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EFFECTS OF BETA-ALANINE SUPPLEMENTATION AND HIGH INTENSITY INTERVAL TRAINING AMONG RECREATIONALLY ACTIVE FEMALES

by

MARY ELIZABETH YARBROUGH

(Under the direction of John Dobson)

ABSTRACT

Beta-alanine is a non-essential amino acid that when combined with L-histidine forms the dipeptide carnosine. Recent research has shown that supplementary intake of beta-alanine can substantially increase carnosine content in muscle fibers and has been associated with attenuating fatigue and enhancing high intensity exercise performance. The aim of this study was to examine the effects of 4 weeks of beta-alanine supplementation combined with high intensity interval training (HIIT) on indices of aerobic and anaerobic performance, rowing performance, and body composition. Twenty-one recreationally active females (22.2 ± 2.2 yrs.) participated in a double blind, placebo controlled study and were randomly assigned to one of 3 groups: beta-alanine (BA, $n = 8$), placebo (PLA, $n = 7$), or control (CON, $n = 6$). Prior to and following 4 weeks of supplementation all groups had anthropometric measurements done and body composition was determined using dual energy x-ray absorptiometry (DEXA). Participants also performed a graded exercise test on the rowing ergometer to determine VO_2 peak, ventilatory threshold (VT), and time to exhaustion (TTE). Additionally, participants came back to the lab to complete a 1,000 m time trial (TT) to determine time for completion and peak power output (PPO). During the 4 week intervention the BA and PLA groups completed a 7 minute 1: 1 (work: recovery) HIIT protocol that was a pre-determined workload whilst consuming either 6.4 g/day of BA or PLA. All three groups showed significant improvements from pre to post testing in VO_2 peak, TTE, VT, TT, PPO and a reduction in total BF% in arms ($p \leq 0.05$). While several of the variables had significant interactions between groups: VO_2 peak, TTE, TT, PPO, BF% legs, and BF% trunk, ($p \leq 0.05$), post hoc testing revealed that peak power output was the only the variable that showed a significant difference between the BA and PLA groups as compared to the CON group during post testing ($p = 0.00$). The results of this study suggest that HIIT can enhance measures of aerobic capacity and increase PPO, while beta-alanine supplementation did not seem to have much of an effect on training.

INDEX WORDS: Carnosine, Beta-alanine, Rowing, Time trial, Peak power output, Aerobic capacity, Ventilatory threshold

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INTERVAL TRAINING AMONG RECREATIONALLY ACTIVE FEMALES

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Fulfillment of the Requirements for the Degree

MASTERS OF SCIENCE

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DEDICATION

I am dedicating this to my daughter Isabel Katherine Yarbrough, who was born during my last year as a graduate student, and my husband Rob Yarbrough. Both provided me with lots of love and support and never let me give up even when I wanted to. I love you both more than anything and cannot ever say thank you enough.

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CHAPTER I

INTRODUCTION

Rowing is a full body workout and when rowers are completing a 2,000 meter race in ~6-8 minutes and is dependent on the capacity of both the aerobic and anaerobic energy pathways. Some training strategies might involve ergogenic aids such as high intensity interval training or supplementing with beta-alanine. Beta-alanine has been shown to increase muscle carnosine content and muscle buffering capacity, while high intensity interval training has been shown to improve anaerobic and aerobic fitness.

Beta-alanine (BA) is a nonessential amino acid that can combine with L-histidine to form the cytoplasmic dipeptide carnosine (β -alanyl-L-histidine). In humans, the rate of carnosine synthesis in skeletal muscle is limited by the availability of beta-alanine. Carnosine is found at highest concentrations in skeletal muscle and can act as an intracellular buffer during high intensity exercise, the mechanism of carnosine presumably relates to its characteristics as a calcium sensitizer (Dutka et al., 2011). There are several determinants of muscle carnosine concentrations including: gender, age, muscle fiber type, diet, supplementation, and exercise training (Sale, 2013). According to Dutka and colleagues (2011), carnosine concentrations in skeletal muscle are almost twice as high in fast-twitch (type II) fibers as slow twitch (type I) fibers. Harris and colleagues (2006) found that oral supplementation of beta-alanine can considerably elevate carnosine concentration in humans. The increases in carnosine concentration within the muscle are associated with performance enhancements, such as buffering capacity, increased power, and delay in time to fatigue. Several studies have shown that beta-alanine supplementation can increase muscle carnosine ~45-60% after 4 weeks of supplementation and can improve anaerobic capacity and muscle function during high intensity

exercise (Harris et al., 2006; Derave et al., 2007). Increases in carnosine concentration through regular supplementation with beta-alanine may improve muscle buffering capacity and delay the onset of fatigue during intense exercise (Walter, 2010). Currently, the only known adverse effects of beta-alanine supplementation are the symptoms of parasthesia, which are triggered by high and acute single doses and typically disappear within approximately an hour after ingestion (Harris, 2006). Parasthesia is defined as a skin sensation, such as burning, prickling, itching, or tingling, with no apparent physical cause. Considering that beta-alanine is an amino acid that naturally plays a relevant role in the human body and that doses studied so far are quite similar to that found in diet, it is likely that this supplement is safe (Artioli, 2010). Based off of previous studies, supplementing with beta-alanine for four or more weeks can evoke significant changes in exercise performance or capacity especially during high intensity exercise or when performance is likely to be limited by hydrogen ions in skeletal muscle (Sale, 2013).

High-intensity interval exercise results in diminished stores of adenosine tri-phosphate (ATP), phosphocreatine (PCr) and glycogenic substrates, and the intracellular accumulation of metabolites (adenosine di-phosphate (ADP), inorganic phosphate (Pi), hydrogen ions (H⁺) and magnesium (Mg⁺), each of which has been implicated as a cause of muscle fatigue (Smith et al., 2009). Depletion of PCr store has frequently been cited as a rate limiting factor for performance of repeated sprint exercises (Bishop, 2004). Different aerobic and anaerobic training modalities have been shown to enhance performance, including 5- to 30-second repeated sprints (Burgomaster, 2006 and Creer, 2004). Recent literature has termed this type of training, high-intensity interval training (HIIT). HIIT is also known to be a more efficient training strategy for individuals who have time constraints and want to see similar outcomes to those who do a traditional endurance training protocol. According to the National Strength and Conditioning

Association, HIIT training can provide benefits such as: aerobic and anaerobic fitness, decreased blood pressure, cardiovascular health, insulin sensitivity (which helps the exercising muscles more readily use glucose for fuel to make energy), lowered cholesterol, lower abdominal fat and body weight while maintaining muscle mass.

Events such as rowing involve both the anaerobic and aerobic energy systems. Aerobic oxidation takes over after about 75 seconds of near maximal efforts and becomes the primary energy system used; it also has the greatest potential for improvement with training, about 2-4% in well trained athletes (Laursen, 2010) and more in untrained individuals. Akca and Aras (2015) found that competitive rowers had significantly improved rowing performance (peak power output, VO₂ Peak, and 2,000 meter time to completion) after 4 weeks of high intensity interval training or supramaximal interval training.

The combination of high intensity interval training and beta-alanine supplementation has been shown to improve ventilatory threshold (VT), this has been highly correlated with lactate threshold (LT). While these represent different metabolic mechanisms they are both representative of anaerobic threshold (AT) (Bishop, 2004). Anaerobic threshold can be defined as the exercise intensity at which performance decrements rapidly ensue and are considered the onset of fatigue (Walter, 2010).

There is a dearth of scientific research that involves recreationally active females, especially using the rowing ergometer as a mode, current research uses the cycle ergometer or treadmill and to our knowledge there is no current research that specifically looks at BA supplementation combined with HIIT using non-competitive female rowers. Therefore, the main purpose of this study was to determine if beta-alanine supplementation combined with four weeks of high intensity interval training on the rowing ergometer would lead to significant

improvements in oxygen consumption (VO_{2peak}), ventilatory threshold, and body fat percentage in recreationally active females. A secondary purpose of this study was to look at time to complete a 1,000 meter time trial and peak power output in pre and post testing as well as segmental body composition changes in the arms, trunk, and legs. It was hypothesized that supplementing with beta-alanine and HIIT for 28 days would lead to improvements in VO_2 peak, ventilatory threshold, body composition, 1,000 meter time trial, and peak power.

CHAPTER II

REVIEW OF LITERATURE

Most research using the rowing ergometer involves competitive rowers, as this mode can provide a full body workout while using both anaerobic and aerobic energy pathways. High intensity interval training (HIIT) has become popular for people who have time constraints, but want the benefits of endurance training, improvements in oxygen consumption and time to exhaustion (NSCA). Beta-alanine supplementation has been shown to help with pH buffering in skeletal muscle and can attenuate muscular fatigue. Combinations of HIIT and BA supplementation on the rowing ergometer could potentially delay the onset of fatigue, increase peak power, increase oxygen consumption, and improve time trial performance, while providing a full body workout.

Beta-alanine and Carnosine

Carnosine (β -alanyl-L-histidine) is a cytoplasmic dipeptide that is synthesized from beta-alanine and histidine; it is found in the highest concentration in skeletal muscle and relatively high concentrations within the central nervous system (Sale, 2013). Carnosine contributes to intracellular muscle pH buffering capacity and among other functions is thought to play a role in slowing the development of acidotic muscle fatigue (Decombaz, 2011; Artioli, 2010; and Derave, 2007). Oral ingestion of beta-alanine (3.2-6.4g/day), the rate limiting precursor in carnosine synthesis, has been shown to elevate the muscle carnosine content by ~45-60% after 4 weeks of supplementation with an aim to improve anaerobic muscle capacity and muscle function during high intensity exercise (Harris, 2006 and Derave, 2007). The endogenous formation of beta-alanine in humans is synthesized in the liver from degradation of uracil, where

exogenous supply mostly depends on the type and amount of animal protein in the diet (Decombaz, 2011). On average an omnivore can ingest ~ 0.8 g/day of carnosine from meat and fish (Harris, 2006) with the highest concentrations found in chicken and turkey (Abe, 2000).

Harris and colleagues (2006) tested three different doses: 40, 20, and 10 mg/per kg of body weight/per day. They found that the highest dose elicited a peak in serum beta-alanine levels, which was related to intense and unpleasant symptoms of paresthesia starting approximately 20 minutes after the capsule ingestion and ending approximately 60 minutes after. Paresthesia is defined as a skin sensation, such as burning, prickling, itching, or tingling, with no apparent physical cause and could potentially cause unpleasant sensory symptoms associated with the ingestion of beta-alanine. The 20 mg dose resulted in a lower peak in serum beta-alanine, but was also accompanied by symptoms of paresthesia, which were less frequent and intense, while the 10 mg/per kg of body weight resulted in no significant symptoms of paresthesia and a discrete peak of serum beta-alanine. Harris et al. (2006) split the amounts of beta-alanine given daily into multiple doses of 800 mg (~10 mg/kg/day) and was successful in reducing the incidence of paresthesia. Numerous studies have also confirmed the efficacy of this supplementation protocol, (Hill, 2007 and Kendrick, 2008), and more recently, data suggest that it is possible to reach the same total daily dose of 6.4 g using a twofold higher single dose when using controlled release capsules (1600 mg instead of 800 mg) (Hoffman, 2008, Stout, 2007, and Zoeller, 2007).

Decombaz et al. (2011) compared the kinetics of plasma beta-alanine and its association with beta-alanine induced paresthesia symptoms following the ingestion of 1.6 g/day of beta-alanine slow release tablets or the pure aqueous solution of beta-alanine. Three different treatments were administered: 1.6g of pure beta-alanine in an aqueous solution, 1.6 g of slow

release beta-alanine, or placebo (Carnosyn™, Natural Alternatives International (NAI), San Marcos CA) each was ingested with 250 ml of water. Each treatment was separated by one week and in a fasted state. Subjects were provided with questionnaires with information on sensations, mood, or anxiety state and blood and urine samples were collected at various intervals to assess beta-alanine concentrations over the course of the next 6 hours. Researchers found that ingesting slow release tablets, rather than the pure solution, improved retention of carnosine and there was no evidence of paresthesia in either group.

Stegen and colleagues (2014) designed an experiment to determine an optimal maintenance dose ensuring that carnosine levels remained elevated by 30-50% above baseline. They wanted to determine if sex, body mass, and fat free mass are factors during muscle carnosine loading, maintenance, and washout. The researchers found that sex and body mass have minimal effects on absolute carnosine increases and are not the major determinants for increases in carnosine content. In order to keep levels ~30-50% above baseline, an average dose of 1.2 g/day is considered optimal to maintain carnosine concentration. The washout period for beta-alanine appears to be a relatively slow process ~2-4% a week, but is also related to the carnosine content; essentially, the more you have, the more you can lose (Stegen, 2014; Baguet, 2009; and Stellingwerff, 2011). Moreover, Baguet and colleagues (2009) showed that there are high and low responders to beta-alanine supplementation. The washout period for high responders is about 15 weeks compared to 6 for low responders, this after supplementing with 4.8 g/day for 6 weeks (Baguet et al., 2009). Evidence seems to indicate that taking beta-alanine is safe in doses from 3.2-6.4 g/day and that the slow release tablets can help reduce the incidence of any unpleasant side effects.

Beta-alanine and Performance

Several researchers have used the non-invasive method called proton magnetic resonance spectroscopy (MRS) to assess carnosine concentrations in skeletal muscle and have determined that it is an effective and practical tool that can be used to assess muscle carnosine content as opposed to muscle biopsies (Bex, 2013 and Derave, 2007). Bex and colleagues (2013) also wanted to determine if carnosine concentrations were higher in trained rather than untrained individuals. The study found that the carnosine levels were much higher (almost double) after supplementation as compared to the untrained individuals (Bex et al, 2013) which is comparable to other studies (Baguet et al., 2010; Derave, et al., 2007; and Saunders, 2012). In contrast Painelli and colleagues (2014) found that beta-alanine, when used as an ergogenic aid for high-intensity exercise, can improve exercise performance regardless of training status. In a similar study Derave and colleagues (2007) aimed to evaluate the use of proton MRS as a non-invasive tool to monitor carnosine concentrations and to determine if supplementation with beta-alanine for 4 weeks would elevate muscle carnosine and affect exercise performance in 400 m sprint among trained athletes. The beta-alanine group significantly increased carnosine content and dynamic knee extension torque significantly improved as compared to the placebo group. Despite conflicting results it would appear that in certain situations beta-alanine can augment carnosine content in trained individuals more than in the untrained.

Jordan and colleagues (2010) were the first researchers to look at running performance and the onset of blood lactate accumulation (OBLA) during a graded exercise test (GXT) after supplementation with beta-alanine. The beta-alanine group did have a delay in the onset of OBLA after 4 weeks of supplementation, but also had a reduced aerobic capacity in comparison to the placebo group. Other research has shown that supplementing with beta-alanine can also

reduce the onset of blood lactate accumulation (OBLA) by increases in heart rate at OBLA, % heart rate max @ OBLA as compared to the placebo groups (Jordan, 2010 and Zoeller, 2007). Both studies also showed no changes in VO_2 peak or decrements with the beta-alanine, these were similar to other findings (Stout, 2006; Baguet, 2010; and Kresta, 2014). Other studies have shown that supplementing with beta-alanine, sodium bicarbonate, or the combination of the two can increase blood lactate levels during exercise (Tobias et al., 2013 and Mero et al., 2013). Saunders and colleagues (2012a) found that both the placebo and beta-alanine groups had increased blood lactate levels during exercise with no effects from supplementation. This is similar to what Baguet et al. (2010) found in a comparable study. While blood lactate levels increase during exercise, the reduction of OBLA improved after supplementing with beta-alanine, sodium bicarbonate, or the combination of the two. VO_2 peak did not significantly improve in the groups, however time to exhaustion did improve. This delayed onset of neuromuscular fatigue was most likely due to the increased carnosine concentration.

Stout et al. (2006) examined the effects of 28 days of beta-alanine supplementation and the onset of neuromuscular fatigue and ventilatory threshold among women. The researchers found that the ventilatory threshold (VT) improved, physical working capacity increased, and time to exhaustion all improved in the beta-alanine group as compared to the placebo group. They did not see any significant improvements in maximal oxygen consumption. In contrast Smith and colleagues (2012) found that both the beta-alanine and placebo groups had significant improvements in maximal oxygen consumption and slight, non-significant changes in time to exhaustion and ventilatory threshold. Kresta et al. (2014) researchers had recreationally active females supplement with creatine and beta-alanine combined, beta-alanine, creatine, or placebo. They found that there were not any significant improvements on body composition, aerobic (VO_2

peak, TTE, or VT), or anaerobic performance (total work or peak power) measures in any of the groups.

Hoffman and colleagues (2014) wanted to determine if supplementation with beta-alanine could improve tactical and cognitive performance among an elite combat unit after a series of exhaustive exercises. The researchers found that cognitive performance was not impacted, but power performance, marksmanship, and target engagement speed improved from pre-ingestion levels. Both the placebo and beta-alanine groups had significant improvements in their 4km run time, but no difference between groups. Peak jump power and number of firing shots at post testing was greater for the participants who consumed beta-alanine as compared to the placebo group.

Painelli et al. (2014) looked at the effects of training status on high intensity intermittent performance after supplementing with beta-alanine. The trained (T) and non-trained (NT) men were split between placebo (PL) and beta-alanine (BA), this provided four experimental conditions total. Researchers found that total work done significantly increased following supplementation in both the TBA and NTBA groups, significantly decreased in the NTPL, and had no change in the TPL. Regardless of training status researchers determined that using beta-alanine as an ergogenic aid for high intensity exercise can be beneficial. These findings are in contrast to previous studies that have shown no effect of beta-alanine supplementation with highly trained individuals (Derave et al. 2007; Baguet et al 2010; and Saunders et al 2012).

Baguet and colleagues (2010) looked at the role of muscle carnosine in rowing performance and found that there was a positive correlation between the two. The beta-alanine group also improved time to exhaustion following supplementation by 4.3 seconds as compared

to the placebo group. Ducker et al. (2013) found that 2,000 meter rowing performance did improve after 28 days of beta-alanine supplementation in well trained rowers, however this only had a significance level of ($p < 0.055$) and was not supported by moderate to large effect size (Ducker, 2013). While both of these studies showed improvements in performance, it should be noted that they both had small sample sizes and both had p- values that were near significance.

During intense anaerobic exercises, one of the major causes of fatigue is believed to be acidosis caused by the accumulation of hydrogen ions $[H^+]$, this corresponds to the decrease of the pH in blood. Along with carnosine being used as a pH buffering agent is sodium bicarbonate, which can also be used as an ergogenic aid. Tobias et al. (2013) had well-trained, experienced judo and jiu-jitsu male competitors complete the study. They were randomly assigned to the placebo (PL+PL), beta-alanine + placebo (BA+PL), placebo + sodium bicarbonate (SB+PL), or beta-alanine + sodium bicarbonate (BA+SB) group. Both BA+PL and SB+PL had gains in the total work done, peak power, and post exercise blood lactate. The group that had co-ingestion (BA+SB) had a significant effect on total work done, peak power, post exercise blood lactate, and ratings of perceived exertion (RPE). Researchers concluded that while sodium bicarbonate and beta-alanine and can elicit improvements in aerobic and anaerobic measures the two combined can significantly lead to greater improvements in performance and RPE. Furthermore, taking the two in combination enhances it's effects as a buffering agent during intense exercise.

Several studies have also investigated the effects of beta-alanine supplementation on body composition (Glenn et al., 2015; Kresta et al., 2014; Kern et al., 2011, and Outlaw et al., 2014). Glenn and colleagues (2015) looked at total body fat percentage as well as regional areas of interest and found that after supplementing with beta-alanine for 28 days female masters

athletes elicited no significant reductions in body fat. Similarly in another study by Kresta et al. (2014) researchers looked at changes in body weight, fat mass, fat free mass, and total body fat and found that after supplementing with beta-alanine, creatine, or the combination for 28 days all three groups had improvements in body weight and other markers of body composition over time, but none were statistically significant. Kern et al. (2011) had in season wrestlers and football players supplement with beta-alanine or placebo for 8 weeks and found that both groups had increases in lean body mass. It should be noted that wrestlers during this phase of training are also cutting weight, their losses in fat mass and weight were higher than those of the football players who actually gained weight. While not significant, both groups increased lean mass as compared to the placebo groups. Outlaw and colleagues (2012) found that after 8 weeks of both supplementing with beta-alanine or placebo and undergoing a resistance training program that both groups elicited improvements in lean body mass, fat mass, and total body fat %. While all of these groups showed improvements or changes over time not all of them were statistically significant and because most were already trained or were doing some sort of intervention we cannot be sure that it was because of beta-alanine or the combination of exercise and BA supplementation.

HIIT

Recurring bouts of very intense exercise with breaks that vary in length are referred to as sprint interval training or high intensity interval training (HIIT). The physiological adaptations to this type of training are similar to those of a more traditional endurance training protocol (longer steady state exercise) and provide benefits such as: cardiopulmonary, metabolic, and neuromuscular adaptations (National Strength & Conditioning Association, 2016). Moreover, this type of workout is often appealing to individuals with time constraints, but is at a very high

intensity and thus not appropriate for an untrained individual because of the dangers associated with high intensities. In addition, the intensities and durations for work to rest ratios are important factors to consider when dealing with different populations. In a review article, Laursen et al. (2010) discusses the energy contributions made from both the anaerobic and aerobic systems and how HIIT and high volume training can both elicit improvements in exercise performance and should both be utilized. Bishop et al. (2004) investigated the relationship between muscle buffer capacity and repeated sprint ability (RSA) among untrained females. Results showed that VO_2 peak and lactate threshold are significant predictors of RSA and the ability to buffer H^+ is important when one is trying to maintain performance during repeated sprints. While this study did not implement a HIIT intervention, it did provide evidence that training status and aerobic capacity of an individual can affect their ability to perform repeated sprints. HIIT can provide anaerobic and aerobic enhancements, similar to a more traditional endurance training program. Furthermore, it can increase muscle buffering capacity.

Burgomaster et al. (2005) aimed to examine the effects of short term (2 weeks) sprint-interval training (SIT) on endurance capacity at 80% of VO_2 peak, maximal oxygen uptake, and endurance time to exhaustion cycling. Results showed that there were no changes in VO_2 peak, but that aerobic endurance capacity improved by almost 100% in the SIT group as compared to the control group. In another study done by Burgomaster and colleagues (2006), researchers found similar results: there were no changes in VO_2 peak, time trial cycling improved by ~10% in the intervention group while also showing an increase in peak power output. This is in contrast to what Talanian and colleagues (2006) found. Their study aimed to determine if two weeks of high-intensity interval training (HIIT) had an effect on skeletal muscle metabolism, aerobic capacity, and fat oxidation. Results showed that VO_2 peak increased by 13% and whole

body fat oxidation increased by 36%. Overall, even over a short period of time HIIT could induce marked increases in time trial performance, VO_2 peak, and endurance capacity.

Esfarjani and Laursen (2007) examined the difference between two different HIIT protocols over 10 weeks on VO_2 max, lactate threshold and 3,000 meter running performance. Both groups had significant improvements in 3,000 meter run time, VO_2 max, and lactate threshold as compared to the control group.

Acka and Aras (2015) looked at differences between HIIT and supramaximal interval training (SMIT) over 4 weeks on indoor rowing performance. The HIIT group was working at 90% of their PPO while the SMIT was working at 150% during each session. Both groups, while not significantly different improved 2,000 meter rowing time, peak power output, and VO_2 peak. These are similar findings to those of Driller et al. (2009) who also looked at the effects of HIIT in well trained rowers. The HIIT group had significant improvements in 2,000 m time, peak power, and relative VO_2 peak. Both of these studies show that even well trained rowers can benefit from HIIT at varying high intensities and that it can be especially beneficial when training for a 2,000 m race when you need the last bout of energy to finish strong.

Beta-alanine and HIIT

High-intensity exercise can result in diminished stores of the adenosine tri-phosphate (ATP), phosphocreatine (PCr) and glycolytic substrates, and the accumulation of intracellular metabolites (adenosine di-phosphate[ADP], inorganic phosphate [P_i], hydrogen ions [H^+] and magnesium [Mg^+]), each of which has been implicated as a cause of muscular fatigue (Smith et al., 2009). As mentioned previously the formation of H^+ can result in a decrease in pH, most of these protons that are formed during exercise are buffered by the sodium bicarbonate system (HCO_3^-). However, during short bursts of intense exercise, such as HIIT, the physiochemical

buffering will exceed what the HCO_3^- system and use other intramuscular stores of phosphates and peptides. Carnosine concentration is high in skeletal muscle and its pKa of 6.83 make it more effective at sequestering protons than either HCO_3^- (pKa 6.73) or P_i (pKa 7.2), this is limited by the availability of beta-alanine. Smith and colleagues (2009) conducted a study to evaluate the effects of beta-alanine supplementation combined with high-intensity interval training (HIIT) on endurance performance and aerobic metabolism in recreationally active college-aged men. Forty-six men were randomly assigned to either the placebo or beta-alanine group and were tested for VO_2 peak, time to exhaustion (TTE), ventilatory threshold (VT), and total work done (TWD) at 110% of pre-training VO_2 peak. The beta-alanine group showed significant improvements in VO_2 peak, TTE, and TWD after the first three weeks of training. Supplementing with beta-alanine may further enhance HIIT, improve endurance capacity, and lean body mass in recreationally active men along with the use of HIIT to induce aerobic improvements.

Saunders and colleagues (2012b) had amateur male footballers supplement with either beta-alanine or placebo for 12 weeks and were asked to perform the YoYo Intermittent Recovery Test 2 (IR2) prior to supplementation and again after 12 weeks of supplementation. This test evaluates an individual's capacity to perform high-intensity exercise while simultaneously stimulating both aerobic and anaerobic energy pathways (Saunders, 2012b). Researchers found that the distance covered by the beta-alanine group was significantly higher than the placebo group after 12 weeks of supplementation, delaying neuromuscular fatigue, most likely due to increased carnosine content. In another study by Kern et al (2011) football players and wrestlers were randomized and placed into either a placebo or beta-alanine group for 8 weeks. All players maintained regular team workout, similar to a periodized HIIT program. The football players

showed greater improvements than the wrestlers in a 300 yard shuttle run and a 90° flexed arm hang, while neither were statistically significant both improved with the groups who supplemented with beta-alanine as to the placebo group.

Walter and colleagues (2010) looked at cardiovascular fitness and body composition changes among women after supplementing with placebo or beta-alanine for 8 weeks. Both groups underwent a rigorous periodized HIIT protocol on the cycle ergometer 3 times a week for 8 weeks. Both the placebo and beta-alanine groups showed improvements in VO_2 peak as compared to the control group, ventilatory threshold improved in all 3 groups, but no improvements other than body mass increases for all groups occurred over 8 weeks. This is similar to what Smith et al. (2009b) found after having men supplement with beta-alanine or placebo for 6 weeks. There were significant changes in VO_2 peak, time to exhaustion, and total work done in both groups, while the group that supplemented with beta-alanine had greater improvements in all three during the second three weeks of training. There were also significant improvements with ventilatory threshold, but the placebo group had larger improvements during the second half of the study. The beta-alanine group also showed gains in lean body mass, which is similar to what Kerns et al. (2011) found.

In another study done by Smith and colleagues (2009a), neuromuscular fatigue and muscle function among men after supplementing with beta-alanine or placebo combined with HIIT over 6 weeks was observed. The researchers in this study used an EMG to identify neuromuscular fatigue by looking for increases in amplitude. Both groups' significantly increased neuromuscular fatigue and physiological functioning of lower body muscle tissue, it would appear that supplementing with beta-alanine had no impact and most positive results were due to the HIIT. Similarly Stout and colleagues (2007) measured physical working capacity at

fatigue threshold and saw significant improvements in neuromuscular fatigue, but no HIIT was prescribed. While both delayed the onset of neuromuscular fatigue their findings show that one is because of HIIT and the other because of beta-alanine supplementation. Despite the conflicting results from both studies, it could be due to the methodological differences, or sample size.

The potential ergogenic effects of beta-alanine supplementation, across a wide range of exercises, has been shown to delay the onset of neuromuscular fatigue, and improve PPO or TWD, while HIIT seems to have more effects on maximal oxygen uptake and buffering capacity. Moreover, when combined BA has been shown to enhance HIIT, improve time to exhaustion and in some cases stimulate lean body mass.

CHAPTER III

METHODOLOGY

Participants

Thirty four females between the ages of 18-35 from Georgia Southern University were recruited to participate in the study (Mean \pm SD for age, height [cm], and weight [kg] are reported in (Table 1). The requirements were that each woman was moderately active (~150 minutes/weekly as recommended by American College of Sports Medicine). Participants who were currently or had taken any sort of ergogenic supplement (i.e. creatine or beta-alanine) in the last 12 weeks were excluded from the study. This was a randomized, double-blind, placebo-controlled study and participants were randomly placed in either the control group (C), the placebo group (PLA), or the Beta-alanine group (BA). The BA and PLA were labeled (A or B) and placed in identical containers by the company (Carnosyn® Natural Alternatives International (NAI), San Marcos, CA, USA) so that the researchers and participants could not tell the difference. Participants were then matched with two other females based off of height, weight, and self-reported hours of physical activity weekly and then randomly placed in either group A, B, or C so that there was an equal distribution and matched participants throughout the groups. The CON group underwent the pre and post testing procedures only, while the PLA and BA groups did the pre and post testing procedures, and a 4 week HIIT intervention on the rowing ergometer. The participants were given an explanation of the procedures, risks, possible benefits from the intervention and/or supplement, and then gave informed consent to participate in the study. The Institutional Review Board of Georgia Southern University approved this study.

Familiarization

This was done during the first week of the study and participants were randomly assigned to the BA, PLA, or CON groups these were labeled as A, B, and C respectively. Participants were then asked to sign the informed consent, and go over testing protocols as well as rowing technique. Participants were asked to fill out a Par-Q+ health questionnaire before any testing takes place, those not contraindicated by Par-Q+ could safely engage in VO_{2max} testing and high intensity interval training. The participants were then asked to watch a short video demonstrating proper rowing technique (concept2.com/technique) to help reduce injury and increase efficiency on the rowing ergometer. During the familiarization period participants also demonstrated to researchers that they were using proper techniques and had proper foot placement on the rowing ergometer. Foot placement and attachment was important for optimal performance and the heels were positioned in the foot stretcher so that the strap crossed over the ball of participant's foot, this was consistent throughout all testing procedures on the rowing ergometer. While there are two parts to the rowing stroke, the drive and the recovery, it is all done in one continuous motion. Participants extended arms forward, body leaning towards the flywheel, and knees bent, this was the catch. The drive began with participants straightening their legs, which did the majority of the work while keeping their arms and shoulders relaxed. After the legs were fully extended the rower used their core and back to swing back to the vertical position. During the last part of the drive the participant pulled the handle of the rowing ergometer with their arms and shoulders into the abdomen, the legs were straight and the body was leaning slightly back. The recovery began by extending the arms forward and swinging the body forward at the hips, this put the handle back in front of the knees to avoid any interference between the knees and the hands as the seat moved forward. The last motion, the catch, is the

exact same as the start position and the participants could begin their next drive in one continuous motion. Participants were reminded that their body should never come to a complete stop (Concept II Product Manual). Once the participants felt comfortable with the equipment and protocols they began pre-testing procedures.

Pre and Post Testing

All three groups underwent all pre-testing protocols: anthropometric measurements, body composition on the dual energy x-ray absorptiometry (DEXA) scanner, a VO₂ max test on a Concept II Rowing Ergometer (Model E, Type II, Concept2 Inc., Morrisville, VT, USA) and a 1,000 meter time trial. The three groups of women all came to the Human Performance Lab during week 2 for all pre testing and during week 7 for post testing procedures. The first day included anthropometric measurements, DEXA scan, and VO₂ max testing and no less than 48 hours later they came back to the lab to complete the 1,000 m time trial on the rowing ergometer. Participants were also asked to keep a food log using My Fitness Pal (MyFitnessPal, Inc.) for two weekdays and one weekend and to share the information with the researchers to keep track of macronutrients in the diet. On testing days, participants were also asked to consume a diet that was similar or the same to be consistent and to maintain their normal dietary and physical activity routines throughout the duration of the study. Participants came to the lab for their first day of actual testing wearing athletic attire. Their mass (kg) and height were measured (cm) using a stadiometer, waist and hips (cm) were measured with a spring loaded Gulick Measuring Tape (Country Technology, Inc., Gays Mills, WI, USA) and were done prior to the DEXA scan and exercise.

Participants then had a whole body scan done using (DEXA) to determine body composition. All scans were performed and analyzed by a trained technician using the AIS

whole-body DEXA protocol (Nana et al., 2012). Prior to testing the DEXA Scanner (Lunar Prodigy Model; GE/Lunar Corp, Madison, WI, USA) was calibrated per manufacturers guidelines using the GE Encore 12.30 Software and phantom. Participants were instructed to wear metal free clothing and to remove all jewelry and other metal before the scan. Each individual was centrally positioned on the scanner and feet were strapped together to maintain a constant distance (~15 cm), hands were placed at the sides within the given parameters. The scan took about 6-8 minutes and the whole body scan mode was used. Researchers looked at regional body fat % for both arms, legs, and trunk researchers had to manually adjust the regions of interest (ROI). Stults-Kolehmainen et al. (2013) defined the regions of interest using the following for parameters. The arm region was comprised of the arm and shoulder area formed by placing a line from the crease of the axilla and through the glenohumeral joint. The trunk region included the neck, chest, abdominal and pelvic areas. Its upper perimeter is the inferior edge of the chin and the lower borders intersect the middle of the femoral necks without touching the brim of the pelvis. The leg region included all of the area below the lines that formed the lower borders of the trunk (Stults-Kolehmainen et al., 2013).

The $\text{VO}_{2\text{max}}$ testing protocol was completed on the rowing ergometer using the ParvoMedics Metabolic cart (True One 2400, Sandy, UT, USA). Prior to testing the metabolic cart was calibrated per manufacturer's guidelines, this included the gas and flowmeter calibration protocols. Participants were then setup on the rowing ergometer (Model E, Concept2 Inc., Morrisville, VT, USA) and a heart rate monitoring strap was placed across the chest (Garmin Model FR70, Olathe, KS, USA). The drag factor was set at 3 for all testing and intervention days. The test began at an initial workload of 75 W for two minutes, and was then increased to 100 W for a further two minutes. The workload was then increased an additional 25 W every

two minutes until the participant could no longer continue, despite verbal encouragement. If the participants could not maintain appropriate Watts (within 15 above or below), or any contraindications to testing arose, the test was terminated. All data was averaged over 15-s intervals and the highest 15-s VO_2 value during the graded exercise test was recorded as $\text{VO}_{2\text{peak}}$, if it coincided with at least two of the following criteria: (a) a plateau in heart rate or heart rate values within 10% of the age-predicted maximum heart rate, (b) a plateau in VO_2 (defined by an increase of no more than 150mL/min (Thompson et al., 2010) or the respiratory exchange ratio has exceeded ($\text{RER} > 1.15$). The ventilatory threshold (VT) inflection point was determined by the True One program using the V-slope method. This eliminated the need for multiple researchers to evaluate and confirm inflection points. Maximal oxygen uptake ($\text{VO}_{2\text{peak}}$) in (L/min and ml/kg/min), power output at the ventilatory threshold (VT), time-to-exhaustion (TTE), maximal heart rate, ratings of perceived exertion (RPE), using a 6-20 numeric scale (Borg, 1970) were measured and recorded.

Within 48-72 hours of maximal testing participants came back to the Human Performance Lab to complete an all-out 1,000 m time trial (TT) on the rowing ergometer. Participants were set up on the Type II Concept Rower in the same fashion that they were on day 1 of pre-testing and a heart rate strap (Garmin Model FR70, Olathe, KS, USA or Polar) was placed across the chest to monitor heart rate. Participants were allowed to warm up for 3-5 minutes at a low intensity before the TT and also allowed a cool down after the TT on the rowing ergometer or treadmill. The distance was programmed into the PM4 monitor and display so that the participant and researcher could see total distance covered, time, heart rate, average watts, and peak power; these values were all recorded. Participants were informed to row “all out” and told to not stop, encouragement was given throughout the test.

Beta-alanine and Placebo Dosing

After being randomly assigned to either the beta-alanine (BA) or placebo (PLA) group, which were called A and B respectively, participants were given weekly doses (56 pills) of supplement or placebo in identical white bottles labeled A or B. Both groups took either 6.4 grams of maltodextrin or beta-alanine daily, these were the time-released capsules that were pre-packaged and blinded by the company Carnosyn® (Natural Alternatives International, San Marcos, CA, USA). Each participant took 2 pills four times daily (8 pills total) for 28 days, they were given a log to keep track of times and doses on a weekly basis to ensure compliance. On training days the participants were asked to consume one dose of their supplement or placebo 30 minutes prior to training and another immediately following. It was expected to observe an increase in muscle carnosine content to be close to 15 mmol/kg dry muscle (an increase of 65% in a participant eating a mixed diet), given that Harris et al. (2006) reported this level of increase following a similar but slightly lower total dose of beta-alanine.

High Intensity Interval Training Protocol

During the next four weeks the PLA and BA groups met with a researcher to begin the high intensity interval training intervention. Participants came to the Human Performance Lab three times weekly on nonconsecutive days. Each session lasted around 15 minutes, participants were asked to maintain a regular workout regimen during the week, but to refrain from workouts on intervention days. These included a warm up and cool down on the rowing ergometer. During the intervention portion of the study, PLA and BA groups were given training and supplement logs to document their type, intensity, and duration of workouts along with their dosing logs to ensure compliance. The intensities were undulating (Figure 1) and were based off

of a similar protocol that Walter et al. (2012) did in their study on a similar population of females. Thirty minutes prior to arriving, participants were asked to take two 800 mg capsules of placebo or beta-alanine and take a second dose immediately following the training session. Participants were setup on the rowing ergometer in the same fashion that they were in the fatiguing and TT protocol; heart rate was monitored using a Garmin strap attached at the chest (Garmin Model FR70, Olathe, KS, USA). The workout included a 5 minute warm-up and cool down that was on the rowing ergometer. Participants warmed up at 75 W for 5 minutes followed by 7 sets of 1-minute exercise bouts at a predetermined percentage of their peak workload using a fractal periodization scheme (Walter et al., 2010). Peak workload was determined during the TT testing and was reported as peak power output (PPO), this varied between participants and was what determined their workload during training days. On all odd days (1, 3, 5, 7, 9, and 11) participants rowed at a workload that was 90% of their predetermined PPO and on the even days (2, 4, 6, 8, 10, and 12) the workload increased by 5% (95%, 100%, 105%, 110%, 110%, and 115%). Training volume (seconds completed at each interval), average Watts, heart rate, and RPE were recorded. One minute of passive recovery was allowed between each set. At the end of the 4 weeks, participants discontinued the placebo or beta-alanine supplementation and followed up the next week for post testing procedures. Post-testing procedures followed the exact same setup as the pre-testing procedures. The CON group also reported back to the lab for post testing procedures.

Statistical Analyses

Eight separate 2 (time) [pre vs. post] \times 3 (treatment) [BA vs. PLA vs. CON] repeated mixed factorial analyses of variance (ANOVAs) were used to evaluate the following variables: relative VO_2 peak (ml/kg/min), ventilatory threshold (VT), time to complete 1,000 m time trial (TT),

peak power output (PPO), total body fat percentage (BF %), body fat of arms (ABF %), body fat of legs (LBF %), and trunk body fat (TBF %). Further one-way ANOVA's were run when there was a significant interaction between groups and time. The alpha level was set at $p \leq 0.05$ to determine statistical significance and a Bonferroni correction was applied. Data was analyzed using SPSS for Windows version 21 (SPSS Inc., Chicago, IL, USA). All values are reported as mean \pm standard deviation (SD).

CHAPTER IV

RESULTS

Subject Characteristics

A total of 21 of the 34 woman completed the entire study (BA = 8, PLA = 7, and CON = 6), five dropped out due to injuries unrelated to the study, one became pregnant, and seven just did not complete the study due to time constraints. The mean \pm SD age for all participants ($n = 21$) was 22.2 ± 2.20 , body mass 63.00 ± 8.52 (kg), and height 163.59 ± 6.69 (cm), and further subject characteristics are shown in (Table 1).

Relative VO₂ Peak (ml/kg/min)

Relative VO₂ peak scores are shown in (Table 2). There was a main effect of time $F(1, 18) = 15.07$, $p = 0.01$, $\eta^2 = 0.46$; the mean \pm SD for all 3 groups was (35.84 ± 4.12 v. 39.24 ± 5.26) pre and post testing. There was no significant interaction between groups and time $F(2, 18) = 3.16$, $p = 0.06$, $\eta^2 = 0.26$ however it should be noted that the observed power was 0.53. There was no difference between groups $F(2, 18) = 0.77$, $p = 0.48$.

Absolute VO₂ Peak (l/min)

There was a main effect for time $F(1, 18) = 22.60$, $p = 0.00$, $\eta^2 = 0.56$; the mean \pm SD for all 3 groups was (2.25 ± 0.39 v. $2.44 \pm .045$) pre and post testing. There was a significant interaction by group and time $F(2, 18) = 3.76$, $p = 0.04$, $\eta^2 = 0.30$, further one way ANOVA's were run on all groups for the pre and post testing values. Post hoc analysis for pre testing values showed nothing significant $F(2, 18) = 1.42$, $p = 0.27$. Further analysis for post testing values between groups also showed no significance $F(2, 18) = 2.74$, $p = 0.09$.

Time to Exhaustion TTE (seconds)

Time to exhaustion scores are shown in (Table 2). There was a main effect for time $F(1, 18) = 13.15$, $p = 0.00$, $\eta^2 = 0.42$; the mean \pm SD for all 3 groups was $(468.38 \pm 98.34$ v. $524.67 \pm 99.89)$ pre and post testing. There were significant interactions by group and time $F(2, 18) = 6.10$, $p = 0.01$, $\eta^2 = 0.40$, observed power was 0.83, further ANOVA's were run for the pre and post testing values. Post hoc results for the pre testing values were not significant $F(2, 18) = 0.55$, $p = 0.59$. Further analysis for post testing values were also not significant $F(2, 18) = 1.61$, $p = 0.23$.

Ventilatory Threshold % of VO₂ Peak (VT)

Ventilatory threshold % data is shown in (Table 2). There was a main effect of time $F(1, 18) = 6.01$, $p = 0.03$, $\eta^2 = 0.23$; the mean \pm SD for all 3 groups was $(53.38 \pm 11.46$ v. $63.95 \pm 7.26)$. There were no significant interactions between groups and time $F(2, 18) = 0.12$, $p = 0.74$, $\eta^2 = 0.03$. There was also no significance between groups $F(2, 18) = 0.14$, $p = 0.35$.

Ventilatory Threshold (l/min)

There was a main effect of time $F(1, 18) = 6.01$, $p = 0.03$, $\eta^2 = 0.25$; the mean \pm SD for all 3 groups was $(1.34 \pm 0.31$ v. 1.54 ± 0.25 l/min). There were no significant interactions between groups and time $F(2, 18) = 0.12$, $p = 0.89$, $\eta^2 = 0.01$. There was also no significance between groups $F(2, 18) = 1.11$, $p = 0.11$, $\eta^2 = 0.11$.

Time Trial (TT)

Time trial data is shown in (Table 2). There was a main effect of time $F(1, 18) = 27.38$, $p = 0.00$, $\eta^2 = 0.60$; the mean \pm SD for all groups was $(266.71 \pm 19.08$ v. $261.83 \pm 14.55)$ seconds. There was an interaction between groups and time $F(2, 18) = 5.17$, $p = 0.02$, $\eta^2 = 0.37$, observed

power = 0.76, further one-way ANOVA's were run for the pre and post testing values. Post hoc results showed that there was not significance between groups in the pre-testing $F(2, 18) = 0.65$, $p = 0.53$. Further analysis between the groups for the post testing showed no significance $F(2, 18) = 0.85$, $p = 0.44$.

Peak Power Output (PPO)

PPO data is shown in (Table 2). There was a main effect of time $F(1, 18) = 60.65$, $p = 0.00$, $\eta^2 = 0.77$; the mean \pm SD for all groups was (244.86 ± 42.15 v. 322.81 ± 68.21 W) measured in Watts. There was an interaction between groups and time $F(2, 18) = 14.07$, $p = 0.00$, $\eta^2 = 0.61$, observed power = 0.10, further one-way ANOVA's were run on the pre and post testing values. The pre-testing post hoc showed no statistical significance between groups $F(2, 18) = 0.05$, $p = 0.95$. Post testing results (Figure 2) revealed significance between groups BA (325.13 ± 22.611 W) and CON (254.00 ± 40.60 W) groups, $p = 0.05$ and PLA (378.71 ± 72.23 W) and CON groups, $p = 0.00$.

Total Body Fat % (BF)

Total body fat % scores are shown in (Table 3). There was no main effect of time $F(1, 18) = 2.64$, $p = 0.12$, $\eta^2 = 0.13$; the mean \pm SD for all groups was (31.10 ± 5.71 v. 30.71 ± 5.50) percent body fat. There were significant interactions between groups over time $F(2, 18) = 4.71$, $p = 0.02$, $\eta^2 = 0.34$, observed power = 0.72. Further one-way ANOVA's were run on pre and post testing values. The post hoc testing for the pre testing values did not show any significance $F(2, 18) = 0.91$, $p = 0.42$. The post testing values also showed no significance $F(2, 18) = 1.26$, $p = 0.31$.

Body Fat % of Arms (ABF)

Arm body fat % scores are shown in (Table 3). There was a main effect of time $F(1, 18) = 10.42, p = 0.01, \eta^2 = 0.37$; the mean \pm SD for all groups was $(27.07 \pm 5.95$ v. $25.95 \pm 5.86)$ percent body fat in the arms. There were no significant interactions between groups over time $F(2, 18) = 1.98, p = 0.17, \eta^2 = 0.18$. There was also no significance between subjects $F(2, 18) = 1.40, p = 0.27$.

Body Fat % of Legs (LBF)

Leg body fat % scores are shown in (Table 3). There was no main effect of time $F(1, 18) = 0.60, p = 0.45, \eta^2 = 0.03$. There was a significant interaction over time between groups $F(2, 18) = 3.75, p = 0.04, \eta^2 = 0.30$, observed power = 0.61. Further one way ANOVA's were run on the pre and post testing values. The pre testing values showed no significance between groups $F(2, 18) = 0.77, p = 0.48$. The post testing values also showed no significance $F(2, 18) = 1.05, p = 0.37$.

Body Fat % of Trunk (TBF)

Trunk body fat % scores are shown in (Table 3). There was no main effect of time $F(1, 18) = 2.28, p = 0.15, \eta^2 = 0.11$. There was a significant interaction between groups $F(2, 18) = 5.28, p = 0.01, \eta^2 = 0.42$, observed power = 0.85. Further post hoc analysis for the pre testing values showed no statistical significance $F(2, 18) = 1.13, p = 0.35$. Further post hoc testing also did not show any significance $F(2, 18) = 1.68, p = 0.21$ in the post testing results.

CHAPTER V

DISCUSSION

The purpose of this study was to determine if supplementing with beta-alanine combined with four weeks of high intensity interval training on the rowing ergometer would elicit improvements in VO_2 peak, ventilatory threshold, peak power output, and body composition. The secondary purpose was to look at time to complete a 1,000 meter rowing time trial and changes in regional body fat. While there is a growing body of evidence that indicates positive effects of beta-alanine supplementation on performance measures, several studies have found no notable impact. This study suggests, however, that supplementation with beta-alanine combined with HIIT can attenuate fatigue and increase peak power output during time trial performance. The main finding of this study indicates that over time both the placebo and beta-alanine groups showed improvements in VO_2 peak, time to exhaustion, ventilatory threshold, peak power output, and reduced arm body fat percentage. The most noteworthy finding was that the placebo and beta-alanine groups had significant improvements in peak power post testing compared with the control group. This was the only variable measured that revealed any changes between groups and was likely due to the HIIT intervention.

VO_2 peak in both the BA and PLA groups improved over time as compared to the CON group, however there was no significant difference between those two groups. These results are comparable with what several other studies found (Smith et al., 2012; Smith et al.2009b; Walter et al., 2010), all of which saw improvements in VO_2 peak and TTE. Both Walter et al. (2010) and Smith et al. (2009b) implemented a HIIT protocol for six weeks combined with the beta-alanine supplementation. In contrast, a number of other studies have found that supplementing with beta-alanine (or combining another nutritional supplement, e.g. creatine or

sodium bicarbonate) and/or implementing HIIT has not shown improvements in VO_2 peak or TTE (Jordan et al., 2010; Zoeller et al., 2007; Outlaw et al., 2014; Baguet et al., 2010b, Stout et al., 2006). Jordan et al. (2010) showed decrements in maximal oxygen uptake, but delays in the onset of blood lactate accumulation (OBLA) in the BA group, which could suggest that increases in carnosine can attenuate fatigue. Similarly, Zoeller and colleagues (2007) noted increases in power output at lactate threshold, while the VO_2 remained unchanged; neither of these studies had an exercise intervention along with supplementation. Outlaw et al. (2014) looked at the combination of resistance training and beta-alanine supplementation over 8 weeks and showed no improvements in VO_2 peak or TTE, but the researchers did suggest that it might lead to increased muscular endurance. Moreover, several studies have found aerobic and anaerobic benefits after short and long term HIIT (Driller et al., 2009; Acka and Aras, 2015; Esfarjani et al., 2006). Both Acka and Aras (2015) and Driller et al. (2009) looked at varying intensities of high intensity training among elite rowers as compared to a more conventional rowing training regimen and found that all groups improved VO_2 as compared to control group.

The results of our study showed VO_2 peak improvements in both the BA and PLA groups over time as compared to the CON group, commensurate with what other researchers found using similar protocols. In reviewing the literature it should be noted that the studies that did see improvements in aerobic capacity typically used a HIIT protocol, while BA supplementation alone did not substantiate any improvements in VO_2 peak. Therefore, we can postulate that the improvements seen were most likely due to the HIIT protocol and training adaptations that occurred on the rowing ergometer and not from BA supplementation. In addition, our data showed that both the BA and PLA groups improved VT as % of VO_2 and VT (l/min) over time, but there were no differences between groups. These results support what other studies found

(Stout et al., 2006; Zoeller et al., 2007; Walter et al., 2010). Stout et al. (2006) indicated that supplementing with beta-alanine can elicit improvements in VT (l/min) and physical working capacity at fatigue threshold (PWC_{FT}), therefore delaying neuromuscular fatigue. Some studies have looked at BA supplementation combined with HIIT and found improvements in VT, total work done (TWD), and power output at VT (Smith et al., 2009b; Walter et al., 2010). In contrast Smith and colleagues (2012) found no differences in ventilatory threshold over time or between groups who supplemented with BA or PLA and did not receive any sort of exercise intervention. The improvements of VT, PWC_{FT} , and TTE might have been due to increases in carnosine concentration through beta-alanine supplementation. These results might suggest that supplementing with beta-alanine can help maintain intramuscular pH during intense exercises when accumulation of H^+ ions is high and delay the onset of neuromuscular fatigue. These muscle carnosine increases must be assumed because carnosine was not directly measured in the current study.

Participants were asked to do an all-out 1,000 m row during the pre and post testing to determine total time and peak power output. While there were no changes over time in any of the groups for completion time there were for peak power out (PPO). There were also differences between groups for TT and PPO. While further testing between groups during pre and post testing for TT did not reveal any significant differences, our data supports the hypothesis that both the BA and PLA groups would significantly improve their PPO as compared to the CON group. However, another hypothesis was that the BA group would be significantly higher than the PLA group, yet there were no differences. There have been a few studies that have examined 2,000 m time trial performance among elite rowers (Ducker et al., 2013; Baguet, 2010) while supplementing with beta-alanine and maintaining their typical training. Both studies

found no significant improvements in TT performance after supplementation with BA; however, it should be noted that while these were not significant there were reductions in overall time to complete a 2,000 m TT. In a competition setting this would be considered a considerable difference. This was similar to what Kern and Robinson (2011) found among football players in season; their 300 m shuttle time improved (1.1 seconds) after supplementing with BA as compared to PLA group (0.4 seconds). While Ducker et al. (2013) did not see improvements in TT after 28 days of beta-alanine supplementation split times and average power did increase in the BA group as compared to the PLA group. In a comparable study Baguet and colleagues (2010) determined that there was a positive correlation between increased carnosine content and rowing performance. Similarly Saunders et al. (2012a) and Derave et al. (2007) found no improvements in performance after supplementing with beta-alanine. While these studies had methodological differences and did not look at a time trial specifically they did look at distance covered during LIST (repeated sprints) and time to complete a 400 m run respectively. In contrast Saunders and colleagues (2012b) found that football players (soccer) significantly increased distance covered during the anaerobic YoYo IR2 (see definition) after supplementing with BA for 12 weeks.

There are a multitude of studies using a variety of modes that found improvements in time trial performance after implementing a HIIT program anywhere from 2-8 weeks long with varying degrees of intensities (Acka and Aras, 2015; Driller et al., 2009; Burgomaster et al., 2005; Esfarjani et al., 2007). Driller et al. (2009) and Acka and Aras (2015) both looked at performance among elite rowers after having groups either use a more conventional training method for rowing or HIIT; both groups 2,000 m time and average power significantly improved during the TT. Burgomaster et al. (2005) and Esfarjani et al. (2007) used the cycle ergometer

and running respectively and implemented varying intensities for the HIIT protocol; both saw significant improvements in time. In a meta-analysis, Hobson et al. (2012) concluded that BA was effective in improving high intensity exercise in durations between 60-240 seconds and in excess of 240 seconds and that the anaerobic energy system contributed significantly. The current study might not have had a large enough sample to determine if the BA was as much of a contribution to performance enhancements as the HIIT.

Results indicated that PPO improved over time and that there were significant differences between the BA and CON and the PLA and CON. In addition, it appears that the increases in PPO were due more to the HIIT rather than the combination of HIIT and BA supplementation which could also be a reflection of low sample size. Several other studies have shown that beta-alanine supplementation alone can elicit improvements in PPO (Tobias et al., 2013; Ducker et al., 2013; Van Thienen et al., 2009; Painelli et al., 2014). Researchers that implemented HIIT on a variety of modes supported these findings (Acka and Aras, 2015; Driller et al., 2015; Burgomaster et al., 2005). Smith and colleagues (2009b) found that TWD improved after supplementing with BA combined with HIIT over 6 weeks. In contrast the study by Outlaw et al. (2014) did not show any significant improvements in PPO after 8 weeks of BA supplementation combined with a resistance training program. Other studies suggest that the combination of BA and HIIT can further delay time to exhaustion and improve TWD or PPO. One can speculate that the current study might have found differences between the BA and PLA groups had more women been recruited and perhaps matched based on VO_2 peak or PPO from the initial pre-testing procedures.

This study did not show any significant differences in total BF%, leg BF%, or trunk BF% over time or between groups. While most studies using DEXA looked only at total body fat

percentage (BF%), lean body mass, and fat mass, our study assessed all of these as well as regional areas of interest (arms, legs, and trunk), similar to (Glenn et al., 2015), which showed comparable results to the current study. A number of other studies have looked at the ergogenic effects of beta-alanine supplementation on body composition and found similar results (Kresta et al., 2014; Kern et al., 2011; Outlaw et al., 2014; Walter et al., 2010). While these studies had methodological differences they all indicated that BF%, fat mass, and fat free mass improved over time for both the PLA and BA groups. It should be noted that all of the aforementioned studies also included some sort of training intervention along with supplementation of BA. Interestingly, Walter et al. (2010) also indicated that the BA group had significant body mass increases, although researchers were unclear as to why. According to Nana et al. (2012) any subjects that are undergoing a whole-body scan, using DEXA, must be in a rested, fasted, and euhydrated state. In the current study, participants were asked to come to the lab well hydrated and to have had some sort of breakfast, because they were engaging in volitional fatigue testing and needed to be well hydrated. One possible limitation of the study was that participants did not eat consistent meals on those days and could have been in a different state of hydration, which could cause variability in the results. Contrary to expectations, the data indicated that the regions of interest (legs, arms, or trunk) showed no significant findings between groups or over time.

Because of the time of year, time commitment, and difficulty of the HIIT protocol this study had very low recruitment. Further, the small sample size of the present study resulted in low statistical power and increased the risk of type II errors and may have contributed to the lack of significant findings that research has demonstrated. Participants were not evenly matched between groups, in part some participants quit the study, which caused the groups to become unevenly balanced. As neither menstrual cycles nor hormonal changes were not accounted for in

the present study, future studies could time training around certain phases of the menstrual cycle for more accuracy.

Conclusions

To the best of our knowledge, this is one of the first studies that utilized the rowing ergometer as a mode for non-elite rowers, and one of few that used recreationally active females. Although there were improvements in performance, it cannot be concluded that supplementing with beta-alanine combined with HIIT provides enhancements. This study determined that HIIT had a significant impact on exercise performance, while the results did not show much from the additional BA supplementation. Moreover, supplementation with beta-alanine may have more impact on exercise capacity (TTE, PPO) while HIIT can offer improvements in aerobic and anaerobic performance. Furthermore, the results of the present study did not reveal any significant changes in body composition. The lack of significance in this study may have been due to how we participants were matched and low sample size. Future researchers should consider using a 3 or 4-c model to analyze body composition to take multiple factors into account and have participants come to the lab in a fasted and euhydrated state on a day when no other testing is taking place. Additionally, a cross-over design would be preferred so that participants would receive the same treatments and matching would not be an issue. Such a design is very time consuming and would require prolonged washout periods of at least 12 weeks. Future studies should also consider matching subjects based off of peak power, VO_2 peak, or total work done. Although changes in muscle carnosine were not measured in this study, the dosing protocol was comparable to other studies (Harris et al., 2006) that showed a ~60% increase in carnosine levels after 4 weeks of supplementation. Given that beta-alanine is rate limiting pre-cursor for carnosine synthesis, it can be assumed that supplementing with this

ergogenic aid will elevate muscle carnosine content. Ideally, future studies would measure carnosine content and blood lactate, as it could provide valuable data. Finally, food diaries and compliance are complicating factors, as they are incredibly difficult to monitor because of honesty and memory recall. Additionally, future studies might want to use the rowing ergometer as a mode for recreationally active males or females as it can provide a whole body workout and consider using combined supplementation (beta-alanine, creatine, or sodium bicarbonate).

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APPENDIX A

Research Questions

1. Was there a combined effect from BA supplementation and HIIT on VO₂ peak, TTE, and body composition?
2. Did supplementation with BA combined with HIIT on the rowing ergometer elicit changes in regional areas of interest (arms, trunk, or legs)?
3. Will the combination of HIIT and BA supplementation reduce the time to complete a 1,000 m time trial and increase peak power output?

Assumptions

1. That all participants will comply with dosing protocols.
2. That all participants will accurately document their physical activity and food diaries.
3. That all participants gave 100% during all pre and post testing procedures and during the training protocol.

Limitations

1. Only using women 18-35
2. Did not control for menstrual cycles or hormonal changes
3. Cannot control diet or exercise outside of the lab setting
4. Compliance with placebo and beta-alanine supplementation

Definitions

1. **ATP-PC System:** metabolic pathway that uses ATP and phosphocreatine to rephosphorylate ADP. It is used at the initial onset of exercise and during high intensity exercise.
2. **ADP (adenosine diphosphate):** a molecule that combines with inorganic phosphate to form ATP.
3. **ATP (Adenosine triphosphate):** high energy phosphate used by cells to release energy during work.
4. **H⁺ (Hydrogen Ions):** free hydrogen ion in a solution that can result in a decrease in pH.

5. **LIST Test (Loughborough Intermittent Sprint Testing):** variable-intensity shuttle running over 20 m, mimics the characteristics of a soccer game.
6. **pKa:** an index used to express the acidity of weak acids.
7. **YoYoIR2:** test to evaluate ability of an individual to repeatedly perform and recover from intense exercise, while stimulating both the aerobic and anaerobic energy systems.
8. **DEXA (Dual energy-ray absorptiometry):** a tool normally used to assess bone mineral content, but has been recently used to assess body composition using a three compartment model (fat mass, fat free mass, and bone mineral content). Moreover, it can also assess whole body and regional estimates of fat %.
9. **BOD POD:** Gold standard device used for body composition measurement. It uses air displacement plethysmography and whole body densitometry to determine body composition (fat and fat free mass).

APPENDIX B



**GEORGIA
SOUTHERN
UNIVERSITY**

COLLEGE OF HEALTH & HUMAN SCIENCES
POST OFFICE BOX 8076
STATESBORO, GEORGIA 30460-8076
TELEPHONE (912) 681-1200

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

Title of Project: Effects of Beta-Alanine supplementation and High Intensity Interval Training among Recreationally Active Females

Investigator's Name: Mary E. Yarbrough **Phone:** (970) 402 - 1980

Participant's Name:

Date:

Data Collection Location: Human Performance Laboratory & Biomechanics Laboratory,
Georgia Southern University Campus Hanner Complex-Fieldhouse 590 Harty Dr

1. This study is being conducted by Mary E. Yarbrough, Masters Student in the School of Health and Human Sciences and Dr. John Dobson, Assistant Professor in the School of Health and Kinesiology, both at Georgia Southern University. This study is an attempt to understand if beta-alanine and high intensity interval training can change outcomes in body composition and maximal oxygen uptake (an indicator of cardiorespiratory endurance and aerobic fitness) among recreationally active females.
2. The purpose of this study is to examine the effects of beta-alanine supplementation combined with high intensity interval training among recreationally active females. Differences in body fat percentage and aerobic fitness will be measured before and after the testing intervention.
3. You will come to the Human Performance and Biomechanics Labs at a minimum of 16 occasions over the course of seven weeks, with the exception of the control group who will meet at a minimum of 5 times. Each participant will also receive a packet with the testing procedures and protocols and any risks associated with exercise, the training intervention or beta-alanine supplementation. Researchers will also explain this to all participants. The first week will be familiarization with the lab, equipment, and protocols that are being used for the study. Participants will be randomly divided into the control, placebo or experimental groups in a double-blind fashion. During this period the placebo and experimental groups will begin supplementing 6400 mg daily of either Maltodextrin (complex carbohydrate) or beta alanine (a nonessential amino acid) over the course of 7 weeks. The second week we will begin pre-testing procedures for all groups, this will include obtaining height, weight, and girth measurements, body composition on the DXA, maximal oxygen uptake on the rowing ergometer, and a 1,800 meter time trial. Over the course of the

next four weeks participants in the placebo and experimental group will begin a high intensity interval training protocol. They will meet in the Biomechanics Lab on three nonconsecutive days a week and be asked to refrain from any sort of exercise on those days. The seventh week will be post testing procedures; all three groups will come back to the Human Performance Lab for anthropometric measurements, body fat percentage on the DEXA, maximal oxygen uptake testing on the rowing ergometer, and a 1,000m time trial.

4. The information we collect on your performance may be sent off campus for analysis, however any information sent will be devoid of identifying characteristics (no one will be able to tell it's you)
5. By participating in this research, there exists a remote possibility of some adverse changes during the fatiguing protocol. Risks will be minimized by excluding those who are contraindicated to participate in physical activity according to a health questionnaire (Par-Q-) provided by the Canadian society of Exercise Physiology. These include dizziness, abnormal blood pressure, irregular heartbeat, or fainting. Participants' physical risks will be minimized by having each testing session conducted by qualified investigators. Those who are not contraindicated can safely engage in high intensity interval training. All additional research staff members directly involved with testing of the participants is familiar with the American College of Sports Medicine standards and has up-to-date CPR/First Aid certifications. All research staff members will have knowledge of signs and symptoms of severe injury and will refer the participant to a physician if the participant presents the symptoms. The risks associated with taking the supplement beta-alanine are skin sensations, such as burning, prickling, itching, or tingling, with no apparent physical cause; this will be minimized by taking the recommended dose of 6.4g/daily in controlled release tablets. The ingredients in this supplement are naturally occurring amino acids. All research staff members will have knowledge of signs and symptoms of severe injury and will refer the participant to a physician if the participant presents the symptoms. Risk will be minimized by excluding those who are contraindicated to participate in physical activity per ACSM (2010). Qualified professionals will carry out the program and participants will be closely monitored to ensure maximum safety possible. You also understand that you are not waiving any rights that you may have against the University for injury resulting from negligence of the University or investigators. Should medical care be required, you may contact Health Services at (912) 478-5641.
6. All participants who complete the testing will be entered into a drawing to win a \$100 gift card after the study. Two names will be drawn and each will win a \$100 gift card. All groups will have the opportunity to win.
7. You understand that all data concerning yourself will be kept confidential and available only upon your written request to either Mary E. Yarbrough or any of the other investigators listed below. You understand that any information about your records will be handled in a confidential (private) manner consistent with medical records. Your identity on all records will be indicated by a case number. You will not be specifically mentioned in any publication of research results. However, in unusual cases your research records may be inspected by appropriate government agencies or released to an order from a court of law.

All information and research records will be kept for a period of five years after the termination of this investigation.

- 8. If you have any questions about this research project, you may call Mary E. Yarbrough at (970) 402-1980. If you have any questions or concerns about your rights as a research participant in this study it should be directed to the IRB Coordinator at the Office of Research Services and Sponsored Programs at (912) 478-6843.
- 9. You understand that you do not have to participate in this project and your decision to participate is purely voluntary. At any time you can choose to end your participation by telling the primary investigator, Mary E. Yarbrough. If participants chose to not complete the study they will not be entered to win the \$100 gift card.
- 10. You understand that you may terminate participation in this study at any time without prejudice.
- 11. You understand there is no deception involved in this project.
- 12. You certify you are 18 years of age or older and you have read the preceding information, or it has been read to you, and understand its contents. Any questions you have regarding the research have been, and will continue to be, answered by the investigators listed at the beginning of this consent form or at the phone numbers given (970) 402 – 1980.
- 13. You have been provided a copy of this form. This project has been reviewed and approved by the GSU Institutional Review Board under tracking number H15311.

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*Both School of Health and Kinesiology P.O. Box 8076

Participant Signature

Date

I, the undersigned, verify that the above informed consent procedure has been followed

Investigator Signature

Date

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous	<input type="checkbox"/>	<input type="checkbox"/>
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Are you currently taking prescribed medications for a chronic medical condition?	<input type="checkbox"/>	<input type="checkbox"/>
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	<input type="checkbox"/>	<input type="checkbox"/>
7.	Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.



- › Start becoming much more physically active – start slowly and build up gradually.
- › Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- › You may take part in a health and fitness appraisal.
- › If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist* (CSEP-CEP) or CSEP Certified Personal Trainer* (CSEP-CPT).
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:



- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PAR med-X for Pregnancy before becoming more physically active OR
- › Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity program.

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?	<input type="checkbox"/> If yes, answer questions 1a-1c	<input type="checkbox"/> If no, go to question 2
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	<input type="checkbox"/>	<input type="checkbox"/>
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you have Cancer of any kind?	<input type="checkbox"/> If yes, answer questions 2a-2b	<input type="checkbox"/> If no, go to question 3
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	<input type="checkbox"/>	<input type="checkbox"/>
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm	<input type="checkbox"/> If yes, answer questions 3a-3e	<input type="checkbox"/> If no, go to question 4
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	<input type="checkbox"/>	<input type="checkbox"/>
3c.	Do you have chronic heart failure?	<input type="checkbox"/>	<input type="checkbox"/>
3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	<input type="checkbox"/>	<input type="checkbox"/>
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	<input type="checkbox"/>	<input type="checkbox"/>
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	<input type="checkbox"/> If yes, answer questions 4a-4c	<input type="checkbox"/> If no, go to question 5
4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	<input type="checkbox"/>	<input type="checkbox"/>
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	<input type="checkbox"/>	<input type="checkbox"/>
4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)	<input type="checkbox"/> If yes, answer questions 5a-5b	<input type="checkbox"/> If no, go to question 6
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
5b.	Do you also have back problems affecting nerves or muscles?	<input type="checkbox"/>	<input type="checkbox"/>

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure	<input type="checkbox"/> If yes, answer questions 6a-6d	<input type="checkbox"/> If no, go to question 7
	6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	6b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	<input type="checkbox"/>	<input type="checkbox"/>
	6c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, labored breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	<input type="checkbox"/>	<input type="checkbox"/>
	6d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	<input type="checkbox"/>	<input type="checkbox"/>
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia	<input type="checkbox"/> If yes, answer questions 7a-7c	<input type="checkbox"/> If no, go to question 8
	7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	7b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	<input type="checkbox"/>	<input type="checkbox"/>
	7c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	<input type="checkbox"/>	<input type="checkbox"/>
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event	<input type="checkbox"/> If yes, answer questions 8a-c	<input type="checkbox"/> If no, go to question 9
	8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
	8b. Do you have any impairment in walking or mobility?	<input type="checkbox"/>	<input type="checkbox"/>
	8c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	<input type="checkbox"/>	<input type="checkbox"/>
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?	<input type="checkbox"/> If yes, answer questions 9a-c	<input type="checkbox"/> If no, read the advice on page 4
	9a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	<input type="checkbox"/>	<input type="checkbox"/>
	9b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, and kidney problems)?	<input type="checkbox"/>	<input type="checkbox"/>
	9c. Do you currently live with two chronic conditions?	<input type="checkbox"/>	<input type="checkbox"/>

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+



If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- › It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- › You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- › As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the follow-up questions about your medical condition:

- › You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.



Delay becoming more active if:

- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- › Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity program.

SECTION 3 - DECLARATION

- › You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- › The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- › If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- › Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness center, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN _____

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

APPENDIX C
TABLES & FIGURES

TABLE 1: Subject Characteristics

GROUP	MEAN +/- STANDARD DEVIATION			
	Age	HEIGHT (cm)	MASS (kg) PRE	MASS (kg) POST
BA (n=8)	21.38 ± 2.33	162.01 ± 6.44	62.56 ± 8.43	61.43 ± 5.84
PLA (n=7)	23.86 ± 2.12	164.97 ± 7.46	66.67 ± 10.46	66.77 ± 10.09
CON (n=6)	21.33 ± 0.82	164.08 ± 6.86	59.31 ± 4.9	59.32 ± 4.77
TOTAL (n=21)	22.2 ± 2.2	163.69 ± 6.92	63 ± 8.52	62.61 ± 7.62

TABLES

TABLE 2: Pre & Post Testing Results

GROUP	Mean +/- Standard Deviation									
	Relative VO ₂ peak (ml/kg/min)		TTE (seconds) during VO ₂		VT as % of VO ₂		TT (seconds)		PPO (Watts)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
BA (n=8)	34.49 ± 4.46	38.58 ± 3.06	441.25 ± 73.25	518.25 ± 80.29	58.13 ± 9.11	65.38 ± 6.70	272.88 ± 17.74	257.00 ± 14.46	241.25 ± 23.63	325.13 ± 22.61
PLA (n=7)	36.50 ± 3.48	41.83 ± 6.58	474.43 ± 135.41	573.86 ± 115.12	58.29 ± 10.48	61.29 ± 7.65	262.86 ± 23.90	249.14 ± 22.97	245.57 ± 53.58	378.71 ± 72.23
CON (n=6)	36.87 ± 4.54	37.10 ± 5.48	497.50 ± 83.21	477.00 ± 94.53	58.83 ± 16.63	65.17 ± 7.94	263.00 ± 15.24	261.82 ± 14.55	248.83 ± 53.28	254.50 ± 40.60
TOTAL (n=21)	35.84 ± 4.12	39.24 ± 5.26 *	468.38 ± 98.34	524.67 ± 99.89 *	58.38 ± 11.46	63.95 ± 7.26 *	266.71 ± 19.08	255.76 ± 17.65 *	244.86 ± 42.15	322.81 ± 68.20 *

* Indicates that post testing values were significantly higher than the pre-testing values ($p \leq 0.05$)

TABLE 3: Body Composition Results

GROUP	Body Composition Mean +/- Standard Deviation							
	Total BF %		Arm BF %		Trunk BF %		Leg BF %	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
BA (n=8)	33.25 +/- 6.53	32.99 +/- 5.98	29.48 +/- 6.58	28.81 +/- 5.88	32.94 +/- 7.24	32.43 +/- 6.64	35.01 +/- 6.38	35.24 +/- 6.02
PLA (n=7)	29.83 +/- 5.48	28.61 +/- 5.30	25.53 +/- 5.90	23.44 +/- 5.33	28.54 +/- 4.55	26.89 +/- 4.96	32.76 +/- 6.54	31.70 +/- 6.23
CON (n=6)	29.70 +/- 4.78	30.13 +/- 4.73	25.67 +/- 4.91	25.07 +/- 5.68	29.32 +/- 5.80	30.22 +/- 5.68	31.13 +/- 4.08	31.43 +/- 4.03
TOTAL (n=21)	31.10 +/- 5.71	30.71 +/- 5.50	27.07 +/- 5.10	25.95 +/- 5.86 *	30.44 +/- 6.10	29.95 +/- 6.05	33.15 +/- 5.82	32.97 +/- 5.63

* Indicates that post testing values were significantly higher than the pre-testing values ($p \leq 0.05$)

FIGURES

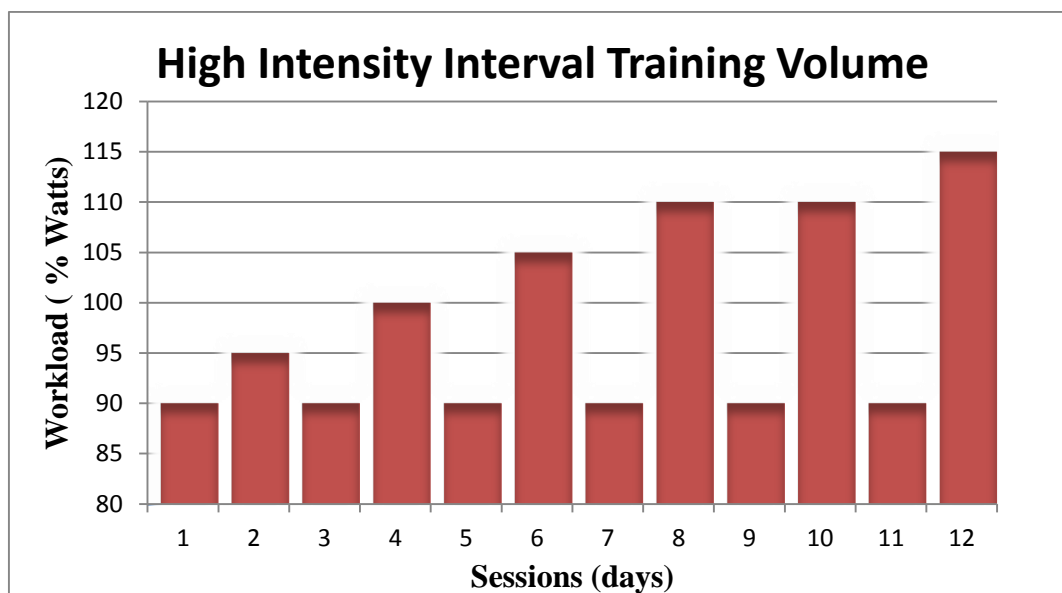


Figure 1 Workload for periodized HIIT Protocol

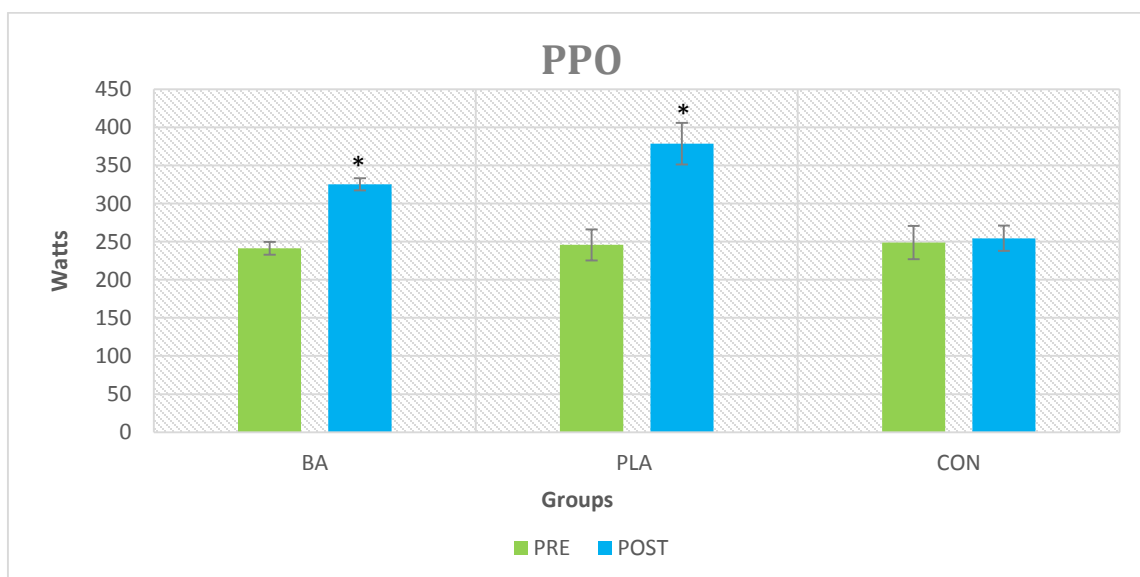


Figure 2 * ($p \leq 0.05$). PPO was significantly higher during post testing for the BA and PLA groups as compared to the CON group