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The Longitudinal Effects of Beta-Alanine Supplementation on Isometric Strength, Time to Exhaustion, and Lower-Body Isometric Torque in Female Masters Athlete Cyclists

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The Longitudinal Effects of Beta-Alanine Supplementation on Isometric Strength, Time to Exhaustion, and Lower-Body Isometric Torque in Female Masters Athlete Cyclists

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctorate of Philosophy in Kinesiology

by

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Abstract

Within the population of aging individuals exists a subset of competitive seniors or masters athletes (MA). As masters-level competition increases in popularity, MA must find methods to enhance individual athletic performance. Beta-alanine (BA) is an amino acid used to enhance physical capability based on ability increase intramuscular carnosine concentrations. Older adults and females have naturally lower carnosine levels compared to age- and gender-matched counterparts and may experience enhanced benefits from BA supplementation. **Purpose:** Therefore, the purpose of this investigation was to examine the longitudinal effects of BA supplementation on isometric handgrip strength (HG), time to exhaustion (TTE), total work completed (TWC), and lower-body isokinetic torque (ISO) in female MA. **Methods:** Twenty-two female MA (age = 53.3 ± 1.0) participated in this double-blind design. Subjects were randomly assigned to BA ($n = 11$; 800mg BA + 8 g dextrose) or placebo (PLA; $n = 11$; 8 g dextrose) groups and supplemented 4 times/day over 28 days. HG, TTE, TWC, and ISO were assessed at baseline and each week throughout the intervention. Blood lactate was measured at baseline, immediate post, and 20-minutes after recovery from TTE. **Results:** No initial significant differences existed between groups for any variable ($p > .05$). By the 28th day, TTE (23% vs 1% change) and TWC (21% vs 2% change) significantly increased in BA compared to PLA ($p < .05$). Lactate clearance rate also significantly increased with BA having 24% greater reductions from peak values after the 20-minute recovery. For ISO, work done during the final third (24.0% vs -16.8% change) and average peak torque (5.4% vs 2.9% change) significantly increased in BA compared to PLA ($p < .05$). When comparing HG and body composition, no significant differences existed at any time point between the two groups ($p > .05$). No differences existed for any variable during intermittent time points. **CONCLUSION:** Four-

weeks of BA supplementation increased exercise performance and lactate clearance in female MA potentially due to increases in intramuscular carnosine concentrations. Future research should evaluate mechanistic properties influencing these factors as carnosine concentrations can only be evaluated via muscle biopsy analysis or proton magnetic resonance spectroscopy.

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Dedication

This dissertation is dedicated to my mother.

After all these years, we finally got the puffy sleeves.

Table of Contents

Introduction.....	1
Purpose of the Study	3
Research Hypotheses	3
Limitations	3
Definition of Terms.....	5
Significance of the Study	6
Review of the Literature	7
Introduction.....	7
Older Adults.....	8
Physiology of Aging	8
Masters Athletes.....	10
Muscular Strength Performance	12
Aerobic Performance	14
Anaerobic (Sprint and Power) Performance	16
Ergogenic Aids.....	18
Beta-Alanine	19
Carnosine and Exercise.....	21
Special Populations.....	22
Beta-Alanine Dosage	25
Conclusion	26
Methodology	27
Introduction.....	27

Subjects	27
Experimental Design.....	28
Controls.....	29
Supplementation Protocol.....	29
Aerobic Capacity Testing Protocol (VO_{2peak}).....	30
Handgrip Testing (HG).....	30
Time to Exhaustion (TTE) Protocol and Total Work Completed (TWC).....	31
Isokinetic Lower-Body Exercise Assessment (ISO).....	32
Blinding Efficacy and Side Effects.....	32
Statistical Analyses	32
Results.....	34
Controls.....	34
Performance Variables.....	35
Time to Exhaustion.....	35
Total Work Completed	35
Isokinetic Lower-Body Exercise.....	36
Handgrip Strength.....	39
Physiological Variables	40
Lactate.....	40
Body Composition	41
Blinding Efficacy and Side Effects.....	41
Discussion.....	43
Time to Exhaustion and Total Work Completed	43

Isokinetic Strength Evaluation	45
Isometric Handgrip Strength.....	47
Lactate Accumulation and Clearance	47
Limitations	48
Conclusion	50
References.....	51
Appendices.....	66
Appendix A: Institutional Review Board Approval Document.....	66
Appendix B: Informed Consent	68
Appendix C: Health History Questionnaire	71
Appendix D: VO _{2peak} Data Collection Sheet.....	74
Appendix E: Time to Exhaustion Data Collection Sheet.....	76
Appendix F: Subject Weekly Training Log.....	78
Appendix G: 3-day Subject Diet Log and Instructions.....	80
Appendix H: Beta-alanine certificate of authenticity provided by third party lab test.....	88

List of Tables

1. Table 1. *Initial subject demographic data* – pg. 28
2. Table 2. *Aerobic capacity between groups* – pg. 34
3. Table 3. *Dietary intake values for supplementation groups* – pg. 35
4. Table 4. *Performance during the lower-body isokinetic strength evaluation* – pg. 37
5. Table 5. *Handgrip strength between groups through the 4-week supplementation intervention* – pg. 39
6. Table 6. *Body composition between groups after supplement intervention* – pg. 41

List of Figures

1. *Figure 1a*. Time to exhaustion and *b*. Total work completed between beta-alanine (BA) and placebo (PLA) groups after 4 weeks of supplementation – pg. 36
2. *Figure 2a*. Average peak torque and *b*. total work completed during the final third of exercise between beta-alanine (BA) and placebo (PLA) groups after 4 weeks of supplementation – pg. 38
3. *Figure 3*. Lactate rates 20 minutes after completion of time to exhaustion evaluation between beta-alanine (BA) and placebo (PLA) groups after 4 weeks of supplementation – pg. 40

List of Abbreviations

1. MA – Masters Athletes
2. USSG – United States Senior Games
3. BA – Beta-alanine
4. PLA – Placebo
5. TTE – Time to Exhaustion
6. TWC – Total Work Completed
7. HG – Isometric Handgrip Strength
8. ISO – Isokinetic Lower-body Strength Evaluation
9. DXA – Dual-energy X-ray Absorptiometry
10. VO_{2peak} – Maximal Cycling Aerobic Capacity

Introduction

The number of aging individuals worldwide is increasing exponentially. By 2030, it is estimated that 20% of the United States population will consist of individuals 65 years or older (Administration on Aging, 2011), reaching 65.7 million seniors (United States Census Bureau, 2008). For older adults, maintaining a physically active lifestyle has been associated with an increased quality of life and ability to perform activities of daily living (Drewnowski & Evans, 2001; McAuley et al., 2006; Rejeski & Mihalko, 2001). Although there is no amount of physical activity that can reverse the aging process, maintaining a consistent exercise regimen can attenuate levels of physical decline and risk of chronic disease. However, certain populations of aging adults have alternative physical goals and are not simply content with maintaining general physical health.

Within the increasing population of aging individuals lies a similarly increasing subset of competitive seniors or masters athletes (MA). MA can begin as early as 35 years of age; however, age ranges defining masters athlete categories are sport specific and age cutoffs vary significantly. Governing bodies of each sport typically set the age of MA participation based upon ages that elite performances are accomplished. Regardless of sport or age, elite MA convene every 4 years to compete in individual and team events at the World Masters Games (WMG). The WMG began in Toronto, Canada in 1975 with 1,427 competitors representing 32 countries (World Masters Athletics, 2014). The first United States Senior Games (USSG) was held in St. Louis, Missouri in 1987 and hosted 2,500 athletes. Since its inauguration, the competitive population of the USSG has risen to over 13,000 in 2013 (National Senior Games Association, 2013). More importantly to MA than the effects of continued training on general health, is the ability to maintain and even increase physical performance.

As masters-level competition continues to increase in popularity, athletes constantly attempt to find methods to enhance individual athletic performance. The understanding and development of legal and safe supplemental aids to augment physical ability can provide a competitive edge for elite masters-level competitors. Currently, the body of literature evaluating the general use of supplements in MA populations is limited (Kavanagh & Shephard, 1977; Striegel, Simon, Wurster, Niess, & Ulrich, 2005) and evidence that evaluates the effect of individual supplement use on performance is non-existent. Certain ergogenic aids have been evaluated in untrained, older populations including protein supplementation (Chale et al., 2013; Paddon-Jones, 2013), creatine-monohydrate supplementation (Candow et al., 2014; Moon, Heywood, Rutherford, & Cobbold, 2013), and beta-alanine (BA) supplementation (del Favero et al., 2012; McCormack et al., 2013; Stout et al., 2008). Although these ergogenic aids increases physical performance in untrained older individuals, it is unknown if the same effects exist in MA.

Research has indicated positive benefits with the individual supplementation of BA as a method to enhance anaerobic physical ability. The individual effects of BA (del Favero et al., 2012; McCormack et al., 2013; Stout et al., 2008) have been evaluated in untrained older adults; however, no data exist in male or female MA populations. Understanding how these ergogenic aids effect performance in masters-level competitors may create a new opportunity for these athletes to continue their sporting careers and perhaps reach new levels in their respective events.

Purpose of the study

Exogenous supplementation of BA effect exercise performance in trained, younger populations; however, these claims remain unsupported when evaluating BA in MA populations. Therefore, the purpose of this study was to examine the longitudinal effects of BA supplementation on isometric handgrip strength (HG), time to exhaustion (TTE), total work completed (TWC), and lower-body isokinetic torque (ISO) in female MA cyclists.

Research Hypotheses

H₁: Longitudinal supplementation of BA will increase time to exhaustion during high intensity cycling when compared to a placebo.

H₂: Longitudinal supplementation of BA will decrease time to exhaustion along with maximal and recovery lactate levels when compared to a placebo.

H₃: Longitudinal supplementation of BA will increase lower-body isokinetic torque when compared to a placebo.

H₄: Longitudinal supplementation of BA will increase isometric grip strength when compared to a placebo.

Limitations

1. Results from these studies can only be generalized to females.
2. Results from these studies can only be generalized to cycling performance.

3. Results from this study can only be generalized to supplementation of BA in female MA and do not imply increased effects from longitudinal supplementation in males.

Definition of Terms

1. Anaerobic exercise – muscle movement that does not require oxygen and primarily utilizes carbohydrates to produce energy from adenosine triphosphate (ATP)
2. Beta-Alanine – 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 (Smith et al., 2009)
3. Carnosine – cytoplasmic dipeptide found in high concentrations in the skeletal muscle of both vertebrates and non-vertebrates, as well as in the central nervous system (Sale, Saunders, & Harris, 2010)
4. 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)
5. Ergogenic aid – any external influences determined to enhance exercise performance
6. Masters athletes – individuals that continue participation in competitive sport-based events past the age records traditionally set for that particular event
7. Older adult – individual over the age of 65 years
8. Paresthesia – transient flushing and/or tingling sensations on the skin (Decombaz, Beaumont, Vuichoud, Bouisset, & Stellingwerff, 2012)
9. Physical fatigue – decline in ability of a muscle to generate force
10. Power – rate at which mechanical work is performed; product of force and velocity (Noffal & Lynn, 2012)

Significance of the Study

As our population grows older, there has been an exponential increase in participation in masters athletics and with concurrent increases in level of competition. Use of legal, supplemental, ergogenic aids are slowly increasing in older athletes and understanding these effects on sport performance could influence methods in which these athletes approach both training and competition. BA supplementation is used in a number of populations including younger athletes and older adults. BA is the precursor to the intramuscular pH buffer carnosine and compared to males, carnosine levels are lower in females. Carnosine levels also naturally decrease with age. Taken together, the use of BA may have an increased effect in female populations and the ability to enhance exercise performance with exogenous supplementation may be further augmented in older females. Longitudinally, BA significantly increases performance in trained and untrained younger individuals, but whether these effects are present in MA are unknown. It cannot be ascertained if longitudinal supplementation of BA will have the same effects in MA as they do untrained older populations; therefore, further study of the dosing and longitudinal effects of ergogenic aids in female and MA populations is warranted.

Review of the Literature

Introduction

Throughout the aging process, certain individuals desire to remain active and continue participation in competitive sporting events. While the aging process itself cannot be stopped, it may be possible to allow these athletes to remain competitive at higher levels for extended periods of time. Many reports support the use of ergogenic aids as a technique to increase athletic ability. Implementing these aids in older athletes could potentially diminish the rate of natural decline in physical ability, ultimately increasing athletic durability. For this review of literature, methodology development, and discussion of results, a search of Medline (PubMed), ProQuest International, and Google Scholar was conducted using the keywords: older adult, masters athlete, beta-alanine, sport, performance, and all related derivatives. When adequate and related literature was identified, follow-up searches were completed to further examine research design and methodology. This review included randomized control studies that assessed the following components: a) general aging, b) MA, c) BA, d) the influence of supplemental aids on physical performance in older adults. All articles were published in peer-reviewed journals and when necessary, textbooks were referenced to explain and clarify general concepts. No restriction was placed on the year or language in which the article was originally published. A total of 782 article titles were found and reviewed. Of those, 169 full-length articles were collected and utilized. The review of literature was divided into the following major sections: 1) older adults and 2) ergogenic aids. Each section was further subdivided as necessary.

Older Adults

Physiology of aging. The number of aging individuals worldwide is increasing exponentially. By 2030, the number of Americans 65 years and older will nearly double to 72 million individuals, accounting for nearly 20% of the United States population (Center for Chronic Disease Prevention and Health Promotion, 2013). Throughout this process, individuals develop functional limitations and physical ability begins to diminish even in the absence of discernible disease (Masoro, 1995). However, even among the healthiest of aging individuals, onset of disease can and usually becomes an inherent issue.

Early increased presence of age-related disorders is defined as “un-successful aging” (Aviv, Levy, & Mangel, 2003). Chronic diseases such as obesity, cardiovascular disease, type 2 diabetes, and even certain types of cancer become more prevalent with increasing age (Bouchard, Blair, & Haskell, 2012; Lakatta & Levy, 2003; Singh & Antoinette, 2004). Estimates indicate 79% of older adults develop at least one chronic disease after 70 years of age (Chodosh et al., 2005). Similarly, older adults experience the highest levels of musculoskeletal conditions, including, but not limited to sarcopenia, osteoporosis, and arthritis (Ostchega, Harris, Hirsch, Parsons, & Kington, 2000; Paterson & Stathokostas, 2002; Shephard, 1997). Development of these conditions can predispose individuals to greater fall risk and ultimately diminished levels of independence (Arnold, Busch, Schachter, Harrison, & Olszynski, 2005; Carter et al., 2001; Henderson, White, & Eisman, 1998).

Two physical factors affected with increased aging include skeletal muscle function (Buchner & De Lateur, 1991) and aerobic capacity (Fitzgerald, Tanaka, Tran, & Seals, 1997). When evaluating longitudinal isokinetic strength in the knee extensors and flexors, declines occurred at 14% and 16% per decade, respectively (Hughes et al., 2001). Decreases in strength

predict all-cause mortality in older adults (Metter, Talbot, Schrager, & Conwit, 2002). Over a 44-year longitudinal study, individuals with the highest levels of strength demonstrated a 2.5 times greater chance of becoming a centurion (Rantanen et al., 2012). For both men and women, decreases in leg strength directly relate ($r = .79$) to decreases in muscle mass (Reed, Pearlmutter, Yochum, Meredith, & Mooradian, 1991). As a result, older adults with decreased levels of skeletal muscle mass have increased risk for sarcopenia and ultimately physical impairment and disability (Janssen, Heymsfield, & Ross, 2002). Other data suggest muscle mass declines at a lesser rate than strength, indicating amount of general muscle mass is not the issue, but that the quality of the remaining muscle seems to wane (Goodpaster et al., 2006). Regardless of the quantity or quality of muscle available, muscular strength and specifically grip strength has been associated with increased longevity and quality of life. Longitudinal data (44 year follow-up) from 2,239 individuals exhibit grip strength among other variables in relation to maintenance of physical health (Rantanen et al., 2012). Among measures taken, grip strength was most significantly related to individuals maintaining independent lifestyles (Rantanen et al., 2012).

When evaluating aerobic capacity, declines are documented at rates of 15%-20% per decade in individuals greater than 70 years of age (Fleg et al., 2005; Trappe, Costill, Vukovich, Jones, & Melham, 1996). As older adults experience reductions in maximal aerobic capacity, this indicates submaximal intensity exercise will require a higher percentage of maximal capacity when compared to younger counterparts (Chodzko-Zajko, Proctor, & Singh, 2009). These decreases also relate to increased levels of all-cause mortality (Blair et al., 1996; Keteyian et al., 2008; Myers et al., 2002). With reference to maximal aerobic capacity, each increase in metabolic equivalent of task (MET) indicates individuals establish a 12% greater rate of survival

(Myers et al., 2002). The most survival benefits associated with aerobic capacity are observed at a maximal capacity greater than five METs (Kokkinos et al., 2010).

When referring to factors such as development of chronic disease, decreased musculoskeletal function, and diminished aerobic capacity, physical activity helps maintain levels of physical health (Bouchard et al., 2012). Consistent physical activity can increase both life quality and expectancy through reduction of chronic disease development. Aerobic and resistance training increase aerobic capacity and muscle strength by 20%-30% in older adults (Huang, Shi, Davis-Brezette, & Osness, 2005; Lemmer et al., 2000). Regardless of age, physical activity has the same effects on mechanistic properties and training adaptations. The ability to maintain control of blood pressure, oxygen delivery, and heat dissipation (at moderate intensity) remains functional in healthy older individuals from both acute and chronic perspectives (Seals, Taylor, Ng, & Esler, 1994). For older adults, maintaining a physically active lifestyle is also associated with an increased quality of life and ability to perform activities of daily living (Drewnowski & Evans, 2001; McAuley et al., 2006; Rejeski & Mihalko, 2001). Although no amount of physical activity can reverse the aging process, prolonged exercise can attenuate levels of physical decline and risk of chronic disease. Adults over 65 years of age can gain extensive health benefits from regular physical activity with the benefits continuing throughout the lifespan. Certain populations of aging adults, however, are not simply content with maintaining general physical health.

Masters athletes (MA). As the aging process continues, many individuals, termed MA, have a desire to continue to participate in sport-based competition. The inauguration of the WMG began in Toronto, Canada in 1975 representing 32 countries and expanded to 93 countries during the 2011 games (World Masters Athletics, 2014). Similar to growth on the international

stage, the United States Senior Games has increased from the inaugural 2,500 athletes to over 13,000 in 2013 (National Senior Games Association, 2013). Defining MA is a difficult task. MA can begin at 35 years of age and older; however, based on each individual sport, age cutoffs vary significantly. Governing bodies of each sport typically set the age of MA participation based upon ages that elite performances are accomplished. Sports such as swimming begin masters level competition at the age of 20 because world records are set at such young ages. In contrast, sports such as sailing and curling begin masters competition at age 55.

Compared to sedentary counterparts, MA perform more physical activity, spend more time training, and have better indicators of health (Rittweger, di Prampero, Maffulli, & Narici, 2009). Originally, changes in physical function and development of functional limitations were associated with the natural aging process. Comparing MA to sedentary age-matched populations has determined that many of the aging related effects are more associated with sedentary behavior and disuse (Coggan et al., 1990; Kasch, 1988; Tanaka & Seals, 2008; Wilmore, 1991). While these effects are not entirely preventable, many of these changes attenuate in MA competitors (Babcock, Paterson, Cunningham, & Dickinson, 1994). More importantly to MA than the effects of aging is the ability to maintain and even increase physical performance.

MA compete in a variety of sports similar to general Olympians. As of the 2013 games, sports included a variety of individual (archery, badminton, bowling, golf, horseshoes, pickleball, racquetball, road races, shuffleboard, table tennis, tennis, track and field) and team (basketball, softball, volleyball) events (National Senior Games Association, 2013). Sports range in physical demand and can include power, strength, aerobic, and anaerobic requirements. Even with intensive training performance variables decline longitudinally, but certain performance variables are more difficult to maintain (Ransdell, Vener, & Huberty, 2009; Trappe

et al., 1996).

Muscular strength performance. As previously described, muscular strength decreases throughout the aging process (Bassey et al., 1992; Fiatarone et al., 1994; Rantanen et al., 2012) and this declination in strength seems to be resultant of quantity and/or quality of muscle available (Frontera, Hughes, Lutz, & Evans, 1991). For example, when comparing two groups of aging adults (age 45-54 and age 65-78) both muscle mass and muscle strength were significantly lower in the older age group, indicating potential issues with quality and quantity of muscle available. Although absolute strength was significantly different between genders, differences disappeared when strength was expressed relatively to body weight.

For trained athletes, muscular strength declines initially then maintained from ages 60-89 with consistent training (Pollock et al., 1997). Baker and Tang (2010) evaluated record performances for masters sporting events comparing results from endurance and strength-based events. While performance in all sports declined with age, the fastest decrement was observed with weightlifting activities (Baker & Tang, 2010). The greatest discrepancies were documented in strength-based events, independent of gender. Relative performances for women during endurance and strength events were 85% and 52%, respectively compared to male counterparts (Baker & Tang, 2010). Comparing age-related declines between traditional weightlifting and powerlifting events revealed both genders significantly decreased for weightlifting- (curvilinear) and power- (linear) based events (Anton, Spirduso, & Tanaka, 2004). Declines in weightlifting performance were significantly greater than powerlifting events. Also interesting is the magnitude of decline for weightlifting was greater in women, but differences in decline between genders disappeared when evaluating powerlifting performance (Anton et al., 2004). Evaluation of MA competing in throwing events further supports the notion that strength declines with age

(Ojanen, Rauhala, & Hakkinen, 2007). Competitors were divided into four groups based on age (40, 50, 60, and 75 years) and 28 untrained men of equal ages were used as controls. Upper- and lower-body maximal strength and muscle size was significantly decreased among each group with advancing age, however when compared to their age-matched controls the MA were significantly higher in each circumstance. These results indicate that even though maximal strength declines throughout the aging process, it can be maintained at a higher level than untrained individuals with appropriate training methods. Furthermore, while absolute strength tends to attenuate throughout the lifespan, the associated effects of fatigue are similar in MA when compared to younger trained athletes (Louis, Hausswirth, Bieuzen, & Brisswalter, 2009). While MA exhibited lower maximal voluntary contractions of the knee extensors, there were no differences in fatigue levels after exhaustive bouts of lower-body exercise. Ultimately, strength appears to decline at faster rates than power and is harder to maintain independent of gender; however, this appears independent to changes in fatigue.

Regardless of event, the most exponential declines in athletic performance occur around the age of 75 years (Wright & Perricelli, 2008). Currently, these aforementioned reductions in athletic performance are inevitable, but proper conditioning and training can attenuate declines compared to untrained individuals. The most significant factors affecting the ability to maintain longitudinal physical performance are related to decreases in not only training volume, but intensity as well (Hawkins, Marcell, Jaque, & Wiswell, 2001; Hawkins & Wiswell, 2003; Wiswell et al., 2000; Young, Medic, Weir, & Starkes, 2008). Moving forward, it is imperative to evaluate the effects of specific training techniques including exercise selection, intensity, duration of training sessions, frequency of training, and sport nutrition. All of these components

will become important in allowing MA to continue to compete and perform at elite levels throughout the aging process.

Aerobic performance. Although aerobic performance naturally declines with advancing age, the ability to reach higher levels of maximal performance has increased. During the inauguration of the Olympic games in 1896, record times of aerobic events such as the 1,500m (4:33.2) and marathon (2:54.5) have since been matched or surpassed not just by elite younger athletes, but individuals well past what are considered prime performance ages (Tanaka & Seals, 2008). The 1,500m record has been surpassed by a 60 year-old individual (4:27.7) and the marathon by a 73 year-old individual (2:54.5). These records indicate that MA currently perform at higher levels than previous competitively active counterparts.

Ransdell et al. (2009) examined cross-sectional performance records from cycling, swimming, and running events for athletes ranging from 35 to >90 years of age. Performance in the 5,000m declined slightly (1% - 14%) from the ages of 35 to 45, but by 85 years, performance declined 98% in men and 157% in women. Similar declines were noticed in the 10,000m (males = 100%, females = 195%) and marathon events (males = 172%, females = 294%). Although physical decreases are visible in all competitions with age, the declines are more augmented in longer duration events. Analysis of gender differences indicated that the decrease in performance is more dramatic among female competitors. In all three previously reported events (5,000m, 10,000m, marathon), the greatest performance declines between genders were documented in the highest age group (≥ 85 years). When analyzing swimming performance, general declines were similar, but discrepancy between genders was reversed. The longest swimming event (1,500m swim) indicated that females 90 years and later declined 129% while

men had a greater decrease (149%). Highest performance declines in the oldest age groups indicate physical ability becomes progressively harder to maintain with increasing age.

Performance decrements are unavoidable, and gender appears to play a significant role in rate of decline. After 55 years of age, female performance declines at a faster level, specifically in running events. This accelerated decline in female performance levels has been supported by previous research (Ransdell & Wells, 1998; Spirduso, Francis, & MacRae, 2005; Tanaka & Seals, 2003), but there is no physiological evidence to mechanistically explain why this occurs. One plausible explanation is that many women currently competing at a masters level are pre-Title IX and may not have had the same exposure to proper training techniques, nutritional information, facility usage, and equipment availability at high school and collegiate levels (Priest, 2003). Regardless of the reason for gender differences in aerobic decline, both genders approach or exceed 100% around 85 years of age. This is in concert with previous findings where similar levels of decline were documented in older athletes by age 80 (Ransdell & Wells, 1998). While it is necessary to document the rate of performance declination in MA, understanding the mechanisms by which these declines are attributed is necessary to longitudinally maintain physical ability.

Maximal oxygen consumption (VO_{2max}) is proposed as the most significant indicator of running performance in MA runners (Wiswell et al., 2000). Masters runners ($n = 168$) were measured for VO_{2max} , lactate threshold, and fat free mass using a longitudinal design. Declines in VO_{2max} , and fat free mass were significantly correlated to performance declines in both men and women, but VO_{2max} alone indicated a stronger direct relationship to age-related changes in running performance (Wiswell et al., 2000). These longitudinal declines mirror previous data in non-competitive older adults indicating performance diminishes 1% per year with similar

declines in VO_{2max} (Spirduso et al., 2005). Conversely, VO_{2max} has been suggested to decline for MA at 0.5% per year from 40-59 with declines increasing to 2.4% per year after age 60 (Hawkins et al., 2001). Some of the factors contributing to these decreases in VO_{2max}^2 include increased body fat and decreased lean mass (Korhonen, Mero, & Suominen, 2003), decreased myocardial contractility and cardiac output (Reaburn & Dascombe, 2008), and decreased maximal heart rate (Hawkins et al., 2001). Maximal heart rate declines at a rate of 0.5 beats per year from age 40-49 and 1.0-1.6 beats per year from 50-70 years of age. Longitudinal data indicate male MA maintaining consistent training regimens over a 20-year period decline in aerobic capacity 5%-7% per decade and have 50% less decline in maximal heart rate compared to non-athletes (Kasch et al., 1995; Pollock et al., 1997; Trappe et al., 1996). In trained females ($n = 49$, ages 35 to 70), cross-sectional analysis suggested although VO_{2max} decreases with increasing age, regular training may prevent age-related decreases in maximal heart rate (Wells, Boorman, & Riggs, 1992). Trained female runners also exhibited increased cardiorespiratory fitness compared to untrained women. Ultimately, the combination of reduced contractility and maximal heart rate along with the natural increases in body fat are the most significant factors related to decreased aerobic capacity.

Anaerobic (sprint and power) performance. Comparable to aerobic records, original sprint world records set at the inaugural Olympic Games by young Olympians have since been surpassed by masters level competitors. The initial 100m sprint (12.0 s), 200m sprint (22.2 s), and 400m sprint (54.2 s) records have been exceeded by individuals of 61 years (11.7 s), 46 years (22.1 s), and 63 years (53.9 s), respectively. However, while performance continues to increase in the athletic populations, individual anaerobic capacity also experiences a magnitude of age-related longitudinal decline. Perhaps most important to anaerobic activity is speed. A

variety of physical (muscle size, muscle strength, reaction time) and biomechanical (stride frequency and length, and ground contact time) factors account for the ability to produce speed (Korhonen et al., 2009). When comparing younger and older Finnish runners for sprint speed, athletes declined in stride length by 4% per decade with an increased ground contact time (Korhonen et al., 2009). However, speed alone is not the only factor important for maintaining an athlete's anaerobic capabilities.

One of the primary issues in maintaining sprint speed lies in an athlete's ability to generate power. Power and speed are related and regression modelling has used speed to significantly predict power production (Glenn, Vincenzo, Gray, & Binns, 2014; Hawley, Williams, Vickovic, & Handcock, 1992; Neptune, McGowan, & Fiandt, 2009). This decrease in power has also been suggested to be independent of both gender and athletic event. When comparing cross-sectional data from males ($n = 295$) and females ($n = 200$) competing at varied running distances at the World Masters Games, declines in power were similar regardless of gender (0.51 and 0.59 decreased watts/kg/year for males and females, respectively) or event (Michaelis et al., 2008). Interestingly, when evaluating physical declines in power, a linear relationship exists similar to declines in aerobic-based performance (Rittweger et al., 2009). Although absolute power was significantly different between cycling and running sprint events, the relative decline from age 40 to 65 has been documented as 25.3% and 25.4%, respectively (Martin, Farrar, Wagner, & Spirduso, 2000; Rittweger et al., 2009). When comparing peak anaerobic power between groups of MA and trained younger athletes using standing vertical jump performance on a force platform, MA competitors generated 50% less absolute and relative power compared to their younger counterparts (Grassi, Cerretelli, Narici, & Marconi, 1991).

These data are in concert with previous research indicating similar reductions in power by the 7th decade of life (Runge, Rittweger, Russo, Schiessl, & Felsenberg, 2004).

Declines in anaerobic capacity are similar to endurance-based declines in MA. As with most other physical characteristics, these declines are not preventable, but can be attenuated with proper training and maintenance of training intensity. Anaerobic performance maintenance and improvement in the MA requires the same commitment to training required from younger athletes, however some modifications may be required.

Ergogenic Aids

The largest determinant of success in athletes of all ages is performance, which can be increased through a number of factors including training, hydration status, diet, and supplementation. When focusing on supplemental ergogenic aids, their use as a method to enhance performance is well documented (Coleman, 1998; Eichner, 1997; Silver, 2001; Tokish, Kocher, & Hawkins, 2004). From 2005-2009, the use of ergogenic aids in children, adolescents, and young adults has increased 10-fold (Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration, 2011). A survey completed with college athletes reported 89% of university competitors were utilizing at least one type of nutritional aid (Froiland, Koszewski, Hingst, & Kopecky, 2004) and recent reports have suggested that supplement use begins as young as 11 years of age (O'Dea, 2003). Data evaluating supplement use are recorded in younger athletes (Calfee & Fadale, 2006; Krowchuk et al., 1989; Metz, Small, Levine, & Gershel, 2001), with minimal data available providing an insight to ergogenic effects in masters-level competitors.

Indications of supplementation in MA are recorded as early as 1977 (Kavanagh & Shephard, 1977). Various MA at the 1977 World Masters Games reported use of vitamins C, B, and E, along with wheat germ oil. The majority of supplementation was documented in long-distance running events; however, these particular supplements are not traditionally utilized for performance enhancement. Recent data by Striegel et al., (2005) provide a more in-depth insight to the level of ergogenic aids in competitive MA. Evaluation of 554 competitors at the 2004 World Masters Athletic Championship Indoors revealed supplement use in 60.5% of all athletes ($n = 230$ and $n = 105$ for males and females, respectively). Vitamins (35.4%), minerals (29.9%), exogenous protein intake (10.6%), carbohydrates (8.8%), and creatine-monohydrate (6.5%) were most popular among athletes (Striegel et al., 2005). Unfortunately, comparisons of ergogenic aids to competition performance were not analyzed in this study. To date, the use of supplemental ergogenic aids on performance benefits in older adults, and specifically MA, is unclear. As of this current report, BA is legal for competition under World Anti-Doping Agency regulations (World Anti-Doping Agency, 2013). Based on this, more data are needed in order to determine the ergogenic effects of BA on performance in MA as it could prove useful when preparing for competition.

Beta-alanine. BA supplementation has empirical support for younger individuals as an ergogenic aid for increasing physical performance. It 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 (Smith et al., 2009). Supplementation of BA is gaining ground as an exercise supplement based on research indicating its effectiveness during anaerobic performance (Derave et al., 2007; Hill et al., 2007). BA functions as the precursor to the cytoplasmic dipeptide carnosine (β -alanylhistidine), which is located in muscle tissue (Culbertson, Kreider,

Greenwood, & Cooke, 2010). After acute supplementation, BA reaches peak plasma concentrations between 30-40 minutes post-consumption with the half-life of disappearance at 25 minutes (Harris et al., 2006).

In human muscle, homeostatic carnosine content typically measures 20-30 mmol·kg⁻¹ of dry weight and intra-individual variations are only 9-15% over a three-month timeline (Baguet et al., 2009). Carnosine is dependent upon the presence of the hydrolyzing enzyme carnosinase which breaks down carnosine into its constituent amino acids (BA and l-histadine) allowing them to transport to other tissues (Asatoor, Bandoh, Lant, Milne, & Navab, 1970; Perry, Hansen, Tischler, Bunting, & Berry, 1967). In vivo, human muscle does not contain carnosinase and requires a specific ratio (2:1:1) of Na⁺, Cl⁻, and beta-amino acids for active transport into the tissue (Miyamoto, Nakamura, Hoshi, Ganapathy, & Leibach, 1990). Resynthesis of carnosine in muscle is limited by very low intramuscular concentrations of BA as l-histadine is available in much larger quantities. As a result, supplementing with exogenous BA should ultimately enhance muscle carnosine concentrations.

When evaluating carnosine levels it is important to understand intramuscular differences based on training state and individual fiber type. Trained muscle responds more effectively to BA supplementation (Bex et al., 2014) and fiber-type has been established as one of the most important determinants of muscle carnosine levels. When compared to slow-twitch fibers, fast-twitch fibers have 30-100% higher levels of carnosine based on single fiber analysis (Harris, Dunnett, & Greenhaff, 1998; Hill et al., 2007; Kendrick et al., 2009). This relationship has been further elucidated in younger, elite aerobic and anaerobic athletes who traditionally have higher levels of slow-twitch and fast-twitch fibers, respectively. Results indicated sprinters had significantly higher carnosine levels compared to marathon runners (Parkhouse, McKenzie,

Hochachka, & Ovalle, 1985). Higher numbers of type-II fibers represent an increased ability to elicit power (Widrick, Trappe, Costill, & Fitts, 1996) and using BA supplementation to augment intramuscular carnosine levels increases power-based performance (Donovan, Ballam, Morton, & Close, 2012; Hoffman et al., 2014). In older adults, fiber type changes with increasing age. As slow-twitch fibers are mostly unchanged, these age-related changes are primarily related to both decreases in size (Lexell, 1995) and number (Lexell, Henriksson-Larsen, Winblad, & Sjostrom, 1983) of type-II fibers. As type-II fibers are the primary sites of carnosine availability, losses of these particular fibers indicate age-associated losses in intramuscular carnosine availability (Stuerenburg & Kunze, 1999; Tallon, Harris, Maffulli, & Tarnopolsky, 2007).

Carnosine and exercise. During exercise, lactic acid is metabolized for energy and the hydrogen ion (H^+) byproducts left behind serve to alter muscular pH and attenuate exercise performance (Westerblad, Bruton, & Katz, 2010). The body utilizes carnosine during moderate- to high-intensity exercise to buffer these increased levels of H^+ in an effort to maintain homeostatic intramuscular pH. Due to an acid dissociation constant ($pK_a = 6.83$) and high concentrations, carnosine is suggested to be a more effective buffer than bicarbonate ($pK_a = 10.30$) as a buffer (Eudy et al., 2013). Although l-histidine is also a precursor to carnosine synthesis, it is readily available in vivo and BA becomes the rate-limiting factor associated with carnosine production (Bakardjiev & Bauer, 1994; Bauer, Hallermayer, Salnikow, Kleinkauf, & Hamprecht, 1982; Dunnett & Harris, 1999). It has also been suggested that in vivo concentrations of carnosine are directly correlated ($r = .97$) to BA availability (Dunnett & Harris, 1999) suggesting that increasing levels of BA will directly increase carnosine concentrations. Increasing the body's carnosine levels will cause an increased buffering capacity of H^+ , resulting in delayed fatigue and increased power output (Kern & Robinson, 2011) along with minimizing

feelings of perceived exertion (Gross et al., 2014; Hoffman et al., 2008). Therefore, increasing carnosine levels via supplementation of exogenous BA would serve to augment physical performance.

However, simply increasing intramuscular carnosine concentrations via supplementation of BA will not increase performance for all physical measures and exercise durations. A meta-analysis of 15 studies spanning 57 exercise measures concluded that exercise lasting: <60 s was unaffected by exogenous beta-alanine supplementation, 60-240 seconds was most effective, and >240 seconds was not as effective as the 60-240 second range, but still significant (Hobson, Saunders, Ball, Harris, & Sale, 2012). However, care must be taken when considering exercise measures >240 seconds in duration as long-term aerobic performance (≥ 60 minutes) does not seem to be affected even with 200% increases in intramuscular carnosine (Chung, Baguet, Bex, Bishop, & Derave, 2014). These data indicate that for BA to have the greatest effects on exercise performance the exercise measures must total a minimum of 60 seconds of high-intensity exercise, but longer lasting aerobic exercise bouts may be unaffected.

Special populations. Levels of intra-muscular carnosine vary amongst specific populations and therefore certain individuals may benefit further from exogenous intake of BA supplementation. When evaluating carnosine levels between gender, animal models have documented the ratio as 3.5/1.0 for males and females, respectively (Penafiel, Ruzafa, Monserrat, & Cremades, 2004) and to a similar extent, this ratio exists in humans with age-matched females having naturally lower levels of carnosine compared to males (Everaert et al., 2011). Baseline carnosine concentrations of the quadriceps femoris are 21% higher in healthy, young males compared to age-matched females (Mannion, Jakeman, Dunnett, Harris, & Willan, 1992); however, females experience greater relative increases in intramuscular carnosine from BA

supplementation compared to males (Stegen et al., 2014). Research evaluating the efficacy of BA supplementation in female populations is controversial. When supplementing female populations with BA it delayed onset of neuromuscular fatigue (12.6%) during submaximal cycling trials and increased time to exhaustion (2.5%) during maximal cycle ergometry performance (Stout, Cramer, Mielke et al., 2006). Females have also improved rating of perceived exertion supplementing with BA compared to a placebo (Smith, Stout, Kendall, Fukuda, & Cramer, 2012). However, in contrast to high intensity measures, when supplementing BA there were no significant effects for increasing maximal oxygen consumption compared to a placebo group (Smith et al., 2012; Walter, Smith, Kendall, Stout, & Cramer, 2010). This indicates that BA improves high intensity exercise parameters, but not aerobic capacity in women.

Although data assessing carnosine concentrations are available in older men, no data are currently available in older female populations concerning exercise performance. Because decreased levels in carnosine concentrations are documented in both females and older adult populations, this indicates a potential for increased effect on exercise performance from supplementation of exogenous BA in older, trained female populations.

As earlier reported, carnosine concentrations decrease with age and these decreases are present within both animal and human models. In rodents, a number of studies have documented a decreased carnosine content of 35-50% with increasing age (Derave, Jones, Hespel, & Harris, 2008; Johnson & Hammer, 1992; Stuerenburg & Kunze, 1999). In humans, longitudinal data are unavailable, but cross-sectional evidence mirrors animal models as elderly individuals have 33-60% lower levels of carnosine compared to younger populations (Stuerenburg & Kunze, 1999; Tallon et al., 2007). When examining subjects aged 9-83 years ($n = 263$), significant declines in

carnosine concentration were observed with increasing age (Baguet, Everaert, Achten, Thomis, & Derave, 2012). These data are in concert with the work of Everaert et al. (2011) indicating significant declines in carnosine concentrations by 47 years when compared to younger populations matched for physical activity. The ability to increase carnosine levels in the aging population could have a significant impact of the ability to maintain physical ability in trained and untrained older populations.

The cause of carnosine declines is potentially associated with decreases in androgens/estrogens, the decline in fiber number of slow twitch and fast twitch fibers, and the decline in size of type II fibers (Boldyrev, Aldini, & Derave, 2013). The role of BA's effects on carnosine concentrations and exercise performance have been reviewed in multiple investigations with reference to younger athletes (Artioli, Gualano, Smith, Stout, & Lancha, 2010; Hobson et al., 2012; Sale et al., 2010; Wilson, Wilson, Zourdos, Smith, & Stout, 2010), but research focused on trained older adult populations is limited. This is important as older adults have decreased levels of carnosine compared to younger populations and as a result, may experience increased benefits from BA supplementation (Everaert et al., 2011).

Recent studies using untrained older adults with regard to BA supplementation demonstrate significant effects for increasing physical ability. Stout et al. (2008) suggested supplementation improved intramuscular pH, therefore delaying the onset of neuromuscular fatigue. Similar results were proposed by del Favero et al. (2012), that supplementation in healthy individuals aged 60-80 years was effective for increasing carnosine concentrations and subsequent exercise capacity. When evaluating physical ability, BA increased physical working capacity, muscle function, and muscle quality (McCormack et al., 2013); however, other parameters of functional fitness (sit-to-stand, grip strength) were not increased from exogenous

supplementation. These particular tasks are less than 30 s in duration and therefore, outside the 60-240 s range suggested for maximum effects (Hobson et al., 2012). Although BA has effects on increasing physical performance in untrained older adult populations, data evaluating effectiveness in trained older populations are unsubstantiated.

Levels of intra-muscular carnosine content have also been evaluated in reference to dietary restrictions. Meat and fish are extremely high in carnosines' rate limiting factor BA (Smith et al., 2009), and restrictions in dietary intakes of these foods may cause deficiencies in BA concentrations. Males ingesting higher and lower levels of dietary BA (via animal protein) exhibited no differences in carnosine concentrations (Everaert et al., 2011), but when adding exogenous BA, individuals were able to increase muscle carnosine concentrations past homeostatic conditions (Baguet et al., 2009; Derave et al., 2007; Kendrick et al., 2009). Although no differences in carnosine concentrations exist for individuals consuming different levels of animal protein, long-term (> 8 years) vegetarianism has an inverse effect on muscle carnosine in the soleus and gastrocnemius (17% and 26% lower, respectively) compared to omnivores (Everaert et al., 2011). Taken together, this implies that it takes very low doses and/or exogenous supplementation of BA intake to influence carnosine concentrations past naturally occurring levels.

Beta-alanine dosage. Safe and uniform dosages of BA are not universally established, but recommendations have been developed based on empirical findings. Data suggest that as the dose of BA increases there is a correspondent decrease in the longitudinal time needed to continue supplementation (Stellingwerff, Decombaz, Harris, & Boesch, 2012). Protocols have been developed using doses that range from 1.6 – 6.4g per day (Stellingwerff et al., 2012). Increased levels of acute supplementation (> 800mg) have been documented to cause paresthesia

(sensation of tingling or pricking on the skin) lasting anywhere from 60-90 minutes (Harris et al., 2006). Although reported as uncomfortable, these effects are temporary and harmless. For longitudinal designs, this has been circumvented by administering multiple 800mg doses (up to 8 times per day) throughout the day (Kendrick et al., 2008; Kendrick et al., 2009).

The connection between BA dosage and performance increases remains unclear. Meta-analysis of BA supplementation protocols found no relationship ($p = .34$) between dosage amount and performance differences between BA and placebo groups (Hobson et al., 2012). The smallest differences in effect size between BA and placebo groups were associated with the largest supplemental doses (Kendrick et al., 2008; Sweeney, Wright, Glenn Brice, & Doberstein, 2010). With respect to supplementation, discrepancies in performance benefits attribute to differences in subject sizes, exercise tests, and measures employed (Hobson et al., 2012). Further research warrants evaluation of similar measures and sample sizes with varying levels of BA dosing.

Conclusion

As competition continues to increase in masters athletics, so does the need for advanced training and nutritional protocols. Research has indicated a positive benefit to the supplementation of BA as a method to enhance exercise performance. Currently, no data are available in MA populations based on BA supplementation. Understanding how this ergogenic aid effects performance in masters level competitors may create a new opportunity for these athletes to continue their sporting careers and perhaps reach new levels in their respective events.

Methodology

Introduction

Throughout the aging process, physical ability attenuates. These effects are commonly evaluated during activities of daily living, but for the portion of the older population that continues participation in competitive sports, the ability to maintain athletic performance becomes equally important. Nutritional supplementation is advocated as a method of increasing physical performance, but evidence supporting their use in MA is inconclusive. One specific supplement that has attracted attention for the benefits in older adults is BA. Currently, there have been no published data evaluating the effect of these supplements on physical performance in MA. Therefore, the purpose of this study was to examine the longitudinal effects of BA supplementation on HG, TTE, TWC, and ISO in female MA cyclists.

Subjects

Based on previous literature, 22 subjects (11 per group; Table 1) participated in this investigation (Stout, Cramer, Zoeller et al., 2006). However, based on an expected 20% attrition rate, 30 females were recruited. MA were classified as competitive masters cyclists based on requirements set forth by USA Cycling and World Masters Cycling. These requirements necessitated that MA a) were at an age ≥ 30 years, b) not be classified as an elite cyclist or competed in an elite event based on Union Cycliste Internationale (UCI) standards, and c) not have been a team member of a registered team disciplined by the UCI. For the purposes of this study, MA must also have had at least 2 years competitive cycling experience and cycle a minimum of 3 days per week (Halsen et al., 2002). Carnosine levels significantly decrease by 47 years (Everaert et al., 2011) and therefore that was used as the age cutoff. Females were utilized because they have naturally lower levels of intramuscular carnosine concentrations compared to

males (Everaert et al., 2011) and may further benefit from exogenous BA supplementation. Participants were recruited via email, fliers, and visits to local cycling clubs and organizations. Each participant read and signed an informed consent approved by the University's Institutional Review Board prior to participation.

Table 1.
Initial subject demographic data

	<u>PLA</u>	<u>BA</u>	<u>p-value</u>
Age (years)	52.6 ± 1.2	54.0 ± 1.5	0.46
Height (cm)	162.33 ± 2.50	163.00 ± 2.08	0.84
Mass (kg)	68.70 ± 5.64	64.01 ± 2.82	0.47
Body Fat (%)	32.97 ± 2.70	28.05 ± 3.88	0.31
Time cycling (years)	5.86 ± 2.20	7.20 ± 2.12	0.67
Distance cycled/week (km)	135.18 ± 7.88	144.76 ± 15.03	0.58
Fast Twitch Fibers (%)	33.20 ± 3.20	41.50 ± 2.70	0.06
Slow Twitch Fibers (%)	66.80 ± 3.20	58.50 ± 2.70	0.06

Note. All data are expressed as mean ± *se*. BA = beta-alanine, PLA = Placebo. Significance level was set at $\alpha < 0.05$.

Experimental Design

This double blind, randomized study consisted of a 28-day supplement intervention using BA as the experimental group and dextrose as the placebo group (PLA). Each participant reported to the Human Performance Lab on the University of Arkansas campus for all trials. Participants completed baseline testing on two separate days. Day one consisted of signing an informed consent and completion of a health history questionnaire to ensure all participants met inclusion criteria. Height and mass measurements were assessed using a stadiometer and weight beam eye-level scale, respectively (Detecto, Webb City, MO). Body fat, lean mass analysis (dual-energy x-ray absorptiometry; DXA), and determination of aerobic capacity (VO_{2peak}) were also assessed on the initial visit. Day two consisted of handgrip strength (HG), time to exhaustion evaluation (TTE) at 120% of the participant's initial VO_{2peak} values, and dominant leg

ISO, in respective order. During the supplementation period, participants ingested the assigned supplementation (PLA = 8g dextrose; BA = 800mg + 8g dextrose) 4 times/day throughout the 28-day period. HG, TTE, and ISO were reconducted at the 7-, 14-, 21-, and 28-week intervals before reassessment of VO_{2peak} and DXA on the final trial. Testing protocols were completed in the same order for all participants and at least 24 hours were allotted between the VO_{2peak} session and HG, TTE, and ISO session at the baseline and 28th day.

Controls

To ensure changes during each supplementation trial were not based on training or detraining effects, participants maintained the same training intensity throughout the study and completed weekly exercise logs at the baseline, 7th, and 21th day. Exercise logs were evaluated between groups to ensure results were not based on increases in training intensity. Food logs were distributed to all participants at baseline, 7th, and 21th day to be completed on two non-consecutive weekdays and one weekend day (Smith et al., 2009). To account for dietary consumption on testing days, participants fasted 3-hours prior to each trial (Stout, Cramer, Zoeller et al., 2006). All participants had never ingested exogenous, supplementary BA and were instructed to refrain from vigorous exercise, alcohol, and caffeine 24-hours preceding each trial. Subjects replicated the same attire for all trials and wore clothes/shoes in which they normally cycled.

Supplementation Protocol

After the familiarization trial, subjects were randomly assigned to either the PLA or BA groups. The BA provided for this investigation was third party lab tested for supplement purity and authenticity (Powder City, York, PA). To maintain a double-blind design, a separate

investigator completed subject supplement assignments. Conditions included PLA (8 g dextrose per day) or BA (800mg BA + 8 g dextrose per day). Individual doses of 800 mg were used to circumvent the potential onset of paresthesia occurring (Kendrick et al., 2008; Kendrick et al., 2009), which would ultimately remove the double-blind design. Participants were instructed to consume supplement doses in 16 ounces of water (Stout, Cramer, Mielke et al., 2006).

Aerobic Capacity Testing Protocol (VO_{2peak})

At the initial and final testing trials, all participants performed a graded exercise test (GXT) on a Velotron Dynafit Pro Ergometer electronically braked cycle ergometer (Racer Mate, Seattle, WA) to determine VO_{2peak} . Prior to testing, seat and handlebar preferences were established for each individual comfort and were recorded to be used for all future testing sessions. Subjects warmed-up at 50 W for 5 minutes at a self-selected cadence. Upon completion of the warm-up, the resistance increased 25 W in 2-minute intervals until the participant could no longer maintain 60 revolutions per minute (RPM). VO_{2peak} was measured using breath-by-breath analysis and analyzed via open-circuit spirometry (PARVO Medics, Sandy, UT). The highest 15 second VO_{2peak} value recorded was used as the maximal measurement provided it met at least two of the following criteria: a) a plateau in heart rate or heart rate is within 10% of the age predicted maximum, b) a plateau in VO_{2peak} (an increase of no more than $150 \text{ ml}\cdot\text{min}^{-1}$), and/or c) the subjects RER value reached > 1.15 (Smith et al., 2009). Test retest reliability ($ICC = .98$) and coefficient of variation (5.18%) for this protocol have been previously demonstrated (Smith et al., 2009).

Handgrip Testing (HG)

HG testing determined overall strength (Lauretani et al., 2003; Rantanen, Era, Kauppinen, & Heikkinen, 1994). All HG measurements were administered by a trained

technician and measured in kg using a handheld dynamometer (Creative Health Products, Ann Arbor, MI). All measurements were performed on the dominant hand with the subject standing, arm down at the side, wrist in neutral position, and interphalangeal joint of the index finger maintained at 90°. Participants maximally squeezed the handle for 5 seconds as standard encouragement was provided. The test was repeated three times on the dominant hand with 60 s rest between trials. The greatest of the three trials was used as the final strength measurement. High test-retest reliability (ICC = .95) for the HG strength test has been previously recorded (Bohannon & Schaubert, 2005).

Time to Exhaustion Protocol (TTE) and Total Work Completed (TWC)

Prior to TTE testing, the predetermined seat height and handlebar settings were adjusted for each participant. After completing the same warm-up as used for the GXT, subjects completed a TTE at 120% of their previously recorded VO_{2peak} (Simmonds, Minahan, & Sabapathy, 2010; Weber & Schneider, 2001). TTE was defined as the time (s) participants could maintain intensity above 40 RPM pedaling cadence (Astorino, Robergs, Ghiasvand, Marks, & Burns, 2000; Vivodtzev et al., 2011). Reliability and coefficient of variation for TTE protocol have been reported as ICC = .71 and 3.8%, respectively (Smith et al., 2009). Lactate measurements were taken from the fingertip (Tobias et al., 2013) at rest, immediately after TTE cessation, and after a 20-minute seated recovery period and analyzed immediately (Accutrend Lactate Monitor, Indianapolis, IN). TWC was calculated by multiplying time (s) during the TTE test and the power output (W), divided by 1,000 to get the final product in kilojoules.

Isokinetic Lower-body Torque Assessment (ISO)

The Biodex system III Isokinetic Dynamometer (Biodex Medical, Inc., Shirley, NY) measured ISO using a 50-repetition protocol with 240° eccentric/180° concentric movement parameters. Participants sat upright with the dynamometer axis of rotation aligned with the axis of rotation of the dominant knee. Secured belts stabilized the trunk, pelvic girdle, and thigh to the Biodex chair to prevent additional body movement. The chair and dynamometer settings were recorded to ensure positioning for all testing remained the same between trials. Before testing, the dominant limb was weighed by the isokinetic dynamometer so that it could be added and subtracted from torque values when working against and with gravity, respectively. Subjects fully extended and flexed the knee and to work maximally during the 50-repetition testing period. To ensure maximal effort was given during the evaluation, strong verbal encouragement was provided throughout the testing sessions (Andreacci et al., 2002). Calibration of the dynamometer was performed according to manufacturer specifications.

Blinding Efficacy and Side Effects

Upon completion of the supplementation intervention, subjects were asked which supplement they believed they had consumed. Subjects were also asked if they experienced any side effects throughout the course of the study related to the supplement ingested.

Statistical Analyses

SAS version 9.4 (Cary, IN.) was used to analyze all data. To assess the effects of supplementation on performance and physiological variables between groups, investigators utilized separate one-way within, one-way between repeated measures ANOVAs (group [BA vs. PLA] x time [baseline, 7th, 14th, 21st, 28th day]) for each variable measured. For statistically

significant F-scores, simple main effects were analyzed with one-way factorial ANOVAs for each time point. An alpha level of $p < .05$ defined significance. Fisher's exact test evaluated subject ability to determine supplement ingestion throughout the study. When appropriate, effect size (η^2) was calculated based on the recommendations by Cohen (Cohen, 1988). All data are reported as mean \pm *se*.

Results

Controls

There were no initial significant differences between groups for demographic variables or aerobic capacity (table 2). When examining dietary logs between BA and PLA, no significant differences were observed for overall total kilocalorie intake or individual macronutrient breakdowns (carbohydrate, fat, protein) within or between groups at any time point (table 3).

Table 2.
Aerobic capacity between groups

	Pre		<i>p</i> -value	Post		<i>p</i> -value
	<u>BA</u>	<u>PLA</u>		<u>BA</u>	<u>PLA</u>	
Vo₂peak (l/min)	2.28 ± 0.65	2.31 ± 0.49	.90	2.45 ± .45	2.28 ± .47	.41
Vo₂peak (ml/kg/min)	39.13 ± 8.46	36.51 ± 8.27	.47	39.82 ± 8.88	36.15 ± 7.84	.32

Note. All data are expressed as mean ± *se*. BA = beta-alanine, PLA = placebo. No significant differences were observed over time between or within groups from the initial to post-testing trials. Reported *p*-values indicate between group analyses. Significance level was set at $\alpha < 0.05$.

Table 3.

Dietary intake values for supplementation groups

<u>PRE</u>	<u>BA</u>	<u>PLA</u>
Total Kilocalories	1802.10 ± 153.18	2189.71 ± 143.94
Carbohydrates	182.82 ± 18.63	249.30 ± 20.59
Fats	69.38 ± 6.68	79.96 ± 7.41
Protein	90.15 ± 8.02	96.96 ± 8.17
<u>MID</u>		
Total Kilocalories	1752.11 ± 104.01	1889.01 ± 160.51
Carbohydrates	175.70 ± 17.23	224.19 ± 28.10
Fats	75.41 ± 6.85	66.78 ± 7.23
Protein	87.96 ± 7.64	85.59 ± 5.54
<u>POST</u>		
Total Kilocalories	1770.83 ± 165.68	1897.91 ± 186.04
Carbohydrates	187.20 ± 17.76	227.81 ± 27.08
Fats	69.08 ± 7.20	68.66 ± 9.36
Protein	100.31 ± 8.60	88.52 ± 8.18

Note. All data are expressed as mean ± *se*. BA = beta-alanine, PLA = Placebo, PRE = pre-testing, MID = 14th day, POST = 28th day. No differences were observed for dietary intake between groups or over time. Significance level was set at $\alpha < 0.05$.

Performance Variables

Time to exhaustion (TTE). No initial significant differences existed between groups at the pre-testing time point [$F(1,22) = 2.301, p = .15$]. Repeated measures ANOVA revealed a significant interaction between groups throughout the 28-day intervention ($p = .002$). Follow-up univariate analysis indicated that by the 28th day, BA significantly [$F(1,22) = 5.706, p = .03$] increased TTE (23%) compared to PLA (1%); however, no differences were observed for any of the intermittent time points (Figure 1a).

Total work completed (TWC). Similar results were observed for TWC via repeated measures ANOVA ($p = .001$) in reference to performance increases (Figure 1b). No initial significant differences were observed between groups [$F(1,22) = .217, p = .65$] and BA did not

elicit increases during the intermittent time points. However, at the 28th day, TWC significantly increased [$F(1,22) = 5.649, p = 0.28$] for BA compared to the PLA (21% vs. 2%, respectively).

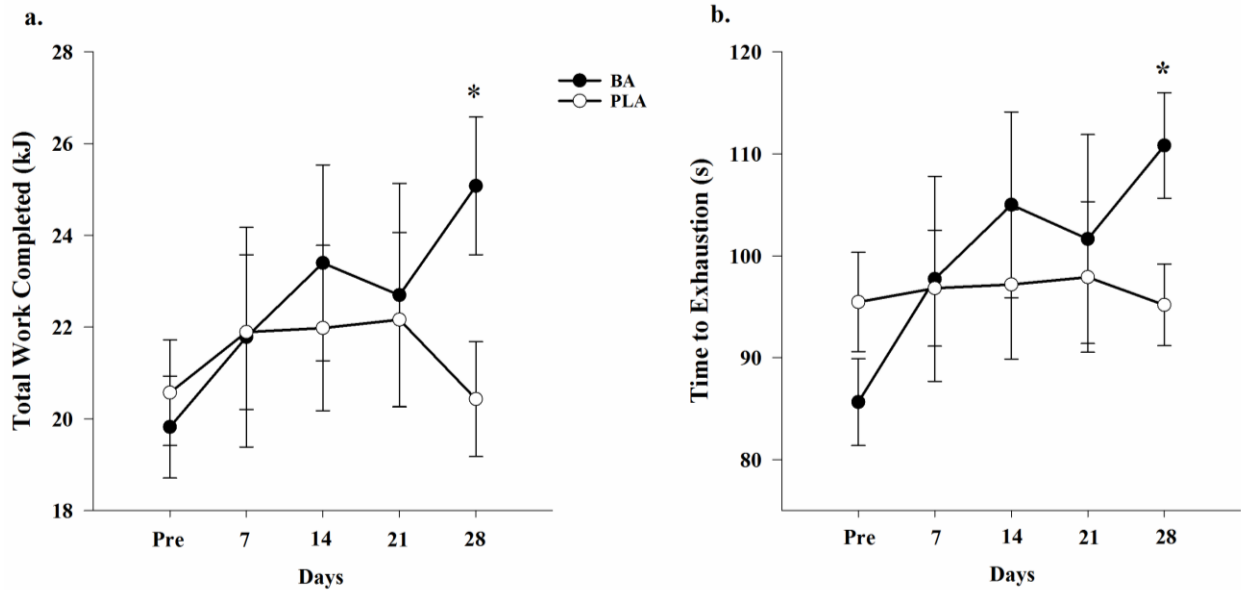


Figure 1a. Time to exhaustion and b. Total work completed between beta-alanine (BA) and placebo (PLA) groups after 4 weeks of supplementation. *Indicates significant differences between BA and PLA ($p < .05$).

Isokinetic lower-body exercise (ISO). There were no initial significant differences between groups for all flexion and extension variables measured during ISO (Table 4, all $p > .05$). Repeated measures ANOVA revealed that there was a significant group by time interaction during the flexion component for total work completed during the final third of exercise ($p = .008$) and the extension component for average peak torque generated throughout the test ($p = .012$). When comparing BA and PLA, at the 28th day, average peak torque ($F = 7.398, p = .014$; 8.1% vs 1.4% change, respectively) and total work completed during the final third of exercise [$F(1,22) = 5.942, p = .024$; 24.0% vs -16.8% change, respectively] significantly increased (Figure 2a, b, respectively). No differences existed for any variable during the intermittent time points (all $p > .05$).

Table 4.
Performance during the lower-body isokinetic strength evaluation

	Flexion		Extension	
	BA	PLA	BA	PLA
Pre				
Peak Torque (N·m)	35.3 ± 3.0	34.8 ± 2.2	68.2 ± 2.8	69.2 ± 5.4
Time to Peak Torque (m/s)	476.7 ± 47.3	342.7 ± 71.0	302.0 ± 7.2	259.1 ± 23.2
Work/Body Weight (%)	18.1 ± 2.8	18.9 ± 2.2	49.9 ± 2.2	50.5 ± 3.6
Total Work Completed (J)	1043.7 ± 201.9	1196.3 ± 142.0	3451.7 ± 114.6	3459.4 ± 288.8
Work During the first 3 rd (J)	436.3 ± 96.8	503.2 ± 68.5	1384.5 ± 70.2	1466.4 ± 130.6
Fatigue (%)	32.4 ± 6.9	39.4 ± 5.9	35.1 ± 2.9	42.6 ± 2.4
Average Power (W)	34.3 ± 6.7	39.0 ± 4.9	100.1 ± 3.7	97.9 ± 7.6
Day 7				
Peak Torque (N·m)	38.6 ± 3.2	38.9 ± 4.2	70.9 ± 3.4	73.0 ± 6.0
Time to Peak Torque (m/s)	488.0 ± 43.5	426.4 ± 50.1	289.1 ± 11.7	279.1 ± 27.7
Work/Body Weight (%)	20.3 ± 2.5	20.8 ± 1.9	51.6 ± 2.8	49.2 ± 2.8
Total Work Completed (J)	1205.0 ± 195.6	1292.7 ± 148.4	3471.0 ± 159.3	3430.5 ± 260.8
Work During the first 3 rd (J)	522.2 ± 100.0	589.2 ± 76.2	1457.3 ± 81.0	1511.4 ± 124.5
Fatigue (%)	37.3 ± 5.2	49.2 ± 5.0	40.2 ± 2.0	47.2 ± 1.6
Average Power (W)	41.1 ± 7.0	43.0 ± 5.1	101.4 ± 5.0	99.5 ± 7.4
Day 14				
Peak Torque (N·m)	37.2 ± 3.4	36.5 ± 2.6	74.9 ± 3.8	78.8 ± 5.3
Time to Peak Torque (m/s)	420.0 ± 45.4	409.0 ± 63.9	281.0 ± 21.5	280.0 ± 18.2
Work/Body Weight (%)	19.8 ± 2.5	21.2 ± 1.6	48.5 ± 3.2	54.5 ± 3.3
Total Work Completed (J)	1207.2 ± 163.2	1288.3 ± 119.3	3492.0 ± 179.9	3600.7 ± 291.3
Work During the first 3 rd (J)	537.4 ± 88.8	429.7 ± 582.6	1453.8 ± 90.2	1596.4 ± 129.5
Fatigue (%)	38.5 ± 6.3	50.8 ± 3.3	39.5 ± 1.8	47.8 ± 1.1
Average Power (W)	42.1 ± 6.2	43.5 ± 4.2	104.4 ± 5.1	104.8 ± 8.0
Day 21				
Peak Torque (N·m)	33.2 ± 1.9	40.0 ± 3.3	71.2 ± 2.0	72.7 ± 5.4
Time to Peak Torque (m/s)	483.8 ± 40.5	342.0 ± 57.6	288.9 ± 13.7	275.9 ± 21.0
Work/Body Weight (%)	19.7 ± 1.6	22.8 ± 2.5	50.4 ± 1.9	50.8 ± 2.8
Total Work Completed (J)	1084.6 ± 106.7	1131.1 ± 171.2	3381.1 ± 86.8	3510.0 ± 272.0
Work During the first 3 rd (J)	467.2 ± 48.2	530.1 ± 78.8	1419.9 ± 26.4	1497.6 ± 123.0
Fatigue (%)	38.0 ± 7.3	52.3 ± 4.2	40.9 ± 1.8	47.0 ± 1.9
Average Power (W)	37.2 ± 4.6	42.1 ± 4.9	100.7 ± 4.0	96.9 ± 6.6
Day 28				
Peak Torque (N·m)	40.0 ± 3.1	36.4 ± 1.7	74.9 ± 3.0	75.0 ± 5.0
Time to Peak Torque (m/s)	415.0 ± 71.0	336.0 ± 50.1	270.9 ± 16.6	268.0 ± 18.4
Work/Body Weight (%)	21.6 ± 1.7	21.5 ± 1.73	51.3 ± 1.9	52.6 ± 3.2
Total Work Completed (J)	1218.8 ± 150.9	1222.8 ± 108.5	3378.6 ± 108.3	3379.7 ± 224.3
Work During the first 3 rd (J)	493.3 ± 78.4	563.6 ± 51.6	1456.5 ± 61.7	1525.6 ± 113.1
Fatigue (%)	22.3 ± 15.9	51.3 ± 4.8	43.8 ± 1.9	49.4 ± 1.7
Average Power (W)	42.7 ± 5.8	40.7 ± 3.8	102.6 ± 3.8	98.9 ± 5.9

Note. All data are expressed as mean ± se. BA = beta-alanine, PLA = Placebo, PRE = pre-testing. All measurements were taken from the subject's dominant limb. No differences were observed between or within groups at any time point (all $p > .05$).

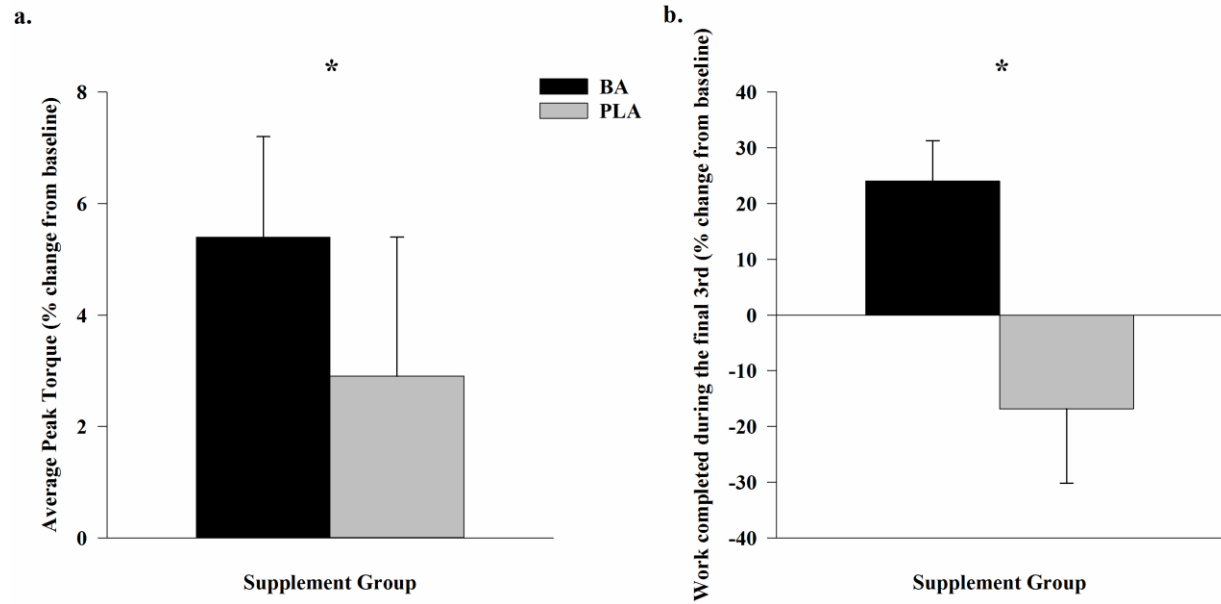


Figure 2a. Average peak torque and b. total work completed during the final third of exercise between beta-alanine (BA) and placebo (PLA) groups after 28 days of supplementation. *Indicates significant differences between BA and PLA ($p < .05$).

Handgrip strength. Handgrip strength was not affected by supplementation (Table 5). Repeated measures ANOVA indicated there were no group by time interactions throughout the course of the 28-day intervention [$F(4,80) = 0.62, p = .65$].

Table 5.
Handgrip strength between groups through the 4-week supplementation intervention

	Pre		Day 7		Day 14		Day 21		Day 28	
	BA	PLA	BA	PLA	BA	PLA	BA	PLA	BA	PLA
Average Grip (kg)	27.8 ± 1.1	28.6 ± 1.8	27.6 ± 1.3	28.6 ± 1.6	28.0 ± 1.3	28.5 ± 1.9	27.6 ± 1.1	27.8 ± 1.7	27.4 ± 1.3	28.3 ± 1.6
Maximal Grip (kg)	29.1 ± 1.2	29.8 ± 1.8	28.9 ± 1.2	29.7 ± 1.7	29.3 ± 1.1	29.5 ± 1.9	28.5 ± 1.1	28.7 ± 1.6	28.4 ± 1.2	29.1 ± 1.7

Note. All data are expressed as mean ± *se*. BA = beta-alanine, PLA = Placebo, PRE = pre-testing. No differences existed between or within groups at any time point (all $p > .05$). Significance level was set at $\alpha < .05$.

Physiological Variables

Lactate. No significant differences existed between groups for blood lactate levels at the rest, immediate post, or 20-minute rest intervals at pretesting (all $p > .05$). Repeated measures ANOVA revealed lactate measures taken at rest ($p = .96$) and immediately after completion of the TTE test ($p = .13$) were not significantly different between BA and PLA over the course of the intervention. However a significant group by time interaction ($p = .01$) was observed between groups for lactate levels after the 20-minute rest interval (Figure 3). Univariate analyses indicated that lactate was significantly lower [$F(1,22) = 4.700, p = .04$] for BA when compared to PLA (24%) by the 28th day, although there was a trend for significance by the 21st day [$F(1,22) = 4.115, p = .056$]. Effect sizes for the 21st and 28th day were $\eta^2 = .19$ and $\eta^2 = .17$, respectively.

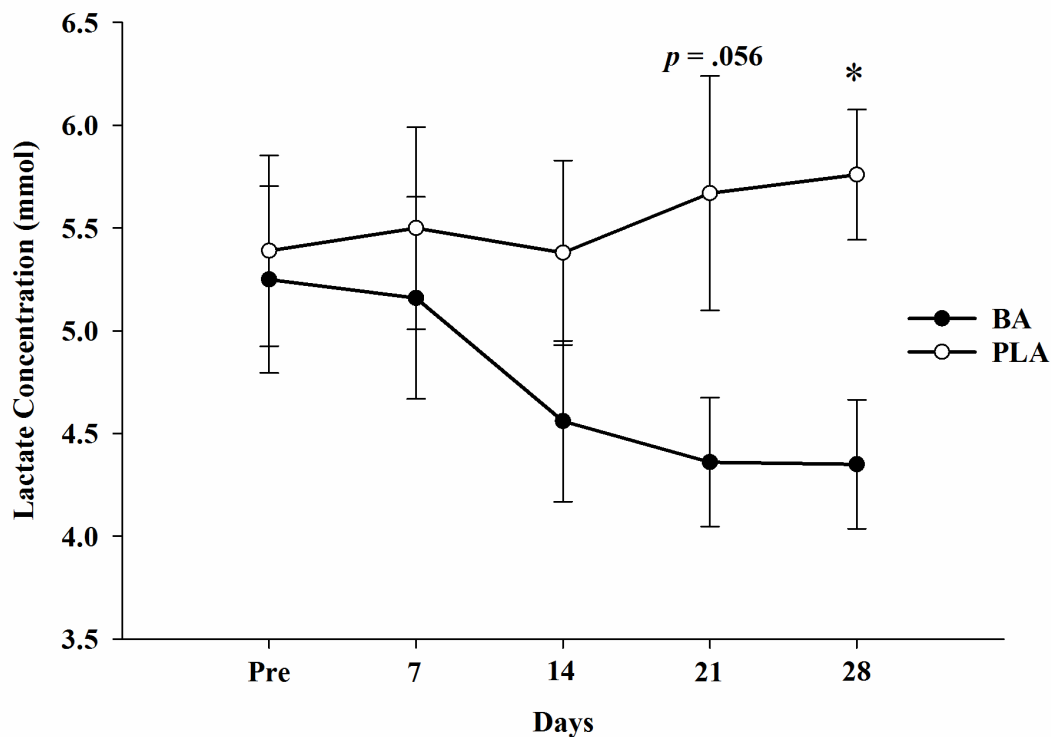


Figure 3. Lactate rates 20 minutes after completion of time to exhaustion evaluation between beta-alanine (BA) and placebo (PLA) groups after 28 days of supplementation. By the 21st day, as trend for significance was observed between groups ($p = .056$). *Indicates significant differences between BA and PLA ($p < .05$).

Body Composition

Body composition (regional and total) was not affected by supplementation (Table 6).

Repeated measures ANOVA indicated there were no group by time interactions throughout the course of the intervention (all $p > .05$).

Table 6.

Body composition between groups after supplement intervention

	PLA		BA	
	Pre	Post	Pre	Post
L Arm BF (%)	30.76 ± 3.29	31.52 ± 3.62	24.38 ± 4.11	24.05 ± 3.65
L Leg BF (%)	36.87 ± 2.45	37.06 ± 2.74	32.15 ± 3.74	31.08 ± 3.30
L Trunk BF (%)	32.56 ± 2.94	33.23 ± 3.35	27.29 ± 4.25	25.75 ± 3.74
L Total BF (%)	33.00 ± 2.70	33.44 ± 3.05	27.96 ± 3.86	26.77 ± 3.40
R Arm BF (%)	30.78 ± 3.29	31.49 ± 3.62	24.33 ± 4.10	24.05 ± 3.64
R Leg BF (%)	36.90 ± 2.45	37.06 ± 2.74	32.17 ± 3.74	31.12 ± 3.30
R Trunk BF (%)	32.85 ± 2.98	33.25 ± 3.35	27.32 ± 4.25	25.77 ± 3.73
R Total BF (%)	32.97 ± 2.70	33.53 ± 3.05	28.12 ± 3.88	26.79 ± 3.38
Arms Total BF (%)	30.77 ± 3.28	31.51 ± 3.61	24.35 ± 4.11	24.05 ± 3.64
Legs Total BF (%)	36.88 ± 2.45	37.05 ± 2.75	32.15 ± 3.73	31.11 ± 3.30
Trunk Total BF (%)	32.57 ± 2.93	33.24 ± 3.35	27.30 ± 4.25	25.76 ± 3.73
Android BF (%)	36.43 ± 3.69	36.37 ± 3.95	28.74 ± 4.71	27.70 ± 4.46
Gynoid BF (%)	41.65 ± 2.13	41.29 ± 2.51	36.10 ± 3.67	35.95 ± 3.20
Total BF (%)	32.97 ± 2.70	33.48 ± 3.05	28.05 ± 3.88	26.78 ± 3.39

Note. All data are expressed as mean ± *se*. BA = beta-alanine, PLA = placebo, L = Left, R = Right, BF = Body Fat. No significant differences were observed over time between or within groups from the initial to post-testing trials. Significance level was set at $\alpha < .05$.

Blinding Efficacy and Side Effects

Fisher's exact test indicated the subjects were unable to accurately assess which supplement they had consumed based on a 2 (supplement guess) x 2 (accuracy) analysis ($p = .31$). Accurate guesses for the BA, and PLA groups were recorded as 23% and 32%, respectively. Only one subject reported feelings of paresthesia throughout the course of the intervention. All analyses were conducted without and with the subject experiencing side effects

during the trials. No changes were detected for any analysis and as a result, the subject was included in the final statistical models. One subject was forced to drop out due to repeated headaches, which she believed was related to the supplement she was ingesting. At the completion of the study, it was established that she was in the PLA group and her data has not been included in the final analyses.

Discussion

The purpose of this study was to examine the longitudinal effects of BA supplementation on HG, TTE, TWC, and ISO in female MA cyclists. These are the first data evaluating the ergogenic effects of BA on performance measures in MA, much less in females. The use of BA to increase intramuscular carnosine plays an important role during exercise (Sale et al., 2010) and as there is an increased reliance on buffering systems during high-intensity exercise, carnosine becomes an integral component for improving performance (Hobson et al., 2012). Baseline levels of intra-muscular carnosine vary amongst specific populations indicating certain individuals may experience enhanced effects from exogenous BA supplementation. When comparing intramuscular carnosine levels between males and females, a 3.5:1.0 ratio is observed respectively between genders (Everaert et al., 2011). Females also require lower levels of BA supplementation to obtain similar relative increases in carnosine compared to males (Stegen et al., 2014). In women, BA increases TTE (Stout, Cramer, Mielke et al., 2006) and decreases feelings of perceived exertion (Smith et al., 2012). These data, taken together with the fact that carnosine loading is further augmented in trained muscles (Bex et al., 2014), indicates trained females may be more sensitive to BA supplementation in reference to increasing intramuscular carnosine concentrations.

Time to Exhaustion (TTE) and Total Work Completed (TWC)

Results from this investigation support this concept as female MA significantly increased TTE and TWC during cycling after 4 weeks of BA supplementation compared to controls. Previous literature has evaluated the effects of BA supplementation on cycling performance (Hill et al., 2007; Smith et al., 2009). Initial work concluded that exogenous ingestion of BA increased total work completed 13% after 4 weeks of supplementation with an additional 3%

increase after a total of 10 weeks (Hill et al., 2007). These performance increases were suggested to be the result of increased carnosine concentrations at each time point (59% and 80%, respectively). Smith et al. (2009) further evaluated the use of BA to increase cycling performance with similar findings. $\text{VO}_{2\text{peak}}$, TTE, and TWC all significantly increased after longitudinal supplementation, potentially due to the increased buffering capacity from increased carnosine concentrations. However, it is important to note that the results from Hill (2006) and Smith (2009) were collected only in males and it cannot be generalized that females respond similarly to BA supplementation.

Cycling performance data involving younger females have suggested similar results as compared to males. After 28 days of BA supplementation, significant increases were observed for working capacity at the onset of fatigue (14%), ventilatory threshold (13%), and TTE (3%) when compared to subjects supplementing with a placebo (Stout, Cramer, Mielke et al., 2006). Although the dipeptide was not measured directly, these results were suggested to be the result of increased carnosine concentrations ultimately resulting in an enhanced buffering capacity. Results from the present investigation also observed significant increases in TTE and TWC; however, these are the first data to evaluate these performance benefits in MA. The female MA participating in this study significantly increased TTE (23%) and TWC (21%) when compared to age-matched controls. These increases are markedly higher compared to previous literature and may be an indication that older females present an ideal population for benefits from longitudinal supplementation. However, it must be noted that this study utilized a TTE evaluation at 120% of the subject's $\text{VO}_{2\text{peak}}$ while earlier studies used either the time exercised during a GXT (Stout, Cramer, Mielke et al., 2006) or an intensity of 110% $\text{VO}_{2\text{peak}}$ (Smith et al., 2009). Therefore, it cannot be ascertained whether these elevated increases from BA supplementation are a direct

reflection of the population tested or if the type of evaluation also had an effect on performance outcomes. Future research needs to be conducted in female MA to determine overall efficacy of BA supplementation for athletic improvements in comparison to younger females or age-matched males.

Isokinetic Strength Evaluation (ISO)

Previous literature has evaluated the effects of ergogenic aids such as creatine-monohydrate (Gilliam, Hohzorn, Martin, & Trimble, 2000) or amino acids (Williams, van den Oord, Sharma, & Jones, 2001) as methods to increase isokinetic exercise performance although no significant improvements occurred in performance variables. BA has also been previously examined as an aid to increase isokinetic strength with similar non-significant results (Kendrick et al., 2008); however, these data were collected in younger, untrained males. In the current investigation, similar to cycling performance variables, female MA experienced significant increases in lower-body isokinetic exercise performance. After the 28-day supplementation intervention, subjects consuming BA increased average peak torque (8%) and total work completed during the final third of exercise (24%) compared to age-matched controls consuming PLA.

Average peak torque is the overall average of the peak torque measurements from each repetition performed throughout the isokinetic testing protocol. Cyclists consuming BA were able to maintain a higher peak torque for each repetition compared to PLA. Cyclists are constantly pedaling throughout competition and this pedaling intensity increases near the race's end (Andez-Garcia, Perez-Landaluce, Rodriguez-Alonso, & Terrados, 2000). The ability to generate increased peak torque during not only an individual repetition, but throughout multiple

pedaling repetitions leading to the finish could potentially lead to an increase in performance times. Work completed during the final third of exercise also significantly increased after 4 weeks of BA supplementation, indicating female MA were able to produce more work during the latter component of ISO. Similar to average peak torque, this is also important for cyclists as they near the end of a race and are attempting to hold or advance in finishing position. The ability to produce more work towards the end of a race may also potentially allow competitors to increase performance times due to increased sprinting ability.

When examining exercise performance, increases in TTE and TWC are directly in line with previous literature indicating BA supplementation increases performance during high-intensity exercise (Hobson et al., 2012); however, the increases in isokinetic exercise indices are unique to the current investigation. Regardless of exercise assessment, increases in performance are most likely due to an increased buffering capacity directly related to elevated carnosine concentrations (Sale et al., 2010). BA supplementation directly increases intramuscular carnosine (Dunnett & Harris, 1999), which ultimately increases the ability to remove byproducts (H^+) of lactate metabolism (Culbertson et al., 2010). Although carnosine was not directly measured in this investigation, it can be theorized that increases in the dipeptide from exogenous BA consumption was responsible for the increases in cycling and isokinetic exercise capacity. However, based on initial gender decreases and natural age-related declines (Everaert et al., 2011), female MA represent a population that may be more sensitive to increases in intramuscular carnosine based on BA supplementation. As a result, it is important future investigations evaluate these how these increases compare to younger female and age-matched male counterparts.

Isometric Handgrip Strength (HG)

Although indices of cycling and isokinetic strength performance improved via longitudinal BA supplementation, there were no significant effects on grip strength among female MA. It has been proposed that increases in intramuscular carnosine can have beneficial physiological effects on exercise outside of increased buffering capacity (Hobson et al., 2012). One of these effects is an increase in Ca^+ sensitivity of the muscle fibers potentially leading to greater force of contraction (Dutka & Lamb, 2004). However, this did not relate to increases in handgrip strength at any time point during the intervention. This may have been because the evaluation was not long enough to illicit the onset of fatigue and future investigations should employ longer-duration isometric evaluations (60-240 s) when examining this component of BA supplementation.

Lactate Accumulation and Clearance

Although BA supplementation increased various indices related to exercise performance, this was independent from changes in peak lactate accumulation immediately after completion of TTE. Previous literature evaluating peak lactate accumulation are equivocal. Although numerous studies indicate BA has no effect on peak lactate accumulation (Baguet, Koppo, Pottier, & Derave, 2010; Kern & Robinson, 2011; Van Thienen et al., 2009), other investigations have proposed otherwise (Ghiasvand et al., 2012; Tobias et al., 2013). However, regardless of peak lactate accumulation, it is important to note these investigations did not measure the effects on lactate clearance during recovery.

When investigating lactate clearance, studies involving BA supplementation have measured blood lactate 5-minutes after completion of high-intensity exercise (Sale et al., 2011;

Tobias et al., 2013). Although Sale et al. (2011) observed no significant differences in lactate clearance when compared to subjects consuming PLA, Tobias et al. (2013) determined that BA supplementation resulted in reduced levels of lactate clearance after 28 days of supplementation. As it can take an hour or more to return blood lactate to baseline levels (Karlsson, 1971), a five-minute measure may not be appropriate to determine the efficacy of BA on post-exercise lactate clearance. In the current study, blood lactate was 24% lower in BA 20 minutes after completion of TTE compared to PLA. From a practical point of view, these findings translate into attractive implications for athletes during competitive races or events. During sanctioned competition, cyclists reach higher levels of exertion during elevated climbs or intermittent sprints when pulling a team. Throughout a longer-duration event (i.e. century rides), cyclists may be forced to endure multiple high-intensity bouts which go beyond steady-state exercise (Andez-Garcia et al., 2000; Rodriguez-Marroyo, Garcia-Lopez, Juneau, & Villa, 2009) leading to elevated lactate accumulation (Gladden, 2004; Wasserman, Beaver, & Whipp, 1986). As increased carnosine levels lead to increased buffering capacity, this may constitute an improved lactate clearance after the athlete returns to a steady-state pace, ultimately leading to improved performance times.

Limitations

The results of this study are based on the premise that performance increases are directly related to increases in intramuscular carnosine concentrations; however, carnosine was not directly measured in this investigation. Several recent studies have indicated significant increases in carnosine concentrations based on at least 28 days of BA supplementation (Hill et al., 2007; Kendrick et al., 2009) and this study utilized dosing strategies previously reported to be sufficient for these increases to occur (Hobson et al., 2012). This indicates these increases in performance are related to elevated intramuscular carnosine levels; however, this cannot be

confirmed based on this investigation. Other limitations included the inability to directly control dietary intake and exercise activity. Although subjects reported maintenance of regular training programs and no differences existed between groups for total kilocalorie or macronutrient intakes throughout the study, this is reliant on subject honesty and accurate recordings.

Conclusion

In this investigation, the first data are presented evaluating the effects of BA on exercise performance in female MA. Four weeks of supplemental BA increased cycling TTE and TWC along with increased rate of lactate clearance after recovery. During ISO, average peak torque and work completed during the final third of exercise were also increased from exogenous BA consumption when compared to age-matched controls consuming PLA. These results have practical applications for cyclists near the end of competition when finishing time and the ability to maintain high-intensity exercise are critical for resultant performance times and ultimately race placement.

It is hypothesized that these performance increases are a direct result of increased intramuscular carnosine concentrations; however, as carnosine was not directly measured in this study this cannot be confirmed. Older females have naturally lower levels of carnosine concentrations compared to age- or gender-matched counterparts and taken together with the fact that trained muscle is more sensitive to carnosine increases, this indicates female MA may experience elevated increases from BA supplementation. In order to determine the efficacy of BA supplementation in female MA, future research should investigate the direct effects of BA supplementation on intramuscular carnosine concentrations.

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Appendix A

Institutional Review Board Approval Document

May 21, 2014

MEMORANDUM

TO: Jordan Glenn
Michelle Gray

FROM: Ro ~~Windwalker~~
~~IRB~~ Coordinator

RE: New Protocol Approval

~~IRB~~ Protocol #: 14-05-717

Protocol Title: *The Longitudinal Effects of Beta-Alanine Supplementation on Isometric Strength, Time to Exhaustion, and Lower-Body Isometric Torque in Female Masters Athlete Cyclists*

Review Type: EXEMPT EXPEDITED FULL ~~IRB~~

Approved Project Period: Start Date: 05/21/2014 Expiration Date: 05/15/2015

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 30 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~~.

Appendix B
Informed Consent

INFORMED CONSENT

***Title:* The Longitudinal Effects of Beta-Alanine Supplementation on Isometric Strength, Time to Exhaustion, and Lower-Body Isometric Torque in Female Masters Athlete Cyclists**

Investigator(s): Jordan M. Glenn & Michelle Gray
University of Arkansas
College of Education and Health Professions
Dept. of Health, Physical Education, and Recreation
155 Stadium Drive-HPER 321Q
479-575-6638

Ro Windwalker, CIP
IRB Coordinator
Office of Research Compliance
210 Administration Bldg.
University of Arkansas
Fayetteville, AR 72701
479-575-2208
irb@uark.edu

Description: The purpose of this study is to evaluate the ability of beta-alanine to increase performance during handgrip, timed cycling, and lower-body power tests. This study involves a 4-week supplementation period in which you will receive either beta-alanine or a placebo to consume with 16oz of water each day for the 4-week period. In total you will be asked to come to the Human Performance Lab 7 times. The first two sessions will include 1) an explanation of the study, VO_{2peak} test, body composition assessment and 2) baseline testing for handgrip, cycling performance, and lower-body power. Each week you will be asked to return to the lab to repeat handgrip, cycling performance, and lower-body power testing. The last week you will be reassessed for VO_{2peak} and body composition.

You will be masked as to which supplement you are consuming each trial until the conclusion of the study. During each cycling performance trial, lactate will be taken via finger stick at 3 different time points. Heart rate will be taken via heart rate monitor and perceived exertion measures will also be taken via the OMNI exercise scale throughout each cycle test. On the first and last testing days, you will receive a dual-energy x-ray absorptiometry (DXA) scan for your participation.

Risks and Benefits: The risks associated with participation in this study are those involved in traditional exercise. Risks associated with exercise include, but are not limited to, muscle or joint injury, changes in blood pressure, and increased heart rate. Risk will be minimized by having trained personnel present during all testing and familiarization sessions. During the DXA scan you will be exposed to a small amount of ionizing radiation. The amount received during a DXA test is about the same as four (4) days of normal background radiation in Northwest Arkansas. If you have an intact uterus and ovaries and there is a chance you may be pregnant (unprotected intercourse within the last 60 days), you may not participate in this testing at this time. Radiation may be harmful to a fetus. There is also a potential side effect of tingling in the extremities associated with beta-alanine ingestion, however this does not always occur and does not occur in all individuals. There are no potential gastrointestinal side effects that may occur as result of supplement ingestion. Benefits include two free DXA scans and two free VO_{2peak} tests offered to all participants during the last testing session along with understanding if there is an

effect of beta-alanine on exercise performance in female masters athletes which could help with future training and competitions.

Voluntary Participation: Testing will take place on seven separate days for about 60 minutes each session. Permission for you to engage in the testing and exercise protocol is voluntary. You are free to deny or withdraw from testing at any time if you so desire. The IRB Coordinator can be contacted at irb@uark.edu if participants have any questions about the research or their rights as a participant.

Confidentiality: All data collected that can be associated with a subject/respondent must remain confidential. Describe the methods to be used to ensure the confidentiality of data obtained. All participants will be assigned a code number. The code number will be used for all analyses. All information collected will be kept confidential to the extent allowed by law and University policy. Only those directly involved in the research project will have access to the data.

Right to Withdraw: You are free to refuse to participate and to withdraw at any time. Your decision to withdraw will bring no penalty to you.

Informed Consent: I, _____, have read the description of this program, including the purpose of the program, the procedures to be used, the potential risks and side effects, the confidentiality, as well as the option to withdraw from the program at any time. The investigator has explained each of these items to me. The investigator has answered all of my questions regarding the program, and I understand what is involved. My signature below indicates that I freely agree to participate in this study and that I have received a copy of this agreement from the investigator.

I understand that participation in all activities related to this project is voluntary on behalf of all participants. I acknowledge and agree that the University of Arkansas does not provide insurance for any of its activities and shall not be liable for any injuries that occur at any time during participation.

Participant: _____

Date: _____

Witness: _____

Date: _____

Appendix C
Health History Questionnaire

Today's Date: _____

Date of Birth _____

What is your current age? _____

Number of miles cycled/week _____

Number of hours cycled/week _____

Number of years training _____

How often do you participate in organized competition? _____

Most recent date in which you competed _____

Are you a vegan and/or vegetarian? _____

Have you ever had any of the following conditions? Check yes or no. If yes, explain.

Heart Disease Yes No

Heart Attack Yes No

Angina (Chest Pain) Yes No

Peripheral Artery Disease Yes No

Stroke Yes No

High Cholesterol (>220) Yes No

High Blood Pressure (>140/90) Yes No

Diabetes Yes No

Rheumatic Fever Yes No

Aneurysm Yes No

Joint Replacement Yes No – *If yes, specify which joints

Have you ever had any of the following conditions? Check yes or no. If yes, explain.

Severe Illness (in the last year) Yes No

Operations (in the last year) Yes No

Broken bone/fracture (in the last year) Yes No

Additional information

Have you experienced any of these symptoms? Check yes or no.

If yes, explain.

Pain and/or discomfort in the chest, neck, jaw, or arms Yes No

Shortness of breath at rest or with mild exertion Yes No

Dizziness Yes No

Ankle edema (swelling) Yes No

Rapid or irregular beating heart Yes No

Leg pain, cramping, or tightness during exercise Yes No

Heart murmur Yes No

Fatigue or shortness of breath during the day Yes No

Do you smoke? Yes No Quit

Please attach a list of all medication (prescription or over-the-counter) you are currently taking or use the form below.

Medication	Reason Prescribed	When do you take this medication? (check all that apply)
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/> Bedtime

Please list all supplements (NOT medications, ex. vitamins, minerals, protein, caffeine, etc.) you are currently taking in the form below.

Supplement	Reason for taking	When do you take this supplement? (check all that apply)
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		
_____	_____	<input type="checkbox"/> Morning <input type="checkbox"/> Mid-Day <input type="checkbox"/> Evening <input type="checkbox"/>
Bedtime		

Appendix D

VO₂ Peak Data Collection Sheet

VO₂ Max Test

Subject #:		Researchers:				
Date:		Age Predict Max HR:				
BM:		Seat Ht:				
Height:		Time at start:				
Age:		Time at end:				
<u>Time</u>	<u>Resistance (W)</u>	<u>RPM</u>	<u>HR</u>	<u>RPE</u>	<u>RER</u>	<u>VO₂</u>
Warm-up	50					
0:00	75					
1:00	75					
2:00	100					
3:00	100					
4:00	125					
5:00	125					
6:00	150					
7:00	150					
8:00	175					
9:00	175					
10:00	200					
11:00	200					
12:00	225					
13:00	225					
14:00	250					
15:00	250					
16:00	275					
17:00	275					
18:00	300					

Appendix E

Time to Exhaustion Data Collection Sheet

Time to Exhaustion Data Sheet

Subject #: Date: BM: Height: Age:	Researchers: 110% VO₂peak (W): Seat Ht: Time at start: Time at end:
--	---

<u>Time</u>	<u>Resistance (W)</u>	<u>RPM</u>	<u>HR</u>	<u>RPE</u>	<u>Lactate</u>
Rest					
Warm-up	50				
Start					
1:00					
2:00					
3:00					
4:00					
5:00					
6:00					
7:00					
8:00					
9:00					
10:00					
Post					
R 5:00					
R 10:00					
R 15:00					
R 20:00					

Appendix F

Subject Weekly Training Log

Training Log

Dates:

Name:

	<u>Monday</u>	<u>Tuesday</u>	<u>Wednesday</u>	<u>Thursday</u>	<u>Friday</u>	<u>Saturday</u>	<u>Sunday</u>
Type of Exercise:							
Duration:							
Intensity:							
Details:							

Appendix G

3-day Subject Diet Log and Instructions

DIETARY FOOD RECORD INSTRUCTIONS

- **ALL foods and beverages (INCLUDING WATER)** that are consumed should be recorded.
- Be very specific in your description of the type, the preparation method, and the amount of each food/beverage you consume.
- Use the label on foods to help you determine portion sizes.
- Save labels from packages and return them with your food record forms (this will greatly assist and enhance our analysis of your true nutrient intake).
- Use nutrient descriptors (e.g., low-fat, fat-free, light, reduced calorie, etc.) and brand names (e.g., Kraft, Nabisco, Planters, etc.) to describe foods.
- Record food/beverage consumption after each meal/snack instead of waiting until the end of the day.

MEATS/CHEESES

Description: Include description of the type, cut, and preparation method.

Portion Sizes:

List cooked (not raw) amounts of meats.

Determine amounts by weighing when possible.

Three ounces of cooked meat is equivalent to approximately a deck of cards or the palm of your hand.

***Listed below are examples of how to document foods**

3oz. Skinless, boneless, chicken breast-roasted

3oz. Ground beef round-fried

3oz. Deli turkey breast slices

3oz. Atlantic cod-baked

3oz. Sirloin steak-grilled

1/2 cup cubed beef stew meat

1 slice ham, 3" x 4" x 1/4"

1 oz colby cheese

1 piece cheddar cheese, 3" x 2" x 1"

STARCH/BREAD

(CEREALS, BREADS, PASTAS, RICES, BEANS)

Description: Include a complete description of the starch/bread including preparation method and brand name if applicable.

Portion Sizes:

List cooked (not raw) amounts of starch/bread products.

Generally, a measuring cup will suffice for cereals, rices, pastas, and beans.

½ cup brown rice (Uncle Bens)

2 slices rye bread-toasted

2 cups spaghetti noodles-boiled

1½ cups dry cereal (Cheerios)

1 cup oatmeal (Quaker Oats)-microwaved

8 animal crackers

Blueberry muffin, small

½ cup canned baked beans

1 corn tortilla, 6" across

FRUITS/VEGETABLES

Description: Include description of fruit/vegetable and whether it was fresh, frozen, or canned.

Include preparation method (e.g., steamed, fried, etc.)

Portion Sizes:

For whole pieces of fruit or vegetables, you may use small, medium, or large.

For many fruits/vegetables, cups may be used also.

- 1 medium Granny Smith apple
- ½ of a large tomato-fresh
- 5 small strawberries-fresh
- ½ cup canned pineapple-canned in water
- 1 cup frozen peas-steamed
- ¾ cup frozen mixed vegetables
- 3 spears steamed broccoli
- 2 medium raw carrots

COMBINATION DISHES

For standard mixed dishes, it is generally acceptable to list the type of dish without trying to list the ingredients separately. If the food is modified (e.g., low-fat), indicate this and try to describe how the food was modified. Provide enough detail to explain the composition of the dish. For tossed salad, list the individual ingredients paying careful attention to salad dressings and other caloric-dense toppings (bacon bits, cheese, ham, chopped egg, etc.).

- 1 cup bean chili w/o meat
- 3 slices thin crust large cheese pizza with pepperoni-frozen

½ cup potato salad
1 cup tuna casserole
1 cup macaroni and cheese (Kraft)
1 slice angel food cake
2 cups tossed salad
 2 cups lettuce greens
 3 slices cucumber
 3 slices tomato
 1 T shredded cheddar cheese
 1 T shredded carrots
 2 T fat-free Italian dressing

BEVERAGES/FLUIDS

Description: Include ALL BEVERAGES INCLUDING WATER complete description of the beverage

Portion Sizes: Use fluid ounces, liters, cups, or tablespoons.

6 oz regular coffee, brewed

12 oz Diet Pepsi

1 cup 2% milk

16 oz unsweetened iced tea

4 oz red table wine

6 oz orange juice (from concentrate)

2 T light olive oil

MISCELLANEOUS

Remember to list all condiments and additions to foods and beverages, such as cream, sugar, butter, jelly, lemon, salad dressing, artificial sweeteners, catsup, etc.

3 T low-fat french salad dressing

2 tsp black raspberry jam

1 packet Sweet'n Low

1 T cream (half and half)

2 tsp margarine spread (Country Crock)

3 T fat-free ranch salad dressing (Kraft)

Day of Week/Date: _____ Name: _____

Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	<i>Total kcal (from label)</i>

Comments:

Appendix H

Beta-alanine certificate of authenticity provided by third party lab testing evaluation

Certificate of Analysis

Product Name: Beta Alanine
Batch Number: 201401005
Manufacturing Date: January, 2014
Re-Test Date: January, 2016
Country of Origin: China

Item	Standard	Result	Method
Appearance	White crystalline powder	White crystalline powder	Visual
Transmittance	≥95.0%	99.87%	AJI97
Assay	98.0% ~ 101.0%	100.28%	AJI97
Loss on Drying	0.3% max	0.06%	AJI97
Residue on Ignition	0.20% max	0.1%	AJI97
Chloride	≤0.04%	<0.04%	AJI97
Iron	30 ppm max	30 ppm max	AJI97
Ammonium	≤0.02%	<0.02%	AJI97
Sulphate	≤0.048%	<0.048%	AJI97
Heavy Metals	10 ppm max	10 ppm max	AJI97
Lead	3 ppm max	3 ppm max	ICP-MS
Mercury	0.1 ppm max	0.1 ppm max	ICP-MS
Arsenic	2 ppm max	2 ppm max	ICP-MS
Cadmium	1 ppm max	1 ppm max	ICP-MS
Melting Point	197°C ~ 202°C	197.1°C ~ 197.4°C	AJI97
Other Amino Acids	Not detectable	Complies	AJI97
pH	6.5 ~ 7.5	6.98	AJI97
Total Plate Count	≤1,000 cfu/g	≤10 cfu/g	AJI
Yeast & Mold	≤100 cfu/g	≤10 cfu/g	AJI
Coliforms	≤10 cfu/100g	≤10 cfu/100g	AJI
E. Coli	Negative	Negative	AJI
Salmonella	Negative	Negative	AJI

Conclusion: The above results meet the factory standard