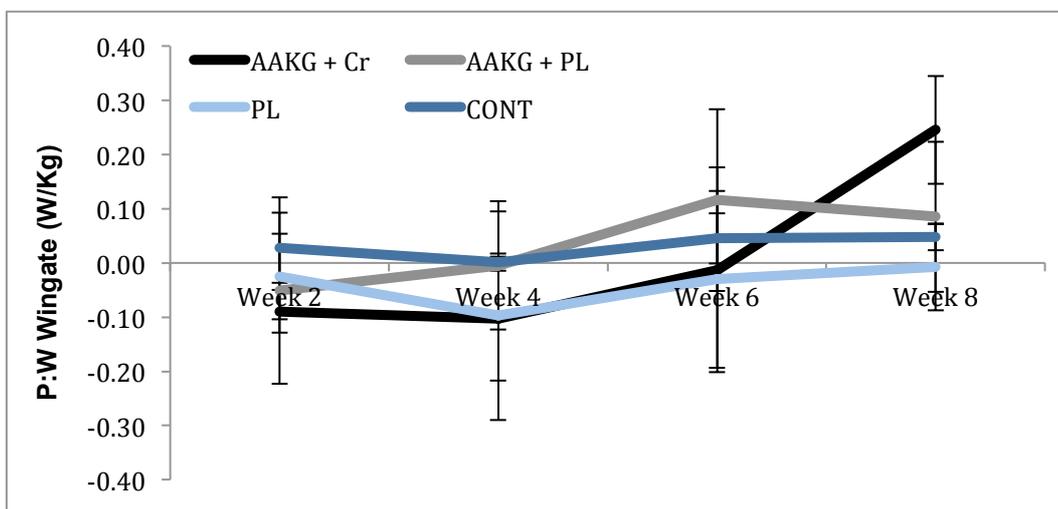


Figure 4.12 P:W of peak power expressed in the anaerobic Wingate performance, no significant differences were noted ($P > 0.05$).

5.0

Discussion

5.1 Discussion of Pre-Experimental findings

This initial section for discussion will endeavour to explain the findings of the pre-experimental study, in particular the reasoning behind selected ingestion timings and collection methods. The primary aim of this study was to determine when a significant peak in L-arginine blood plasma concentration was prevalent, obtaining specific results for oral administration of 6 g.d⁻¹ AAKG mixed with 10 g.d⁻¹ Cr to a healthy population. This led to the secondary aim, determining the viability of capillary sampling for L-arginine concentration analysis.

5.1.1 Bioavailability of L-arginine

The most significant peaks were noted at hour 2 and 4 compared to baseline values (P = 0.012 and P = 0.037 respectively), both outside of the first hour, as was expected prior to the study. This would suggest that by taking the supplement at least 2 hours before training would result in elevated L-arginine levels during this period. The basis for requesting that the participant was to ingest the intervention drink at 2 hours prior to training was deduced through this. These findings result in the acceptance of the null hypothesis, L-arginine plasma concentration will not peak between zero and one hour post consumption.

Although limited, past research would suggest that these findings fall outside that expected through oral administration of 6 g.d⁻¹ L-arginine. Bode-Boger et al. (1998) and Tang et al. (2011) have both experimented with varying dosages of L-arginine but concluded similar statements in regards to oral administration of this strength. Baseline concentrations of L-arginine would be considered average if they were approximately 80 – 120 $\mu\text{mol.L}^{-1}$, well below the average value of 154.075 $\mu\text{mol.L}^{-1}$ found by this study (Tang et al., 2011). In addition, a peak value of 203.617 \pm 8.403 $\mu\text{mol.L}^{-1}$ at 4 hours post consumption is far removed from 310 \pm 152 $\mu\text{mol.L}^{-1}$ at 90 minutes based on the time taken (Bode-Boger et al., 1998).

The differing factor in this study in comparison with other studies to knowledge is the recipe of the supplement administered. When looking at bioavailability, supplementation is usually given individually to help best understand how it behaves physiologically, reducing as many variables as possible. Although unlikely, the addition of Cr to the formula may have had an impact on the metabolic process of L-arginine.

L-arginine, along with glycine, guanidinoacetate and ornithine, are derivatives for creatine synthesis (Wyss & Kaddurah-Daouk, 2000). It would be irresponsible to state that the addition of Cr to this supplement has offset this need for L-arginine to produce creatine endogenously; the many metabolic fates of L-arginine mean it is difficult to exactly determine that nature of this process. If the need for creatine synthesis outweighed the endogenous production of L-arginine then a more readily available 'salt' form may prove invaluable. It is thought that approximately 2 g.d⁻¹ creatine is consumed within the diet and a further 2 g.d⁻¹ is synthesized by the body (Walker, 1979). The difference in an average diet today, compared with the time this research was conducted may account for less being provided by food and more being required through synthesis, thus accounting for lower peak concentration values.

In comparison with contemporary research it would appear that the larger the dose the quicker the response time. Those studies investigating 30 g.d⁻¹ administered intravenously found there to be a 40-minute time to peak with concentrations as large as 822 $\mu\text{mol.L}^{-1}$ being reported

(Bode-Boger et al., 1994; Bode-boger et al., 1998; Kanno et al., 1992). Where as studies utilising dosages between 5 and 10 g.d⁻¹ found it to take significantly longer to reach peak values, Tang et al. (2011) recorded peak values at 60 minutes and this remained elevated for around 180 minutes. Both of these studies exhibited earlier peaks than those observed in this study but the larger dosages used may have had an impact on timing.

5.1.2 Blood sampling efficacy

As previously stated the results found in the bioavailability testing are vastly dissimilar to those seen in past research, this has led to the acceptance of the null hypothesis, capillary blood sampling will not provide indubitable results relative to other collection methods.

Capillary sampling was chosen as the most viable method for blood collection for a couple of reasons; one being the amount of blood required by the ELISA kit for analysis, the other a matter of comfort for the participants. The kit used only required the supply of 50 µl of EDTA treated plasma per test well, working on the assumption that around 50 % of whole blood could be drawn off as plasma as little as 100 µl could have been collected. For the purpose of this 600 µl was collected to allow for multiple tests if needed and accounting for any waste that may occur during the process. This is well below the 4 – 7 ml that would have been collected via venous sampling resulting in less unwanted sample. A secondary reason for selection was the comfort of the participant, sampling four times over a 4-hour period was deemed to cause unnecessary, excessive discomfort.

Studies into the correlation of capillary sampling and venous sampling all show to have varied conclusive evidence that appears to be dependent upon the variable being measured. A study by Kupke, Kather and Zeugner (1981) emerges as one that has determined this correlation on the largest scale of plasma samples; even so, out of all of the samples that they tested only Glutanyl Transferase exhibited no difference between capillary and venous sampling, all others showed favour towards one method or the other. To knowledge no study exists that demonstrates the correlation when studying L-arginine or the correlation of any substance over time. However, research would suggest that capillary sampling exhibits a greater value than venous sampling in the majority of cases, differences in some being as high as 32.8 % (Kupke, Kather & Zeugner, 1981). Although, more recently a study on human growth hormone (hGH) correlation has found there to be no significant difference between capillary sampling and venous sampling ($r = 0.986$) but concluded that once a method was chosen it should be stuck with as they do not appear to be interchangeable (Godfrey, Whyte, McCarthy, Nevill & Head, as cited in Campbell et al., 2006). This study shows closest relevance as it is believed that L-arginine increases hGH and therefore may have been a contributing factor to this data.

5.1.3 Limitations of this study

There were multiple limitations and delimitations associated with this experimental study, the following section will highlight these and look to offer where possible a solution that may alleviate these in future. One major issue with the study was the use of ELISA testing for L-arginine analysis in place of more commonly used high performance liquid chromatography (HPLC) and mass spectrometry (MS). Studies by Bode-Boger et al. (1998) and Tang et al. (2011) both utilised the HPLC method of plasma analysis, while Schwedhelm et al. (2007) used HPLC in combination with

MS to determine dimethylated arginine derivatives. These experiments all concluded similar findings in regards to peak measurements and timing, the body of research suggesting that when taking 5 – 10 g.d⁻¹ L-arginine a peak of around 300 µmol.L⁻¹ was noted between 60 and 120 minutes. The similarities between these studies; compared with the differences in this mean it would be unjust to fully compare these studies with the results obtained from this. HPLC would have been the preferred method of analysis had the costs involved not have escalated above the capabilities of this study.

A further limitation was ensuring the validity and reliability of the ELISA kit. ELISA testing requires extremely accurate pipetting skills and basic biochemistry knowledge. The start of this study was only the first time that the researcher had pipetted amounts as small as 50 µl, pipetting with such a fine accuracy requires practice of which the constraints of deadlines could not afford. Incorrect amounts or inadequate pipetting skills could have resulted in cross contamination of materials from well to well, thus skewing the data and providing inadequate results. If cost and time had permitted then a further ELISA kit would have been bought in order to compare across wells in different kits, providing analysis of reliability, validity and accuracy.

Furthermore, the lack of a standardised pre-test meal before the fast period may have influenced the data. Whilst the instruction was given to eliminate protein from the diet in the period leading up to the experiment and an in depth 3-day food diary was collected beforehand; it is still possible that each participants diet may have had an effect on the results that were obtained. The inclusion of a structured diet plan in the 24-hour period leading to the testing date; eliminating sources known to be high in L-arginine such as seafood, meat and leafy green veg in particular, may aid in eliminating this as an extraneous factor.

Finally, a lack of data to suggest there has been a certain peak has limited the usefulness of the data. Any conclusions made around peak concentration times can only be assumed due to the lack of collection points. Studies showing a peak between 60 – 120 minutes have all used venous sampling as the blood collection method. There is nothing at current that suggests that capillary sampling may exhibit lower peaks or longer periods of absorbance. Although this study would suggest that this is perhaps the case; a further collection of data past the 4-hour mark may have provided clearer conclusions.

5.1.4 Future research

Upon further inspection of this investigation, the flaws highlighted have led to potential future research ideas. In addition to determining changes that may succor the production of clearer L-arginine bioavailability studies, recommendations are also noted for comparison of sampling methods.

One such idea for future research studies would be to assess the different reaction to altered oral dosages over time. Many of the current studies have either used 6 g.d⁻¹ (Bode-Boger et al., 1998) or 10 g.d⁻¹ (Tang et al., 2011) via oral ingestion. To further understand the correlation between dosage and response it would be useful to assess a much larger range of samples. Along with this, as this data has shown, a curve may continue well past the 4 hour mark in some of the dosages. Therefore, it would be wise to collect data over a much greater period to further the understanding and response to oral administration.

Determining the correlation between capillary sampling and venous sampling would also go a long way to furthering the knowledge base surrounding L-arginine consumption. As previously stated, although data on the subject is hardly sparse, very little has been done on individual amino acids. This study demonstrated no obvious peak over a 4-hour period, however, it can be seen that the concentration was still rising at the end point giving argument to a slower response shown in capillary sampling. Should data show that there is no significant difference between each of these sampling types then this argument would be invalid. It would also provide evidence that either collection method proves viable when analysing plasma L-arginine.

Finally, as an addition to the previous point it may also be beneficial to determine the correlation between HPLC-MS and ELISA protocols. There is already data to suggest that the two share a correlation of $r = 0.982$, nonetheless correlation data on capillary sampling specifically may broaden scientific knowledge. As was the case here, it is not always possible or ethically viable to collect blood samples through venous sampling; in these cases data like previously mentioned may prove invaluable and help to further the understanding of L-arginine bioavailability.

5.1.5 Summary of findings

The experiment intended to determine the bioavailability of L-arginine when taken orally as 6 g.d^{-1} L-arginine alpha ketoglutarate combined 10 g.d^{-1} creatine monohydrate, both dosages seen commonly in literature. The testing period lasted a total of 4-hours from initial consumption to the final blood collection point, with collection periods at 0, 1, 2 and 4 hours. Data was incomparable with results seen in past studies as baseline values fell well above those seen previously, time to peak concentrations was elongated and failed to reach levels expected. There was a significant difference in L-arginine concentration at hour 2 and 4 only compared with baseline ($P = 0.012$ and $P = 0.037$ respectively). The main conclusive statement from this is that consumption of L-arginine, in this form particularly, results in elevated levels from 2 hours post consumption; remaining elevated up to at least 4 hours post, possibly further.

5.2 Discussion of Main Experimental findings

This study aimed to determine whether there were any beneficial effects of taking L-arginine alpha ketoglutarate and Creatine monohydrate combined on fat free mass, peripheral blood flow, strength, power and power to weight ratio in comparison to AAKG and placebo, placebo only and control groups. The following section has been split to show the effect of each variable individually, determining whether to accept or reject the null hypothesis, physiological mechanisms as to why it may have happened and offering comparisons with other studies that are found in the current literature.

This research has been conducted to determine whether there is any substantial evidence to suggest that AAKG and Cr exhibits the benefits that are purported due to physiological mechanisms when administered as a 'pre-workout' training drink; and whether these physiological mechanisms translate to a performance improvement. The rationale behind this leads back to the original aim of the research project that was to determine whether there was any evidence to suggest the claims by marketing companies selling 'pre-workout' formulas were valid. Most companies use a form of arginine in their formulas due to the proposed benefits that have previously been described in the

review of literature section, however, it is becoming much more prevalent in today's market to see this combined with other supplements in varying concoctions. One such addition is Cr, despite the limited research to state that they are synergistic when combined together it is becoming increasingly common for manufacturers to combine the two, both as a standalone product or combined with other supplements that are purported to be performance enhancing.

5.2.1 Effect of intervention on weight and fat free mass

Comparison of weekly changes, and overall variance found no significant differences in weight ($P = 0.618$) and despite a strong trend, no significant difference in FFM ($P = 0.061$). Over the 8-week period, weight increased by 1.0 ± 0.7 kg in the AAKG + Cr group, 0.4 ± 0.5 kg for AAKG + PL and 0.2 ± 0.1 kg in CONT; a decrease in weight was seen for the PL group of -0.1 ± 0.5 kg. Fat free mass exhibited changes of 1.3 ± 0.8 kg for the AAKG + Cr, 0.3 ± 0.6 kg for the AAKG + PL, -0.4 ± 0.5 kg for the PL group and -0.5 ± 0.4 kg for the CONT group. There were no significant differences between any of the groups at any stage other than baseline possibly due to the large standard error of the mean, baseline differences were seen as the pool of subjects was recruited from a large variety of different people. These results led to the acceptance of the null hypothesis, a formula containing AAKG and Cr will not increase total body mass and not decrease fat free mass greater than all other groups when compared.

Much of the data associated with body composition change is in favour of Cr supplementation; there is little research to suggest that AAKG is the main stimulating factor in body composition improvements. However, the research that does exist in to the theoretical implications of AAKG on body composition improvement has been focussed around its stimulation of hGH and how that may be effective. It has been found that AAKG and exercise can increase hGH production almost 3 fold compared with exercise alone (Tang et al., 2011). Fujiwara, Morgan and Bichet (as cited in Campbell et al., 2006) determined that hGH will effect the way in which fat and carbohydrates are used as an energy substrate in exercise; It was thought that the promotion of free fatty acids (FFA) as an energy source occurred as hGH inhibited glycolysis and increase the mobilisation of FFA from triacylglycerol stores (Godfrey, Whyte, McCarthy, Nevill & Head, as cited in Campbell et al., 2006). If this were to be the case then it would be expected that FFM would increase in the absence of weight gain, however this has not happened as previously stated.

Creatine on the other hand, a more supported method for altering body composition, is more likely to bring about change as a result of its use and storage as opposed to hormones that it may stimulate. Around 90 – 95 % of bodily creatine stores are found in skeletal muscle, this allows the fastest production of energy during exercise without delay in mobilisation from other storage sites. The main explanation given by research on Cr is the fact it may give a greater aid when performing energetic challenges, in turn reducing cell death or damage and improving cellular function (Persky, Brazeau & Hochhaus, 2003). This means that individuals should theoretically be able to train harder and for longer. Fatty acids would be a preferred source of energy in most cases, as they will provide the greatest amounts of ATP. However they are not mobilised during most strength work, the preferred source of energy being PCr in this case (Persky, Brazeau & Hochhaus, 2003). This generally results in a net fat mass increase due to less fat being used for energy as more PCr is readily available, therefore, it would be expected to note a weight increase and a FFM decrease if Cr

was taken alone in this study. As it was, the additive effects of AAKG and Cr together appear to have increased weight entirely as lean mass.

It is most likely that this is a combined result of a decrease in protein catabolism, associated with both supplements, thus a decrease in muscle wastage. It also may be associated with an increase in waste product removal that AAKG expedites; one of the predecessors in L-arginine metabolism is urea, the way in which Cr waste products, creatinine, are excreted (Persky & Brazeau, 2001). A quicker excretion rate may result in an improved reduction in cell damage and death, and further improve cellular functions, the benefits of which increase the use of FFA to maintain these functions.

Many studies support the fact that AAKG is not known to have any particular effect on body composition, specifically for the goal of increasing total body mass (TBM). Campbell et al. (2006) and Liu et al. (2009) reported there to be no significant increase in TBM whilst taking AAKG supplementation alone, this complies with the results obtained in this study. The group taking an AAKG and placebo sample in this study showed an increase of 0.4 ± 0.5 kg, due to the high standard deviation no significance was found; similar results have been found by all three studies, 0.6 ± 0.3 kg, 0.3 ± 0.1 kg and 0.3 ± 0.5 kg respectively. A potential benefit however of all these results is the reduction in body fat; although TBM has not risen it was observed by these studies that body fat (BF) percentage showed a decrease across trials although no significance was found at any point. This would coincide with those results detailed here as FFM mass increased by 0.3 ± 0.6 kg in a TBM of only 0.4 ± 0.5 kg, thus resulting in a lower BF percentage.

On the other hand, Cr shows less favourable results when trying to increase weight through lean muscle mass, potentially due to the reasons previously spoke about. It is commonly recorded that approximately 50 % of TBM increase is associated with an increase in FFM whilst the other 50 % is associated with fat mass (FM) gains (Arciero et al., 2001; Little et al., 2008). Arciero et al. (2001) reported an increase in TBM of 1.7 ± 1.0 kg of which 0.9 ± 0.7 kg of this was FFM; Little et al. (2008) reported similar results in a 10 day trial, FFM improved by 0.5 ± 0.6 kg whilst FM increased 0.9 ± 0.0 kg. The group co ingesting AAKG and Cr did exhibit a TBM increase of 1.0 ± 0.7 kg giving rise to a comparison with past results, however it can be attributed that the vast majority of this increase was FFM as this increased by 1.3 ± 0.8 kg. The increase in percentage gain associated with FFM as opposed to FM increase during ingestion resulted in lower BF percentage across both groups taking AAKG in some form. Co ingestion with AAKG may benefit the process of weight gain for those hoping to increase without the associated addition of fat.

Although limited, reports of a decreased BF with the addition of AAKG have been supported. Little et al. (2008) not only studied Cr benefits but also the added benefits of taking a combined supplement with AAKG. Over a period as short as 10 days those in the Cr group increased TBM by 1.2 ± 0.6 kg of which BF percentage increased from 15.8 ± 5.8 % to 16.8 ± 5.4 %; in contrast, those in the AAKG + Cr group only increase TBM by 0.4 ± 0.6 kg but BF percentage actually dropped from 14.9 ± 5.7 % to 14.1 ± 6.2 % and FFM increased 1.1 ± 0.2 kg. This supports the findings in this study as it was seen that more than 100% of the TBM increase was attributed to FFM, thus resulting in a lower BF percentage.

Therefore, it would appear that co ingestion of AAKG and Cr would benefit an individual hoping to increase TBM without any gains in BF percentage. However, a result of Cr alone is an

exaggerated increase in TBM, therefore if the goal was to simply increase weight, this study along with other past research would suggest that taking AAKG in combination may actually result in lower total gains thus hampering that particular outcome.

5.2.2 Effect of intervention on peripheral blood flow

Results indicate that there would be no beneficial effects on peripheral blood flow when supplementing with AAKG and Cr compared with all other groups. Throughout the intervention period it was apparent that post test, blood flow measurements exhibited the greatest difference between groups, however no significance was noted at any point. Prior to commencement of the exercise tests blood flow was measured and recorded as blood flow PRE, across the 8-week period there was a change of $0.35 \pm 0.29 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the group taking AAKG + Cr, $-0.07 \pm 0.21 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the AAKG + PL group, $0.08 \pm 0.12 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the PL group and $0.27 \pm 0.17 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the CONT group. Immediately after the isometric strength exercises the INTER measurement was observed, this showed changes of $0.10 \pm 0.29 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for AAKG + Cr, $-0.03 \pm 0.25 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for the AAKG + PL, $0.04 \pm 0.10 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for the PL and 0.30 ± 0.22 for the CONT group. Finally, a further measurement labeled POST was collected at the end; results show changes of $0.19 \pm 0.32 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for AAKG + Cr, $-0.11 \pm 0.22 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for AAKG + PL, $0.12 \pm 0.09 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ for PL and CONT changed by $0.32 \pm 0.14 \text{ ml.100ml}^{-1}.\text{min}^{-1}$. Consequently, the lack of significance has led to the acceptance of the null hypothesis; a formula containing AAKG and Cr will not improve peripheral blood flow greater than all other groups when compared.

Unlike the association between Cr and body composition there is no evidence to suggest the Cr alone may increase blood flow capacities. In contrast to this there is vast research that suggests that physiologically L-arginine, in the form of AAKG among others, may increase blood flow. Research to prove this may have the desired impact when tested in vivo is lacking but still suggests that intended changes are observed when dosages are high enough. The mechanism behind these associations is production of NO; NOSs, in particular eNOS and nNOS produce nitric oxide within the skeletal muscle fibres signaling the vasodilatory mechanisms (Lau et al., 2000; McConell & Kingwell, 2006; McConell, 2007; Nisoli et al., 2003). Without direct measurement of this gas it is impossible to determine whether this has had the effect proposed in this study.

L-arginine is known to have many metabolic fates, NO production is just one of many fates of L-arginine but still the only one that is thought to have a direct effect on blood flow. The exact fate would depend upon the underlying state of the individual, poor nutrition or underlying health issues may have a massive impact on which L-arginine fate is favoured. It is currently known that L-arginine is used in production of urea, nitric oxide, ornithine agmatine and protein synthesis; however, perhaps most important in relation to this study is its role in Creatine synthesis. Persky & Brazeau (2001) and Wyss & Kaddurah-Daouk (2000) are the most recent studies to knowledge that have discovered that the primary metabolic fate of L-arginine is Creatine synthesis via enzymatic action of AGAT; the latter determining that approximately 70 % of available methylated groups are consumed in its production.

The potential benefits of an addition of Cr to a formula containing AAKG were two fold; the first benefit being a decrease in demand of L-arginine for creatine synthesis, which in turn will reduce

homocysteine levels (Taes et al., 2003). By decreasing demand of L-arginine in this pathway it would theoretically increase the amount of free arginine destined for a different fate; however, it is difficult to determine what exact pathway would be favoured over creatine synthesis should more be available. As stated previously, it would more likely depend upon other factors within the individual that purely the need for creatine synthesis. This study would suggest that more available arginine might not result in greater NO production as there has been no significant associated blood flow responses. The other benefit of a reduced synthesis is the role of homocysteine in endothelial function; Jahangir et al. (2009) discerned the prevalence of homocysteine in creatine synthesis through the process of donating methyl groups to guanidinoacetate resulting in S-adenosyl methionine (SAM) being metabolised to S-adenosyl homocysteine (SAH) and eventually homocysteine. Decreasing endogenous production of creatine through additional dietary intake would decrease homocysteine production, reducing adverse effects of endothelial function.

When offering comparisons between this study and those in the past it is immediately prevalent that there is a dosage response in regards to AAKG and its effect on blood flow. It would appear that the higher the dose the larger and more significant the subsequent blood flow response is, with all studies to knowledge showing no significant increase in blood flow when supplemented with 10 g.d⁻¹ L-arginine or less, other than exercise induced. The most common form of analysis has been Doppler ultrasound techniques that have been used by Billinger & Kluding (2009), Hotoleanu, Fodor & Suci (2010), Matthiessen, Zeitz, Richard & Klemm (2004) and Thomson et al. (2001) all found that there was no significance in peripheral blood flow when taking between 5 – 10 g.d⁻¹. More closely related to this research are studies by Arciero et al. (2001), Preli, Klein & Herrington (2002) and Tang et al (2011) whereby strain gauge plethysmography was utilised, who in turn found there to be no significant difference with this dose.

From the studies closely related to this it would seem that exercise is the main contributing factor in blood flow changes. Arciero et al. (2001) found that blood flow in the forearm without resistance training was $2.99 \pm 0.38 \text{ ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ and $3.68 \pm 0.38 \text{ ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ with resistance training at baseline, this then signified a change of $1.1 \pm 0.5 \text{ ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ over a 28 day period. All participants of this study were resistance trained for a minimum of one year prior to beginning; and then actively participated in further resistance training throughout the study meaning comparisons can be made with the group in this study. Arciero et al. (2001) may have noted greater change in forearm blood flow over the testing period due to the difference in methodology; their study occluded the upper arm with a 10 cm occlusion cuff and then pumped to 60 mmHg, no wrist occlusion was given. This study not only occluded the upper arm to 80 mmHg, it also occluded the wrist to approximately 180 mmHg. The greater occlusion may have resulted in a tighter restriction on blood flow hence giving lower results than those of this study. Despite this overall blood volume appears to very similar supporting the use of this method.

It is inferred that any blood flow related benefits of AAKG are related to the inclusion of exercise. However, the results from this and other studies would suggest that different types of exercise are affected individually during supplementation. It is known that aerobic exercise will increase blood flow regardless of AAKG supplementation due to the demand of oxygen for the working muscles. However, a study by Preli, Klein & Herrington (2002) found there to be an exaggerated increase in peripheral blood flow when supplemented with AAKG; in contrast to this

Tang et al. (2011) determined that anaerobic exercise, in particular leg press and knee extension, did not induce any greater blood flow response with AAKG. It has not been analysed by this study as to whether there is a potential to further improve blood flow in aerobic exercise, but it can be inferred that anaerobic exercise; particularly in strength trained individuals does not, inline with those results seen in the latter study mentioned. The results obtained show no significant increase either after the isometric strength exercise or the more power based jump and wingate.

Current research has used similar timings to this study with blood flow analysis occurring prior to any exercise and then again after a resistance protocol; the difference between the two are found after testing; where as this study analysed blood flow immediately post exercise, Willoughby et al. (2011) undertook a final sampling period 30 minutes post testing. Results of the first and second analysis periods would appear concurrent with this study; in that by Willoughby et al. (2011) the placebo group changed by $4.16 \pm 2.14 \text{ cm.s}^{-1}$ and $3.03 \pm 2.12 \text{ cm.s}^{-1}$ respectively and the group taking a form of AAKG changed by $-2.30 \pm 2.76 \text{ cm.s}^{-1}$ and $-2.20 \pm 0.00 \text{ cm.s}^{-1}$ respectively. When comparing that with a change of $0.26 \pm 0.11 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ and $0.25 \pm 0.12 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the placebo group and $0.14 \pm 0.18 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ and $0.03 \pm 0.18 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the group consuming AAKG. Although the units of measurement are different resulting in much different data values the same trend can be seen; those on placebo had a greater increase in blood flow than those taking AAKG at the first and second blood sampling time points.

Conversely, the final time point in this study showed there to still be a favourable increase in the placebo group; $0.22 \pm 0.08 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ compared with $0.04 \pm 0.19 \text{ ml.100ml}^{-1}.\text{min}^{-1}$ in the AAKG group. However, Willoughby et al. (2011) determined that AAKG show improvements at a later period, specifically 30 minutes post exercise; their results showed the placebo group to have changed by $3.84 \pm 1.09 \text{ cm.s}^{-1}$ and the AAKG group to show an exaggerated improvement of $6.51 \pm 13.22 \text{ cm.s}^{-1}$, despite this results still show no significance ($P > 0.05$). Coupling this data with our own it would emerge that there may be a delayed effect on L-arginine effecting blood flow potentially due to other external or internal factors.

Analyses of the results lead towards the conclusion that AAKG has no physical benefit on peripheral blood flow at this dosage. Any changes in blood flow would be greater attributed to an effect of the exercise modality chosen as opposed to an accentuated effect due to AAKG and Cr supplementation. Using this data and past it is difficult to argue that supplementation induces the peripheral blood flow improvements that individuals would often consume it for.

5.2.3 Effect of intervention on strength variables

Upper limb strength in the form of an isometric bench press documented an increase of $83.28 \pm 20.68 \text{ kg}$ in the AAKG + Cr group, $48.33 \pm 15.76 \text{ kg}$ for AAKG + PL, $26.63 \pm 15.24 \text{ kg}$ for PL only and $15.51 \pm 6.35 \text{ kg}$ for CONT over the 8-week trial. Statistical analysis shows a significant difference between the AAKG + Cr and CONT groups only at week 8 ($P = 0.026$) and therefore the null hypothesis, a formula containing AAKG and Cr will not increase isometric strength variables greater than all other groups when compared, was accepted as there were only significant differences between two groups. Lower limb strength noted comparable changes in the isometric half squat, an increase of $117.50 \pm 59.72 \text{ kg}$ for AAKG + Cr, $95.33 \pm 27.27 \text{ kg}$ for AAKG + PL, $33.39 \pm 47.88 \text{ kg}$ for PL and $-0.34 \pm 9.48 \text{ kg}$ for CONT. Statistical analysis this time indicated that there was

no significant difference ($P < 0.05$) between any of the groups, leading to acceptance of the null hypothesis in this case.

At the outset of this study it was expected that there would be an improvement in strength gains even should nothing else be successful in the process. This was due to two reasons, the current supporting literature on the benefits of Cr supplementation and the documented physiological mechanisms of both creatine and arginine. It is well recognized that different sports require different energy system input dependent on its nature; a marathon relies predominantly on the aerobic system whereas a 100 m sprint relies on the adenosine triphosphate – phosphocreatine (ATP-PC) system and lactic acid system. The sports predominance will be based upon the length of time the energy requirement lasts, events under 15 seconds will favour the ATP-PC system (Mackenzie, as cited in Willoughby et al., 2011). As stated around 90 – 95 % of creatine is stored in the muscle as PCr (Walker, 1979). Therefore the predominant energy system in strength training is the ATP-PC system as each repetition lasted around 3 seconds. Taking this into account an increase in muscle PCr levels means there would be more available for energy production resulting in a greater work capacity leading to greater strength improvements.

Although no significant differences were seen between AAKG with Cr and AAKG without, the results lean favourably towards taking the two together; AAKG with Cr presented an increase of 83.28 ± 20.68 kg compared to AAKG without only increasing by 48.33 ± 15.76 kg across the 8 week period in upper body strength, similar results were seen in lower body strength. It is most likely that the addition of Cr has meant there is more available L-arginine; either for further creatine synthesis, resulting in higher muscular PCr, or waste removal via urea production. These two pathways are the most likely reason for an improvement in combination as opposed to alone (Paddon-Jones, Borsheim & Wolfe, 2004). It would be reasonable to believe that taking AAKG with Cr may not have alleviated the dominance of creatine synthesis from arginine as there was no significant difference between the two groups taking AAKG; it may have in fact just resulted in greater waste with more being excreted via urea.

Prior to this study it was expected that a loading period of 14 days and then a maintenance phase for the remainder of the study could have resulted in strength improvements as quickly as 7 days (Kreider et al., 1998). However, as can be seen there was no significant difference between those taking the supplement containing Cr and all other groups until week 8, and even then there was only a difference between this and control. Work by Greenhaff (1997); found that a limiting factor for energy production in the ATP-PC system is PCr, synthesis of which will occur through aerobic production of ATP. Although it was not monitored the delayed reaction to supplementation may be a result of insufficient recovery thus inadequate PCr production.

A purported benefit of both creatine and arginine supplementation is an increased protein synthesis and a decreased protein degradation (Greenhaff, Bodin, Soderlund & Hultman, 1994). Taken separately, each compound has been found to favour one role more than the other, Cr is usually seen as a promoter of protein synthesis whilst inhibition of protein degradation is usually associated more with L-arginine. A significant increase in strength gains with AAKG compared to without, yet no significant increase with the addition of Cr would suggest that a decreased protein degradation has played a greater role in total net protein than protein synthesis has. An increase in net protein turnover will result in an increase in muscle fibre size (Vandenberghe et al., as cited in

Little et al., 2008). Put simply, the larger the muscle fibre, the more sites there are for the actin and myosin to bind; two of the most important proteins strands found within the sarcomere, actin and myosin produce muscular force, the more binding sites there are the greater the force production.

As previously mentioned studies showing ergogenic benefits of Cr supplementation are vast, the majority of them have shown significant differences in strength over the trial period in either upper limb, lower limb or total body (Izquierdo et al., 2001; Jahangir et al., 2009; Pearson, Hamby, Russel & Harris, 1999; Persky, Brazeau & Hochhaus, 2003). In contrast, there is a much larger disparity in evidence to suggest AAKG supplementation has the desired effect on strength variables (Campbell, Bounty & Roberts, 2004; Campbell et al., 2006). If significance has been found in studies on AAKG then it has usually only been recognized as having an effect on the upper limbs.

Izquierdo et al. (2001) and Pearson, Hamby, Russel & Harris (1999) have both used a bench press and a squat variety as their chosen variables, the former choosing to use the half squat; despite selecting similar exercise choices the results obtained have offered different views. Similar to this this study Izquierdo et al. (2001) used the bench press and half squat exercise; determining that there were only increase in lower limb action, in contrast to the methodology used in this study however have only used a concentric action. Pearson, Hamby, Russel & Harris (1999) on the other hand found that there was significant differences in both upper and lower limb strength for the same movement patterns; the difference between them being that the latter used an eccentric and concentric action as opposed to excluding one or the other. Using these studies alone it would appear that eccentric muscle action promotes a greater force hence the difference in significance. Applying this to the current study it can be assumed that isometric strength recruits a similar number of muscle fibres as both the concentric and eccentric actions as significance was seen in both upper and lower limb.

No study to knowledge has currently investigated at isometric strength, opting for the more common concentric and eccentric actions. This makes it difficult to compare raw data and instead making it easier to opt for determining trends in the research. Campbell et al. (2006), in similar vain to Izquierdo et al. (2001), found there to be only significant differences in one of the two variables; although they found this to be for upper limb strength only ($P = 0.03$). Data from this study shows that there is a significant difference in both upper and lower limb when taking a form of AAKG offering an argument for both the previous studies mentioned. However, when looking at the administration protocol for each study it is seen that Campbell et al. (2006) used $4 \text{ g}\cdot\text{d}^{-1}$ AAKG, whereas Izquierdo et al. (2001) used $5 \text{ g}\cdot\text{d}^{-1}$ Cr; implying that the results seen by this study were as a result of the two combined together as opposed to one or the other.

The selection of previously trained individuals may have had an impact on the results that were seen, it would be expected that a participant that already has a strength training base would perform better due to better adaptation of the nervous system and muscle function (Sale, 2008). As can be seen there is a significant difference between those taking AAKG against those without for both bench press and half squat, if that is compared with data from past research using untrained individuals then there may have been no significance. Bednarz et al. (2004) and Paddon-Jones, Borsheim & Wolfe (2004) both found no significant differences when testing untrained subjects in 1RM of the bench press and leg press ($P > 0.05$). In addition to this it was also found that 1RM deadlift when supplemented with AAKG was not significant, AAKG: $4.57 \pm 0.37 \text{ kg}$ and PLAC: $3.72 \pm$

0.89 kg, $P = 0.546$ (Siani et al., 2000), AAKG: 6.79 ± 1.24 kg and PLAC: 6.02 ± 1.46 kg, $P = 0.734$ (Nagaya et al., 2001). Data such as this suggests that the significant differences noted in this trial may not have been as a result of combined supplementation, but in fact reflect the training status of the participants coupled with the ingestion of supplementation.

The nature of this topic results in a vast variety of results that tend to depend on individual factors that are difficult to control. It would appear that over an extended period of time combined AAKG and Cr may have a heightened effect on upper and lower limb strength but that is not to say that one or the other taken alone may have the same desired effect. If the goal is to increase upper or lower limb strength individually then there is evidence to suggest that one or the other may work. However, should a total body effect be wished it would appear that taking AAKG along with Cr could be beneficial.

5.2.4 Effect of intervention on power variables

Results indicate that there is no significant benefit of supplementation on the power indices chosen in this study. Both variables revealed no significant difference ($P > 0.05$), countermovement jump in those taking AAKG + Cr increased by 1.43 ± 0.72 cm and AAKG + PL by 1.47 ± 0.97 cm, there was a decrease however in the PL group by -0.53 ± 2.23 cm and in the CONT group -2.41 ± 1.84 cm; peak wingate on the other hand exhibited a change of 23.92 ± 9.40 W for AAKG + Cr, 10.25 ± 13.99 W for AAKG + PL, -0.58 ± 8.53 W for PL and 5.25 ± 2.38 W for CONT. This resulted in the acceptance of the null hypothesis; a formula containing AAKG and Cr will not increase power variables greater than all other groups when compared.

Determination of power is done by multiplying the force produced with distance divided by time, therefore should one variable change and the others remain the same it can be presumed that power will be. Surprisingly there have been no significant changes in power output despite a significant increase in lower limb strength. Therefore power attribution in this case has most likely been altered by a factor outside of the research limitations. Other factors that may have affected power output could be the nervous system firing rate, technique and fibre type predominance (Mackenzie, 1998). However, given the time period of the study it would have been expected that technique was no longer an issue as there had been adequate time for most to learn (Mackenzie, as cited in Campbell et al., 2006).

The factor that is more difficult to regulate is fibre type predominance, this is determined within the genetics of the individual and is perhaps the most difficult aspect to train for power. It has hitherto been mentioned that the preferred source of energy for ATP production in periods of exertion 15 seconds or less is PCr; it is also shown in research that the highest amounts of PCr are stored within type 2 muscle fibres (Casey & Greenhaff, 2000; Meyer, Brown & Kushmerick, 1985). Knowing this, if the participants were predisposed genetically to have more type 2 fibres then they could potentially have greater stores of PCr thus producing greater power output. Lieber, Mackenzie and then later Andersen, Schjerling & Saltin (as cited in Little et al., 2008) discovered that training of the muscular tissue did not change the fibre type but instead allows them to take on the characteristics of the desired fibres. Studies on endurance athletes found that muscle fibre diameter and number of capillaries increased in type 2b fibres showing characteristics of the slower twitch type 2a fibres, conversely type 2a fibres were shown to take on the characteristics of type 2b fibres with weight

training (McArdle, Katch & Katch, 1996). Without invasive measurement techniques it is difficult to tell whether the participants of this study were more suited for one event over another, it can be said though that a lack of significant changes in power disregard of the strength increases, may be a direct result of genetic predisposition.

Most studies to date have found an increase in power when undertaking a strength training protocol along with AAKG or Cr supplementation. The countermovement jump selected was most comparable to the movement that would be seen in a sporting environment. Hence providing a cross over from lab based testing to field based testing providing evidence to suit both the general gym user and a sporting athlete. Pearson, Hamby, Russel & Harris (1999) found there to be a 7.58 ± 1.08 kg increase in the power clean when taking Cr supplementation rivalled by a decrease 2.16 ± 9.32 kg in the placebo group. A power clean and a countermovement jump are closely matched in terms of technique; the difference is the weight being carried at time of maximum force production. The aforementioned study may have seen significant differences as the participants were being trained particularly for power, whereas the main focus of the training plan in this study was strength. Training specifically for power may have resulted in faster motor units and a better adapted nervous system allowing the participant to produce maximal force in the quickest time possible.

Preceding study has determined a direct correlation between strength and power in the lower limbs (Greenhaff et al., 1996; Pearson et al., 1999). Consequently a strength-training programme should elicit the desired power improvements that have not been found in this study. Further study by Campbell et al. (2006) confirms this, as participants of their study were required to complete a training protocol 4 days per week, this was split into two upper sessions and two lower sessions. Similar to the weight chosen for this study each participant was required to lift at 85 % of their 1RM, this is the weight known to be the minimum weight needed in order to predominate strength gains over hypertrophic gains. They then found there to be a significant difference in anaerobic wingate performance at week 8 compared with week 0, peak power increased by 80 ± 6 W for those taking AAKG and decreased by 79 ± 16 W for those on placebo ($P = 0.005$). These differences are concurrent with those found in this study albeit they were much lower this time, the reason for this is most likely to be an inadequate weight during training resulting in inhibited performance benefits.

Findings of this research would indicate that there is no performance benefit when taking AAKG with or without Cr on power variables. However, the factors that affect power output are vast therefore conclusively stating there is no benefit would be unwise as given the correct scenario there have been improvements in power output. The use of a more closely matched cohort would prospectively give a much greater answer to this question.

5.2.5 Effect of intervention on power to weight ratio

Significant difference can again be seen at week 8 of the bench press power to weight variable between AAKG + Cr and CONT ($P = 0.016$), results showed changes of 1.20 ± 0.31 N.kg⁻¹ for bench press, 1.60 ± 0.86 N.kg⁻¹ for squat and 0.25 ± 0.10 N.kg⁻¹ for wingate in the AAKG + Cr group. For the AAKG + PL group there were changes of 0.55 ± 0.18 N.kg⁻¹ for bench press, 1.07 ± 0.30 N.kg⁻¹ for squat and 0.09 ± 0.14 N.kg⁻¹ for wingate. The changes observed in the PL group were 0.39 ± 0.23 N.kg⁻¹ for bench press, 0.58 ± 0.73 N.kg⁻¹ for squat and -0.01 ± 0.08 N.kg⁻¹ for wingate.

Finally, the CONT group showed changes of $0.18 \pm 0.08 \text{ N.kg}^{-1}$ for bench press, $-0.02 \pm 0.12 \text{ N.kg}^{-1}$ for squat and the wingate changed by $0.05 \pm 0.02 \text{ N.kg}^{-1}$. As there has only been a significant increase at one time point during the trial the null hypothesis, a formula containing AAKG and Cr will not improve power to weight ratio greater than all other groups when compared, will be accepted for all variables.

Investigation into weekly changes uncover that there was only one point where AAKG with Cr performed better than any other group, at week 8 there was a significant difference ($P = 0.016$) between this group and the control group, again this is expected as this matches the single time point in which strength improvements were seen. Power to weight ratio provides a level field for analysis, as it will score each individual on merit, reducing the variety seen in base strength. Unless specifically trained to the contrary, an individuals weight usually reflects their strength; a lighter participant tends to have less strength than a heavier individual. Should weight have increased in this study but strength or power not increase to match this then there would be a decrease in P:W.

As strength was seen to increase across the testing period for the majority of groups P:W ratio was also seen to increase, the difference in weight compared to strength changes becomes apparent when discussing these results. Those taking AAKG with Cr exhibited increases in each of the exercises; bench press, half squat and anaerobic wingate, $1.20 \pm 0.31 \text{ N.kg}^{-1}$, $1.60 \pm 0.86 \text{ N.kg}^{-1}$ and $0.25 \pm 0.10 \text{ W.kg}^{-1}$ respectively. Similar increments are noted in the AAKG with PLA group, $0.55 \pm 0.18 \text{ N.kg}^{-1}$, $1.07 \pm 0.30 \text{ N.kg}^{-1}$ and $0.09 \pm 0.14 \text{ W.kg}^{-1}$ respectively. The only decreases were seen in the control group for squat performance ($-0.02 \pm 0.12 \text{ N.kg}^{-1}$) and the placebo group during the anaerobic wingate performance ($-0.01 \pm 0.08 \text{ W.kg}^{-1}$). The culmination of little TBM changes, along with insignificant strength changes for most groups have resulted with similar differences lacking in the power to weight variables.

5.2.6 Cardio-Vascular Response to Resistance Exercise

An aspect of resistance exercise that is usually overlooked due to its relatively small role is the effect on the cardio-vascular system. The effects that will be discussed shortly may account for the changes in certain variables more than the supplementation itself does; in particular the effect on HR, BP and blood flow, although this does not rule out effects that it may have had on other variables as a result.

When resistance exercise is completed correctly the pushing phase is usually done whilst holding their breath, known as a valsalva manoeuvre. Coupled with an increase in intramuscular pressure this can momentarily block the blood getting to the working muscle. This has the effect of increasing blood pressure and increasing heart rate, additionally stroke volume may also decrease (Pollock et al., 2000). The resting measurements will unlikely to have been affected by this response however the data collected in the middle and at the end would have been influenced, thus conclusions that have been drawn in regards to the supplements effect may be incorrect. It can be expected that blood pressure would rise sharply in order to pump the blood past the barrier it faces, as the blood vessels are restricted. In support of this Fleck and Kramer (1997, p. 195) found that the lockout phase of a lift demonstrated the highest cardiac output and highest stroke volume compared with the concentric and eccentric phases of the lift. As the isometric exercises of the bench press and

half squat were intended to create this 'lockout' effect it can be assumed that it will have put the greatest demand on the cardio vascular system.

5.2.7 Limitations of this study

Upon reflection there have limitations and delimitation of the study that will have affected the validity of the data collected, should similar study be completed again the following would be addressed in order to improve validity and reliability of the data. The first delimitation is the age of the equipment that was used for blood flow analysis. Although every effort is made in the upkeep of equipment over time items became worn and damaged. The main issue being damage to the mercury bands used to determine forearm volume change, the constant stretching and shortening of the bands may have affected their accuracy. If the accuracy was affected then it can be assumed that this may have impacted the results collected for peripheral blood flow. If cost was not an issue then regular replacement of the mercury bands may increase accuracy provided calibration was followed stringently.

A further limitation was the set up of the laboratory; due to clashes with other research studies it was necessary to use every available space. This unfortunately resulted in the equipment being spread out. The time taken to move between each area had no been accounted for in initial preparation therefore this may have had an effect on the results obtained. Only a handful of studies have researched blood flow using the strain gauge plethysmographer meaning there is little evidence to support the best time to analyse this, due to the set up there was a gap of approximately 2 minutes while participants were moved to the strain gauge and attached. This may have given blood flow time to return to baseline levels thus affecting the results of this study. Ideally all equipment would be situated in the same place to reduce this time as much as possible.

The penultimate limitation that has been identified from this study is the inconsistent set up for the bench press and half squat. Other researchers required the use of the power rack meaning the MIE myometer had to be removed at the end of each testing day. There was no measure of the pressure that this was tightened to each time it was reattached which may have had a role in strength output. The tighter the straps around the bar the more difficult it is to create movement and produce force, conversely the lighter the pressure around the bar there is possibility for greater force production. In future it would be ideal to have the straps attached and not have to move or tighten them, this would then increase reliability and perhaps show a difference in the strength and P:W variables.

The final delimitation was the room temperature, it is known that temperature has an effect on vascular vasodilation due to the need to release heat through the skin. This investigation was undertaken over the spring and summer months (April – July). Over this period the room temperature fell within a range of 20.6 – 23.4 °C. The warmer the room the higher the expected vasodilatory response would be, this would then make it impossible to determine whether it was supplementation or temperature that was mostly affecting blood flow. Should this research be conducted again and there were no restraints on laboratory sighting it would be better to conduct it within a temperature controlled room to help reduce the effect of this variable.

5.2.8 Future research

During this study potential future research ideas were brought to the researchers attention, the following section will highlight these areas. The first, and perhaps most pertinent area for future research, is determination of L-arginine fates during oral administration. There are many metabolic fates documented in current research, however during ingestion for performance benefits there is still much debate as to the exact mechanism of action that may aid performance. Research appears to state that there are signs of a performance benefit, however the exact reasons to why this helps is equivocal. An extension of this could also be to determine the fates of L-arginine when supplemented with other compounds similar to creatine. Creatine synthesis is just one of the fates of L-arginine within the body, it may be useful to investigate whether the addition of glutamine or agmatine further increases available L-arginine. This may then promote a greater production of NO and potentially increase blood flow as physiological mechanisms state.

Furthermore, 24-hour monitoring of blood flow may help further understand this mechanism surrounding L-arginine. This study found there to be a dampening effect on blood flow with L-arginine similar to those results by Willoughby et al. (2011); the differentiation between the two though show there to be an increase in blood flow 30 minutes after exercise. This could suggest that there is a delayed response in blood flow or that L-arginine increases blood flow outside of exercise to aid recovery rather than for performance benefits during exercise. Twenty-four hour monitoring will give a larger picture of fluctuations in blood flow perhaps finding a heightened response far removed from exercise.

Finally, it was chosen for this research that the dosage would be administered at one time 2 hours prior to exercise. Offering a split dosage may further increase performance benefits. It would be useful to compare a single dose of both combined with each one individually at different times of the day. It may further knowledge as well if this was to be compared to with a split dosage of both AAKG and Cr combined. Smaller amounts may aid diffusion through the intestine wall reducing waste and potentially increase muscle use.

6.0

Conclusion

6.1 Pre-Experimental Conclusion

To date there have only been a handful of studies into the effect of creatine on L-arginine bioavailability, therefore the findings of the pre-experimental work were of particular interest. It was unexpected that there would be such a delay in time to peak plasma concentration, with no definite peak being seen within the four hour period. It can be seen that a significant peak compared to baseline began at 2 hours and continued on to 4 hours but may have potentially continued further. The main limitation of this stage of the research was the lack of sufficient data points giving no definitive peak plasma concentration. More stringent rules around dietary intake in the 48 hour period leading up to the testing date may improve these results in future studies.

6.2 Main experimental Conclusion

Previous research papers on the actual performance benefits of taking AAKG with Cr are limited, despite the large body of research that has previously reported potential benefits base on physiological mechanisms. Despite no significant results for any of the body composition, blood flow, or power variables there is still strong trends to suggest that there may be a small performance benefit if taken together. An example of this is seen in TBM and FFM changes, significantly there is no difference, however the group taking AAKG with Cr increased TBM an average of 0.6 kg more than all other groups and FFM an average of 1.0 kg more than all other groups. Strength was the only factor that did see a significant difference when taking AAKG; bench press exhibited an increase of on average 40 kg more than all other groups in addition to half squat increasing by an average of 20 kg more than all other groups. The major limitation of this stage of the research was control of laboratory conditions, the need to share with other research interrupted the reliability of equipment, in particular the tightening of the MIE myometer straps. Future research would benefit from further knowledge around the metabolic fate of L-arginine when combined with other different compounds.

7.0

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