The effects of 28 days of beta-alanine supplementation on the physical working capacity at heart rate threshold (PWC_{HRT}).

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ABSTRACT

MARY NINA WOESSNER: The effects of 28 days of beta-alanine supplementation on the physical working capacity at heart rate threshold (PWC_{HRT}).
(Under the direction of Abbie Smith-Ryan, Ph.D.)

Beta-alanine (BA) supplementation has proven to be an effective means of delaying fatigue. The purpose of this study was to determine the effect of 28 days of BA supplementation on the physical working capacity at heart rate threshold (PWC_{HRT}), a submaximal aerobic fatigue measure. Testing included eight-nine total visits: an enrollment day, resting EKG, physical screening, peak oxygen consumption (VO_{2peak}), and two PWC_{HRT} assessments over four days. Thirty subjects (mean ± SD; age 21.0 ± 2.1 years; body mass 72.7 ± 14.5kg; height 170.1 ± 7.9 cm) were randomly assigned to BA (n=15) or placebo (PL, n=15) groups. Significant differences existed between BA and PL for PWC_{HRT} (p=0.000; BAΔ=+24.2, PLΔ=+11.2), but not for VO_{2peak} (p=0.222), TTE (p=0.562), or VT (p=0.134). Change scores with 95% confidence intervals showed significant improvements in PWC_{HRT} and TTE for BA only. BA may increase heart rate training threshold.

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CHAPTER I

INTRODUCTION

The ability to delay fatigue may play a crucial role in performance outcomes. The point of fatigue onset itself is difficult to pinpoint as there are multiple factors, both central and peripheral, that attribute to the body's inability to produce a maximum force. Central fatigue results from the brain reducing neural activation to the working muscles during exercise, subsequently causing a decrease in power output, motivation, and the up-regulation of the neurotransmitter serotonin (1). Peripheral fatigue is defined by mechanisms that affect the muscle directly and that are distal to the neuromuscular junction (1, 2). While mechanisms of peripheral fatigue include depletion of substrate availability, excitation-contraction coupling failure and oxidative stress, during high intensity exercise one of the largest contributors to fatigue is metabolite accumulation, specifically hydrogen ions (H^+) (3). During exercise, an increase in metabolism leads to excess accumulation of H^+ by-products from the hydrolysis of ATP (3). An increase [H^+] combined with a corresponding decreasing in intramuscular pH, has been identified as one of the primary causes of peripheral fatigue (3). Correspondingly, research has demonstrated that delaying the accumulation of H^+ through buffering mechanisms can delay fatigue onset (4-7).

The human body has natural systems to buffer metabolite accumulation and the resulting acidosis. During intense exercise, the lactate and excess H^+ produced can be

monitored by the bicarbonate buffer system by combining the free H^+ with bicarbonate to form a the weaker carbonic acid (8). While this system works to keep the blood pH from rising, eventually the H^+ accumulation overwhelms the system and the pH drops. Another endogenous mechanism for buffering the H^+ accumulation is muscle carnosine. Muscle carnosine acts to maintain acid-base homeostasis by being a H^+ acceptor (9). Carnosine is synthesized in the body naturally by L-histidine and beta-alanine (BA). Research has shown that supplementing with BA can significantly increase muscle carnosine levels and consequently increase the muscle's buffering capabilities and delay fatigue onset. (4, 9-11).

To pinpoint fatigue onset, various performance tests were developed utilizing fatigue variables such as: electromyography (EMG) activity, ventilatory threshold and heart rate threshold, to name a few. Originally developed in 1981 by Moritani et al. (12), the physical working capacity (PWC) test was designed to determine the point of muscular fatigue onset. This category of tests are based on the initial work by Monod and Sherrer (13), who developed a relationship between total work done and time to fatigue. The earliest PWC test was based on the physical working capacity at fatigue threshold (PWC_{FT}), where the rate of rise in EMG activity as a function of time was calculated at four different power outputs. The slopes were plotted against the workloads and the linear plot was extrapolated to the point of zero slope which was considered the fatigue threshold (12, 14). Studies have previously shown the PWC test to be sensitive to changes from training and supplementation (15, 16). Another PWC test utilizing heart rate as a fatigue variable may provide additional means for quantifying BA's buffering effect on fatigue.

The physical working capacity at heart rate threshold (PWC$_{HRT}$) test was developed by Wagner and Housh (17) to determine the maximum workload an individual can maintain on a cycle ergometer without a significant increase in HR. The test involves four eight minute cycle rides at different workloads, while measuring the change in HR over time. An aerobic training study by Weir et al. (18) demonstrated that the PWC$_{HRT}$ is sensitive to adaptations as a result of aerobic training. Another study by Mielke et al. (19) aimed to validate the PWC$_{HRT}$ by comparing it to PWC$_{VO2}$ and ventilatory threshold (VT). Findings indicated that the power output at the PWC$_{HRT}$, PWC$_{VO2}$ and VT thresholds were not significantly different, validating the PWC$_{HRT}$ as an indirect measure of fatigue.

Utilizing HR as a fatigue measure in the current study provides an additional variable to quantify fatigue, contributing to the existing body of BA research. A study by Smith et al. (20) examined the effects of BA supplementation on women and found that following 28 days of supplementation, HR and ratings of perceived exertion (RPE) were lower during a 40 minute treadmill run compared with placebo subjects. This suggests that BA could have a positive effect on HR and RPE during submaximal exercise.

Beta-alanine research has shown supplementation has a positive effect on delaying fatigue, by way of increasing intramuscular carnosine levels and thereby augmenting H^+ buffering (10, 15, 16). Results from a study by Stout et al.(16) indicated that 90 days of BA supplementation in an older population can effectively increase the PWC$_{FT}$. Another study by Stout et al. (15) showed that 28 days of BA supplementation in a similar population can significantly increase the PWC$_{FT}$. These studies indicate that physical working capacity tests are sensitive enough to assess the effects of BA supplementation,

and that BA is effective at increasing fatigue thresholds. No studies to date have assessed the effects of BA supplementation on the PWC_{HRT}.

In addition to previous literature exploring BA's effect on fatigue thresholds, several studies have also examined its influence on aerobic fitness measures such as VO2peak, ventilatory threshold (VT) and total time to exhaustion (TTE). Due to BA's main influence being on delaying acidosis, studies have consistently shown it to have no effect on VO_{2peak} (a measure than does not mainly rely on anaerobic sources of energy)(10, 11, 15). Research has given conflicting results for BA's effect on TTE with some indicating BA increasing TTE (10, 21) and some indicating no effect (11). VT has demonstrated increases with BA supplementation (10, 21) indicating that BA has a potential positive influence on aerobic physiological measures that occur at moderate intensities or when anaerobic systems begin to play a role (TTE in BA literature is typically taken during higher intensity exercises or during VO_{2peak} tests).

While the focus of BA research involves mechanisms pertaining to peripheral fatigue, with the highest levels of carnosine located intramuscularly, there are concentrations within the brain and heart tissue as well (22). It is suggested that BA supplementation may increase carnosine levels within the brain, thereby affecting the onset of central fatigue, as well as peripheral fatigue in the active muscles. It is also well established that an increase in H^+ leads to a decrease in pH which stimulates afferent pain receptors (23). The presence of pain could inhibit a person's willingness and/or motivation to continue exercise, thereby initiating volitional fatigue. Therefore, an increase in carnosine can delay peripheral as well as central fatigue. While HR accounts for both central and

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peripheral factors (24) , it is not the aim of the present study to differentiate between the two.

The primary purpose of this study was to determine the effects of 28 days of BA supplementation on the PWC_{HRT}. A secondary purpose was to determine if the PWC_{HRT} can be used as a predictor for VO_{2peak}.

Purpose

1. The primary purpose of this study was to determine the effect of 28 days of beta-alanine supplementation, a non-essential amino acid, on the physical working capacity at heart rate threshold (PWC_{HRT}).
2. A secondary purpose was to assess the effect of beta-alanine supplementation on aerobic measures- VO_{2peak}, TTE and VT.

Research Questions

1. Does four weeks of beta-alanine supplementation increase PWC_{HRT}?
2. Does four weeks of beta-alanine supplementation increase VO_{2peak}, TTE and VT?

Hypotheses

1. PWC_{HRT} will significantly increase following 28 days of beta-alanine supplementation.
2. VO_{2peak} will not significantly increase following 28 days of beta-alanine supplementation.

3. TTE will significantly increase following 28 days of beta-alanine supplementation

4. VT will significantly increase following 28 days of beta-alanine supplementation

Delimitations

1. Thirty-eight recreationally active men and women were recruited to participate in this study.

2. Participants were between the ages of 18-35.

3. The duration of the study per subject was approximately six weeks including four weeks of supplementation.

4. During a VO_{2peak} oxygen consumption test, VO_{2peak} was measured.

5. A physical working capacity at heart rate threshold test was completed in order to calculate PWC_{HRT}.

6. Participants had not consumed any performance enhancing supplements containing beta-alanine or creatine within the last three months.

Limitations

1. Subject recruitment was from exercise classes on campus and through fliers located in fitness areas on UNC's campus. Therefore subject population was not truly random.

2. Due to the time-commitment, participant withdrawal occured.

Assumptions

1. Subjects provided accurate health, medical, nutritional and physical activity history.

2. Subjects gave maximal effort during VO_{2peak} testing.

3. Subjects adhered to guidelines stipulating no change in normal nutritional and exercise patterns.

4. Subjects complied with the supplementation protocol.

Operational Definitions

VO$_{2peak}$ – A VO$_{2max}$ is the maximal capacity of an individual's ability to utilize oxygen during exercise and can be used as a measure of physical fitness. It is determined to be the point at which oxygen consumption does not increase despite an increase in workload. All VO$_{2max}$ tests performed on a cycle ergometer are considered to be peak tests (VO$_{2peak}$).

Physical working capacity at heart rate threshold (PWC$_{HRT}$)- This assessment is used to identify the power output associated with the onset of fatigue, as determined by heart rate. Theoretically the PWC$_{HRT}$ represents the highest power output that can be maintained for an extended period of time without signs of fatigue, as identified by an increase in heart rate.

Recreationally Active – 1-5 hours per week of structured and/or recreational exercise.

CHAPTER II

LITERATURE REVIEW

Fatigue can be defined as an inability to continually produce maximum force, or

inability to maintain a given exercise intensity (8). While fatigue can be identified by a

decrement in force, often the cause of fatigue involve smaller factors that simply lead to a

disturbance in the body's ability to maintain homeostasis (8). Fatigue is attributed to

combined central and peripheral attributes. Central fatigue describes the influence within

the brain and spinal cord, whereas peripheral fatigue refers to mechanisms that affect the

muscle directly, distal to the neuromuscular junction (1, 2). Mechanisms of peripheral

fatigue are numerous and can be traced to excitation-contraction coupling failure,

depletion of substrate availability, oxidative stress and metabolite accumulation (25).

During intense exercises, increases in ATP hydrolysis yields an accumulation of

inorganic phosphate (P_i) (3). The hydrolysis of ATP also results in the release of a proton

in the form of a hydrogen ion (H^+). While P_i acts as a buffer to the increasing H^+

accumulation, the increase in intracellular P_i is much less than the total amount of ATP

hydrolysis. Due to the hydrolysis of ATP, there is also an accumulation of ADP, which

combines with P_i to further produce ATP and lactate (3). While other factors of

metabolite accumulation act on muscular pH and play a role in peripheral fatigue, H^+

accumulation is the focus of the current study. An increased $[H^+]$, and a corresponding

decrease in pH, is identified as one of the most influential aspects of peripheral fatigue

(4). Animal studies have shown a decrease in pH from 7 to 6 leads to a significant

decrease in isometric tension in the muscle, resulting in a reduced force production (5).

Literature has shown that delaying the accumulation of H^+ may concomitantly delay the

onset fatigue (4-7).

Central fatigue is typically defined as a person's inability to maintain voluntary force

due to the subconscious brain reducing neural activation to contracting muscles during a

fatiguing exercise (1, 26). Fatigue in many instances results from an individual's

unwillingness to continually produce adequate central drive to maintain a given force or

power output (1). One identified source of decreased motivation (central drive) is the up-

regulation of the neurotransmitter serotonin during exercise (1). An increase in serotonin,

and corresponding decrease in dopamine levels, is associated with central fatigue. The

combination of central and peripheral fatigue has been shown to account for the total loss

of force during sustained muscle contractions (25). The autonomic nervous system

(ANS), is composed of both a sympathetic nervous system (SNS) and parasympathetic

nervous system (PNS) that act as complimentary systems. Both the SNS and PNS

influence heart rate by varying impulses in nerve fibers in the sinoatrial node (27). The

heart is dually innervated by the PNS and ANS, and is up- and down-regulated by the

two respectively. The central control of heart rate can be demonstrated by the anticipatory

rise in heart rate seen in athletes prior to race events. Thus without any muscle

contraction or energy, there is a distinct increase in heart rate. In addition, during exercise

the CNS stimulation modulates the ANS, and the peripheral sensors then regulate the

effects according to muscle needs. Previous literature has demonstrated that central

factors, as opposed to muscular fatigue, are the limiting factors for performance amongst

chronic fatigue patients (28). At lower VO_2peak values, but higher ratings of perceived exertion (RPE), chronic fatigue patients previously demonstrated significantly lower heart rate, indicating a centrally mediated increase in effort perception. This increased perception of effort led to subjects' unwillingness or inability to achieve maximal performance. Extending this to normal fatigue processes during intense or long duration exercise, central factors may play a limiting role in a person's ability or willingness to achieve high intensity exercise. The central command theory suggests that at the onset of exercise centrally controlled signals set the cardiovascular response, while peripheral feedback loops are responsible for the fine tuning adjustments as exercise continues. Despite the overlap between the two systems, it is clear that both central and peripheral factors influence heart rate during exercise (26).

Nutritional supplementation may be an effective way to delay fatigue onset. Recently, beta-alanine (BA), a non-essential amino acid has been shown to effectively delay neuromuscular fatigue (10, 11). The direct effect of BA supplementation is to increase carnosine levels within the muscle (4, 9). Beta-alanine supplementation has been shown to significantly increase intramuscular carnosine levels by as much as 60% (9) and also improve exercise performance by delaying the drop in pH (29). To date, research suggests carnosine's most effective role is delaying peripheral muscle fatigue by maintaining acid-base homeostasis through buffering H^+ (9) . With a dissociation content (pKa) of 6.83 , carnosine is highly effective at accepting excess H^+ in the muscle in order to maintain intramuscular pH (30). Carnosine is synthesized from L-histidine and BA, but due to the high concentration of L-histidine within the plasma, beta-alanine has been shown to be the rate-limiting step of muscle carnosine production (9, 31).

While beta-alanine's positive effect on peripheral fatigue via an increase in skeletal muscle carnosine are well documented (4, 9, 32), its direct effect on central fatigue has not yet been examined. While the highest concentrations of carnosine are in skeletal muscle, carnosine has also been located within the brain (22). High concentrations of carnosine are found in the olfactory lobe, which has been shown to influence both central and peripheral characteristics (22). One of the side effects experienced acutely with BA supplementation, paresthesia (or prickling sensations on the skin), is linked to BA activated strychnine-sensitive glycine receptor sites located within the spinal cord (33, 34). It is still undetermined what effect carnosine could have on the central nervous system, and, more specifically, delaying central fatigue.

Various fatigue tests have been utilized to examine the effectiveness of BA supplementation. The physical working capacity test has been used to show significant increases in physical working capacities following supplementation(10, 15, 16). The physical working capacity tests were originally developed by Moritani et al. 1981 and Devries et al. 1982 to measure the point of onset of muscular fatigue (12, 14). The researchers utilized electromyographic (EMG) fatigue curves to identify an EMG fatigue threshold. Prior to this, however, Monod and Scherrer (1963) found a relationship between total work done at varying workloads and the duration of time to fatigue. The slope of this relation was termed critical power and represented the power output a muscle could maintain without fatigue (13). However, these original measures required subjects to perform supramaximal tests, such as a VO_{2max}. Therefore, in 1987, Devries et al. completed a follow-up study to submaximally estimate physical working capacity at the fatigue threshold (PWC_{FT}) (35). Theoretically a physical working capacity threshold

represents the highest power output that can be maintained for an extended period of time without evidence of fatigue (19). Utilizing submaximal loads and the same principles developed by Devries et al. (1987), the physical working capacity at heart rate threshold test (PWC_{HRT}) was developed by Wagner and Housh (17) in 1993 in order to identify the highest cycle workload an individual can maintain without a significant increase in heart rate over an extended period of time (17). Studies utilizing the PWC_{HRT} have found it to be highly sensitive to the effects of aerobic training programs (18), as well as an accurate estimate of the maximal power output that can be produced without a significant rise in heart rate (17).

The purpose of this study is to determine the effect of four weeks of BA supplementation on the physical working capacity at heart rate threshold in men and women. By utilizing heart rate as the main measure of fatigue, this study will be indirectly examining BA's effect on both central and peripheral fatigue. Previous research on BA has shown that it is effective in delaying fatigue peripherally by increasing muscle carnosine, but its effect on central mechanisms is still unknown. The physical working capacity tests offer an easy, cost sensitive way to quantify a fatigue threshold. The following annotated article review will examine previous research describing fatigue, heart rate response to exercise, BA, and physical working capacity tests; in order to support the purpose and hypothesis of the present study.

Central and Peripheral Fatigue and Heart Rate

Cooke, Franks, Luciani, and Pate 1988 (5)

The purpose of this study was to gain a more comprehensive understanding of the effects of phosphate and hydrogen ions on parameters of contraction by varying their concentrations in rabbit psoas muscles. Dissected psoas fibers from the rabbits were fully activated utilizing $Ca2^+$ at a constant temperature of 10ºC. The velocities of induced contractions were measured utilizing load clamps. Fiber ATPase was also monitored utilizing enzyme coupling of ADP production to reduced NADH, and then measuring the depletion of NADH. Results showed that increasing phosphate concentration from 3 to 20mM at a pH of 7.0 decreases ATPase activity, does not affect maximum contraction velocity (V_{max}), and causes a 25% decrease isometric tension (P_0). Changing the pH from 7 to 6, while maintaining the 3mM concentration of phosphate, showed a 45% decrease inP_0. These results are similar to those of other studies which indicated that a decrease in muscular pH from 7 to 6 results in a decrease in isometric tension. The main conclusion drawn from this study is that most of the fiber contraction inhibition seen in fatiguing muscles is explained by product inhibition (H^+ and Pi).

Robergs, Ghiasvand, and Parker 2004 (3)

The focus of this review article was to compile the previous and current literature available on metabolic acidosis to form a comprehensive review. Once thought to be a major source of metabolic acidosis, lactate is known to play an important role in initial buffering of H^+ accumulation to the lactate dehydrogenase reaction (pyruvate to lactate) consuming a proton, thus lactate production acts to alkalinize the cell, and not acidify.

The lactate has a further use as it can be transported from the producing cell to be used as a substrate for metabolism in other skeletal cells, the liver or kidneys. The real source of H^+ accumulation has been more correctly identified as ATP hydrolysis. A phosphate (P_i) is produced and has the ability to buffer the free proton that is released from the water. During ATP hydrolysis, the ADP and P_i go on to act as substrates for glycolysis to produce more ATP, which leaves the extra proton to accumulate if the other buffering systems cannot meet the necessary removal rates. Another source of increased H^+ could be from an accumulation of NADH + H^+ produced via the glyceraldehydes 3-phosphate dehydrogenase reaction step of glycolysis. This could occur when the rate of substrate efflux from glycolysis is greater than the mitochondrial activity to accept the products. The conclusions of this review were that lactate facilitates proton removal from the muscle, lactate production is essential to supporting ATP regeneration from glycolysis, and metabolic acidosis results from an increased reliance on non-mitochondrial ATP turnover (glycolysis, etc).

Davis, 1995 (1)

The purpose of this review article was to explore the causes central fatigue, specifically the role that brain serotonin (5-HT) plays in leading to central fatigue. This article proposes that central fatigue plays a crucial role in limiting overall exercise performance. Increased 5-HT is suggested to be the main contributor to central fatigue in prolonged exercise due to its known effects on arousal, lethargy and sleepiness. Increases in brain 5-HT are due to increases in blood-borne tryptophan (TRP) delivery to the brain. Since most TRP is bound to albumin in the blood, it is the free tryptophan (f-

TRP) that is transported to the brain. The transport mechanism occurs with other amino acids, specifically the branched-chain amino acids (BCAAs). Synthesis of brain 5-HT increases when the ratio of f-TRP to total plasma BCAAs increases. Animal studies have indicated an association between increase levels of brain 5-HT and fatigue during continuous prolonged exercise. Another study utilizing rats found that drug-induced increases in brain 5-HT lead to faster onset of fatigue whereas drug-induced decreases in brain 5-HT lead to a delay in fatigue onset.

The article also explores the effectiveness of nutritional supplementation to delay central fatigue. Studies have indicated that consumption of the large doses of BCAAs needed to significantly change the ration of f-TRP to BCAAs would also increase plasma ammonia which could lead to toxic effects on the brain and negative changes to metabolism. A study utilizing carbohydrate supplementation found that supplementing with 6 or 12% carbohydrate-electrolyte drink solutions lead to greatly reduced plasma f-TRP levels as compared to water supplementation, and that the carbohydrate-electrolyte supplement was successful in delaying fatigue. This study contributes to the current body of fatigue literature by highlighting the role that central fatigue can play in limiting exercise performance, and by indicating the importance of further research on mechanisms of central fatigue.

Kent-Braun, 1999 (7)

The purpose of this study was to estimate the contribution of peripheral and central factors to human skeletal muscle fatigue. Nine people (five men, and four women, mean age=30) with physical activity levels ranging from sedentary to recreationally

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active were recruited for this study. Subjects performed a baseline MVC of the dorsiflexor muscles, then a sustained isometric MVC for 4 minutes with a titanic stimulation at the end of the 4 minutes. An EMG was used to quantify muscle activation during maximal voluntary isometric contractions (MVC) of the ankle dorsiflexor muscles. Fatigue was quantified by the change in force during the MVC. Measures of intramuscular metabolism were also made during the exercise utilizing magnetic resonance spectroscopy.

The results of the study showed a significant difference between the titanic and voluntary fatigue (15%), and an overall 78% fall in MVC. Data indicated that approximately 20% of the fatigue developed during the sustained MVC was due to central fatigue, and the other 80% was due to intramuscular sources such as an increase proton concentration. There was a decrease in central activation ratio (CAR), defined as the peak MVC/peak total force, which is indicative of a slight central activation failure during exercise.

The study also found significant changes in the intramuscular metabolism during exercise. The fall in pH was correlated with the observed fall in force, and the lack of peripheral activation failure is indicative of metabolic inhibition leading to fatigue. Other studies cited have indicated that there is a linear relationship between $[H^+]$ and fatigue.

Robinson, Epstein, Beiser, and Braunwaldet, 1966 (27)

The purpose of this study was to identify the roles of the efferent pathways that control heart rate during supine exercise by observing the effects of β-adrenergic blockade and parasympathetic blockade on cardiac acceleration. Five people (age 19-28

years) were recruited for this study: four normal volunteers and one patient who had undergone a successful mitral commissurotomy. All subjects were men who were in good physical condition, but were not trained athletes. After familiarization with the equipment, the subjects were placed on a tilt table in the supine position and their heart rate (HR) was recorded via ECG. The subject was then tilted to 45º and 80º and HR was again recorded. The subjects were then moved to a supine bicycle where their HR was recorded at rest as well as during the fourth minute of exercise at each series of work levels. Oxygen uptake was recorded continuously throughout. The protocol was performed 3 more times: one where the cardiac sympathetic nerves were blocked, one where the parasympathetic nerves were blocked, and one where both were blocked. Sympathetic blockade has been found to lower HR during exercise, suggesting sympathetic stimulation is responsible for cardiac acceleration. Results of the study indicated that the increase in HR during moderate exercise was due to withdrawal of parasympathetic stimulation. At higher workloads, increases in HR were more due to sympathetic stimulation. These results were determined by comparing the double-blockade response to the single blockade responses of HR.

Beta-Alanine Supplementation

Derave, Ozdemir, Harris, Pottier, Reyngoudt, Koppo, Wise, and Achten, 2007 (32)

The purpose of this study was to determine whether 28 days of BA supplementation would increase muscle carnosine levels in the calf and improve performance in a 400 meter sprint. Fifteen male (age 18.4 ± 1.5 yrs) track-and-field athletes, each recording a 400 meter under 53 seconds, were recruited for this study. The

study was a placebo-controlled, double-blind study where 7 athletes consumed a placebo (maltodextrin) for the duration of the study, and 8 consumed six divided doses of 400mg capsules BA CarnoSyn® time release capsules.2.4g/day for the first 4 days, 2.6g/day for the next 4 days, and 4.8g/day for the remaining days. A week prior to supplementation, pre-testing was performed. A proton magnetic resonance spectroscopy (MRS) was utilized to determine initial muscle carnosine concentrations. Isokinetic and isometric fatigue tests using a Biodex isokinetic dynamometer was utilized to evaluate the contractile performance of the knee extensors. The isokinetic test was performed on the right leg that consisted of 5 sets of 30 maximal voluntary isokinetic knee extensions at a velocity of 180º/second. Subjects had a one minute recovery between each set of 30 repetitions. Peak torque data was measured and used to calculate an average peak torque for each set of exercise. The left leg was then use for isometric testing. The knee was fixed at 45º, and the maximum voluntary contraction (MVC) torque was recorded. The highest of three trials was recorded as their final MVC. Finally, subjects completed a 400 meter sprint on a separate day, following a self-constructed warm-up routine lasting ~45 minutes.

The results of this study showed substantial increases in muscle carnosine (+47% in the soleus, +37% in the gastrocnemius). Since the subjects were highly trained athletes, these increases were similar to, but slightly lower than other studies that utilized untrained subjects. Even subjects with high initial levels of muscle carnsoine saw increases in concentration, which suggests a lack of a supplement ceiling. The results of this study also showed that in spite of improvements in muscle torque following supplementation, there was no significant effect on the 400 meter spring time. Dynamic

knee extension torque was significant increased in all 5 bouts following supplementation, and increased in the first 2 bouts following a placebo. This suggests that BA supplementation can attenuate fatigue in the later stages of exercise. There was no significant effect on isometric endurance.

Zoeller, Stout, O'Kroy, Torok, and Mielke, 2006 (11)

The purpose of this study was to determine the effect of four weeks of BA supplementation alone or in combination with creatine monohydrate (Cr) on endurance performance. Fifty-five men (age 24.5 ± 5.3 years) were recruited for this study. Subjects were randomly assigned to one of four supplementation groups: placebo (PL, 34g of dextrose; n=13), creatine (Cr; 5.25g of creatine monohydrate plus 34g of dextrose; n=12), beta-alanine (Carnosyn®, NAI, San Marcos, CA) (B-Ala; 1.6g beta-alanine plus 34g of dextrose; n=14), or beta-alanine plus creatine (Phosphagen Elite™, EAS, Golden, CO) (CrBA; 5.25g of creatine monohydrate; 1.6g beta-alanine plus 34g of dextrose; n=16). For pre-testing, subjects performed a graded exercise test (GXT) on a cycle ergometer to determine VO_{2peak}, ventilatory threshold (VT), and lactate threshold (LT). For the GXT protocol, initial power was set to 30 watts, and was increased by 30 watts every 2 minutes as the subjects maintained a pedal cadence of 70rpm. The test was stopped when the subject could no longer maintain the rpm, or until volitional fatigue. To confirm the test was a VO_{2peak}, subjects had to meet the following criteria: a plateau in heart rate (HR) values or attainment of HR within 10% of age-predicted HR_{max}, a plateau in oxygen uptake (defined by an increase of no more than150ml/min), and an RER value greater

than 1.15. Subjects then completed 28 days of their assigned supplementation protocol and finished with post-testing consisting of the same GXT. Results of this study demonstrated that the Cr group showed improvements in power at VT and total time to exhaustion (TTE), while B-Ala only had improvements in power output at LT. In contrast, the combination group, CrBA showed increases in power output at LT, VO_2 at LT, VO_2 at VT, power output at VT, and percent VO_2 at VT. This data indicates that supplementation with the combination of creatine and beta-alanine may dely the onset of fatigue, via LT and VT measures during incremental cycle exercise in men.

Artioli, Gualano, Smith, Stout, and Lancha 2009 (31)

The purpose of this review paper was to discuss the current information on carnosine and beta-alanine metabolism and to highlight the effects of beta-alanine supplementation on exercise performance. For purposes of the present study, the important findings will be limited to the effects of beta-alanine supplementation. Beta-alanine has been shown to effectively increase muscle carnosine after only 28 days of supplementation. Harris et al. in 2006 (9) demonstrated that 800mg is the maximum tolerable single dose of beta-alanine, but that this dose could be repeated throughout the day if there was a three hour time window between dosings. Later studies showed that using controlled release capsules could be utilized in order to double the dose from 800mg to 1600mg. A study by Zoeller et al. in 2007 (11) reported that beta-alanine improves the anaerobic threshold. Stout et al. in 2007 (10) showed a significant increase of 13% on ventilatory threshold in women supplementing with beta-alanine. The conclusion from these studies was that the increased buffering capacity created by beta-alanine supplementation allowed subjects to achieve higher levels of power output with a

smaller lactate accumulation. Another study by Stout et al. in 2006 (15) utilized 55 men with a mean age of 24.5 to demonstrate beta-alanine's ability to delay fatigue by increasing the physical working capacity at fatigue threshold after 28 days of beta-alanine supplementation. Overall, studies utilizing exercise protocols that elicited extreme acidosis demonstrated increases in performance linked with beta-alanine supplementation. Protocols that elicit less of an acidosis response, (such as those having a single bout of exercise lasting less than 60 seconds, or those using small muscle groups) resulted in little or no effect from beta-alanine supplementation. Another focus of this review was the adverse effects of beta-alanine supplementation. The only known one is the symptoms of paresthesia, or tingling sensations, that are triggered by ingesting a high single dose of beta-alanine, and tend to disappear within an hour. Controlled time-release capsules or smaller dosages can avoid the symptoms altogether. The conclusions of this review were that 28 days was a significant amount of time to increase muscle carnosine; beta-alanine supplementation can improve fatigue thresholds; and in order to avoid paresthesia, controlled time-release capsules should be utilized.

Stout, Graves, Smith, Hartman, Cramer, Beck, and Harris, 2008(16)

The purpose of this study was to determine the effects of 90 days of beta-alanine supplementation on the onset of neuromuscular fatigue when measured by the physical working capacity at fatigue threshold (PWC_{FT}) test in elderly men and women. Twenty-six untrained men and women were recruited for this study. For pre-testing, subjects completed a PWC_{FT}. Subjects were fitted to a cycle ergometer and began pedaling at 50 rpms with an initial power output determined by the researchers based on participant's self-reported physical fitness. Power output was increased 10-20 watts every two

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minutes. After each two minutes there was a rest interval long enough to return heart rate (HR) to within 10bpm of the arrival HR. During each workload, EMG samples were recorded on the vastus lateralis. PWC_{FT} was determined to be the highest power output that resulted in a non-significant slope value for the EMG amplitude verse time relationship. Following pre-testing, subjects were randomly assigned to either the beta-alanine group (BA, 2.4 grams/day of beta-alanine, CarnoSyn[®], n=12) or the placebo group (PL, 2.4 grams/day of microcrystalline cellulose, n=14). All subjects ingested one capsule (containing 800mg) three times a day for 90 days. Within three to five days following supplementation subjects completed a post-supplementation PWC_{FT}. Results showed a significant increase in PWC_{FT} (+28.6%) in the BA group, but no significant change in the PL group. The results of this study indicate that 90 days of beta-alanine supplementation in the elderly can significantly increase PWC_{FT}.

Hill, Harris, Kim, Harris, Sale, Boobis, Kim, and Wise, 2007 (4)

The purposes of this study were to further evaluate the effect of four weeks of beta-alanine supplementation on the accumulation of muscle carnosine, to determine if changes were fiber specific, and to see if the effects altered results of a high intensity cycling capacity test. Twenty-five male subjects who were physically active but not in structured training programs were recruited. Following a cycle performance test, subjects were split into two groups; one that supplemented with beta-alanine (CarnoSyn[®]) for either 4 or 10 weeks (n=13), and one that had a matching placebo (PL).Beta-alanine (BA) was divided into 8 daily doses with total dosage per day increasing each week (4.0g-6.4g over the study). For determination of muscle carnosine content shifts, a percutaneous

muscle biopsy of the vastus lateralis was administered 1-2 days following the cycle test, and 1-2 days following the 4 and 10 week tests. For muscle fiber assessment, 20-40 single muscle fibers were dissected from the 0 and 10 week biopsies of subjects in the beta-alanine group. Results showed muscle carnosine concentrations were significantly increased at both the 4 (19.9 ± 1.9 mmol/kg dm) and 10 (30.1 ± 2.3 mmol/kg dm) week markers for the BA group with no significant changes observed in the control. Muscle fiber content changes were not significantly different between type IIa and type I muscle fibers. Total muscle carnosine content was greater in type IIa fibers, but the increases over the 10 weeks were not significantly different. For the cycling test, total work done (TWD) was significantly increased following 4 (+13%) and 10 weeks (16.2%) of BA supplementation, with no significant change seen in the control group. The findings of this study support BA supplementation as an effective enhancer of muscle carnosine at both 4 (+58%) and 10 (+80.1%) weeks. Most importantly, this study showed a relationship between greater muscle carnosine content and increased power output via the changes seen in TWD in the cycle tests. The authors conclude that this is one of the first studies linking improvements in whole body exercise capacity to an increase in intracellular H^+ buffering content.

Harris, Tallon, Dunnett, Boobis, Coakley, Kim, Fallowfield, Hill, Sale and Wise, 2006 (9)

The purpose of this study was to investigate the absorption of BA and the effect of supplementation on muscle carnosine synthesis. Six healthy male subjects (age 33.5 ± 9.9 years) were recruited for this study. All subjects underwent four of the five supplementation treatments following an 12 hour overnight fast. Treatment A involved

ingesting chicken broth containing 40mg/kg of body weight (bwt) of BA. For treatments B, C, D, and E subjects ingested 3ml/kg bwt of a drink containing either 0, 10, 20, or 40 mg/kg bwt of beta-alanine (CarnoSyn[®]), followed by 5ml/kg bwt of water. Blood samples were taken at 10 minute intervals for the first 90 minutes following supplementation and then at 120, 180, 240 and 360 minutes. Complications with subjects administered 40mg/kg bwt of beta-alanine arose when they rapidly experienced symptoms of flushing (prickly sensations) which began at 20 minutes and lasted an hour. As a result of this, two subjects refused the dose and were given 10 and 20 mg/kg bwt instead. Symptoms were seen with the 20mg/kg bwt again, but to a much lesser extent. There was a significant increase in plasma BA following the chicken broth ingestion, but the increase was only half the increase seen in subjects consuming 40mg/kg bwt of beta-alanine. Important findings in this study indicated that administration of BA above 10mg/kg bwt was associated with severe flushing symptoms. The chicken broth which resulted in similar BA concentrations to those elicited by 20mg/kg bwt supplementation, did not lead to any symptoms.

Smith, Stout, Kendall, Fukuda, and Cramer, 2011 (20)

The purpose of this study was to determine the effects of BA supplementation on oxidative stress in women. Twenty-four moderately trained (3-7 days per week of aerobic, resistance or recreational activities) women were used in this study. Each subject had a total of four laboratory visits. On visit one subjects completed an initial run to determine VO_2max and peak velocity (PV). No more than 3 days later, subjects came in for baseline blood testing and an oxidative stress run. For the oxidative stress test,

subjects ran at 70-75% of their PV for 40 minutes. Post run blood samples were collected

at two and four hours post-exercise. Upon completion of all pre-testing, participants were

randomly assigned to a placebo (PL) group (800mg/tablet of maltodextrin, 2 tablets 3

times daily) or a BA group (800 mg/tablet, 2 tablets 3 times daily; CarnoSyn®). Subjects

then supplemented for 28 days, reporting to the lab after two weeks to report intake and

side effects. Subjects then returned to the lab for post-testing in the form of a VO2 max

and the 40 minute oxidative stress run. Results of this study showed that, while non-

significant, 28 days of BA supplementation still had a slight influence on aerobic

performance defined by total time to exhaustion (TTE) and ventilatory threshold (VT).

Supplementation had no significant effect on the oxidative stress markers- antioxidant

capacity, superoxide dismutase, glutathione, and 8-Isoprostane. Rating of perceived

exertion decreased (non-significantly) in the BA group at 30 and 40 minutes of the

oxidative stress run. Ventilatory threshold also increased non-significantly in the BA

group suggested an increased buffering capacity and improvement in submaximal

performance.

Physical Working Capacity Tests

Stout, Cramer, Mielke, O'Kroy, Torok, and Zoeller, 2006 (15)

The purpose of this study was to determine the effects of 28 days of beta-alanine

(BA) and creatine monohydrate (CrM) supplementation on the physical working capacity

at neuromuscular fatigue threshold (PWCFT). Fifty-one men (age 24.5± 5.3 years), who

had not ingested creatine or other dietary supplements for the last 12 weeks, were

recruited for this study. Subjects were fitted with EMG electrodes on their vastus lateralis

to use as the measure for neuromuscular fatigue. Subjects were asked to ride an electronically braked cycle ergometer at a pedal frequency of 70rpm starting at a resistance of 60 watts (W). The power output was increases by 30W every 2 minutes until the pedal cadence could not be maintained. At every 2 minute interval six 10-second EMG samples were recorded. The PWC_{FT} was calculated later by averaging the highest and lowest power output that had non-significant slope values for the EMG amplitude vs. time relationship. Following pre-testing, subjects were randomly assigned to one of four treatment groups: 1) placebo (PLA; 34g dextrose; n=13), 2) creatine (CrM; 5.25g CrM plus 34g dextrose; n=12), 3) b-Ala (1.6g b-Ala plus 34g dextrose; n=12), 4) b-Ala plus CrM (CrBA; 5.25 g of CrM plus 1.6g b-Ala plus 34g dextrose; n=14). Subjects took the supplements 4 times per day for 6 days, and then twice per day for the last 22. Subjects completed the same tests in post-testing as in pre-testing. Results of this study showed that b-Ala supplementation could delay the onset of neuromuscular fatigue by significantly increasing the PWC_{FT} (+14.5%). The combination of CrBA did not have an additive effect to the delay in fatigue, but did significant increase the PWC_{FT} (+11%), while Cr alone had no significant effect. These findings suggest that beta-alanine supplementation, with or without creatine, may delay the onset of neuromuscular fatigue during incremental cycle ergometry.

Wagner and Housh, 1993 (17)

The purpose of this study was to introduce the physical working capacity at heart rate threshold (PWC_{HRT}) test that could be used to estimate the maximal power during cycle ergometry that can be maintained for an extended period of time without fatigue.

Eight sedentary men (age 22 ± 2 years) were recruited for this study. The subjects had

nine laboratory visits, each separated by at least 48 hours. On the first four visits subjects

completed four different workloads for determination of the PWC_{HRT}, and on the last five

subjects completed hour long rides in order to validate the $PWC_{HRT.}$ For determination of

PWC_{HRT} subjects completed four different workloads ranging from 105-200 watts (W).

Upon reporting to the lab, subjects were fitted to the electronically braked cycle

ergometer and completed a five minute warm-up at 70rpm with a resistance of 50W.

Following the warm-up, subjects completed the eight minute work bout at a workload

chosen so that they could complete the full eight minutes, but would also elicit a positive

slope in the heart rate verses time relationship. Heart rate was recorded every 15 seconds,

but only the last five minutes were used to allow for stabilization of the HR and to ensure

the rise in HR was due to the demands of the workload and not cardiac adjustment. After

all four workloads were completed, the power outputs were plotted as a function of the

slope for the HR versus time relationships. The PWC_{HRT} was determined to be the y

intercept of the plot, or the point of zero slope. For validity of the test, one hour

continuous work bouts were performed at randomly assigned power outputs equivalent to

80, 100, 120, 140 and 160% of PWC_{HRT}. Subjects were blinded from the workload until

completion of all workloads. Warm-up procedures were identical to the PWC_{HRT} trials,

and following the warm-ups subjects completed an hour long ride at the prescribed

intensity. Statistical analysis included regression analyses using least squares method and

t tests were used to determine if there were significant differences between the mean

slope coefficients for the five one hour rides. Results of the study showed that the HR

increased significantly ($p \leq .05$) at all one hour workloads. However, the mean slope

coefficients corresponding to workloads equal to or less than the PWC_{HRT} had HR increases less than $0.1bpm \cdot min^{-1}$. This minimal rise indicates that the PWC_{HRT} can provide an accurate estimate of the power output that can be maintained for an hour without significant fatigue. The authors concluded that the PWC_{HRT} would be an effective way to assess the effects of interventions designed to increase work capacities.

DeVries, Tichy, Housh, Smyth, Tichy, and Housh, 1987 (35)

The purpose of this study was to evaluate the physical working capacity at fatigue threshold (PWC_{FT}) test on a cycle ergometer with fatigue being determined by EMG fatigue curves in the quadriceps muscle. Thirty-two healthy men (age 23.4 ± 3.1 years) with fitness levels ranging from sedentary to highly trained athletes were recruited for this study. For determination of the PWC_{FT}, subjects completed four pre-determined workloads on the cycle ergometer ranging from 420 kp m/min to 1260 kp m/min. Each workload lasted two minutes and was followed by a rest period long enough to bring the heart rate back to within 10bpm of the initial heart rate. EMG was recorded on the quadriceps throughout the two minute work bouts and PWC_{FT} was determined to be the lowest workload producing a slope of the EMG amplitude verse time relationship that was significantly different than a zero slope. Subjects were also tested for lactate threshold (OBLA) via blood draws within 1-2 minutes of each exercise bout, and percentage heart rate range at PWC_{FT}. Seventeen subjects were retested for PWC_{FT} a week following initial testing to determine reliability. Fifteen subjects were retested for OBLA a week following initial testing as well. Results of this study showed that the PWC_{FT} was high reproducible with a test re-test correlation of r=0.947. For the unfit

quartile of the subjects there was a small non-significant difference between the mean

power of OBLA and the mean power of PWC_{FT}. However, for the fit quartile, there was a

significant difference ($p \leq .05$) between the two mean powers. The authors conclude that

the results suggest that fit subjects are limited by central factors, whereas in unfit

subjects, muscular (or peripheral) fatigue factors are the limiter. From this study it can be

concluded that the PWC_{FT} is an effective and reliable way to evaluate the physical status

and to monitor training progress in individuals. It is ideally suited for individuals who are

unable to complete maximal workloads, as the PWC_{FT} utilizes short, submaximal and

discontinuous workloads.

Weir LL, Weir JP, Housh, and Johnson, 1997(18)

The purpose of this study was to determine the effect of an eight week aerobic

training program on the physical working capacity at heart rate threshold (PWC_{HRT}).

Nineteen college-aged men and women who had not participated in a regular aerobic

exercise program for at least one year were recruited for this study. For pre- and post-

testing, subjects completed both a VO_{2max} and a PWC_{HRT} on a cycle ergometer. For the

VO_{2max} subjects completed a five minute warm-up at 30 watts (W). The first stage of the

test had a resistance of 50W and a required pedal cadence of 70rpm. The W was

increased by 30W every two minutes until voluntary exhaustion. The test was considered

a true VO_{2max} if the subjects met two of the following criteria: a plateau of VO_2 with an

increase in W, maximal heart rate within 15bpm of the age predicted max heart rate, or a

respiratory exchange ratio (RER) ≥ 1.1. For determination of PWC_{HRT}, subjects again

completed a five minute warm-up consisting of a resistance of 50W and a pedal cadence

of 70rpm. Each subject then completed four eight minute workloads (on separate days) ranging in resistance from 55-215W. The rate of rise in heart rate as a function of time for each workload was determined for each subject. The PWC_{HRT} was calculated by plotting the workloads as a function of the slope for the heart rate verse time relationship and taking the y-intercept. Following pre-testing, subjects were randomly assigned to either a training or control group. The training group completed three exercise sessions a week for eight weeks, while the control group had no training sessions and simply maintained their normal activities. The training sessions consisted of a five minute warm-up on a cycle, followed by 30 minutes of cycling at intensities between 70 and 90% of max heart rate at 70rpm. Results of this study showed a significant increase in PWC_{HRT} (30.1 W) following training indicating that the PWC_{HRT} is sensitive to an eight week aerobic training program. The study also showed only a moderate relationship between VO_{2max} and PWC_{HRT} ($r^2 = 0.31$). This indicates that VO_{2max} and PWC_{HRT} are controlled by different mechanisms and cannot be explained by similar variables. There was also a decrease in heart rate slope coefficients following training. The authors speculate this drop could be due to increased stroke volume, decreased sympathetic drive or an increase in parasympathetic activity. Regardless of the mechanism, the decrease itself refutes previous research suggesting that heart rate slopes remain stable with training.

Mielke, Housh, Malek, Beck, Hendrix, Schmidt, and Johnson, .2008 (19)

The purpose of this study was to further validate the PWC_{HRT} and the PWC VO_2 tests by utilizing the results of the tests to find an estimated time to exhaustion (ETTE) and then compare the power outputs and estimated time to exhaustion (ETTE) to

ventilatory threshold (VT). Ten men and women with a mean age of 23 were recruited for the study. Their physical activity status ranged from sedentary (not currently participating in aerobic or resistance training) to moderately active (4-5 hours of exercise per week). They performed an incremental test to exhaustion on an electronically braked cycle ergometer in order to determine VO_{2peak} and VT. VO_{2peak} was identified as the highest VO_2 value in the last 30 seconds of the test if the subject met two of the termination criteria: 1) 90% of age-predicted max HR, 2) respiratory exchange ratio >1.1, 3) a plateau of oxygen uptake (defined as <150 ml/min increase in VO_2 during the last 30 seconds). VT was defined was identified as the VO_2 value corresponding to the intersection of two regression lines derived from points below and above the breakpoint in the VCO_2 vs. VO_2 relationship. On a separate visit, subjects completed a physical working capacity test which included four randomly ordered workloads to exhaustion at different power outputs (98-246 watts), to determine the PWC_{HRT} and the PWC VO_2. Power curve analyses were used to estimate the ETTE at PWC_{HRT}, PWC VO_2, and VT. Results showed no significant mean differences between the three fatigue thresholds suggesting that fatigue thresholds determined by HR and VO_2 could be used for estimating VT. The power-curve analyses also indicated, contrary to previous studies, that the subjects could only maintain the power outputs for the PWC_{HRT}, PWC VO_2, and VT for 29 ± 6, 21 ± 3, and 27 ± 11 minutes respectively. The authors speculate the differences in this study as compared to previous ones could be due to the fatiguing protocol used in this one as well as differences in subject training status. The findings of this study for the similar power thresholds between PWC_{HRT}, PWC VO_2, and VT support the PWC_{HRT} as being a valid measure of fatigue threshold.

CHAPTER III

METHODS

Experimental Design

All subjects reported to the lab a total of eight or nine times. Prior to enrollment, subjects were given an overview of the study and asked to sign an informed consent approved by the University's Institutional Review Board. They were then given a physical and a 12-lead resting electrocardiogram (EKG). Depending on the subject's availability the EKG and physical screen were sometimes on separate days (leading to some subjects completing the study in 8 visits, and some taking 9). A familiarization trial occurred for the peak oxygen consumption test (VO_{2peak}) prior to performing the first VO_{2peak}trial on a cycle ergometer, with 24-48 hours between sessions. The PWC_{HRT} testing occurred on visits four and five where subjects performed four randomized workloads on two separate days. Following pre-testing, participants were randomly assigned to supplementation group A or B (beta-alanine (BA) or placebo (PL)) and underwent twenty eight days of supplementation. Following completion of supplementation, subjects returned for three post-testing days consisting of a VO_{2peak}, and two separate days of PWC_{HRT}.

Subjects

Thirty eight men and women between the ages of 18 and 31 were recruited for this study (Table 1). A total of 30 (16 women and 14 men) subjects completed the study.

Eight individuals that were enrolled were not included in the final analysis: one subject was not cleared medically, one subject had taken beta-alanine within the last 3 months, two suffered outside injuries within the pre-testing phase, and four subjects did not come back following enrollment for unknown reasons. Participants were recreationally active (accumulating 1-5hrs of exercise per week) and cleared through a physical and resting EKG. Participants were excluded if they had taken performance enhancing supplements within the previous 3 months, including beta-alanine and creatine. Before pre-testing and at the start of post-testing subjects turned in a three day food log to account for any dietary changes in consumption of non-essential amino acids throughout the duration of the study. During the study the participants were asked to maintain their normal dietary and exercise habits.

Peak Oxygen Consumption Test

Participants performed a total of three VO_{2peak} tests, two pre-supplementation and one post-supplementation, on an electronically-braked cycle ergometer (Corival Lode, Gronigen, The Netherlands) in order to determine peak oxygen uptake. Upon arrival subjects were fitted on a cycle ergometer with the handlebars adjusted to a comfortable position and the seat height adjusted so that there was no more than an approximate 15 degree bend in the knee on full extension. Heart rate (HR) was monitored throughout the test using a Polar telemetry system (Polar Electro Inc., Lake Success, NY) Pedal cadence was maintained between 60-80 rpms, with an initial power output set at 20 watts (W). The workload was increased by 1 W every 3 seconds until the participant could no longer maintain the 60-80rpm pedal cadence or volitional fatigue. Respiratory gases were

analyzed throughout the test breath by breath via open-circuit spirometry (Parvo Medics TrueMax 2400, Salt Lake City, UT) to determine $VO_{2\ peak}$. This data was averaged over 15 second increments and the highest 15 second VO_2 value elicited during the test was considered VO_{2peak} if two of the following criteria are met: 1) Either a plateau in the HR or an HR within 10% of the age-predicted max HR, 2) a plateau in VO_2 (an increase of no more than 150 $ml \cdot min^{-1}$), 3) a respiratory exchange ratio (RER) value greater than 1.15 (ACSM, 1991). The higher of the two pre-supplementation VO_{2peak} tests was recorded as the subject's VO_{2peak} in order to allow for familiarization. There were 24-48 hours between each testing session.

Assessment of the physical working capacity at heart rate threshold (PWC_{HRT})

For determination of PWC_{HRT}, participants completed four different workloads on a Lode electronically-braked cycle ergometer over a period of two days. Workloads were randomly assigned each day with two workloads being completed on each of the two days. Upon arrival, subjects were fitted with a Polar telemetry system and asked to sit passively for five minutes in order to obtain resting HR. Once fitted for seat and handlebar height, subjects completed a three minute warm-up at a self-selected pace and resistance. Following the warm-up, the subjects completed the eight minute test at one of the predetermined workloads (60% of Max Watts, 60%-20W, 60%+20W and 60%+40W) .Heart rate was recorded every 30 seconds over the first three minutes, and then every 15 seconds for the last five minutes of the test (17). Only the last five minutes of HR data were used for later computations as the first three minutes were allotted for the cardiovascular measures to stabilize. When the eight minute test was completed, the subject had a cool down at a self-selected pace. Following the cool down, participants

were asked to recover passively for 15 minutes or until they reached 10% of their post warm-up HR. The second workload was completed in a similar fashion but without a warm-up. Day two of testing occurred 24-48 hours after day one.

Supplementation

Following pre-testing, participants were randomly assigned to either the placebo group (PL: 6.4g/day, 2 tablets 4 times per day maltodextrin) or the beta-alanine group (BA: 6.4g/day, 2 tablets 4 times per day; CarnoSyn®, Natural Alternative Inc) using a double-blind placebo controlled design (Groups A and B). Placebo and beta-alanine supplements were identical in appearance and taste. Two capsules were consumed three times per day (with at least two hours between each ingestion) for 28 days. Participants were required to log daily supplement intake and check in with the study-coordinator halfway into supplementation to report consumption and side-effects. Any remaining product and dosing logs were returned at post supplementation.

Statistical Analysis

An analysis of covariance (ANCOVA) was utilized to assess PWC_{HRT} outcomes by treatment, corrected for baseline differences in the PWC_{HRT}. Four separate two-way mixed factorial ANOVAs [2×2; treatment (PL vs. BA) $\times$ time (pre vs. post)] were used to analyze VO_{2peak}, ventilatory threshold (VT) and total time to exhaustion (TTE). One three-way mixed factorial ANOVA [$4 \times 2 \times 2$; treatment (PL vs. BA) $\times$ final HR at each workload (60% VO_{2peak} vs. 60% VO_{2peak}-20W vs. 60% VO_{2peak}+20W vs. 60% VO_{2peak}+40W) $\times$ time (pre vs. post)] was used to evaluate the final HR at each of the four

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PWC workloads. Ninety-five percent confidence intervals were also used to evaluate

PWC$_{HRT}$ mean differences, final HR differences, TTE and VT using Excel (Microsoft

Office Professional Plus 2010). All statistics were run utilizing SPSS Version 20 (IBM;

Chicago, IL). An alpha level of 0.05 was set *a priori.*

CHAPTER IV

MANUSCRIPT

INTRODUCTION

Beta-alanine (BA) supplementation has been shown to significantly increase muscle carnosine levels (9, 32), thereby augmenting muscle buffering capacity (9). Previous research suggests that exercise-induced decreases in intramuscular pH, due to an accumulation of H^+, can lead to decreases in power output and a corresponding increase in fatigue onset (2). An endogenous mechanism for buffering H^+ accumulation during exercise is attributed to muscle carnosine, which assists in maintaining acid-base homeostasis by being a H^+ acceptor (9). Carnosine is synthesized in the body naturally by L-histidine and BA (4, 9, 32). Due to naturally high plasma concentration of L-histidine, BA acts as the rate limiting component of carnosine synthesis (9). Direct carnosine supplementation in humans is inefficient due to the activity of carnosinase, which immediately breaks down carnosine into its substituents upon ingestion (36, 37). Therefore, BA supplementation is supported to be the most effective way to augment muscle carnosine concentration.

Though the highest levels of carnosine lie within skeletal muscle, there are sizeable concentrations within brain and cardiac tissues (22). The majority of existing BA research has focused on peripheral contributions to fatigue (32, 38). However, there may be a need to evaluate the effect of BA on central fatigue mechanisms as well. Previous

animal data has suggested augmented carnosine concentrations within the brain can lead to significant positive changes in neurological control mechanisms (39, 40). If BA can also increase carnosine levels within the brain in humans, it has the potential to assist in delaying both peripheral and central aspects of fatigue; however this has yet to be evaluated. One indirect measure of central fatigue is heart rate as it is controlled by both central and peripheral factors (24). During exercise, an increase in H^+ and a corresponding decrease in pH can stimulate pain receptors(23). These painful afferent signals from the muscles inhibit the person's willingness/motivation to continue exercising (23). Heart rate increases correspondingly with the stress placed on the cardiovascular system. So as workload increases, so does HR and eventually fatigue onset occurs (23). In this case, peripheral factors, combined with central motivation, pain, potential sympathetic control, and oxidative stress, may lead to a non-linear increase in HR. If BA can increase carnosine levels within the brain and decrease acidosis, it has the potential to assist in delaying fatigue both peripherally and centrally.

Physical working capacity (PWC) tests have been utilized to quantify fatigue onset (12, 14, 17), as well as a tool to examine the influence of potential ergogenic aids, such as creatine (11, 15), arginine (41), and BA (10, 15, 16). The PWC test has been shown to identify a theoretical point of fatigue onset by regressing a time-distance linear relationship (35, 38). Specifically, the physical working capacity at heart rate threshold (PWC$_{HRT}$) utilizes HR as its fatigue quantifier. Wagner and Housh developed the PWC$_{HRT}$ in order to estimate a workload that can be sustained on a cycle ergometer for an extended period of time without fatigue (a steady-state HR) (17). Previous studies have shown it to be sensitive enough to assess aerobic fitness changes (18), but have also

found it to over-estimate the maximal power output that can be sustained for an hour ride without a significant increase in HR (42). A more recent study found the PWC_{HRT} to be both reliable and valid at calculating a $PWCH_{RT}$ that could be maintained over a 45 minute ride without a significant increase in HR (43).

Previous PWC tests have been used to quantify the peripheral effects of BA. In 2006, Stout et al. (10) demonstrated 28 days of BA supplementation (1.6g/day) significantly increased the PWC_{FT} of young males (15) and females (10). In a follow-up study, increases in PWC_{FT} resulted from 90 days of 2.4g BA in an elderly population (16). No studies have directly examined BA's effect on central fatigue. However, a recent study reported decreases in HR and rating of perceived exertion (RPE) during a moderate intensity 40 minute run following 28 days of BA supplementation (4.6g/day) (20). While the PWC_{HRT} cannot differentiate between the potential effect of BA on central and peripheral fatigue, an increased PWC_{HRT} following BA supplementation may indicate an influence on both central and peripheral factors of fatigue. Thus this study has the potential to determine if future studies, utilizing more sophisticated laboratory procedures, should evaluate the central effects of BA. Therefore the purpose of this study is to determine the effects of twenty eight days of beta-alanine supplementation on the physical working capacity at heart rate threshold.

METHODS

Subjects

Thirty-eight men and women were recruited for the study. Participants were excluded from the study if they had consumed beta-alanine or creatine within the previous three months, or were not approved following a physical exam. From the initial

cohort, 30 subjects completed testing and supplementation (Table 1). Eight individuals

that were enrolled were not included in the final analysis: one subject was not cleared

medically, one subject had taken beta-alanine within the last 3 months, two suffered

outside injuries within the pre-testing phase, and four subjects did not come back

following enrollment for unknown reasons.

Experimental Design

All subjects underwent an initial screening which included a resting EKG

reading, a medical history and a physical exam. If cleared, subjects underwent a VO_{2peak}

familiarization trial, and then pre- (baseline) and post-testing for PWC_{HRT} and VO_{2peak}

using an electronically braked cycle ergometer. Following pre-testing, subjects were

randomized, in a double-blind manner, to either a placebo (PL; 6.4g/day maltodextrin) or

beta-alanine (BA; 6.4g/day) group. Subjects returned to complete post-testing following

28 days of supplementation.

Determination of VO_{2peak}

Subjects were fitted with a heart rate monitor and attached to a metabolic cart

(Parvo Medics TrueMax 2400, Salt Lake City, UT) to measure expired gas samples.

Subjects maintained a cadence of 60-80 rpm with the workload starting at 20 watts of

resistance and increasing by one watt every three seconds until the required cadence

could not be maintained or until volitional fatigue (44). The VO_{2peak} was established as

the highest VO_2 value when at least two of the following criteria were met: 1) Either a

plateau in the HR or an HR within 10% of the age-predicted max HR; 2) a plateau in VO_2

(an increase of no more than 150 ml·min^{-1}); or 3) a respiratory exchange ratio (RER)

value greater than 1.15 (ACSM, 1991). Following 24-48 hours of recovery, subjects

returned for a second VO$_{2peak}$ test following the same methodology, and their second

VO$_{2peak}$ value was recorded as their peak. A familiarization VO$_{2peak}$ was included to

account for any learning effect. However, the initial two trials were still determined to be

reliable, ICC: 901; SEM: 3.64 ml·kg·min^{-1}, p=0.427.

Determination of PWC$_{HRT}$

Subjects completed four different workloads on an electronically-braked cycle

ergometer (Corival Lode, Gronigen, The Netherlands) over a period of two days. Pre-

determined workloads at percentages of max workload (MW) achieved in the higher of

the two VO$_{2peak}$ trials (60%MW, 60%-20W, 60%+20W and 60%+40W) were calculated.

Subjects reported to the lab and were fitted with a heart rate monitor. After sitting

passively for 5 minutes, their resting heart rate was recorded and they were fitted on a

cycle. Following a three minute warm-up (self-selected pace and resistance), subjects

completed an eight minute ride at one of the pre-determined workloads. Workload order

was randomized for each subject utilizing a random number generator. Heart rate was

recorded following the warm-up, every 30 seconds for the first three minutes, and then

every 15 seconds for the last five. Following a self-selected cool down, participants were

asked to recover passively for 15 minutes or until they reached 10% of their post warm-

up HR. The second workload was completed in a similar fashion but without a warm-up.

The rate of rise (slope) in heart rate as a function of time for each of the four workloads

was determined for each subject (Figure 1A). The PWC$_{HRT}$ was then calculated by

plotting the workloads as a function of the slope for the heart rate verse time relationship

(Figure 1B) (17-19, 42). The y-intercept, or point of zero slope was defined as the

PWC_{HRT}, or the predicted maximum workload that person could maintain.

Supplementation

     Subjects were given a three day food log to complete during pre-testing, prior to

beginning supplementation in order to account for their current dietary intake. Following

pre-testing, participants were randomly assigned to either the placebo group (PL:

6.4g/day, two 800mg tablets four times per day maltodextrin) or the beta-alanine group

(BA: 6.4g/day, two 800mg tablets four times per day; CarnoSyn®, Natural Alternative

Inc) using a double-blind placebo controlled design. Participants were given supplements

for 14 days and required to check-in with the primary investigator in order to receive the

other half of the supplement. Upon completion subjects returned both the dosing logs and

any leftover pills. During post-testing subjects completed a second three day food log in

order to ensure no significant dietary changes occurred that could mitigate the potential

effects of BA.

Statistical Analysis

     An analysis of covariance (ANCOVA) was utilized to assess PWC_{HRT} outcomes

by treatment, corrected for baseline differences in the PWC_{HRT}. Four separate two-way

mixed factorial ANOVAs [2×2; treatment (PL vs. BA) $\times$ time (pre vs. post)] were used

to analyze VO_{2peak}, ventilatory threshold (VT) and total time to exhaustion (TTE). One

three-way mixed factorial ANOVA [$4 \times 2 \times 2$; treatment (PL vs. BA) $\times$ final HR at each

workload (60% VO_{2peak} vs. 60% VO_{2peak}-20W vs. 60% VO_{2peak}+20W vs. 60%

42

VO$_{2peak}$+40W) × time (pre vs. post)] was used to evaluate the final HR at each of the four

PWC workloads. Ninety-five percent confidence intervals were also used to evaluate

PWC$_{HRT}$ mean differences, final HR differences, TTE and VT using Excel (Microsoft

Office Professional Plus 2010). All statistics were run utilizing SPSS Version 20 (IBM;

Chicago, IL). An alpha level of 0.05 was set *a priori.*

RESULTS

Compliance was reported and reviewed via supplementation logs. All subjects

met the minimum required dosage (4.8g/day). The average dose per day for all subjects

was 6.0 ± 0.42 g/day. There were no reported side effects in either group. Data from three

day dietary records, analyzed using The Food Processor (esha Research, Salem OR, v.

10.12), demonstrated no significant differences from pre to post-testing for caloric intake

or macronutrients (Table 2). There were no significant differences between groups

(p=0.282-0.722).

Physical Working Capacity at Heart Rate Threshold

ANCOVA results demonstrated a significant time × treatment interaction

(p=0.000; f=10.732; η$_p$^{2}= 0.479) (Table 3). Mean change scores from pre to post

supplementation with 95% confidence intervals showed a significant increase in PWC$_{HRT}$

(W) for the BA group, and a significant decrease for the PL group (Figure 2). For

individual change scores, 67% of the BA participants saw a positive change score,

whereas 60% of the PL participants saw a positive change score (Figure 5).

Final HR at Each PWC$_{HRT}$ Workload

There was no significant three-way interaction (final HR × time × treatment, p= 0.732; η_p^2= 0.016). There were no significant two-way interactions (final HR × time; p=0.238, η_p^2= 0.052; final HR× treatment; p=0.963, η_p^2= 0.004: time × treatment; p=0.574, η_p^2= 0.012). There was no main effect for time (p=0.065; η_p^2=0.125) or treatment (0=0.071; η_p^2= 0.120), there was a main effect for final HR between workloads (p=0.001 η_p^2= 0.854). Heart rate was significantly different between all workloads (p= 0.001). Each progressive workload produced significant increases in final HR (Table 1). Mean change scores with 95% confidence intervals showed significant decreases in final HR for the first three PWC$_{HRT}$ workloads for both BA and PL groups (Figure 2). The highest workload showed a significant increase in final HR for the BA group and a significant decrease for the PL group (Figure 2).

Aerobic Capacity

VO$_{2peak}$ (L/min)

There was no significant two-way interaction (time × treatment, p=0.222; η_p^2=0.053), and no main effect for time (p= 0.987; η_p^2= 0.001) or treatment (p= 0.536; η_p^2= 0.014). The mean change score with 95% confidence intervals demonstrated a significant decrease in VO$_{2peak}$ in the BA and a significant increase for PL (Figure 3).

VO$_{2peak}$ Time to exhaustion (TTE, sec)

There was no significant two-way interaction (time × treatment, p= 0.562; η_p^2= 0.012), and no main effect for time (p=0.140; η_p^2=0.076) or treatment (p=0.072; η_p^2= 0.005). The

mean change score with 95% confidence intervals demonstrated a significant increase for TTE for both BA and PL groups (Figure 4).

Ventilatory threshold (VT; L/min)

There was no significant two-way interaction (time × treatment, p=0.134; η_p^2=0.078), and no main effect for time (p= 0.422; η_p^2=0.023) or treatment (p= 0.119; η_p^2=0.084). The mean change score with 95% confidence intervals showed a significant increase in VT for PL and a significant decrease for BA (Figure 3).

DISCUSSION

The primary findings of the present study suggest that 28 days of BA supplementation may increase PWC_{HRT} in men and women. Similar results with BA supplementation have been reported, demonstrating an increase in physical working capacities in women and elderly (10, 15, 16). Most previous literature relied on EMG readings as the fatigue quantifier while the present study utilized heart rate as the fatigue variable to examine the effect of BA using a submaximal testing approach, and included both male and female participants.

The PWC_{HRT} has been shown to be sensitive to changes in aerobic fitness (18) and both reliable and valid as a measure of a HR fatigue threshold (43). The present study found significant differences between the BA and PL groups in the ANCOVA testing of the raw data measures, aligning well with previous results indicating BA's positive effect on PWC tests (10, 15, 16). This PWC_{HRT} study differs from pervious literature due to the use of HR as a fatigue variable and the submaximal nature of the protocol. The ergogenic

effects of BA on submaximal workloads have been mixed with most showing a positive effect at submaximal workloads,(10, 15, 45), but other results from a study showing no effect at submaximal intensities (11). The current study contributes to the current body of literature by demonstrating BA's positive effect on submaximal workloads of short duration. Mielke et al. also (19) previously reported similar workloads at VT, PWC_{VO2} and PWC_{HRT}; thus, the PWC_{HRT} is applicable as a training threshold that can be utilized outside of the laboratory, similar to VT.

Studies examining the effects of BA supplementation alone on aerobic measures have demonstrated no effect on VO_{2peak} (10, 11, 15); mixed results for TTE, with some showing an increase in TTE (10, 21), and others indicating no significant changes (11). Similar to previous literature, the present study found no significant treatment effect on VO_{2peak}. Due to the progressive aerobic nature of VO_{2peak}, it is most dependent on oxygen delivery and not likely to be affected by muscle buffering capabilities (46). Since BA's main effect is on muscle buffering, it is therefore not surprising that BA had no effect on the outcome. Time to exhaustion in the present study demonstrated significant positive change scores for both the BA (4%) and PL (1%) groups (Figure 4), although not significantly different from each other. Stout et al, saw an increase (2.5%) in TTE during a continuous incremental cycle test following BA supplementation (10). Other studies examining TTE however saw no significant improvements following BA supplementation (11, 45). Results from TTE demonstrate the need for more research in this area due to the conflicting results of both groups increasing TTE, and the conflicting literature available. The present study demonstrated no significant change in VT (p=0.134). This is in contrast with previous literature suggesting BA may increase VT

following 28 days of supplementation (10, 11, 15, 21). Previous research has proven BA to be effective at increasing anaerobic thresholds measured by short bouts of intense exercise (31, 32).

The results of this study indicate that twenty eight days of BA supplementation is effective at increasing PWC_{HRT} without an exercise intervention. This finding supports the concept that BA supplementation is useful for improving fatigue thresholds and thereby increasing performance at submaximal workloads. The study, however, had a few limitations. Muscle carnosine was not directly measured, so actual percent increases in carnosine content are unknown. Previous studies have shown doses of 4.8-6.4 grams/day increases carnosine concentration by 60% in 4 weeks (4, 9). It is therefore assumed that BA supplementation increased muscle carnosine concentration in the BA subjects of this study. Future research should continue to examine the effects of BA on other submaximal workloads, as the literature is lacking agreement on BA's effect on aerobic exercise.

CHAPTER V

CONCLUSION

Four weeks of supplementation with BA demonstrated significant increases in change scores for the PWC_{HRT}, TTE and VT. Additionally, final heart rates from each workload were significantly different from each other indicating that the HR slopes for each workload were significantly different. Since the PWC_{HRT} is calculated utilizing four unique HR slopes from the workloads, it is crucial for calculation that the workloads illicit different slopes. The study also is the first that used an easily reproducible methodology for workload watt determination for the PWC_{HRT}. Future research should continue to examine in more depth BA's effect on central as well as peripheral fatigue, since HR has components of both. Further studies are also needed in order to continue to validate the use of PWC_{HRT} as a viable measure of fatigue in a variety of populations. Being a submaximal measure of fatigue and minimally invasive as well as cost-efficient, it lends itself well as a field test for special populations. Finally, studies should continue to examine BA's effect on aerobic measures of fatigue. There is an abundance of research focusing BA's effect on short-term high-intensity exercise, but the present study and others indicate BA supplementation may have an effect on moderate duration submaximal exercise.

Table 1

	Age (years)	Height (cm)	Weight (kg)
BA (n=15)	20.7 ± 1.1	169.4 ± 7.7	74.5 ± 15.8
PL (n=15)	21.1 ± 2.8	171.5 ± 7.8	72.3 ± 13.1

Table 2

	BA		PL	
	Pre	Post	Pre	Post
Total Avg	2900 ± 1107	2583 ± 1099	2339 ± 920	2305 ± 863
Fat Avg	995 ±424	872 ± 470	797 ± 416	829 ± 487
Pro Avg	519 ± 279	447 ± 239	439 ± 161	396 ± 164
Cho Avg	1182 ± 473	1031 ± 402	1103 ± 548	933 ± 297

Table 3

	Pre PWC$_{HRT}$ (W)	Post PWC$_{HRT}$ (W)	Δ Final HR 60%-20W	Δ Final HR 60%	Δ Final HR 60%+20W	Δ Final HR 60%+40W
BA	138.6 ± 63.0	146.3 ± 44.9	-2 ± 9	-3 ± 10	-3 ± 6	1 ± 8
PL	142.0 ± 56.7	134.0 ± 32.5	-5 ± 6	-2 ± 11	-4 ± 8	-1 ± 7

Table 4

	Pre PWC$_{HRT}$	Post PWC$_{HRT}$	Sig.	Partial Eta Squared
BA	118.298	142.469	<0.001	0.479
PL	118.298	129.536		
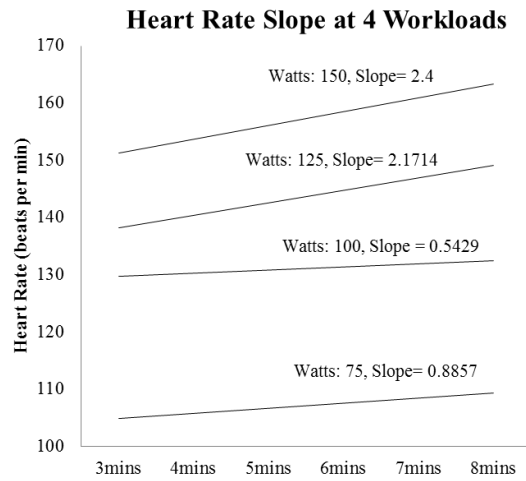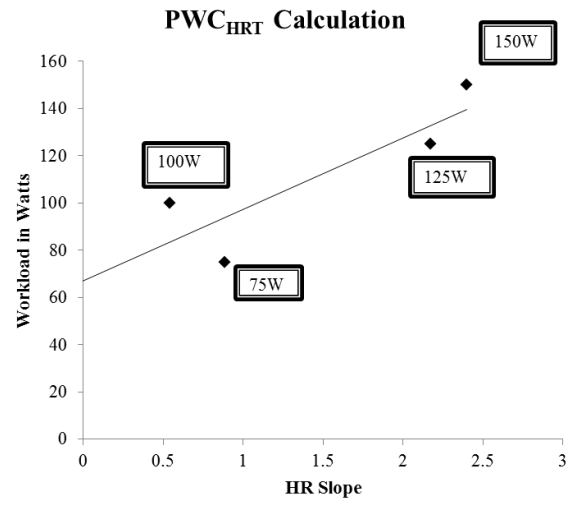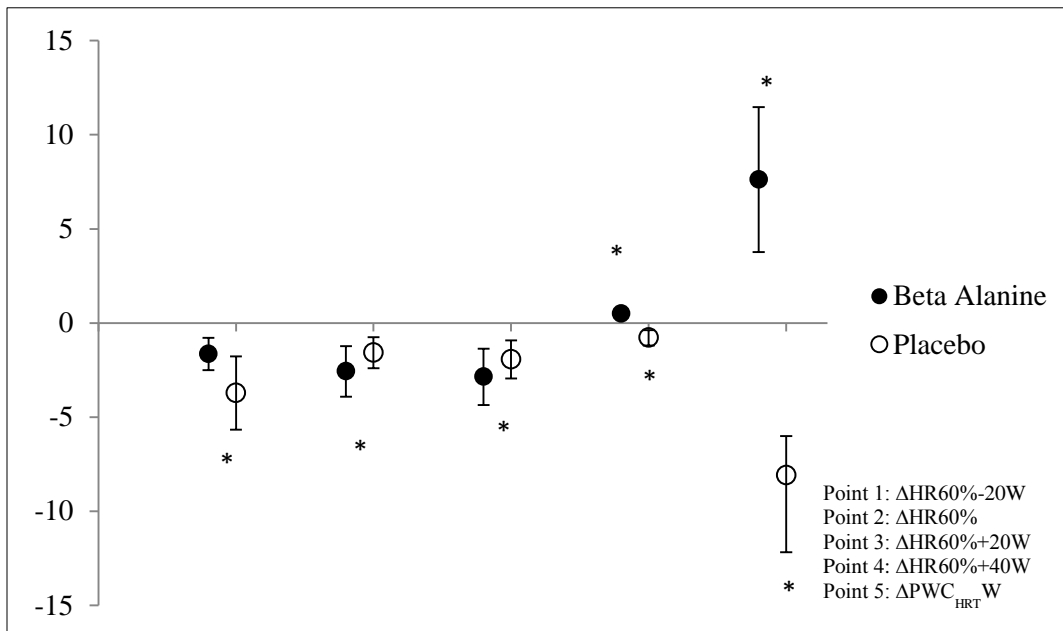
Figure 1

Figure 2

Figure 3

Figure 4

Individual BA PWC$_{HRT}$ Change Scores (W)

Individual PL PWC$_{HRT}$ Change Scores (W)

Watts

Watts

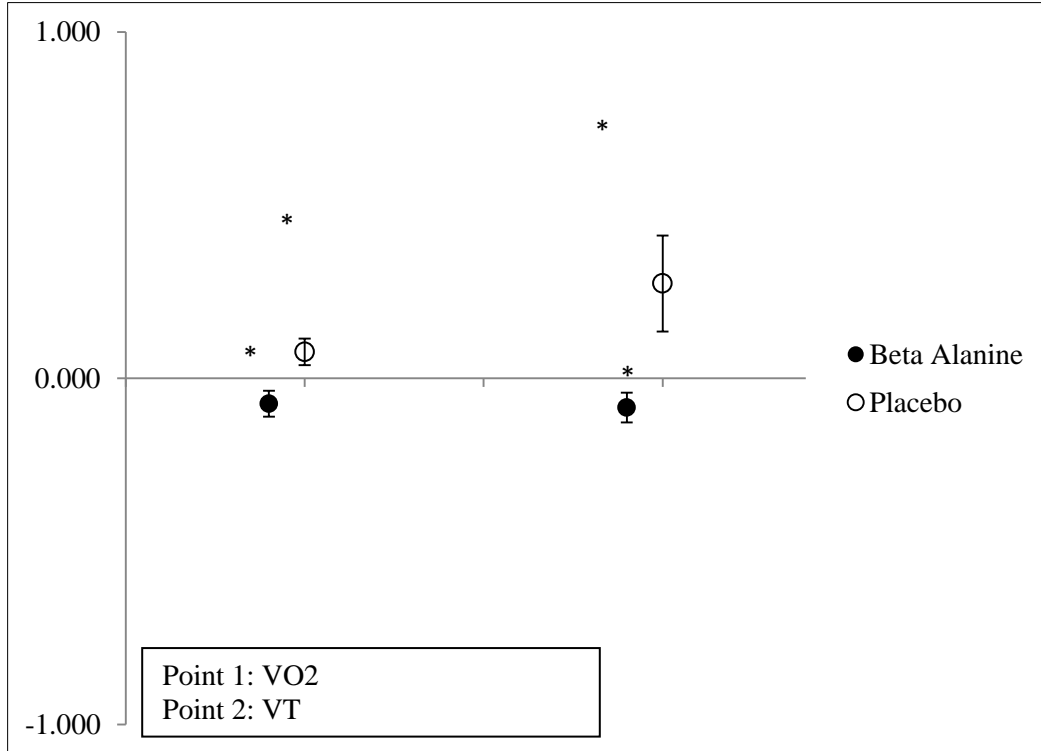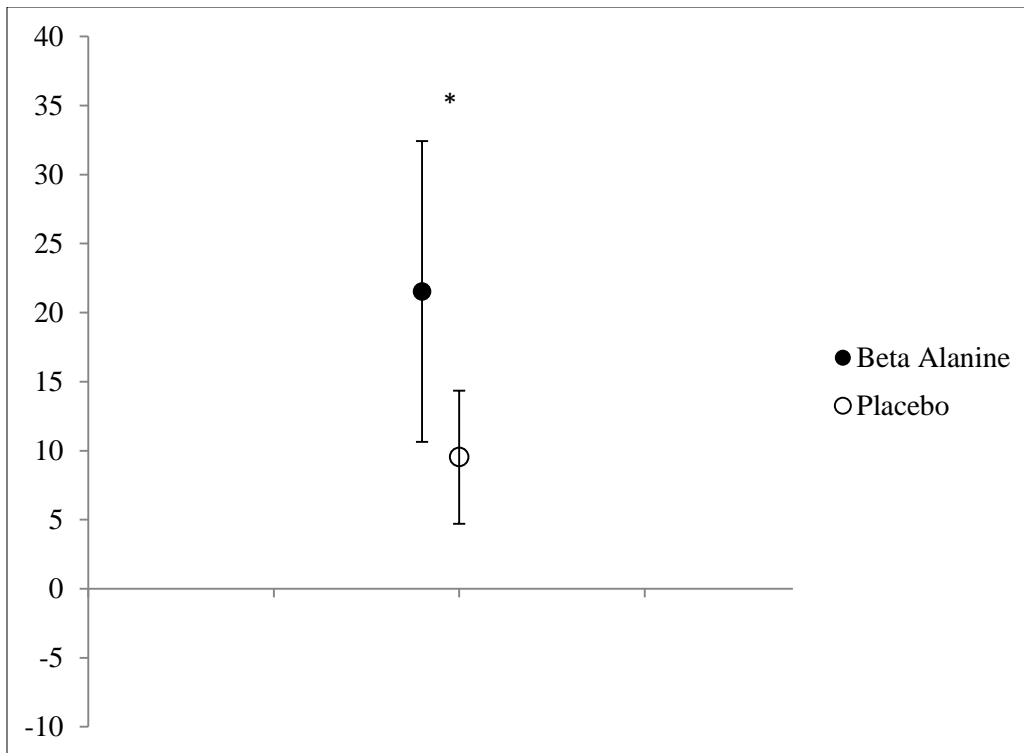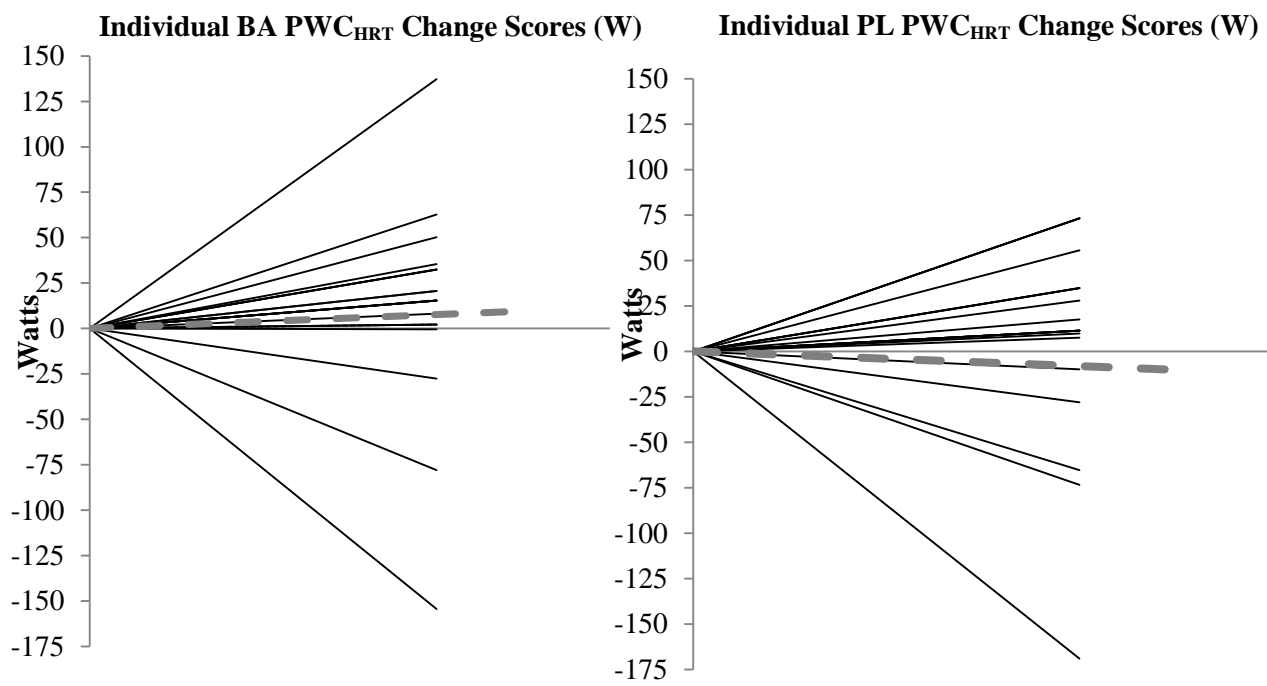**Figure 5**

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