

THE EFFECT OF REPEATED SPRINT TRAINING IN HYPOXIA AND BETA-ALANINE  
SUPPLEMENTATION ON EXERCISE PERFORMANCE

by

RAN WANG

B.Ed. Beijing Sport University, 2010

M.Ed. Beijing Sport University, 2013

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Major Professor: Jay Robert Hoffman

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## ABSTRACT

The primary objective of this study was to evaluate the synergistic effects of repeated sprint training in hypoxia (RSH) and beta-alanine supplementation on performance in recreationally active men. Participants were randomly assigned to one of the following groups: hypoxia + beta-alanine (HB, n = 10), hypoxia + placebo (HP, n = 9), normoxia + beta-alanine (NB, n = 11) and normoxia + placebo (NP, n = 8). All participants completed a total of 8 training sessions (each consisting of 3 sets of 5 × 10-s sprints at a resistance of 7.5% of body mass, with 20-s rest intervals between sprints) over 4 weeks on a cycle ergometer either in hypoxia (Oxygen fraction:  $FiO_2 = 14.2\%$ ) or normoxia ( $FiO_2 = 20.9\%$ ). Participants were instructed to consume a daily dosage of 6.4g (two 800 mg tablets ingested 4 times per day at 3-4 hour intervals) of either beta-alanine or placebo. Changes in performance in a graded exercise test (GXT), repeated sprint test (RST) and 3-min all-out test (3MT) were examined before and after 28-days of training and supplementation. Aerobic performance was measured by maximal oxygen consumption ( $VO_{2max}$ ), peak power output (PPO). Exercise intolerance was assessed from critical power (CP), oxygen consumption ( $VO_{2RCP}$ ) and power output (PRCP) at respiratory compensation point. Exercise capacity was measured by total work (TW) during 3MT. Anaerobic capacity was evaluated via anaerobic working capacity (AWC), heart rate response to RST (RST\_HR60) and lactate responses to RST (RST\_La) and 3MT (3MT\_La). Repeated sprint performance was estimated through average power output of the last sprint (RST\_AP5) and all sprints (RST\_AP). No between-group differences were observed for training volume or supplementation compliance. Anthropometric and hematological measures remain unchanged before and after intervention in all groups. A main effect of altitude was shown for  $VO_{2RCP}$ , PRCP, RST\_AP5,

RST\_HR60, and TW, with post-intervention values in the hypoxia groups significantly ( $p < 0.05$ ) higher (lower for RST\_HR60) than the normoxia groups. A main effect of beta-alanine was detected in AWC, with post-intervention values in the beta-alanine groups being significantly ( $p < 0.05$ ) higher than the placebo groups. Results of this investigation demonstrated that RSH and beta-alanine benefit performance from different perspectives. RSH improved aerobic performance, exercise tolerance, cardiovascular recovery and exercise capacity, while beta-alanine supplementation maintained anaerobic working capacity in recreationally-trained men during the four-week repeated sprint training intervention.

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## CHAPTER ONE: INTRODUCTION

Muscle buffering capacity is a key mechanism related to high intensity exercise performance. Skeletal muscle acidosis impedes several metabolic processes, and as a result, force production is reduced (Spriet, Lindinger, McKelvie, Heigenhauser, & Jones, 1989). These processes include the disruption of phosphocreatine (PCr) resynthesis, inhibition of glycolysis, and dysfunction of the muscle contractile machinery (Hobson, Saunders, Ball, Harris, & Sale, 2012). Muscle buffering capacity derives from several physiological buffers, such as proteins, inorganic phosphate, bicarbonate, and the histidine-containing dipeptide carnosine (Parkhouse & McKenzie, 1984). In humans, carnosine contributes 7-8% of total muscle buffering capacity (Harris, Marlin, Dunnett, Snow, & Hultman, 1990; Hill et al., 2007; Mannion, Jakeman, Dunnett, Harris, & Willan, 1992). Due to the importance of buffering capacity on anaerobic exercise performance, many training programs and dietary supplementation strategies have been focused on elevating muscle buffering capacity and improving anaerobic performance.

Altitude and simulated altitude training has been used in the pursuit of performance enhancement when returning to sea level for many years (Millet, Roels, Schmitt, Woorons, & Richalet, 2010). This type of training has been implemented in a variety forms, including live high-train high (LHTH), live high-train low (LHTL), and live low-train high (LLTH). More specifically, LLTH is usually incorporated with intermittent hypoxic training (IHT). Traditionally, it is has been hypothesized that endurance athletes benefited from LHTH or LHTL owing to an increase in hematological capacity (Levine & StrayGundersen, 1997). However, other factors such as muscle buffering capacity also play a significant role (Gore, Clark, & Saunders, 2007). Performance benefits and associated mechanisms of LHTH have been

discussed thoroughly in several review articles (Bailey & Davies, 1997; Fulco, Rock, & Cymerman, 2000; Millet et al., 2010). More recently, interest has focused on the potential of repeated sprint training in hypoxia (RSH), which combines repeated sprint training and intermittent hypoxic training. The advancement and development of hypoxic technologies have made RSH more convenient and affordable (Girard, Brocherie, & Millet, 2013).

Several studies have demonstrated the effectiveness of RSH (Faiss et al., 2013b; Galvin, Cooke, Sumners, Mileva, & Bowtell, 2013; Puype, Van Proeyen, Raymackers, Deldicque, & Hespel, 2013). RSH is based on the repetition of ‘all-out’ efforts of short ( $\leq 30$ -second) duration activity interspersed with brief incomplete recoveries (Girard, Mendez-Villanueva, & Bishop, 2011). This model differs from standard IHT due to its use of maximal intensity as the training stimulus, which relies heavily on the recruitment of fast-twitch (FT) muscle fibers recruitment under hypoxic conditions (Hautier, Linossier, Belli, Lacour, & Arzac, 1996). Millet and Faiss (2012) reported that mRNA genes for oxygen signaling, oxygen carrying capacity, and pH regulation were up-regulated, whereas mRNA genes involved in mitochondrial biogenesis were down-regulated, reflecting more effective recruitment of FT fibers and the anaerobic energy system following RSH. The advantage of RSH over IHT can be further explained by the greater fractional oxygen extraction from tissue due to decreased oxygen delivery during exercise (McDonough, Behnke, Padilla, Musch, & Poole, 2005). Holliss, Fulford, Vanhatalo, Pedlar, and Jones (2013) reported that the addition of a hypoxic stimulus to training modulates phosphocreatine resynthesis during exercise. Furthermore, Calbet and Lundby (2009) suggested that RSH promotes the utilization of a “functional reserve in the muscle oxygen diffusing capacity”. Thus, RSH has the potential to stimulate beneficial adaptations in terms of PCr

resynthesis and oxygen utilization mediated by hypoxia-inducible factors in skeletal muscle (Faiss et al., 2013b).

In addition to a well-designed and efficacious training program, the appropriate consumption of dietary nutrients is of vital importance to maintain optimal training and maximize competitive performance. Beta-alanine is commonly employed as an ergogenic aid (Artioli, Gualano, Smith, Stout, & Lancha, 2010; Derave, Everaert, Beeckman, & Baguet, 2010; Sale, Saunders, & Harris, 2010) and has been identified as the rate-limiting precursor to carnosine which acts as a physiological buffer (Dunnett & Harris, 1999). Elevating muscle carnosine enhances the intracellular buffering system, attenuating fatigue (Abe, 2000). Numerous studies have demonstrated the effectiveness of beta-alanine supplementation on improving muscle carnosine content (Baguet, Bourgois, Vanhee, Achten, & Derave, 2010; Derave et al., 2007; Hill et al., 2007; Hoffman et al., 2015a; Stellingwerff, Decombaz, Harris, & Boesch, 2012), which may improve performance during high intensity intermittent exercise. However, most studies have focused on prolonged high intensity exercises, and relatively little is known regarding the potential for ergogenic aids, especially with prescribed training programs, to improve physiological and performance responses to multi-effort, short-duration exercises such as repeated sprint training.

Of particular interest is whether the integration of RSH and beta-alanine supplementation is superior to each intervention by itself, in regards to improving exercise performance during various testing modalities. Saunders, Sale, Harris, and Sunderland (2014) examined the effects of beta-alanine supplementation on an acute RSH protocol and found no differences in performance when compared to placebo. Moreover, a recent study demonstrated that combining sprint interval

training in hypoxia and nitrate ingestion increased the proportion of type IIa fibers in muscle, which may contribute to enhanced 30s Wingate performance (De Smet et al., 2016). However, there were several methodological limitations associated with these studies. Saunders et al. (2014) did not include any type of hypoxic training, but only examined intermittent exercise performance in hypoxia. De Smet et al. (2016) failed to blind the participants for normoxia/hypoxia conditions and employed inappropriate statistical procedures. It can be concluded from previous studies that RSH primarily enhances metabolic reactions involved with pH regulation and glycolysis (Faiss et al., 2013b), while the most likely mechanism of action of beta-alanine supplementation is to increase intracellular buffering capacity (Hoffman, Stout, Harris, & Moran, 2015b). Therefore, the combination of RSH and beta-alanine supplementation may provide independent but additive ergogenic effects on muscle buffering capacity through a variety of potential mechanisms and has yet to be evaluated with regard to exercise performance. Considering the lack of research examining the interaction between RSH and beta-alanine supplementation, the purpose of this study was to examine the effects of RSH and beta-alanine supplementation on aerobic, anaerobic, and repeated sprint performance in recreationally trained men.

## **CHAPTER TWO: LITERATURE REVIEW**

### **Acute Effects of Repeated Sprint Training in Hypoxia**

Several investigations have compared acute and training-induced responses to repeated sprint training in hypoxia (RSH) and normoxia (RSN) on performance enhancement, endocrine function, metabolic stress, and immune function (Alvarez-Herms, Julia-Sanchez, Gatterer, Viscor, & Burtscher, 2015; Billaut & Buchheit, 2013; Billaut et al., 2013; Born et al., 2016; Bowtell, Cooke, Turner, Mileva, & Sumners, 2014; Brickley, Hodkinson, & Hortal, 2014; Girard, Brocherie, & Millet, 2016; Goods et al., 2016; Kon, Nakagaki, Ebi, Nishiyama, & Russell, 2015; Morrison, McLellan, & Minahan, 2015) Various modes of exercise, such as cycling, running, swimming and skiing were used in these studies. A comprehensive analysis of the acute effects of repeated sprint training in hypoxia is provided below.

#### **Performance responses**

Bowtell et al. (2014) examined the acute physiological and performance responses to repeated sprints on a non-motorized treadmill with varying levels of inspired oxygen fraction (12.0%, 13.0%, 14.0%, 15.0%, 21.0%) in well-trained athletes. Increased heart rate, minute ventilation, blood lactate, and muscle deoxygenation, together with decreased blood oxygen saturation, pulmonary oxygen uptake and muscle recruitment were observed under all hypoxic conditions. As for performance, peak running speed was well maintained in hypoxia, while fatigue index and speed decrements were significantly greater in only the 12.0% vs 21.0% comparison. These findings were supported by Girard et al. (2016), who assessed neuromuscular changes during knee extension after repeated treadmill sprints in normoxia, moderate (16.8% O<sub>2</sub>) and severe (13.3% O<sub>2</sub>) hypoxia. In this study, a larger sprint decrement score and a shorter

cumulative distance covered was only noted under severe hypoxia, but not moderate hypoxia, when compared to normoxia. Furthermore, post-sprint reduction in isometric maximal voluntary contraction was greater in severe hypoxia compared to normoxia. However, post-sprint alterations in the rate of torque development were similar among the three conditions examined.

Peak and mean power output have been reported to be significantly lower in the last two out of three sets during repeated sprints when performed in hypoxia (14.5% O<sub>2</sub>) compared to the same training session performed in normoxia (Goods et al., 2016). Moreover, blood lactate increased significantly after the training sessions, but no group difference was observed. This is in accordance with a previous study (Brickley et al., 2014), which revealed similar blood lactate concentrations after a single session of repeated cycling sprints performed in hypoxia (14.2% O<sub>2</sub>) and normoxia. The authors also reported that critical power, but not repeated sprint performance, was reduced after RSH when compared with RSN. Likewise, Alvarez-Herms et al. (2015) reported that both blood lactate (measured immediately after exercise) and creatine kinase (measured 24 hours after exercise) were similar among normoxia, and both moderate and severe hypoxia conditions. They also reported that neither moderate nor severe hypoxia affected jumping performance after six sets of 15s all-out consecutive jumps. In contrast, Morrison et al. (2015) reported that blood lactate was significantly higher after repeated running sprints in hypoxia than normoxia. The results of their investigation also indicated that hypoxia impaired the average acceleration in set four, and peak speed and running distance during the last two out of four total sets of running sprints.



## **Physiological responses**

Kon et al. (2015) examined the acute hormonal and metabolic responses to repeated cycling sprints under different simulated altitudes. Glucose, free fatty acids, blood lactate, growth hormone, epinephrine, norepinephrine, and insulin concentrations were evaluated before and 15 min after hypoxia exposure, as well as before and up to 180 min after exercise performed in hypoxia. The results demonstrated that all endocrine and metabolic measures increased significantly after exercise without any between-group differences. However, when performing area under the curve analysis, all-out sprints under severe normobaric hypoxia (13.6% O<sub>2</sub>) induced greater elevation in circulating growth hormone, when compared to moderate hypoxia (16.4% O<sub>2</sub>) and normoxia. The investigators also suggested that the elevated growth hormone may contribute to the increase of free fatty acids observed in circulation after these sprints. Born et al. (2016) examined a group of competitive cross-country skiers and assessed the circadian variation of biomarkers of mucosal immune function, together with mood, in response to an acute session of repeated double-poling sprints performed under normoxic or hypoxic conditions. Similar responses were noted between the experimental conditions. Furthermore, Goods et al. (2016) investigated the acute oxidative stress (F<sub>2</sub>-Isoprostane) and inflammatory (interleukin-6) responses to a single session of repeated running sprints in hypoxia (14.5% O<sub>2</sub>) in Australian footballers. They reported that repeated sprint training at simulated altitude induced greater inflammation at 60-min after exercise than the same training session performed at sea level.

## **Training Adaptations to Repeated Sprint Training in Hypoxia**

With the advancement and development of simulated hypoxic devices, the ‘live low-train high’ methods have become more convenient and affordable. Particularly, the combination of repeated sprint training and intermittent hypoxic training has received considerable attention. Several recent studies compared the effects of RSH and RSN on various testing modalities, including those related to aerobic, single-effort, and repeated-effort performance. In a newly published meta-analysis, Brocherie, Girard, Faiss, and Millet (2017a) concluded that RSH improved average repeated sprint performance to a significantly greater extent than RSN. Though not significant, additional beneficial effects on the best repeated sprint performance during a given session and maximal oxygen consumption were also reported after RSH. The following section contains primarily contemporary studies (see Table 1) while providing a brief review on the effects of RSH on three main performance aspects related to aerobic, anaerobic, and repeated sprint abilities. Furthermore, physiological mechanisms that may mediate the adaptations to exercise performance are also summarized.

### **Training programs**

RSH is characterized by the repetition of several short all-out efforts in simulated or natural altitude environments interspersed with incomplete recoveries (Faiss, Girard, & Millet, 2013a). Reflective of the heterogeneity of participants recruited, various training programs have been employed to accommodate differences related to sporting environment (soccer, rugby, cycling, skiing, field hockey, Australian football), training status (untrained, recreationally-trained, and well-trained), and gender (women, and men). Generally, the duration of RSH interventions ranged between two to five weeks with training frequencies of two to three sessions

per week. However, training protocols differ considerably with regard to modality (overground/treadmill running, cycling ergometer, double-poling ergometer, swimming), sprint times (5-25 s efforts), as well as recovery time within sets (20-45 s) and between sets (5-10 min). As for the environment, RSN is usually conducted at sea level with an O<sub>2</sub> concentration around 20.9%, while the O<sub>2</sub> concentration for RSH ranged from 13.0% to 14.8%, to simulate altitudes between 2,500 and 3,500 m. Most studies chose SpO<sub>2</sub> and heart rate, which serves as a reflection of training intensity, to monitor training response for RSH and RSN.

### **Aerobic performance**

Aerobic performance can be assessed using both laboratory-based (graded exercise testing with gas analysis) and field-based (expressed as total distance or work rates) measurements. Regarding field-based measure, RSH has shown to result in significantly greater improvements in the Yo-Yo intermittent recovery (YYIR) test than RSN (Galvin et al., 2013). However, Gatterer et al. (2015) found that microcycles of maximal shuttle running at simulated altitude and sea level improved YYIR performance to the same extent. This is in accordance with Faiss et al. (2015b), who reported that no differences in mean power output during a sport-specific aerobic test were found between RSH and RSN. Faiss et al. (2013b) indicated that average power output during the three-minute all-out test remained unchanged from pre to post in RSH and RSN. Kasai et al. (2015) found that time to exhaustion, but not maximal oxygen consumption, had a greater increase in RSH compared to RSN in collegiate female athletes. In contrast, Montero and Lundby (2016) reported no additional impact of RSH on endurance performance, as determined by an incremental exercise test and time to exhaustion trials, at sea level in endurance-trained participants. Hamlin, Olsen, Marshall, Lizamore, and Elliot (2017)

also found that YYIR performance was improved throughout the post-training period following both RSH and RSN without any between-group differences. Brocherie, Girard, Faiss, and Millet (2015a) reported that maximal aerobic speed remained unchanged after RSH and RSN in youth soccer players. In another study, Brocherie et al. (2015b) combined RSH and RSN with traditional live-high train-low methods and reported similar improvement in YYIR performance immediately, and three weeks following intervention.

### **Anaerobic performance**

The effects of RSH and RSN on anaerobic performance, such as vertical jump, isometric strength and isokinetic power, has been relatively under-investigated. Due to the recruitment of fast-twitch muscle fibers to meet the neuromuscular demand during RSH (Buchheit & Laursen, 2013a, 2013b), adaptations in short-duration, single-effort performance would be expected. Surprisingly, only a limited number of studies have examined this issue. Faiss et al. (2013b) reported that both RSH and RSN improved 10s single sprint and 30s Wingate performance with no differences between experimental conditions. Brocherie et al. (2015a) found that countermovement jump and sprint performance improved similarly between RSH and RSN, while the best and mean repeated agility time decreased to a greater extent in RSH than RSN. A study by Gatterer et al. (2015) also demonstrated identical change in long jump performance between running-based RSH and RSN.

### **Repeated sprint ability**

Galvin et al. (2013) found that RSH training tended to result in greater distance covered with a smaller speed decrement and greater ventilation compared to RSN during repeated sprint testing. Similarly, Kasai et al. (2015) examined the effects of RSH and RSN on repeated sprint

performance in women and reported that RSH showed significantly greater improvement in repeated sprint performance than RSN. In addition, Faiss et al. (2013b) stated that the average power of all sprints during repeated sprint ability (RSA) testing increased to the same extent in RSH and RSN. Nevertheless, the number of sprints prior to exhaustion was extended only after RSH, but not RSN. However, not all studies have shown beneficial effects of RSH with respect to RSN. Lundby and Robach (2016) first questioned the study by Faiss et al. (2013b) pertaining to the approach used to determine exhaustion, and later reported no difference between RSH and RSN in regards to RSA performance enhancement (Montero & Lundby, 2016). It is worth mentioning that participants in this study (Montero & Lundby, 2016) performed 12 different tests within three days following the intervention period. Therefore, such a condensed schedule may have induced residual fatigue while potentially confounding the training adaptations from RSH and RSN (Millet, Brocherie, Faiss, & Girard, 2017).

As discussed elsewhere (Brocherie et al., 2017a), the exercise modality used to examine RSH and RSN has not been limited to cycling. Faiss et al. (2015b) extended RSH to cross-country skiing, and their results indicated that RSH resulted in the completion of more sprints exceeding 70% of peak power. In a study by Trincat, Woorons, and Millet (2016), 16 competitive swimmers completed six training sessions of either RSH or RSN over two weeks. RSA was tested before and after the intervention via 25m all-out sprints with 35s rest until volitional fatigue. The results demonstrated a greater increase in maximal blood lactate concentration in and a greater number of sprints completed in RSH than in RSN. Though underpowered, Gatterer et al. (2014) first introduced soccer specific shuttle-run sprint training in a simulated altitude and reported that RSH elicited a lower fatigue slope than RSN in a group of

youth male soccer players. A follow-up study in adult male soccer players found a small effect of hypoxia training for RSA performance when compared with training performed at sea level (Gatterer et al., 2015). Brocherie, Millet, and Girard (2016) compared physiological and perceptual responses to six sessions of running-based RSH and RSN over two weeks in field hockey athletes. Results indicated that changes in heart rate were similar between the hypoxic and normoxic conditions; however, ratings of overall perceived discomfort, difficulty breathing and lower-limb discomfort were higher after the first session and reduced to a larger extent during subsequent sessions in RSH compared to RSN. Recently, Hamlin et al. (2017) demonstrated similar RSA performance improvement after three weeks of RSH and RSN with only RSH maintaining improved RSA performance two weeks following the intervention. Thus, RSH may have long-lasting beneficial effects compared to RSN. This is partially in line with Brocherie et al. (2015b), who combined a traditional hypoxia exposure protocol with RSH or RSN and reported both immediate and extended RSA performance improvement after RSH. More specifically, RSA improvement was twice as large in RSH than RSN and was well maintained for three weeks after the intervention.

### **Potential physiological mechanisms**

The beneficial effects of RSH are generally believed to be the result of a hypoxia-induced, non-hematological molecular regulation, mediated by hypoxia inducible factor-1 $\alpha$  subunit (HIF-1 $\alpha$ ), within skeletal muscle. HIF-1 $\alpha$  is an oxygen sensitive transcriptional activator that stabilizes in the nucleus in low oxygen fraction environments (Wang & Semenza, 1995). It has been previously reported that HIF-1 $\alpha$  controls the expression of a series of hypoxia-induced genes in response to decreased oxygen availability (Semenza, Shimoda, & Prabhakar, 2006). Faiss et al.

(2013b) reported that RSH resulted in higher mRNA expression of factors related to pH regulation and glycolysis, and a greater downregulation of factors involved in mitochondrial biogenesis, than RSN. Their findings suggested a potential enhancement of glycolytic metabolism in muscle. In a more recent study, Brocherie et al. (2017b) examined whether RSH induces greater beneficial adaptations in HIF-1 $\alpha$  pathway and its target genes than RSN, when combined with chronic hypoxic exposure. Muscle biopsies were obtained before, immediately after, and three weeks of the intervention. Compared to RSN, RSH elicited greater increases in mRNA levels related to the regulation of oxygen signaling and carrying, mitochondrial biogenesis, as well as certain enzymes implicated in mitochondrial metabolism (Brocherie et al., 2017b).

Several mechanisms have been proposed to explain the physiological adaptations mediating athletic performance improvement as a result of RSH (Brocherie et al., 2017a). Galvin et al. (2013) suggested that performance enhancement associated with RSH was due to decreased cerebral deoxygenation during training. Gatterer et al. (2015) proposed that the altered redox status might explain the difference in performance improvement between RSH and RSN. They reported an identical decrease in markers of oxidative stress at rest with a concomitant increase in plasma antioxidant activity between RSH and RSN. However, increased redox status, expressed as a ratio between the oxidative stress and antioxidant potential, was associated with improvements in RSA mean time. In addition, improvements in YYIR and RSA could be attributed to the diminished resting oxidative stress. Born et al. (2016) assessed changes in mood and mucosal immune function before and after six RSH or RSN training sessions conducted over two weeks, and found increased mucosal immune function with reduced mood for RSH when

compared with RSN. This is particularly important for athletes competing in winter sports since reduced mucosal immune function may be associated with higher risk of chronic fatigue, impaired performance, and overreaching (Papacosta & Nassis, 2011), as well as an elevated incidence of upper respiratory tract infections (Mortatti et al., 2012).



Table 1. Summary of studies examined training adaptations to RSH.

Study	Design	Gender, n (RSH, RSN)	Training Status	O <sub>2</sub> Fraction	Intervention Duration	Training Protocol sets × reps × time	Recovery time within/between sets
Faiss et al. (2013)	Parallel, single-blind	Male (20, 20)	Moderately trained cyclists	14.6%	8 sessions over 4 wks.	3 × 5 × 10s, cycling	20s / 5min
Galvin et al. (2013)	Parallel, single-blind	Male (15, 15)	Collegiate rugby players	13.0%	12 sessions over 4 wks.	1 × 10 × 6s, treadmill running	30s / NA
Gatterer et al. (2014)	Parallel, single-blind	Male (5, 5)	Youth soccer players	14.8%	7-8 sessions over 5 wks.	3 × 5 × 10s, overground running	20s / 5min
Gatterer et al. (2015)	Parallel, single-blind	Male (7, 7)	Amateur soccer players	14.8%	8 sessions over 12 days	3 × 5 × 10s, overground running	20s / 5min
Kasai et al. (2015)	Parallel, single-blind	Female (16, 16)	Collegiate lacrosse players	14.5%	8 sessions over 4 wks.	2 × 10 × 7s, cycling	30s / 10-20min
Faiss et al. (2015)	Parallel, double-blind	Mixed (9, 8)	Elite cross-country skiers	13.8%	6 sessions over 2 wks.	4 × 5 × 10s, double- poling	20s / 5min
Brocherie et al. (2015a)	Parallel, double-blind	Male (8, 8)	Youth soccer players	14.3%	10 sessions over 5 wks.	Varied sets & reps of 15s, treadmill running	15s / 5min
Brocherie et al. (2015b)	Parallel, double-blind	Male (11, 12)	Elite field hockey players	14.2%	6 sessions over 2 wks.	4 × 5 × 5s, overground running	25s / 5min
Goods et al. (2015)	Parallel, single-blind	Male (9, 10)	Trained Australian footballers	Equal to 3000m	15 sessions over 5 wks.	3 × 7 × 5s, cycling	15-35s / 3min
Brocherie et al. (2016)	Parallel, double-blind	Male (11, 12)	Elite field hockey players	14.5%	6 sessions over 2 wks.	4 × 5 × 5s, overground running	25s / 5min
Trincat et al. (2016)	Parallel, unblinded	Male (8, 8)	Highly trained swimmers	Equal to 2000m	6 sessions over 2 wks.	2 × 16 × 15m, front crawl swimming	30s / 20min
Montero et al. (2016)	Crossover, double-blind	Male (15, 15)	Moderately trained cyclists	13.8%	12 sessions over 4 wks.	4 × 5 × 10s, cycling	20s / 5min
Hamlin et al. (2017)	Parallel, single-blind	Male (9, 10)	Highly trained rugby players	14.5%	6 sessions over 3 wks.	4 × 5 × 5s, cycling	25s / 5min

Note. n = sample size; RSH = repeated sprint training in hypoxia; RSN = repeated sprint training in normoxia.

## **Effects of Beta-alanine Supplementation on Athletic Performance: An Update**

Carnosine is an intramuscular dipeptide that is stored within skeletal muscle, which primarily functions as an intracellular buffer (Suzuki et al., 2006). Additionally, carnosine has been shown to act as a calcium regulator by increasing calcium sensitivity during contraction while benefiting calcium release during relaxation (Dutka, Lamboley, McKenna, Murphy, & Lamb, 2012), and as an antioxidant by scavenging free radicals (Klebanov et al., 1998) and chelating transition metals (Kohen, Yamamoto, Cundy, & Ames, 1988). As a non-essential, non-proteogenic amino acid, beta-alanine plays a vital role in carnosine synthesis since its availability is the rate-limiting factor for carnosine synthesis in skeletal muscle (Harris et al., 2006). Although limited by its low synthesis rate endogenously in the liver, chronic beta-alanine supplementation has been shown to dramatically increase the intramuscular carnosine content (Harris et al., 2006). Moreover, there appears to be a dose-response for beta-alanine ingestion and muscle carnosine synthesis. Changes in muscle carnosine from beta-alanine supplementation ranged from 20 to 87%, depending upon dose and duration of the use, method of analysis, muscles examined, and training level of the participants (Hoffman et al., 2015b). Reflecting its popularity as an ergogenic aid, numerous reviews have been conducted to elaborate the physiological mechanisms and performance benefits of beta-alanine supplementation across a wide range of exercise protocols (Bellinger, 2014; Blancquaert, Everaert, & Derave, 2015; Derave et al., 2010; Hobson et al., 2012; Hoffman et al., 2014; Hoffman et al., 2015a; Hoffman et al., 2015b; Quesnele, Laframboise, Wong, Kim, & Wells, 2014; Sale et al., 2010; Trexler et al., 2015).

## **Relationship between beta-alanine supplementation and repeated sprint performance**

It is a widely held view that 1.6 - 6.4 g·day<sup>-1</sup> of beta-alanine supplementation over 4 - 6 weeks can improve exercise performance, especially for open end-point tasks and time trials lasting one to four minutes in duration (Hoffman, Emerson, & Stout, 2012; Trexler et al., 2015). In a recent meta-analysis, Saunders et al. (2016) concluded that high intensity exercise between 30 seconds and 10 minutes resulted in the greatest performance gains from beta-alanine supplementation, while those lasting less than 30 seconds did not appear to benefit from supplementation. Repeated efforts of high intensity exercise invoke muscle acidosis (Bishop, Edge, Davis, & Goodman, 2004) due to the considerable reliance on glycolytic ATP resynthesis (Belfry et al., 2012). Increases in muscle carnosine content resulting from beta-alanine supplementation has been shown to enhance muscle buffering capacity (Abe, 2000), potentially improving repeated sprint performance. However, little attention has been paid to the benefits of beta-alanine supplementation on repeated-effort, short-duration exercises. Therefore, the purpose of the following sections is to review the most recent publications (see Table 2) and provide an update on the effects of beta-alanine supplementation on athletic performance with a focus on repeated sprint ability.

## **Effects of beta-alanine supplementation without prescribed training on repeated sprint performance**

Derave et al. (2007) reported that elevated muscle carnosine levels attenuated fatigue during the later stages of repeated, exhaustive bouts of isokinetic knee extensions. However, it should be noted that isokinetic movement can only be accomplished using a dynamometer, therefore, this testing modality has limited practical implications. Sweeney, Wright, Glenn Brice,

and Doberstein (2010) extended the testing protocol to treadmill running sprints and found no ergogenic effect of beta-alanine supplementation on repeated sprint performance. The authors speculated that their repeated sprint protocol may have required a greater reliance on PCr resynthesis rather than the removal of  $H^+$ . Although the average lactate concentration after testing was about  $12 \text{ mmol}\cdot\text{L}^{-1}$ , it should be noted that the blood sample was collected five minutes after exercise. Therefore, it is difficult to exclude the possibility that the exercise intensity utilized during testing was insufficient to challenge muscle buffering capacity. Saunders et al. (2014) also employed treadmill running and examined the effects of beta-alanine supplementation on repeated sprint performance in hypoxia, which should increase the reliance on anaerobic glycolysis and muscle buffering capacity. However, repeated sprint performance was not improved following beta-alanine supplementation. This was consistent with their previous study (Saunders, Sale, Harris, & Sunderland, 2012), which showed no effect of beta-alanine on repeated 15m sprints during the Loughborough intermittent shuttle test. Again, the reported low lactate levels ( $3\text{-}6 \text{ mmol}\cdot\text{L}^{-1}$ ) after testing indicated that the intensity of the test employed may not have been sufficient to challenge muscle buffering capacity and elicit performance decrements. Similarly, Ducker, Dawson, and Wallman (2013) found that beta-alanine only marginally improved performance during repeated sprints. Noteworthy, the testing protocol (Ducker et al., 2013) only resulted in limited elevation of blood lactate concentrations ( $7\text{-}8 \text{ mmol}\cdot\text{L}^{-1}$ ) and decrease in blood pH (about 7.3). Hoffman et al. (2015a) reported no effect of beta-alanine ingestion on repeated sprint performance in soldiers. The authors suggested that the short duration (less than 10s) and low number (e.g., five) of sprints was unlikely to induce a significant decline in muscle pH.

Recently, two studies reported ergogenic benefits of beta-alanine supplementation on repeated front crawl swimming sprints in water polo athletes (Brisola, Artioli, Papoti, & Zagatto, 2016; Claus et al., 2017). More specifically, Brisola et al. (2016) found a likely beneficial effect on repeated sprint performance in trained athletes, whereas Claus et al. (2017) reported a significant improvement in mean time and total time during repeated front crawl swimming sprints, as well as an increased throwing velocity and fatigue resistance in a specific water polo repeated sprint testing. This is consistent with Tobias et al. (2013), who demonstrated that beta-alanine supplementation significantly increased total work during a repeated upper-body Wingate test. In addition, the investigators also noted increased peak and mean power during testing accompanied with an augmented blood lactate response (increased from 11 to 13 mmol·L<sup>-1</sup>) immediately after testing. Likewise, de Andrade Kratz et al. (2016) demonstrated that beta-alanine supplementation successfully improved performance in a repeated judo specific fitness test, and elicited greater blood lactate concentrations (increased from 9 to 12 mmol·L<sup>-1</sup>) immediately after testing.

### **Effects of beta-alanine supplementation combined with prescribed training on repeated sprint performance**

All of the aforementioned studies involved participants maintaining their regular training or physical activity as opposed to incorporating training interventions with beta-alanine supplementation. Only two studies have provided prescribed short-duration training involving repeated efforts with evaluation of changes in repeated sprint performance. Cochran et al. (2015) reported that beta-alanine supplementation prior to and during six weeks of sprint interval training increased muscle carnosine level by about 50% in active men, but did not enhance

training workload or repeated Wingate performance. Blood lactate levels were not reported. The authors explained that the ergogenic benefits of beta-alanine may have been masked by the increased skeletal muscle buffering capacity elicited by the sprint interval training program. In contrast, Bellinger and Minahan (2016a) reported that beta-alanine supplementation enhanced sprint interval training intensity and resulted in additional benefits to repeated sprint performance in trained cyclists than sprint interval training only. The investigators also reported that blood lactate concentrations in the beta-alanine group were significantly higher than in the placebo group following the last two bouts of 4 × 1 km sprint cycling sessions. The inconsistency in these findings may be attributed to differences in training status of the participants, training interventions, and testing protocols. Therefore, future studies should address the underlying mechanisms of these discrepancies.

### **Co-ingestion of beta-alanine and other supplements on repeated sprint performance**

Muscle buffering capacity refers to the ability to buffer or regulate proton accumulation resulting from muscle acidosis (Edg, Bishop, Hill-Haas, Dawson, & Goodman, 2006). Acid buffers within the human body are generally categorized into intracellular and extracellular buffers. In theory, repeated sprint performance could be enhanced if any of these buffering systems are enhanced. The additive effects of beta-alanine and sodium bicarbonate co-ingestion on repeated sprint performance has been previously examined, with conflicting results. Saunders et al. (2014) reported no additive effects of co-supplementation on repeated treadmill running sprints, while Ducker et al. (2013) suggested that an acute dose of sodium bicarbonate ingestion improved repeated sprint performance more than beta-alanine supplementation alone and co-ingestion. Interestingly, Tobias et al. (2013) demonstrated that chronic beta-alanine and sodium

bicarbonate supplementation separately improved repeated upper-body Wingate performance to a similar extent, whereas co-ingestion elicited an additive ergogenic effect.

### **Conclusion**

Both RSH and beta-alanine supplementation have been shown to benefit performance. Previous research indicates that these interventions have different mechanisms of action. Consequently, a potential additive effect may exist if RSH and beta-alanine supplementation are combined. To the best of our knowledge, no study has examined the additive effect of RSH and beta-alanine supplementation. Therefore, the aim of this investigation was evaluate whether RSH plus beta-alanine supplementation elicited greater performance and physiological adaptations when compared to individual interventions involving only RSH or beta-alanine supplementation. The expected outcome of these comparisons was that both groups would improve performance after intervention, and that greater muscle buffering capacity in the combined group would result in greater improvements in working capacity, exercise tolerance, and recovery in comparison to RSH or beta-alanine supplementation alone.

Table 2. Summary of studies examined ergogenic effects of beta-alanine on repeated sprint performance.

Study	Parallel design	Gender (BA, PL)	Training status	Total dosage	Training protocol	Performance testing
Derave et al. (2007)	Double-blind, placebo-controlled	Male (8, 7)	Trained track & field athletes	134.4g	Non-prescribed training	5 × 30 isokinetic knee extensions at 180°/s with 1min rest
Sweeney et al. (2010)	Double-blind, non-placebo controlled	Male (9, 10)	Active individuals	154.0g	Non-prescribed training	2 × 5 × 5s treadmill running with 45s/2min rest intra/inter sets
Saunders et al. (2012)	Double-blind, placebo-controlled	Male (8, 8)	Elite hockey players	179.2g	Non-prescribed training	Loughborough intermittent shuttle test
Saunders et al. (2012)	Double-blind, placebo-controlled	Male (10, 10)	Active individuals	179.2g	Non-prescribed training	Loughborough intermittent shuttle test
Ducker et al. (2013)	Single-blind, placebo-controlled	Male (6, 6)	Competitive athletes	168.0g	Non-prescribed training	3 × 6 × 20m overground running with 25s/4min rest intra/inter sets
Tobias et al. (2013)	Double-blind, placebo-controlled	Male (10, 9)	Trained judo & jiu-jitsu athletes	179.2g	Non-prescribed training	4 × 30s upper-body Wingate test with 3min rest
Saunders et al. (2014)	Double-blind, placebo-controlled	Male (8, 8)	Active individuals	201.6g	Non-prescribed training	5 × 6s treadmill running with 24s rest
Cochran et al. (2015)	Double-blind, placebo-controlled	Male (12, 12)	Active individuals	224.0g	Prescribed sprint interval training	4 × 30s Wingate test with 4min rest
Hoffman et al. (2015)	Double-blind, placebo-controlled	Male (9, 9)	Elite Soldiers	180.0g	Non-prescribed training	5 × 30m sprints with 5s shooting
Bellinger et al. (2016)	Double-blind, placebo-controlled	Male (7, 7)	Trained cyclists	179.2g	Prescribed sprint interval training	4 × 1km cycling sprints with 4min rest
Brisola et al. (2016)	Double-blind, placebo-controlled	Male (11, 11)	Trained water polo athletes	163.2g	Non-prescribed training	6 × 10m front crawl swimming sprints with 17s rest
de Andrade Kratz et al. (2016)	Double-blind, placebo-controlled	Male (12, 11)	Trained judo athletes	179.2g	Non-prescribed training	3 bouts of judo specific fitness test with 3min rest
Claus et al. (2017)	Double-blind, placebo-controlled	Male (7, 7)	Youth water polo athletes	268.8g	Non-prescribed training	8 × 15m front crawl swimming sprints with 30s rest

Note. n = sample size; RSH = repeated sprint training in hypoxia; RSN = repeated sprint training in normoxia.



## **CHAPTER THREE: METHODOLOGY**

### **Participants**

Fifty-two healthy men were recruited by word of mouth and flyers. Participants with exposure to altitude over 1,500m during the previous 6 months were excluded. Among the 52 eligible participants initially recruited, two individuals withdrew from the study due to training intolerance, 12 individuals did not finish the training protocol due to reasons not directly related to the study. Consequently, 38 participants (see Table 3) completed all testing, training and supplementation protocols and their data were included in the final analysis. Participants were recreationally active. Participants were non-smokers and did not use any supplements that contained beta-alanine for at least 9 weeks prior to the study, or during the study period. They were instructed to maintain their habitual physical activity level (5-7 hours resistance/endurance training per week) and normal diet throughout the study. This investigation was approved by the University of Central Florida Institutional Review Board. All participants provided written informed consent after clearing any physical and medical limitations, as determined by Medical and Activity History Questionnaire and Physical Activity Readiness Questionnaire, and being fully informed about the content of the study and the risks involved.

Table 3. Participant characteristics.

	HB ( $n = 10$ )	HP ( $n = 9$ )	NB ( $n = 11$ )	NP ( $n = 8$ )
Age (y)	22.5 ± 2.7	22.7 ± 2.8	22.6 ± 2.9	22.6 ± 2.9
Height (cm)	174.9 ± 6.9	174.8 ± 7.2	175.2 ± 6.8	174.5 ± 7.4
Body Mass (kg)	76.9 ± 8.4	76.3 ± 9.2	76.7 ± 8.9	76.5 ± 9.3

*Note.* Data are mean ± standard deviation (SD) and represent baseline characteristics of the participants training in hypoxia while receiving beta-alanine (HB) or placebo (HP) supplementation and participants training in normoxia while receiving beta-alanine (NB) or placebo (NP) supplementation.  $n$  = sample size.

### Experimental Design

This investigation employed a randomized, double-blind, placebo controlled design, and a single-blind manner for training. All participants completed a resting blood draw, body composition assessments, and graded exercise testing on day 1, a lower body repeated sprint testing on day 2, and a 3-min all-out cycling testing on day 3. Following the pre-testing (PRE) assessment, participants were evenly divided into 4 groups: normoxia + placebo group (NP), hypoxia + placebo group (HP), normoxia + beta-alanine group (NB) and hypoxia + beta-alanine group (HB). The fraction of inspired oxygen and simulated altitude were 14.5-14.2% (e.g., 2800-3000 m) for hypoxia, and 20.9-20.1% and (e.g. 0-300 m) for normoxia, respectively. To compare changes in anaerobic performance, each group completed a 4-week training program (two sessions per week) and supplementation protocol (6.4 g per day) under the direct supervision of certified strength and conditioning specialists (CSCS). Participants completed at least 95% of their respective repeated sprints and 95% of the supplementation protocol over the course of 4-week period. Post-testing (POST) occurred following the completion of the 4-week training program. The study design is illustrated in Figure 1.

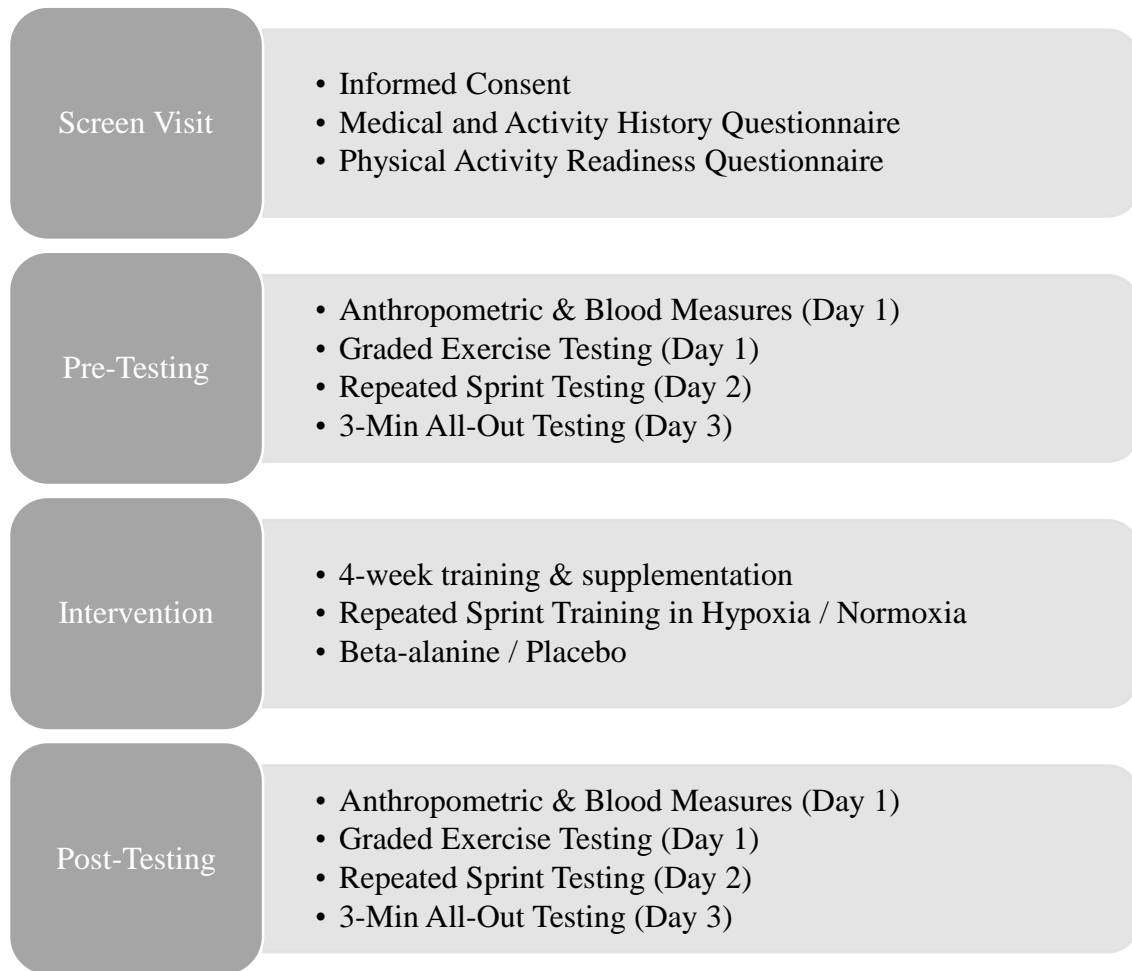


Figure 1. Illustration of study design.

### **Anthropometric assessments**

Anthropometric measurements for all participants were conducted in the following sequence: height, body mass and body composition. Height ( $\pm 0.1$  cm) and body mass ( $\pm 0.1$  kg) were determined using a Health-o-meter Professional (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA) with the participants standing barefoot, with feet together, in their normal daily attire. Body composition were determined using air displacement plethysmography. Participants dressed down to their undergarments, removed their footwear, including socks, put on a swim cap provided and sit in the device (BOD POD, COSMED, Rome, Italy) for measurement to determine body composition. Values for body fat percentage were recorded.

### **Graded exercise testing**

An incremental test to volitional exhaustion was performed by each participant on a cycle ergometer to determine maximal oxygen consumption ( $VO_{2peak}$ ), gas exchange threshold (GET), and respiratory compensation point (RCP). Prior to testing, each participant completed a standardized warm-up consisting of 5-min cycling at 120W and a dynamic stretch which included 10 repetitions of body weight squats, alternating lunges, walking knee hugs and walking butt kicks. Following the warm-up, each participant was fitted with a heart rate monitor to record the participants' heart rate. Participants maintained a pedaling cadence of 70–75 revolutions per minute (RPM) at an initial workload of 60 W. The workload increased 1 W every 3 s until the participant was unable to maintain a cadence above 70 RPM for 10 s despite verbal encouragement, or volitional fatigue. Prior to each graded exercise test, metabolic gas analyzer (Quark CPET, COSMED, Rome, Italy) was calibrated with room air and gases of known concentration. Oxygen ( $O_2$ ), carbon dioxide ( $CO_2$ ), ventilation (VE), and respiratory exchange

ratio (RER) will be monitored continuously and expressed breath-by-breath.  $\text{VO}_2$  peak was determined as the highest  $\text{VO}_2$  value. The GET was defined as the  $\text{VO}_2$  value corresponding to the intersection of two linear regression lines derived separately from the data points below and above the breakpoint in the carbon dioxide production rate ( $\text{VCO}_2$  -versus-  $\text{VO}_2$  relationship). The RCP was defined as the  $\text{VO}_2$  value corresponding to the point of departure from linearity of the  $\text{VE}$  -versus-  $\text{VCO}_2$  relationship.

### **Repeated sprint testing**

Each participant performed a total of six 10-second lower-body cycling Wingate tests with a 7.5% body mass loading which was interspersed with 60 s active recovery periods. Besides the standardized warm-up described previously, the participants also completed several short sprints on the ergometer (894E, Monark Exercise AB, Vansbro, Sweden) at the same resistance to be used during the test. Following the warm-up and prior to each successive sprint, the participants commenced with maximal pedaling after a standardized 10 s countdown. Verbal encouragement was provided throughout the sprints. At the end of the test, the load was removed and the participant continued to exercise at 60 RPM on the unloaded ergometer for at least 5 min. Peak power, mean power and fatigue index was recorded. Using a metabolic gas analyzer (Quark CPET, COSMED, Rome, Italy), metabolic data during the testing was also collected for later analysis.

### **3-min all-out testing**

Each participant performed the 3-min maximal effort cycling test using a calibrated electronically braked cycle ergometer (Excalibur, Lode, Groningen, the Netherlands). Following the aforementioned standardized warm-up, the participant completed 60 s of unloaded cycling at

90 RPM, followed by an all-out 3-min effort with resistance being set as a function of pedaling rate. Participants was asked to accelerate to approximately 110 RPM over the last 5 s of the baseline period. The resistance was adjusted during the all-out effort using the pedaling rate dependent linear mode on the cycle ergometer that used a linear factor (power/cadence<sup>2</sup>) based on the power output at a given pedaling rate (70 RPM) being equal to 50% of the difference between the power output at GET and VO<sub>2</sub>peak assessed during the graded exercise test described previously (Wang et al., 2017). To prevent pacing and ensure an all-out effort, the participant was not aware of the elapsed time and strong verbal encouragement was provided. Critical power was calculated as the average power output during the final 30 s of the test and anaerobic working capacity was calculated as the work-time integral above critical power. Additionally, metabolic data during the testing was also collected via a metabolic gas analyzer (Quark CPET, COSMED, Rome, Italy).

### **Repeated sprint training intervention**

A repeated sprint training program (see Figure 2) consisting of eight training sessions (2 sessions per week for 4 weeks) was employed and each session was separated by at least 48 h. The HP and HB groups trained under hypoxia, while the NP and NB groups trained under normoxia. Following a standardized warm-up, the participants completed three sets of 5 × 10 s all-out repeated sprints with a 7.5% body mass loading which was interspersed with 20 s active recovery periods. A 5-min recovery period at was given between sets and each training protocol ended with a 10-min recovery period. Subjects was instructed to perform all-out sprints trying to reach and maintain the highest power output for every sprint, and strong verbal encouragement was given during each sprint. Heart rate from a wireless device (BioHarness 3, Zephyr

Technology Corporation, Annapolis, MD) and arterial oxygen saturation ( $SpO_2$ ) via pulse oximeter ( $GO_2$ , Nonin Medical, Inc., Plymouth, MN) was monitored throughout each training session.  $SpO_2$  decline was calculated as the average value of the delta changes between the  $SpO_2$  value at the beginning and the end of each training session.

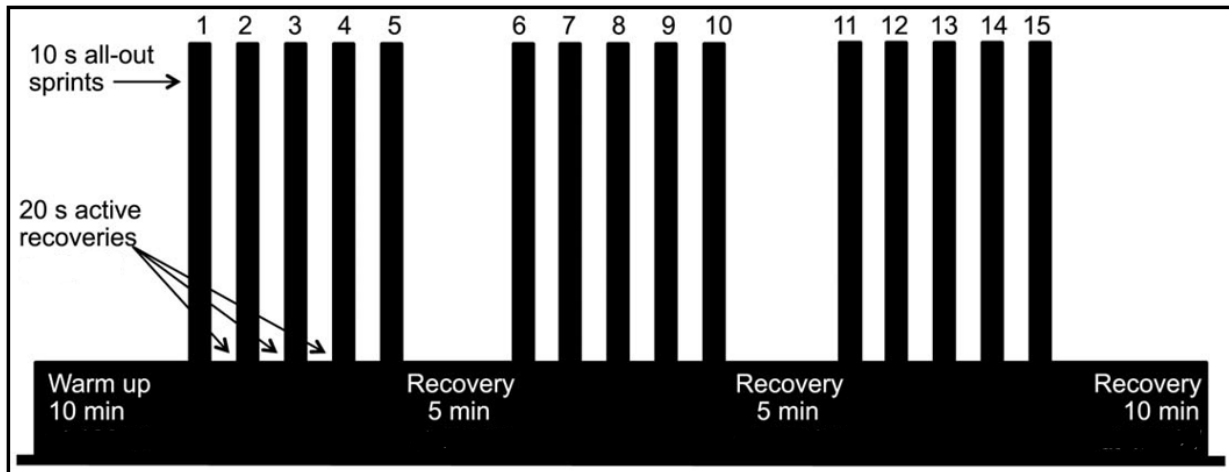


Figure 2. Illustration of a repeated training session.

### **Beta-alanine supplementation**

The NB and HB groups received 6.4 g of sustained release beta-alanine (Natural Alternative Inc, Carlsbad, CA) per day during the 4-week period, while the HP and NP groups received the same amount of placebo (rice powder) throughout the study. The dosing regimen was similar to that reported by Saunders et al. (2014) consisting of two 800 mg beta-alanine or placebo tablets ingested four times per day at 3-4 hour intervals for 28 days. Compliance was monitored using supplementation logs. Additionally, participants completed a 3-day dietary recall before and after the supplementation period.

### **Blood samples**

Blood samples was collected during the testing sessions before and after the 4-week training period. During day 1, a resting blood sample was obtained by venipuncture of the forearm vein following a 15-min equilibration period. All blood samples were collected into a Vacutainer® tubes containing K<sub>3</sub>EDTA (4 ml) for determination of hematocrit and hemoglobin concentrations. In addition, blood samples were also obtained by finger-prick before and after the repeated sprint test and the critical power test to determine blood lactate concentration.

### **Biochemical analysis**

The hemoglobin and hematocrit was measured immediately after the blood draw via a hematology analyzer (Ac·T diff2™, Beckman Coulter, Brea, CA), which was calibrated every day using control reagents. Coefficients of variation for each assay was 6.3 % for hemoglobin, and 6.1 % for hematocrit. Blood lactate concentrations were analyzed using a portable device (Lactate Plus, Nova Biomedical, Waltham MA) from 20- µl fingertip capillary blood.



## **Statistical analysis**

One-way (Group) analysis of variance (ANOVA) was performed to compare training volume, supplementation compliance, and SpO<sub>2</sub> during training. Since there were no baseline differences, one-way (Group) ANOVA with repeated measures (pre-test vs post-test) was performed to examine pre-post changes for anthropometric and hematological measures. To account for baseline differences between groups, two-way (Altitude x Supplement) analysis of covariance (ANCOVA) was performed on post-test values for all performance measures between groups with pre-test values serving as the covariate. The assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurement of the covariate were verified. For effect size, the partial eta squared statistic was calculated, and 0.01, 0.06, and 0.14 was interpreted as small, medium, and large effect sizes, respectively. An alpha of  $p < 0.05$  was established a priori. All data were reported as mean  $\pm$  SD. In addition, post-test performance measures were reported as mean  $\pm$  95% confidence intervals to indicate meaningful changes as compared with covariate adjusted pre-test values. Statistical software (IBM SPSS Statistics for Windows, Version 22.0; Armonk, NY: IBM Corp) was used for all analyses.

## CHAPTER FOUR: RESULTS

### Training Volume and Supplementation Compliance

No significant difference was observed between groups for training volume ( $F_{3,33} = 1.268$ ,  $p = 0.301$ ) or supplementation compliance ( $F_{3,33} = 1.541$ ,  $p = 0.222$ ). NB, NP, HB, and HP consumed  $179.2 \pm 0.0$  g,  $175.7 \pm 4.3$  g,  $178.6 \pm 1.1$  g, and  $177.2 \pm 4.2$  g out of 180 g of the provided supplement and completed,  $120.0 \pm 0.0$ ,  $115.9 \pm 10.5$ ,  $119.1 \pm 1.7$ , and  $119.8 \pm 0.7$  of the 120 required sprints, respectively.

### Arterial Oxygen Saturation during Training

Significant differences were noted in SpO<sub>2</sub> measures at rest ( $F_{3,33} = 185.299$ ,  $p = 0.01$ ); during training ( $F_{3,33} = 64.695$ ,  $p = 0.01$ ) and the difference ( $\Delta$ ) from training and rest ( $F_{3,33} = 19.763$ ,  $p = 0.01$ ). Resting SpO<sub>2</sub> values for NB ( $96.4 \pm 0.9$  %) and NP ( $97.2 \pm 0.7$  %) were significantly higher ( $p = 0.01$ ) than that observed for HB ( $88.8 \pm 1.1$  %) and HP ( $89.6 \pm 1.1$  %). Training SpO<sub>2</sub> values for NB ( $94.1 \pm 3.5$  %) and NP ( $95.7 \pm 0.8$  %) were significantly greater ( $p = 0.01$ ) than that for HB ( $78.6 \pm 2.7$  %) and HP ( $81.5 \pm 4.6$  %). SpO<sub>2</sub> decline for NB ( $2.3 \pm 3.5$  %) and NP ( $1.5 \pm 0.9$  %) were significantly lower ( $p = 0.01$ ) than that for HB ( $10.2 \pm 2.4$  %) and HP ( $8.0 \pm 3.8$  %). No difference ( $p > 0.05$ ) were noted between NB and NP, or between HB and HP.

### Anthropometric and Hematological Measures

No significant interaction was observed between time and group ( $F_{3,33} = 0.632$ ,  $p = 0.600$ ,  $\eta^2 = 0.054$ ) for body mass, nor was any significant main effect for time noted ( $F_{3,33} = 0.654$ ,  $p = 0.424$ ,  $\eta^2 = 0.019$ ). No significant interaction was observed between time and group ( $F_{3,33} = 1.158$ ,  $p = 0.341$ ,  $\eta^2 = 0.095$ ) for percent body fat, nor was there a main effect for time

noted ( $F_{3,33} = 0.717$ ,  $p = 0.403$ ,  $\eta^2 = 0.021$ ). No significant interaction was observed between time and group ( $F_{3,33} = 0.291$ ,  $p = 0.832$ ,  $\eta^2 = 0.026$ ) for hemoglobin, and no main effect for time was noted ( $F_{3,33} = 1.026$ ,  $p = 0.319$ ,  $\eta^2 = 0.030$ ). No significant interaction was observed between time and group ( $F_{3,33} = 0.093$ ,  $p = 0.963$ ,  $\eta^2 = 0.008$ ) for hematocrit, and no main effect for time was noted ( $F_{3,33} = 2.507$ ,  $p = 0.123$ ,  $\eta^2 = 0.071$ ). Results indicated that anthropometric and hematological measures were unchanged before and following intervention for all groups (see Table 4).

### **Graded Exercise Testing**

Unadjusted raw data for graded exercise testing variables are shown in Table 5.

ANCOVA results with covariate values are shown in Figure 3.

#### **Maximal oxygen consumption (VO<sub>2</sub>max)**

No significant altitude x supplement interaction was noted ( $F_{1,32} = 0.864$ ,  $p = 0.360$ ,  $\eta^2 = 0.026$ ). In addition, no main effects for altitude ( $F_{1,32} = 3.842$ ,  $p = 0.059$ ,  $\eta^2 = 0.107$ ) or supplement ( $F_{1,32} = 0.050$ ,  $p = 0.824$ ,  $\eta^2 = 0.002$ ) were observed.

#### **Peak power output (PPO)**

No significant altitude x supplement interaction was noted ( $F_{1,32} = 0.305$ ,  $p = 0.584$ ,  $\eta^2 = 0.009$ ). In addition, no main effects for altitude ( $F_{1,32} = 0.417$ ,  $p = 0.523$ ,  $\eta^2 = 0.013$ ) or supplement ( $F_{1,32} = 0.275$ ,  $p = 0.604$ ,  $\eta^2 = 0.009$ ) were observed.

#### **Oxygen consumption at RCP (VO<sub>2</sub>RCP)**

No significant altitude x supplement interaction was noted ( $F_{1,32} = 0.020$ ,  $p = 0.888$ ,  $\eta^2 = 0.001$ ). In addition, no main effect for supplement ( $F_{1,32} = 0.014$ ,  $p = 0.908$ ,  $\eta^2 = 0.001$ ) was noted as well. However, a main effect for altitude ( $F_{1,32} = 5.029$ ,  $p = 0.032$ ,  $\eta^2 = 0.136$ ) was

observed, with the collapsed values for HB and HP ( $38.44 \pm 3.33 \text{ ml}\cdot\text{min}\cdot\text{kg}^{-1}$ ) being greater than that of NB and NP ( $35.96 \pm 3.36 \text{ ml}\cdot\text{min}\cdot\text{kg}^{-1}$ ).

### **Power output at RCP (PRCP)**

No significant altitude x supplement interaction was noted ( $F_{1, 32} = 1.754$ ,  $p = 0.195$ ,  $\eta^2 = 0.052$ ). In addition, no main effect for supplement ( $F_{1, 32} = 0.044$ ,  $p = 0.834$ ,  $\eta^2 = 0.001$ ) was noted as well. However, a main effect for altitude ( $F_{1, 32} = 5.091$ ,  $p = 0.031$ ,  $\eta^2 = 0.137$ ) was observed, with the collapsed values for HB and HP ( $2.91 \pm 0.18 \text{ W}\cdot\text{kg}^{-1}$ ) being greater than that of NB and NP ( $2.77 \pm 0.19 \text{ W}\cdot\text{kg}^{-1}$ ).

### **Repeated sprint testing**

Unadjusted raw data for repeated sprint testing variables are shown in Table 6. ANCOVA results with covariate values are shown in Figure 4.

### **Average power output of all sprints (RST\_AP)**

No significant altitude x supplement interaction was noted ( $F_{1, 32} = 1.252$ ,  $p = 0.272$ ,  $\eta^2 = 0.038$ ). In addition, no main effects for altitude ( $F_{1, 32} = 2.158$ ,  $p = 0.152$ ,  $\eta^2 = 0.063$ ) or supplement ( $F_{1, 32} = 0.864$ ,  $p = 0.360$ ,  $\eta^2 = 0.026$ ) were noted as well.

### **Average power output of the last sprint (RST\_AP5)**

No significant altitude x supplement interaction was noted ( $F_{1, 32} = 0.091$ ,  $p = 0.765$ ,  $\eta^2 = 0.003$ ). In addition, no main effect for supplement ( $F_{1, 32} = 0.008$ ,  $p = 0.930$ ,  $\eta^2 = 0.001$ ) was noted. However, a main effect for altitude ( $F_{1, 32} = 4.107$ ,  $p = 0.050$ ,  $\eta^2 = 0.114$ ) was noted, with the collapsed values for HB and HP ( $8.66 \pm 0.51 \text{ W}\cdot\text{kg}^{-1}$ ) being greater than that of NB and NP ( $8.32 \pm 0.51 \text{ W}\cdot\text{kg}^{-1}$ ).

### **Lactate concentration after RST (RST\_La)**

No significant altitude x supplement interaction was observed ( $F_{1,32} = 0.053$ ,  $p = 0.819$ ,  $\eta^2 = 0.002$ ). In addition, no main effects for altitude ( $F_{1,32} = 0.284$ ,  $p = 0.598$ ,  $\eta^2 = 0.009$ ) or supplement ( $F_{1,32} = 2.664$ ,  $p = 0.112$ ,  $\eta^2 = 0.077$ ) were noted.

### **Heart rate at 60s after RST (RST\_HR60)**

No significant altitude x supplement interaction was noted ( $F_{1,30} = 1.802$ ,  $p = 0.190$ ,  $\eta^2 = 0.057$ ). In addition, no main effect for supplement ( $F_{1,30} = 1.154$ ,  $p = 0.291$ ,  $\eta^2 = 0.037$ ) was observed. However, a main effect for altitude ( $F_{1,30} = 5.370$ ,  $p = 0.027$ ,  $\eta^2 = 0.152$ ) was noted, with the collapsed values for HB and HP ( $144.79 \pm 7.52$  bpm) being smaller than that of NB and NP ( $150.29 \pm 7.62$  bpm).

## **3-Min All-Out Testing**

Unadjusted raw data for 3-min all-out testing variables are shown in Table 7. ANCOVA results with covariate values are shown in Figure 5.

### **Critical power (CP)**

No significant altitude x supplement interaction was noted ( $F_{1,32} = 0.124$ ,  $p = 0.727$ ,  $\eta^2 = 0.004$ ). In addition, no main effects for altitude ( $F_{1,32} = 4.103$ ,  $p = 0.051$ ,  $\eta^2 = 0.114$ ) or supplement ( $F_{1,32} = 0.691$ ,  $p = 0.412$ ,  $\eta^2 = 0.021$ ) were observed.

### **Anaerobic working capacity (AWC)**

No significant altitude x supplement interaction was observed ( $F_{1,32} = 1.561$ ,  $p = 0.221$ ,  $\eta^2 = 0.047$ ). In addition, no main effect for altitude ( $F_{1,32} = 2.627$ ,  $p = 0.115$ ,  $\eta^2 = 0.076$ ) was noted. However, a main effect for supplement ( $F_{1,32} = 5.570$ ,  $p = 0.025$ ,  $\eta^2 = 0.148$ ) was noted,

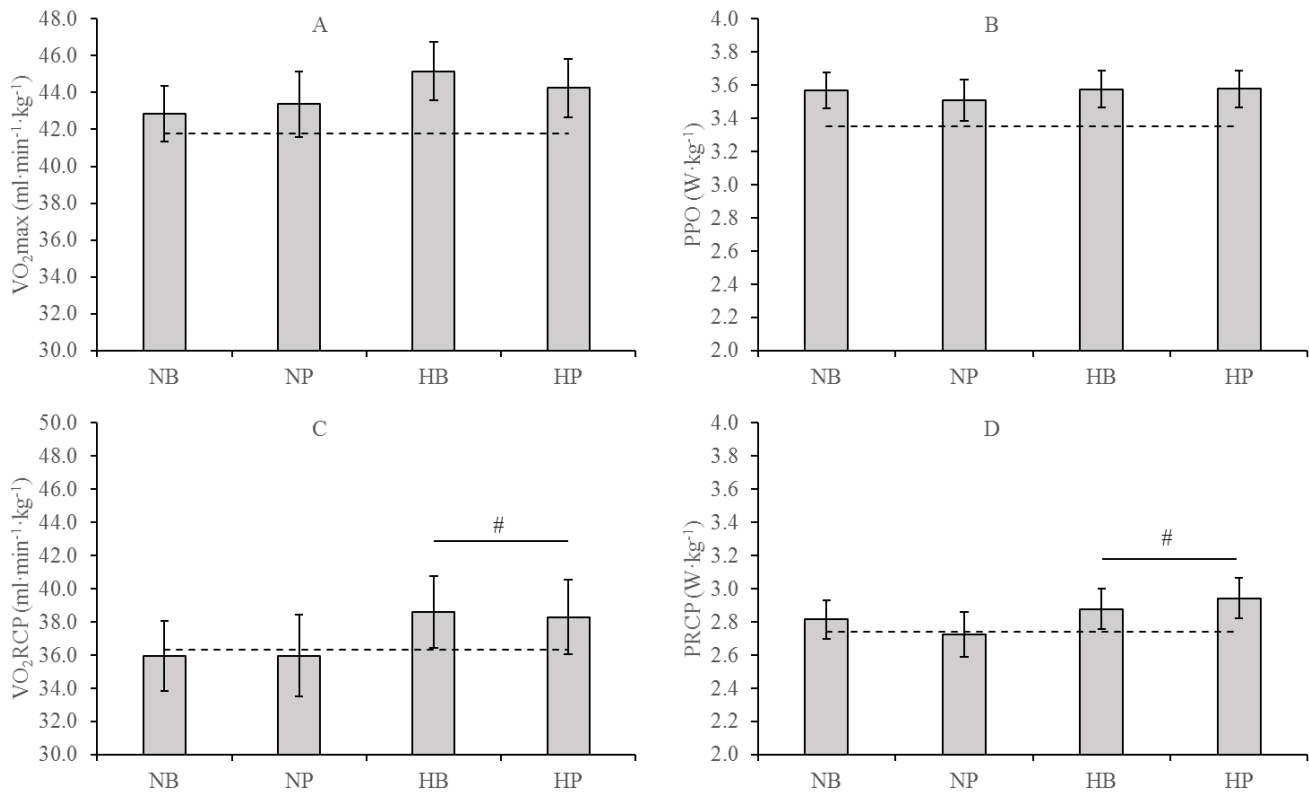
with the collapsed values for HB and NB ( $0.24 \pm 0.04 \text{ kJ}\cdot\text{kg}^{-1}$ ) being greater than that of HP and NP ( $0.21 \pm 0.03 \text{ kJ}\cdot\text{kg}^{-1}$ ).

### **Total work (TW)**

No significant altitude x supplement interaction was noted ( $F_{1, 32} = 1.640$ ,  $p = 0.209$ ,  $\eta^2 = 0.049$ ). In addition, no main effect for supplement ( $F_{1, 32} = 1.866$ ,  $p = 0.182$ ,  $\eta^2 = 0.055$ ) was observed. A main effect for altitude ( $F_{1, 32} = 9.402$ ,  $p = 0.004$ ,  $\eta^2 = 0.227$ ) was noted, with the collapsed values for HB and HP ( $0.73 \pm 0.03 \text{ kJ}\cdot\text{kg}^{-1}$ ) being greater than that of NB and NP ( $0.70 \pm 0.03 \text{ kJ}\cdot\text{kg}^{-1}$ ).

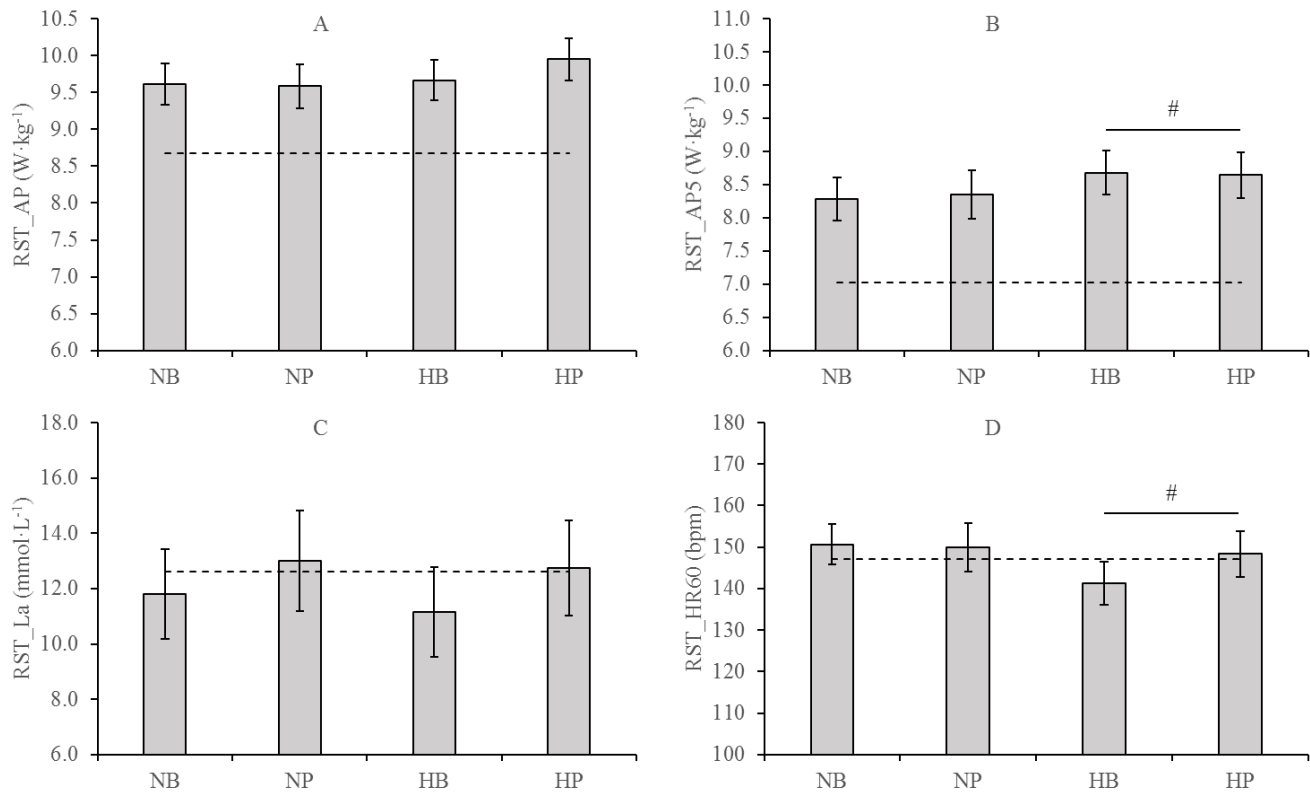
### **Lactate concentration after CP (CP\_La)**

No significant altitude x supplement interaction was observed ( $F_{1, 32} = 0.109$ ,  $p = 0.744$ ,  $\eta^2 = 0.003$ ). In addition, no main effects for altitude ( $F_{1, 32} = 0.022$ ,  $p = 0.883$ ,  $\eta^2 = 0.001$ ) or supplement ( $F_{1, 32} = 1.024$ ,  $p = 0.319$ ,  $\eta^2 = 0.031$ ) were noted as well.



*Note.* Mean values ( $\pm 95\%$  confidence interval) for posttest adjusted for initial differences in pretest (dash line) for normoxia + beta-alanine group (NB), normoxia + placebo group (NP), hypoxia + beta-alanine group (HB) and hypoxia + placebo group (HP): A. Average power output of all sprints (RST\_AP; covariate: adjusted pretest mean =  $8.68 \text{ W}\cdot\text{kg}^{-1}$ ); B. Average power output of the last sprint (RST\_AP5; covariate: adjusted pretest mean =  $7.03 \text{ W}\cdot\text{kg}^{-1}$ ); C. Lactate concentration after repeated sprint testing (RST\_La; covariate: adjusted pretest mean =  $12.61 \text{ mmol}\cdot\text{L}^{-1}$ ); D. Heart rate at 60s after repeated sprint testing (RST\_HR60; covariate: adjusted pretest mean =  $147.94 \text{ bpm}$ ); # indicates main effect for altitude.

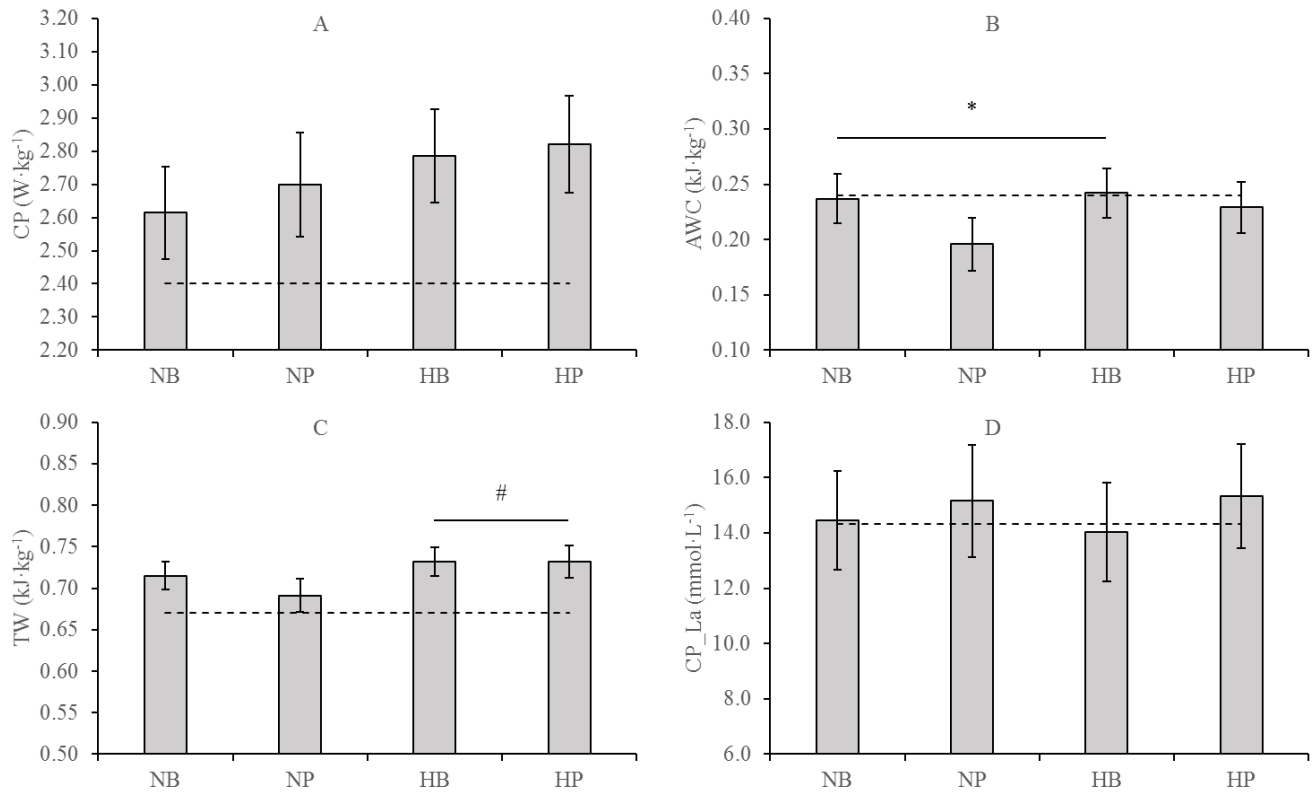
Figure 3. Graded exercise testing variables after 4 weeks of training.



*Note.* Mean values ( $\pm 95\%$  confidence interval) for posttest adjusted for initial differences in pretest (dash line) for normoxia + beta-alanine group (NB), normoxia + placebo group (NP), hypoxia + beta-alanine group (HB) and hypoxia + placebo group (HP): A. Average power output of all sprints (RST\_AP; covariate: adjusted pretest mean =  $8.68 \text{ W}\cdot\text{kg}^{-1}$ ); B. Average power output of the last sprint (RST\_AP5; covariate: adjusted pretest mean =  $7.03 \text{ W}\cdot\text{kg}^{-1}$ ); C. Lactate concentration after repeated sprint testing (RST\_La; covariate: adjusted pretest mean =  $12.61 \text{ mmol}\cdot\text{L}^{-1}$ ); D. Heart rate at 60s after repeated sprint testing (RST\_HR60; covariate: adjusted pretest mean =  $147.94 \text{ bpm}$ ); # indicates main effect for altitude.

Figure 4. Repeated sprint testing variables after 4 weeks of training.





*Note.* Mean values ( $\pm 95\%$  confidence interval) for posttest adjusted for initial differences in pretest (dash line) for normoxia + beta-alanine group (NB), normoxia + placebo group (NP), hypoxia + beta-alanine group (HB) and hypoxia + placebo group (HP): A. Critical power (CP; covariate: adjusted pretest mean =  $2.40 \text{ W}\cdot\text{kg}^{-1}$ ); B. Anaerobic working capacity (AWC; covariate: adjusted pretest mean =  $0.24 \text{ kJ}\cdot\text{kg}^{-1}$ ); C. Total work (TW; covariate: adjusted pretest mean =  $0.67 \text{ kJ}\cdot\text{kg}^{-1}$ ); D. Lactate concentration after 3-min all-out testing (CP\_La; covariate: adjusted pretest mean =  $14.33 \text{ mmol}\cdot\text{L}^{-1}$ ); # indicates main effect for altitude; \* indicates main effect for supplement.

Figure 5. 3-min all-out testing variables after 4 weeks of training.

Table 4. Anthropometric and hematological variables before (pre-) and after (post-) intervention.

	NB ( <i>n</i> = 11)		NP ( <i>n</i> = 8)		HB ( <i>n</i> = 10)		HP ( <i>n</i> = 9)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
BM (kg)	78.2 ± 11.6	77.8 ± 10.2	81.4 ± 7.9	81.7 ± 7.8	72.4 ± 4.3	73.3 ± 4.8	72.3 ± 8.4	72.5 ± 7.5
%BF	20.1 ± 8.1	19.2 ± 8.4	18.8 ± 8.4	18.7 ± 9.3	14.5 ± 4.5	14.8 ± 4.3	15.6 ± 4.8	15.3 ± 5.1
Hb (g·dl <sup>-1</sup> )	15.1 ± 0.8	14.9 ± 0.8	14.2 ± 0.9	14.1 ± 1.0	14.5 ± 0.8	14.5 ± 0.9	14.7 ± 1.1	14.5 ± 1.1
Hct (%)	42.8 ± 3.0	44.0 ± 2.5	41.2 ± 2.7	42.2 ± 2.5	41.5 ± 2.2	42.5 ± 2.3	42.2 ± 2.7	42.7 ± 2.6

*Note.* Data are mean ± standard deviation (SD) and represent raw data measured before and following intervention for the participants training in normoxia while receiving beta-alanine (NB) or placebo (NP) supplementation and participants training in hypoxia while receiving beta-alanine (HB) or placebo (HP) supplementation. *n* = sample size. BM = body mass; %BF = percent body fat; Hb = hemoglobin; Hct = hematocrit.

Table 5. Graded exercise testing variables before (pre-) and after (post-) intervention.

	NB ( <i>n</i> = 11)		NP ( <i>n</i> = 8)		HB ( <i>n</i> = 10)		HP ( <i>n</i> = 9)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
VO <sub>2</sub> max (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	41.7 ± 4.8	42.8 ± 4.8	38.0 ± 7.3	40.4 ± 6.3	45.1 ± 6.0	47.8 ± 5.2	41.6 ± 3.1	44.1 ± 2.6
PPO (W)	257 ± 45	272 ± 39	247 ± 32	264 ± 28	262 ± 44	279 ± 43	244 ± 21	260 ± 21
VO <sub>2</sub> RCP (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	37.0 ± 3.9	36.4 ± 5.3	33.6 ± 6.6	34.3 ± 4.7	38.3 ± 6.3	39.8 ± 4.4	35.8 ± 4.4	38.0 ± 4.0
PRCP (W)	211 ± 37	217 ± 35	205 ± 30	210 ± 21	213 ± 38	222 ± 34	195 ± 18	211 ± 21

*Note.* Data are mean ± standard deviation (SD) and represent raw data measured before and following intervention for the participants training in normoxia while receiving beta-alanine (NB) or placebo (NP) supplementation and participants training in hypoxia while receiving beta-alanine (HB) or placebo (HP) supplementation. *n* = sample size. VO<sub>2</sub>max = maximal oxygen consumption; PPO = peak power output; VO<sub>2</sub>RCP = oxygen consumption at respiratory compensation point; PRCP = power output at respiratory compensation point.

Table 6. Repeated sprint testing variables before (pre-) and after (post-) intervention.

	NB ( <i>n</i> = 11)		NP ( <i>n</i> = 8)		HB ( <i>n</i> = 10)		HP ( <i>n</i> = 9)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
RST_AP (W)	649 ± 115	728 ± 123	700 ± 105	780 ± 119	650 ± 94	726 ± 87	642 ± 114	733 ± 110
RST_AP5 (W)	523 ± 121	629 ± 125	559 ± 103	674 ± 99	538 ± 114	656 ± 87	517 ± 106	631 ± 91
RST_La (mmol·L <sup>-1</sup> )	13.2 ± 3.1	12.0 ± 3.3	12.2 ± 3.2	12.9 ± 2.6	13.0 ± 1.9	11.3 ± 1.8	12.0 ± 3.3	12.6 ± 2.6
RST_HR60 (bpm)	147 ± 20	150 ± 19	148 ± 5	150 ± 12	145 ± 18	139 ± 17	150 ± 14	151 ± 10

*Note.* Data are mean ± standard deviation (SD) and represent raw data measured before and following intervention for the participants training in normoxia while receiving beta-alanine (NB) or placebo (NP) supplementation and participants training in hypoxia while receiving beta-alanine (HB) or placebo (HP) supplementation. *n* = sample size. RST\_AP = average power output of all sprints in repeated sprint testing; RST\_AP5 = average power output of the last sprint in repeated sprint testing; RST\_La = lactate concentration after repeated sprint testing; RST\_HR60 = heart rate at 60s after repeated sprint testing.

Table 7. 3-min all-out testing variables before (pre-) and after (post-) intervention.

	NB ( <i>n</i> = 11)		NP ( <i>n</i> = 8)		HB ( <i>n</i> = 10)		HP ( <i>n</i> = 9)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
CP (W)	189 ± 51	203 ± 33	177 ± 46	210 ± 30	186 ± 38	212 ± 35	171 ± 22	202 ± 17
AWC (kJ)	16.8 ± 3.6	17.9 ± 4.4	19.2 ± 6.3	16.0 ± 4.1	18.0 ± 3.9	18.0 ± 3.1	18.3 ± 3.7	17.2 ± 3.7
TW (kJ)	50.8 ± 10.3	54.5 ± 9.5	51.1 ± 7.9	53.7 ± 7.5	51.4 ± 7.5	56.2 ± 8.0	49.0 ± 5.3	53.5 ± 4.7
CP_La (mmol·L <sup>-1</sup> )	15.6 ± 2.5	15.0 ± 3.2	12.6 ± 2.7	14.5 ± 2.2	15.5 ± 2.9	14.5 ± 3.3	13.1 ± 3.7	14.8 ± 2.7

*Note.* Data are mean ± standard deviation (SD) and represent raw data measured before and following intervention for the participants training in normoxia while receiving beta-alanine (NB) or placebo (NP) supplementation and participants training in hypoxia while receiving beta-alanine (HB) or placebo (HP) supplementation. *n* = sample size. CP = critical power; AWC = anaerobic working capacity; TW = total work; CP\_La = lactate concentration after 3-min all-out testing.

## **CHAPTER FIVE: DISCUSSION**

To the best of our knowledge, the present study is the first randomized, placebo controlled investigation, conducted in a double-blind manner for supplement and a single-blind manner for altitude, examining the additive effects of combining repeated sprint training in hypoxia with beta-alanine supplementation on sea-level performance in recreationally-trained men. The present study demonstrated that RSH and beta-alanine benefit performance from different perspectives. RSH resulted in greater post-intervention values for fatigue threshold, exercise tolerance, cardiovascular recovery and overall working capacity than RSN, while beta-alanine supplementation resulted in greater anaerobic working capacity compared to placebo following the repeated sprint training intervention.

### **Training Program Comparisons**

The current study employed oxygen fractions of 14.2% and 20.9% for hypoxia and normoxia, respectively. We observed that SpO<sub>2</sub> was significantly lower in the hypoxia groups than in the normoxia groups. This is in agreement with previous studies (Billaut et al., 2013; Girard et al., 2016; Morrison et al., 2015), which reported that SpO<sub>2</sub> was significantly lower in hypoxia during an acute repeated sprint training session. Generally, lower SpO<sub>2</sub> indicates a reduction in O<sub>2</sub> availability at the cellular level, particularly during recovery. Therefore, reduced SpO<sub>2</sub> values are usually accompanied by increased heart rate, minute ventilation and oxygen debt (Bowtell et al., 2014). Previous training studies have consistently reported lower SpO<sub>2</sub> when exercising in hypoxia throughout the training period (Brocherie et al., 2015a; Faiss et al., 2015b; Goods, Dawson, Landers, Gore, & Peeling, 2015; Hamlin et al., 2017; Kasai et al., 2015). Similar to previous investigations (Bowtell et al., 2014), the present study also observed a greater

SpO<sub>2</sub> decline in hypoxia than normoxia. A greater SpO<sub>2</sub> desaturation has been shown to induce greater metabolic perturbations and performance declines during repeated-effort, short-duration exercise interspersed with incomplete recoveries (Smith & Billaut, 2010, 2012). Decreased repeated high intensity efforts have been reported in rugby athletes at relatively low altitudes (1,550 – 1,700m) (Hamlin, Hinckson, Wood, & Hopkins, 2008; Weston, Mackenzie, Tufts, & Mars, 2001). Smith and colleagues (2010, 2012) reported that arterial hypoxemia inhibited pre-frontal cortex oxygenation during repeated sprint training and resulted in restricted muscle recruitment and performance.

### **Graded Exercise Testing Performance**

Greater post-intervention values for VO<sub>2</sub>max and PPO were noted in all groups, without any between-group differences. However, a trend ( $p = 0.059$ ) for a main effect for altitude was observed for VO<sub>2</sub>max. Results from previous studies have reported contradictory findings. Millet et al. (2014) reported elevated PPO with training regardless of altitude, while VO<sub>2</sub>max did not improve after training at either altitude or sea-level. Kasai et al. (2015) also reported that VO<sub>2</sub>max did not improve significantly in either RSH or RSN. Conflicting results have also been shown for field-based tests. No significant improvements were noted in an incremental field test for either RSH or RSN (Brocherie et al., 2015a). The investigators suggested that the total duration of hypoxic exposure was too short to induce any positive hematological adaptations. The present study demonstrated no changes in hemoglobin and hematocrit values in any group regardless of intervention.

Hamlin et al. (2017) reported no substantial between group changes in YYIR, which is consistent with some studies (Faiss et al., 2015b; Gatterer et al., 2014; Goods et al., 2015), but

contrasts with others (Galvin et al., 2013). The variation in aerobic adaptation between studies is likely due to the training status of the participants. Untrained individuals are more likely to benefit from RSH than well-trained individuals. The current results indicated a greater post-intervention values for  $\text{VO}_2\text{RCP}$  and  $\text{PRCP}$  following training at altitude than the groups training in normoxia. It is generally believed that RCP demarcates heavy and severe exercise domain (Whipp, Ward, & Rossiter, 2005), and relies on the accumulation of metabolic by-products signaled by exercise induced lactic acidosis and hyperventilation (Meyer, Faude, Scharhag, Urhausen, & Kindermann, 2004). Therefore, our results indicated that participants were able to better maintain steady-state  $\text{VO}_2$  and lactate at high intensity exercise following four weeks of RSH compared to RSN.

### **Repeated Sprint Testing Performance**

Results of this study indicated significant improvements in  $\text{RST\_AP5}$ , but not  $\text{RST\_AP}$  when exercising at altitude compared to the groups training in normoxia. Previously reported changes in repeated sprint performance after RSH and RSN are inconsistent. Our results are supported by several previously published investigations that showed beneficial changes in repeated sprint performance after RSH compared to RSN (Faiss et al., 2013b; Faiss et al., 2015b; Galvin et al., 2013; Gatterer et al., 2015; Gatterer et al., 2014; Kasai et al., 2015; Trincat et al., 2016). However, others reported no advantage of RSH over RSN (Goods et al., 2015; Montero & Lundby, 2016). Investigations by Brocherie et al. (2015b) and Hamlin et al. (2017) both indicated a residual effect for repeated sprint performance in RSH, but not RSN.

Recently, there has been considerable debate over performance improvement associated with RSH (Faiss, Holmberg, & Millet, 2015a; Lundby & Robach, 2016; Millet et al., 2017;

Montero & Lundby, 2015). Methodological differences, including but not limited to the training status of the participants, the selection and sequence of performance testing, and the calculation of fatigue scores may contribute to this variation in performance change. We did not observe any ergogenic effects for beta-alanine, which appears to confirm previous findings (Saunders et al., 2014; Sweeney et al., 2010). It is possible that training-based performance enhancement may have masked any potential ergogenic effects from beta-alanine supplementation. The lower limit of 95% confidence intervals far surpassed the adjusted pre-test values for RST\_AP and RST\_AP5, indicating the efficacy of the repeated sprint training itself. In support of this notion, Bishop, Edge, and Goodman (2004) previously reported that repeated sprint ability was significantly correlated with muscle buffering capacity measured from  $\Delta[\text{La}^-]/\Delta\text{pH}$ , but not from titration method. Their results suggested that non-physiochemical buffering such as metabolic reactions that consume  $\text{H}^+$  may have a greater influence on muscle buffering capacity than physiochemical buffering, which would be provided by augmented carnosine concentrations. Additionally, the recovery time (60 seconds) utilized during our repeated sprint protocol may not have provided a sufficient stimulus to effectively challenge muscle buffering capacity. Considering the half-time for PCr resynthesis has been reported to be about 1 minute (Bogdanis, Nevill, Boobis, & Lakomy, 1996), it is plausible to deduce that the extended recovery time allowed for a greater reliance on the PCr energy pathway, lessening the reliance on glycolytic metabolism.

Heart rate recovery (HRR) refers to the rate at which heart rate declines within a certain time frame, generally ranging between 30 seconds to 2 minutes, after the termination of physical exercise (Daanen, Lamberts, Kallen, Jin, & Van Meeteren, 2012; Shetler et al., 2001). Reduced

HRR was previously reported after maximal exercise, primarily due to the continued sympathetic activation during the early stages of recovery (Kaikkonen, Rusko, & Martinmaki, 2008). Research also showed that athletes competing in intermittent sports had faster HRR than endurance athletes (Ostojic et al., 2010). A main effect for altitude was demonstrated for RST\_HR60 in the present study with the two groups training in hypoxia showing a significantly lower post-intervention values for heart rate during recovery than those training in normoxia. It has been suggested before that HRR can be used to predict changes in training status, as well as to monitor the accumulation of fatigue (Daanen et al., 2012). Results of the current investigation indicated a beneficial effect of RSH that may have facilitated physiological adaptations from repeated sprint training and reduced the accretion of fatigue during repeated sprint testing.

### **3-Min All-Out Testing Performance**

Critical power is considered to represent the highest sustainable rate of oxidative metabolism (Jones, Vanhatalo, Burnley, Morton, & Poole, 2010). Commonly used to demarcate different exercise intensity domains (Francis, Quinn, Amann, & LaRoche, 2010), critical power has been shown to effectively track changes in aerobic capacity before and after high intensity intermittent training (Wang et al., 2017). Our results indicated that critical power improved in all groups, with a trend ( $p = 0.051$ ) towards a main effect of altitude. Theoretically, the fatigue at or above critical power is based on the interaction of anaerobic capacity,  $VO_{2max}$ , and the  $VO_2$  slow component (Burnley & Jones, 2007), and may be related to the availability of high-energy phosphates and cross-bridge dysfunction resulting from the accumulation of metabolites (Jones, Wilkerson, DiMenna, Fulford, & Poole, 2008). It was hypothesized that critical power should benefit from beta-alanine supplementation. However, our results indicated no effect of beta-



alanine on critical power. While the underlying mechanism is still unknown and needs to be addressed, performance improvements resulting from beta-alanine supplementation may have been masked by repeated sprint training. The lower limit of 95% confidence intervals all exceeded the adjusted pre-test value for CP. In addition, it has been suggested that open-end point exercise tasks, such as time to exhaustion trials, are more sensitive to beta-alanine supplementation (Trexler et al., 2015). The 3MT is a fixed-end point test that may be subject to the influence of intrinsic pacing (Hinckson & Hopkins, 2005). In fact, a recent study (Abbiss, Thompson, Lipski, Meyer, & Skorski, 2016) suggested that participants showed a more conservative pacing strategy during duration-based cycling trials than distance-based cycling trials, potentially masking any ergogenic potential derived from beta-alanine supplementation. Saunders et al. (2016) suggested that exercise capacity measures, such as open-end point exercise tasks, demonstrate a greater effect size than exercise performance measures, such as fixed-end point exercise tasks when examining the efficacy of beta-alanine supplementation. During the 3MT, critical power is estimated by averaging the power output during the last 30 seconds, where the slow component drives  $\text{VO}_2$  to its maximum and reflects the development of muscular fatigue (Turner et al., 2006). It has previously been suggested that critical power, as a reflection of exercise tolerance, is rate but not capacity-limited (Chidnok et al., 2012).

The work-time integral above critical power, termed AWC (or  $W'$ ), theoretically represents the maximum amount of work that can be performed above critical power (Fukuba et al., 2003). A closer examination of our results showed that the post-intervention values for AWC in the beta-alanine groups were significantly higher than in the placebo groups. The differential responses are likely due to a greater improvement in CP in the placebo groups. While anaerobic

glycolysis is the major contributor to anaerobic capacity during high intensity exercise (Medbo et al., 1988), the accumulation of  $H^+$  ions has been shown to inhibit glycolytic enzyme activity, including phosphofructokinase (Spriet, Soderlund, Bergstrom, & Hultman, 1987). It is likely, that elevated muscle carnosine content from beta-alanine supplementation contributes to improved glycolytic energy production by buffering  $H^+$  ions and fostering phosphofructokinase activity (Baguet, Koppo, Pottier, & Derave, 2010). When comparing 95% confidence interval of post-test values with the adjusted pre-test value, AWC appeared to be maintained in the two beta-alanine groups, and potentially decreased in the normoxic placebo group. Our finding is not consistent with Bellinger and Minahan (2016a), who reported an increased anaerobic capacity from an open-end point task following beta-alanine supplementation when compared with placebo. The underlying mechanism for this inconsistency is unknown and should be addressed in future studies.

The utility of the ratio between AWC and CP has been previously discussed (Jones et al., 2010). This ratio indicates the proportion of anaerobic and aerobic contribution to total work, and originated from a two-component mathematical model examining bioenergetics (Jones et al., 2010). Calculating this ratio from the mean raw data values provided in Table 7 revealed that it remained unchanged in the normoxic beta-alanine group, but decreased in the placebo groups. Our results indicated that the repeated sprint training protocol utilized may have caused a disproportionate reliance on the aerobic energy supply yielding a potential deficit in the anaerobic energy supply; however, beta-alanine supplementation may have alleviated or offset this effect. This finding may have important implications for intermittent team sports like rugby, which tend to prioritize anaerobic capacity.

In agreement with the  $\text{VO}_2\text{RCP}$  and  $\text{PRCP}$  results from the graded exercise test, a main effect for altitude was demonstrated when examining the total work completed during the 3MT. The hypoxia groups had greater post-intervention values in total work than the normoxia groups. Interestingly, Faiss et al. (2013b) reported no differences in average power from 3MT following RSH and RSN. Since the time duration is fixed for 3MT, the lack of differences in average power reported would be synonymous with total work as reported in the current investigation. The discrepancy is likely due to the variation in 3MT protocol, which was not reported in detail in their study (Faiss et al., 2013b).

No difference was noted in the lactate response between groups. Previous studies reported conflicting results, with some reporting that beta-alanine supplementation increased lactate level after exercise (Bellinger & Minahan, 2016a, 2016b; de Andrade Kratz et al., 2016), while others reported no effect on post-exercise lactate concentration (Ducker et al., 2013; Saunders et al., 2012). Blood lactate concentration is the result of lactate production and removal, therefore it does not necessarily reflect muscle acidosis (Moxnes & Sandbakk, 2012). Previous investigations have reported that beta-alanine supplementation can significantly reduce exercise-induced acidosis without affecting blood lactate concentrations. The increased lactate production following beta-alanine supplementation might be attributable to a lower intramuscular  $\text{H}^+$  concentration, allowing for a less inhibited glycolytic metabolism and higher rate of glycogen usage (Tobias et al., 2013). It has been suggested that the monocarboxylate transporter (MCT) system is mainly responsible for lactate utilization from the circulation (MCT1) and lactate efflux from glycolytic-dependent tissues (MCT4), respectively (Dimmer, Friedrich, Lang, Deitmer, & Broer, 2000; Ullah, Davies, & Halestrap, 2006). Faiss et al. (2013b)

demonstrated an upregulation of MCT4 mRNA and a downregulation of MCT1 mRNA in RSH, but not RSN. Although speculative, the discrepancy between our study and previous investigations is likely related to the differences in the ratio between MCT1 and MCT4. However, more detailed mechanistic study is needed.

In summary, the results of this study indicate that normobaric hypoxia improved fatigue threshold, exercise tolerance, cardiovascular recovery, and overall working capacity in recreationally-trained men following the repeated sprint training intervention. Furthermore, it appears that beta-alanine supplementation maintained the anaerobic working capacity following the repeated sprint training intervention. The present study suggested that beta-alanine did not provide additional benefits with respect to attenuating fatigue or enhancing repeated sprint performance. However, beta-alanine supplementation maintained anaerobic working capacity, which tended to decrease as a result of repeated sprint training, especially in normoxia.

**APPENDIX: UCF IRB LETTER**



University of Central Florida Institutional Review Board  
 Office of Research & Commercialization  
 12201 Research Parkway, Suite 501  
 Orlando, Florida 32826-3246  
 Telephone: 407-823-2901 or 407-882-2276  
[www.research.ucf.edu/compliance/irb.html](http://www.research.ucf.edu/compliance/irb.html)

### Approval of Human Research

From: UCF Institutional Review Board #1  
 FWA00000351, IRB00001138

To: David Fukuda and Co-PIs: Jay R. Hoffman, Jeffrey Ray Stout, & Ran Wang

Date: May 26, 2016

Dear Researcher:

On 05/26/2016 the IRB approved the following human participant research until 05/25/2017 inclusive:

Type of Review: UCF Initial Review Submission Form  
 Expedited Review

Project Title: Effects of Hypoxic Sprint Interval Training and ?-Alanine  
 Supplementation

Investigator: David Fukuda

IRB Number: SBE-16-12148

Funding Agency: National Strength and Conditioning Association(NSCA)

Grant Title: Effects of Hypoxic Sprint Interval Training and ?-Alanine  
 Supplementation

Research ID: N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form cannot be used to extend the approval period of a study. All forms may be completed and submitted online at <https://iris.research.ucf.edu>.

If continuing review approval is not granted before the expiration date of 05/25/2017, approval of this research expires on that date. When you have completed your research, please submit a Study Closure request in iRIS so that IRB records will be accurate.

Use of the approved, stamped consent document(s) is required. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a signed and dated copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the [Investigator Manual](#).

This letter is signed by:



Sophia F. Dziegielewski, Ph.D., LCSW  
IRB Chair

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