EFFECTS OF 28 DAYS OF BETA-ALANINE AND CREATINE MONOHYDRATE SUPPLEMENTATION ON MUSCLE CARNOSINE, BODY COMPOSITION AND EXERCISE PERFORMANCE IN RECREATIONALLY ACTIVE FEMALES

A Dissertation

by

JULIE YONG KRESTA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Kinesiology

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ABSTRACT

Effects of 28 Days of Beta-Alanine and Creatine Monohydrate Supplementation on Muscle Carnosine, Body Composition and Exercise Performance in Recreationally Active Females. (May 2012)

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Chair of Advisory Committee: Dr. Richard Kreider

Early research with beta-alanine (β-ALA) supplementation has shown increases in muscle carnosine as well as improvements in body composition, exercise performance and blood lactate levels. Creatine monohydrate supplementation has been extensively researched for its effects on anaerobic exercise performance. Recently, a new line of studies have examined the combined effects β-ALA and creatine supplementation on anaerobic exercise performance and lactate threshold. The purpose of the present study is to examine the acute and chronic effects of β-ALA supplementation with and without creatine monohydrate on body composition, aerobic and anaerobic exercise performance, and muscle carnosine and phosphagen levels in college-aged recreationally active females.

Thirty-two females were randomized in a double-blind placebo controlled manner into one of four supplementation groups including β-ALA only, creatine only, β-ALA and creatine combined and placebo. Participants supplemented for four weeks and

reported for testing at baseline, day 7 and day 28. Testing sessions consisted of a resting muscle biopsy of the vastus lateralis, body composition measurements, a graded exercise test on the cycle ergometer for VO_2max and lactate threshold, and multiple Wingate tests for anaerobic exercise performance.

Results showed all supplementation strategies increasing muscle carnosine levels over placebo after four weeks, but not between groups. Muscle creatine increased for all groups after four weeks, but not between groups. There were improvements for all groups with body composition after four weeks, despite the present study not including a specific training protocol. There were no group differences observed for aerobic exercise, blood lactate levels, lactate threshold, ventilatory threshold, peak power, mean power, total work or rate of fatigue. There were some trends for anaerobic exercise indicating groups supplementing with creatine may have greater improvements, however, these findings were not statistically significant.

The present study failed to show any additive effects of β-ALA and creatine supplementation for body composition, aerobic exercise, lactate threshold or anaerobic exercise measures. This could be due to the small sample size resulting in low power and effect sizes. Previous research has demonstrated that four weeks of β-ALA and creatine supplementation was enough time to increase muscle carnosine and phosphagen levels. However, perhaps more time is needed for performance adaptations to occur, especially without the addition of an exercise training component.

DEDICATION

To my husband and son, Kurt and Roman Kresta.

Kurt, you have been by my side through this entire process. You have supported me through every decision and hard time that I have encountered. I can honestly say that without your encouragement, I would not have been able to be where I am now. I know that you will always be proud of me and push me to be a better person. Thank you for being the amazing person that you are and for always having my back.

Roman, I hope that I have made you proud and am able to show you that with hard work and dedication, you can accomplish anything your heart desires. You amaze me everyday and I am truly lucky to have you as my son.

ACKNOWLEDGEMENTS

There are many people that I would like to thank for their help and support through this process. I have learned that this is not an individual experience, but a group effort with fellow peers that I respect and trust. Specifically, I would like to express my gratitude to Jonathan Oliver and Andrew Jagim. Jonathan, your help and countless hours in various labs is truly appreciated. I also value your dedication to your work and true friendship that you have showed me. Andrew, thank you for spending many early mornings in the biopsy lab and for all of your work with my project in my absence. I honestly could not have completed this project without both of you and I am truly grateful.

Dr. Fluckey and Dr. Riechman, you were not only valuable committee members on my dissertation, but you also donated many early and long mornings in the biopsy lab helping with data collection. Thank you for not only your time, but your guidance throughout my graduate career.

I would also like to thank my dissertation committee chair, Dr. Richard Kreider. He has allowed me to have a wide array of opportunities throughout my doctoral experience that has resulted in a high level of confidence that I will carry with me in my career. He has taught me to be an independent thinker and was always helpful in making my ideas and research interests come to life.

Finally, I would like to thank my family. My husband and son, Kurt and Roman Kresta, will always be my rocks and biggest fans. My parents and sister, Kenneth and Yong Culbertson and Lisa Culbertson, and my husband's family, Kenneth and Jeanette

Kresta and Kyle, Tonya and Kaden Kresta, have provided encouragement and motivation to push through when the going got tough. I know they are proud of me, and I hope that I continue to make them proud.

NOMENCLATURE

β-ALA – beta-alanine

LT – lactate threshold

MP – mean power

PCr – phosphocreatine

PP – peak power

PWC_{FT} – physical working capacity at the fatigue threshold

TBW – total body water

TTE – time to exhaustion

TW – total work

VT – ventilatory threshold

VO_2max – highest oxygen consumption attained during a graded exercise test

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CHAPTER I

INTRODUCTION AND RATIONALE

Previous studies with beta-alanine (β-ALA) supplementation have shown increases in muscle carnosine levels as early as 2 weeks, with greater increases as the duration of supplementation increases. The amount of carnosine elevation ranges from around 34% after two weeks [1], up to 80.1% after ten weeks [2]. A recent study examined the effects of three weeks of supplementation on power athletes, but failed to note any significant increases in anaerobic performance as a result of the β-ALA supplementation [3]. They suggested the 4.5 g/day dose used may have been too low for the population studied and duration of the study. Therefore, the proposed study will use a more individualized dosing strategy to elicit effects on muscle carnosine. This dose will be 0.1 g/kg of body weight. This value was calculated from previous studies that showed significant increases in muscle carnosine using males [2, 4, 5] so each person will be receiving a more standardized amount of the supplement.

Additionally, the effects of creatine have been extensively researched in recent years regarding its effects on anaerobic exercise performance. High intensity exercise bouts require a faster rate of ATP resynthesis, which is most quickly attained by breaking down phosphocreatine (PCr) [6, 7]. PCr is stored in limited amounts in the skeletal muscle, however supplementation has been shown to increase these muscle stores to aid in ATP resynthesis during high intensity exercise [8].

This dissertation follows the style of *The Journal of the International Society of Sports Nutrition.*

Recently, studies have been examining the combined effects of creatine and β-ALA supplementation on anaerobic performance and muscle carnosine levels. Results have shown improvements in performance variables such as VO_2peak, lactate threshold and time to exhaustion with the combined supplementation [9]. The acute effects of the combined supplementation has not yet been examined for its effects on anaerobic performance, short term recovery or muscle carnosine concentrations. Creatine monohydrate is typically supplemented using a loading phase between five to seven days of a larger dose around 20 g/day followed by a maintenance load of a smaller amount [10]. Since β-ALA supplementation is a relatively new field of research in regards to exercise performance, there has not been a standard supplementation protocol developed. Typically, the β-ALA dose ranges from 3.2 g/day to 6.4 g/day for anywhere between two to ten weeks of supplementation. Previous studies have tapered and/or increased the dose as the duration increased. The present study will utilize the loading phase dose of creatine with an individualized dose of 0.1 g/kg body weight for β-ALA for four weeks.

There is also a lack of research related to this supplementation method in females as most studies direct their focus to males. Thus, the present study will examine the effects of four weeks of supplementation in college-aged females.

Statement of the Problem

Will four weeks of supplementation with β-ALA alone, creatine alone, combination of β-ALA and creatine or placebo supplementation exhibit effects on

muscle carnosine or anaerobic power markers in college aged, recreationally active females?

Purpose

The purpose of this study is to examine the acute and chronic effects of β-ALA and creatine supplementation on body composition, aerobic capacity, lactate threshold, ventilatory threshold, total body water, muscle carnosine, creatine, and phosphocreatine levels, anaerobic exercise markers and short term recovery for multiple sprint performances in college-aged, recreationally active females.

General Study Overview

This study will be a randomized, double-blind placebo controlled trial. This study will include four supplementation groups including β-ALA only (BA), β-ALA plus creatine monohydrate (BC), creatine monohydrate only (CR) and a placebo (PL). Each group will supplement for 28 days with muscle creatine and carnosine being assessed at baseline and days 7 and 28 to determine acute and chronic effects. In addition, lactate threshold and anaerobic power variables will be assessed and compared between groups using a graded exercise test on the cycle ergometer as well as a multiple sprint and time trial protocol also on the cycle ergometer at the same time points.

Hypotheses

Ho1: Muscle carnosine concentration will be significantly greater with β-ALA supplementation

Ho2: Muscle creatine and phosphagen stores will be significantly greater with creatine supplementation.

Ho3: Anaerobic power will be significantly greater with β-ALA and creatine supplementation.

Ho4: There will be no significant difference observed for muscle carnosine, creatine or phosphagen stores for combined supplementation of β-ALA and creatine.

Ho5: There will be no significant difference observed in anaerobic power for the combined supplementation compared to the other supplementation groups with multiple maximal sprints.

Ho6: There will be no significant improvements observed in fat mass (FM) or fat-free mass (FFM) for β-ALA alone, creatine alone or the combined supplementation.

Ho7: There will be no significant difference observed in VO_2max on the cycle ergometer for β-ALA alone, creatine alone or the combined supplementation.

Ho8: There will be no significant difference observed in resting or post-exercise blood lactate for β-ALA alone, creatine alone or the combined supplementation.

Ho9: There will be no significant difference observed for ventilatory threshold (VT) or lactate threshold (LT) as measured by percent VO_2max for β-ALA alone, creatine alone or the combined supplementation.

Ho10: There will be no significant difference observed for total body water (TBW) as determined by BIA for β-ALA alone, creatine alone or the combined supplementation.

Delimitations

The study will be conducted under the following guidelines:

1. 60 recreationally active females between the ages of 18 and 30 years will be recruited from the Texas A&M University and the College Station community to participate.
2. Eligible participants will take part in a familiarization session where they will be informed of all testing protocols and requirements, complete paperwork including an informed consent and be scheduled for testing.
3. Participants will refrain from strenuous exercise for 24 hours prior to baseline testing.
4. Participants will not have consumed any nutritional supplementation that may affect muscle mass or metabolism for at least three months prior to the start of the study.
5. Participants will not have participated in an anaerobic training program for at least three months prior to the start of the study.

Limitations

1. The participants will be individuals of the Texas A&M University and College Station community that respond to advertisements and therefore the selection process will not be truly random. This may affect the generalizability of the results to the general population.
2. There may be variations in testing times, dietary intake and hormonal status in the participants that are unavoidable.
3. There are innate limitations of the laboratory equipment that will be used for data collection and analysis.

Assumptions

1. Participants will be fasted for the 8 hours prior to testing on each of the testing days.
2. Participants accurately answered the entrance criteria screening questions and the health and activity history form.
3. Participants adhere to all of the regulations during the study involving the supplementation and exercise.
4. All laboratory equipment will be calibrated and functioning properly for all testing sessions.
5. The population, which the sample is drawn from, is normally distributed.
6. The variability among the samples will be approximately equal.
7. The sample will be randomly selected and assigned into the different supplement groups.

Definition of Terms

1. Peak Power (PP) – the highest mechanical power achieved during any stage of the Wingate test. This represents the explosiveness of an individual's muscle power.
2. Mean Power (MP) – the average local muscle endurance throughout the entire 30 second Wingate test.
3. Rate of Fatigue – the drop in power from peak power to the lowest power. This is expressed as a percent.

4. Anaerobic Capacity – calculation of adding each 5-second peak power output over the entire 30 second exercise test, expressed as kg-J.
5. Ventilatory Threshold (VT) – The point during the graded exercise test in which ventilation increases at a disproportional rate compared to oxygen uptake.
6. Lactate Threshold (LT) – the point during the graded exercise test in which the blood lactate levels increase non-linearly and lactate begins to accumulate in the blood. Expressed as percent VO_2max.
7. Onset of Blood Lactate (OBLA) – the point during the graded exercise test in which blood lactate levels are ≥ 4.0. Expressed as percent VO_2max.
8. Wingate Anaerobic Capacity Test – a 30 second supermaximal exercise test on a cycle ergometer against a set resistance of 0.075 kg per kg of body mass. The participant will continue to pedal at a maximal rate throughout the entire test.

CHAPTER II

REVIEW OF LITERATURE

Introduction

During moderate to high-intensity exercise, hydrogen ions (H+) begin to accumulate leading to a drop in intramuscular pH and ultimately influencing muscle performance [11]. The greater the reliance on glycolysis as the primary energy system (as seen with high-intensity exercise), the greater production of lactic acid and H+, thus leading to further decreases in intramuscular pH. This decrease in intramuscular pH has been suggested to be linked to fatigue-induced increases in muscle activation and electromyographic (EMG) amplitude [12, 13]. Thus, if the intramuscular pH decline can be prevented or delayed, the fatigue induced EMG increase may also be delayed [14]. β-ALA supplementation has been shown to increase muscle carnosine levels, which can act as a buffer to reduce the acidity in the active muscles during high-intensity exercise [2, 4, 5]. β-ALA supplementation has been shown to have beneficial effects on exercise performance variables such as cycling capacity [2], ventilatory threshold, and time to exhaustion [15]. For this reason, β-ALA has become a widely used nutritional supplement for improving high-intensity exercise performance [2, 4, 9, 14, 16]. Creatine monohydrate supplementation has also been shown to have ergogenic effects by increasing the availability of PCr and total creatine concentrations in the muscle and improving high-intensity exercise performance, and training adaptations [17]. For this reason, several studies have assessed whether co-ingesting β-ALA with creatine may

have synergistic and/or additive effects on exercise capacity and/or training adaptations [9, 14, 18].

Carnosine

Carnosine (β-alanyl-L-histidine) is a naturally-occurring histidine-containing compound found in many animal tissues including skeletal muscle, which is the most abundant source. Carnosine is a multifunctional dipeptide with many roles including buffering [19, 20], fighting free radicals [21, 22], enzyme regulation [23] and sarcoplasmic reticulum calcium (Ca^{2+}) regulation [24, 25]. Carnosine is broken down in the body by carnosinase, which is found in most tissues except skeletal muscle, partially explaining why carnosine concentrations are highest in this tissue [25]. Figure 1 shows the chemical structure of carnosine.

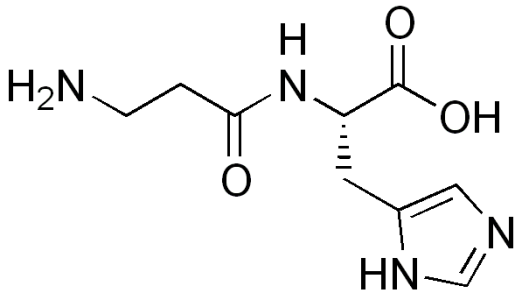

Figure 1: Chemical structure of carnosine

The development of dipeptides, such as carnosine, occurs in the body's muscle tissue soon after birth, but at differing rates between species. It appears to follow a similar timeline as the development of the skeletal muscles under nervous control [26]. It can be synthesized from muscle and nerve tissues in the body from the precursors β-

ALA and histidine with the assistance of the enzyme carnosine synthetase [26]. The chemical reaction responsible for the production of carnosine is as follows: [27]

ATP + L-histidine + beta-alanine ↔ AMP + diphosphate + carnosine.

Carnosinase is an enzyme involved in cleaving carnosine into β-ALA and histidine; however, the exact role remains unclear. It is mainly found in the kidney, liver and blood serum. Carnosinase is actually a group of intra- and extracellular dipeptidases that are part of a large family of metalloproteases. Human tissue carnosinase performs as a non-specific dipeptidase [28].

The mechanisms explaining the protective effects of carnosine are still being established. In vitro studies have shown that carnosine is able to prevent membrane damage when related to lipid peroxidation. It also protects the functionality of the sarcoplasmic reticulum against oxidative damage [29, 30]. One study showed that at a carnosine concentration of 10 mM, which is typical for the muscle cytoplasm, the rate of peroxidation is significantly reduced. When concentrations are raised to 50 mM, oxidation is almost completely stopped. Other studies relate peroxidation to the inactivation of the calcium pump in the sarcoplasmic reticulum membrane. When carnosine was present, it prevented the accumulation of thiobarbituric acid-reactive products, thus ultimately preventing the inhibition of the calcium pump [26].

Carnosine in human skeletal muscle generally ranges between 5-10 mM wet weight or 15-40 mmol/kg dry weight [4]. Concentrations differ among animal species, in part due to the differences in muscle mass [26]. For example, horses have been reported to have higher carnosine concentrations than Greyhound dogs [31]. Carnosine levels are

typically higher in fast-twitch muscle fibers compared to slow-twitch, which corresponds to the observation that animals exposed to frequent sprints, explosive flight behaviors and prolonged hypoxic dives have higher initial carnosine concentrations [4, 31, 32]. Humans athletes involved in anaerobic sports such as sprinters [33, 34] and bodybuilders [35] have also been found to have higher concentrations of carnosine. Exercise training has been reported to increase resting muscle carnosine concentrations in these athlete types. For example, Gardner and colleagues [36] reported that exercise training increased plasma carnosinase activity and decreased carnosine excretion leading to greater muscle carnosine concentrations [36]. Moreover, Suzuki and colleagues [37] examined the effects of sprint training on muscle carnosine concentrations. Six male subjects performed sprint training twice a week for a total of 16 training sessions. Each session involved either single (for weeks one and two) or a double (for weeks three through eight) bout of 30 seconds of maximal sprinting on a cycle ergometer with 20 minutes of rest between sprints on the double bout days. Muscle samples were collected from the vastus lateralis one week before training and again two days following the training protocol. Results revealed that muscle carnosine content and mean power output significantly increased after the eight weeks of training [37]. Tallon and coworkers [35] suggested the greater muscle carnosine content in bodybuilders may be due to the chronic exposure to lower pH environments due to their training, differences in their diet such as increased protein intake where carnosine can be found, supplementation use, and/or possible anabolic androgenic steroid use [35].

Effect of Carnosine as an Intracellular Buffer

Carnosine was first discovered as an intracellular pH buffer in 1953 by Severin and colleagues [38] using frog muscle tissue. Subsequent studies examining this relationship in human muscle tissue followed thereafter [19, 20, 39-42]. When skeletal muscles are involved in moderate to intense exercise, there is typically a generation of lactic acid and subsequent dissociation into lactate and H+, which can alter the pH levels. It had previously been reported that the majority of protons produced during exercise in the blood were buffered by the bicarbonate buffering system [43]. The pKa of this system is 6.1, which is less than that of carnosine (pKa of 6.83), and thus a greater pH change is needed to elicit benefits from this system. Since the pKa of carnosine is closer to the physiological pH, it is likely that this is utilized sooner as a buffer during high-intensity exercise [11]. The imidazole group on the histidine containing molecules, such as carnosine, makes it especially effective as a buffer. This group has a pKa value close to that of the intracellular pH, therefore one of the nitrogens from the imidazole ring can be used to accept a proton [44].

Early studies examined the role of carnosine in animal models. One study, utilizing chromatography methodology to analyze rabbit and pigeon muscle samples, reported muscle dipeptides (mainly carnosine and anserine) accounted for approximately 40% of the pH buffering capability in skeletal muscle [19]. Later, Bump and colleagues [45] examined the carnosine concentrations in different breeds of horses. They compared Quarter horses (QH), Thoroughbreds (TB) and Standardbreds (SB) in order to correlate buffering capabilities of the muscle to fiber type composition. The QH demonstrated less

slow-twitch muscle fibers, greater fast-twitch glycolytic fibers, and fewer fast-twitch oxidative muscle fibers compared to the other horses. Results showed QH had significantly greater amounts of carnosine in their muscle. The researchers reported a positive correlation between carnosine concentrations and fast-twitch glycolytic fibers and a negative correlation between carnosine and fast-twitch oxidative fibers. The investigators inferred that intramuscular carnosine acted as an intracellular buffer, although this was not directly measured. A later study conducted by Sewell and associates [46] specifically examined the buffering capability of carnosine in different fiber types of horses. These researchers found that carnosine contributed about 20% of the buffering in type I fibers, and up to 46% in Type IIb fibers. These findings are consistent with the findings that less lactic acid is accumulated in Type I fibers due to the lower intensity muscle activity involved with this fiber type.

An early study in humans utilizing carnosine supplementation by Kraemer and associates [47] reported no effect on acid-base status or exercise performance using four subsequent 30 second Wingate tests with only two minutes of rest between exercise bouts. In this regard, the researchers evaluated ten trained and ten untrained males who consumed a total of 15 capsules of a supplement containing 1000 mg dibasic sodium phosphate, 204 mg potassium bicarbonate and 12.5 mg L-carnosine over a 3.5 day period. Placebo capsules were matched in sodium and potassium content. Blood samples were taken at baseline prior to any exercise, immediately after each Wingate test, and at three minutes after all exercise was completed. Though intramuscular carnosine levels were not measured, the authors suggested that the amount of carnosine provided to

subjects (about 185 mg) may have been too low to have an impact on intramuscular carnosine levels [36, 48, 49] particularly since previous animal studies had shown increases following a daily dose between 50-200 mg/kg of body weight [50, 51].

Human studies have shown that lowered pH levels can also negatively affect the excitation-contraction coupling in the skeletal muscle [48, 52]. The buffer efficacy in human muscle was examined by calculating the buffering ability over the physiological pH range of 7.1 – 6.5. This study involved 50 healthy active individuals who underwent a muscle biopsy from the lateral portion of the quadriceps femoris muscle. Anserine and carnosine were analyzed in neutralized perchloric acid extracts using high-performance liquid chromatography (HPLC) methods. The Henderson-Hasselbach equation was then used to indirectly calculate the buffer contribution across the pH range of 7.1 to 6.5. It was estimated that carnosine was able to buffer between 2.4 and 10.1 mmol $H^{+} \cdot kg^{-1}$ dry mass, which corresponded to about 7% of the total muscle buffering [16]. Therefore, these results indicated that carnosine played a minimal role in buffering pH.

Suzuki and coworkers [53] examined the effects of the nonbicarbonate buffers carnosine and anserine. They had eight active males supplement with either a placebo or chicken breast extract (CBEX) soup that contained 1.5 g carnosine and anserine. Subjects then performed ten sets of five second maximal cycle sprints at 7.5% of their body weight as resistance. Blood samples were collected at rest, one minute before exercise, after each exercise set, and immediately after the intervals to measure blood-gas parameters, blood lactate and concentrations of carnosine and anserine. The researchers found that supplementing the diet with CBEX delayed the decrease in

bicarbonate during intense exercise, but did not improve performance. These results support the initial use of carnosine as a buffer instead of the bicarbonate system [53].

Early studies with carnosine supplementation noted plasma carnosine levels failed to elevate due to the high activity of carnosinase [36]. The researchers were able to measure only 14% of the ingested carnosine in urine suggesting this was due to the absorption in the gastrointestinal tract [36]. Later, research pointed towards supplementing with β-ALA and L-histidine instead to raise carnosine levels since these are the precursors to carnosine. Dunnett and Harris [20] discovered that β-ALA was able to increase carnosine in muscle tissue. In their study, they supplemented horses with both β-ALA and L-histidine and found β-ALA to have an additive response suggested to be due to the increase in β-amino acid transport across the gastrointestinal tract. This was not observed for L-histidine, thus speaking to the efficacy of β-ALA instead to increase carnosine levels [20]. However, Tamaki et al. [54] was able to show an increase in carnosine with histidine in rats [54].

Aside from buffering effects, carnosine has shown to have other physiological roles, including that of an effective antioxidant against oxidative stress [55]. Reactive oxygen species (ROS) can arise from exercise in several proposed mechanisms including: an increase flow of electrons in the electron transport system from increased respiration [56] or a decrease in pH can lead to oxygen being released from hemoglobin and a subsequent increase in pO_2 in the tissues [57]. Some believe the development of ROS to be related to muscle fatigue during activity [58, 59].

Carnosine is also linked to enzyme regulation related to activation of myosin ATPase, which is used to help maintain ATP stores [60]. Finally, carnosine has been noted to have a role in electron-contraction (E-C) coupling in skeletal muscle. An early study by Lamont and Miller [61] showed 15 mM of carnosine resulted in a significant increase in Ca^{2+} sensitivity in muscle fibers of Rana temporaria [61]. More recently, Dutka and Lamb [62] examined if carnosine affects E-C coupling in functional fibers under physiological conditions. They used mechanically skinned rat extensor digitorum longus muscle fibers. Their results showed that carnosine did not affect Ca^{2+} release from the sarcoplasmic reticulum; however, carnosine was able to increase the Ca^{2+} sensitivity of the contractile components of the muscle fibers. Authors suggested the assistance in Ca^{2+} sensitivity could help maintain force production in the later stages of fatigue once Ca^{2+} release begins to decrease. Therefore, higher levels of carnosine can help offset the decrease in Ca^{2+} as well as the accumulation of H^{+} ions during high-intensity exercise [62].

Since carnosine has a number of physiological roles, there are many future research opportunities available. Specifically, the exact mechanism of carnosine in its role to improve exercise performance and/or reduce muscular fatigue needs to be studied. It will also be important to examine how different nutritional strategies to increase carnosine levels in the muscle may optimize physiological activity and/or exercise capacity.

Beta-Alanine

β-ALA is a naturally occurring amino acid that is one of the precursors to carnosine, along with L-histidine. Carnosine synthetase is the enzyme used to synthesize carnosine from β-ALA and L-histidine. β-ALA is also likely to be the rate limiting step in the synthesis of carnosine [20, 63, 64]. Carnosinase is the enzyme present in cells and serum that breaks down carnosine into β-ALA and L-histidine [28].

β-ALA supplementation in doses greater than 10 mg/kg of body weight has shown to cause a short period of paraesthesia with increasing severity as the dose increases. However, when a large dose around 40 mg/kg of body weight is ingested with CBEX, the paraesthesia did not occur. It is hypothesized that this side effect is a result of the rapid high peak blood plasma concentrations of β-ALA with supplementation alone, since it is not experienced when β-ALA is ingested through the diet with histidine containing dipeptides such as carnosine in meat products [5].

Beta-Alanine and Muscle Carnosine

As previously mentioned, β-ALA supplementation has recently been shown to significantly increase intramuscular carnosine levels, which then corresponds to improvements in exercise performance [15]. Harris and colleagues [5] examined the effects of β-ALA supplementation on human skeletal muscle carnosine concentration in a series of studies. In one study, investigators examined the effects of four weeks of β-ALA or carnosine supplementation on muscle carnosine concentrations. The supplementation protocol included consuming 800 mg of β-ALA four times a day (average 2.3 g/day) for a total intake of approximately 90 g over the four week period

(group I) or increasing doses of β-ALA through the supplementation period (average 6.4 g/day) for a total intake of about 146 g over the four week period (group II). The carnosine supplementation group involved consuming increasing doses of L-carnosine through the supplementation period for a total intake of 364 g of L-carnosine over the four week period, which corresponded to an intake of about 143 g of β-ALA. A final group supplemented with maltodextrin as a placebo in the same frequency as the β-ALA and L-carnosine supplementation groups. A muscle biopsy was taken before and after supplementation. Results revealed that each supplement group showed significant increases in carnosine content. Mean carnosine content increase (measured in $mmol \cdot kg^{-1}dm$) was greatest with L-carnosine and was followed by groups II and I of β-ALA with values of 16.37±3.03 ($p<0.05$), 11.04±2.68 ($p<0.05$) and 7.80±0.36 ($p<0.05$) $mmol \cdot kg^{-1}dm$, respectively. There was no change in the placebo group (1.87±1.73, $p<0.05$ $mmol \cdot kg^{-1}dm$). This corresponded to percent changes of 66%, 64%, 42% and 10% for L-carnosine, group II, group I and placebo group, respectively. They also indirectly calculated the contribution of carnosine to buffing capacity between pH levels of 7.1 and 6.5 using the Henderson-Hasselbach equation. They found that after four weeks of supplementation, carnosine accounted for 14.2%, 14.3% and 12.6% of the total muscle buffering capacity in L-carnosine, and groups II and I, respectively [5].

Studies have also suggested that there does not appear to be an upper limit on increasing muscle carnosine concentrations. For example, Derave and colleagues [4] supplemented trained male sprinters with β-ALA or placebo (maltodextrin) for four to five weeks. The supplementation protocol included six daily doses of 400 mg capsules

of either β-ALA or maltodextrin totaling 2.4 g/day for the first four days, 3.6 g/day for the next four days, and 4.8 g/day for the duration of the study. Interesting, muscle carnosine levels were increased even in individuals with high resting muscle carnosine concentrations [4].

While β-ALA and carnosine supplementation have been reported to increase muscle carnosine levels, less is known about the time course of carnosine degradation. Carnosinase is responsible for the hydrolyzation of carnosine and is mainly present in human plasma, which is why carnosine levels are much lower in the blood than in skeletal muscle, where this enzyme is not present (26). β-ALA supplementation in doses of 4-6 g/day over time has been shown to increase carnosine by 20-30% after two weeks, by 40-60% after four weeks, and up to 80% by ten weeks [2, 65]. A study by Baguet and colleagues [66] sought to determine the loading phase of carnosine and the time course of removal. They included 20 males who supplemented with either β-ALA or maltodextrin as a placebo for five to six weeks. The investigators provided doses of 2.4 g/day for days one and two, 3.6 g/day for days three and four, and 4.8 g/day for the remainder of the study duration. Using a proton magnetic resonance spectroscopy (MRS), they measured the carnosine content in three different muscles (soleus, tibialis anterior and gastrocnemius) at four time points (pre-supplementation, during the last week of supplementation and at weeks three and nine following the cessation of supplementation). They determined that carnosine elimination occurs relatively slowly and in a linear pattern at an average rate of 0.21 mM/week in both type I and II fibers. Authors suggest the slow clearance of carnosine is indicative of the high stability of the

metabolite [66]. Table 1 provides a summary of recent studies examining the effects of β-ALA supplementation on carnosine concentrations.

Table 1: Summary of the effects of β-ALA supplementation on muscle carnosine concentrations

Authors	Population	Supplementation Protocol	Muscle Carnosine Concentration Effects	Performance Results
Baguet et al., 2009 [66]	20 physically active males	5-6 weeks of β-ALA or placebo (maltodextrin) 2.4 g/day – first 2 days 3.6 g/day – days 3-4 4.8 g/day to end of study	• Soleus: carnosine ↑ 30% (p=0.003) with β-ALA; remained stable with placebo (p=0.867) • Tibialis anterior: carnosine ↑ 27% (p=0.005) with β-ALA; ↓ 17% (p=0.05) with placebo • Gastrocnemius: carnosine ↑ 23% (p=0.038) and did not change with placebo (p=0.740). • Carnosine elimination was measured at 3 and 9 weeks after supplementation • At 3 weeks, only 26.1% (soleus), 20.1% (tibialis anterior) and 44.7% (gastrocnemius) of the increase had disappeared. There	• None measured

Table 1: Continued

Authors	Population	Supplementation Protocol	Muscle Carnosine Concentration Effects	Performance Results
			was no difference between β-ALA and placebo at this point (p=0.431) • At 9 weeks, carnosine levels in all 3 muscles returned to initial values	
Harris et al., 2006 [5]	Study 3: 21 physically active males Ages 26.1±5.6 yrs	4 weeks, 4 groups (I – IV): I) 800mg β-ALA x 4 daily (avg. 3.2g daily and 89.6g 4wk total) II) 8 daily doses of either 400 or 800mg β-ALA (avg. 6.4g daily and 145.6g 4wk total) III) 8 daily doses of 1000 or 2000 mg L-carnosine (364g 4wk total L-carnosine, corresponding to 143.3g β-ALA) IV) Placebo of maltodextrin at doses to match groups II and III	• ↑ in carnosine concentration greatest with carnosine supplementation, followed by group II, then group II β-ALA protocols. • Mean ↑ over 4 weeks (mmol·kg^{-1}dm) I) 7.80±.36 (p<.05) II) 11.04±2.68 (p<.05) III) 16.37±3.03 (p<.05) IV) 1.87±1.73 (p>.05)	None measured

Table 1: Continued

Authors	Population	Supplementation Protocol	Muscle Carnosine Concentration Effects	Performance Results
Derave et al., 2007 [4]	15 male track athletes (sprinters) 18-24 yrs	4-5 weeks β-ALA or placebo (maltodextrin) 2.4 g/day – first 4 days 3.6 g/day – days 5-8 4.8 g/day to end of study	Soleus: • ↑ 47% with β-ALA • No change with placebo Gastrocnemius: • ↑ 37% with β-ALA • No change with placebo	No difference between groups for 400m running performance
Hill et al., 2007 [2]	25 physically active males	10 weeks β-ALA: 4 g/day – wk 1 4.8 g/day – wk 2 5.6 g/day – wk 3 6.4 g/day – wk 4-10	• β-ALA group, ↑ from 19.0 to 30.1 mmol/kg (58.8%) at 4 weeks and up to 34.7 mmol/kg (80.1%) at 10 weeks • Not significant between weeks 4 and 10	• No effect on body mass • ↑cycling capacity time at 110% with β-ALA

Beta-Alanine and Exercise Performance

Increases in muscle carnosine due to β-ALA supplementation have resulted in significant effects on several variables related to exercise performance. Some of these include improved time to fatigue on a maximal cycle test [2], increased ability to sustain power output in the final ten seconds of the Wingate test [42], delayed onset of neuromuscular fatigue during incremental cycle ergometry tests as noted by increased physical working capacity (PWC_{FT}), increased ventilatory threshold (VT) and time to

exhaustion (TTE) [15], and improvements in muscle torque during repeated bouts of intense dynamic contractions [4].

Since studies have reported that muscle carnosine levels are typically higher in fast-twitch muscle fibers, which are most predominantly used in high-intensity anaerobic exercise bouts, it has been hypothesized that β-ALA supplementation could aid in anaerobic performance. In 2002, Suzuki and colleagues [42] performed a study that did not involve any nutritional supplementation, but simply analyzed muscle biopsy samples from the vastus lateralis before and after a 30-second maximal cycle sprint Wingate test. The muscle samples were analyzed for carnosine content. Analysis showed a direct relationship between carnosine concentration in skeletal muscle and performance on the 30 second Wingate exercise test. This relationship lends itself to the question of efficacy of β-ALA supplementation in further improving anaerobic exercise performance.

Hill and coworkers [2] examined the effects of four and ten weeks of β-ALA supplementation on muscle carnosine concentration and high-intensity cycling capacity. They also sought to discover whether the effects were muscle type specific. Physically active males supplemented with either β-ALA or maltodextrin as a placebo. β-ALA was given in eight doses per day with increasing dose amounts during the first four weeks ranging from 250-750 mg per dose. Subjects underwent muscle biopsies and maximal cycle performance tests at various points during the study. The group supplementing with β-ALA had significantly greater muscle carnosine concentrations at four and ten weeks from 19.9±1.9 to 30.1±2.3 (30.4%) and 34.7±3.7 (35.1%) $mmol \cdot kg^{-1}dm$. There was no significant change with placebo. The change between four and ten weeks with β-

ALA was not significant despite the small increase (p~0.07). The results also indicated no difference between fiber types, in that each showed similar increases in carnosine as measured by HPLC with fluorescence detection. The authors suggested that the possible benefits from β-ALA supplementation may be limited to four weeks, which is in agreement with previous findings by Suzuki and coworkers [42] who showed an increase in the ability to sustain power output after four weeks of supplementation with no additional benefits observed at ten weeks [42].

Limited research has examined the effects of β-ALA on sport-specific anaerobic performances. Derave and colleagues [4] studied the effects of a four week supplementation period on athletic performance, using a 400 m running race time trial. The researchers found no significant differences in performance after supplementation, but suggested this may have been due to the short time period of supplementation since it takes several weeks to induce carnosine loading. Using a proton MRS to detect muscle carnosine concentrations, investigators showed an increase in carnosine concentrations of 47% in the soleus muscle after β-ALA supplementation with no significant increase after placebo supplementation (8%). Both groups showed significant increases in carnosine concentrations in the gastrocnemius, but subjects supplementing their diet with β-ALA observed a greater increase (37% versus 16%) [4]. This is in contrast to the previously discussed study that reported performance improvements after four weeks of supplementation [2]. The researchers suggested that this may be due to the possibility that in trained athletes, a 400 m running performance is not necessarily limited by the intracellular pH decrease, and therefore the buffering

capabilities of the increased carnosine concentrations would not be as critical of a component [4].

Another recent study sought to determine whether β-ALA supplementation would affect endurance cycling performance. Van Thienen and colleagues [67] evaluated whether β-ALA supplementation would enhance the final sprint performance during endurance cycling since many competitions are won in the final seconds of the race after an all-out sprint. They studied 21 trained males who supplemented their diet with either β-ALA or a maltodextrin placebo for eight weeks. The dose gradually increased from 2 g/day for the first two weeks, 3 g/day for weeks three and four, to 4 g/day for weeks five to eight. The exercise test involved 110 minutes of cycling in ten minute stages with increasing intensity between 50-90%. Following this, the subjects performed a 30 second all-out sprint. The researchers reported that β-ALA supplementation increased sprint peak power after a two hour endurance exercise bout by 11 – 15% ($p=0.0001$) and mean power output by 5 – 8% ($p=0.005$) [67].

In contrast to trained individuals, Smith and colleagues [68] recently examined the combined effects of six weeks of β-ALA supplementation and high-intensity interval training on endurance performance in recreationally active males. In this study, 46 participants were randomly assigned to either β-ALA or placebo supplementation groups. Both groups trained at 90-110% of their peak oxygen utilization (VO_2peak) for the first three weeks, followed by three weeks of training at 115 %VO_2peak. During the training, they continually supplemented with 6 g/day of β-ALA or a dextrose placebo for the first three weeks and 3 g/day for the second three weeks. They showed increases in

both groups for VO_2peak, time to reach VO_2peak, and total work done. However, the group ingesting β-ALA observed a greater increase in VO_2peak and time to reach VO_2peak during the second three weeks of the training protocol ($p<0.05$), with no change in the placebo group. They also noted a significant increase in lean body mass for the β-ALA group after the first three weeks. These results suggest that β-ALA supplementation may enhance the effects of high-intensity interval training and improve endurance performance in untrained individuals. Additionally, Smith and colleagues [69] examined the effects of the same high-intensity interval training and β-ALA supplementation protocol described above on neuromuscular fatigue and function. The researchers reported that three weeks of the interval training was sufficient to result in a significant increase in the EMG fatigue threshold (EMG_{FT}). However, β-ALA supplementation did not promote greater benefits [69]. Table 2 presents a summary of recent studies examining the effect of β-ALA supplementation and carnosine loading on exercise performance.

Table 2: Summary of recent β-ALA supplementation and exercise performance studies

Authors	Population	Supplementation Protocol	Exercise Testing Protocol	Performance Results
Baguet et al., 2009 [70]	14 physically active males	4 weeks of β-ALA or placebo (maltodextrin) 2.4 g/day – first 2 days 3.6 g/day –days 3-4 4.8 g/day to end of	Maximal ramp exercise test on cycle ergometer to determine VO_2peak, VT and gas exchange threshold	• Exercise-induced acidosis was 19% lower with β-ALA • No difference in VO_2 throughout exercise before or after

Table 2: Continued

Authors	Population	Supplementation Protocol	Exercise Testing Protocol	Performance Results
		study	Pre and Post supplementation: 3 x 6min cycle exercise bouts at 50% Δ power output	supplementation in either group • Time delay in the fast component was significantly shorter with β-ALA than placebo • Does not support a role for acidosis in O_2 deficit or the slow component of VO_2 kinetics
Stout et al., 2006 [14]	51 males	4 groups: • Placebo – 34 g dextrose • Creatine – 5.25 g creatine monohydrate and 34 g dextrose • β-ALA – 1.6 g β-ALA plus 34 g dextrose • β-LA+Creatine – 5.35 g creatine monohydrate, 1.6 g β-ALA and 34 g dextrose 28 days of supplementation: • 4 doses/day - days 1-6 • 2 doses/day - days 7-28	PWC_{FT} test with EMG measurements on a cycle ergometer	• β-ALA may delay the onset of neuromuscular fatigue, but no additive effects of creatine • Significant increase in PWCFT with β-ALA (14.5%) and creatine plus β-ALA (11%) compared to placebo

Table 2: Continued

Authors	Population	Supplementation Protocol	Exercise Testing Protocol	Performance Results
Stout et al., 2007 [15]	22 females Ages: 28.9±8.1 yrs (β-ALA) 25.8±4.0 yrs (placebo)	• 4 weeks β-ALA or placebo • 4 divided doses/day for totals of: • 3.2 g/day–wk 1 • 6.4 g/day–wk 2-4	Continuous graded exercise test on cycle ergometer for VO_2max, VT, PWC_{FT} and TTE	β-ALA delays onset of NMF during incremental cycle ergometry (↑ PWC_{FT}, ↑VT, ↑TTE)
Stout et al., 2008 [71]	26 elderly males and females	90 days supplementation with β-ALA or placebo (microcrystalline cellulose) 3 doses/day of: 2.4 g β-ALA or 2.4 g placebo	Continuous graded exercise test on cycle ergometer for PWC_{FT} with EMG measurements	• 28.5% increase in PWC_{FT} after 90 days of β-ALA
Sweeney et al., 2009 [72]	19 physically active college-aged males	5 weeks β-ALA or placebo (rice flour) • 4 g/day – week 1 • 6 g/day – weeks 2-5	2 sets of 5x5-sec sprints with 45- sec recovery between sprints and 2 min between sets performed on non-motorized treadmill at 15% body weight as resistance	• No between group difference for peak or mean horizontal power • No difference in % fatigue • No difference in blood lactate pre- and post-testing between groups
Van Thienen et al., [67]	17 healthy young males	8 weeks β-ALA or placebo (maltodextrin) • 2 g/day – wks 1-2 • 3 g/day – wks 3-4 • 4 g/day – wks 5-8	Simulated road race of 110 min intermittent endurance with intensity between 50% and 90% of the maximal lactate steady state	• β-ALA enhanced sprint power output at the end of the endurance race compared to placebo

Table 2: Continued

Authors	Population	Supplementation Protocol	Exercise Testing Protocol	Performance Results
			(MLSS) in 10 minute stages. Immediately after this, they started a 10 minute time trial at 100% MLSS with voluntary increase of intensity at each minute.	
Zoeller et al., 2007 [9]	55 males ages 24.5±5.3 yrs	4 weeks, 4 groups (4 doses/day for first 6 days, then 2 doses/day • Placebo – 34 g dextrose • Creatine – 5.25 g creatine monohydrate and 34g dextrose • β-ALA – 1.6 g β-alanine and 34 g dextrose • β-ALA plus Creatine – 5.25 g creatine monohydrate, 1.6 g β-ALA and 34 g dextrose	Continuous graded exercise test on cycle ergometer	• ↑ in 5 cardio-respiratory endurance variables with creatine + β-ALA • Combined supplementation may delay the onset of VT and lactate threshold during incremental cycle exercise

Beta-Alanine and Exercise Training

Many athletes incorporate resistance exercise as part of their training. Resistance-exercise has been reported to lower pH levels to around 6.8 during an

exercise session [73, 74]. Thus, β-ALA supplementation may provide ergogenic value to athletes engaged in resistance training due to the heavy reliance on glycolytic systems in the exercises [16]. Several recent studies have examined this hypothesis. For example, Kendrick and coworkers [16] examined the effects of ten weeks of resistance training with and without β-ALA supplementation on muscle carnosine concentration and performance measures. Subjects consumed 6.4 g/day of β-ALA or a maltodextrin placebo for ten weeks. Results revealed that β-ALA supplementation increased muscle carnosine levels by 12.8±8 mmol/kg dry muscle weight in agreement with previous research [2, 5]. However, the researchers reported that β-ALA supplementation had no effects on whole body strength, isokinetic force production, muscular endurance, or body composition [16].

In a follow-up study, Kendrick and colleagues [75] examined the effects of four weeks of β-ALA supplementation on isokinetic training adaptations and muscle carnosine content in type I and II fibers. Fourteen male subjects were divided into two supplementation groups. Subjects ingested 800 mg of β-ALA or a maltodextrin placebo eight times per day for four weeks (6.4 g/day). Subjects trained three times a week for the first two weeks and four times a week for weeks three and four. Each session consisted of ten sets of ten maximal 90° knee extension and flexion contractions at 180°/sec on the right leg using a Kin-Com isokinetic dynamometer with one minute of rest between sets. The left leg acted as the untrained control. Muscle biopsies were obtained from the trained and untrained legs prior to and following the training and supplementation period. Results revealed that carnosine content was increased in the

trained (9.6±3.9 mmol/kg dry muscle) and untrained legs (6.6±2.4 mmol/kg dry muscle) with no significant differences observed between groups. In addition, no significant differences were observed between carnosine concentrations in type I and type II fiber types. The researchers concluded that four weeks of isokinetic training is not effective in increasing carnosine content and that β-ALA supplementation serves to increase muscle carnosine concentration in both untrained and trained type I and type II muscle fibers [75]. Other recent studies support contentions that β-ALA supplementation can enhance training adaptations [18, 68, 69]. Table 3 provides a summary of recent studies on β-ALA supplementation and exercise training.

Table 3: Summary of recent β-ALA supplementation and exercise training studies

Authors	Population	Supplementation Protocol	Exercise Protocol	Muscle Carnosine Concentration Effects	Performance Results
Hoffman et al., 2006 [18]	33 male strength power athletes	• 10 weeks • Creatine β-ALA (CA) – 10.5 g/day creatine monohydrate and 3.2g/day β-ALA • Creatine (C) – 10.5 g/day • Placebo (P) – 10.5 g/day dextrose	Resistance training program 4 days/week for 10 weeks	Not measured	• ↓ fatigue rate in CA • ↑ Δ lean body mass and % body fat • No change in power measures • ↑ training volume in CA

Table 3: Continued

Authors	Population	Supplementation Protocol	Exercise Protocol	Muscle Carnosine Concentration Effects	Performance Results
Kendrick et al., 2008 [16]	26 healthy males, 19-24 yrs	800 mg x 8/day for 4 weeks of β-ALA or placebo (maltodextrin)	Resistance training 4days/wk for 10 weeks	• β-ALA – 23.96 ± 5.94 to 36.77 ± 8.26 (p<0.0001) • Placebo – 29.17 ± 9.82 to 27.29 ± 9.52 (p >0.05)	No difference in whole body strength or isokinetic force
Kendrick et al., 2009 [75]	14 Vietnamese college aged students	4 weeks β-ALA or placebo (maltodextrin) 800 mg x 8/day	Single legged isokinetic training 3 sessions: weeks 1-2 4 sessions: weeks 3-4 10 x 10 maximal 90° extension and flexion contractions at 180°/sec on Kin-Com	• Carnosine ↑ in both trained and untrained legs with β-ALA • Training alone had no effect on carnosine levels	None measured
Smith et al., 2009[69]	46 recreationally active young males	6 g/day for 3 weeks, then 3 g/day for 2nd 3 weeks of β-ALA or placebo (dextrose)	High intensity interval training	Not measured	Training increased EMG_{FT}, no additive effect with β-ALA

Table 3: Continued

Authors	Population	Supplementation Protocol	Exercise Protocol	Muscle Carnosine Concentration Effects	Performance Results
Smith et al., 2009 [68]	46 recreationally active young males	6 g/day for 3 weeks, then 3 g/day for 2nd 3 weeks of β-ALA or placebo (dextrose)	High intensity interval training	Not measured	• ↑ VO_2peak and time to reach VO_2peak with β-ALA • ↑ lean body mass with β-ALA

Beta-Alanine and Muscular Fatigue

There are several factors that play a role in muscular fatigue with high-intensity exercise. Some common theories include a disruption of the neuromuscular junction; a decrease in Ca^{2+} release and uptake leading to the inability of muscles to contract; a depletion of fuel stores such as ATP; production of free radicals due to oxidative stress; and, the accumulation of metabolites such as H^+ [48]. Carnosine has been implicated to play a role in each of these proposed mechanisms of fatigue, but is most commonly researched for its effect on metabolite accumulation as a buffer.

The previously mentioned study by Derave et al. [4] also examined the effects of β-ALA supplementation on isokinetic and isometric fatigue. The isokinetic protocol involved performing five sets of 30 maximal voluntary isokinetic knee extensions at 180°/sec with one minute of recovery between sets on the right leg. The isometric

protocol was performed on the left leg and involved a maximal static voluntary contraction (MVC) at 45°. Once the MVC was determined, subjects performed isometric contractions at 45 % of the MVC for as long as possible. Results indicated that carnosine loading significantly improved the latter stages of exercise (sets four and five of the isokinetic test). The researchers noted that the observed response with β-ALA supplementation had similar results as muscle creatine loading on muscle fatigue [76]. The authors also suggested the increase in carnosine attenuated fatigue by not only its buffering capacities, but also by its ability to improve myofibrilar Ca^{2+} sensitivity.

Neuromuscular fatigue is defined as an increase in electrical activity of a working muscle over time [77-79]. The increase in electrical activity is observed by the increase in EMG amplitude and is indicative of the recruitment of more motor units and/or the increase in firing rate of the active motor units in order to attempt and sustain the given activity [79]. The accumulation of H^+ ions is one possible explanation for this EMG response. Other possible explanations include depleted energy stores and impaired regulation of muscle cations [12, 80]. deVries and coworkers [77] developed a protocol to assess neuromuscular fatigue threshold. It was termed the PWC_{FT} and examines the relationship between EMG amplitude and fatigue during cycle ergometry. This specifically measures the power output at the point of neuromuscular fatigue [15]. Subsequent studies have shown relationships between PWC_{FT} and VT as well [79, 81].

Since it has been established in previous research that β-ALA supplementation has enhanced buffering capabilities during exercise by the subsequent increase in muscle carnosine content [2, 4, 5, 16, 42], it has been hypothesized that β-ALA

supplementation may delay fatigue [15]. Until recently, this had only been shown in trained and untrained men [5]. Stout and coworkers [15] examined the effects of 28 days of β-ALA supplementation in women on PWC_{FT}, VT, VO_2max, and TTE during a cycle ergometry protocol. Subjects were assigned to supplement with either β-ALA or placebo (maltodextrin) in doses of 3.2 g daily for days one through seven and 6.4 g daily for days eight through 28. Subjects were tested prior to and following supplementation. Results showed β-ALA supplementation increased PWC_{FT} by 12.6%, VT by 13.9% and time to exhaustion by 2.5%.

Stout and colleagues [71] also recently examined the effects of three months of β-ALA supplementation on PWC_{FT} in elderly men and women. Participants supplemented with either 2.4 g β-ALA or placebo (microcrystalline cellulose) three times per day for the duration of the study. Results revealed that β-ALA supplementation increased physical working capacity in an elderly population by 28.5%. The researchers attributed these findings to an increase in muscle carnosine concentrations leading to an enhanced buffering capacity, although carnosine was not directly measured in this study [71]. The data related to by β-ALA and muscular fatigue show promise for improvements with supplementation, but still requires future research.

Creatine Monohydrate

Approximately 95% of the total creatine found in the body is located in skeletal muscles, of which 40% is free creatine and 60% is phosphorylated creatine [82]. Creatine has several roles in the body during exercise, with one of the most important being as an energy source for high-intensity exercise bouts. Performances that require

immediate energy (such as maximal sprints) utilize high energy phosphate, ATP and PCr that are stored in the muscles. The reversible reaction in which this energy is released is: PCr + ADP ← creatine kinase → ATP + creatine [83]. Creatine supplementation enhances the initial stores and availability of PCr and therefore, theoretically would enhance mechanisms of the phosphagen system used in high-intensity exercise and improve the shuttling of high-energy phosphates in the creatine phosphate shuttle that may potentially improve anaerobic and aerobic capacity [84, 85].

During short duration high-intensity exercise, ATP is rapidly consumed to provide energy for the given activity. In order to continue at the same intensity, the body must quickly resynthesize ATP from its byproducts. At maximal intensities, this is primarily achieved by anaerobic degradation of PCr and glycogen. The main function of PCr breakdown in this case is to act as an initial buffer and delay the reliance on glycogenolysis [76]. The decrease in maximal force production has been linked to PCr stores in a direct relationship [86]. Creatine supplementation in doses of 20-30 g/day have shown to increase skeletal creatine content by about 20% where 20-30% of this is as PCr [87]. Creatine supplementation also shows to speed the PCr resynthesis within the first minute of recovery from intense muscular activity [88].

Creatine supplementation has been extensively studied and is known to have ergogenic properties in power and strength athletes, with recent studies showing supplementation resulting in increases in muscular strength, anaerobic power, and body mass [10, 76, 89, 90]. In fact, the majority of long term training studies with creatine suggests an ergogenic effect with supplementation in a variety of populations including

trained adolescents, adults and the elderly [17]. For example, Kreider and colleagues [91] examined the effects of 28 days of creatine supplementation during training for college football players. Subjects supplemented their diet with either a carbohydrate electrolyte placebo or this same supplement containing 15.75 g/day creatine monohydrate for 28 days while engaged in resistance-training and agility exercises. The researchers reported that the group supplementing with creatine had greater gains in fat free mass, bench press lifting volume and repetitive sprint performance on a cycle ergometer compared to the placebo [91].

Creatine supplementation has several proposed physiological mechanisms of action. It increases the PCr concentrations in the skeletal muscle, which is used during recovery to rephosphorylate ADP back into ATP via the creatine kinase (CK) reaction [10, 92-95]. Creatine can also improve the capacity for high-energy phosphate diffusion between the myosin heads and mitochondria, which aids in the binding during the cross-bridge cycle [10, 92, 96, 97]. Another function of creatine supplementation is its action as a buffer against the increased acidosis during exercise. Creatine uses the hydrogen ions during the CK reaction and rephosphorylation of ADP to ATP to improve cellular homeostasis [92, 96]. A final mechanism for creatine is to increase the rate of glycolysis to raise the production of ATP. Declining levels of PCr increases the need for rephosphorylation, thus stimulating phosphofructokinase (PFK), which is the rate limiting enzyme for glycolysis. Therefore, supplementation increases the PCr levels and prevents the stimulation of PFK [92, 96].

When PCr levels are elevated, it has been shown to improve PCr resynthesis during exercise recovery, thus improving successive exercise bouts [76, 88, 98-100]. Greenhaff and colleagues examined the effects of creatine supplementation on muscle PCr resynthesis after an electrical stimulation of the muscle to deplete PCr. Participants underwent electrical stimulation of the thigh muscles with blood occluded to the limb, which has been shown to degrade PCr stores. They then had muscle biopsies at 20, 60 and 120 seconds after stimulation. The participants supplemented with 20 g daily of creatine monohydrate before retuning to the lab for the post-supplementation testing. Their results indicated that for the participants with increased creatine uptake from supplementation, there was also accelerated rates of PCr resynthesis after 60 seconds of recovery [88]. Another study examined the effects of creatine supplementation on multiple six second running sprints with 30 seconds rest. They found supplementation to significantly improve sprints four through six in regards to work capacity [98]. The authors of this study suggested that due to supplementation, the PCr levels were at a higher level, thus delaying complete depletion during exercise. PCr resynthesis during exercise recovery is somewhat aided by creatine kinase (CK), which would link oxidative ATP production with PCr resynthesis [101-103]. Therefore due to their necessity in maintaining CK equilibrium, the factors that influence this resynthesis rate include free ATP, SDP, H^+ and creatine concentrations [104].

There are conflicting results regarding the acute effects of creatine monohydrate. Some studies do not show the same effects as previously described. Green et al. examined the effects of creatine supplementation on consecutive upper and lower body

Wingate tests with two minutes of recovery between tests. Participants supplemented with either 20 g daily of creatine or a placebo of sucrose and maltodextrin for six days. They observed no difference in mean power between pre- and post-testing during any of the Wingates for either group, therefore suggesting no benefit from the creatine supplementation. There was also no difference seen for peak power between the groups [105].

An earlier study also sought the effects of creatine monohydrate supplementation on muscular power and strength in weight trained male subjects. They again looked at anaerobic performance measures with the Wingate test. Participants supplemented for 28 days, but performed the Wingate test at baseline, day 14 and day 28. After day 14, there was already a significant increase in total anaerobic work for the Wingate test in the creatine group compared to a glucose placebo [99].

Creatine and Beta-Alanine Supplementation

Recently, creatine supplementation has been shown to increase skeletal muscle carnosine levels in 25-week old mice. Derave and colleagues [106] examined the relationship between creatine supplementation and histidine-containing dipeptide (carnosine and anserine) concentrations as well as the contribution to contractile fatigue and recovery from muscle contractions. The mice received a dose of 2% creatine in their food pellets for 15 weeks, which resulted in an 88% increase in carnosine concentration compared to the age-matched controls. They proposed two explanations for this increase. First, there could be an increased level of β-ALA already in circulation. Second, creatine could play a role in suppressing the degradation of carnosine by acting as an antioxidant.

The present study also showed attenuation of fatigue with creatine supplementation. They suggested this is due to the increase in carnosine resulting from the supplementation causing an increase in the Ca^{2+} sensitivity in the muscle in addition to the buffering capabilities.

More recently, studies have examined the effects of supplementing the diet with creatine monohydrate and β-ALA on exercise performance and training adaptations. Since β-ALA has been shown to have buffering capabilities in skeletal muscle, the addition of creatine may increase the ergogenic benefit by potentially withstanding the fatigue of high-intensity anaerobic exercise bouts.

A study by Hoffman and colleagues [18] used male power athletes and supplemented with creatine or a combination of both β-ALA and creatine. The supplementation doses were 10.5 g daily of creatine monohydrate; 10.5 g daily of creatine monohydrate in combination with 3.2 g daily of β-ALA; or 10.5 g daily of dextrose as a placebo. In addition to supplementation, subjects were also involved in a ten week detailed resistance training program with workouts four days a week. The researchers reported significant improvements in body composition after ten weeks of the combined supplementation of β-ALA and creatine in conjunction with resistance training compared to creatine alone or placebo. Additionally, they showed the addition of β-ALA to creatine was able to reduce fatigue rates during training compared to creatine alone. These findings suggest that there may be additive effects of supplementation of creatine and β-ALA [18].

Stout and coworkers [14] examined the effects of 28 days of β-ALA and creatine supplementation on neuromuscular fatigue and PWC_{FT}. In the study, 51 men supplemented their diet with either 34 g of a dextrose placebo; 5.25 g of creatine with 34 g of dextrose; 1.6 g of β-ALA with 34 g of dextrose; or, 1.6 g of β-ALA with 5.25 g of creatine and 34 g of dextrose. Subjects ingested this dose four times a day for the first six days, and then only twice a day for the remainder of the study. Results revealed that PWC_{FT} increased in the β-ALA group, with no additive effect of creatine. The researchers suggested that 28 days of β-ALA supplementation was able to delay neuromuscular fatigue during incremental cycling, but this was independent of the inclusion of creatine [14].

A study by Zoeller and associates [9], examined the effects of four weeks of creatine and β-ALA supplementation on VO_2peak, LT, VT and TTE. This study had four supplementation groups including a placebo of 34 g dextrose; 5.25 g creatine monohydrate plus 34 g dextrose; 1.6 g β-ALA plus 34 g dextrose; and, a combination of 5.35 g creatine monohydrate and 1.6 g β-ALA plus 34 g dextrose. Subjects ingested these supplements four times a day for six days and then twice a day for the duration of the study. The combined creatine and β-ALA supplementation resulted in significant increases in five of the eight cardiorespiratory endurance variables tested (VO_2 and power output at LT and VT, and percent VO_2peak at VT). Individually, results revealed improvements in power output at VT and total TTE for creatine alone, and improvements in power output at LT for β-ALA alone. However, no significant effects were noted between groups. Therefore, it was concluded that the combination of creatine

and β-ALA supplementation may potentially be beneficial in improving submaximal performance when measured at the lactate and ventilatory thresholds [9]. Collectively, these findings suggest that there may be benefit of supplementing the diet with creatine and β-ALA, but it is unclear whether these benefits are independent or additive in nature.

Summary of Beta-Alanine Supplementation

The use of β-ALA in recent research has shown to increase muscle carnosine concentrations in as short as two weeks, with increasing levels with longer supplementation periods [2, 65]. However, although there is strong support that β-ALA supplementation during training possesses ergogenic value, the specific mechanism of action and ergogenic value remains to be fully examined. Some studies show that β-ALA supplementation can improve high intensity exercise capacity, delay VT and/or neuromuscular fatigue, promoted greater gains in lean body mass during training, and increase VO_2peak or time to exhaustion. On the other hand, other studies show limited effects of β-ALA supplementation on exercise performance. The combination of β-ALA and creatine monohydrate supplementation is still a new field of research with conflicting results. Additive effects were shown in one study for improving fatigue rates with a resistance training program as well as for increasing lean body mass [18]. Combined supplementation was also shown to improve VT and LT during incremental cycle exercise [9]. Other studies failed to show additive effects for variables such as anaerobic power [18] and PWC_{FT} [14]. However, dosing patterns differed in these studies so it is difficult to draw definitive conclusions.

Future Directions

Future research is needed to examine the effects of β-ALA supplementation on muscle carnosine concentrations as well as the physiological effects of increasing muscle carnosine. In this regard, more research should be conducted to understand the effects of β-ALA supplementation and corresponding increases in muscle carnosine concentrations on muscle buffering capacity, antioxidant properties, enzyme regulation, calcium regulation, exercise capacity, performance outcomes, and neuromuscular fatigue. An important direction for future research is the determination of an optimal dosing strategy of β-ALA in order to optimize increases in muscle carnosine concentrations, physiological adaptations, and performance. The current literature shows many variations in the amount and length of β-ALA supplementation; therefore, a standard strategy is still pending. Studies should also examine whether different types of exercise training may influence muscle carnosine to a greater degree in order to determine the most effective method of raising carnosine levels. Determining the correct combination of training and supplementation dose may be especially important in the athletic populations. It will also be important to study the long-term safety and efficacy of β-ALA supplementation.

Further research is clearly warranted to assess the efficacy of β-ALA and other ergogenic nutrients such as creatine. Creatine loading significantly increases muscle phosphagen levels within a few days whereas it has been determined that β-ALA supplementation takes several weeks to increase muscle carnosine concentrations. Therefore, future research should examine effective dosing strategies to optimize the

benefits of both supplements. It is also possible that different types of athletes may benefit from both β-ALA and creatine supplementation. Therefore, studies need to be conducted to examine the potential ergogenic value in trained athletes with supplementation. In addition, studies examining the effects on exercise recovery may be useful since β-ALA and creatine supplementation has been reported to delay fatigue. The majority of current research has focused on the effects in young men, with the exception of the studies by Stout and associates [15, 71] which examined the effects in women and the elderly. Nevertheless, additional research is needed to examine whether age and/or gender may influence results. Another area that should be investigated is supplementing the diet with β-ALA may provide some therapeutic benefit for patients with various neuromuscular and/or muscle wasting diseases as has been reported with creatine supplementation. Finally, additional research should examine the possible synergistic effects of β-ALA with other nutrients.

Experimental Techniques

Wingate Anaerobic Test

The Wingate anaerobic test is a well established and validated measure of anaerobic capacity. Results from this test have been correlated with metabolic variables such as oxygen debt and lactate concentration [107]. The Wingate is also highly correlated with the proportion of type IIa and IIb muscle fibers [108]. This test is typically 30 seconds in duration, which has been correlated with anaerobic work capacity in well trained sprint and endurance athletes [109]. Several previous studies have utilized a protocol with repeated Wingate tests to examine recovery and anaerobic

capacity. The test has shown to be a valid measure of anaerobic capacity compared to several anaerobic performances in the field and laboratory, yielding an r value of 0.75 or more in most cases [110].

Multiple sprints are often used to measure fatigue and recovery rates. Short term recovery is measured with a protocol of multiple Wingate tests with minimal rest between tests. A previous study utilized two Wingate tests with three minutes of rest between tests, which served as the model for the present study protocol [111]. Results from this study showed this protocol was able to inducing fatigue. Subjects had a decreased power output of 60-73% between tests and a decrease in total work of 23-25% between sprints. In addition, blood lactate increased six fold [111].

Biochemistry Methods

Muscle carnosine will be measured in the present study to show the effects of supplementation. Most studies have shown an increase with supplementation as explained earlier, however to ensure the efficacy of the supplementation protocol as well as to examine the difference between types of supplementation, the carnosine concentration will be directly measured from muscle samples. It is determined using high-performance liquid chromatography (HPLC). The method developed by Dunnett and Harris [112] explained in the subsequent chapter has been utilized by several research groups thereafter to measure muscle carnosine concentrations [2, 5].

Muscle phosphagens (PCr and creatine) will also be measured in the current study in order to determine the effects of the supplementation protocol. The dose of creatine supplementation to be used in the present study (20 g/day) has been shown

previously to increase ATP, PCr and creatine levels, however in order to ensure the efficacy of the current supplementation, these variables will be directly measured from muscle biopsy samples using techniques utilized by previous researchers [113-115].

Summary

As it has been discussed, β-ALA supplementation is a relatively recent and growing area of research. It carries beneficial effects with high intensity exercise including anaerobic sprints and resistance training, especially when combined with creatine monohydrate. Future research will help to further explain the exact effects of β-ALA and muscle carnosine on the buffering capabilities as well as improvement in athletic performance under a variety of conditions in both men and women. Most of the research has focused on the effects in young males, with the exception of the studies by Stout et al. [15, 71], which examined the effects in females. This can potentially open the door to more research of the effects in females. Another area that can be further examined is the potential difference in effects with varying baseline physical activity status of the participants. The future of β-ALA may potentially open the door to further improvements in high intensity exercise and sport performance in a wide range of individuals.

CHAPTER III

METHODS

Participants

Thirty-two apparently healthy, moderately active females between the ages of 18 and 35 years were recruited to participate in this study. They were recreationally active and had not been involved in an anaerobic exercise training program for at least the last three months. They were asked to not change their current diet or physical activity level during the course of the study, to consume 8 glasses of water per day, and refrain from any caffeine or other nutritional supplements from the 24 hours prior to the start of the study and throughout the duration of their participation. Subjects were not allowed to participate if they had taken ergogenic levels of nutritional supplements that may have affected muscle mass or anaerobic exercise capacity (i.e. creatine, beta alanine, ergogenic levels of caffeine, HMB, etc.), anabolic/catabolic hormone levels (i.e. androstenedione, DHEA, etc.), or weight loss (i.e. ephedra, thermogenics, etc.) for at least three months prior to the start of the study. Subjects meeting entrance criteria signed informed consent statements in compliance with the Human Subjects Guidelines of the Texas A&M University and the American College of Sports Medicine.

Study Site

All testing took place in either the Exercise and Sport Nutrition Laboratory or the Human Countermeasures Laboratory in the Department of Health and Kinesiology at Texas A&M University in College Station, Texas.

Experimental Design

Table 4 shows the research design for the participants in this study. They were randomly assigned to one of four supplementation groups in a double-blind manner. They followed the schedule described in the table on each of the testing days. Figure 2 shows the time course for each muscle biopsy (days 0, 6 and 27) and full testing session on days 1, 7 and 28. All participants underwent the same procedures, regardless of their assigned supplementation group.

Table 4: Experimental schedule

FAM	Day 0	Day 1 Baseline Testing	Day 6	Day 7 Week 1 Testing	Day 27	Day 28 Post-Testing
Familiar-ization Session Complete Paper-work Review Medical History Random-ized assign-ment Practice Wingate Protocol	Muscle Biopsy	Body Weight DEXA Scan BIA Cycle Protocol for VO_2max and lactate threshold 2 x Wingate Anaerobic Tests Begin Supplemen-tation	Muscle Biopsy	Body Weight DEXA Scan BIA Cycle Protocol for VO_2max and lactate threshold 2 x Wingate Anaerobic Tests Continue Supplemen-tation	Muscle Biopsy	Body Weight DEXA Scan BIA Cycle Protocol for VO_2max and lactate threshold 2 x Wingate Anaerobic Tests

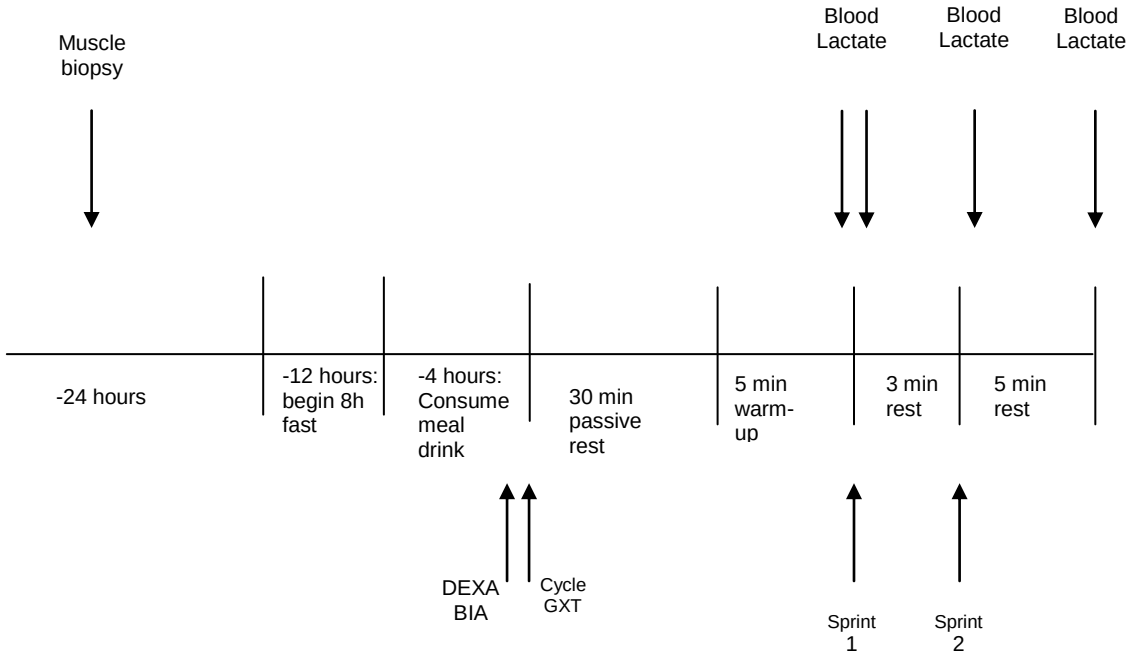

Figure 2: Testing session timeline – days 0, 7 and 28

Independent and Dependent Variables

The independent variable throughout the study is the supplementation group of either β-ALA only, creatine only, β-ALA combined with creatine, or placebo.

The dependent variables measured include: muscle carnosine concentration, creatine and PCr concentration, whole body fat mass, whole body fat free mass, TBW, VO_2max, blood lactate, VT, LT, and anaerobic power measures (PP, MP, rate of fatigue and TW) from each of the Wingate exercise bouts.

Entry and Familiarization Session

Participants expressing interest in the study were interviewed to determine if they were qualified to participate. Participants meeting eligibility criteria attended a familiarization session with the study investigator. During this session, participants signed informed consent statements and completed activity and medical histories. Participants were then familiarized to the study protocol by verbal and written explanation outlining the study design and requirements. This included the supplementation and exercise protocol and an introduction to the tests and equipment used. They were weighed using a standing scale and practiced the exercise tests that were part of each of their testing sessions. They were asked to refrain from any vigorous physical activity during the non-testing days of the study, but encouraged to continue with their regular exercise routine. They were given guidelines to follow regarding appropriate physical activity to engage in for the duration of the study. They remained recreationally active and continued their normal activity without beginning any new exercise or diet regime.

Pre-Supplementation / Baseline Testing

Prior to the pre-supplementation and baseline testing, participants were asked to abstain from exercise for 24 hours and fast for at least eight hours. They were asked to record their exercise activity and food intake for the five days prior to testing. Participants reported to the Human Countermeasures Lab the day before exercise testing to receive a percutaneous muscle biopsy obtained from the vastus lateralis muscle of the right leg using standard procedures for the Bergstrom method [104]. Muscle samples

were immediately frozen at -80°C until assayed. Muscle creatine, PCr and carnosine concentrations were determined from samples.

The morning after the biopsy, participants were asked to fast for at least eight hours before reporting to the Exercise and Sport Nutrition Lab where they will be provided with a standard meal replacement drink four hours prior to the start of testing to control for nutrition for a total of 12 hours prior to testing. Participants then reported back to the Exercise and Sport Nutrition Lab for the initial exercise testing. They were first be weighed using a free standing scale and had body composition determined by a Dual Energy X-ray Absorptiometer (DEXA) (Discovery QDR Series, Hologic Inc., Waltham, MA). This involved the participant lying on their back on the exam table for approximately six minutes. A low dose x-ray radiation scanned the entire body to determine the amount of fat mass, lean mass and bone density. The participants then had their total body water measured using bioelectrical impedance analysis.

Following resting measures, the participant was prepped for exercise testing. They first performed a maximal graded exercise test (GXT) using an incremental protocol on the Lode Excalibur Sport 925900 cycle ergometer (Lode BV, Groningen, The Netherlands) with metabolic measurements recorded on the ParvoMedics True One 2400 Metabolic System (ParvoMedics, Sandy, Utah). The protocol began at 50 W while maintaining 70 rpm. The intensity increased by 25 W every three minutes until a pedaling rate of 70 rpm could no longer be maintained. During this test, the participant wore a Polar heart rate monitor. Heart rate at each stage was recorded. This test was used to determine their VO_2max, VT and LT. Blood samples were taken from the fingertips in

the final minute of each stage of exercise and 5 minutes into recovery to determine the LT. Lactate was determined using a Lactate Scout (Sports Resource Group, USA) handheld analysis device. The LT was defined as the point where the blood lactate concentration rises more than 1.0 mM/l from the previous recorded value. LT was reported as a percent of the VO_2max [83]. The onset of blood lactate accumulation (OBLA) was also noted as a secondary method of determining LT. This is the point in which blood lactate concentrations are equal to or greater than 4.0 mM [83]. Ventilatory threshold was defined as the point during the incremental exercise test where pulmonary ventilation increased at a disproportional rate with VO_2. This was also recorded as a percent of VO_2max [83].

The participants then rested passively for 30 minutes upon completion of the maximal test. Following recovery, the multiple sprints protocol was performed to assess anaerobic power variables such as PP, MP, TW and rate of fatigue. This involved two Wingate Anaerobic Tests with a 3 minute passive rest between tests. The Wingate is a 30 second sprint on a cycle ergometer to measure anaerobic capacity. This test was also performed on the Lode Excalibur Sport 925900 cycle ergometer (Lode BV, Groningen, The Netherlands) at a standardized work rate of 7.5 J/kg/rev. The seat position was standardized between trials. Data was collected and downloaded using the Lode Ergometry Manager Expansion Module Software. Following a short five minute warm up period, the first test began and the participant was asked to pedal as fast as possible for the entire 30 seconds. Pedal revolutions were recorded in five second intervals to determine work performed, which was then used to determine power for the entire 30

seconds as well as each five second interval. The participant performed two 30 second Wingate tests with three minutes of passive rest between tests. They also had 400-500 µl of blood taken before the start of Wingate 1, immediately post Wingate 1, immediately after Wingate 2, again five minutes after the after the 2nd Wingate. This was used to measure lactate concentrations.

Supplementation Protocol

The supplementation protocol was modified from that used by Hoffman et al. in 2006[18] and Zoeller et al. in 2007[9]. Participants were randomly assigned to one of four supplementation groups. The first group (BA, n=8) received β-ALA alone in a dose of 0.1g/kg body weight per day for the 28 days with 0.3 g/kg/day of dextrose for week 1 and 0.1 g/kg/day of dextrose for weeks 2-4, the second group (BAC, n=9) consumed a combined β-ALA and creatine supplementation in the dose of 0.1 g/kg body weight per day of β-ALA with 0.3 g/kg/day of creatine for week 1 and 0.1 g/kg/day of creatine for weeks 2-4, and 0.3 g/kg/day of dextrose for week 1 and 0.1 g/kg/day dextrose for weeks 2-4, the third group (CRE, n=8) received creatine alone in a dose of 0.3 g/k/day of creatine and dextrose for week 1 and 0.1 g/kg/day for weeks 2-4 with 0.1 g/kg/day maltodextrin for the 28 days, the final group (PLA, n=9) was given 0.1 g/kg/day of maltodextrin for all 28 days, and 0.3 g/kg/day of dextrose for week 1 and 0.1 g/kg/day for weeks 2-4 as a placebo. The β-ALA dose and matched maltodextrin placebo was rounded to the nearest 800mg amount to correspond to the supplement capsules. The table below explains the supplementation protocol. Subjects were provided enough of their designated supplement at each testing session. They were asked to take a

supplement dose four times a day for the entire 28 days as close to the times of 8:00am, 12:00pm, 4:00pm and 8:00pm as possible. The β-ALA supplement was in the form of capsules identical in appearance, and was taken with water. The creatine was a powder that was mixed with water for consumption. They returned the empty supplement containers to ensure compliance as well as completed a daily supplementation log. At this time, they also reported any side effects or problems with the supplementation, if applicable. Table 5 shows the dosing strategy for each of the groups.

Table 5: Dosing strategy for each supplement group

Supplement Group	Dosing Schedule	Total Daily Dose
β-ALA *(BA)* *n=8*	4 x 0.025 g/kg – β-ALA 4 x 5 g - Dextrose	0.1 g/kg/day β-ALA 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
β-ALA + *Creatine (BAC)* *n=9*	4 x 0.025 g/kg – β-ALA 4 x 0.75 g/kg – Creatine (wk 1) 4 x 0.025 g/kg – Creatine (wks 2-4) 4 x 5 g - Dextrose	0.1 g/kg/day–β-ALA 0.3 g/kg/day Creatine (wk 1) 0.1 g/kg/day Creatine (wks 2-4) 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
Creatine (CRE) *n=8*	4 x 0.75 g/kg – Creatine (wk 1) 4 x 0.025 g/kg – Creatine (wks 2-4) 4 x 0.025 g/kg – Maltodextrin 4 x 5 g – Dextrose	0.1 g/kg/day Maltodextrin 0.3 g/kg/day Creatine (wk 1) 0.1 g/kg/day Creatine (wks 2-4) 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
Placebo (PLA) *n=7*	4 x 0.025 g/kg – Maltodextrin 4 x 5 g – Dextrose	0.1 g/kg/day Maltodextrin 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)

Week 1 and Post Supplementation Testing

Participants returned to the lab for testing at one week (day 7) and on day 28 at the completion of the study at the same time of day as the previous testing sessions. Testing followed the same procedures as the baseline testing days. Participants came in fasted for at least eight hours before having a muscle biopsy on alternating legs. They then consumed the standard meal replacement drink at four hours prior to testing. They again recorded their dietary intake for the previous 5 days. The testing session began with resting measures of body weight, body composition on the DEXA and total body water with the BIA.

Following resting measures, the participants performed the same exercise tests as before. They began with a maximal GXT on the cycle ergometer using an incremental protocol to determine VO_2max, VT and LT. Blood samples were taken from their fingertips each minute of testing to determine lactate levels. Metabolic data was collected in order to determine VO_2max and VT values.

Next, the participant passively rested for 30 minutes before performing two subsequent Wingate tests with three minutes of passive rest between. They also had blood taken at the same time points to be analyzed for lactate.

Muscle Biopsies

Three resting muscle biopsies were taken on the majority of the subjects, one during their baseline testing on day 0, another on day 6, and the final at the start of the post-supplementation testing session on day 27.

In this procedure, a small piece of muscle less than the size of a pea (60-100 milligrams) was taken from the large outside (lateral) muscle of the thigh (vastus lateralis). The actual site was midway between the patella and the greater trochanter, at the anterior border of the iliotibial band. The subjects were asked to contract their thigh to assist in identifying this location. The muscle biopsy procedure was done with a special needle designed for obtaining a small piece of muscle. In order to perform the biopsy procedure, the subject was asked to lie in a supine (on back) position for sampling the thigh on a padded examination table. When the vastus lateralis was biopsied, the leg remained straight and relaxed. This position releases the tightness in the muscles and permits optimal relaxation.

After the area of the skin above the muscle to be biopsied was identified, it was shaved if necessary and then the skin area was cleaned by iodine sterilization. After cleaning, the skin area over the muscle was numbed with the anesthetic xylocaine. This was done by injecting a small amount (3 ml) of the anesthetic approximately 1.5 cm under the skin and 1.5 cm above the muscle fascia. During this part of the procedure, the subject probably felt a slight burning sensation as the anesthetic entered the skin and the area under the skin just above the muscle. Following injection of the anesthetic, the injected biopsy area on the skin was further cleaned by application of a fluid antiseptic (Betadine) and the cleansed area was enlarged by swabbing the antiseptic several centimeters around the anesthetic injection site in a circular fashion. When the injected area over the muscle was numb (after five to ten minutes), a small incision (about the width of the end of the fingernail on your little finger, approximately 1 cm or a little over

one quarter inch) was made with the pointed end of a #11 scalpel. The incision was made down through the skin and slightly through the covering (fascia) of the muscle. Since the area had been numbed, the subject should not have felt any pain (there are no sensory pain endings in the muscle covering or muscle itself). Because the sensory endings in the skin had been numbed, the subject was likely to experience only slight pressure against the skin.

After this preparation, the subject was instructed to relax the muscle to be biopsied and try to relax all over. The biopsy needle was then slipped through the small opening in the muscle's skin and fascia covering prepared by the incision. Following a brief suction applied to the upper outer-end of the biopsy needle, a small piece of muscle was cut off, and this piece was removed as the needle was withdrawn. This takes 5-15 seconds. During insertion of the needle into the muscle and cutting the small piece from it, the subject may have experienced some moderate pressure, but usually no pain. In addition slight localized cramping followed by brief and minor aching may have been experienced by the subject, but these symptoms usually went away when the needle was withdrawn. Frequently, subjects felt little or no sensation at all. It must be remembered, however, that skeletal muscle tissue is electrically excitable and that when an object is inserted it responds by contracting or shortening, thus the cramping and mild aching.

After the needle was withdrawn, pressure was applied to the site of the incision to prevent any unwarranted bleeding (there is usually little bleeding). The muscle sample was then be placed into a small, pre-labeled storage container and rapidly frozen in liquid nitrogen and subsequently stored in a freezer at -80°C for future analysis.

The subject was continuously informed of the specific procedures during the progress of the biopsy. The incision was then closed with a band-aid applied in a special way. Written instructions for post-biopsy care were given to each subject. The subject was instructed to leave the pressure bandages in place for the remainder of the day and report back to the laboratory within 24 hours. At this time, the incision will be inspected, and new Band-Aid applied for three days. The subject was advised against all vigorous activity during the first 48 hours post-biopsy and not to shower or get the incision wet during this time period. These suggestions should minimize pain and unwarranted bleeding. If localized post-biopsy pain should occur, it was advised to apply ice to the area by means of a plastic bag while keeping the bandages intact. Taking a mild non-prescription pain medication such as Tylenol, providing the subject can tolerate this medication, was also recommended for pain. Medications such as aspirin, Nuprin, Bufferin, or Advil were not recommended since they may contribute to bleeding and/or bruising during the post-biopsy period. After the biopsy, the muscle was likely to be moderately sore for about 24 hours, similar to muscle soreness following unusually vigorous exercise or a muscle injury especially if muscle is compressed against a bone (e.g., "charley horse"). Subjects would naturally feel nervous before this procedure and were probably apprehensive of any pain or discomfort associated with it.

Complications accompanying this procedure are rare. The primary concern would be prolonged bleeding which could produce a bruise in the area. This would extend the period of muscle soreness, but is adequately treated with rest, ice, compression, and elevation. Although the muscle selected for biopsying (vastus

lateralis) has no major blood vessels or nerves in the areas where the biopsy needle will be inserted, there is the rare occurrence of compressing or cutting small nerve branches which can sometimes cause temporary tingling and numbness in the skin. These responses, when they have occurred, have dissipated in a few days or weeks. In all these procedures, care is taken to employ special precautions to avoid infection, including the "universal precautions" for the handling of blood and infectious materials [104, 116-121].

Lactate Analysis

Blood lactate was analyzed at rest, at the end of each stage and at VO_2max during the incremental cycle ergometer test. Blood lactate was taken prior to the Wingate protocol, immediately after Wingate 1, immediately after Wingate 2, and after five minutes of rest.

The lactate was analyzed using a handheld Lactate Scout analyzer. At each time point, a technician punctured the top of the participant's finger and collected about 0.5-0.7 µl of blood that was directly placed onto a lactate strip and analyzed by the Lactate Scout.

Biochemical Analysis for Muscle Creatine and Phosphocreatine

The following procedures were followed to determine muscle creatine and PCr levels based on previous studies [113-115]. Once the biopsy was taken, it was frozen in liquid nitrogen and stored at -80°C until analysis. The sample was then freeze dried and crushed between tweezers and repeatedly rubbed together to pulverize the muscle into a fine powder. It was observed to ensure all visible connective tissue was removed. Five to

10mg of powder will be weighed out into a 1.5 mL polyethylene tube, for perchloric acid extraction. Muscle metabolites will be extracted using 0.5 M perchloric acid containing 1mM EDTA at a ratio of 800 μL to every 10mg of powder for five minutes on ice while periodically vortexing. They were then centrifuged for 5 minutes at 7000 rpm and neutralized using 2M $KHCO_3$ for five minutes while periodically vortexing. After a final 15 minute centrifuge at 7000 rpm, the supernatant will be stored in a 1.5 mL polyethylene tube at -50°C.

The PCr assays were done in the presence of 50 mM Tris buffer, pH 7.4; 1 mM magnesium chloride, 0.5 mM dithiothreitol, 100 μM glucose, 50 μM NADP+, 350 U/mL glucose-6-phosphate dehydrogenase. The assay was carried out in 13 x 75 glass screw-top tubes using 10 μL of sample to 1mL of reagent. The reactant solution was vortexed and read using a fluorometer with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. 25 mL of hexokinase solution was added to 1 mL of reagent and stabilized. For PCr, 20 μL of CK/SDP solution was added to the tubes, vortexed and incubated in the dark at room temperature for 60 minutes when samples were read again. All results were expressed as mmol/kg dry mass (dm).

For the creatine analysis, samples were assayed in the presence of 50 mM imidazole buffer, pH 7.4; 5 mM magnesium chloride; 30 mM potassium chloride; 25 μM phosphoenolpyruvate; 200 μM ATP; 45 μM NADH; 1250 U/m: lactate dehydrogenase; 2000 U/mL pyruvate kinase. 5 mg CK (25 u/mg) was added to 1 mL of the above buffer and stabilized using 10% bovine serum albumin. Assay was carried out in 13 x 75 glass screw-top tubes using 10 μL of sample in 1 mL of reagent. After the sample was added

to the reagent, the reactant solution was vortexed, incubated at room temperature in the dark for 15 minutes, and read in the fluorometer. Creatine kinase buffer solution (25 µL) was added to the sample, vortexed, and incubated at room temperature in the dark for 30 minutes and read again on the fluorometer.

Biochemical Analysis for Muscle Carnosine

Muscle carnosine was analyzed using the HPLC procedures developed by Dunnett and Harris [112]. Chromatography was performed using a Thermo Scientific Hypersil ODS (150 mm x 4.6 mm ID) analytical column protected by a Hypersil ODS guard column. Solvents were filtered to 0.45 µm. Compounds were eluted using a solvent gradient at ambient temperature with the following mobile phases: LINE A: Solvent A:20 mM Phosphate buffer [(20 m*M* Na_2HPO_4 (2.84g/l) + 20 m*M* $NaH_2PO_4.2H_2O$ (3.12g/l)] , pH 6.8 – tetrahydrofuran (995:5 v/v); LINE B: Solvent B: 20 mM Phosphate buffer, pH 6.8 – methanol - acetonitrile (500:350:150, v/v); LINE C: 100% methanol; LINE D: 100% water; 2 litres 20 m*M* Na_2HPO_4 = 5.68g; 2 litres 20 m*M* $NaH_2PO_4.2H_2O$ = 6.24 g. Table 6 shows the current method of chromatography at a flow rate of 0.8 $ml \cdot min^{-1}$.

Table 6: Current method of carnosine chromatography

Time	A	B	C	D
0-3 min	100%			
3-35 min linear change to	50%	50%		
35-39 min linear change to		100%		
39-39.5 min		100%		
39.5- 41 min linear change to		50%	50%	
41-43 min			100%	
43-45 min linear change to		50%	50%	
45-46 min linear change to		100%		
46-47 min linear change to	50%	50%		
47-49 min linear change to	100%			
50 min	100%			

For the utilization of HPLC to determine muscle carnosine concentrations, there needed to be an inclusion of an internal standard. This was used to compensate for variations in injection volume and derivitisation. Beta-alanine can be used as the internal standard with human and murine muscle since it runs in an area beyond carnosine, and these species do not contain beta-alanine. A solution of 100 uM of β-ALA was prepared. The target was 30.3 mg/l, 15.15 mg/500 ml or 7.58 mg/250 ml. The appropriate weight was measured in a flask. This was not exactly equal the target but the exact weight was known and recorded. The beta-alanine was dissolved in the appropriate volume of water. 1.8 ml Flip top Eppendorf tubes were filled to 30 – 50 ml. They were then bagged and frozen. This served as the internal standard for the next 6-12 months.

When running the carnosine analysis, the following solutions were added (in this order):

63 ul extract or standard or 90 ul of either

7 ul β-ALA	or 10 ul of β-ALA
70 ul OPA	or 100 ul OPA

The same pipette was used for the β-ALA standard (suggest the 10 ul Finnpipette). It was not important that the volume added is exactly 7 or 10 ul, only that the volume was always the same. When plotting the standard curve, it was read off the areas and those of the internal beta-alanine standard and the plot $Area_{Tau}$ / $Area_{Bal}$ vs. concentration was completed. Areas on sample chromatograms were divided by $Area_{Bal}$ generated from the internal standard. By referring everything to the internal standard then variations in injection volume were compensated for. For plasma, carnosine can also be used as the internal standard. The extracts and standards were filtered through a 0.2 μm centrifugal filter (Nanosep MS 0.2 μm, Pall Life Sciences).

Statistics

All data was analyzed using SPSS 16.0 software. A 4 x 3 (supplement x time) repeated measures multivariate analysis of variance (MANOVA) was used to analyze all muscle phosphagens, body composition, aerobic exercise variables, blood lactate related variables and VT. Baseline demographics were analyzed with a one-way analysis of variance (ANOVA). Total body water was analyzed using a 4 x 3 (supplement x time) repeated measures ANOVA. Muscle carnosine content was analyzed using a 4 x 2 (supplement by time) repeated measures ANOVA. The anaerobic exercise variables were analyzed with a 4 x 3 x 2 (supplement x time x Wingate test) repeated measures MANOVA. If significant interactions or main effects existed, Tukey's least significant difference post hoc analyses were performed. A significance level of 0.05 was accepted

for all analyses. Cohen's d calculations for effect size were performed on select variables with large mean differences, but non-significant results. Missing data for performance variables was treated using the previously recorded value. The sample mean was used to replace missing data for the muscle related variables. Delta values were calculated and analyzed on select variables by repeated measures ANOVAs to determine changes from baseline.

CHAPTER IV

STUDY OUTCOME

Introduction

Early research with beta-alanine (β-ALA) supplementation has shown increases in muscle carnosine levels as early as two weeks, with greater increases as the duration of supplementation increases. The amount of carnosine elevation ranges from around 34% after two weeks [1] to around 80% after ten weeks [2]. Recent studies have also sought to examine the relationship of β-ALA supplementation on exercise performance. The results have leaned towards a beneficial effect of β-ALA on body composition [68], anaerobic exercise markers such as ventilatory threshold [15], as well as blood lactate levels [9].

The effects of creatine monohydrate have been extensively researched over recent years regarding the effects on anaerobic exercise performance. High intensity exercise bouts require a faster rate of ATP resynthesis, which is most quickly attained by breaking down phosphocreatine (PCr) [6, 7]. PCr is stored in limited amounts in skeletal muscle, however supplementation with creatine monohydrate has been shown to increase the muscle stores to assist in ATP resynthesis during high intensity exercise [8].

More recently, a new line of studies have examined the combined effects of creatine monohydrate and β-ALA supplementation on anaerobic exercise performance and muscle carnosine levels. Results have shown improvements in exercise performance variables such as VO_2peak, lactate threshold and time to exhaustion with a combined supplementation strategy [9]. The acute effects of the combined supplementation has not

yet been examined for its affects on anaerobic performance, short term recovery or muscle carnosine concentrations. Creatine monohydrate is typically supplemented using a loading phase between five and seven days of a larger dose around 20 g/day followed by a maintenance phase of a smaller amount [10]. Since β-ALA supplementation is a relatively new line of research in regards to exercise performance, there has not been a standard supplementation strategy developed. Typically, the β-ALA dose ranges from 3.2 g/day to 6.4 g/day for anywhere between two and ten weeks of continuous supplementation. Previous studies have tapered and/or increased the dose as the duration increased. The present study utilized a loading and maintenance phase dosing strategy for creatine monohydrate with an individualized dose of 0.1 g/kg body weight of β-ALA for four weeks.

The purpose of this study is to examine the acute and chronic effects of β-ALA supplementation with and without creatine monohydrate on body composition, aerobic and anaerobic exercise performance, and muscle carnosine and phosphagen levels in college-aged recreationally active females.

Methods

The present study is a randomized, double-blind placebo controlled trial that recruited apparently healthy, moderately active females between the ages of 18 and 35 years to participate in the study. Subjects were not allowed to participate if they had taken ergogenic levels of nutritional supplements that may have affected muscle mass or anaerobic exercise capacity (i.e. creatine, beta-alanine, ergogenic levels of nutritional caffeine, HMB, etc.) for at least three months prior to the start of the study. Subjects

meeting the entrance criteria signed informed consent statements in compliance with the Human Subjects Guidelines of Texas A&M University and the American College of Sports Medicine. Participants were randomly assigned to one of four supplementation groups following a familiarization session.

Familiarization Session

Prior to beginning the study, all participants met with the principal investigator to obtain information about the study and all testing procedures. They then signed informed consent statements and completed activity and medical histories. Participants were familiarized to the study protocol with verbal and written explanations of the study requirements. They were also weighed using a standing scaled and asked to perform a practice Wingate exercise test on the cycle ergometer. They were given guidelines to follow for physical activity during their involvements in the study and scheduled for all subsequent testing sessions.

Resting and Exercise Testing

Resting and exercise testing was performed at baseline prior to any supplementation, at one week of supplementation, and after four weeks at the completion of the study. Subjects were asked to abstain from exercise for 24 hours and fast for at least 8 hours prior to baseline testing. One day prior to exercise testing, participants received a percutaneous muscle biopsy from the vastus lateralis muscle of the right leg using standard procedures for the Bergstrom method [104]. Muscle sample were immediately frozen at -80° until analyzed.

The morning after the biopsy, participants were asked to fast for at least eight hours before being asked to consume a standard meal replacement drink and report to the lab four hours later to begin exercise testing. They were weighed using a free standing scale and had body composition determined using a Dual Energy X-Ray Absorptiometer (DEXA) (Discovery QDR Series, Hologic Inc., Waltham, MA). They then had their total body water measured using bioelectrical impedance analysis. Following the resting measures, participants began exercise testing starting with a maximal graded exercise test (GXT) using an incremental protocol on the Lode Excaliber Sport 925900 cycle ergometer (Lode BV, Groningen, The Netherlands) with metabolic measurements recorded on the ParvoMedics True One 2400 Metabolic System (ParvoMedics, Sandy, Utah. The protocol began at 50 W maintaining 70 rpm and the intensity was increased by 25 W every three minutes until a pedaling rate of 70 rpm was no longer maintained. Blood samples were taken from the fingertips in the final minute of each stage of exercise and five minutes into the recovery to determine lactate threshold (LT). Lactate was determined using a Lactate Scout (Sports Resource Group, USA) handheld analysis device. The LT was calculated two different ways including the point at which blood lactate concentrations rises more than 1.0 mM/l from the previously recorded value (LT) and the point at which blood lactate level was greater than or equal to 4.0 (also termed the onset of blood lactate, OBLA). All values were reported as a percent of the VO_2max [83]. Ventilatory threshold was determined as the point during the GXT where pulmonary ventilation increased at a disproportional rate with VO_2, and was also recorded as a percent of VO_2max. Following the GXT, participants rested

passively for 30 minutes and then performed two Wingate Anaerobic Tests with 3 minutes of passive rest in between. Blood was taken from the fingertips before the start of Wingate 1, immediately post Wingate 1 and 2, and finally after 5 minutes of passive recovery following the completion of both Wingates.

Supplementation Protocol

The supplementation protocol was modified from those used by Hoffman et al. in 2006 [18] and Zoeller et al. in 2007 [9]. The creatine monohydrate (Creapure®, AlzChem Trostberg GmbH, Germany) supplementation was provided in the form of a powder that the subjects were instructed to mix with water. Individual doses were rounded to the nearest 0.1 g. The β-ALA (CarnoSyn®, Natural Alternatives International, Inc., San Marcos, CA) came in the form of 800 mg capsules that subjects were instructed to take at 4 intervals throughout the day with water and/or food, as close to 8:00am, 12:00pm, 4:00pm and 8:00pm. Individual doses were rounded to the nearest 800 mg for β-ALA. The four groups included β-ALA alone (BA, n=8), creatine alone (CRE, n=8), a combination of creatine and β-ALA (BAC, n=9), and placebo (PLA, n=7). The dosing strategy is depicted in Table 7. The β-ALA only group received a dose of 0.1 g/kg body weight per day for the entire 28 days with 0.3 g/kg/day of dextrose for week 1 and 0.1 g/kg/day of dextrose for weeks 2-4. The creatine only group was given a dose of 0.3 g/kg/day of creatine for week 1 and 0.1 g/kg/day for weeks 2-4, with 0.1 g/day maltodextrin for the 28 days. The β-ALA and creatine combined group consumed a 0.1 g/kg/day of β-ALA for the entire 28 days with 0.3 g/kg/day of creatine for week 1 and 0.1 g/kg/day of creatine for weeks 2-4. Finally, the placebo group was given 0.1

g/kg/day of maltodextrin for all 28 days with 0.3 g/kg/day of dextrose for week 1 and 0.1 g/kg/day for weeks 2-4 as a placebo. The β-ALA and matched placebo doses were rounded to the nearest 800 mg capsule. The creatine and matched placebo doses were rounded to the nearest 0.1 g. Participants were given supplements one week at a time and were asked to return the empty containers to ensure compliance. They also completed supplementation logs each week to monitor compliance of supplementation.

Table 7: Supplementation protocol for each group

Supplement Group	Dosing Schedule	Total Daily Dose
β-ALA *(BA)* *n=8*	4 x 0.025 g/kg – β-ALA 4 x 5 g - Dextrose	0.1 g/kg/day β-ALA 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
β-ALA + *Creatine (BAC)* *n=9*	4 x 0.025 g/kg – β-ALA 4 x 0.75 g/kg – Creatine (wk 1) 4 x 0.025 g/kg – Creatine (wks 2-4) 4 x 5 g - Dextrose	0.1 g/kg/day–β-ALA 0.3 g/kg/day Creatine (wk 1) 0.1 g/kg/day Creatine (wks 2-4) 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
Creatine (CRE) *n=8*	4 x 0.75 g/kg – Creatine (wk 1) 4 x 0.025 g/kg – Creatine (wks 2-4) 4 x 0.025 g/kg – Maltodextrin 4 x 5 g – Dextrose	0.1 g/kg/day Maltodextrin 0.3 g/kg/day Creatine (wk 1) 0.1 g/kg/day Creatine (wks 2-4) 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)
Placebo (PLA) *n=7*	4 x 0.025 g/kg – Maltodextrin 4 x 5 g – Dextrose	0.1 g/kg/day Maltodextrin 0.3 g/kg/day Dextrose (wk 1) 0.1 g/kg/day Dextrose (wks 2-4)

Muscle Analysis

The muscle samples from the biopsies were analyzed for creatine, PCr and carnosine levels. The creatine and PCr procedures were based on those from previous studies [113-115]. Once the biopsy was taken, the sample was immediately frozen in liquid nitrogen and stored at -80° until assayed. Upon time to prepare the muscle for analysis, they were freeze dried and crushed to pulverize the muscle into a powder. Five to 10 mg of powder was weighed out for perchloric acid extraction. Muscle metabolites were extracted using 0.5 M perchloric acid containing 1 mM EDTA at a ratio of 800 µL to every 10 mg of powder for five minutes on ice while periodically vortexing. They were then centrifuged for 5 minutes at 7000 rpm and neutralized using 2M $KHCO_3$ for five minutes while periodically vortexing. After a final 15 minute centrifuge at 7000 rpm, the supernatant was stored in a 1.5 mL polyethylene tube at -50°C. The PCr assays were done in the presence of 50 mM Tris buffer, pH 7.4; 1 mM magnesium chloride, 0.5 mM dithiothreitol, 100 µM glucose, 50 µM NADP+, 350 U/mL glucose-6-phosphate dehydrogenase. The assay was carried out in 13 x 75 glass screw-top tubes using 10 µL of sample to 1mL of reagent. The reactant solution was vortexed and read using a fluorometer with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. 25 mL of hexokinase solution was added to 1 mL of reagent and stabilized. For PCr, 20 µL of CK/SDP solution was added to the tubes, vortexed and incubated in the dark at room temperature for 60 minutes when samples were read again. All results were expressed as mmol/kg dry mass (dm).

For the creatine analysis, samples were assayed in the presence of 50 mM imidazole buffer, pH 7.4; 5 mM magnesium chloride; 30 mM potassium chloride; 25 μM phosphoenolpyruvate; 200 μM ATP; 45 μM NADH; 1250 U/m: lactate dehydrogenase; 2000 U/mL pyruvate kinase. 5 mg CK (25 u/mg) was added to 1 mL of the above buffer and stabilized using 10% bovine serum albumin. Assay was carried out in 13 x 75 glass screw-top tubes using 10 μL of sample in 1 mL of reagent. After the sample was added to the reagent, the reactant solution was vortexed, incubated at room temperature in the dark for 15 minutes, and read in the fluorometer. Creatine kinase buffer solution (25 μL) was added to the sample, vortexed, and incubated at room temperature in the dark for 30 minutes and read again on the fluorometer. Creatine and phosphagens were analyzed using a SpectraMax 250 (Molecular Devices, Sunnyvale, CA).

Muscle carnosine was analyzed using the HPLC procedures developed by Dunnett and Harris [112]. The muscle samples were prepared using the same drying methods as before. Muscle was analyzed using an Aquity-UPLC system (Waters, Milford, MA). Chromatography was performed using a Thermo Scientific Hypersil ODS (150 mm x 4.6 mm ID) analytical column protected by a Hypersil ODS guard column. Solvents were filtered to 0.45 μm. Compounds were eluted using a solvent gradient at ambient temperature with the following mobile phases: LINE A: Solvent A:20 mM Phosphate buffer [(20 m*M* Na_2HPO_4 (2.84g/l) + 20 m*M* $NaH_2PO_4.2H_2O$ (3.12g/l)] , pH 6.8 – tetrahydrofuran (995:5 v/v); LINE B: Solvent B: 20 mM Phosphate buffer, pH 6.8 – methanol - acetonitrile (500:350:150, v/v); LINE C: 100% methanol; LINE D: 100% water; 2 litres 20 m*M* Na_2HPO_4 = 5.68g; 2 litres 20 m*M* $NaH_2PO_4.2H_2O$ = 6.24g.

Statistical Analysis

All data was analyzed using SPSS 16.0 software. A 4 x 3 (supplement x time) repeated measures multivariate analysis of variance (MANOVA) was used to analyze all muscle phosphagens, body composition, aerobic exercise variables, blood lactate related variables and ventilatory threshold. Baseline demographics were analyzed with a one-way analysis of variance (ANOVA). Total body water was analyzed using a 4 x 3 (supplement x time) repeated measures ANOVA. Muscle carnosine content was analyzed using a 4 x 2 (supplement by time) repeated measures ANOVA. The anaerobic exercise variables were analyzed with a 4 x 3 x 2 (supplement x time x Wingate test) repeated measures MANOVA. If significant interactions or main effects existed, Tukey's least significant difference post hoc analyses were performed. A significance level of 0.05 was accepted for all analyses. Cohen's d calculations for effect size were performed on select variables with large mean differences, but non-significant results. Missing data for performance variables was treated using the previously recorded value. The sample mean was used to replace missing data for the muscle related variables. Delta values were calculated and analyzed on select variables by repeated measures ANOVAs to determine changes from baseline.

Results

A total of 32 apparently healthy, recreationally active females completed the protocol for the present study (age 21.42±2.77 years, height 65.35±2.31 inches, weight 60.82±6.21 kg, lean mass 38.55±3.64 kg, and percent body fat 26.96±5.94 %). There

were no significant differences between groups at baseline for age, initial weight, lean mass or initial body fat percentage.

Muscle Biochemistry

Muscle samples were obtained from 31 total participants. There was sufficient sample to analyze 27 samples for carnosine and 19 samples for phosphagen levels. Table 8 shows the results from analyses of both phosphagens and carnosine. Cohen's d effect size calculations were performed to compare supplementation group means to placebo at week four after participants had completed all supplementation and differences were expected to be seen. The results of these calculations are seen in Table 9. Unfortunately, the sample sizes for the muscle analyses were very small resulting in low power values. A larger sample size would be needed to have more accurate comparisons.

Table 8: Muscle biochemistry results

		BA (n=7)	BAC (n=8)	CRE (n=7)	PLA (n=5)	Total (All Groups)	P Value
Carnosine (mmol/g muscle)							
	Baseline	19.74 ± 8.69	20.81 ± 7.66	20.80 ± 2.81	15.70 ± 4.70	19.58 ± 6.50	T=0.224
	4 Weeks	23.68 ± 1.56	24.23 ± 4.09	21.04 ± 7.00	16.53 ± 4.80	21.83 ± 5.31	G=0.042[a]
							T x G=0.817
		BA (n=4)	**BAC (n=7)**	**CRE (n=4)**	**PLA (n=4)**	**Total (All Groups)**	**P Value**
Creatine (mmol/kg DW)							
	Baseline	47.13 ± 19.88	59.82 ± 37.37	72.96 ± 29.59	59.85 ± 7.79	59.92 ± 27.60	T=0.191
	1 Week	50.73 ± 26.83	65.49 ± 15.25	88.55 ± 38.72	68.17 ± 7.74	76.80 ± 24.90	G=0.136
	4 Weeks	42.33 ± 16.24	59.90 ± 9.77	67.72 ± 15.94	57.19 ± 8.07	57.28 ± 14.34	T x G=0.980
Phosphocreatine (mmol/kg DW)							
	Baseline	22.18 ± 4.28	22.94 ± 18.02	31.69 ± 16.54	21.35 ± 4.44	24.29 ± 13.26	T=0.134
	1 Week	25.91 ± 9.88	32.61 ± 19.62	23.43 ± 4.40	24.08 ± 4.23	27.47 ± 12.96	G=0.976
	4 Weeks	34.75 ± 7.38	26.87 ± 7.04	30.51 ± 6.26	31.43 ± 9.39	30.25 ± 7.49	T x G=0.250
Total Creatine (mmol/kg DW)							
	Baseline	63.04 ± 22.30	82.75 ± 37.19	105.11 ± 26.57	80.76 ± 11.18	82.89 ± 29.81	T=0.724
	1 Week	59.47 ± 27.07	94.79 ± 8.30	111.98 ± 37.99	85.53 ± 4.39	89.02 ± 26.75	G=0.021[b]
	4 Weeks	74.28 ± 11.40	88.89 ± 5.32	84.66 ± 13.41	85.91 ± 18.07	84.66 ± 13.41	T x G=0.733

Data are means ± SD. BA signifies beta-alanine only group; BAC is beta-alanine and creatine combined supplementation group; CRE is creatine only group; and PLA is placebo.

[a] PLA significantly lower than BA (p=0.019), BAC (p=0.007) and CRE (p=0.042)

[b] PLA significantly greater than BA (p=0.003)

Table 9: Effect size and magnitude calculations for select muscle biochemistry variables

	BA		BAC		CRE	
Creatine	1.16	(large)	-0.30	(low)	-0.83	(large)
Phosphocreatine	-0.39	(low)	0.55	(low)	0.12	(low)
Total Creatine	0.77	(moderate)	-0.22	(low)	0.08	(low)
Carnosine	-2.00	(large)	-1.73	(large)	-0.75	(moderate)
Cohen's d calculations compared each group mean to PLA.						
All calculations used data from week 4.						

Carnosine did not show any time x group interaction or time effect, but there was a significant group effect (p=0.042). Post hoc analysis showed the placebo group to have lower concentrations than β-ALA alone (p=0.019), β-ALA and creatine combined (p=0.007) and creatine alone (p=0.042). After four weeks of supplementation, the groups receiving β-ALA showed trends of more increases in carnosine than the groups without β-ALA (3.93±9.10 μmol/g muscle for β-ALA alone and 3.41±10.50 μmol/g muscle for β-ALA and creatine combined vs. 0.24±5.84 μmol/g muscle for creatine alone and 0.83±7.60 μmol/g muscle for placebo). The percent change values for each group after four weeks were 35.3±44.8% for β-ALA only, 42.5±99.3% for β-ALA and creatine combined, 0.7±27.1% for creatine only and 13.9±44.0% for placebo. This change in carnosine content was not significant, which is likely a result of the large variation and small sample size again. The effect sizes were large enough to imply the differences seen between groups may be large enough to counteract the small sample size and these results may better reflect the outcomes for the larger population. Hypothesis 1 stated that carnosine would be greater with β-ALA supplementation; therefore, with the present

study results, this hypothesis is accepted as it was greater than placebo, just not the other supplementation groups.

Muscle creatine did not show any significant time x group interaction, time effect or group effect. Although not significant, after the first week's loading phase of supplementation, the group taking creatine alone showed trends towards having the greatest increase in muscle creatine compared to β-ALA only, β-ALA and creatine combined and placebo (15.59±14.85 mmol/kg DW vs. 3.59±15.58 mmol/kg DW, 5.67±31.67 mmol/kg DW and 8.32±12.75 mmol/kg DW, respectively). This relationship was not maintained after four weeks however. The percent change in muscle creatine after four weeks for each group was 4.6±71.4% for β-ALA only, 154.0±375.0% for β-ALA and creatine combined, 1.7±41.6% for creatine only and -4.1±10.9% for placebo. The effect size calculations showed large values for β-ALA only and creatine only supplementation groups compared to placebo, indicating those relationships are strong based on the present data.

Phosphocreatine did not show any time x group interaction, time effect or group effect. The effect size calculations were low for all groups compared to placebo, therefore the relationship between groups is quite weak in this case. The delta values in this case did show a trend in the β-ALA and creatine combined group with greater PCr values after the first week of supplementation (9.67±6.69 mmol/kg DW vs. 3.73±12.09 mmol/kg DW for β-ALA alone, -8.27±12.74 mmol/kg DW for creatine alone and 2.73±1.70 mmol/kg DW for placebo). This relationship did not hold up after four weeks. The percent change for each group after the first week was 22.4±56.1% for β-ALA only,

77.9±119.7% for β-ALA and creatine combined, -17.7±24.7% for creatine only and 13.7±11.2% for placebo. The percent change after four weeks for PCr in each group was 61.9±48.0% for β-ALA only, 77.0±149.2% for β-ALA and creatine combined, 13.0±53.5% for creatine only and 51.5±46.5% for placebo. The variation seen in the standard deviations are large for each group, which may have affected the group differences from being significant. Larger sample sizes may have helped show more significant results.

Total creatine also did not show any time x group interaction or time effect; however, it did show a significant group effect (p=0.021). The post hoc analysis for total creatine indicated that the β-ALA and creatine combined group was greater than β-ALA only (p=0.043) and creatine only is greater than β-ALA only (p=0.003). In examining the delta values, the groups receiving creatine showed trends of greater increases in total creatine after week one, but not after four weeks (12.04±36.01 mmol/kg DW for β-ALA and creatine combined and 6.83±26.03 mmol/kg DW for creatine only vs. -3.57±31.09 mmol/kg DW for β-ALA alone and 4.76±7.94 mmol/kg DW for placebo). The percent change for each group after the first week of supplementation was 5.9±67.7% in β-ALA only, 103.5±258.2% in β-ALA and creatine combined, 7.1±24.1% in creatine only and 6.9±10.0% in placebo. The percent change for each group after four weeks was 27.3±35.3% for β-ALA only, 80.5±233.5% for β-ALA and creatine combined, -3.4±28.7% for creatine only and 6.4±15.4% for placebo. Note the standard deviations are very large, which may have affected these results from being significant like the overall group effect explained earlier. The effect sizes for total creatine between groups

were mostly low, indicating a small and weak relationship between the groups. A larger sample size may have been necessary to improve effect size and show more significant relationships.

The creatine results failed to accept Hypothesis 2, which stated that creatine and phosphagen stores would be greater with creatine supplementation. The results from all muscle analyses led to accepting the null Hypothesis 4 that stated that there would be no differences seen in carnosine, creatine or phosphagen stores for the combined β-ALA and creatine supplementation group.

Body Composition

The body composition variables included in the MANOVA analysis included body weight (kg), fat mass (kg), fat free mass (kg) and percent body fat. Total body water was analyzed separately. Table 10 shows the results from all body composition variables. Cohen's d effect sizes were calculated to compare groups, which are shown in Table 11. The majority of the effect sizes were low, indicating a low relationship between the present results and what would be expected in a larger population. Perhaps a larger sample size would have allowed for or significant results and larger effect sizes.

Table 10: Body composition results

		BA (n=8)	BAC (n=10)	CRE (n=8)	PLA (n=7)	Total (All Groups)	P Value
Body Weight (kg)							
	Baseline	63.16 ± 7.48	60.23 ± 6.08	61.15 ± 5.68	58.60 ± 5.81	60.82 ± 6.21	T=0.021
	1 Week	63.33 ± 7.30	60.37 ± 5.98	61.38 ± 5.94	59.22 ± 6.07	61.09 ± 6.21	G=0.605
	4 Weeks	63.60 ± 7.28	61.11 ± 5.61	61.40 ± 6.00	59.44 ± 5.88	61.40 ± 6.00*	T x G=0.629
Fat Mass (kg)							
	Baseline	16.49 ± 4.93	14.92 ± 4.03	14.30 ± 4.88	14.76 ± 3.94	15.12 ± 4.32	T=0.033
	1 Week	15.52 ± 4.30	14.08 ± 3.77	13.86 ± 4.79	13.84 ± 4.07	14.32 ± 4.08*	G=0.672
	4 Weeks	16.59 ± 4.67	13.92 ± 4.08	13.44 ± 3.00	14.02 ± 4.39	14.47 ± 4.07	T x G=0.675
Fat Free Mass (kg)							
	Baseline	39.35 ± 4.02	38.26 ± 4.05	39.55 ± 3.25	36.87 ± 3.06	38.55 ± 3.64	T=0.000
	1 Week	40.45 ± 3.47	39.25 ± 4.23	40.26 ± 3.12	38.34 ± 3.26	39.59 ± 3.53*	G=0.625
	4 Weeks	39.68 ± 4.20	40.01 ± 4.04	40.72 ± 3.73	38.38 ± 2.96	39.76 ± 3.72*	T x G=0.335
Percent Fat (%)							
	Baseline	28.23 ± 6.72	26.94 ± 5.71	25.25 ± 6.62	27.51 ± 5.34	26.36 ± 5.94	T=0.011
	1 Week	26.51 ± 5.08	25.47 ± 5.47	24.34 ± 6.52	23.46 ± 5.23	25.45 ± 5.39*	G=0.722
	4 Weeks	28.25 ± 6.34	24.89 ± 6.03	23.88 ± 4.11	25.60 ± 5.94	25.61 ± 5.66*	T x G=0.635
Total Body Water (%)							
	Baseline	51.31 ± 4.09	52.88 ± 6.72	48.86 ± 5.48	50.90 ± 4.48	51.11 ± 5.34	T=0.841
	1 Week	51.27 ± 3.83	51.79 ± 4.58	50.53 ± 3.61	50.01 ± 3.39	50.96 ± 3.80	G=0.593
	4 Weeks	50.61 ± 3.06	52.47 ± 4.51	48.80 ± 10.08	50.53 ± 3.08	50.73 ± 5.69	T x G=0.876

Data are means ± SD. BA signifies beta-alanine only group; BAC is beta-alanine and creatine combined supplementation group; CRE is creatine only group; and PLA is placebo.
*Significantly different from baseline.

Table 11: Effect size and magnitude calculations for select body composition variables

	BA	BAC	CRE
Body Weight	-0.63 (moderate)	-0.29 (low)	-0.33 (low)
Fat Mass	-0.37 (low)	0.02 (low)	0.15 (low)
Fat Free Mass	-0.36 (low)	-0.46 (low)	-0.69 (moderate)
Percent Fat	-0.43 (low)	0.12 (low)	0.34 (low)

Cohen's d calculations compared each group mean to PLA.
All calculations used data from week 4.

Body weight did not show any time x group interaction or group effects. There was a significant time effect seen for weight (p=0.021) with values increasing over time. There were trends of increasing weight through the course of the four week supplementation protocol in all groups when comparing delta values (0.66±2.74 kg for

β-ALA only, 0.98±1.23 kg for β-ALA and creatine combined, 0.11±0.69 kg for creatine only and 0.92±2.03 kg for placebo). The effect sizes for all groups compared to placebo were low, indicating a weak relationship.

There were no significant time x group interactions or group effects for fat mass. There was however, a significant time effect (p=0.033). Fat mass tended to decrease over time for most groups with the greatest reduction seen in the β-ALA and creatine combination supplementation strategy (-1.11±1.61 kg vs. -0.86±2.70 kg in creatine only, 2.25±5.92 kg in β-ALA only and -0.40±1.25 kg in placebo). The effect sizes for each group compared to placebo are low, thus indicating a weak relationship for this variable. Larger sample sizes may have been helpful in resulting in more significant results.

Fat free mass showed a similar trend as fat mass in that there was only a significant time effect (p=0.000) and no time x group interaction or group effect. When examining delta values, it shows trends towards more improvement after four weeks in the β-ALA and creatine combined group (1.94±1.32 kg vs. 1.16±1.85 kg in creatine only, -0.32±1.13 kg in β-ALA only and 1.51±1.33 kg in the placebo group. The effect sizes were low for the groups supplementing with β-ALA, indicating weak relationships. There was a moderate relationship seen with the effect size for the creatine only group compared to placebo. These results allow for the failure to reject the null for Hypothesis 6, which stated there would be no improvement in fat mass or fat free mass with supplementation.

There were no time x group interactions or group effects for percent body fat either. The time effect was significant (p-0.011). Looking at the delta values, there

shows to be a trend with the β-ALA and creatine combined group having greater reductions compared to creatine only, β-ALA only and placebo (-2.3±2.6%, -1.4±4.5%, 0.2±1.8% and -1.3±2.2%, respectively). The group trends are not statistically significant however, which may be due to the large variation depicted in the standard deviations, and the small sample sizes.

The results of the ANOVA for total body water showed no time x group interactions, group effects or time effects. There were no trends seen between groups when examining delta values either. These results led to the acceptance of the null for Hypothesis 10 stating supplementation will not elicit a difference in total body water between groups.

Aerobic Exercise Performance

Table 12 shows the results from the MANOVA that included VO_2max, time to VO_2max, maximal METs achieved and ventilatory threshold. Table 13 depicts select effect size calculations for non-significant group effects. It also interprets the values, which in this case are almost all low. This indicates most likely the sample size used for this study was too small to decipher significance since the relationship of the data to the population is low in this case.

Table 12: Aerobic performance results

		BA (n=8)	BAC (n=9)	CRE (n=8)	PLA (n=6)	Total (All Groups)	P Value
VO_2max (ml/kg/min)							
	Baseline	41.50 ± 5.60	39.43 ± 7.79	34.20 ± 5.73	35.88 ± 9.65	37.93 ± 7.44	T=0.527
	1 Week	41.58 ± 5.96	38.58 ± 8.14	36.10 ± 6.04	33.75 ± 10.49	37.78 ± 7.80	G=0.274
	4 Weeks	41.53 ± 6.12	38.10 ± 7.51	35.34 ± 2.98	37.90 ± 9.03	38.23 ± 6.68	T x G=0.093
Max Time (sec)							
	Baseline	1249.38 ± 209.64	1143.11 ± 310.88	962.88 ± 289.05	1093.00 ± 324.22	1114.32 ± 290.45	T=0.093
	1 Week	1293.88 ± 246.16	1152.67 ± 361.05	1019.75 ± 251.49	1031.83 ± 313.61	1131.42 ± 304.19	G=0.275
	4 Weeks	1293.38 ± 240.11	1132.11 ± 322.62	1045.88 ± 198.35	1083.00 ± 310.06	1141.97 ± 275.16	T x G=0.324
MET							
	Baseline	11.88 ± 1.60	11.23 ± 2.23	9.79 ± 1.64	10.27 ± 2.77	10.84 ± 2.13	T=0.274
	1 Week	11.88 ± 1.70	11.03 ± 2.34	10.31 ± 1.74	9.63 ± 3.00	10.79 ± 2.24	G=0.277
	4 Weeks	11.85 ± 1.74	10.89 ± 2.15	10.09 ± 0.85	10.83 ± 2.58	10.92 ± 1.91	T x G=0.095
Ventilatory Threshold							
(%VO_2max)	Baseline	86.81 ± 8.73	87.19 ± 10.29	77.01 ± 6.46	85.78 ± 10.64	84.19 ± 9.66	T=0.001
	1 Week	84.06 ± 7.34	86.22 ± 10.14	78.61 ± 10.53	85.78 ± 11.03	83.62 ± 9.79	G=0.351
	4 Weeks	78.59 ± 9.75	79.92 ± 13.15	76.50 ± 11.21	75.30 ± 9.63	77.80 ± 10.78*†	T x G=0.539
Data are means ± SD. BA significies beta-alanine only group; BAC is beta-alanine and creatine combined supplementation group;							
CRE is creatine only group; and PLA is placebo.							
* Significantly different from baseline. † Significantly different from 1 week.							

Table 13: Effect size and magnitude calculations for select aerobic performance variables

	BA	BAC	CRE
VO_2max	-0.47 (low)	-0.02 (low)	0.38 (low)
MaxTime	-0.76 (moderate)	-0.16 (low)	0.14 (low)
Ventilatory Threshold	-0.34 (low)	-0.4 (low)	-0.11 (low)
Cohen's d calculations compared each group mean to PLA.			
All calculations used data from week 4.			

VO_2max showed no significant time x group interaction, group effect or time effect. There was a trend for the time x group interaction (p=0.093) that may suggest some effect of the supplementation. However, the power and effect sizes are quite low, which may have affected this trend not being significant. In examining the delta values for VO_2max, participants in the creatine only group showed trends towards

improvements after one week of supplementation compared to β-ALA only, β-ALA and creatine combined and placebo, who all showed slight decreases (1.90±1.87 ml/kg/min vs. -0.01±3.30 ml/kg/min, -0.96±3.84 ml/kg/min and -2.13±5.14 ml/kg/min, respectively). At four weeks, this relationship still exists with the exception of the placebo group that seemed to show greater trends towards improvement (1.14±4.48 ml/kg/min for creatine only, -0.07±4.18 ml/kg/min for β-ALA only, -1.50±3.79 ml/kg/min for β-ALA and creatine combined and 2.02±1.78 ml/kg/min for placebo).

Time to VO_2max did not result in a significant time x group interaction, group effect or time effect. There was a trend for the time effect (p=0.093), however, low statistical power and wide variation within the data may have affected significance. The effect sizes were also low for this variable indicating a weak relationship and low strength of the data. In looking at delta values, the creatine group showed the greatest change towards improvement over the four weeks compared to other groups (83.00±125.17 sec vs. 41.14±110.51 sec for β-ALA only, -12.38±77.16 sec for β-ALA and creatine combined and -10.00±224.61 sec for placebo). This trend was not significant however, possibly due to the large standard deviation values associated with each group's data.

There was no significant time x group interaction, group effect or time effect for the maximal MET units achieved during the GXT. The time x group interaction shows a slight trend in the data (p=0.095), but with the small sample size and large variation within the data, significance was not reached.

Ventilatory threshold showed to have a significant time effect (p=0.001), but no group effect or time x group interaction. The direction of this result was not expected in that there was actually a decrease in ventilatory threshold over the four weeks of supplementation in each group (-9.77±7.85 %VO_2max for β-ALA only, -8.18±5.29 %VO_2max for β-ALA and creatine combined, -0.51±9.04 %VO_2max for creatine only and -10.48±13.27 %VO_2max for placebo. There is large variation within each group, which could explain the lack of significance and low effect size between groups.

The present results led to accepting Hypothesis 7, which stated there would be no difference in VO_2max levels between groups. Results also led to accepting Hypothesis 9 stating no differences would be seen in ventilatory threshold between groups.

Blood Lactate and Lactate Threshold

Table 14 reports the results from the MANOVA that included resting lactate, peak lactate, lactate threshold, OBLA and the difference in blood lactate between resting and maximal effort on the GXT. The power analysis showed low power values for most variables, thus making the interpretations of the statistics difficult. Because of this, effect size calculations were performed on select variables. Table 15 shows the results of the effect size calculations.

Table 14: Blood lactate and lactate threshold results

	BA (n=8)	BAC (n=10)	CRE (n=8)	PLA (n=7)	Total (All Groups)	P Value
Resting Blood Lactate (mmol/L)						
Baseline	1.44 ± 0.64	1.46 ± 0.45	1.33 ± 0.24	2.03 ± 0.96	1.54 ± 0.63	T=0.503
1 Week	1.60 ± 0.61	1.39 ± 0.56	1.18 ± 0.28	1.54 ± 0.46	1.42 ± 0.50	G=0.124
4 Weeks	1.58 ± 0.43	1.32 ± 0.47	1.53 ± 0.47	1.66 ± 0.45	1.50 ± 0.45	T x G=0.448
Peak Lactate (mmol/L)						
Baseline	12.91 ± 4.48	8.21 ± 3.43	9.71 ± 1.83	7.54 ± 2.26	9.57 ± 3.70	T=0.177
1 Week	10.43 ± 2.27	8.48 ± 3.33	9.20 ± 2.15	8.76 ± 1.25	9.18 ± 2.48	G=0.051
4 Weeks	9.85 ± 1.89	7.68 ± 2.70	8.99 ± 1.46	8.64 ± 2.28	8.73 ± 2.23	T x G=0.043[a]
Lactate Threshold (%VO_2max)						
Baseline	73.05 ± 8.44	79.05 ± 16.53	82.08 ± 8.55	79.59 ± 11.13	78.44 ± 11.94	T=0.825
1 Week	77.70 ± 10.16	80.64 ± 10.78	77.49 ± 8.95	72.26 ± 10.51	77.38 ± 10.13	G=0.665
4 Weeks	76.59 ± 4.69	77.71 ± 14.48	79.16 ± 11.59	76.13 ± 10.32	77.45 ± 10.70	T x G=0.655
Onset of Blood Lactate (%VO_2max)						
Baseline	77.50 ± 9.89	84.51 ± 11.37	81.71 ± 8.82	83.81 ± 8.82	81.98 ± 9.85	T=0.139
1 Week	78.50 ± 10.11	86.27 ± 9.78	83.48 ± 7.67	75.17 ± 17.43	81.35 ± 11.75	G=0.363
4 Weeks	84.14 ± 8.35	87.87 ± 8.86	83.76 ± 11.68	84.77 ± 11.93	85.31 ± 9.84	T x G=0.578
Blood Lactate Difference Baseline to Max (mmol/L)						
Baseline	11.48 ± 4.19	6.73 ± 3.52	8.39 ± 1.75	5.51 ± 2.15	8.02 ± 3.71	T=0.143
1 Week	8.83 ± 2.64	7.03 ± 3.37	8.03 ± 2.03	7.20 ± 1.59	7.74 ± 2.57	G=0.045[b]
4 Weeks	8.28 ± 1.88	6.29 ± 2.76	7.46 ± 1.26	6.81 ± 2.25	7.17 ± 2.19	T x G=0.014[c]

Data are means ± SD. BA significies beta-alanine only group; BAC is beta-alanine and creatine combined supplementation group; CRE is creatine only group; and PLA is placebo.

[a] BAC significantly greater than BA (p=0.010); BA significantly greater than PLA (p=0.026)

[b] BA significantly greater than BAC (p=0.014)

[c] BA significantly greater than PLA (0.016)

Table 15: Effect size and magnitude calculations for select blood lactate variables

	BA	BAC	CRE
Peak Lactate	-0.58 (moderate)	0.38 (low)	-0.18 (low)
Lactate Threshold	-0.05 (low)	-0.12 (low)	-0.28 (low)

Cohen's d calculations compared each group mean to PLA.

All calculations used data from week 4.

Results showed no time x group interaction, group effect or time effect for resting blood lactate. There were no trends seen when examining the delta values either. This led to accepting the first part of Hypothesis 8, which states there would be no difference in resting lactate between groups.

Peak lactate showed a significant time x group interaction (p=0.043) and a trend for a group effect (p=0.051). Post hoc analyses indicated that β-ALA supplementation alone had a lower peak lactate than the group supplementing with β-ALA and creatine combined (p=0.010) and a greater peak lactate than placebo (p=0.026). There was no time effect seen for peak lactate. In looking at delta values, the β-ALA only group seemed to show the greatest trend towards improvement with lower peak lactate values compared to the other groups after four weeks (-3.54±4.20 mmol/L vs. -0.59±2.36 mmol/L for β-ALA and creatine combined, -0.73±1.62 mmol/L in creatine only and 0.72±1.89 mmol/L in placebo). The effect sizes for each group compared to placebo were mostly low, indicating a weak relationship with this data. Increasing the sample size may help decrease the variation within each group, which may also help increase effect sizes and statistical power. These results led to accepting the latter part of Hypothesis 8, which states there would be no difference in peak lactate between groups.

There was no significant time x group interaction, group effect or time effect for lactate threshold after four weeks of supplementation. When looking at delta values after four weeks, the β-ALA only group shows a trend towards increasing lactate threshold over the other groups (4.6±10.38 %VO_2max vs. -1.49±15.06 %VO_2max for β-ALA and creatine combined, -2.91±13.96 %VO_2max in creatine only and -1.77±14.18 %VO_2max in placebo). The trend was not significant, which may be due to the large standard deviation values or small effect sizes noted between groups. Statistical power was also low, which may have been improved with larger sample sizes. These results led to accepting Hypothesis 9, stating no difference in lactate threshold between groups.

The onset of blood lactate did not result in a significant time x group interaction, group effect or time effect either. However, when looking at delta values after four weeks of supplementation, there was a similar trend to peak lactate with the β-ALA only group showing a greater difference in $\%VO_2max$ compared to other groups (8.91±13.97 $\%VO_2max$ vs. 3.73±8.09 $\%VO_2max$ in β-ALA and creatine combined, 2.05±14.18 $\%VO_2max$ in creatine only and 3.98±7.95 $\%VO_2max$ in placebo). Again, the standard deviations are quite large, which may be a factor as to why these relationships are not significant.

The final blood lactate variable examined was the difference in blood lactate between maximal effort on the GXT and baseline levels. Results showed a significant time x group interaction (p=0.014) and group effect (p=0.045) with post hoc analyses indicating that this lactate difference was larger in the β-ALA only group compared to placebo. There were no time effects for this variable. When comparing delta values after four weeks of supplementation, the β-ALA only group also showed trends towards greatest improvement by lowering this difference compared to other groups (-3.2±3.69 mmol/L vs. -0.44±1.91 mmol/L in β-ALA and creatine combined, -0.93±1.65 mmol/L in creatine only and 1.30±2.63 mmol/L in placebo). This relationship was not significant though, perhaps due to the large variation noted in the standard deviations and small sample sizes.

Anaerobic Exercise Performance

Table 16 shows the results of the MANOVA that included peak power, mean power, total work and rate of fatigue. Due to low statistical power values for group comparisons, effect size calculations were performed with results shown in Table 17.

Table 16: Anaerobic exercise performance results

			BA (n=8)	BAC (n=9)	CRE (n=8)	PLA	Total (All Groups)	P Value
Peak Power (W)								
	Wingate 1	Baseline	947 ± 359	895 ± 378	908 ± 210	651 ± 163	858 ± 283	T=0.297
		1 Week	986 ± 232	840 ± 177	891 ± 155	800 ± 169	881 ± 190	G=0.417
		4 Weeks	938 ± 142	796 ± 213	823 ± 178	851 ± 307	850 ± 211	T x G=0.60
								W=0.202
	Wingate 2	Baseline	857 ± 162	796 ± 186	773 ± 191	804 ± 211	807 ± 181	W x G=0.480
		1 Week	938 ± 65	905 ± 235	815 ± 215	755 ± 223	849 ± 191	T x W x G=0.037
		4 Weeks	869 ± 125	832 ± 245	946 ± 288	750 ± 170	852 ± 220	
Mean Power (W)								
	Wingate 1	Baseline	282 ± 35	349 ± 50	359 ± 47	318 ± 75	353 ± 55	T=0.368
		1 Week	393 ± 48	357 ± 51	369 ± 58	319 ± 47	361 ± 55	G=0.282
		4 Weeks	383 ± 48	352 ± 41	348 ± 69	338 ± 57	356 ± 54	T x G=0.592
								W=0.000§
	Wingate 2	Baseline	352 ± 62	329 ± 56	318 ± 41	298 ± 60	325 ± 56	W x G=0.390
		1 Week	345 ± 59	333 ± 54	330 ± 54	307 ± 56	330 ± 54	T x W x G=0.396
		4 Weeks	334 ± 59	328 ± 44	318 ± 52	306 ± 68	322 ± 54	
Total Work (J)								
	Wingate 1	Baseline	11467 ± 1048	10476 ± 1499	10764 ± 1420	9541 ± 2262	10591 ± 1653	T=0.368
		1 Week	11793 ± 1438	10719 ± 1531	11081 ± 1726	9566 ± 1422	10826 ± 1660	G=0.282
		4 Weeks	11494 ± 1430	10561 ± 1223	10437 ± 2071	10152 ± 1698	10674 ± 1621	T x G=0.592
								W=0.000§
	Wingate 2	Baseline	10565 ± 1862	9878 ± 1678	9545 ± 1235	8939 ± 1800	9761 ± 1678	W x G=0.390
		1 Week	10363 ± 1767	9986 ± 1617	9903 ± 1605	9220 ± 1673	9892 ± 1633	T x W x G=0.396
		4 Weeks	10019 ± 1785	9835 ± 1320	9548 ± 1556	9168 ± 2041	9663 ± 1619	
Rate of Fatigue (%)								
	Wingate 1	Baseline	107.37 ± 13.86	104.05 ± 14.32	103.74 ± 21.01	92.42 ± 9.35	102.26 ± 15.59	T=0.609
		1 Week	105.84 ± 14.05	102.00 ± 9.94	96.20 ± 16.62	108.42 ± 9.43	102.91 ± 13.06	G=0.199
		4 Weeks	109.84 ± 10.88	103.39 ± 10.34	104.20 ± 15.92	93.08 ± 12.40	102.70 ± 13.14	T x G=0.231
								W=0.015§
	Wingate 2	Baseline	91.65 ± 12.01	101.87 ± 11.38	95.99 ± 14.84	92.89 ± 13.68	95.88 ± 13.00	W x G=0.925
		1 Week	102.42 ± 9.53	97.87 ± 13.45	99.56 ± 11.22	90.30 ± 20.99	97.77 ± 14.14	T x W x G=0.113
		4 Weeks	108.52 ± 13.81	95.25 ± 16.57	99.56 ± 15.89	92.81 ± 11.12	99.11 ± 15.20	

Data are means ± SD. BA significies beta-alanine only group; BAC is beta-alanine and creatine combined supplementation group; CRE is creatine only group; and PLA is placebo.
§ Wingate #1 significantly greater than Wingate #2

Peak power did not show any time x group interaction, time x Wingate interaction, time effect, group effect or Wingate effect. There was however, a significant time x group x Wingate interaction noted (p=0.037). The creatine only group showed a trend towards improvement over other groups during the second Wingate test after four

weeks (138±14 W vs. 38±123 W in β-ALA alone, 41±131 W in β-ALA and creatine combined and -61±114 W in placebo). The effect sizes were low for most peak power group comparisons indicate weak relationship within the data. This could be due to the wide variation noted or small sample sizes for each group. When comparing delta values for the differences between the two Wingates at each testing session, the creatine only group also showed trends towards greater improvements as seen by a smaller gap between peak power values compared to other groups after four weeks (-258±177 W vs. -22±461 W in β-ALA alone, -135±379 W for β-ALA and creatine combined and 254±253 W in placebo). This relationship is not significant due to the large variation within groups. These results led to the failure to accepted Hypothesis 3, which states that power will be greater with β-ALA alone and creatine alone supplementation strategies. These results also allowed for the acceptance of Hypothesis 5 that stated there would be no difference in power with the combined β-ALA and creatine supplementation protocol compared to the other groups.

There were no time x group x Wingate or Wingate x group interactions for mean power. There were also no significant time or group effects. There was a significant Wingate effect (p=0.000) with the first Wingate having greater values than the second, which is to be expected. When comparing the change over time for the differences in mean power between Wingates, the creatine group shows trends of the most improvement over four weeks compared to other groups (-11±28 W vs. 19±39 W in β-ALA alone, 4±28 W in β-ALA and creatine combined and 13±38 W in placebo). This

relationship was not significant, possibly due to the large standard deviations within each group.

Total work showed no time x Wingate x group, time x group or Wingate x group interactions, and no time or group effects. There was only a significant Wingate effect (p=0.000) with the first test having higher values than the second, which is again expected. In looking at the delta values for the difference between Wingates, the creatine group showed trends towards greatest improvement by decreasing the difference in total work after four weeks (-331±835 J vs. 573±1168 J in β-ALA alone, 127±856 J in β-ALA and creatine combined and 382±1140 J in placebo). This relationship was not significant though, most likely due to the large standard deviations within groups and low effect sizes for most group comparisons.

Rate of fatigue on the Wingates did not elicit any time x Wingate x group, time x group or Wingate by group interactions. It also did not show any group effects or time effects. There was only a Wingate effect (p=0.015) with the first test being greater than the second. The delta values show the β-ALA and creatine combined supplementation group having a trend towards improvement after four weeks on the second Wingate compared to other groups (-15.8±30.7% vs. 18.0±15.1% in the β-ALA only group, 3.2±11.9% in the creatine group and 3.0±10.9% in the placebo group). The effect size calculations indicated strong values for the β-ALA only and β-ALA and creatine combined groups, therefore suggesting a strong relationship with the data for those groups.

The effect size calculations shown in Table 17 compared group means at week four to the placebo group. The effect sizes were mostly low in magnitude. However, a few were moderate to large, indicating reliable results from the MANOVA with the given sample size.

Table 17: Effect size and magnitude calculations for select anaerobic performance variables

	BA		BAC		CRE	
Peak Power Wingate 1	-0.36	(low)	0.21	(low)	0.11	(low)
Peak Power Wingate 2	-0.80	(moderate)	-0.39	(low)	-0.83	(moderate)
Total Work Wingate 1	-0.85	(large)	-0.28	(low)	0.15	(low)
Total Work Wingate 2	-0.44	(low)	-0.39	(low)	-0.21	(low)
Rate of Fatigue Wingate 1	-1.44	(large)	-0.90	(large)	-0.78	(low)
Rate of Fatigue Wingate 2	-1.25	(large)	-0.17	(low)	-0.49	(low)
Cohen's d calculations compared each group mean to PLA.						
All calculations used data from week 4.						

CHAPTER V

DISCUSSION AND CONCLUSION

The present study sought to examine the effects of β-ALA and creatine monohydrate supplementation on body composition, aerobic and anaerobic exercise performance and muscle carnosine and phosphagen levels in recreationally active females. The results did not show many differences between supplementation protocols for the majority of body composition, exercise performance or muscle biochemistry variables. However, there were trends in the data that implied some possible positive effects of β-ALA and creatine supplementation in this given population. The details of the findings are explained in the subsequent sections.

Muscle Biochemistry

The present study showed a significant difference in muscle carnosine content between supplementation groups, all being greater than the placebo group. This is an expected result based on previous studies of similar β-ALA supplementation strategies and muscle carnosine content. Harris et al. [5] showed an increase of about 42% after four weeks with a supplementation protocol of 3.2 g/day of β-ALA. The present study showed an increase of about 35% in the β-ALA alone group and an increase of about 42% in the β-ALA and creatine combined group with an average dose of 6.1±0.7 g/day of β-ALA. The present study used an individualized dosing strategy that corresponded to 0.1 g/kg/day for the entire four weeks. Other studies have used a supplementation strategy that increased in dose over the course of the study to 4.8 g/day [4], or 6.4 g/day [2, 75] and have shown greater increases in muscle carnosine levels of about 47% [4],

52% [75] and 59% [2]. Longer supplementation protocols of up to 10 weeks have also shown significantly greater increases in carnosine content of about 80% over baseline values [2]. Currently, there are no studies that have looked at the effects on muscle carnosine content with a combined supplementation protocol of β-ALA and creatine. The present study directly compared these supplementation strategies; however, there was no significant difference seen between the two groups, despite the combined strategy showing a slightly greater increase in carnosine levels. Although there was an increase in muscle carnosine, this did not translate into improved performance measures as a result of supplementation, as will be discussed in subsequent sections.

There were some differences between groups observed for muscle creatine over the four weeks as well with the two groups with creatine having greater values than the β-ALA only group. It is important to note that the sample size for muscle creatine and phosphagens was quite small due to prioritizing muscle carnosine assays as well as some samples not being large enough to run the appropriate assays. The present study did not show significant differences between groups for muscle creatine variables; however, there were some trends after the first week of loading with creatine monohydrate in the creatine and β-ALA plus creatine supplementation groups. This agrees with previous studies that indicate significant increases in muscle creatine after a loading phase [10]. Previous studies have reported that the typical loading phase of creatine supplementation (20 g/day for 5 days) results in an increase in PCr of about 10-40% [122-124]. The present study showed conflicting results after one week of supplementation with percent changes for PCr of about -18% with creatine supplementation alone and about 78%

when creatine was combined with β-ALA. The combined supplementation group showed the greatest increase in PCr after the first week, implying there may be a synergistic effect of the two supplements. It is unknown why the creatine group in this study reported the lowest percent change of all the groups. When examining the raw data, the creatine group did have the highest PCr levels at baseline, although not statistically different from the other groups, which may have played a role in not seeing as much of an increase with supplementation. However, this does not explain the negative percent change after one week of loading. After four weeks, the percent change increased, but was still the lowest of the four groups. Granted, the large variation within each group is also a factor that must be noted as this may affect the significance of the data. The literature has also reported increases in total creatine of around 10-30% [122-124]. These values again do not agree with previously reported results in the literature since creatine supplementation alone only increased levels by about 7% and the combined supplementation with β-ALA increased levels by about 104%. Like with PCr, the combined supplementation group had the greatest increase after one week, however with such a large standard deviation (±258.2), this result may not be accurate to interpret.

Although the present study failed to show significant increases in muscle creatine or phosphagens as a result of four weeks of supplementation, it is well reported in the literature that this relationship does exist for creatine monohydrate supplementation after four weeks [91, 125]. Since there were increases after the first week of loading with creatine, which were not seen after four weeks, it may be safe to say that the

supplementation was adequate for loading, but the maintenance dose was not sufficient to maintain the levels statistically. Vandenberghe and colleagues [90] examined the effects of creatine supplementation after 10 weeks in women with a loading dose for the first four days and a maintenance dose of 5 g/day for 65 days. He reported a significant increase in PCr over baseline levels with supplementation over placebo of 5.2%. Another study by Volek and colleagues [126] however, failed to show that a longer duration of lower amounts of supplementation maintained increases in muscle creatine and PCr levels. This study examined the effects of supplementation after 12 weeks and reported increases in PCr and total creatine of approximately 5.3% and 10.6%, respectively, but these were not significant compared to baseline. The present study did use an individualized supplementation strategy of 0.3 g/kg/day for the loading phase in week one followed by 0.1 g/kg/day for the following three weeks. This corresponded to an average of 18.3±2.0 g/day for the loading phase and 6.1±0.7 g/day for the maintenance phase of the study. Despite the trends towards increased muscle creatine levels seen in the first week of supplementation, this did not seem to correspond to performance improvements as will be discussed in later sections.

This study also examined the effect of supplementation in females, which is different from most studies pertaining to β-ALA and creatine supplementation. Most studies have examined the effects of β-ALA in males and/or trained athletic populations. β-ALA is a supplement suggested for more athletic populations due to the potential performance enhancing benefits; therefore, the population of the present study may not have been the optimal target for this specific supplementation protocol. Also, there is

little information comparing gender differences and β-ALA supplementation. The data is undecided as to whether there is a difference in carnosine, creatine and PCr levels between men and women. Fosberg and colleagues [127] showed females to have greater total creatine amounts relative to tissue weight; however other studies show there is no difference between genders [128].

There is also the issue of responders and non-responders in relation to creatine supplementation as well as β-ALA supplementation. According to Greenhaff et al., [88], an increase in total creatine of at least 20 mmol/kg DW is needed in order to see ergogenic effects in exercise performance. The present study did not show that any group had that great of an increase after four weeks of supplementation, which may explain the lack of performance related effects in the present study. There is less evidence supporting the issue of responders and non-responders for muscle carnosine as a result of β-ALA supplementation, however, the concept may be similar to creatine, thus partially explaining the lack of ergogenic responses in the present study.

Body Composition

The results of the present study agreed with previous studies showing body composition measures of body weight, fat mass, fat free mass and percent fat improving over time. However, in the present study, this could not be directly attributed to the supplementation protocol since there were no group differences. Therefore, the reason for these improvements in body composition is in question since the protocol did not involve an exercise training program. The participants were all recreationally active and maintaining their own exercise program from before the start of the study. The addition

of the supplementation may have allowed for improved workouts, which may in turn affect their body composition. In a study by Hoffman et al. [3], it was reported that training volume was improved with β-ALA supplementation in college football players. Although the present study included only moderately active females, the same may have been the case with supplementation, although this was not directly measured and only trends were found with improvements between supplementation groups. Hoffman and colleagues [18] showed an increase in lean body mass and decrease in percent body fat over the course of 10 weeks of supplementation with a combination of β-ALA and creatine compared to placebo. Their supplementation strategy was similar to the present study, but included male strength power athletes who continued with their strength training routine during the study. Smith and colleagues [68] found an increase in lean body mass with β-ALA supplementation compared to placebo in recreationally active males. This study included a high intensity interval training protocol in addition to the supplementation, which may have contributed to the differences in body composition. The present study focused on the influence of supplementation alone, as participants were not involved in any exercise training as part of the study design, only asked to continue their normal exercise routine under specific guidelines.

Total body water was also examined in this study and reported to have not changed over the course of the four weeks of supplementation for any group. This suggests that any gains in fat free mass were not due to hyperhydration.

Aerobic Exercise Performance

β-ALA and creatine supplementation are believed to have a mild effect on aerobic exercise markers, although the literature is torn on this relationship. The results of the present study are in line with most studies in that there did not seem to be any improvements with four weeks of supplementation. There are not many studies that examine VO_2 variables with β-ALA and creatine, as they are known more for their anaerobic effects. However, Baguet et al. [70], used a similar supplementation protocol for β-ALA as the present study with physically active males and did not show any differences in VO_2 throughout exercise as a result of supplementation. On the other hand, Smith and colleagues [68] reported significant differences in VO_2peak and time to reach VO_2peak as a result of β-ALA supplementation for six weeks in recreationally active males. This study however, included a high intensity interval training program, which may have played a role in seeing significant performance differences. Zoeller et al. [9] examined the effects of β-ALA and creatine supplementation on aerobic exercise performance and was able to show that the combined supplementation strategy significantly improved five markers of cardiorespiratory endurance including: VO_2 and power output at lactate threshold and ventilatory threshold, and percent VO_2peak at ventilatory threshold. When compared to creatine only, there was only an improvement in time to exhaustion and power output at ventilatory threshold for this group.

Stout and colleagues [15] measured the effects of β-ALA supplementation on ventilatory threshold in females. They supplemented for 28 days and found that ventilatory threshold and time to exhaustion were increased in the β-ALA group. The

present study was unable to show similar results with β-ALA supplementation groups. There was a slight trend with the creatine only group towards improvement in time to VO_2max, but this was not significant. The lack of significance in the present study is partially due to the low power and effect size of the data, which may have been improved with a larger sample size. The ventilatory threshold values of the present study actually decreased over time for all groups, which is difficult to explain aside from there being a familiarity effect to the GXT or large variation in the data resulting in unreliable statistics for this variable. It is unlikely that familiarity was a major factor as all participants underwent familiarization tests on the cycle ergometer prior to starting the study protocol.

Blood Lactate and Lactate Threshold

The increase in muscle carnosine following supplementation would theoretically affect blood lactate levels and lactate threshold since one of the main functions of carnosine is as an intramuscular pH buffer. The present study was able to show a significant difference in peak lactate achieved during the maximal aerobic capacity test for the group supplementing with β-ALA over the combined supplementation and placebo. However, the study failed to show any differences with lactate threshold between the groups, only a trend of β-ALA supplementation improving levels after four weeks.

Previous studies have reported mixed results pertaining to the effect of β-ALA and creatine supplementation on blood lactate accumulation and lactate threshold. Van Thienen and colleagues [67] reported no difference between groups in blood lactate

levels in healthy males after an incremental maximal cycle ergometer test followed by a 30 second all out sprint after eight weeks of supplementation with β-ALA or placebo. Zoeller et al. [9] studied 55 men who supplemented with β-ALA, creatine, a combination or placebo for 28 days and reported a greater VO_2 at lactate threshold for the combined supplementation group, suggesting that this supplementation protocol may delay the onset of lactate threshold during incremental exercise.

Stoud and colleagues [129] examined the change in blood lactate levels during a GXT on the treadmill with creatine supplementation and found no change in blood lactate concentrations as a result of a loading phase of five days of 20 g/day of creatine. Greenhaff et al. [76] also reported no difference between creatine and placebo supplementation groups for blood lactate accumulation with an isokinetic exercise protocol of 5 x 30 maximal voluntary unilateral knee extensions at 180°/s.

The present study may have failed to show improvements in lactate accumulation and lactate threshold with β-ALA alone or the combined β-ALA and creatine supplementation strategy, despite the increase in muscle carnosine content that was observed, for various reasons. First, the power analysis and effect size calculations were low, which indicates the strength of the data could be improved, possibly with a larger sample size. Also, the present study examined the effects of supplementation in recreationally active females, who did not engage in a training program during the four weeks of the study. Perhaps with a training program, like one seen in other studies, there may have been training effects seen for lactate variables.

Anaerobic Exercise Performance

The present study failed to show any significant differences between groups for peak power, mean power, total work rate of fatigue. However, there were trends in the data that suggest creatine only supplementation may have led to greater improvements in peak power, mean power and total work. Another trend suggested that the combined β-ALA and creatine supplementation may have led to an improvement in rate of fatigue after four weeks.

The support for creatine supplementation is greater than β-ALA since it has been more extensively researched. Van Thienen et al [67] showed a significant increase in mean power with β-ALA supplementation during a 30 second all out sprint on the cycle ergometer after eight weeks of supplementation. Wiroth and colleagues [130] showed creatine supplementation improved maximal power and work during a set of 5 x 10 second sprints on the cycle ergometer. Green and colleagues [105] specifically examined the effect of creatine supplementation of 20 g/day for six days on peak power, mean power and percentage decline during multiple leg and arm Wingate tests. They were able to conclude that peak power increased with creatine supplementation during the first arm Wingate test, and percentage decline was lower with supplementation after the second leg Wingate test. Ziegenfuss et al. [131] also showed that creatine supplementation in college athletes resulted in increased total work and peak power during multiple maximal 10-sec sprints on a cycle ergometer.

The literature for β-ALA supplementation and anaerobic measures is not as available, and actually tends to lean towards a lack of differences between groups.

Hoffman et al. [3] studied the effects of 3 weeks of β-ALA supplementation in college football players. They used a modified 60 second Wingate anaerobic test and did not show any difference between supplementation groups for peak power, mean power or total work. They showed a trend with rate of fatigue that suggested β-ALA supplementation may have improved values over placebo. The authors suggested that the supplementation protocol may not have led to a great enough increase in muscle carnosine to cause performance effects in this group of highly trained athletes. Another study by this group examined the effects of creatine alone, β-ALA and creatine combined and placebo supplementation in strength power athletes. This study did not show any improvement for any supplementation group with the Wingate test, and no differences were observed between groups [18]. Although these results are similar to the present study, the reasoning could be somewhat opposite since the present study included only recreationally active females instead of trained athletes. The participants in the present study may not have shown any improvements due to the lack of training associated with their supplementation.

Although these studies have not shown significant improvements with β-ALA supplementation and power measures, β-ALA has been linked to strength gains [16] and improved training volume [3]. Therefore, there is a definite need for more research in this area.

Conclusion

The results of the current study showed increases in muscle carnosine and phosphagen levels with supplementation compared to placebo, but not between specific

supplementation strategies. These overall increases with supplementation in the muscle were not associated with improved body composition or exercise performance, therefore failing to show beneficial outcomes of β-ALA and creatine supplementation.

The present study failed to show any additive effects of creatine and β-ALA supplementation for body composition, aerobic exercise, lactate thresholds or anaerobic exercise measures. This could be due to the small sample size of the present study resulting in low power and effect sizes. Previous research has demonstrated that four weeks of creatine and β-ALA supplementation was sufficient to increase muscle carnosine and phosphagen levels. However, perhaps more time is needed for performance adaptations to occur, especially without the addition of an exercise training component. Also, both of these supplements may have had a greater effect on a more trained population, or if combined with a specific anaerobic training program, since previous research has shown success when taken alongside a training program.

This is one of the first studies to use an individualized dosing strategy for β-ALA supplementation instead of a standardized amount for all participants. This may have also played a role in the lack of significant findings between groups in that there may be a gender effect with females needing a different amount compared to males to elicit performance effects. The current study utilized physically active females who had an average intake of 6.1 g/day, compared to other studies that supplement with up to 6.4 g/day mostly in males, that corresponded to approximately 0.1 g/kg/day in most studies, which was the amount utilized in the present study as well. However, there may be

gender differences in the storage or utilization of carnosine once in the muscle that prevented the present study from seeing more significant results.

Despite the results of the present study, it is still believed that β-ALA and creatine play a role in improving anaerobic exercise markers, lactate threshold as well as body composition when combined with an exercise program. Further studies should be conducted to show the potential effects of a combined supplementation strategy in athletic populations. Additionally, future studies should examine the effects of combined supplementation on muscle carnosine and phosphagen levels in a larger and/or more active population.

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PUBLICATIONS

- **Culbertson J**, Kreider RB, Greenwood M, Cooke M. Effects of beta-alanine on muscle carnosine and exercise: a review of the current literature. *Nutrients*. 2(1):75-98, 2010.

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- **Kresta JY**. In Press. Energy Demands: Sedentary vs. Active Individuals. In *Nutritional Guidelines for Athletic Performance: The Training Table*, edited by L. Taylor. Oxford, UK: Taylor and France.