

THE EFFECT OF BETA-ALANINE
SUPPLEMENTATION ON AEROBIC AND
ANAEROBIC CAPACITY IN TRAINED CYCLISTS

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Abstract

Beta-alanine supplementation has been shown to increase skeletal muscle carnosine concentration resulting in the delay of neuromuscular fatigue and an increased aerobic and anaerobic capacity. The current study investigated the effects of beta-alanine supplementation on aerobic and anaerobic capacity in trained cyclists. Fourteen highly-competitive (sprint, endurance, road and track) cyclists underwent an 8 week 6.4g/day protocol (beta-alanine and maltodextrin). Pre and post supplementation testing included a VO_2max test (familiarization and characterization), maximum aerobic power test (aerobic capacity), and 30s wingate anaerobic test (anaerobic capacity). Aerobic capacity parameter measures included aerobic and anaerobic thresholds, and maximum aerobic power, while anaerobic capacity parameters included fatigue index, average power, peak power, watts per kilogram, and final lactate concentration.

There was a lack of change in aerobic and anaerobic capacity parameters post supplementation for both groups. Assuming an increase in skeletal muscle carnosine concentration, results suggest 8 weeks 6.4g/day beta-alanine does not increase aerobic and anaerobic capacity in trained cyclists. This lack of change has 3 potential explanations; carnosines' physicochemical H^+ buffering ability was not substantially elevated to prevent muscular fatigue via acidosis, pH decrease is only one limiting factor in aerobic and anaerobic capacity, or other factors (neuromuscular junction failure, contractile failure, substrate depletion, metabolite accumulation, oxidative stress) influence muscular fatigue.

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CHAPTER ONE

Introduction

Performance

Striving for excellence in sport is achieved in several ways; naturally, through training and adaptation, artificially, through the use of performance enhancing drugs/supplements, and most importantly, genetics. Such an example of artificial enhancement is the East German regime of the 1970s, where in the need to succeed, hormone doping became a natural part of the training regime and led not only to extraordinary adaptation, but to a substantial improvement in performance (Franke and Berendonk, 1997). Performance enhancing supplementation is a common tool for pushing the boundaries of human physiological development. In the body-building world where bigger is better, it is common practice to abuse the wide variety of synthetic hormones available in today's market (Jazayeri and Amani, 2004) that act on muscle hypertrophy stimulation (increase muscle protein synthesis and anabolism) (Kadi et al., 1999). This brought about the development of the World Anti Doping Agency (WADA) who have not only established a prohibited list of substances, but developed testing procedures to combat such use.

Natural performance enhancement, although not as dramatic and effective, comes through the means of training adaptation and mental preparation. Adaptation requires stressing the physiological system into an unaccustomed state. With the appropriate rest, adaptation of the body (heal) to the new stress will result in an increase in performance/capacity (Radak et al.,

2001). A question posed by athletes, coaches and scientists is whether legal sports supplements can help improve aerobic/anaerobic performance/capacity associated with sporting success.

Cycling Disciplines

Cycling (road, track, and mountain) includes a range of anaerobic (track sprint) and aerobic events (road race). Each discipline requires specific training to achieve optimal performance (Table 1). For example, the Tour de France, the event most commonly associated with cycling, covers approximately 3500km and is dominated by aerobic capacity and lactate threshold (Kenefick et al., 2002, Lucía et al., 2003). In comparison, several disciplines are track based and more commonly associated with power, dominated by riders with large quadriceps, explosive high power outputs, and a heavily developed anaerobic system. Events of this nature include the match sprint, keirin, and the kilometer time trial (kilo). The match sprint suits riders with explosive power, size, and strength over 5-10s (Dorel et al., 2005), while the keirin and kilo are governed by the ability to maintain the same power for a sustained duration (30-65s). Thus, in order to succeed at such events, a tolerance to pain is a necessity (muscular acidosis).

The scratch race, points race, Madison, teams pursuit, and individual pursuit however, are typically based upon aerobic capacity (Schumacher and Mueller, 2002), a high lactate threshold, periods of explosive power, and an ability to recover from numerous anaerobic efforts. What is thought to govern success in these events is the ability to manage and control lactate concentrations.

Table 1. The type, duration and training associated with the cycling disciplines. (Based on the programs of the current New Zealand sprint and endurance squads, 2010)

Event	Duration of event	Training Zones
Road race	2-7 hours	Aerobic (2-7 hours), VO ₂ max and lactate threshold (LT) training
Road time trial	50-65 minutes	Aerobic, VO ₂ max and LT training
Scratch	15-25 minutes	Aerobic, LT and sprint training
Points/Madison	20-30 minutes	Aerobic, LT and sprint training
Pursuit	3:30-4:30 minutes	Aerobic, LT and LTol training
Kilo/Keirin	30-65 seconds	Aerobic, LTol, VO ₂ max and sprint training
Sprint	10-20 seconds	Aerobic, LTol, and sprint training

A cyclist's control of threshold, characterized by the highest intensity at which lactate production and elimination are in equilibrium (Faude et al., 2009) (main determinant in road cycling success), and/or tolerance to lactate is an important aspect to success. Lactate production, and therefore H⁺ production (decreases muscle pH and therefore muscle activation), is an aspect of fatigue that determines cycling performance (Messonnier et al., 2007). Lactate control has 2 aspects: lactate management, where managing levels of lactate above and below the threshold

determines the outcome in pursuit and time trial events, and lactate tolerance, where the production is not the determinant of performance, but rather the athlete's tolerance to the increasing lactate concentration (pain) (success in events like the keirin and kilo). It is therefore important that the cycling fraternity receive unbiased, independent advice regarding the control of acidosis toward aerobic and anaerobic metabolism/capacity.

Energy Systems

Cycling is sustained through the aerobic and anaerobic metabolic energy systems that provide chemical energy in the form of adenosine triphosphate (ATP). Initially, all cycling events utilize the readily available store of ATP within the muscle. Following depletion, specific events utilize specific pathways to produce the required energy for muscle contraction. For explosive events (sprint and keirin), the ATP-PC energy system is employed to provide rapidly utilizable ATP (Wells et al., 2009) during high intensity exercise such as track sprint cycling (Flyger, 2008).

Through the chemical breakdown of phosphocreatine (PCr) (a phosphorylated creatine molecule that serves as a rapidly mobilizable reserve of high energy phosphates used to form ATP from ADP during the first 2-8 seconds (Karlsson and Saltin, 1970) of intense exercise) a phosphate group is liberated and provided to ADP. The creatine molecule itself is formed from arginine, methionine and glycine through a series of catalyzed chemical reactions (creatine kinases) in the liver, kidneys, and pancreas (Williams and Branch, 1998) and transported via the bloodstream to the skeletal muscle (Walker, 1979). Creatine concentration within striated human muscle is approximately $4\mu\text{mol g}^{-1}$ (Clark, 1997), with evidence indicating this concentration can increase following supplementation and extend the duration of the ATP-PC system (Birch et al., 1994). Following PCr consumption, activation of the anaerobic metabolic pathway (fast-rate glycolysis)

occurs. The production of ATP sustains muscle contraction for events of 2 minutes or less (kilo) (Robergs et al., 2004) and is discussed shortly.

Fast rate glycolysis is heavily relied upon in the keirin and kilo (2 minutes or less), and to a lesser extent in the scratch and points race. Through the breakdown of glycogen to glucose in the absence of oxygen, and the subsequent enzymatic reactions to produce pyruvate and lactic acid (glycolysis) enough ATP is created to provide chemical energy for muscle contraction (Gastin, 2001). Muscular acidosis (decrease in muscle pH) arises due to the automatic dissociation of lactic acid at neutral pH (lactate (La^-) and hydrogen (H^+) ion) (Cooke et al., 1988, and Lamb et al., 1992). At low pH, it remains in its undissociated form (lactic acid pKa 3.86 (Graves et al., 2006)), however under physiological conditions (7.4), dissociation into the ion salt occurs. The consequent drop in pH alters muscle mechanics, resulting in fatigue and decreased performance. If, however, sustained muscular contractions are required (road and pursuit events), the oxidative phosphorylation pathway provides an indefinite sustainable supply of ATP and is described in the following paragraph.

The aerobic system (Ebert et al., 2006) is composed of three stages and is heavily relied upon in almost all disciplines of cycling. Digestion is followed by the first stage incorporating glycolysis (carbohydrate breakdown) which breaks down glucose through a series of 10 enzyme catalyzed reactions to yield 2 ATP molecules, 2 molecules of NADH, and 2 molecules of pyruvate (Garret and Grisham, 2005), beta-oxidation, which breaks down fats in the mitochondria to acetyl-CoA, the entry molecule into the citric acid cycle, and deamination, which breaks down proteins in the

liver through amine group removal (deaminases). In the presence of oxygen, slow rate glycolysis is utilized and the pyruvate molecules are fed into the second stage (Kreb's cycle), where 1 ATP molecule, 1 carbon dioxide molecule, 3 NADH molecules, and 1 FADH molecule are produced per pyruvate molecule (Garret and Grisham, 2005). Further progression of the NADH and FADH molecules into the electron transport chain (third stage) yields a total of 10 NADH⁺ molecules and 2 FADH⁺ molecules, which equates to 34 ATP molecules (Garret and Grisham, 2005). The production of 38 ATP molecules per molecule of glucose provides enough sustainable energy for muscular contraction during cycling events of long duration (road).

In my study, progressive acidosis during cycling activity is under investigation with beta-alanine supplementation and cycling capacity. The following section combines the structure and composition of skeletal muscle (which utilizes the production of chemical energy) in relation to cycling performance.

Muscle Physiology

Muscle physiology genetics is one determinant of cycling success. Muscle composition (fibre type) pre-determines event specificity of an individual, so following is a discussion of the various aspects of muscle composition and structure in relation to cycling performance.

Skeletal muscles are dominated by the presence of numerous myofibrils, each consisting of a highly regulated arrangement of cytoskeletal elements of thick (12-18nm assemblies of myosin)

and thin (5-8nm assemblies of actin) filaments, which combined make up the contractile region of the muscle (sarcomere) (Sherwood et al., 2005).

Neural stimulation governs muscle contraction (Lomo, 2009), and release of acetyl choline (ACh) at the neuromuscular junction (between the motor neuron terminal and the muscle fibre) was identified as the key initiation step (Brown et al., 1936). Subsequent post-synaptic binding of the molecule stimulates an action potential that is propagated across the entirety of the muscle cell membrane (Axelsson and Thesleff, 1959; Katz, 1961). A propagated release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum follows (SR, modified endoplasmic reticulum consisting of fine interconnected tubules surrounding the myofibrils) (Sherwood et al., 2005) when the action potential reaches the transverse tubules (T-tubules) which run primarily into the central portions of the muscle fibre. The propagated release is achieved through the ryanodine calcium channels that are activated through coupling with dihydropyridine proteins (Bellinger et al., 2009), with structural and/or functional defects of this channel causing dystrophic muscle (Bellinger et al., 2009). The molecular regulation of the contraction couples the binding and dissociation of Ca^{2+} on troponin (Tn) to the movement of tropomyosin (Lehman et al., 2009) on the actin filaments exposing the actin binding sites allowing the formation of a cross-bridge (Craig and Lehman, 2001). Activation of the “power stroke” (pulling of the actin filament) through the chemical energy supplied from ATP causes the muscle to contract (Geeves and Holmes, 1999). Cytoplasmic Ca^{2+} is actively absorbed into the SR (SR Ca^{2+} -ATPase pump) (Toyoshima, 2007) following the completion of the action potential, resulting in muscle relaxation, with a further ATP molecule liberating the cross bridge.

Cycling success is determined through the rate, durability, and susceptibility of muscle fibres to fatigue. The 3 muscle fibre types can be distinguished by rate and force of contraction (Westerblad et al., 2010); slow-oxidative (type I), fast-oxidative (type IIa), and fast glycolytic (IIx) cover the fibre distribution among human skeletal muscles. Depending on the training, type IIc, an undifferentiated fibre, has shown an ability to convert to either of the type II fibres (Staron et al., 1990). Endurance events (such as road or pursuit) predominantly utilize type I and occasionally type IIa fibres (Greig and Jones, 2010), whereas the anaerobic power events (match sprint) predominantly utilize type IIx fibres. Table 2 differentiates between fibre types based on rate of contraction and durability.

Oxidative phosphorylation is the dominant metabolic pathway associated with type I fibres, whereas type IIa fibres rely on both mechanisms to produce ATP. Minimal contractile activity in the absence of oxygen makes them less susceptible to the accumulation hypothesis (discussed in following section) and therefore predominantly used during road and aerobic track cycling events. This is in accordance with a study in 2004 (Wang et al.) where mice genetically enhanced through activation of a form of peroxisome proliferator-activated receptor δ (PPAR δ) in skeletal muscle, allowed the mice to run twice the distance compared to that of a wild-type littermate through the increased numbers of type I muscle fibres. However, the glycolytic fibres (type IIx), due to their design and contractile ability in the absence of oxygen, are susceptible to fatigue (metabolite accumulation) but produce great force and high contractile rates (Sherwood et al., 2005), essential to success in sprint orientated events. Knowledge of muscle fibre fatigue is essential (prolonging the maximal activity of each fibre), and the following section discusses how skeletal muscle fibres are affected.

Muscular Fatigue

Fatigue is the reduction in force generating capacity of a muscle through neuromuscular junction failure, contractile failure, substrate depletion, metabolite accumulation and/or oxidative stress (Fig. 1) (Bigland-Ritchie and Woods, 2004). Skeletal muscle contractile activity can be maintained indefinitely at low intensity, however maintaining anaerobic activity for extensive periods is impossible. As fatigue materializes, muscle fibre tension declines, decreasing cycling performance.

Table 2. Characteristics of skeletal muscle fibres. (Adapted from Sherwood et al., 2005)

Characteristic	<u>Type of Fibre</u>		
	Type I	Type IIa	Type IIx
Myosin ATP-ase Activity	Low	High	High
Speed of contraction	Slow	Fast	Fast
Resistance to Fatigue	High	Intermediate	Low
Oxidative phosphorylation capacity	High	Intermediate	Low
Enzymes for anaerobic glycolysis	Low	Intermediate	High
Mitochondria	Many	Many	Few
Contraction rate	Low	Intermediate	High

Muscular fatigue is thought to occur through several mechanisms (Fig. 1). Inhibition of the axon terminal (Taylor et al., 2000), depletion of ACh, and altered neurotransmitter receptor binding (Luckin et al., 1991), result in fatigue of the neuromuscular junction (NMJ). Contractile muscle failure (decreased force and rate) however, is based upon altered Ca^{2+} release and absorption from the SR. These include an inability to release Ca^{2+} from the SR, an inability to absorb the Ca^{2+} following reduction of an action potential (Favero, 1999; and Rubtsov, 2001; Westerblad et al., 1991), and/or the inability of Ca^{2+} /troponin binding and the formation of a cross bridge (Fitts, 1994).

The 2 primary hypotheses commonly associated with muscular fatigue are the depletion (exhaustion) and accumulation hypotheses first characterized in 1989 (McClaren et al.). Depletion of key metabolites (PCr, glycogen and ATP) (McClaren et al., 1989) characterize the depletion hypothesis, more commonly associated with the oxidative fibres and endurance events (road and pursuit). The accumulation of metabolic by-products that interrupt the contractile mechanics of the skeletal muscle (lactate, inorganic phosphate (Pi), ADP, inosine monophosphate (IMP), ammonia (NH_3), magnesium (Mg^{2+}) and H^+ ions) (McClaren et al., 1989) characterize the accumulation hypothesis, more commonly associated with the glycolytic fibres.

Molecules identified in relation to the accumulation hypothesis are lactate ions (La^-), H^+ , ADP, Pi, IMP, NH_3 , Mg^{2+} , and inorganic phosphate (PO_4^-). The high-energy splitting of PCr and ATP during cross-bridge formation (Robergs et al., 2004) produces an excess toxic level of PO_4^- that contributes to muscular fatigue (Westerblad et al., 2002). This action mechanism prevents the

release of Ca^{2+} from the SR (Allen and Westerblad, 2001), and a low to high force transition rate of the contractile elements (diminishing force production) (Westerblad et al., 2002).

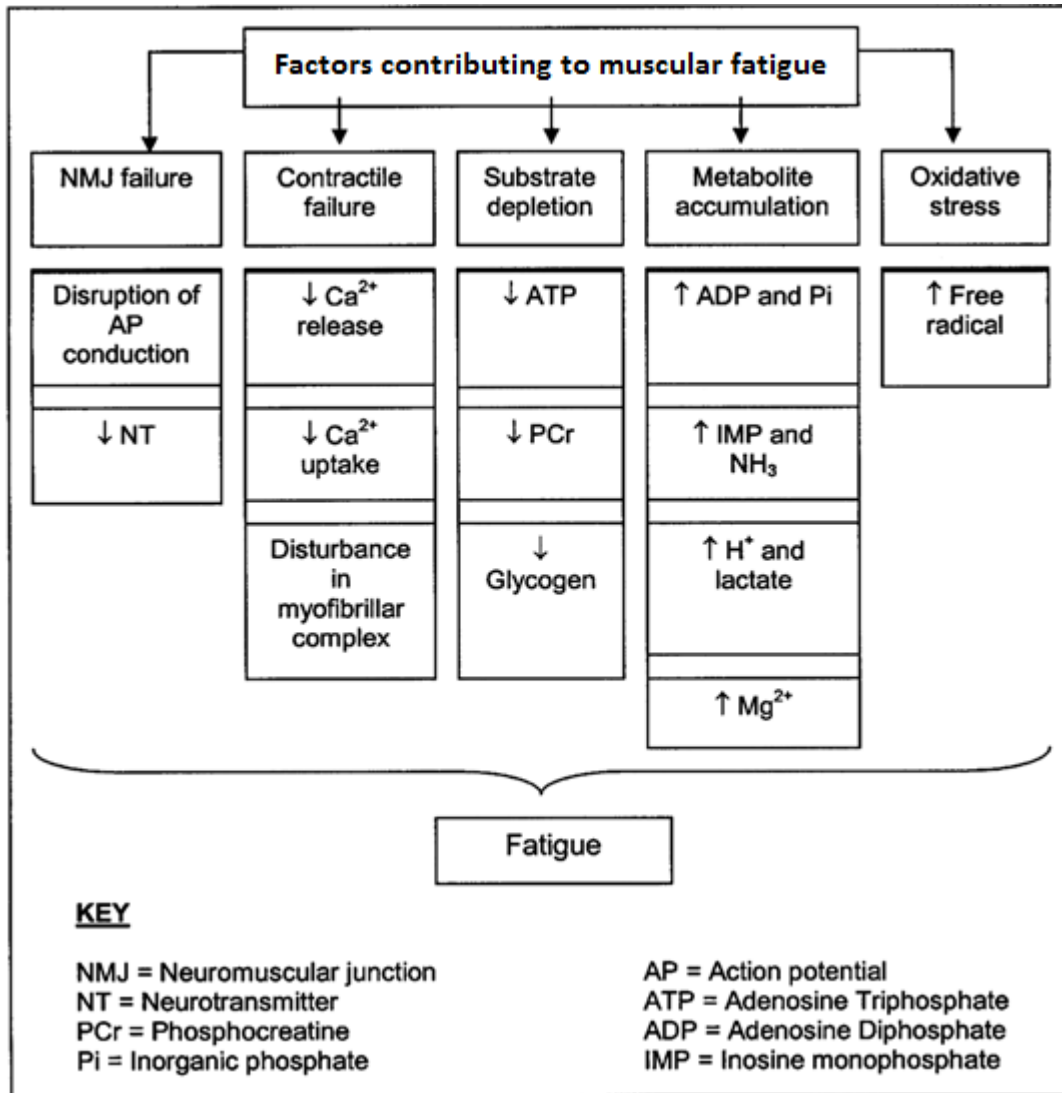


Figure 1. Factors contributing to muscular fatigue (adapted from Begum et al., 2005)

ADP, also the result of the high energy splitting of ATP, in conjunction with Mg^{2+} (MgADP), has been shown to decrease maximum muscle fibre velocity and increase muscle isometric tension (Cooke and Pate, 1985). While ADP and Mg^{2+} affect muscle fibre tension and velocity, IMP and NH_3 (correlation in concentration with ADP, adenosine monophosphate (AMP), and depletion of PCr) (Sahlin et al., 1990), have been shown to decrease muscular force during continuous isometric contractions, reduce Mg^{2+} -stimulated acto-myosin-ATPase activity (up to 60% at 10 mM IMP), and prevent the formation of the actin-myosin complex (Westra et al., 2001). This accumulation is thought to prevent an “energy crisis” during exhaustive exercise, by down regulating the contractile machinery (Westra et al., 2001).

Excess lactic acid is produced during anaerobic metabolism (fast-rate glycolysis) (Marcinek et al., 2010), which further dissociates into the lactate (La^-) and H^+ (Brooks, 1986) at neutral pH (7-7.4). Increased La^- concentration plays a minor, albeit significant role in muscular fatigue through interference with cross-bridge formation (Hogan et al., 1995). Metabolic acidosis arises when H^+ production exceeds the chemical and physiological mechanisms available to buffer it (Robergs et al., 2004). In relation to lactate production, questions have been raised about the effect of H^+ in relation to metabolic acidosis at physiological pH. A direct correlation between lactate production and H^+ accumulation has been identified (Marcinek et al., 2010), which suggests during intense exercise, concentrations will be similar. A decrease in ATP production directly linked to enzyme structural changes, structural changes to membrane transport mechanisms, and changes to substrate availability (Robergs et al., 2004) identifies how important it is to manage lactate and H^+ levels (an accumulation of lactate and H^+ is correlated to a drop in ATP). Phosphofructokinase (PFK) (the rate-limiting enzyme in glycolysis) is a significant example, as the rate of reaction decreases following H^+ ion adherence. Subsequent reactions of

glycolysis fail to occur because of the alteration to the conformation of the enzyme, and the subsequent failure of its substrate (D-Fructose-6-phosphate) to recognize its binding site.

The fundamental mechanics of muscle contraction are disrupted through the production of excess H^+ . Inhibition of actomyosin ATPase (solely responsible for the breakdown of ATP and cross-bridge formation (Cooke et al., 1988), and relaxation), interference with Ca^{2+} release, absorption from the SR (Lamb et al., 1992), and alterations to calcium-troponin binding are a result of increased H^+ ion concentration. In association with metabolite accumulation, a combination of these problems has shown to result in decreased force and rate of contraction in skeletal muscle. Therefore, it is imperative to delay fatigue, naturally or artificially, to improve capacity/performance.

Natural Fatigue Controlling Mechanisms

There are a range of natural fatigue controlling mechanisms available to delay the onset of muscular fatigue. The human body is dependent on several biochemical pathways to alleviate increased levels of toxic products (accumulation hypothesis) and/or the depletion of key metabolites (depletion hypothesis).

The issue during anaerobic metabolism is the accumulation of toxic metabolites and metabolic by-products. First shown in 1953 (Harris), thwarting the detrimental effects of PO_4^- (ATP and PCr splitting) becomes increasingly difficult due to the muscle membrane having little or no

direct permeability to phosphate. Transportation of phosphate ions out of a cell via a Na^+ transporter is conceivable, with evidence showing a high concentration of phosphatase at the surface of secreting cells (Danielli, 1951). In conjunction, the control of La^- and H^+ ion accumulation during anaerobic metabolism becomes crucial to muscle function. Naturally buffering H^+ is accomplished in part through sodium bicarbonate, with evidence showing a direct correlation between rising levels of lactate and decreasing levels of the compound (Beaver et al., 1986).

In conjunction with sodium bicarbonate, limiting the decrease in pH and accumulation of H^+ in supramaximal exercise is accomplished through the function of the Na^+/H^+ exchanger, carbonic anhydrase (CA) enzymes (CA, CAII, CAIII, CAIV, CAXIV), and the monocarboxylate transporters (MCTI and MCT4) (Messonier et al., 2007). Additionally, the evolution of an intracellular non-bicarbonate proton buffering system aids skeletal muscle buffering abilities. The imidazole rings found in histidine containing compounds (Abe, 2000) maintains a neutral pH, made possible through protonation of these compounds due to their pKa being closely associated with the intracellular pH of the cell (Abe, 2000).

The body's natural mechanisms can be enhanced by the use of substances/supplements that provide the athlete with a much greater resistance to fatigue than can be accomplished naturally (due to the demands of professional sport, and the drive to succeed). In the following section, analysis of several illegal and legal compounds will be covered.

Ergogenic Aids – Cycling and Supplements

It is becoming increasingly important to develop advanced mechanisms of fatigue resistance that allow individuals to compete at the highest level and succeed. Millions of dollars worth of research has developed several ergogenic aids, some legal and some illegal, to cope with the demand of performance enhancement.

Anabolic steroids are common illegal ergogenic aids (Calfee and Fadale, 2006, and Juhn, 2003). Synthetic testosterone (made famous by Floyd Landis in the Tour de France), anadrol, and nandrolone are prime examples. Their ability to mimic the effects of testosterone and dihydrotestosterone (increased cell protein synthesis and muscle anabolism through cortisol blockage (Sheffield-Moore, 2000)) draws significant attention to these compounds in a range of sports, from bodybuilding to cycling. Other illegal ergogenic aids include erythropoietin (EPO), human growth hormone (HGH), and to a lesser extent, amphetamines (stimulants), and painkillers.

EPO, a glycoprotein hormone produced primarily in the kidney and liver, stimulates the production of red blood cells (erythropoiesis) within the bone marrow (Mairbaurl, 1994). There is substantial evidence to conclude that an intravenous injection of EPO enhances aerobic performance (Thomson et al., 2007), making it popular among endurance athletes. Red blood cell proliferation eventuates, increasing oxygen delivery to the working muscle following administration. More commonly associated with strength/power sports (weightlifting) (Saugy et al., 2006), HGH, a naturally occurring polypeptide hormone, is responsible for growth, cell

reproduction, cell regeneration, and muscular physique, a trait highly sought after in strength/power sports. Amphetamines, however, are primarily used as a stimulant to increase awareness, concentration, alertness, energy, and self esteem through its actions as a psychostimulant by increasing monoamine and dopamine levels (Fleckenstein et al., 2007). Painkillers on the other hand, are used extensively to achieve analgesia, allowing an athlete to push their performance beyond their natural pain barrier (Tscholl et al., 2009).

There is a fine line between the legality of compounds that have been shown to increase performance. Legal compounds include beta-alanine, creatine, sodium bicarbonate, colostrum and caffeine. Because of its ergogenic properties in repetitive anaerobic cycling sprints (Calfee and Fadale, 2006; Juhn, 2003), creatine is becoming one of the most commonly used legal supplements among cyclists. Creatine's use was developed to improve the availability of the compound within skeletal muscles during intense exercise. Through increased creatine availability (supplementation of 4 weeks or more), ATP synthesis duration can be extended (Bemben et al., 2001; Nelson et al., 2000). At the opposing end of the supplementation spectrum, sodium bicarbonate (discussed previously) is commonly orally administered ninety minutes prior to an event, increasing blood sodium bicarbonate concentrations substantially, offsetting the acidity produced in the muscles during intense exercise, and essentially leading to an increase in blood pH (McNaughton et al., 1999). Colostrum, however, (a form of milk produced by the mammary glands containing immunoglobulins, cytokines and growth factors) has been shown to decrease recovery time and prevent sickness during peak performance (Buckley et al., 2002). Caffeine, one of the more popular legal performance enhancing supplements, has been shown to bind to the adenosine receptors as a competitive inhibitor, resulting in increased cognitive

function (process of thought) (Foskett et al., 2009) correlating to an increase in performance (Graham and Spriet, 1991). Beta-alanine and carnosine's performance enhancing capabilities will be discussed in the following sections.

Carnosine

There is extensive literature on the function and mechanism of carnosine. This review will encompass its absorption, relation to muscular fatigue (specifically as a pH buffer), action as an antioxidant, and action as a skeletal muscle regulator, all of which relate to performance.

Carnosine is a naturally occurring histidine containing dipeptide also known as β -alanyl-L-histidine (Hill et al., 2007) (Fig. 2). It is commonly found in muscle and nerve cells (Hill et al., 2007) and has effects not only on muscular fatigue resistance, but also roles in quenching free radicals (Kurihara et al., 2009), enzyme regulation (Johnson and Aldstadt, 1984), and calcium regulation from the SR (Batrukova and Rubtsov, 1997). Its buffering potential was first established in 1953 (Severin et al.) using isolated frog muscle, which showed that muscles in the presence of carnosine could accumulate excessive amounts of lactate without hindrance to function. However, in its absence, considerable acidosis occurred, hindering contractile function. This is commonly known as the Severin Phenomenon.

Carnosine Absorption

Information about carnosine absorption is equivocal. Oral administration of carnosine as a dietary supplement, or through foods rich in the dipeptide (beef, pork), have been shown to not increase skeletal muscle concentrations (Kraemer et al., 1995) due to degradation of the molecule

within the gastrointestinal tract (Gardener et al., 1991). Furthermore, evidence has shown (Chan et al., 1994) that rats who were fed a semi-purified diet containing carnosine and/or α -tocopherol showed no increase in carnosine concentration in heart muscle when fed only carnosine, however in combination with α -tocopherol, a 1.5 fold increase was observed. However, recent research (Maynard et al., 2001) has shown that an increase in dietary carnosine by as little as 1.8% over an 8 week period is sufficient to increase the concentration of carnosine in the soleus and lateral gastrocnemius muscles of rats.

Oral supplementation of carnosine's constituents (beta-alanine or histidine) has identified significant increases in muscle concentration (Harris et al., 2006) through the process depicted in Figure 2. Histidine dietary deficiency can result in a carnosine concentration decrease within skeletal muscles of rats (Tamaki et al., 1984), while beta-alanine supplementation significantly elevates carnosine's concentration (Harris et al., 2006). Short-term increases have been shown to dramatically decrease following supplementation cessation (Boldyrev and Severin, 1990). As described by Baguet et al. (2009), carnosine washout (carnosinase, Fig. 2) takes on average 6-15 weeks depending upon the exercise status of the individual (Gardener et al., 1991). Based on the evidence presented, skeletal muscle carnosine concentration following oral carnosine supplementation is equivocal, although significant elevations are observed following histidine and beta-alanine supplementation.

Carnosine and Muscle Buffering

During high intensity exercise, skeletal muscle cells become saturated with lactate and H^+ (Livingstone et al., 2001), causing the interruption of muscle function. The effect of various pH

levels on the excitation-contraction-coupling mechanism (ECC) of muscle contraction were investigated (Lamb et al., 1992). Initial depolarization elicited a large response at all pH levels (6-8), however the subsequent responses decreased as pH became more acidic. Following a decrease in pH (accomplished via H^+ ion increments), poor operation of the Ca^{2+} -ATPase pump and subsequent decrease in ECC are observed.

The presence of the imidazole group (Fig. 2) found in carnosine (and other histidine based compounds, such as anserine) is thought to play an important role in muscle buffering capacity (Abe, 2000); however the percentage of total muscle buffering capacity has yet to be determined. It was suggested over the physiological pH range (7.1-6.5), carnosine had a 7% role (Mannion et al., 1992) in total muscle buffering (2.4 and 10.1 mmol H^+ kg^{-1}). In combination with anserine, an estimated 40% of total muscle buffering may be covered (Davey, 1960). If the percentages were as high as suggested, one could significantly increase muscular performance in type I and type II fibres, by increasing skeletal muscle carnosine concentration.

During high intensity exercise, elevated carnosine concentrations have been shown to increase the skeletal muscle buffering capacity (Suzuki et al., 2001). A direct correlation between superior sprint performance and buffering capacity were observed in a group with significant elevations in vastus lateralis type II muscle fibre carnosine concentration (Suzuki et al., 2001). Carnosine concentration is directly dependent upon muscle fibre type, highlighting the importance of its use in endurance and strength/power sports. With twice the carnosine concentration in type II fibres compared to type I fibres (23.2 ± 17.8 and 10.5 ± 7.6 mmol kg^{-1} dry weight, respectively) (Harris et al., 1998), it is suggested that there is a direct correlation between carnosine concentration and type II fibre H^+ ion concentration during anaerobic exercise.

Furthermore, studies (Mannion et al., 1992) have shown an average carnosine concentration of $20 \pm 4.7 \text{ mmol.kg}^{-1}$ in the type II fibres of the quadriceps femoris in humans, with further evidence suggesting sex plays a significant role (significantly higher concentration in the male population).

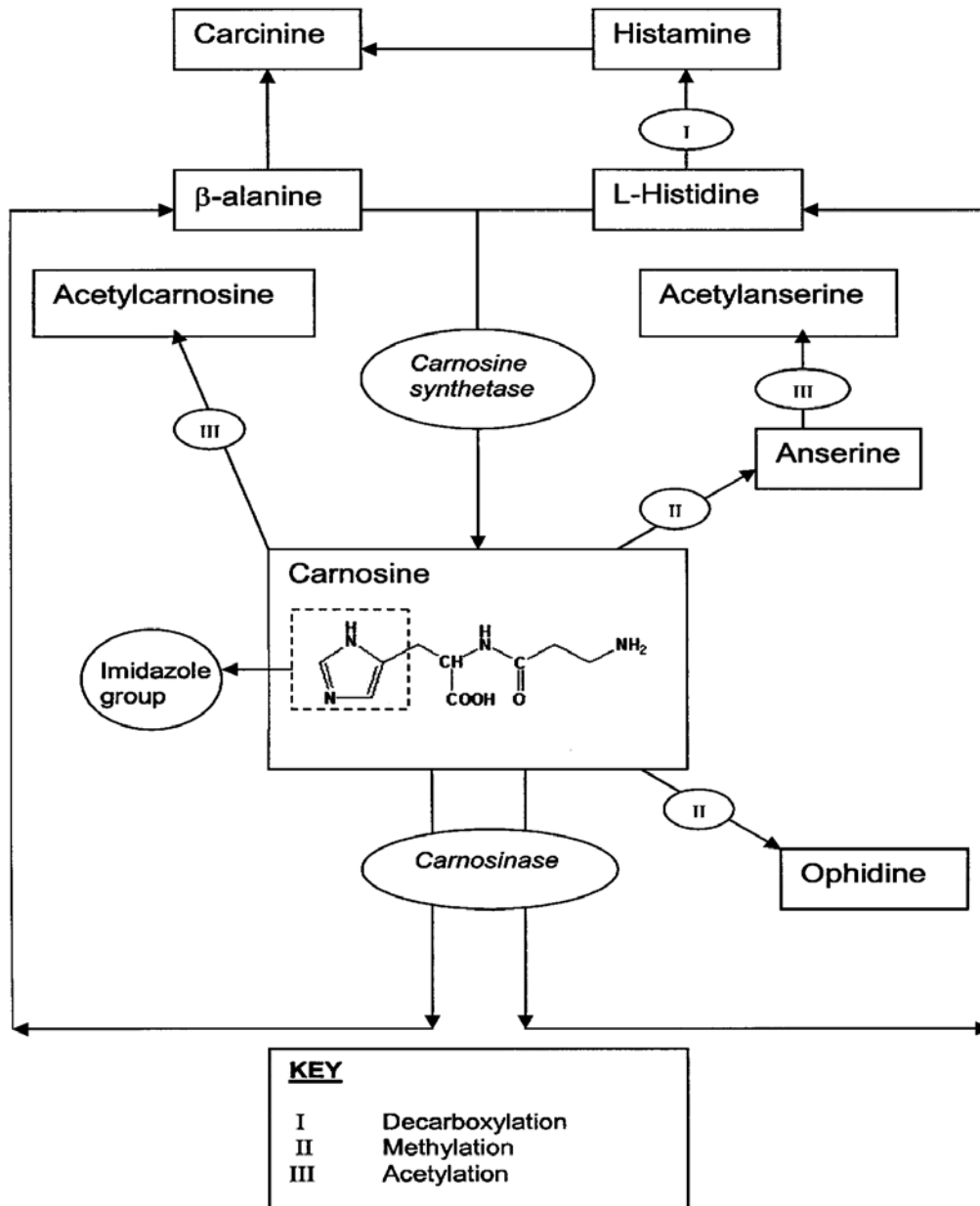


Figure 2. Metabolism of carnosine (Begum et al., 2005)

Carnosine as an Anti-oxidant

Besides its effective role as a muscle buffering agent in skeletal muscles, there is evidence to suggest that carnosine plays an effective and important role as an anti-oxidant, through its biological function of quenching singlet molecular oxygen, and scavenging hydroxyl and superoxide radicals (Gariballa and Sinclair, 1992; Kohen et al., 1988; Pavlov et al., 1993). It is well established that muscle contraction increases oxidative metabolism and the level of oxidative stress associated with it (Ji, 1995). Elevations in reactive oxygen species (ROS) levels may occur (Mastaloudis, 2001) through increased respiration and an increase in electron flow through the electron transport chain. In conjunction, ROS levels may rise due to depletion of ATP pools, leading to a higher intracellular level of ADP which triggers catabolism of ADP, and converts xanthine dehydrogenase to the superoxide generating enzyme xanthine oxidase (Moller et al., 1996). Furthermore, a decrease in pH may promote oxygen release from hemoglobin. (Ebbeling and Clarkson, 1989).

An increase in ROS has been shown to interrupt the SR Ca^{2+} -ATPase (Moreau et al., 1998). The specifics of interruption were examined (Xu et al., 1997) on isolated SR containing the Ca^{2+} -ATPase. Further analysis identified complete inhibition of the pump through the attack of the ATP binding site by hydroxyl radicals.

Carnosine's antioxidative properties have been investigated several times. An investigation into the effect of carnosine's antioxidative properties was conducted on senile cataracts (formed from lipid peroxidation) in dog eyes (Boldyrev et al., 1987). Further peroxidation of the cataracts was prevented through the interaction of carnosine, both *in vitro* and *in vivo*, with the lipids commonly associated with the issue. Furthermore, the minimum effective concentration of

carnosine required to inhibit lipid and protein oxidation was determined to be 2.5mM and 1mM respectively, observed through exposure of isolated rat muscle tissue to free radicals (Nagasawa et al., 2001). Further demonstration (Kang et al., 2002; Kohen et al., 1988) identified other histidine derivatives (homocarnosine and anserine) having similar antioxidative properties (imidazole group) (Fig.2). Therefore, during endurance based sport where the body produces excess ROS, increased skeletal muscle carnosine concentration may prevent the consequent damage associated with ROS and improve performance/capacity.

Carnosine as a Skeletal Muscle Regulator

As discussed previously, muscle contraction is dependent upon SR release and absorption of Ca^{2+} . Inhibition of Ca^{2+} release and/or absorption is achieved through the concentration increase in H^+ . Prohibiting the alteration of the ryanodine receptor transport mechanisms is preventable through the identification of carnosine's H^+ absorption properties. It is suggested however, that there is a carnosine binding site on the SR Ca^{2+} ryanodine transporter for rapidly inducing Ca^{2+} release (carnosine concentration dependent) (Batrakova and Rubtsov, 1997). Increased isolated rat heart contractility has been observed when saturated in carnosine (Roberts and Zaloga, 2000), suggesting that during high intensity cycling, carnosine may induce Ca^{2+} release and delay fatigue.

Further documentation has shown that a reduction in action potential propagation is experienced when there is a loss of K^+ and Na^+ potential during repeated muscular contractions (Fitts and Balog, 1996). The effects of muscular fatigue on Na^+ and K^+ concentrations, as well as membrane potential, were investigated on isolated semitendinosus frog muscle (Fitts and Balog,

1996). Alterations in the concentrations led to 3 possible mechanisms of muscle fatigue. 1. It completely blocked the propagation of the action potential; 2. It caused depolarization-induced inactivation of T-tubular charge movement; and 3. It caused a reduction in magnitude of the t-tubular charge due to the lower action potential spike potential. This phenomenon occurs through disturbance of the membrane potential in the T-tubules and subsequent Ca^{2+} release from the SR (Fitts and Balog, 1996). Severin's phenomenon was justified when carnosine's introduction restored muscular function of a neuromuscular preparation under fatigue (Petukhov et al., 1976). Further analysis indicated that neither synaptic processes nor contractile mechanisms were responsible for muscle contraction continuation, but rather carnosine's ability (properties) to restore transmembrane potential depolarized by exhaustion (Begum et al., 2005).

Carnosine analysis in relation to absorption, muscular fatigue, antioxidative properties, and skeletal muscle regulation, classify how important an increase in its concentration may be to cycling performance/capacity. In the succeeding sections, a discussion of carnosine's rate-limiting constituent, beta-alanine, and what evidence there is to suggest that it improves athletic performance will be conducted.

Beta-alanine

Beta-alanine (3-aminopropionic acid) (Fig. 3) is a naturally occurring, non-essential beta amino acid that is obtained through foods rich in dipeptides, such as carnosine, anserine and balenine (commonly found in chicken, pork or beef). Specific β -amino, sodium, and chloride transporters (Bakardjiev and Bauer, 1994) are responsible for transport of the amino acid across the gut wall.

The body can also synthesize beta-alanine in the liver through catabolism of pyrimidine nucleotides. These are broken down into uracil and thymine, and further metabolized into beta-alanine and B-aminoisobutyrate (Zöllner, 1982).

Beta-alanine supplementation may indirectly increase muscular endurance through its physicochemical buffering ability of H^+ . The H^+ buffering ability of the di-peptide carnosine (beta-alanine and histidine) is responsible and was discussed in the previous section. Several studies have investigated the effects of beta-alanine supplementation on skeletal muscle carnosine concentration and muscular fatigue. The following section highlights experimental design (dosage and timeline), subject, and the effect of beta-alanine supplementation on carnosine concentration and performance.

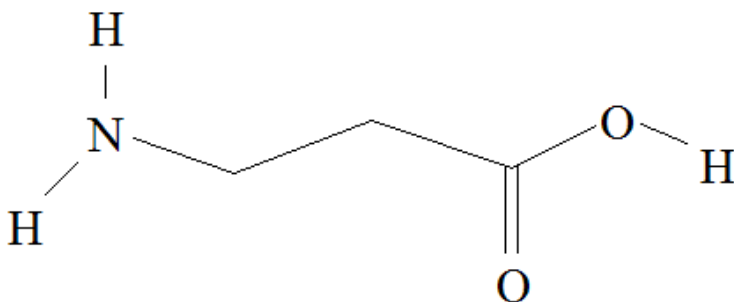


Figure 3. Structure of beta-alanine.

Beta-alanine Supplementation on Skeletal Muscle Carnosine Concentration

Beta-alanine is the rate-limiting constituent in the formation of carnosine. The concentration in which it is produced, which fibre type produces the highest concentration, and which muscles

(depending upon the fibre type) produce the highest concentration may be a determinant of sporting success.

There is a consensus that oral beta-alanine supplementation increases skeletal muscle carnosine concentration (Table 3). Muscle carnosine concentrations are analysed via a non-invasive technique known as proton magnetic resonance spectroscopy (proton MRS). However, several other studies (Dunnett et al., 1999, Harris et al., 2006, Hill et al., 2007, Kendrick et al. 2009) have successfully utilized the use of a muscle biopsy (invasive) followed by analysis using high performance liquid chromatography (HPLC).

To date, there has been no evidence of a “ceiling effect” on skeletal muscle carnosine concentrations (Derave et al., 2007), with significant elevations in carnosine concentration above basal level still being observed among individuals with an initial high concentration (12mmol kg^{-1}). A very similar protocol (Baguet et al., 2009) investigated human skeletal muscle carnosine loading and washout and identified a mere 1.7% increase in the soleus muscle of the control group and a 39% increase in the beta-alanine group following supplementation. Following on from a previous gastrocnemius study (Maynard et al., 2001), a smaller, but still significant elevation in carnosine concentration was recognized in the beta-alanine group (23% increase). Fast twitch muscles have a significantly higher carnosine concentration (due to a higher expression of beta-alanine transporters (Bakardjiev and Bauer, 1994)), carnosinase activity (Bakardjiev and Bauer, 2000), and/or carnosine synthase activity (Horinishi et al., 1978) than slow twitch muscles (soleus). The increase however, was directed evenly among the two fibre types, which agrees with previous research (Hill et al., 2007) that fibre types respond equally within the same muscle.

Table 3. Pre and post carnosine concentrations following beta-alanine supplementation protocols on human skeletal muscle.

Study	Dosage (g/day)	N	Duration (weeks)	Carnosine concentration pre (mmol kg ⁻¹)	Carnosine concentration post (mmol kg ⁻¹)
Derave et al. 2007	2.4-4.8	15	5	7.76±1.36	11.39±1.38
Baguet et al. 2009	2.4-4.8	20	6	5.63±0.94	7.83±1.74
Hill et al. 2007	4.0-6.4	25	10	21.8±1.6	34.7±3.7

The carnosine concentration distribution among the different muscle fibre types is of particular importance for sporting performance. The glycolytic fibres that utilize the ATP-PC and anaerobic systems are responsible for producing the necessary power during a cycling sprint. However, evidence suggests that, regardless of fibre type, carnosine concentrations increase equally (Kendrik et al., 2009) among type I and type II fibres.

There is significant evidence to suggest that beta-alanine supplementation increases carnosine synthesis within skeletal muscle fibres. The distribution of this increase is controversial, with some studies lending evidence to an equal balance among fibre types for carnosine concentration increase, and others stating the glycolytic fibre increases are substantially higher. Both scenarios are beneficial to endurance (type I) and strength/power (type II) sports.

Performance, Capacity, and Physiological Mechanisms

Until recently, the effect beta-alanine supplementation had on athletic performance was essentially ignored. Only within the last decade has the effectiveness of this carnosine

concentration increase come to light. It is important to relate the performance changes associated with an elevated carnosine concentration to trained individuals who utilize the substance substantially more than the sedentary population.

In 2003, significant improvements in 4 minute cycling power (38 Watts) were observed (Harris et al.); it was further identified that this change was due to increased H^+ buffering activity observed within the first minute of exercise. Similar research (Baguet et al., 2009) identified the physiological changes associated with beta-alanine supplementation in high-intensity cycling. Results yielded significant results including a difference in pH change between the beta-alanine and control groups after 6 minutes of high intensity exercise (exercise-induced acidosis). Pulmonary gas exchange values (VO_2 , ventilation, and CO_2 output) between the 2 groups were found to be non-significant, lending evidence to believe that beta-alanine has no effect on aerobic parameters.

Beta-alanine supplementation has been shown to significantly improve peak power (11.4%) and mean average power (5%) after exhaustive exercise (110 minute cycle race (van Thienen et al., 2009). Indications suggest beta-alanine supplementation has a positive effect on the aerobic system, the anaerobic system, and the crossover effect (switching from one to the other) when it comes to exercise capacity. The significance this has on the outcome of a cycle stage such as that of the Tour de France is promising, with approximately half the stages contested by a sprint finish.

Beta-alanine supplementation in power/strength sports is becoming a frequent occurrence. In combination with creatine, beta-alanine supplementation (CBA) has shown significant increases

in 1 repetition max (1-RM) squat strength and bench press (Hoffman et al., 2006). However, it has failed to improve a 20-jump test, and more importantly the anaerobic power measures from a wingate anaerobic test. Results of this nature make it hard to believe that supplementation improves anaerobic power even though strength levels may increase.

Once again, results following beta-alanine supplementation on anaerobic power measures are equivocal. Significant differences in 300 shuttle (sprinting to and from cones) time and flexed arm-hang time between groups have been identified (Kern et al., 2010), while other results (Hoffman et al., 2008) using college football players had contradictory results. Although no significant differences in peak power, mean power, and total work between groups have been observed, an insignificant trend ($P=0.07$) in fatigue rates occurred, suggesting there may be an increase in hydrogen buffering capacity. Similarly, repeated isokinetic contractions of 30s for a maximum of 5 repetitions, showed significantly higher average torque in the beta-alanine group, and more specifically in bouts 4 (6.1%) and 5 (3.8%) (Derave et al., 2007). Further analysis of the knee extensors in question during a 45% maximum voluntary contraction (MVC) turned up insignificant results, suggesting isometrically at least, beta-alanine supplementation does not cause a performance effect.

With respect to performance improvement, 400m running times have been shown to significantly decrease following beta-alanine supplementation. However, these observations occurred in both groups, suggesting that the changes in gastrocnemius carnosine concentration did not correlate with decreases in 400m times (Derave et al., 2007). With respect to cycling events, the keirin and kilo closely resemble 400m in terms of time to complete and metabolic energy system

requirements. This evidence would suggest that beta-alanine supplementation would not elevate performance/capacity.

The ability to repeat maximal sprints in cycling is essential for success (points and scratch races). Evidence has shown there is no significant improvement following beta-alanine supplementation on power output during repeated sprint activity (10x5s sprints separated by 45s recovery). A lack of change for peak power, average power, fatigue rates, and blood lactate levels further indicates increased carnosine concentration does not increase power output during repeated sprint bouts, and thus would not aid in recovery (muscular fatigue) during the points or scratch races.

Neuromuscular fatigue (NMF) is characterized by an increase in the electrical activity of working muscles over time. It is a fatigue-induced increase in electromyographic amplitude as a result of progressive recruitment of additional motor units and/or an increase in the firing frequency of the active motor units (Moritani et al., 1993). Based on this, a term known as PWC_{FT} (physical working capacity at fatigue threshold) was developed (deVries et al., 1987; deVries et al., 1990), and is the basis for neuromuscular studies. Therefore, it was postulated that because of the properties of carnosine, an increase in its skeletal muscular concentration may delay the onset of NMF.

In relation to cycling, evidence (Stout et al., 2007) justified the use of beta-alanine toward improving ventilatory threshold (VT) (13.9%), and more importantly PWC_{FT} (12.6%). In terms of VO_{2max} and time to exhaustion, no significant differences have been observed between groups; however a study by Kim et al. (2006) on highly trained male cyclists showed significant improvement in VT and TTE (time to exhaustion) with the same effect being found on the elderly

(55-92 years) (Stout et al., 2008). If indeed carnosine concentrations increase, then beta-alanine supplementation must cause the same effect independent of age and sex and possibly improve endurance related cycling events. However, Smith et al. (2009) investigated the effects of beta-alanine supplementation on neuromuscular fatigue and muscle function in active men (19-26 years of age). Electromyographic fatigue threshold (EMG_{FT}) and efficiency of electrical activity (EEA) were analyzed, and contrary to the results mentioned by Stout et al. (2007 and 2008), EMG_{FT} and EEA values for both mid and post supplementation indicated no significant difference between the groups. This begins a debate about why active healthy males fail to decrease neuromuscular fatigue following increased carnosine concentration.

Addressing the issue of training in conjunction with beta-alanine supplementation on performance is essential for justified use of the supplement. Subjects who performed training 5 hours a day/6 days a week, plus 3 days a week performing weights followed by 12 weeks of supplementation, showed significant elevations in carnosine concentration. This resulted in significant increases in VT, TTE, and isokinetic flexion (Kim et al., 2007).

Based on the contradictory evidence outlined above, it is hard to definitively state that the effect beta-alanine supplementation has on carnosine concentration does or does not affect performance and/or aerobic or anaerobic parameter improvement. Because beta-alanine research is relatively new, a greater understanding of the definitive role of increased carnosine concentration is needed both in terms of performance increase and physiological and biochemical mechanisms as discussed in the following section.

Physiological Mechanisms Responsible for Improving Athletic Performance

There is evidence to suggest that beta-alanine supplementation improves performance both aerobically and anaerobically. The underlying mechanisms, both physiologically and biochemically, will be reviewed in this section. It has to be noted that there is a large group of studies which showed little or no effect following supplementation. These differences may be attributed to differences in dosing strategies, subject training status, duration of exercise stimulus, unfamiliarity with tests, and supplementation duration. Experimental protocol (4-6g/day for 6 weeks) and thus skeletal muscle carnosine concentration was a suggestion for failure to observe an improvement in power through repeated sprint performance (Sweeney et al., 2010). It has been clearly shown, however, that 6 weeks of supplementation of 4-6g/day is more than enough to significantly increase carnosine concentration above basal levels (Harris et al., 2006; Hill et al., 2007).

As fast-twitch muscle fibres produce excess H^+ during repeat sprint activity, it would seem sensible to assume that carnosine concentration increase would delay the onset of muscular fatigue. The authors suggest the reason for the absence of this phenomenon is due to the excessive amount of H^+ produced during the activity (not actually calculated via blood or muscle pH) exceeding the capacity of carnosine to act as an intramuscular buffer. This may be because a pH drop from 7.2 to 6.5 during high-intensity exercise (Harris et al., 2006), or repeated sprint activities which are purely based on the ATP-PC system, may not be affected by carnosine concentrations.

Studies state PCr stores and fast glycolysis make up approximately half of the required energy for a short bout (6s) of high intensity exercise (Sweeney et al., 2010). It is postulated that for

repeated sprint activities with incomplete recovery (sprint or points race), the rate of PCr resynthesis is governed by H^+ concentration, blood, and ATP concentration within the muscle (Silverthorn, 2004). This lack of PCr resynthesis may be responsible for the decrease in sprint performance, and not pH change (Bishop., 2004). The inadequate recovery time may have prevented full resynthesis of PCr as its half life is approximately 57s (Bogdanis et al. 1996). Further evidence (Bishop et al. 2004) suggests that metabolic reactions that absorb H^+ , such as sarcolemmal lactate/ H^+ or Na^+/H^+ exchange mechanisms, capillarization, changes in the intracellular strong ion difference, and muscle blood flow may be more responsible for the physiochemical buffering in muscle.

In relation to performance improvement, Derave et al. (2007) observed no significant differences between groups for 400m times (approximately 50s) as stated previously. This leads to an argument with the previous points of view (Sweeney et al. 2010), that maximal exercise for a longer duration (excess of 45s) should be significantly affected by increased carnosine concentration. The discrepancy is believed to be associated with the increased intracellular buffering capacity being insufficient to bring about an increase in 400m time, or that decreasing pH is not a limiting factor to 400m performance. Interestingly, blood and muscle pH were not calculated, so the pH drop is therefore purely speculative.

McClaren et al. (1989) suggest the decrease in muscle pH may be responsible for fatigue-induced increases in muscle EMG amplitude, resulting in neuromuscular fatigue. Furthermore, an increase in lactate concentration, and thus H^+ accumulation, increased EMG amplitude during cycle ergometry (Taylor et al. 1997), lending evidence to speculation by Moritani et al. (1993) who suggested EMG_{FT} activity may be closely related to lactate increase and pH decrease in

active muscle. Based on this statement, beta-alanine supplementation should prevent a rise in EMG amplitude, delay neuromuscular fatigue, and improve cycling performance.

Aside from neuromuscular fatigue, significant improvements in TTE and VT have been observed using beta-alanine supplementation (Stout et al., 2007) based on research by Svedahl and MacIntosh (2003). In a follow-up study, (Stout et al., 2008) similar observations were made that were attributed to an increased carnosine concentration, and its ability to delay the onset of intracellular acidosis via H^+ buffering (Marsh et al., 1993).

Through absorbance of H^+ and increased troponin sensitivity to Ca^{2+} in fast-twitch fibres (Dutka and Lamb., 2004), carnosine improves performance. Dunnett et al. (1999) highlighted the buffering capabilities of carnosine in a study on 6 thoroughbred horses, where following 30 days of beta-alanine supplementation, skeletal muscle non-bicarbonate intracellular physiochemical buffering capacity was significantly increased above that of the control. Furthermore, an increase (9.2-14.3%) in total muscle buffering following beta-alanine supplementation was directly related to an elevated carnosine concentration (Harris et al., 2006). These results were derived from the Henderson-Hasselbach equation and represent underestimated values for carnosine's contribution.

The evidence presented about carnosine's ability to increase performance through beta-alanine supplementation is equivocal. Carnosine's ability to buffer H^+ has been shown, but whether H^+ plays a significant role in fatigue, or carnosine's concentration is not significantly high enough to observe an improvement, is yet to be determined.

Beta-alanine Supplementation Mimicking Lactate Tolerance Training

Where anaerobic power is essential for success, athletes will often incorporate lactate tolerance training. Improving the lactic acid clearance ability of the muscle is characterized by sustained efforts at or above the threshold, known as lactate threshold training. Lactate tolerance training however, is characterized by maximal exercise for 20-60s in duration. By inducing muscular fatigue, the skeletal muscles become accustomed to the pain and discomfort, increasing pain tolerance, buffering mechanisms, and capacity.

For professional cyclists who utilize the ATP-PC and fast rate glycolysis systems, lactate tolerance training is a necessity (extend maximal performance duration). It has been shown to significantly increase muscle buffering capacity in anaerobic orientated sports, with subjects exposed to an 8 week lactate tolerance training protocol (sprint) experiencing a 16% increase in muscle buffering capacity in the vastus lateralis (lactate concentration $21.41 \pm 1.65 \text{ mmol kg}^{-1}$ to $25.61 \pm 2.38 \text{ mmol kg}^{-1}$). The increased lactate concentration signifies a higher production of muscle H^+ ion concentration indicating increased buffering capabilities (Sharp et al., 1986). Further research (Bell and Wenger 1988) discovered that one legged cycle ergometer training (60s maximally, 4 days a week for 7 weeks) significantly increased muscle buffering capacity (49.9 to $57.8 \text{ } \mu\text{mol HCl g}^{-1} \text{ pH}^{-1}$), as well as peak and average power.

With increased muscle buffering capacity developed independently through beta-alanine supplementation and lactate tolerance training, the combined effects of the 2 methods may cause a synergistic effect and substantially increase intramuscular hydrogen buffering. It is known that significant increases in carnosine concentration lead to an increase in muscle buffering capacity (4 to 12 weeks following beta-alanine supplementation), with lactate tolerance training often

commencing 8 to 12 weeks before competition (based on the current New Zealand sprint track cycling program, 2010). Therefore, it may be beneficial for athletes competing in sprint orientated events to combine the use of lactate tolerance training and beta-alanine supplementation.

There is overwhelming evidence to suggest that oral beta-alanine supplementation significantly increases skeletal muscle carnosine concentration. The majority of studies used a 4-12 week duration, and a dosing strategy of 2.4-6.4g/day. The most beneficial experimental protocol for increasing skeletal muscle carnosine concentration is yet to be determined due to the lack of a “ceiling effect”. However, there is contradictory evidence as to what effect, if any, this increase will have on performance, both aerobically and anaerobically. Carnosine is an intracellular physiochemical buffering molecule that is capable of binding free H^+ preventing their detrimental effect on muscle mechanics. The percentage of total buffering capacity remains unclear until further research is conducted via long term studies on a variety of sports.

Aim of the Study

Previous research has identified the effects of beta-alanine supplementation on performance increase in sedentary individuals, college wrestlers, football players, and physically active individuals, however, there is little to no research into its effects on elite cyclists. The aim of this study was to identify any possible ergogenic effects of beta-alanine supplementation for elite cyclists. Due to the prevalence of beta-alanine supplementation by elite cyclists since its introduction a few years ago, it would be beneficial to understand the possible ergogenic effect on cycling capacity in trained cyclists, and whether its continued use is supported scientifically.

CHAPTER TWO

Methodology

Subjects

Fifteen competitive cyclists (8 road, 2 road/track endurance, and 4 track sprinters) were approached and volunteered for this study because of their calibre in the Canterbury region. At the conclusion of the study, one track sprint cyclist had withdrawn. The withdrawal was due to work commitments and they were unable to attend the required dates. All subjects had been at representative level (regional, national or international) for at least four years.

The characteristics of the subjects who completed the study are presented in Table 4. There was no statistically significant difference between the groups for weight, height, age or $VO_2\max$ ($p>0.05$). Both groups completed the required supplementation protocols of their respective supplement.

The majority of the subjects ($N=12$) were recruited via personal contact with the researcher. Two cyclists had received services through the University of Canterbury Sports Science Laboratory in the past, and 1 other was recruited through word-of-mouth. Information (Appendices A and B) regarding the aims of the project, subject requirements, use of results, contact details, and a brief overview of the supplement involved were provided to all subjects in the recruitment process. It was highlighted that any subject was able to withdraw from the experiment at any time for any

reason and they were encouraged to ask questions about the study. The subjects were not required to pay for any part of the study and received no incentives or payment for participating.

At the time of the experiment, all subjects were in an endurance phase and were randomly assigned to either the beta-alanine group (N=7) (those receiving beta-alanine crystals orally) or the control group (N=7) (those receiving the placebo (maltodextrin)).

Table 4. Physical characteristics of the subjects who completed the study. Data are representative of 14 subjects and include both male and female data. (Mean \pm standard deviation)

	Beta-alanine	Control
Height (m)	1.79 \pm 0.08	1.78 \pm 0.07
Weight (kg)	76.3 \pm 7.2	73.9 \pm 4.0
Age (yrs)	20.0 \pm 1.6	21.9 \pm 2.1
VO ₂ max (ml.kg ⁻¹ .min ⁻¹)	60.7 \pm 4.8	56.7 \pm 5.8

During the supplementation phase, subjects continued with their previously planned training and competition program. Typical training consisted of 500 to 1000 kilometres in volume and encompassed a combination of flat and hill rides, ergometer training, gym work, standing start practice, and races varying from club rides to national championships. Training diaries were to be completed for the supplementation block to account for any differences observed in the results.

Prior to the study (10 weeks), each subject was sent information regarding training, nutrition and the use of sporting supplements (Appendices A & B). Each subject was advised to consume the same meal the night prior to and the morning of testing (on each occasion) and to refrain from consuming alcohol.

To ensure a “clean” experiment, subjects were asked to cease the use of sporting supplements ten weeks prior to testing. Ten weeks was chosen specifically because it enabled the complete washout (beta-alanine (9 weeks) and creatine (4 weeks) of the most common sporting supplements. Caffeine however, has a half-life in smokers or heavy caffeine users of 3.2-4.1 hours and 5.1-5.3 hours in non-users (Whitsett et al., 1984). Each subject was subsequently instructed to refrain from drinking any liquid or consuming any food or pills containing caffeine twenty-four hours prior to testing. One subject failed to comply with this and consumed caffeine (1 cup of tea) on the morning of initial testing. To standardize the results, the subject was instructed to consume 1 cup of tea before each test.

A summary of the health issues and physical stress precautions associated with the experiment (Appendix C) was provided. Before the experiment proceeded, informed, written consent (Appendix D) was obtained in accordance with regulations of the University of Canterbury Human Ethics Committee.

Experimental Overview

The study was approximately 10 weeks in length which encompassed 2 weeks of testing and a 8 week supplementation block as seen in Figure 5. The duration of the beta-alanine supplementation period was chosen specifically because it is associated with significantly elevated carnosine concentrations above basal level within the skeletal muscle (Harris et al., 2006). With all subjects having previous exposure to the testing procedures associated with the experiment, familiarization testing was not conducted.

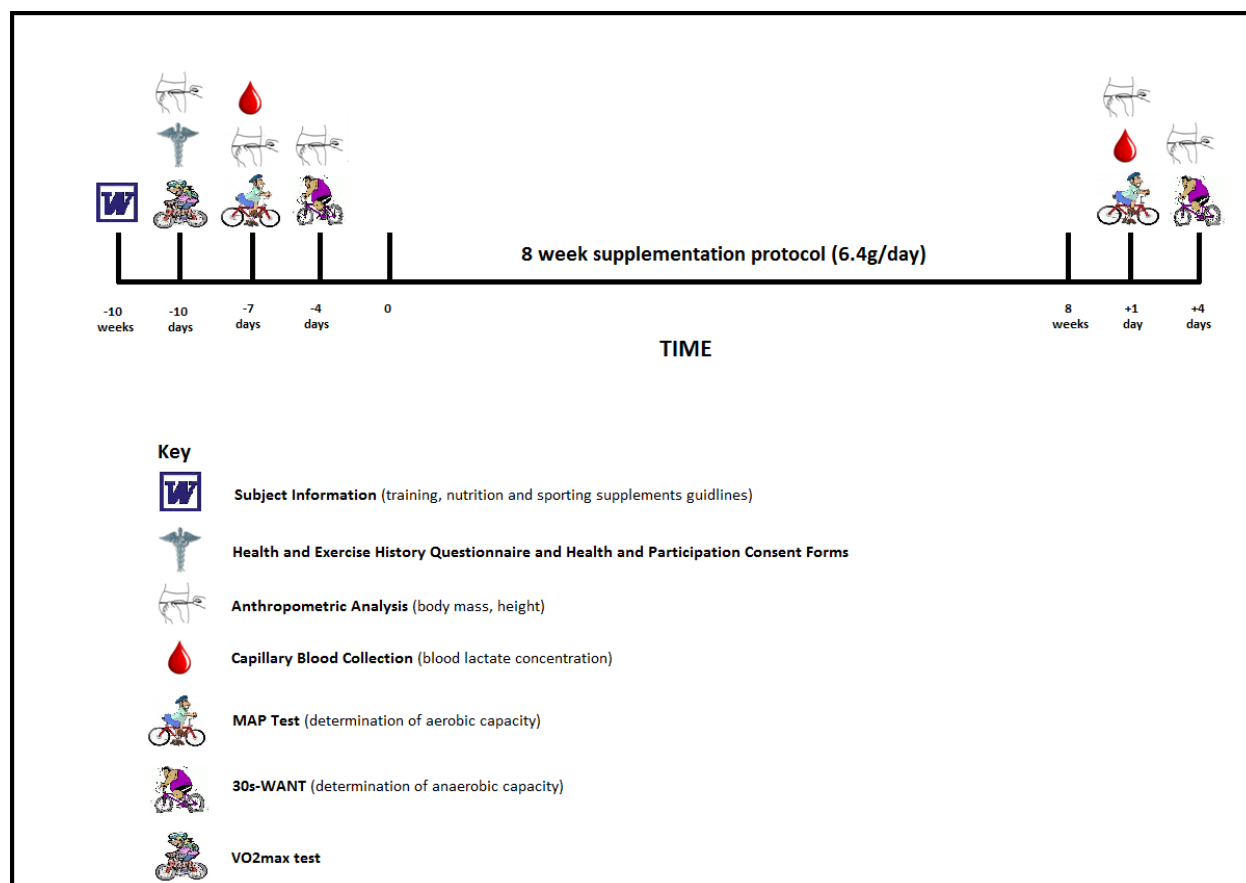


Figure 5. The experimental overview.

Anthropometric Analysis and Ergometer Set-up

Subjects reported to the University of Canterbury Sports Science Laboratory for anthropometric analysis and testing. Testing was conducted using the Velotron Cycle Ergometer (Figure 6) or Repco Cycle Ergometer (Figure 7) at approximately the same time of day to account for any variability in performance in time-of-day (Wyse et al., 1994). Fluid was consumed *ad lib* before, during and after the testing (except during the VO_2max testing due to mouthpiece restrictions and the WAnT as it was an all-out 30 second effort), and a 30cm oscillating, 3-speed fan (Model 1231, Mistral, China) was placed in front of the subject to simulate real life cycling and the air-cooling affect.

Testing took place in an indoor, temperature controlled (19-23°C) laboratory. While relative humidity was not controlled, it was measured before every test and was constantly between 50-60%. Subjects were instructed to bring in their own bikes for specifications and individualized set-up. The Velotron Ergometer was chosen over the Kingcycle due to its stability, power output control (copper flywheel controlled by magnetic force), a consistent testing set-up for all subjects, and familiarization of the apparatus to many of the subjects.

On arrival, anthropometric analysis of the subject was conducted. Height for descriptive purposes, and weight in cycling clothing using digital scales (Seca scales, model 770).

Collection of the subject's bike specifications followed to individualize the set-up of the Velotron or Repco Ergometers for the subject. Crank length (mm), seat height (cm), seat to floor (cm), front of the seat behind the bottom bracket (cm), handlebar to seat (cm) and handlebar to floor

(cm) measurements were all taken. In order to convert these values to the Velotron, a calculation had to be made to account for the stationary height of the Velotron (equation 1). The same specifications were used to calculate the required lengths and heights on the Repco Ergometer.

Both the Velotron and Repco Ergometers were connected to a PC computer.

Equation 1. Adjustment to calculate handlebar height on the Velotron.

Velotron seat to floor.....cm – Subject seat to floor.....cm =cm (A)

Subject handlebar to floor.....cm + (A).....cm =cm (required Velotron handlebar height)



Figure 6. Velotron Cycle Ergometer used for the VO₂max and MAP tests to determine aerobic capacity.



Figure 7. Repco Cycle Ergometer used for the 30s-WAnT for determination of anaerobic capacity.

VO₂max protocol

Prior to the supplementation period (10 days), a VO₂max test was chosen as a characterization test. It is commonly used as an indicator of cycling performance, although VO₂max has not always been shown to be a good predictor of endurance capacity (Mahood et al., 2001). VO₂max protocol was taken from that described by Paton (2000).

Using a PC computer and Velotron software, Velotron Ergometer calibration was conducted. The subject was instructed to initiate calibration by pedaling until the desired speed of 23km/h was attained. Instructions to cease pedaling allowed the flywheel to decrease in speed to 22km/h.

This deceleration period allowed the software to calibrate power outputs. If the power output percentage change was greater than 0.2%, the calibration process continued.

A heart rate monitor strap was fitted around the chest of the subject. Heart rate was displayed via a wristwatch (PE1500, Polar, Finland) that was mounted onto the aerobars of the Velotron. A neoprene face mask that incorporates a hole for the mouthpiece (encloses both the mouth and the nose to allow for a more effective analysis) was attached to the subject's face. The mouth piece (preVent flow sensor) was then connected to the VO_2 max mass spectrometer as seen in Figure 8 (Ultima CPX, Medgraphics). Ventilation rate and breath-by-breath analysis of oxygen and carbon dioxide was analysed and averaged every 20s. The mass spectrometer was calibrated for oxygen, carbon dioxide and nitrogen using an automated process as described by the manufacturer's instructions (Medgraphics).



Figure 8. VO_2 max mass spectrometer (Ultima CPX, Medgraphics) used for gas analysis during an incremental VO_2 max test to determine aerobic capacity.

A 20 minute warm-up preceded the test at a steady cadence and power output that was comfortable for the subject. Sprinting activities were strictly prohibited to prevent the build-up of toxic by-products. The test began with an initial power output of 100W and subsequently increased by 33W every minute until the subject was unable to continue pedaling (muscle exhaustion), could not physically continue because of breathing constraints associated with lung capacity, or could not hold the required power output at a cadence above 60rpm. The subject was given extensive encouragement throughout the test entirety. All subjects lasted between 9 and 13 minutes and absolute VO_2max was taken as the highest oxygen uptake reading (averaged over 20s) in liters, per kg, per minute.

Maximum Aerobic Power Test Protocol

Subjects completed the Maximum aerobic power (MAP) test before (7 days) and after (1 day) the supplementation period to determine the effect of beta-alanine on aerobic capacity. Lactate analysis and the point of exhaustion determined the subject's aerobic threshold, anaerobic threshold, and MAP.

Pre, during, and post test blood analysis for lactate concentrations were conducted. The lactate analyzer (1500 Sport, Yellow Springs Instruments Inc, Ohio, USA) as shown in Figure 9 was calibrated prior to each test in line with the automated procedure that requires 0 and 5mmol^{-1} lactate reference points. A known sample (5mmol^{-1}) was injected and run through twice and if the reading was within the manufacturers specified error limit of $\pm 0.1\text{mmol}^{-1}$, testing began.

Pre warm-up, the subject's blood was analyzed to determine resting lactate levels. Blood was removed via a heparanized capillary tube from the index fingertip of the left hand. If it became increasingly difficult to remove blood from the initial prick, a further incision was made to the same finger or middle finger of the same hand. A 25 μ L sample was drawn via pipette (Model 1501, Yellow Springs Instruments Inc, Ohio, USA) from the capillary tube and inserted into the lactate analyzer according to the manufacturers' instructions.

MAP test subject set-up was in accordance with the VO₂max protocol. The test began at a power output of 100W and had forty watt increments every 3 minutes. Instructions were given to the subject to keep the cadence above 60rpm otherwise the test would cease. Encouragement was given to the subject throughout the entirety of the test. Thirty seconds before the completion of each power output, a blood sample was drawn and analyzed for lactate concentration. To establish MAP, the subject was given instructions to continue to exhaustion (respiratory exhaustion or a drop in cadence below 60rpm).

MAP is determined from the penultimate power output stage completed and the time (s) at the stage they failed to complete. Aerobic threshold, anaerobic threshold and MAP were calculated using workload (W), heart rate (bpm) and lactate concentration values. Aerobic threshold was defined as a lactate concentration above "baseline" levels (the low, steady-state, lactate reading), and the anaerobic threshold was determined as the point at which lactate concentration suddenly increased above clearance levels.



Figure 9. The 1500 sport lactate analyzer used for determination of blood lactate concentrations.

30s-Wingate Anaerobic Test Protocol

The 30s-Wingate anaerobic test (WAnT) was conducted prior to (4 days) and after (4days) the supplementation period. The Repco Ergometer calculates the power profile of a cyclist and is specifically designed to predict the anaerobic power and capacity of an individual.

Calibration of the Repco Ergometer is necessary to account for day to day variation. Calibration was an engineering project by the University of Canterbury using a motor/calibration rig. The calibration was carried out as described in the methods by Raine (1993).

The subject was given instructions to cycle maximally for 30s. This is imperative because the test is used to gauge a cyclist's anaerobic power and capacity. Anything short of their maximum performance can cause misleading results. During the test, the subject was not allowed to look at

their graph of power data; however they were given verbal encouragement throughout the entirety of the test including a 10s to go call and a 5s to go call.

Once the Repco Ergometer had been specifically set-up, the subject was asked to pedal at a comfortable cadence and power output for 20 minutes. This allowed for adequate blood flow through the skeletal muscles of the legs (Bishop, 2003) which is an important aspect of any cyclist's ability to perform at any level (Burnley et al., 2005). Following the completion of the warm-up, the subject was asked to stop pedaling (to allow the hand held brake to be applied) and then perform a 4 second maximum effort. This stimulates the neurological pathway associated with muscle activation and relaxation. It is crucial because of the high cadence activity associated with maximum effort on the Repco Ergometer and allows the subject to activate their central nervous system (CNS) and stimulate maximum firing patterns. Following the 4 second effort, the subject pedaled lightly for 5 minutes. This gave the muscles adequate time to recover and expel any toxic by-products. The theory behind CNS activation in repeated cycling ergometer training has been difficult to prove; however results have shown that the second maximum effort produces greater power (Glaister et al., 2003).

Gear selection is an important aspect. The subject was instructed to choose which gear they felt comfortable with. Because most of the subjects had completed such a test before, they chose on past performances. For the minority (N=5) of the group who had not completed a WAnT test, advice from the researcher was given. The ergometer has 7 gears to choose from. Each subject used gears 2-5, 5 being the hardest and associated with a slower cadence. It was explained to the

subject that the gear chosen for the first 30s-WAnT would have to be the same gear used for the second. A hand-held mechanical brake held the subject and flywheel (Figure 8) in place. With a 5s countdown the subject proceeded to cycle maximally for 30s. At the completion, the subject was instructed to pedal lightly for 5-10 minutes to allow for the release of any anaerobic by-products. Data collected from the test provided the subject's power over the 30s test as indicated by Figure 10. Peak power, finishing power, watts per kilogram, and average power were all provided. Fatigue Index was calculated using the following equation.

Equation 2. Fatigue Index calculation using data from the WAnT.

Peak power – Finishing power / Peak power = Fatigue Index (% change)

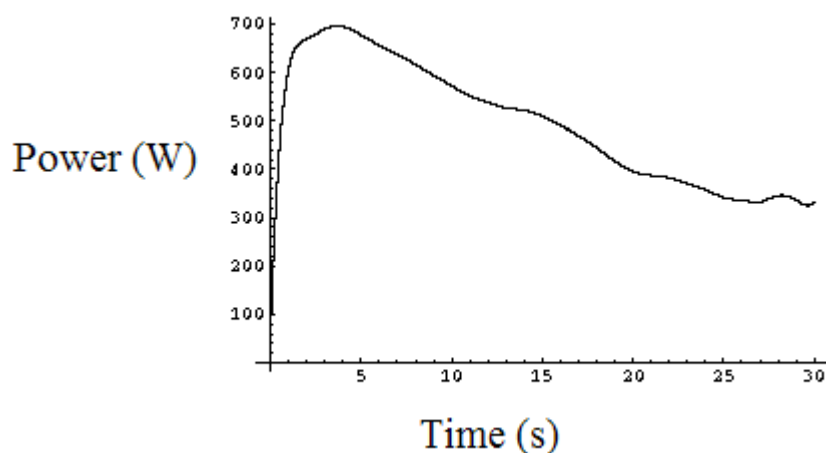


Figure 10. Repco Ergometer data analysis readout example following a 30s-WAnT.

Experimental Protocol

At the conclusion of the first set of testing, the 8 week supplementation protocol began. Beta-alanine is a commercially available substance and is commonly used throughout the sporting community for performance increase. Beta-alanine was ordered in ten one kilogram bags and was subsequently split into 7 separate containers labeled carefully with the subjects' names and the required dosage. Five ml vials were used to carefully provide the supplement in the required amount (3.2g per serving) and transfer to a 200 ml glass of water. Subjects were instructed to stir in the powder until it had dissolved and then consume. Consumption was twice per day for the duration of the experiment. The same protocol was associated with the control group (maltodextrin). Maltodextrin is commonly used as a control (Derave et al., 2007) because of its close resemblance (white crystalline powder) to beta-alanine.

Prior to the study (10 weeks), subjects were provided with written and verbal information from the researcher regarding potential risks associated with the consumption of the substance. Such risks commonly associated with beta-alanine supplementation is paresthesia or the “tingling sensation” and can last anywhere from 10 to 45 minutes.

Statistical Analysis

To gain a better understanding of the results, mean analyses and both within and between groups variances were conducted. The results are presented as mean \pm SD. This method was employed to take into account the types of training each of the subjects had done previously to see if that had had any effect on the results that the ANOVA may not have picked up.

The mixed design ANOVA was used to detect any differences within group and between groups for treatment and the statistical significance was set to 5% ($p < 0.05$). VO_2max , weight, age, height, maximum aerobic power (MAP), anaerobic threshold, aerobic threshold, watts per kilogram (W/KG) of MAP, fatigue index, average power, average W/KG, peak power, finishing power, training volume, training intensity and final lactate concentrations were all analyzed using this method. Data were analyzed using the Statistical Package for Social Sciences (SPSS) and Microsoft excel 2007.

Funding

Beta-alanine production companies funded no part of this study nor took part in any planning or implementing the design of the study. This policy protects the study from alleged bias toward results that could be used for commercial gain by the manufacturer.

The study was supported with the funding from The Biological Sciences Department at The University of Canterbury and with help from the University of Canterbury Recreation Centre, in particular the Sports Science Laboratory.

CHAPTER THREE

Results

In this chapter the descriptive data and inferential analysis findings for the study are presented.

The descriptive data are presented as mean \pm SD. Subjects completed a MAP test and 30s-WAnT on separate days pre and post supplementation. The MAP test and 30s-WAnT were used to analyze whether 8 weeks of 6.4g/day beta-alanine influenced aerobic and/or anaerobic capacity parameters respectively in trained cyclists.

Training Volume and Intensity

Training intensity and training volume for the control and beta-alanine groups are shown in Table 5. During the 8 week supplementation block, there was no significant difference between the control and beta-alanine groups for training volume ($F_{(1,9)} = 0.599$, $p = 0.459$) and training intensity ($F_{(1,9)} = 0.218$, $p = 0.652$). Training intensity was characterized as one of three levels. Flat terrain training session from 1 to 4 hours in duration were classified as a 1, an ergometer session or long (3 hours +) hill ride was classified as a 2, and sprint training exercises or gym work was classified as a 3. Following the supplementation period, intensity values were added up and averaged over the total number of training sessions completed.

Table 5. Average training volume and intensity for the control and beta-alanine groups during the 8 weeks of supplementation. (mean \pm SD)

Parameters	Control	Beta-alanine
Training volume (minutes/week)	746 \pm 134.2	760 \pm 160.1
Training intensity	1.71 \pm 0.4	1.95 \pm 0.4

MAP Test

The control and beta-alanine groups completed a MAP test pre and post supplementation. As can be seen in Table 6, there was minimal change for both groups over time for maximum aerobic power (MAP), aerobic threshold (AeT), and anaerobic threshold (AnT). The trend in the data also shows very little change in the final lactate concentration and watts per kilogram (W/KG) of MAP for both groups. AeT for the control group decreased following the experimental protocol (Table 6), whereas the beta-alanine group saw a rise of 8.6W following the supplementation period. Of interest is the change in final lactate concentration of the beta-alanine group. The 1.63mmol⁻¹ decrease in average lactate concentration was caused by subject 9 whose concentration of 5.83mmol⁻¹ was low in comparison to the 13.57mmol⁻¹ attained in the first MAP test. The lack of change in the parameters (mean) resulted in there being a non-significant difference between groups for MAP ($F_{(1,12)} = 1.398$, $p = 0.261$), AeT ($F_{(1,12)} = 0.759$, $p = 0.401$), AnT ($F_{(1,12)} = 1.070$, $p = 0.321$), final lactate concentration ($F_{(1,12)} = 1.983$, $p = 0.184$), and W/KG of MAP ($F_{(1,12)} = 1.054$, $p = 0.325$). Based on these results, 8 weeks of 6.4g/day beta-alanine does not significantly change the MAP, AeT, AnT, and W/KG of MAP in trained cyclists.

Table 6. Average aerobic capacity parameters following the MAP test pre and post supplementation for the control and beta-alanine groups. (mean \pm SD)

Parameters	Control		Beta-alanine	
	Pre	Post	Pre	Post
MAP (W)	358.6 \pm 38.0	358.0 \pm 34.6	395.7 \pm 72.2	384.3 \pm 54.2
AeT (W)	221.4 \pm 27.9	212.9 \pm 30.4	235.7 \pm 67.8	244.3 \pm 61.3
AnT (W)	295.6 \pm 37.8	293.6 \pm 31.7	322.1 \pm 72.3	321.4 \pm 57.3
W/KG of MAP	4.8 \pm 0.4	4.9 \pm 0.3	5.2 \pm 0.8	5.2 \pm 0.7
Final [La ⁻] (mmol ⁻¹)	10.1 \pm 1.5	10.0 \pm 2.4	12.3 \pm 1.7	10.6 \pm 3.2

30s-WAnT

Subjects completed a 30s-WAnT pre and post supplementation 3 days after the completion of the MAP test. The trend in the data was as shown in Table 7 and indicates minimal change occurred for both groups over time for peak power, minimum power, fatigue index (FI), average 30s maximum power, and average watts per kilogram (avge W/KG) of 30s maximum power. The minimum power decreased in the control group (45.3W) and increased in the beta-alanine group (26.3W), however both were non-significant. There was a non-significant difference between the control and beta-alanine groups for peak power ($F_{(1,12)} = 0.533$, $p = 0.479$), minimum power ($F_{(1,12)} = 0.907$, $p = 0.360$), average 30s maximum power ($F_{(1,12)} = 1.122$, $p = 0.310$), avge W/KG of 30s maximum power ($F_{(1,12)} = 0.827$, $p = 0.381$), and FI ($F_{(1,12)} = 0.004$, $p = 0.953$). Based on

these results, 8 weeks of 6.4g/day beta-alanine does not significantly change any of the above anaerobic capacity parameters.

Table 7. Average anaerobic capacity parameters following the 30s-WAnT pre and post supplementation for the control and beta-alanine groups. (mean \pm SD)

Parameters	Control		Beta-alanine	
	Pre	Post	Pre	Post
FI (%)	59.9 \pm 6.4	58.7 \pm 5.7	61.0 \pm 6.9	57.2 \pm 7.8
Avge 30s max power (W)	835.3 \pm 137.6	789.6 \pm 149.8	899.1 \pm 142.2	884.7 \pm 134.5
Avge W/KG of 30s max power (W)	11.2 \pm 1.7	10.8 \pm 1.8	11.9 \pm 1.4	11.7 \pm 1.5
Peak power (W)	1339.0 \pm 251.2	1280.4 \pm 268.8	1434.3 \pm 321.5	1412.3 \pm 321.4
Minimum power (W)	555.3 \pm 86.8	510.0 \pm 78.5	556.3 \pm 84.2	582.6 \pm 72.3

CHAPTER FOUR

Discussion

Effect of Beta-alanine on Aerobic and Anaerobic Capacity

The aim of the study was to examine the effect of an 8 week 6.4g/day beta-alanine supplementation protocol on aerobic and anaerobic capacity in trained cyclists. My study did not detect any significant difference between the beta-alanine and placebo groups following supplementation over time for any of the parameters measured. The lack of change in aerobic parameters is supported by previous evidence (Stout et al., 2007), while anaerobic capacity parameter evidence provided by Sweeney et al. (2010) and Hoffman et al. (2006) is similar to the current study also. Discrepancies in relation to Derave et al. (2007) and Kern et al. (2010) may be related to experimental protocol length, dosage, and/or the training status of the subjects.

With approximately two decades of research into the potential ergogenic effect of increased carnosine concentration through beta-alanine supplementation, evidence presented in this field is equivocal. Some suggest there is an ergogenic effect (Hoffman et al., 2006), while other studies offer little proof of such an event (Derave et al., 2007). It has to be recognized that there is substantial evidence (Harris et al., 2006; Hill et al., 2007) of increased skeletal muscle carnosine concentration following a beta-alanine supplementation protocol. A 37-47% increase in skeletal muscle carnosine concentration in the soleus and gastrocnemius has been identified (Derave et al., 2007), with an increase as high as 80% following 10 weeks of supplementation (Hill et al.,

2006). Both studies were associated with an increase in performance suggesting there may be a potential ergogenic effect, however, my study did not detect such a change assuming an increase in skeletal muscle carnosine concentration.

Due to the inability to conduct a cross over design (scheduling issues associated with elite athletes), I cannot conclude that beta-alanine supplementation does not improve capacity.

Identifying capacity change was not aided by the statistical power of this experiment (7 subjects per group); therefore a future study examining the same protocol on a significantly greater number of subjects would be beneficial.

Experimental Protocol

Previous research (Harris et al., 2006) identified significant increases in skeletal muscle carnosine concentrations (42.1-65.8%) following 4 weeks beta-alanine supplementation (3.2-6.4g/day).

Due to the significant cost of proton MRS (\$1000/hr) and the objection of subjects to invasive muscle biopsies (prevent training), identifying the possible rise in skeletal muscle carnosine concentration was not possible. Therefore, based on assumption and previous evidence (Harris et al., 2006, Hill et al., 2007), an increase in skeletal muscle carnosine concentration in the beta-alanine group would have been expected.

The current study utilized a 3.2g twice a day strategy, unlike previous studies (Kendrick et al., 2009) which subjected subjects to a 400-800mg dose. The 400-800mg dose is thought to prevent the effect of paraesthesia, and any associated problems with the current strategy are specific to timetable constraints and transport issues. All subjects involved were current students or full-time employees, which would have caused issues with administering the correct dose at the correct time. Therefore, a 3.2g dosage was employed every morning and evening to ensure an uncomplicated effective protocol. Some of the subjects (N=4) commented on paraesthesia, but as a result of previous consumption, all were well aware of the ensuing feeling, and were able to cope.

The 3.2g serve may have caused alterations to the rate and total increase in skeletal muscle carnosine concentration. There is a rate limit to beta-alanine absorption (number of β -amino transporters) due to the identification of a beta-alanine saturation level within the gastrointestinal tract (Harris et al., 2006). Therefore, the absorption rate and percentage of the 3.2g dose may alter final carnosine concentration, and thus capacity.

With evidence showing $10\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}$ stimulates 10-70% V_{max} of transporters over time (Harris et al., 2006), it is possible that the increased dosage may in fact enhance carnosine synthesis. However, following initiation of carnosine synthesis (carnosine synthase), the degradation (carnosinase) may alter total carnosine concentration. Due to the stability of carnosine, the degradation timeline is increasingly slower than other molecules studied thus far (Baguet et al., 2009). Approximately 10 weeks will be required for carnosine levels to return to baseline in

individuals who increase their concentration by 55%, and 20 weeks for individuals who increase their levels by as much as 80%.

Subject Responsibility

Subject responsibility is a major discussion point for study reliability. Based on data published concerning beta-alanine (Baguet et al., 2009), and creatine washout (Greenhaff, 1995), subjects were asked to refrain from beta-alanine and creatine supplementation 10 weeks prior to the study. With creatine significantly enhancing sprint performance (Volek et al., 1997), failure to comply could significantly alter results. Subjects may also have unknowingly ingested a significant portion of beta-alanine naturally through the consumption of chicken, beef, or pork. Increases in carnosine concentration following the ingestion of chicken broth were identified (Harris et al., 2006), equivalent to $10\text{mg}\cdot\text{kg}^{-1}\cdot\text{bwt}$ per day. However, it is unlikely that this had an effect, as the aerobic and anaerobic capacity parameters for both the control and beta-alanine group did not change (although consumption of beta-alanine through foods was not controlled).

Kern et al. (2010) and Stout et al. (2008) observed significant improvements in performance following beta-alanine supplementation, while others (Smith et al., 2009) failed to see such an effect. There was a lack of change in aerobic and anaerobic capacity following beta-alanine supplementation in the current study. It has been established that elite athletes have a carnosine concentration baseline that is significantly elevated above sedentary individuals (Parkhouse et al., 1985), suggesting 8 weeks of $6.4\text{g}/\text{day}$ was insufficient to substantially increase carnosine concentration above this. Similar findings were published (Derave et al., 2007), suggesting 400m

running times were not significantly increased in sprint athletes due to the subjects' training status which correlates well to the kilo.

An investigation into the effects of repeated creatine supplementation on total PCr stores concluded that following initial supplementation, total PCr stores rose by 45%, followed by a 22% decrease after a 30 day washout period, and rose again significantly by 25% following another 5 day cycle (Rawson et al., 2004). This indicates 1 of 2 possibilities; there is either a PCr store maximum limit, or the second dosing cycle failed to raise PCr stores to the achieved level of the first cycle. In relation to the current study (in which the majority of the subjects had used beta-alanine previously), a mechanism preventing carnosine concentrations achieving the level attained by initial supplementation may be involved. I can only speculate as to what this mechanism may be, but I suspect that it may be a similar response to that observed in multiple creatine exposure studies. Creatine transporters responsible for extracellular creatine absorption become down-regulated (Guerrero-Ontiveros and Wallimann, 1998) with continual creatine supplementation. It is when the upper limit within skeletal muscle is attained ($150\text{-}160\text{mmol}\cdot\text{kg}^{-1}$ of dry muscle) that creatine transporter synthesis declines and/or stops completely. In relation to continual beta-alanine supplementation (which may have been the case for the subjects involved in this study), a decline in β -amino transporter synthesis (responsible for beta-alanine absorption) may occur, although to date, a "ceiling effect" has yet to be determined.

A training effect, placebo effect, and/or carbohydrate ingestion may have played a role in the current study. The timing of the study was specifically chosen to try and control training volume

and intensity. A training effect may not have been a causative factor influencing results as indicated by Table 4 (training volume and intensity). With relation to tiredness, accumulation of metabolic by-products, and/or dehydration, all of which have been known to disrupt exercise performance (Cheung et al., 2003; Maughan, 2003; Shirreffs, 2005), training in the week prior may have caused varying results because of test intensity.

The current research was a blind study. However, prevention of subjects determining treatment was impossible leading to the lack of a placebo effect due to paraesthesia accompanying beta-alanine supplementation. Controlling this situation meant tests independent of effort and motivation were conducted (Step test). Subjects could not unduly influence any of the blood parameters utilized within this test. With relation to the use of beta-alanine as a sports supplement in elite cycling, it is not to say it does not significantly improve capacity indirectly. By telling the cyclist population that consumption of beta-alanine will improve performance, they subsequently train harder and improve performance, a true placebo effect.

Beta-alanine supplementation studies have failed to identify any possible effect hydration and carbohydrate ingestion may play on performance. Hypohydration and dehydration can degrade exercise capacity (Maughan, 2003) and may alter results. Carbohydrate ingestion was only controlled for the evening prior to and morning of the tests with subjects receiving instructions regarding food consumption (eat exactly the same food prior to both sets of testing). It has been shown (Sparks et al., 1998) that pre-exercise carbohydrate intake does not alter performance, however, evidence of repeated solid carbohydrate feedings maintaining blood glucose levels,

reducing muscle glycogen during prolonged exercise, and enhancing sprint performance at the end of such activity have been provided (Hargreaves et al., 1984). If not intentionally kept constant, carbohydrate and hydration levels may have influenced aerobic capacity parameters due to the length of the current study (including warm-up) at 33-45 minutes in duration.

Fatigue and Buffering Mechanisms

A lack of change following 8 weeks beta-alanine supplementation may be two-fold based on previous observations (Derave et al., 2007). Either assumed elevations in carnosine concentration were not sufficiently high enough to increase the physicochemical buffering ability of the skeletal muscles, or an increase in H^+ plays one of many roles in fatigue.

Due to its acidic properties and the subsequent acidosis that arises, excess H^+ structurally change enzymes linked to ATP production and alter transport mechanisms (Hultman and Sahlin, 1980) and substrate availability. When pH drops as low as 6.5 (Davey, 1960), the fundamental mechanics of muscle contraction are hampered with the accumulation of excess H^+ . This occurs through inhibition of the actomyosin ATPase (which is solely responsible for the breakdown of ATP), prevention of cross bridge formation (Cooke et al., 1988) and relaxation, and decreased Ca^{2+} release/absorption from the SR (Lamb et al., 1992). Excess concentration can also alter calcium-troponin binding, and induce neuromuscular fatigue via EMG amplitude increase (Taylor et al., 1997). In association with metabolite accumulation, performance/capacity can decrease.

It would seem safe to assume that acidosis (pH decrease) significantly influences fatigue during glycolytic fibre activation (sprint cycling (Ross et al., 2001)). With no change in aerobic and anaerobic capacity in the experimental group, it has to be assumed that either the assumed increase in carnosine concentration was insufficient to buffer this H^+ ion concentration increase, baseline carnosine concentrations were already significantly elevated with supplementation having little to no effect, or other factors are associated with fatigue. With carnosine and anserine making up to 40% of total muscle buffering (Davey, 1960), it could be assumed that the carnosine concentration was not sufficiently high enough to buffer the increased H^+ ion concentration. With respect to recent evidence, however, (7% role in total muscle buffering, (Mannion et al., 1992)), it would suggest irrespective of carnosine's concentration increase, the ability to improve capacity via physicochemical buffering is not solely reliant on the H^+ buffering mechanisms of carnosine.

Other possible physicochemical buffering options are employed in the muscle, and suggested to play a significant role in H^+ ion buffering. Metabolic reactions that remove H^+ such as sarcolemmal lactate/ H^+ and Na^+ / H^+ exchange mechanisms, as well as capillarization, muscle blood flow, and changes in the intracellular strong ion difference, may be more responsible for the physicochemical buffering in muscle (Bishop et al., 2004). Based on this evidence, increasing carnosine concentration may only play one of many roles associated with fatigue.

In a review by Allen et al. (2008), it was suggested that human muscle fatigue often occurs without a large increase in H^+ . With a minor drop in muscle pH (6.9), rat gastrocnemius muscle tetanic force has been shown to significantly decrease by 60% (Baker et al., 1994) clearly

indicating the association of muscular fatigue with other factors. Furthermore, when fatigued muscle undergoes a rest period, force production regenerates substantially faster than pH levels. This indicates that low pH *per se* is not solely responsible for force reduction (Baker et al., 1993; Cady et al., 1989).

Isolated mammalian muscle research at physiological temperatures and significantly low pH levels (6.3), has highlighted the true effect of increased H⁺ ion concentration on anaerobic and/or aerobic capacity. At 37°C and pH 6.3, cat muscle force production decreases by 5-10% (Adams et al., 1991), while at 32°C and pH 6.67, mice muscle maximal tetanic force decreases by a mere 10% with no significant slowing of maximum velocity shortening (Westerblad et al., 1997). Based on the evidence, a lack of change in aerobic and anaerobic capacity during the current study may be associated with other factors other than an increase in H⁺ ion concentration.

As H⁺ ion concentrations increase, Ca²⁺ affinity for the SR Ca²⁺ATPase pump decreases (Donaldson et al., 1978; Allen et al., 2008). During high intensity exercise, this concentration increase would be expected to reduce skeletal muscle force production and rate of contraction. Therefore, with an assumed increase in carnosine concentration, anaerobic capacity should improve. Results however, indicate anaerobic capacity did not change following beta-alanine supplementation. This lack of change may be because evidence has suggested H⁺ increase skeletal muscle force production (removal may decrease force production). Reasoning is twofold; firstly, H⁺ compete for troponin C's binding site reducing Ca²⁺ affinity (twofold) (Baker et al., 1995), and secondly, Ca²⁺ affinity for the SR Ca²⁺ATPase pump is reduced.

Demonstration of such events was shown to occur when pH reduction (7 to 6.3) identified a fivefold decrease in affinity (Ca^{2+} for SR Ca^{2+} ATPase pump) (Wolokser et al., 1997). Due to this vast affinity difference, at acid pH, Ca^{2+} concentration is consequently higher, leading to an increased skeletal muscle force response (Baker et al., 1995). Therefore, a pH decrease during intense exercise may be responsible for maintaining force production. Based on the information provided, lack of capacity change in the experimental group may be associated with absorption of excess H^+ (assumed carnosine concentration increase) decreasing skeletal muscle force production.

Increasing capacity/performance through beta-alanine supplementation and subsequent carnosine concentration is well established (Derave et al., 2007; Kern, 2010; Stout et al., 2007 and 2008). However, the dominant energy system and duration of maximal effort determines the total ergogenic effect of the supplement.

A 16% increase in total work performed during 150s of exhaustive exercise at 110% max power output was demonstrated (Hill et al., 2007). Similarly, significant fatigue improvement following 5 bouts of maximum voluntary contraction (45s each) was observed also. The current study utilized a 30s-WAnT, the standard gauge of anaerobic capacity. In this case, test duration may not have been long enough to utilize the H^+ buffering abilities of carnosine, indicating other factors influencing capacity. Future carnosine research should incorporate maximal effort duration of 45s or more to allow substantial incorporation of the anaerobic system (fast rate glycolysis) and subsequent metabolite accumulation.

The accumulation of toxic metabolites may be a significant cause for muscular fatigue during maximal exercise (less than 45s in duration). Alterations to muscle machinery may prevent the necessary skeletal muscle force production being accomplished. Accumulation of La^- , H^+ , ADP, Pi, IMP, NH_3 , Mg^{2+} , and inorganic phosphate (PO_4^-), a decrease in PCr synthesis, and an increase in extracellular K^+ all result in muscular fatigue. Based on this accumulation, it would seem safe to suggest a decrease in pH is only one of many limiting factors involved in aerobic and anaerobic capacity of maximal contraction (45s duration or less).

An example of such is ADP, the result of the high energy splitting of ATP (Westerblad et al., 2002) during cross-bridge formation (McLester Jr, 1997). During fatigue, this accumulation can cause a slowing of the rate constants (decreased velocity of muscle shortening), and thus power output. In combination with ADP, PO_4^- accumulation reduces skeletal muscle force production through the prevention of Ca^{2+} release (SR Ca^{2+} -ATPase pump) (Allen and Westerblad, 2001), and through its profound effect on skeletal muscle force reduction (Phillips et al., 1993). During a cycling sprint, PCr stores become depleted resulting in an increase in phosphate ions (Hirvonen et al., 1987), decreasing force production, and subsequently affecting anaerobic capacity. A combination of ADP and PO_4^- has been shown to decrease force production, and may explain why aerobic and anaerobic capacity parameters did not change following beta-alanine supplementation.

Due to the WAnT duration (30s), a decrease in pH may not have been responsible for a lack of change in anaerobic capacity as shown during short repeated exercise bouts (Sweeney et al.,

2010). PCr stores and fast glycolysis each make up approximately half of the required energy during short exercise (6s) (Sweeney et al., 2010). It is therefore postulated for repeated sprint activities with incomplete recovery (scratch and points races), that PCr resynthesis rate governs muscular fatigue. In conjunction, a lack of PCr resynthesis may be responsible for a decrease in sprint performance, not H^+ ion accumulation (Bishop, 2004). The toxic effects of metabolite accumulation (H^+) may not solely be responsible for fatigue in terms of the current study (a 30s non-repeat test) based upon other physicochemical buffering mechanisms mentioned previously.

The current study found there to be no significant difference between groups for any of the anaerobic capacity parameters (Table 6). It is possible without controlling for these other previously discussed mechanisms; one or more may have been responsible for a lack of change between the 2 groups. This lends evidence to the belief that H^+ accumulation may only play one of many roles in muscular fatigue.

Genetic Profile on Carnosine Synthesis

Pharmacogenetics is the study of how genetic variability impacts on desired and undesired drugs, and a legitimate reason why there was a lack of change in aerobic and anaerobic capacity following beta-alanine supplementation. In relation to beta-alanine absorption and carnosine synthesis rate, there is a real possibility that a lack of change is associated with this phenomenon using evidence presented in a creatine study (Syrotuik and Bell, 2004). Irrespective of the supplementation protocol, skeletal muscle creatine concentrations can vary between individuals based upon their biological profile (initial creatine levels, number of type II muscle fibres and

greatest preload muscle fibre cross sectional area) and explain why there is equivocal evidence in creatine supplementation studies.

A favourable preexisting biological profile determines the final extent to which individuals respond to supplements (Syrotuik and Bell, 2004). Subjects are less likely to gain the full benefits of creatine supplementation if they have a higher pre-load level of creatine, less type II fibres, small pre-load muscle cross sectional area, and a lower fat-free mass. In regard to this theory, extracellular concentrations of glucose have been shown to influence inter subject creatine stores and capacity variability (Harris et al., 1992; Terjung et al., 2000; Williams et al., 1999).

Even with a high pre-existing skeletal muscle carnosine concentration, subjects supplementing with beta-alanine can still significantly elevate concentrations (Derave et al., 2007). Although a lack of research on beta-alanine response is available, a number of biological variables underlie the carnosine concentration increase among individuals. Such variables may include β -amino transporter abundance and activity within the gastrointestinal tract, Na^+ channel abundance and activity (beta-alanine transportation), carnosine synthase and carnosinase activity, and histidine abundance within the body. Individuals who can maximize all of these activities will increase their carnosine concentration by the greatest amount.

When a beta-alanine group increased skeletal muscle carnosine concentrations significantly (Hill et al., 2007), there was a percentage increase variability within the group. Following 4 weeks beta-alanine supplementation, concentrations varied from 7-13.4mmol kg⁻¹. After 10 weeks,

concentrations were 11.9-18.5mmol kg⁻¹ above pre-supplementation level. It is clear some individuals utilize beta-alanine more efficiently than others.

The current study identified subjects 9, 14, and 15 showing substantial fatigue index decreases (64.4 to 57.3%, 64.7 to 58.1%, and 65.6 to 49.7% respectively), further highlighting the importance of a biological profile. Furthermore, carnosine washout rate in “responders” has been identified at 3.5%/wk and 2.5%/wk in “non-responders” (Baguet et al., 2009); suggesting carnosinase enzyme activity is different between individuals. This evidence may indicate why a lack of change in aerobic and anaerobic capacity in the beta-alanine group was observed, and the need for a biological profile among subjects for future studies.

Study Limitations

There were several study limitations associated with the current study. A lack of statistical power (14 subjects) may have prevented beta-alanine’s true effect on aerobic and anaerobic capacity.

Study nature (elite athletes) and scheduling issues (training/racing commitments) restricted subject availability in comparison to similar research (Hill et al., 2007); whose 25 subjects significantly increased statistical power.

Training between individual subjects was not controlled for two reasons. Firstly, each subject did not want to conform to a modified training schedule which may have disrupted their capacity and/or volume, or their intensity. Secondly, the true effect of beta-alanine supplementation on elite cyclists would not have occurred (problematic issue studying elite athletes of any variety, is

the response the training effect has on results). Conducting the study in this nature gave the true effect of beta-alanine supplementation on elite athletes (response to the substance may vary among types of athletes and the stage of training). However, training in the week leading up to testing (both occasions) may have caused varying results due to tiredness, accumulation of metabolic by-products within the muscles, muscle soreness, or even dehydration, which have been known to disrupt exercise performance (Cheung et al., 2003; Maughan, 2003; Shirreffs, 2005).

Conducting a quantification test via proton MRS or muscle biopsy (skeletal muscle carnosine concentration analysis) would have been advantageous. Refusal to undergo an invasive procedure and cost related issues meant performing these tests was impossible. Analysis would have indicated skeletal muscle carnosine concentrations following the experimental protocol. Based on previous research (Harris et al., 2006), it can only be assumed the study protocol significantly increased skeletal muscle carnosine concentrations which did not change aerobic or anaerobic capacity parameters in the experimental group.

If subjects underwent a biological profile, this would have further enhanced the validity and overall power of the research. Analysis would have incorporated muscle fibre analysis (type and distribution), baseline carnosine concentration analysis, and carnosinase and carnosine synthase enzymatic activity. Identification of “responders” and “non-responders” to beta-alanine supplementation and carnosine synthesis could have explained why this study, and others like it

(Sweeney et al., 2010), did not observe a change capacity and/or performance in comparison to others (Derave et al., 2007).

Neglecting blood and/or muscle pH measurement has been a common theme of beta-alanine research thus far. Cases where lactate ion concentrations have increased (exercise) following beta-alanine supplementation (Zoeller et al., 2007), leads to the assumption that subjects with an increased skeletal muscle carnosine concentration produce more lactate ions during exercise. This would be made possible through the increased physicochemical buffering ability of carnosine (absorbing excess H^+ maintaining an alkaline pH). In this study, blood and muscle pH measurements were not conducted, and the subsequent acidosis effect can only be assumed once again. It would therefore be advantageous to analyze blood and muscle pH, pre and post 30s-WAnTs. This would determine whether an increase in lactate ion concentration corresponded to a decrease in pH, or whether the increased skeletal muscle carnosine concentration utilized its H^+ buffering ability keeping pH neutral for increased exercise capacity. It has to be noted however, that final lactate concentrations did not significantly differ between groups.

Subject motivation and reliability is a study limitation. Elite subject use brought into account the law of diminishing effects. The law of diminishing effects would suggest significant improvement observations are hard to come by. It states that the closer an athlete gets to their biological limit, the less effect can be gained from the same training compared to someone far from their biological limit. It has to be noted that elite athletes have been shown to be more reliable than non-elite athletes in tests used to simulate performance (Hopkins et al., 2001). In

comparison with other studies (Stout et al., 2008), use of elite athletes ($\text{VO}_{2\text{max}} = 60.7 \pm 6.8 \text{mmol.kg}^{-1}.\text{min}^{-1}$) meant there was a chance the subsequent increase in skeletal muscle carnosine concentration following beta-alanine supplementation may not have been sufficient to significantly increase aerobic and/or anaerobic capacity. This may be because elite athletes already have effective buffering systems in place simply because their training regimes demand this, and the addition of more carnosine to their skeletal muscle might not elicit much, if any change.

Future Research

Beta-alanine supplementation future research needs to address a few aspects to further the knowledge of this supposed performance enhancing supplement. Consideration of a biological profile will give us greater insight into not only the synthesis rate and percentage increase, but determine why or why there was not a change in capacity or performance. An example may include subjects significantly improving their performance while 2 others failed to show such change. Further analysis of their biological profile might give insight into why this was.

Experimental protocol is an important aspect into performance/capacity improvement. Future research needs to identify the rate of carnosine synthesis as there appears to be no “ceiling effect” for this molecule within skeletal muscle. A study at least 24 weeks in duration utilizing both elite and sedentary subjects should suffice. In combination, proton MRS or muscle biopsy analyses needs to be conducted every 2 weeks to gauge the synthesis rate. In conjunction with duration, dosing protocol needs to be carefully considered. The maximum dosage utilized thus far is 6.4g/day. Because paraesthesia occurs with $10 \text{mg.kg}^{-1}.\text{bwt}$, increasing the dosage could be

considered difficult. If, however, multiple 800mg doses several times a day were directed, 10g/day could be achieved. In conjunction with a 10g/day, 24 week study, beta-alanine's complete effect on skeletal muscle carnosine synthesis can be identified.

Elite and world class athletes dominate the use of beta-alanine supplementation. It would seem beneficial for future research to identify beta-alanine supplementation effects on a variety of world class athletes, taking into account the law of diminishing effects (subjects close to their biological limit). Furthermore, the effects of supplementation should be considered on actual sporting events. For example, the use of a kilometer time trial or 4 kilometer pursuit should be considered for anaerobic capacity over a 30s-WAnT. Furthermore, studies should include a variety of tests on both men and women as the physiological effects of beta-alanine supplementation may differ between sexes.

Conclusion

Various experimental protocols have changed duration and dosing strategy to examine the effects of beta-alanine supplementation on aerobic capacity, anaerobic capacity, neuromuscular fatigue, carnosine synthesis, and performance improvement. Results, however, have been equivocal thus far. The data from this study identified that aerobic and anaerobic capacity did not change following beta-alanine supplementation in highly competitive cyclists who underwent a similar training regime. The lack of a significant improvement suggests that the assumed increase in carnosine concentration was insufficient to utilize its physicochemical buffering ability, subject's basal level carnosine concentrations were significantly elevated where beta-alanine

supplementation would not alter its concentration, and/or pH only plays one of many roles associated with muscular fatigue.

Based on the data provided, it would seem like a waste of time for elite cyclists to spend money on beta-alanine to elicit a performance/capacity improvement. The law of diminishing effects states that being in close proximity to their biological limit, an increase in carnosine within their muscles will not improve their muscles buffering abilities. On the contrary, a true placebo effect may occur to the elite population who are naïve about beta-alanine supplementation, believing it will improve their performance/capacity, therefore training harder, and subsequently improving performance/capacity.

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APPENDIX A

Information Sheet for Participants

Beta-alanine study

Researcher: Angus Lindsay

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Supervisors:

- Bill Davison

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- Steve Rickerby

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Course: This study is a requirement of a Master's degree in Biological Sciences

Confidentiality: All data will be locked within the sports science lab of the University of Canterbury, offices of Bill Davison, Stephen Rickerby and Nick Draper, as well as password protected computers.

Risks: There are no risks of participating in this study besides the physical demands of the testing themselves.

Addresses: Angus Lindsay – University of Canterbury Sports Science Lab – University of Canterbury Recreation Centre, 22 Kirkwood Ave, Christchurch, New Zealand

Purpose: The purpose of this study is to determine whether oral supplementation of beta-alanine affects aerobic and anaerobic capacity in elite cyclists.

Introduction: Beta-alanine is an amino acid that is used in several medical fields for different purposes. However, the purpose that will be focused on is its ability of it to form a dipeptide with another amino acid called histidine. The combination of these two, of which beta-alanine is the rate limiting component, forms carnosine, which acts as the lactate buffer and what will be the focus of this study.

Carnosine, which is located within the cells of the muscles, is able to absorb hydrogen ions from the breakdown of glucose via anaerobic glycolysis. Most of you will probably think that when you exercise at or near maximum, that when you get the burning sensation and fatigue sets in you can no longer perform at your best. This is because of the production of lactic acid. Forget what you've heard about lactic acid being harmful, because the body uses this lactic acid as fuel. In

actual fact, it is the hydrogen ions, the by-product of lactate production, which causes this burning sensation. So the science behind this burning sensation is what prevents your muscles functioning at their optimum, and to allow this to occur, the pH of your blood (which is 7.4 at neutral) has to remain at or around this number. When the hydrogen ions are produced it decreases the pH of your blood and muscles to around 6.5 depending on how hard you are pushing, and it is this acidosis of your blood that causes the burning sensation and fatigue.

Therefore, the proposed hypothesis of carnosine is that when these hydrogen ions are formed, rather than causing this acidosis, **the carnosine absorbs the hydrogen ions**, and allows your blood to remain at a neutral pH and subsequently your muscles can function at their optimum for longer.

Previous research has found that oral supplementation of beta-alanine both improves aerobic and anaerobic capacity in athletic sprinters, middle distance runners and sedentary people in cycling. However, there is no definitive evidence on the effect it has on elite cyclists, and this is where using you as subjects will give definitive results in this area.

So, the use of beta-alanine to produce carnosine will help improve your cycling performance in many ways. Whether you are a sprinter or pursuiter, the use of this substance can help you overcome the fatigue effect of the hydrogen ions you produce when you cycle. It's quite often seen that when a sprinter tries to hold their maximum speed for a long duration, they will eventually fatigue. Even as a pursuiter or road rider, you are always producing some hydrogen ions in a race, and when it comes down to a bunch sprint or the last 2 laps of a pursuit, the increased carnosine concentration in your muscle will help combat this. For the road riders, in case you think it won't have much of an effect, a recent study produced significant results indicating that supplementing on beta-alanine significantly increased sprinting performance in road cyclists after a simulated race.

Therefore, when you supplement on beta-alanine, the level of carnosine within your muscles begins to increase over time. Past research has indicated that 4 weeks can lead to an increase up to 50% above baseline levels and after 12 weeks up to 80%!

Before the study: Before the study commences, classification has to be conducted to prove that you are indeed elite cyclists which is based on previous data. It has widely been said that $VO_2\text{max}$ is the best predictor of aerobic performance. Therefore, before any test is started, a $VO_2\text{max}$ test will be done a few days prior to the initial testing.

Aerobic test: This will include a step test, whereby you bring in your own road bike and we set it up your specifications onto a velotron ergometer. Therefore, we get better indicators of your capacity and better specificity. The step test will include a 20 minute warm up (everyone has to do this) and then will commence at 100 watts. Every 3 minutes, you have to increase your power output by 40 watts. This 3 minute window allows your blood to settle and for the toxins you produce to plateau. At the point where you increase your power, a small blood sample from

the tip of your finger will be taken to analyze the level of lactate in your blood. This is done by giving you a small prick at the tip of your finger and then the blood is analyzed. This will be done every 3 minutes. Heart rate and lactate concentrations will be measured along the course of the test, and we will determine your final finishing power, and also your power (anaerobic threshold) when your blood lactate concentrations rise to 4mmol.L^{-1} . Therefore, it is essential that you push yourself to the limit as it will be these two indicators that will prove if you are indeed an elite aerobic cyclist.

Anaerobic test: This test is more for the sprinters whose aerobic performance won't be that of the aerobic athletes, however all endurance cyclists have to participate. The test will include a 30 second Wingate test to measure peak power, watts per kg, average power, work done, and fatigue index. It is a very simple test where you cycle maximally for 30 seconds as all the necessary data is fed to a computer.

These tests will be conducted within 5 days of each other. Each person will be given a time and date to come in to the lab to perform these tests.

Now after getting back many replies, most seem to be able to/or want to do the testing in the October-December group. Because of this, the testing will have to be done in this period so that all cyclists are doing approximately the same sort of training. If it were in April-June, training would have changed too dramatically and the results would vary on to big of a scale. Therefore, it is up to the people who wished to do it in April to do the testing in October.

The testing will be conducted at Canterbury University. Therefore it will be up to you to make arrangements and a decision if you are able to make the testing on a specific day.

Now it is very important that before you commence testing, that you have no performance enhancing substances in your system. Therefore, **no creatine** can be taken a month before the testing, and if you are taking **beta-alanine already, it is essential to stop supplementation immediately**. Protein shakes and vitamins etc. are fine to continue with. Also, please try and avoid any caffeinated drinks 2 days prior to the testing.

When each of you arrives to do the classification testing, you will be asked to sign a declaration form giving your consent to allow me to perform these tests. I need to state clearly, that once you have begun the study, you are able to withdraw from the study for any reason at anytime.

What also is important in these tests, is that if indeed this product does have a significant effect, I need to be able to quantify the increased concentration of carnosine in the muscle. This is to prove that it is the increased carnosine concentration that is having the effect and not the type of training that you are doing. There are two ways that can accomplish this. Firstly, there are muscle biopsies which I am NOT going to subject you to, and then there is the use of an MRI machine that is able to detect concentrations of the carnosine within your muscle. Therefore, I

will be indicating a date that you will have to meet me at the hospital to undergo a short MRI test to quantify the level of carnosine in your muscles.

The study: The study will involve an 8 week block of oral supplementation of beta-alanine. Depending upon how many subjects are involved, you will be split up randomly into two groups. A beta-alanine group and a placebo group. Of course, you will be blinded as to which one you are on. I will also be blinded to this to give an unbiased results and it will be my supervisors who will randomly assign you to a group.

Each group will come in and do both the aerobic and anaerobic tests on days within 5 days of each other, and then given a container of either of the substances and instructed to orally supplement on their substance for a given number of times per day for 8 weeks. Following the 8 weeks of oral supplementation, each of you will be instructed to go back to the lab that you had the classification and first testing conducted, and re-do the testing again.

From here, I will analyze all of the data and write it up in a thesis and once complete, will report to all of you the findings and what sort of an impact beta-alanine has on elite cyclists both in aerobic and anaerobic capacity in a summary sheet.

If I can also get you to fill in a rough training log for the 8 week period that you do during the study as the type of training might influence the results depending on what it is that you are doing.

There will also be a slight time difference between the MRI test at the hospital and your second set of testing which will go over the 8 week period, but keep using the substance up until the tests are over.

APPENDIX B

Information Sheet for Participants on Testing Procedure

Researcher: Angus Lindsay

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Considerations

While there have been a number of studies undertaken examining the effects of beta-alanine on sporting performance such testing for differences in power output on a cycle, changes in maximal oxygen uptake, and blood lactate concentrations, to date I have not found significant research examining the effects of beta-alanine on one kilometer time trial performance and seated 30 second Wingate tests.

Due to the physical nature of the tests, all subjects will be required to complete a questionnaire prior to any testing that covers their medical history including any medication they may be on, current and/or past injuries/medical conditions, and any family history of medical conditions. In addition to this, the subjects will all have their blood pressure and resting heart rate taken and recorded to ensure they are physically suitable to take part.

Finally, subjects will be informed of the College's complaints procedure if have any concerns regarding the study or researchers and wish to raise an issue. Also, the subject consent form will ensure that all subjects are willing to take part and confidentiality of subjects data will be ensured.

V₀2 Max Test Procedure

You will be required to be at the University of Canterbury sport science laboratory at least 60 minutes prior to the start of the V₀2 Max test. This is to make sure there is adequate time to do preliminary tests on resting heart rate, blood lactate, and blood pressure levels, as well as an adequate warm-up of 30 minutes.

If results from the preliminary results are within acceptable ranges and you are willing to continue, you will be fitted with the necessary monitoring equipment such as heart rate monitor and CosMed K4B² (this includes a small pack that will sit on your back and a face mask that will measure oxygen consumption) ready to begin the test. If the preliminary test results show anything of possible concern, you must gain written clearance from your GP to take part in further testing.

The test will be completed on a king cycle beginning at a wattage that is reasonably comfortable and then increasing by 100Watts every 3 minutes until you choose to cease the test. At each 3 minute interval, blood lactate levels will be recorded using Accujet and Lactate Pro equipment. This involves a very small needle pricking the end of a finger, and is virtually painless.

Following the completion of the test, you will complete a brief warm-down on the king cycle.

For this test could you please adhere to the following guidelines:

- S Try and keep as fresh as possible for the tests by refraining from vigorous training in the 48 hours prior to the testing. A period of complete rest from any training should be observed 12 hours prior to testing.
- S Normally a high carbohydrate diet, low in fat should be followed in the days leading up to testing.
- S Refrain from tea, coffee, or any other caffeine containing beverages (coke, V, Lift Plus, Redbull, etc.) for 7 days leading up to the test. This is very important as consuming caffeine close to the time of testing may affect the results.
- S Alcohol should be avoided for at least 12 hours prior to testing and no large meals should be eaten 3 hours prior to testing.

You may choose to bring a high carbohydrate snack or sports drink to consume on completion of both tests to aid recovery.

Wingate Procedure

You will be required to be at the University of Canterbury sport science laboratory at least 60 minutes prior to the start of the 30 second Wingate test. This is to make sure there is time for an adequate warm-up of 30 minutes.

If results from the preliminary results found before the VO₂ max test are within acceptable ranges and you are willing to continue, you will be fitted to the Wingate machine. If the preliminary test results show anything of possible concern, you must gain written clearance from your GP to take part in further testing.

The test will be completed on a Wingate cycle machine which gives accurate data output into a computer. You will be asked to remain seated during the effort and go as hard as you can for the entire 30 seconds while getting verbal encouragement.

Ramp Test Procedure

You will be required to be at the University of Canterbury sport science laboratory at least 60 minutes prior to the start of the ramp test. This is to make sure there is time for an adequate warm-up of 30 minutes.

If results from the preliminary results found before the VO₂ max test are within acceptable ranges and you are willing to continue, you will be fitted to the king cycle machine. If the preliminary test results show anything of possible concern, you must gain written clearance from your GP to take part in further testing.

The test will be completed on a king cycle which gives accurate data output into a computer. You will be asked to remain seated during the effort. You will begin the test at 40W at a cadence which suits you. Every 3 minutes you will be asked to increase the wattage by 40W. At each 3 minute interval, blood lactate levels will be recorded using Accujet and Lactate Pro equipment. This involves a very small needle pricking the end of a finger, and is virtually painless.

Risks of Maximal Exercise Testing

With any type of vigorous exercise comes a remote chance of both injury and death. It is also logical to assume that such risks are increased when the exercise is pushed to maximal effort, particularly if the supervision of the subject is inadequate. Conversely the risks diminish perhaps to vanishing point, when full medical precautions are taken including things such as a pre-exercise questionnaire and preliminary testing (e.g. blood pressure and heart rate testing).

With this in mind, there are several criteria that are used to stop any test immediately:

- Chest pain
- Severe dyspnoea (difficulty in breathing)
- Noticeable changes in pallor
- Cold moist skin
- Cyanosis (skin turning blue)
- Confusion of the subject
- Stumbling-uncoordinated
- Desire by subject to stop

The information you divulge during pre-test screening and the actual test results will remain confidential between the testers and yourself and is necessary to determine any risks during testing. In addition, you will be provided with a summary of your results and an interpretation of the data.

APPENDIX C

Participant Consent Form

Researcher: Angus Lindsay
Contact Details: Phone – 027 320 5373
Email – ajl103@student.canterbury.ac.nz

Supervisors:
- Bill Davison
Contact Details: Phone – 364 2029
Email – bill.davison@canterbury.ac.nz

- Nick Draper
Contact Details: Phone – 364 2987 ext 4193
Email – nick.draper@canterbury.ac.nz

- Steve Rickerby
Contact Details: Phone – 364 2987 ext 8418
Email – Stephen.rickerby@canterbury.ac.nz

<p><i>Informed Consent & Release of Liability for</i> Participation in a Fitness Testing Programme</p>

1. Explanation of Testing Procedure

I, the undersigned, consent to voluntarily participate in the fitness testing and fitness program at University of Canterbury. I understand that I will be exercising from sub-maximal to maximum levels depending on the test certain data may be obtained (this could include; blood pressure, blood samples, oxygen and heart rate). All testing will be directed and monitored by a qualified trainer of University of Canterbury who will give me exact instructions of any activities to be conducted.

I have already informed University of Canterbury of any medical conditions or prescribed medications I am taking, and will inform University of Canterbury of any changes to these medical conditions or medications made by myself or my doctor prior to testing.

2. Description of Potential Risks

I understand and have been informed that my participation in fitness testing may have risks associated with the cardiorespiratory system that cannot always be predicted. These include, but are not limited to, abnormal changes to heart rate or blood pressure, ineffective functioning of the heart, and in very rare instances, heart attack, stroke or even death.

I fully understand the risks that are associated with my testing programme including physical injury, heart attack, or in rare instance death, and knowing these risks I hereby indicate a desire to participate, and accept any and all risks of injury or death.

3. Confidentiality

I have been informed that any information pertaining to me obtained during the testing is confidential and will be kept on project leader's personal computer in a locked office and will be held securely for two years and then destroyed.

I have read the preceding information in its entirety and fully understand it.

Any questions that have occurred to me regarding the procedures of this programme have been answered to my full satisfaction.

I understand that I can withdraw from the testing programme at any time without prejudice.

I express full voluntary consent, release all liability and accept all risks associated with all services and procedures of University of Canterbury as explained herein.

Date:.....

Participant Signature:

University of Canterbury

Representative:

Pre-Exercise Medical/Health Evaluation

In order for University of Canterbury to design you the safest and most effective exercise programme we need information regarding your health. This information

is required purely for your personal safety, and remember that everything you record is strictly confidential.

Name: _____ Date: _____

Are you taking any medication or drugs?

- No
- Yes: Name of medication _____
Reason for using medication _____

Dose _____

Name and address of family GP: _____

Does your doctor know you are participating in this exercise program?

- No
- Yes

Do you now, or have you had in the past: (tick)	Yes	No
1. Asthma		
2. History of heart problems, chest pain or stroke.	—	—
3. Increased blood pressure.	—	—
4. Any chronic illness or condition.	—	—
5. Difficulty with physical exercise.	—	—
6. Advice from a physician not to exercise.	—	—

7. Recent surgery (last 12 months).

— —

8. Pregnancy (now or within last 3 months).

— —

9. History of breathing or lung problems.

— —

10. Muscle, joint, or back disorder, or any other previous injury still affecting you.

— —

11. Diabetes or thyroid condition.

— —

12. Cigarette smoking habit.

— —

—

13. Increased blood cholesterol.

— —

14. History of heart problems in immediate family.

— —

15. Hernia or any condition that may be aggravated by

lifting weights. — —

16. Do you have any other medical/health condition not

detailed above. — —

If you answer yes to any of the pre-exercise medical questions you may need to be excluded from the study. The tester will discuss with you the risks associated with your condition and make a decision based upon this discussion. This decision will be based upon the tester's assessment of the additional potential health risks associated with your condition. Additionally, if you answer yes to questions 2, 6, 8, 10, or 15 you would automatically have to be excluded from the study.

Please give details below of any of the questions above to which you answered yes:

APPENDIX D

Participant Health

Consent Form

Angus Lindsay
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch
8140

October 1st 2009

CONSENT FORM

The effect of beta-alanine supplementation on aerobic and anaerobic capacity in trained cyclists.

I have read and understood the description of the above-named project. On this basis I agree to participate as a subject in the project, and I consent to publication of the results of the project with the understanding that anonymity will be preserved.

I understand that comments I make may be written down and used in the report discussion.

I understand that my name will not be written down next to my comments and that my name will not be used in any part of the results, data, and final report.

I understand also that I may at any time withdraw from the project, including withdrawal of any information I have provided.

I understand that my participation is part of the required field toward a Master's Degree.

I note that the project has been reviewed *and approved* by the University of Canterbury Human Ethics Committee.

NAME (please print):

Signature:

Date:

Researcher: Angus Lindsay

Contact Details: Phone – 027 320 5373

Email – ajl103@student.canterbury.ac.nz

Supervisors:

- Bill Davison

Contact Details: Phone – 364 2029

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