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The Longitudinal Effects of Beta-Alanine on Punch Power, Punch Frequency, and Fatigue in
Female Amateur Fighters

A Master's Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Kinesiology

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Southern Arkansas University
Bachelors of Science in Exercise Science, 2013

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University of Arkansas

This Master's Thesis is approved for recommendation to the graduate school

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Abstract

Beta-alanine is a non-essential amino acid typically obtained in the diet via high-protein foods (beef, chicken, pork, and fish) and produced in the confines of the liver. BA is a precursor to the cytoplasmic dipeptide carnosine (beta-alanylhistidine). Carnosine is found in high concentrations in human skeletal muscle where it plays a key role in intracellular pH buffering. Research indicates BA supplementation increased punch power, punch frequency, and lactate concentrations in male amateur fighters after 28-day supplementation. There are currently no studies evaluating the effects of BA on boxing performance variables in women. Therefore, the purpose of this study was to examine the longitudinal effects of exogenous supplementation of BA on punch power, punch frequency, fatigue, and lactate accumulation in female amateur fighters. Twelve females (age = 23.25 ± 3.84) with at least three months of training volunteered to participate in this randomized double-blind study. Each participant was supplemented with either beta-alanine (800 mg + 8 g of dextrose) or placebo (8 g of dextrose) 4 times per day (at least three hours apart) over the course of 28 days. Participants took part in a simulated boxing contest at three time points (pre supplementation, day 14, and day 28). Participants completed three, 3-minute rounds of continuous punching on a punching bag, a one minute punch frequency test, and finished the trial with a 10-second maximal punch power test. Punch power, punch frequency, RPE, and fatigue were all assessed during the trials. Blood lactate measures were taken prior to exercise, immediately post exercise, and after 20-minutes of seated passive recovery. Repeated measures ANOVA revealed there were no significant differences pre- to post-supplementation on any of the boxing performance (punch power, punch frequency, and fatigue index), perceived variables (RPE), or lactate measures ($p > .05$). Previous results evaluating the effects of beta-alanine supplementation on punch power, punch frequency, and

blood lactate measure in men demonstrated significant increases for each variable. However, the results from the current study indicate that there were no significant differences in performance in female fighters.

Keyword: ergogenic aids, beta-alanine, boxing, exercise performance

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Dedication

I would like to dedicate the current research study to my mom, dad, and older sister. My parents have been my biggest supporters over the last 24 years and I know I would not be where I am today without them. To my sister who is the strongest person that I know in that she has cancer and still pushes through. I look up to your daily drive and strive to be as strong as you in everything. Your situation reminds me that things could always be worse and that you have to live every day to the fullest and without regret. For that I appreciate you for always being there for me and being my motivation.

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Introduction

Gonzalez et al. (2011) suggested both aerobic and anaerobic athletes may benefit from using pre-workout supplements because of their potential ergogenic effects and this has attracted the attention of many competitive and recreational athletes as a legal ergogenic aid. Many of the pre-workout supplements that are currently on the market are composed of different amino acids; some which have been linked to enhanced exercise and sports performance. One of the more well-known amino acids found in pre-workout supplements is beta-alanine (BA). BA is a non-essential amino acid typically obtained in the diet via high-protein foods (beef, chicken, pork, and fish) and is produced in the confines of the liver (Smith et al., 2009). Culbertson et al. (2010) suggested that BA supplementation may lead to improvements in high-intensity exercise and sport performance in a wide range of individuals.

Throughout the last two decades, a trend has developed for incorporating unconventional training methods to elicit optimum performance (Santana & Fukuda, 2011). Fighters, both amateur and professional, follow specific training programs because the sport is a combination of several styles of grappling and striking events (Amtmann, 2004). These workouts can be very strenuous and proper training can potentially change the outcome of an event. However, there are few research studies involving the fighting population (Donovan et al., 2012; Siegler & Hirscher, 2010), and data evaluating the effects of ergogenic aids on performance in this population are limited. Currently, only two studies involving sports performance supplementation in mixed martial artist (MMA) fighters and Boxers (Donovan et al., 2012; Siegler & Hirscher, 2010). Of the two studies that currently exist neither investigated the effects of the supplementation in women.

Purpose of the Study

Research has shown that BA has been linked to increases in punch power, punch frequency, and blood lactate measures in male amateur fighters after longitudinal supplementation. There are currently no studies evaluating the effects of BA on these variables in women. Therefore, the purpose of this study was to examine the longitudinal effects of exogenous supplementation of BA on punch power, punch frequency, fatigue, and lactate measures in female amateur fighters.

Hypothesis

H₁: The longitudinal exogenous supplementation of BA will increase mean punch power in female amateur fighters during three, three minute fighting rounds

H₂: The longitudinal exogenous supplementation of BA will increase punch frequency in female amateur fighters during three, three minute fighting rounds.

H₃: The longitudinal exogenous supplementation of BA will decrease fatigue in female amateur fighters during three, three minute fighting rounds.

H₄: The longitudinal exogenous supplementation of BA will decrease ratings of perceived exertion (RPE) during three, three minute fighting rounds.

H₅: The longitudinal exogenous supplementation of BA will increase the accumulation of blood lactate during three, three minute fighting rounds.

H₆: The longitudinal exogenous supplementation of BA will increase the rate of blood lactate clearance from immediately post exercise to 20 minute post exercise.

Operational Definitions

Amateur Fighter – Any person having at least three months experience in either boxing or MMA training.

Beta-Alanine - non-essential amino acid typically obtained in the diet via high-protein foods (beef, chicken, pork, and fish) and is produced in the confines of the liver (Smith et al., 2009)

Carnosine - a cytoplasmic dipeptide located in skeletal muscle tissue that acts as an intramuscular buffer of hydrogen ions (Eudy et al., 2013)

Dual Energy X-ray Absorptiometry (DXA) – radiation-based equipment used to measure the three principle components of soft tissue mass, lean mass and total body bone mineral density (Svendsen, Haarbo, Hassager, & Christiansen, 1993)

Ergogenic Aid - is a technique or practice that serves to increase performance capacity, the efficiency to perform work, the ability to recover from exercise, and/or quality of training thereby promoting greater training adaptations (Kreider, 2003)

Fatigue- the decline in the ability of an individual to maintain a level of performance (De Luca, 1983)

Mixed martial arts – full contact sport that consists of a combination of several styles of grappling and striking events (Amtmann, 2004).

Paresthesia - transient flushing and/or tingling sensations on the skin (Décombaz, Beaumont, Vuichoud, Bouisset, & Stellingwerff, 2012).

Power - defined as the rate at which mechanical work is performed; product of force and velocity (Noffal & Lynn, 2012).

Simulated Fighting Contest- three rounds each lasting three minutes in duration with one minute of rest in between (Donovan et al., 2012).

Assumptions

During the initial familiarization each participant was asked to complete a medical history questionnaire to determine if they qualified for the study. It was assumed that each participant was truthful with the answers provided. This study is applicable to only female amateur fighters that have at least three months of experience. Prior to each trial, participants were asked to refrain from vigorous exercise for 24 hours. They were also asked to refrain from consuming caffeine at least eight hours before and alcohol 24 hours prior to each testing trial. Each participant was also be asked to complete an overnight fast prior to all testing trials. It was assumed that each participant had taken all doses of the assigned supplementation consistently. At the end of each trial, questions pertaining to these variables were asked and it was assumed that each participant followed the guidelines and that they were honest with the researchers when answering these questions.

Limitations

Participants were required to self-report data multiple times this included but was not limited to health history, diet logs, and exercise logs. Failure to comply with any of the set guidelines prior to the testing can greatly skew the data. This study can only be generalized to trained female amateur fighters. The results from this study are also limited; they can only be generalized to the effects of longitudinal supplementation on muscular power and fatigue in female amateur fighters.

Delimitations

This study only involved female amateur fighters who had at least three months of fighter style training from the Northwest Arkansas area. This study included only trained female fighters between the ages of 18-35. This study also included only physically healthy individuals that were required to complete a medical history prior to the start of the data collection. The data collection for each of the participants in the study lasted 28 days.

Review of Literature

Supplementation

Over the years the importance of performance enhancing supplementation has increased in popularity. Much of this increase in performance enhancing supplementation is due to the increase in pre-workout supplements on the market. Pre-workout energy supplements continue to gain popularity among recreational and competitive athletic populations (Gonzalez, Walsh, Ratamess, Kang, & Hoffman, 2011). Gonzalez et al. (2011) found that up to 70% of adolescents and young adults have reported using at least one nutritional supplement, further research suggests that 89% of college athletes report using these pre-workout nutritional supplements (Froiland, Koszewski, Hingst, & Kopecky, 2004). These numbers indicate emphasis put on the use of sport supplementation for exercise and performance enhancement.

Supplementation research. The Federal Drug Administration (FDA) does not test the purity or potency of any performance enhancing supplement (Eudy et al., 2012). Over the last five years there have been studies examining the effects of multi-ingredient performance supplements on muscular power, muscular strength, fatigue, and lean mass (Kedia et al., 2014; Spradley et al., 2012). In a double-blind study, an over-the-counter pre-workout supplement with a multi-ingredient proprietary blend significantly improved subjective levels of energy and concentration, increased focus, and lessened fatigue (Kedia et al., 2014). Research also investigated the longitudinal effects of the pre-workout supplement Assault™ (MusclePharm, Denver, CO), with results suggesting that the supplement significantly improved muscular endurance and choice reaction time (Spradley et al., 2012). Though these particular studies did not administer the purest forms of the supplements, they results suggest that supplementation can further enhance exercise and sports performance, when compared to placebo groups.

Beta-alanine and exercise. BA has become increasingly investigated recently due to the benefits for increasing exercise performance. During exercise, lactic acid is metabolized for energy and the hydrogen (H⁺) byproducts left behind serve to increase muscular fatigue and attenuate exercise performance (Westerblad, Bruton, & Katz, 2010). BA is a precursor to the cytoplasmic dipeptide carnosine (beta-alanylhistidine), which is found in high concentrations in human skeletal muscle where it plays a key role in intracellular pH buffering (Harris et al., 1990; Smith, 1938). Carnosine is utilized during moderate to high-intensity exercise as a buffer to the increased H⁺ to maintain a homeostatic pH. This increase in buffering capacity reduces the acidity in the active muscles during high-intensity exercise (Culbertson et al., 2010). Research suggests that BA, when supplemented (eg. 4-6 g/day) increases carnosine concentration in skeletal muscle by 20%-30% after two weeks, by 40%-60% after four weeks, and up to 80% by ten weeks (Culbertson et al., 2010). This, in turn, has the ability to give athletes increased performance benefits during exercise on a variety of physiological measures. Sale, Saunders, and Harris (2010) suggested that acute supplementation of BA increases exercise performance. Their findings provide evidence to suggest that BA supplementation can sufficiently increase intramuscular carnosine concentrations, therefore enhancing exercise performance (Sale et al., 2010). This increase in the concentration of muscle carnosine has indicated improvements in performance variables such as cycling capacity, ventilatory threshold, and time to exhaustion (Culbertson et al., 2010). Through the increase in the body's intramuscular carnosine concentrations, the buffering capacity of hydrogen ions will increase, resulting in delayed fatigue and increased power output (Kern & Robinson, 2011). This suggests that increasing carnosine levels via the supplementation of BA would serve to trigger increases in physical performance for a large range of individuals. However, the increase of intramuscular carnosine levels

through the supplementation of BA does not reflect increases in performance for all variables or exercise durations. A meta-analysis of 15 studies with 57 different exercise variable measurements suggested that exercise lasting <60 seconds was unaffected by BA supplementation, exercise lasting between 60-240 seconds was the most effective timeframe, and exercise lasting >240 seconds was not as effective as the 60-240 second time frame, but still indicated performance improvements. (Hobson, Saunders, Harris, & Sale, 2012). Hobson et al. (2012) indicated that for BA to have the greatest effect on exercise performance, the exercise being performed must last a minimum of 60 seconds.

When evaluating carnosine levels it is important to understand the intramuscular differences between the different muscle fiber types. When based on single fiber analysis, compared to slow twitch fibers, fast twitch fibers have 30%-100% higher levels of carnosine (Harris, Dunnett, & Greenhaff, 1998; Hill et al., 2007; Kendrick et al., 2009). Therefore, the increase in the number of fast-twitch fibers represents an increased ability to elicit muscular power (Widrick, Trappe, Costill, & Fitts, 1996).

When supplementing BA it is important that participants ingest between 1.6 – 6.4g per day (Sellingwerff et al., 2012). Harris et al. (2006) suggested that levels of supplementation (>800mg) have been documented to cause paresthesia (tingling on the skin) lasting anywhere from 60-90 minutes. For longitudinal design, this paresthesia can be avoided by administering multiple 800mg doses (up to eight) throughout the day (Kendrick et al., 2008; Kendrick et al., 2009).

Boxing and MMA

Boxing and other fighting styles, both amateur and professional are unique from other sports. The rules do not allow for any type of nutrition, except water, to be consumed during a contest (Donovan et al., 2012). Since there are strict rules governing MMA and Boxing matches regarding nutrition. Donovan et al. (2012) suggests that any nutritional strategy or ergogenic aid designed to enhance performance must focus on nutrition hours, days, and even weeks leading up to the contest. When examining variables important for fighters, punch power, punch frequency, fatigue, and lactate measures rise to the top. Fighters must complete at least three rounds of fighting of at least three minutes each. Research suggest that a boxers anaerobic threshold is strongly related to boxing performance (Guidetti et al., 2002) and that boxers must be able to tolerate the build-up of lactate (Ghosh et al., 1995). Guidetti et al. (2002) and Ghosh et al. (1995) suggests that boxers are more likely to fatigue during contests due to the build-up of muscle acidosis, especially in the upper limbs. The fall in skeletal muscle pH, which accompanies lactic acid metabolism may impair the capacity to continually produce high force punches given that metabolic acidosis can directly or indirectly cause local muscle fatigue (Allen et al., 2008; Spriet et al., 1989). Therefore, any strategy to reduce this acidosis could play a huge role in improving fight performance (Donovan et al., 2012).

Boxing and supplementation. To date, there have only been two studies investigating sport supplementation within the fighting populations (Donovan et al., 2012; Siegler & Hirscher, 2010). The first was a double-blind (counter balanced) study examining the effects of sodium bicarbonate (NaHCO_3) ingestion prior to sparring sessions on blood pH and bicarbonate (HCO_3) levels. The subjects had baseline blood samples taken before consuming the assigned bicarbonate drink or placebo. After resting for one hour post consumption a second blood

sample was taken prior to the fighters taking part in four boxing rounds. At the completion of the four rounds the boxers gloves were removed to take the final blood sample. This study observed the potential for improved boxing performance after the ingestion of a standard 0.3g/kg load of sodium bicarbonate (Siegler & Hirscher, 2010). As hypothesized, NaHCO₃ ingestion resulted in an elevated blood buffering status prior to the boxing match, with that elevation being sustained throughout the four rounds (Siegler & Hirscher, 2010). Results indicated a significant increase in HCO₃ for the NaHCO₃ supplemented group when compared to the placebo. There were no significant differences found for blood pH.

The second study, tested the hypothesis that supplementation of BA would improve punch power, punch frequency, and lactate measures in male amateur fighters (Donovan et al., 2012). The study involved a 28 day single-blind design supplementing with BA (*n*=8) or placebo (*n*=8). Donovan et al. (2012) found that 28-day BA supplementation significantly increased punch power, punch frequency, and blood lactate measures in male amateur fighters during a simulated contest. It was reported that there was an increase of approximately 20 kg of punch force and an additional five punches in a 10 second period (Donovan et al., 2012). This study used the longitudinal design to investigate the effects of BA supplementation on punch power, punch frequency, and lactate measures. The results derived from this study suggest that a 28 day intervention of BA can increase punch power, punch frequency, and blood lactate in men. However, these data did not evaluate the effects of BA supplementation on punch power, punch frequenc, and lactate measures in women.

Methodology

Research Design

This study consisted of a randomized, placebo controlled, 28-day supplementation intervention using BA. Each of the participants was required to report to the Human Performance Lab on the University of Arkansas campus for all testing. The outcome variables of this study include performance (punch power, punch frequency, and fatigue index), perceptual (rating of perceived exertion), and physiological (blood lactate, heart rate) measures. Each trial consisted of three boxing rounds on a heavy bag, each lasting three minutes in duration. The participant also completed one minute punch frequency test and a 10-second maximal punch power test. The rest period between each of the rounds, the punch frequency test, and the maximal punch power test were one minute.

Subjects

The participants for this study were 12 female fighters (age = 23.25 ± 3.84) who participated in boxing and/or mixed martial arts training. There were 14 participants that completed testing but two had to drop out before data collection was completed. The participants for this study were required to have participated in boxing or martial arts training for at least three months.

Measures

For this study performance (punch power, punch frequency, and fatigue index), perceptual (RPE), and physiological (blood lactate, and heart rate) measures were taken. Muscular power (punch power) was measured using a TENDO weightlifting analyzer (TENDO Sport Machines, Trencin, Slovak Republic) in conjunction with the Kistler Force Plate (Kistler

Holding AG, Winterthur, Switzerland). The TENDO weightlifting analyzer was used to calculate the power, frequency, and velocity of the punches of each round. While the Kistler Force Plate calculated the participants ground reaction forces each of punch. Fatigue was calculated based on a decrease in power, expressed as a percentage using the fatigue index formula (Fatigue Index % = [Highest P (W) – Lowest P (W)/ Highest P (W)] X 100). A metronome was used and set to steady rate of 168 beats per minute during each of the rounds. This allowed the researcher to keep the number of punches between each round and each trial consistent between participants. A standardized punch combination (standard jab, standard cross) was performed during each of the repeated rounds (Donovan et al., 2012). RPE was taken after each minute of steady exercise using the Borg RPE scale. Heart rate was also assessed after each minute of exercise at the same time that RPE was taken. The heart rate was assessed with a standard heart rate monitor (Polar Heart Rate Monitor, Lake Success, NY) that was attached across the chest of each of the participants.

Procedures

Before testing procedures begin, Institutional Review Board (IRB) approval was granted. Once IRB approval was achieved recruitment of participants began. All recruitment for the study was completed out of various boxing and/or mixed martial arts gyms in northwest Arkansas (UFC Gym, TK Boxing Gym, etc.), on the University of Arkansas campus, and by word of mouth. During the initial visit, signing of the informed consent and the completion of a health history questionnaire were required to ensure all participants met the inclusion guidelines. Height, weight, body fat, and lean mass analysis were also completed during the initial visit. Height and weight was taken using a stadiometer and weight beam eye-level scale (Detecto, Webb City, MO). Body fat and lean mass were assessed using dual energy x-ray absorptiometry

(DXA, General Electric, Fairfield, CT). The initial visit was also used to answer any questions that the participants may have regarding the supplementation. Each participant also completed one of the three simulated boxing contest rounds to familiarize themselves with the procedures. All participants were instructed to keep a week-long exercise log. The exercise logs were given to the participants at baseline, two weeks, and final testing day at the completion of testing trials. The logs ensured that physical activity did not change pre- to post- supplementation. All participants also completed a diet log (two non-consecutive weekdays, and one weekend day) at the beginning of testing, at the two week mark of testing, and at the end of testing. This allowed researchers to assess whether changes in performance could have been due to a change in the participants diet. During the supplementation period, participants were randomly assigned one of the two supplementations of either BA (BA = 800mg + 8g Dextrose) or placebo (8g Dextrose). Participants were instructed to take the supplementation four-times per day (at least three hours apart) throughout the 28-day period. A simulated fighting contest was performed by the participants at baseline, two weeks, and on week four to measure punch power, punch frequency, and fatigue. This testing allowed for researchers to assess the increases between baseline, midpoint, and end of the 28-day supplementation. All testing sessions were scheduled to be chronobiological (± 1 hour) and on the same day each week. During each testing trial blood lactate measures were assessed via finger stick prior to exercise, immediately post exercise, and 20minutes post exercise. These were performed to assess lactate accumulation during exercise and clearance after 20 minutes post completion of the simulated boxing contest. The DXA scan was assessed at baseline then again at week four to track changes in body composition. To assess punch power, punch frequency, and fatigue in this study researchers used the TENDO Weightlifting Analyzers and the Kistler Force Plate. The participants

completed three; 3-minute simulated fighting rounds, a one minute punch frequency testing, and a 10-second maximal power test on a 70lb Training bag (Everlast Worldwide, Bronx, NY). A metronome was used during the three rounds and was set to 168 beats per minute. The participants were instructed to punch and be in contact with the punching bag on each beat. After each minute of the three rounds RPE and heart rate were taken. After the third round the participants then completed a one minute punch frequency test. The participants were asked to throw as many punches as they could in one minute. Punches were calculated using a handheld clicker and were assessed by the principle investigator. After the punch frequency test each participant was asked to complete a 10 second maximal punch power test after one minute of rest. The participants were instructed to throw the same standard punching combination (standard jab, standard cross) as hard as they could for ten seconds. Punch power was found using the Watt formula ($W = N \cdot m/s$). The average velocity (m/s) was taken from the TENDO weightlifting analyzer at 18 different time points throughout each of the three minute rounds or every 14 punches. Researchers then took the ground reaction force (N) every ten seconds from the force plate to calculate the watts that are being produced by the punching in the simulated boxing contest. The ground reaction force represented the force that is being produced from the contact of the back foot on the force plate and is based off of the participant's body weight and dominant boxing hand. The standard fatigue index formula was used in assessing fatigue. The fatigue index was calculated as the percent decrease in power from the highest power to the lowest power (Beams & Adams, 2010), and was expressed as a percentage.

Data Analysis

The independent variable for this study were the supplementation ingested. The dependent variables were the changes for all performance, perceptual, and physiological

measures. In order to assess the effects of supplementation on performance, perceptual, and physiological variables between-trials, researchers used repeated measures analysis of variance (ANOVA). To evaluate supplement effects within-trials, one-way repeated measures ANOVA were used. All data were presented as mean \pm standard error ($\alpha < .05$).

Results

There were no initial differences between the BA and PLA on demographics (Table 1) and diet analysis data (Table 2).

Boxing Performance

Punch power. When comparing the jab and cross highest punch powers pre to post supplementation there were no significant differences between the BA or placebo groups. For the cross, neither the time ($p = .22$) nor the time x group interactions ($p = .53$) were significant (Figure 1). Similar non-significant results were found with the jab for time ($p = .34$) and time x group interactions ($p = .37$) (Figure 2). In trial one the placebo group's punch power on the cross was 155.55 ± 91.98 watts compared to 134.03 ± 50.93 watts for the BA group ($p = .63$). After the 28-day supplementation the placebo group's punch power was 205.98 ± 100.72 watts compared to the BA groups 152.30 ± 48.77 watts ($p = .31$). When assessing the jab in trial one, the placebo group had a punch power of 138.37 ± 79.42 watts compared to the BA groups 122.32 ± 44.97 watts ($p = .70$). Post supplementation the placebo group had a punch power of 140.34 ± 41.95 watts compared to the BA groups 196.23 ± 162.60 watts ($p = .40$). There was a moderate effect size between the BA and the placebo groups for highest punch power after the 28-day supplementation for both the cross (0.65) and the jab (0.50). There were no significant differences between the BA and PLA for punch power percent change from pre to post 28 day supplementation on time ($p = .96$) and time x group ($p = .35$) interactions (Figure 3). For the cross, the BA group had a change of 0.22 ± 54.68 percent for highest punch power while the placebo group changed 20.53 ± 25.74 percent ($p = .48$). Between trials one and three on the jab, the BA group had a change of -20.81 ± 31.48 percent for highest punch power compared to 5.00 ± 34.30 percent for the placebo.

Punch frequency. There were no significant differences between the BA or placebo groups on punch frequency, based off of time ($p = .94$) and time x group interactions ($p = .64$) (Figure 4). For trial one, the BA group had a mean punch frequency of 294.20 ± 25.90 punches in the one minute time period when compared to the placebo groups 276.33 ± 13.31 punches ($p = .17$). After the 28-day supplementation, the BA group had a mean punch frequency of 293.83 ± 32.70 punches when compared to the placebo groups 279.50 ± 25.67 punches ($p = .42$). There was a large effect size of 0.85 prior to supplementation and a moderate effect size of 0.49 after the 28-day supplementation between the BA and placebo groups for punch frequency.

Perceptual Variables

Ratings perceived exertion. RPE was taken during the final minute of the final round for both trial one and trial three. There were no significant differences between the BA and placebo groups from pre to post supplementation on RPE, based off of time ($p = .13$) and time x group interactions ($p = .25$) (Figure 5). Prior to supplementation, the BA group reported a mean RPE of 14.83 ± 2.64 compared to 15.00 ± 1.67 for the placebo group ($p = .90$). Post supplementation the BA group reported a mean RPE of 14.67 ± 2.16 with the placebo group reporting a mean of 13.92 ± 2.49 ($p = .59$). There were also no significant differences found between the BA and the placebo groups on the change in RPE from pre to post supplementation. The BA group had a mean change of -0.17 ± 0.75 compared to the placebo groups mean change of -1.08 ± 1.69 ($p = .26$).

Physiological Variable

Fatigue index. There were no significant differences between the BA or placebo groups for fatigue index neither the cross nor the jab. This was based off of time ($p = .32$) and time x

group interactions ($p = .64$) for the cross (Figure 6) and the time ($p = .25$) and time x group interactions ($p = .30$) for the jab (Figure 7). Prior to supplementation, the BA group showed a mean fatigue percentage of 41.06 ± 21.04 compared to the placebo's 47.33 ± 9.68 ($p = .56$). After the 28-day supplementation, the BA groups mean fatigue percentage of 30.39 ± 7.46 for the cross compared to the placebo groups 41.22 ± 14.33 ($p = .16$). Prior to supplementation, the placebo groups fatigue percentage for the jab was 41.92 ± 5.14 when compared to the BA groups 43.33 ± 13.44 ($p = .83$). After the 28-day supplementation, the placebo groups fatigue percentage was 40.70 ± 6.69 when compared to the BA groups 34.09 ± 11.08 ($p = .25$). There was a high effect size between the BA and placebo groups for the percentage of fatigue for both the cross (0.86) and the jab (0.72) after the 28-day supplementation.

Blood lactate. There were no significant differences between the BA or placebo groups on blood lactate concentration both pre and post exercise. Based off of a time ($p = .83$) and time x group interactions ($p = .40$) prior to exercise, and time ($p = .53$) and time x group interactions ($p = .35$) immediately post exercise (Figure 8). In trial one it was found that prior to exercise the BA group had a mean blood lactate of 2.10 ± 1.35 mmol/L compared to the placebo groups $2.05 \pm .63$ mmol/L. It was found that the BA group had post exercise blood lactate of 9.00 ± 2.63 mmol/L compared to the placebo groups 7.17 ± 2.66 mmol/L ($p = .28$). After the 28 days of supplementation, prior to exercise the BA group had a mean blood lactate of 2.32 ± 1.05 mmol/L compared to the placebo groups $1.83 \pm .82$ mmol/L. The BA groups post exercise blood lactate was 8.63 ± 2.94 mmol/L while the placebo group had a post exercise lactate of 8.08 ± 3.73 mmol/L ($p = .78$). When comparing pre to post exercise blood lactate measures from baseline to post 28-day supplementations there were no significant differences between the BA or placebo groups (Figure 9). Prior to supplementation the BA group had a change pre to post trial of 6.90

± 2.80 mmol/L compared to the placebo groups 5.11 ± 3.24 mmol/L ($p = .35$). After 28 days of supplementation, the BA group reported a change pre to post trial of 6.31 ± 2.63 mmol/L compared to the placebo groups 6.25 ± 3.86 mmol/L ($p = .97$). These values were found by subtracting the pre exercise lactate measure from the post exercise lactate measure. There were also no significant differences between groups on the rate of lactate clearance on time ($p = .24$) and time x group interactions ($p = .99$) (Figure 10). Lactate clearance was measured from immediately post exercise to post 20-minute passive seated recovery. When comparing the rate of clearance from trial one to trial three the BA group's blood lactate clearance in trial one was 5.20 ± 2.70 mmol/L compared to the placebo groups 3.33 ± 2.07 mmol/L ($p = .23$). Post supplementation the BA groups blood lactate clearance was 3.80 ± 2.20 mmol/L and the placebo group's rate of clearance was 3.78 ± 4.03 mmol/L ($p = .99$). There was a large effects size of 0.75 between the BA and placebo groups rate of clearance prior to any supplementation being administered.

Discussion

The aim of the current study was to investigate the effects of 28-day supplementation of BA on punch power, punch frequency, fatigue, RPE, and blood lactate measures in trained female fighters. Researchers utilized a supplementation protocol of 3.2 grams per day of a free powder form of BA. Over the course of 28 days 89.6 grams of BA were consumed by the participants. In previous research it was found that BA supplementation increased mean punch power, punch frequency, and blood lactate concentrations in men when compared to the placebo group. This is the first study evaluating the effects of BA supplementation on sports-specific combat related variables in women in a double-blinded fashion. In the current study there were no significant differences between the BA or placebo groups on punch power, punch frequency, RPE, fatigue, or blood lactate concentrations pre to post supplementation.

Hobson et al. (2012) reported from a meta-analysis of 15 published studies that BA had a median overall positive performance effect of 2.85%. These studies included a wide range of exercise modes such as cycling (Hill et al., 2007; Van Thienen et al., 2009), rowing (Baguet et al., 2010), knee extensor exercise (Derave et al., 2007; Kendrick et al., 2008), and previously boxing performance in men (Donovan et al., 2012). It has been observed that exercise enhancement via the supplementation of BA is especially evident during exercise lasting between 60 – 240 seconds (Hobson et al., 2012). The exercise performed in the current study followed these recommendations in that each round of the simulated boxing contest lasted 180 seconds. However, there were no significant differences found between the BA or placebo groups on any of the boxing performance, perceptual, or physiological variables assessed in women.

The main mechanism by which BA increases exercise performance is via the increase of intra-muscular carnosine concentrations in both type I and type II muscle fibers (Harris et al., 2006). This, in turn, enhances muscle buffering capacity and reduces acidosis in the active muscle. Through this process BA supplementation improves the capacity for glycolytic energy production given that intra-muscular acidosis may impair exercise capacity (Donovan et al., 2012). Previous research indicated that 28-day BA supplementation increased punch power, punch frequency, and blood lactate concentrations in male amateur fighters (Donovan et al., 2012). Results from the current study oppose these results in that there were no significant differences found for punch power, or punch frequency in women. This was the first study evaluating the effects of 28-day BA supplementation on RPE and percentage of fatigue in combat style sports. Results from the study showed that there were no significant differences found for either RPE or percentage of fatigue pre- to post- supplementation in female fighters. It was also observed in the previous research that BA increased blood lactate concentrations from pre to post supplementation while there was no change in the placebo group (Donovan et al., 2012). The current study showed that there was no significant difference on blood lactate concentrations in females from pre to post supplementation. However, it is difficult to make accurate conclusions based off of blood lactate, especially considering that other researchers (using cycling as the exercise modality) have observed post-exercise blood lactate concentration to be unaffected by BA supplementation (Sale et al., 2011; Van Thienen et al., 2009). However, the current study utilized upper limb exercise and it is known that arms exhibit greater contractile-induced glycogen utilization and increased lactate release when compared to the legs (Ahlborg et al., 1991).

When comparing the previous study evaluating boxing performance in men (Donovan et al., 2012), and the current study evaluating boxing performance in women there are many differences. For the current study a dosing protocol of 3.2 grams per day was administered to the participants over the 28-day supplementation. For the study in men a dosage of 6 grams per day was administered to the participants (Donovan et al., 2012). This allows researchers to suggest that the higher dosage (6 grams per day) from the previous study increased carnosine to a higher degree than the smaller dosage (3.2 grams per day) used for this study. This could be attributed to men having naturally higher baseline carnosine concentrations in conjunction with the higher dosing protocol used in the previous study. The training status of the individuals involved was also varied between the two studies. In the previous study in men each participant had at least six years of experience, and were considered to be an elite amateur boxer (Donovan et al., 2012). In the current study the participants were required to have at least three months of previous boxing or mixed martial arts experience, and training status of these individuals varied. The BA group had 4.94 ± 6.14 years of boxing or MMA training compared to the placebo groups 1.24 ± 1.38 years ($p = .18$).

Donovan et al. (2012) found that 28-day BA supplementation significantly increased boxing performance variables in men. Based off of these results it was hypothesized that BA supplementation would increase similar boxing performance variables in females. Physiological differences between men and women show that women have naturally lower levels of intramuscular carnosine (Everaert et al., 2011). Therefore, women would require lower levels of BA supplementation to obtain similar results men do for exercise performance from increased intramuscular carnosine concentrations (Stegen et al., 2014). A limitation to this investigation is the lack of invasive measurements that would provide insight as to the potential mechanism for

performance enhancement, particularly when looking at intra-muscular carnosine concentrations (Donovan et al., 2012). Discrepancies in the literature regarding the magnitude of increased intra-muscular carnosine from BA supplementation is likely due to differing biochemical methods used to assess muscle carnosine, duration of supplementation, dose administered, and training status of the individuals (Donovan et al., 2012; Sale et al., 2010). Based off of the results from this study it can be hypothesized that though intra-muscular carnosine concentrations may have potentially been increased, they were not increased enough to yield significant performance enhancement on the boxing performance, perceptual, and physiological variables in the female fighters.

In conclusion future research should focus on the administration of a greater dose of BA supplementation in women, most likely of slow release capsule form. It could also be valuable to look at the effects of BA supplementation in females that have a higher training status than those represented in the current study. Future studies employing invasive measurement of intra-muscle carnosine concentrations could be beneficial in determining the benefits of BA supplementation, and the magnitude that which BA supplementation can increase intra-muscular carnosine concentrations.

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Tables and Figures

Table 1
Demographics Data

Group	Age (years)	Height (cm)	Weight (kg)	Fighter experience (years)	Exercise (days/week)
BA (n=6)	24.33 ± 3.67	164.11 ± 8.14	58.57 ± 7.91	4.94 ± 6.14	5.83 ± .98
Placebo (n=6)	22.17 ± 4.02	168.20 ± 9.03	63.96 ± 15.18	1.24 ± 1.38	4.83 ± 1.47
p-value	.35	.43	.46	.18	.20

Note. Data are presented as mean ± SE. There were no initial differences found between BA and PLA. Significance was set at $p < 0.05$.

Table 2
Diet Log Analyses

Subjects	Total Kilocalories	Protein	Carbohydrates	Fats
BA (n=6)	1611.36 ± 202.57	74.51 ± 19.86	176.54 ± 40.77	65.20 ± 19.41
Placebo (n=6)	1537.86 ± 423.49	73.25 ± 25.49	177.51 ± 69.49	61.75 ± 24.46
p-value	.71	.93	.98	.79

Note. Data are presented as mean ± SE. There were no initial differences found between the BA and PLA. Significance was set at $p < 0.05$.

Figure 1
Punch Power Cross

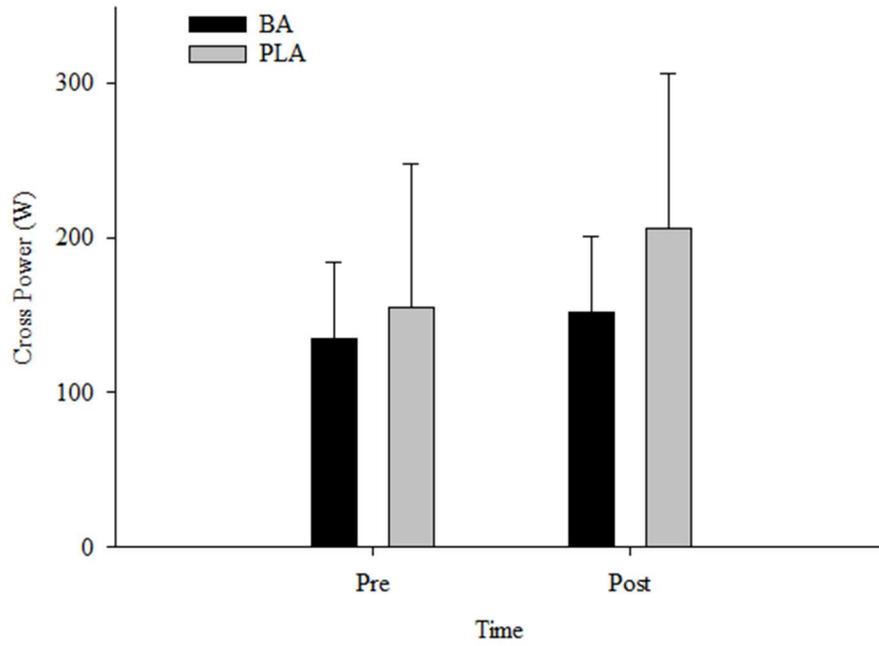


Figure 2
Punch Power Jab

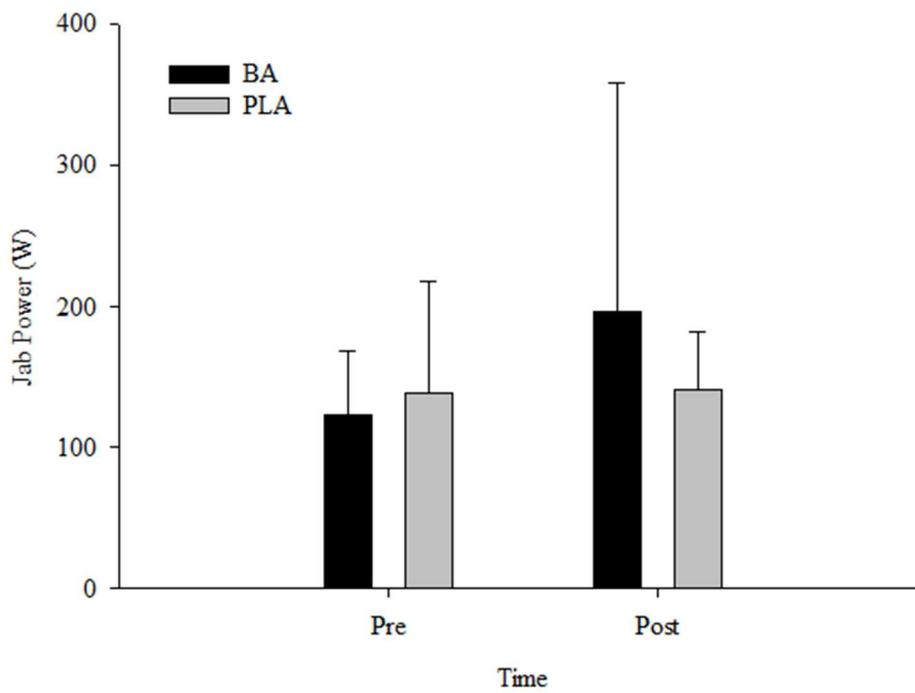


Figure 3
Percent Power Change

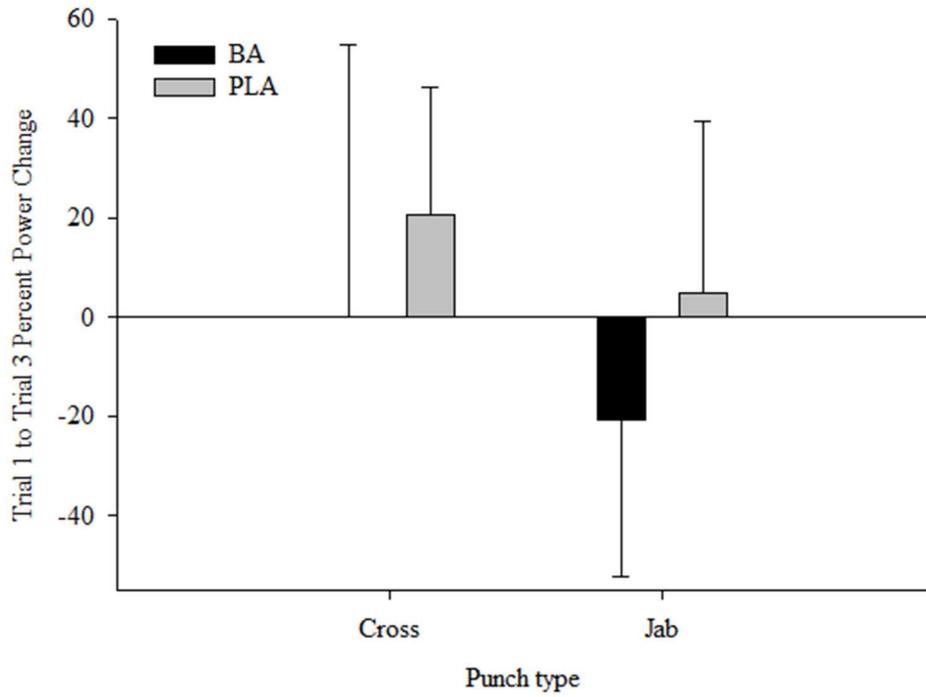


Figure 4
Punch Frequency

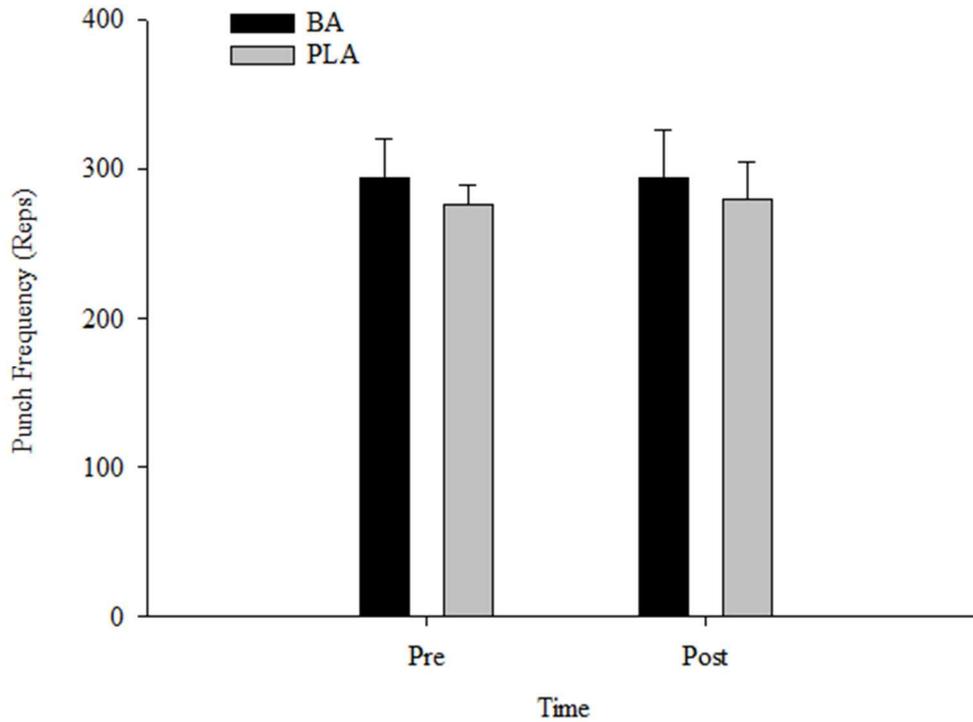


Figure 5
RPE

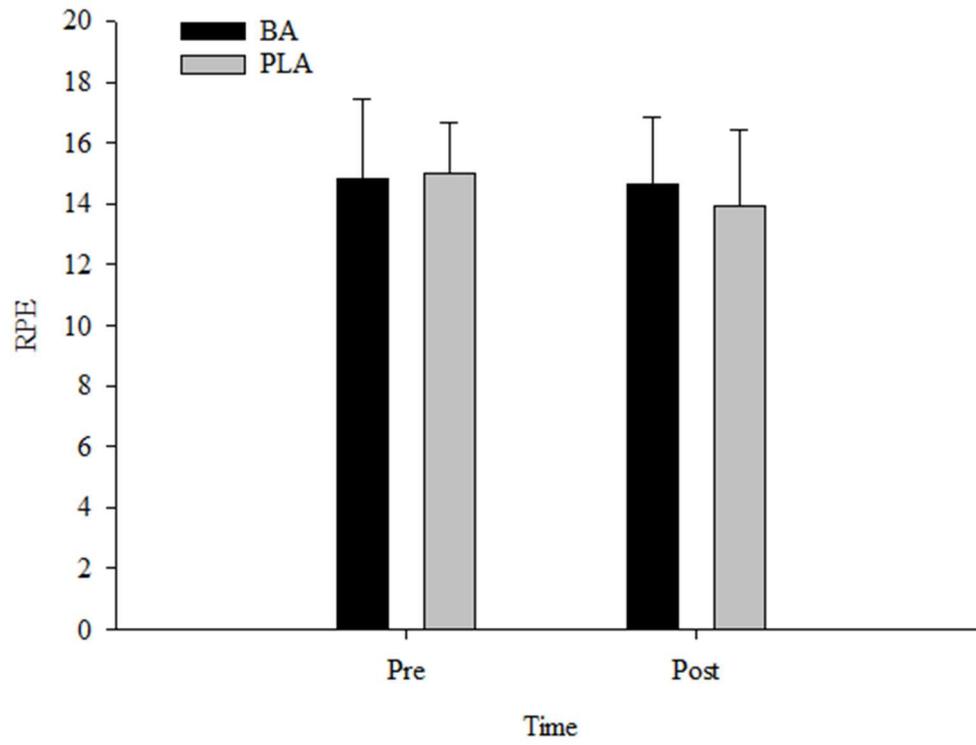


Figure 6
Fatigue Index Cross

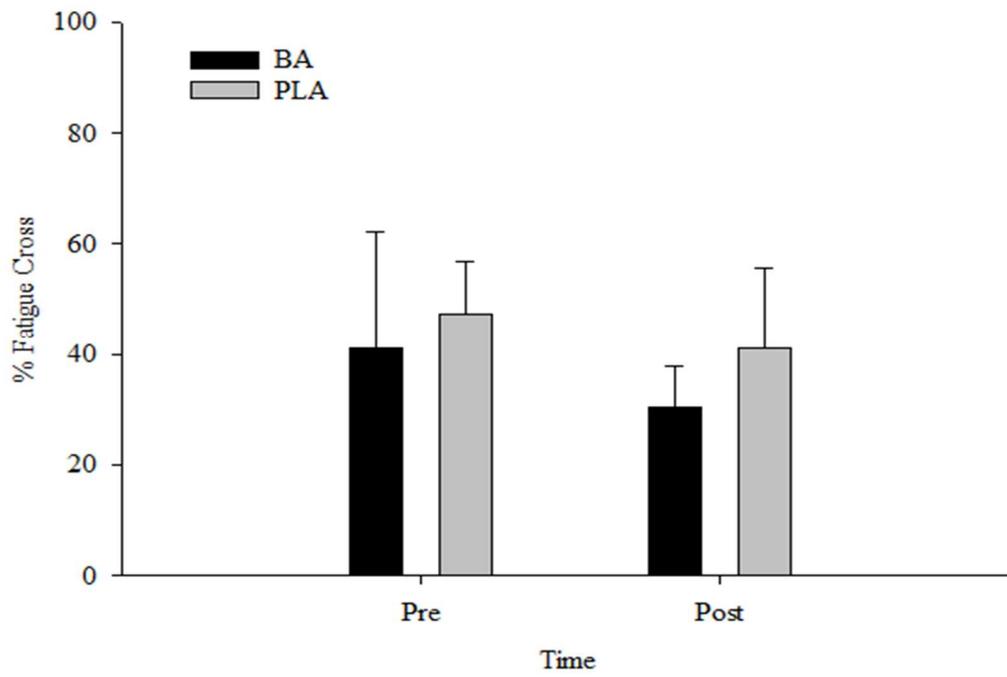


Figure 7
Fatigue Index Job

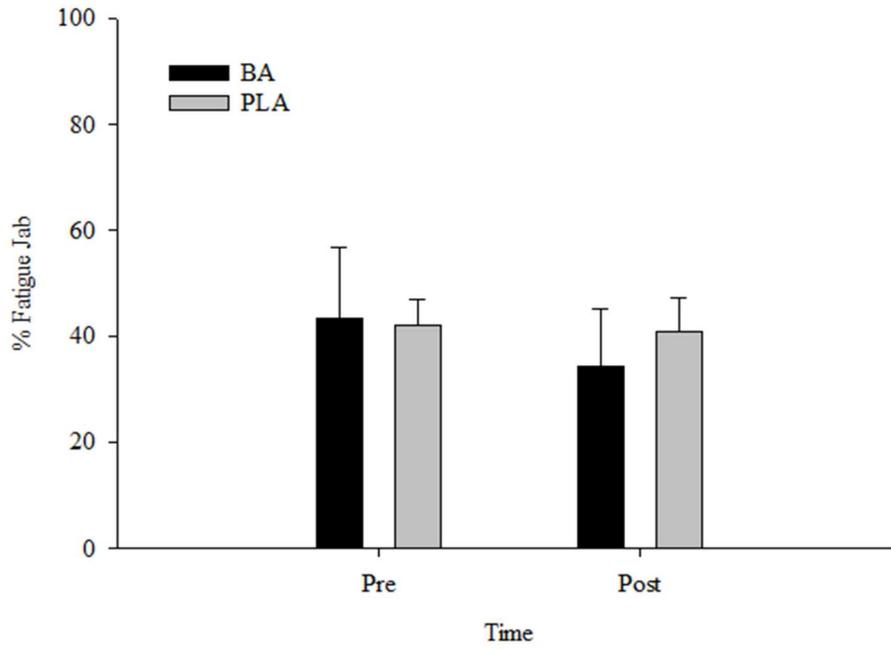


Figure 8
Post Exercise Lactate Concentrations

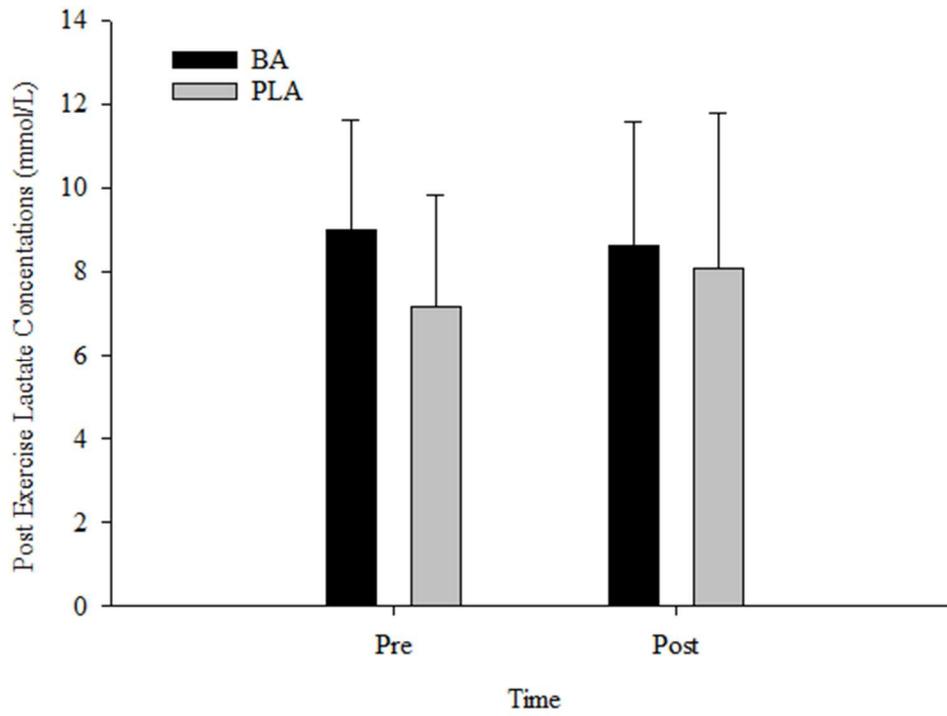


Figure 9
Lactate Change within Trial

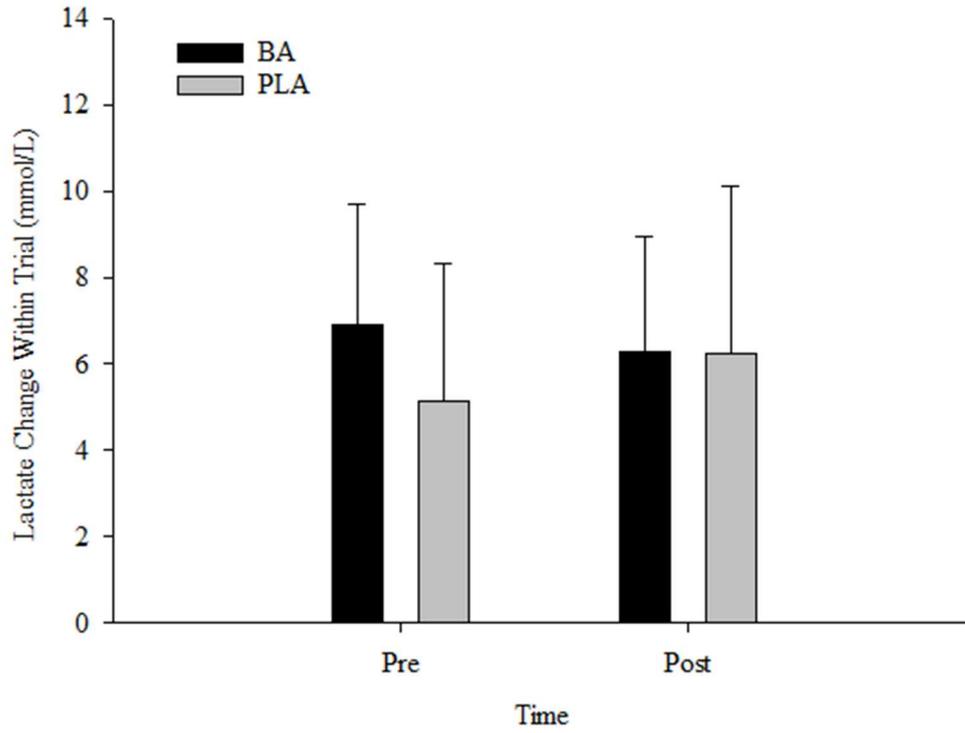
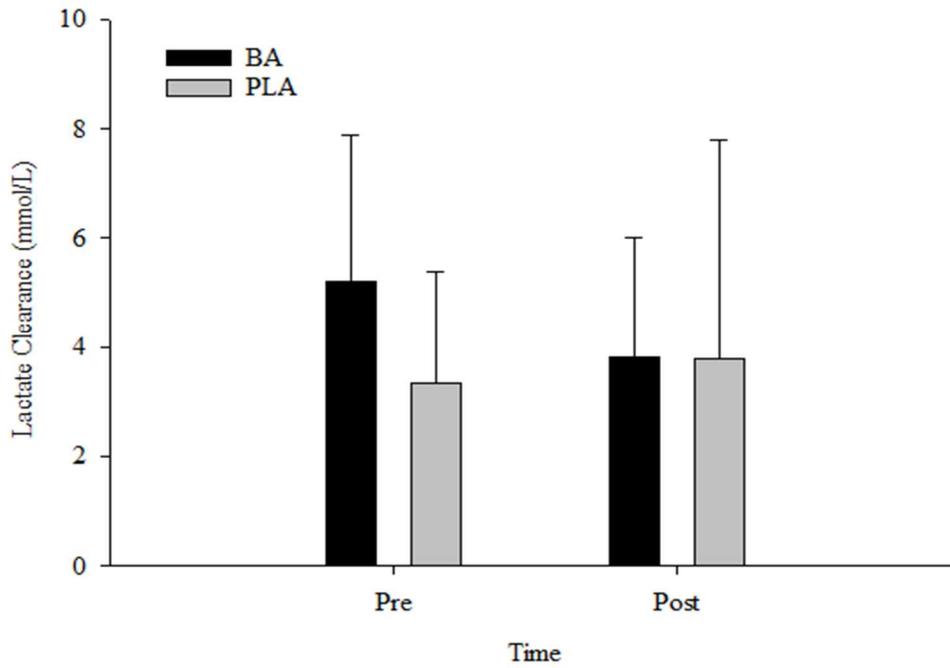


Figure 10
Lactate Clearance



Appendix



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October 14, 2014

MEMORANDUM

TO: Rodger Stewart
Jordan Glenn
Michelle Gray

FROM: Ro Windwalker
IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 14-09-129

Protocol Title: The Longitudinal Effects of Beta-Alanine on Punch Power, Punch Frequency, and Fatigue in Female Amateur Fighters

Review Type: 0 EXEMPT 0 EXPEDITED 1 FULL IRB

Approved Project Period: Start Date: 10/14/2014 Expiration Date: 10/09/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (<http://vpred.uark.edu/210.php>). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 60 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.