NOTTINGHAM TRENT UNIVERSITY

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Effects of Buffering Agents on High-Intensity Exercise Performance and Capacity

By

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Abstract

High-intensity exercise results in hydrogen ion accumulation, which can have a deleterious effect on muscle function, and thus exercise tolerance. Buffering agents are commonly used to enhance exercise performance and capacity. Two such agents, β-alanine and sodium bicarbonate, increase intracellular and extracellular buffering capacity, which could contribute to an improved performance and capacity during exercise limited by increasing acidosis. Despite this, studies on the ergogenic effects of β-alanine are still in their infancy, and research on sodium bicarbonate remains equivocal. The aim of this thesis was to investigate the separate and combined effects of β-alanine and sodium bicarbonate on high-intensity exercise performance and capacity using various exercise modalities. The $CCT_{110\%}$, a cycling capacity test, was shown to be reliable (Chapter 4A), and subsequently employed to investigate the effect of sodium bicarbonate (Chapter 4B), β-alanine and co-supplementation of the two (Chapter 4C). Sodium bicarbonate supplementation was shown to improve total work done during the $CCT_{110\%}$ (+4.8%), only when those experiencing gastrointestinal discomfort were removed from analyses, as was β-alanine (+14.6%); co-supplementation of the two did not confer any further benefits above β-alanine alone. Neither sodium bicarbonate (Chapter 5A) nor β-alanine or co-supplementation of the two (Chapter 5B) improved 5 x 6 s repeated running sprints (all $P > 0.05$). Sprint performance during the Loughborough Intermittent Shuttle Test was unaffected by β-alanine supplementation in elite ($P = 0.63$) and non-elite ($P = 0.58$) games players (Chapter 6), although YoYo Intermittent Recovery Test Level 2 performance was improved (+34.3%) with β-alanine in amateur footballers during a competitive season (Chapter 7). High-intensity match activities during competitive match play were unaffected by β-alanine supplementation (Chapter 8). The results in this thesis showed that β-alanine was effective at improving exercise capacity but not exercise

performance. Results suggest sodium bicarbonate, and co-supplementation with β-alanine, may improve exercise tolerance although further research is warranted.

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Dedicated to my mother

Karin Wage

18th May 1949 – 28th May 2011

X

'As we slowly slide down the razor-blade of life,

carnosine may delay some of the unkindest cuts of all'

Alan R. Hipkiss

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Chapter 1.0 Introduction

Team sports such as football, hockey and rugby consist of periods of low and high-intensity exercise. High-intensity exercise increases energy demand on the working muscles; to meet the increased energy demand, adenosine-5'-triphosphate (ATP) is hydrolysed to adenosine-5'-diphosphate (ADP), although the ATP store is limited and must be continually replenished. The aerobic rate of ATP resynthesis is quickly exceeded by the rate of ATP hydrolysis, meaning that the shortfall in ATP must be met by the hydrolysis of phosphorylcreatine (PCr) and anaerobic glycolysis (Hultman and Sjöholm, 1983). When the glycolytic rate in muscle is higher than the rate of pyruvate oxidation, lactic acid is produced to facilitate the continuation of muscle contraction but this causes acidification following dissociation to the lactate anion (Lac^-) and hydrogen cation (H^+). Muscle pH may drop to as low as 6.0, with a concomitant drop to ~7.0 seen in both arterial and venous blood during high-intensity exercise (Pan et al., 1991; Bogdanis et al., 1996). In order to maintain homeostasis, the body must keep an equilibrium between H^+ production and H^+ removal, which is mediated by buffering systems, a process which can be enhanced by supplementation with buffering agents.

Carnosine (β-alanyl-L-histidine) is a histidine containing dipeptide found in high concentrations in skeletal muscle of vertebrates and non-vertebrates (Harris et al., 2006; Hill et al., 2007). Carnosine has been attributed various roles, although its role as intramuscular pH (pHi) buffer is undisputed due to its molecular structure and pKa of 6.83 (Tanokura et al., 1976), which makes it a suitable buffer over the physiological pH range (Bate-Smith, 1938). The synthesis of carnosine within the muscle is limited by the availability of β-alanine (Dunnet and Harris, 1999). Harris et al. (2006) demonstrated that 4 weeks of supplementation with β-alanine (4.0 $g \cdot d^{-1}$ in the first week rising to 6.4 $g \cdot d^{-1}$ in the fourth) increased muscle carnosine by ~60%.

An increase in muscle carnosine content would increase muscle buffering capacity, potentially mediating an improvement in exercise performance and capacity that is limited by the accumulation of H^+ and a subsequent drop in pHi. Indeed, following 4 weeks of β-alanine supplementation, total work done was improved during a high intensity cycle capacity test designed to induce large amounts of H^+ accumulation (Hill et al., 2007). In a meta-analysis of the literature, Hobson et al. (2012) showed that β-alanine supplementation was effective in improving high-intensity exercise of durations between 60 – 240 s ($P = 0.001$) and in excess of 240 s ($P = 0.05$), but not less than 60 s ($P = 0.3$). A timeframe between 60 and 240 s is a period when anaerobic energy sources can contribute between 20 – 60% of the total energy requirement (Maughan et al., 1997), resulting in a large accumulation of H^+. It has been suggested that an exercise duration of less than 60 s may not be sufficient to induce reductions in pHi that will limit exercise (Sale et al., 2010), although repeated short duration exercise may increase the sensitivity to reduced pHi (Katz et al., 1984).

The rate of efflux of H^+ out of the muscle is dependent on the buffer concentration in the surrounding interstitium (Mainwood and Worsley-Brown, 1975). Sodium bicarbonate has been widely researched as an ergogenic aid designed to increase extracellular buffering concentration. Pre-exercise alkalosis has been shown consistently following sodium bicarbonate supplementation (for review see McNaughton et al., 2008), although the reported effects on exercise performance and capacity are inconsistent. These conflicting results can be attributed to a variety of factors, including differing sodium bicarbonate doses and exercise protocols, GI disturbance and individual variation in blood responses to supplementation (Matson and Tran, 1993). In a meta-analysis of the literature, Carr et al. (2011) showed that sodium bicarbonate was effective at improving a 1 min all out sprint by 1.7 ± 2.0% when ingested at a dose of 0.3 $g \cdot kg^{-1}$Body Mass (BM) prior to exercise. The

ergogenic effects of sodium bicarbonate were enhanced with increasing doses and sprint bouts, although McNaughton (1992) showed increased gastrointestinal disturbance with doses above 0.3 $g \cdot kg^{-1}$BM, which may negatively impact upon performance.

The ability to recover from and repeat high-intensity efforts during team sports is a fitness component that has been termed repeated sprint ability (RSA; Bishop et al., 2001), and is of importance to performance (Rampinini et al., 2007). Fatigue during this type of exercise, defined as the inability to maintain speed or power output during subsequent high-intensity sprints (Girard et al., 2011), has been associated with the accumulation of H^+ (Rampinini et al., 2009), suggesting that performance during this type of exercise can be improved by increasing buffering capacity. Hoffman et al. (2008) and Sweeney et al. (2010) showed no effect of β-alanine supplementation on repeated sprint performance, although these studies did not investigate performance during simulated match play. Similarly, Bishop et al. (2004a) used a single 5 x 6 s repeated sprint protocol to show that sodium bicarbonate could increase total work done and peak power output during cycle sprints. Although these studies address the issue of repeated sprint exercise, more research needs to be performed on RSA during actual or simulated games play to identify whether buffering agents can improve performance during high-intensity intermittent exercise.

Research into the effects of β-alanine supplementation on exercise performance and capacity has developed rapidly since Harris et al. (2006) showed that muscle carnosine concentrations could be increased following 4 weeks of supplementation. A range of exercise and capacity tests have been employed, from single bout high-intensity performance and capacity tests (Hill et al., 2007; van Thienen et al., 2009; Baguet et al., 2010) to repeated sprint protocols (Hoffman et al., 2008; Sweeney et al., 2010). However, these repeated sprint studies did not

determine performance during simulated or actual games play and, thus, lack ecological validity for team sports as they do not consider the implications of the additional metabolic demand of the entire activity. Furthermore, no study to date has investigated the effects of co-supplementing β-alanine and sodium bicarbonate, increasing both intracellular and extracellular buffering capacity, theoretically resulting in an increased protection against acidosis during high intensity exercise which may contribute to a further improvement in exercise performance and capacity above that shown following either sodium bicarbonate supplementation or β-alanine supplementation alone.

The aim of this thesis was to investigate the separate and combined effects of two buffering agents, β-alanine and sodium bicarbonate, on high-intensity exercise performance and capacity using various carefully chosen exercise modalities. Exercise capacity tests require individuals to exercise to exhaustion and are an indication of the maximum amount of exertion an individual can sustain at a fixed intensity (Goldstein, 1990); tests of this nature are mechanistic and allow steady-state measurements to better understand physiological responses at the point of fatigue. Performance tests do not require the individual to exert themselves to the point of fatigue, and encourage them to perform as much work within a set time period or distance. A test of this nature is more applicable to a sporting setting, where individuals are rarely required to exercise until the point of fatigue. Outlines of the experimental chapters reported in this thesis are as follows:

Chapter 4 reports on several studies using the same cycling capacity test ($CCT_{110\%}$) as Hill et al. (2007) which investigated, A) the reliability of the $CCT_{110\%}$, B) the effects of sodium bicarbonate on cycling capacity and C) the effects of four weeks of β-alanine

supplementation on high-intensity exercise capacity, and whether there was an additive effect of acute co-supplementation with sodium bicarbonate.

Chapter 5 reports on two studies using a 2 x 45 minute intermittent exercise protocol that replicates running trends seen in football (Greig et al., 2006), with participants performing 5 x 6 s repeated sprints before, at half-time and immediately following the treadmill protocol. Exercise was performed at a simulated altitude of 2500 m, designed to exacerbate the metabolic demand of the exercise, thereby increasing the production of H^+ and the reliance on intracellular and extracellular buffering. This altitude was chosen because, in 2007, FIFA introduced a ban on international football matches being played at an altitude over 2500 m due to an unfair advantage to home teams and health reasons, although the ban is currently suspended. The effects of A) acute sodium bicarbonate supplementation and B) five weeks of β-alanine supplementation, with and without sodium bicarbonate supplementation, on repeated sprint performance throughout football specific intermittent exercise were investigated.

Chapter 6 reports on the effect of four weeks β-alanine supplementation on repeated sprint performance during the Loughborough Intermittent Shuttle Test, a protocol designed to simulate the demands of football (Nicholas et al., 2000) and incorporates timed 15 m sprints which represents the average sprint distance during team sports (Spencer et al., 2005).

Chapter 7 reports on the effect of β-alanine supplementation on YoYo Intermittent Recovery Test Level 2 (YoYo IR2) performance in amateur footballers during a competitive season. The YoYo IR2 evaluates a player's ability to perform repeated bouts of high-intensity

exercise interspersed with periods of low intensity activity and is used as a measure of team sport fitness.

The effect of long term β-alanine supplementation in an applied setting is reported in Chapter 8. Games players were supplemented over the course of an entire season, monitoring in-game performance during competitive match play in amateur footballers playing for an English league football team. Players were placed into one of three supplementation groups; placebo only, placebo then β-alanine, and β-alanine only.

Chapter 2.0 Review of Literature

2.1 **Outline of the Review of the Literature**

Research into team sports has thrived over the past decade with the emergence of laboratory based protocols (Nicholas et al., 2000; Drust et al., 2000; Greig et al., 2006) and technologies that have facilitated in-match data capture. There is a growing database of research into the effects of buffering agents, such as β-alanine and sodium bicarbonate, on exercise performance and capacity, though few employ high-intensity intermittent protocols, and fewer still have ecological validity to relate the findings to team sport performance. This review aims to first provide an overview of the demands of high-intensity intermittent exercise, with specific reference to football, techniques to analyse performance, and limitations to performance and capacity. It will then develop onto the issue of acid-base balance during exercise, the implications of H^+ accumulation and the intracellular and extracellular buffering systems in the body. Finally, this review will discuss the relative merits of two buffering agents, β-alanine and sodium bicarbonate, their ability to increase baseline concentrations, and the benefits to exercise performance and capacity.

2.2 **Games Play**

2.2.1 Performance Characteristics

Team sports, such as football, hockey and rugby, are characterised by periods of high-intensity exercise (>15 $km \cdot h^{-1}$; Abt and Lovell, 2009) interspersed with periods of low intensity exercise and rest. The nature of team sports, specifically football, are such that changes of speed occur approximately once every 4 – 6 s, and players perform more than 1300 changes in speed during a game (Mohr et al., 2003). Upwards of 200 of these changes in speed are performed at high-intensity (Mohr et al., 2003), including up to 60 sprints (Reilly and Thomas, 1976).

Reilly and Thomas (1976) showed that English league players covered an average of 8680 m during a competitive football match. More recently, Italian league players were shown to cover an average of 10860 ± 180 m per game (Mohr et al., 2003). These total distances covered vary depending on playing position, midfielders covering the greatest distance (~11000 m) and goalkeepers the least (~4000 m) (Reilly and Thomas, 1976; Mohr et al., 2003; Barros et al., 2007; Bradley et al., 2009 & 2010). The total distance covered is performed in several motion categories, with most distance covered in the low intensity zones (Reilly and Thomas, 1976; Bangsbo et al., 1991).

Mohr et al. (2003) showed that distance covered at high-intensity speeds was higher for top-class versus moderate standard football players, while successful teams have been shown to perform more high-intensity running during games than their unsuccessful opponents (Mohr et al., 2003). Therefore, high-intensity performance appears to be the most appropriate marker of football performance during competitive match play. Abt and Lovell (2009) highlighted the need to individualise the high-intensity speed threshold due to the differing speeds at which players reach high-intensity. However, it is not always appropriate to test individual games players due to their limited availability during a competitive season. The most appropriate absolute threshold for high-intensity running has been shown to be 15 $km \cdot h^{-1}$, as this was associated with the median second ventilatory threshold in professional football players (Abt and Lovell, 2009).

The average sprint during games play is between 10 and 20 m, and lasts approximately 2 – 3 s (Spencer et al., 2005). Competitive match play requires players to continually perform these maximal or near maximal sprints; the most common recovery time separating subsequent sprints is less than 20 s or in excess of 121 s (Spencer et al., 2005). Although a recovery

period in excess of 30 s has been shown to be sufficient to maintain 15 m sprint performance up to forty sprints (Balsom et al., 1992a), a timeframe of less than 30 s is likely to have a more deleterious effect on subsequent sprint performance (Balsom et al., 1992b). The term RSA is used to define the fitness component that requires players participating in team sports to perform repeated sprints (Dawson et al., 1993; Bishop et al., 2001). RSA is considered an important aspect of team sport performance as the ability to recover from and repeat high-intensity exercise during competitive match play is crucial to performance (Rampinini et al., 2007). Therefore, an intervention designed to delay fatigue during repeated sprints would be of benefit to games play performance.

2.2.2 Physiological Characteristics

Mean heart rates during a football match have been shown to be around 85% of maximum (Ali and Farrally, 1991; Bangsbo, 1994a), with a player's individual heart rate rarely falling below 65% of maximum (Bangsbo et al., 2006). These heart rate values can be used to estimate oxygen uptake (Esposito et al., 2004), which suggest that, on average, during a competitive soccer match, players will work at an energy expenditure of approximately 70% of their individual VO_{2max} (Bangsbo et al., 2006). Although mean work rate data from a football match can give some insight into the physical demands placed upon players, it is important not to over interpret these data due to the intermittent nature of the game. There will be periods in a game during which players will be performing at maximal capacity, though it would be impossible to maintain this for the entire duration of the exercise due to several contributing factors, including an increase in intracellular H^+ (Rampinini et al., 2009).

Muscle lactate during games play can reach concentrations in excess of 30 $mmol \cdot kg^{-1}$ dry mass (dm) in certain individuals following an intense period of play (Krustrup et al., 2006a),

with a wide range of concentrations seen between players (2.6 – 36.4 mmol·kg^{-1}dm). A rise in muscle lactate increases the co-transport efflux of lactate and H^+ into the blood (Juel 1988; Bangsbo et al., 1993), thereby increasing circulating concentrations of lactate. Blood lactate concentrations between 2 and 10 mmol·L^{-1} have been shown during team sport activity, with individual concentrations as high as 12 mmol·L^{-1} (Ekblom, 1986; Bangsbo, 1994a). Higher concentrations are seen during the first half than the second (Bangsbo et al., 1991; Bangsbo 1994a; Krustrup et al., 2006a); likely reflecting a reduced distance covered during the second half of match play. However, blood lactate has been shown to be a poor indicator of muscle lactate (Krustrup et al., 2006a), likely due to the simultaneous uptake and release of lactate by muscles, and the uptake of lactate into adjacent (Ren et al., 1988) or inactive (Bangsbo et al., 1995) fibres, meaning lactate concentrations in single muscle fibres do not necessarily reflect the amount produced in these fibres during exercise. This could also be due to the rate of lactate clearance from the blood being slower than from the muscle (Bangsbo et al., 1995). This means that the measured blood lactate concentrations are likely to be more representative of the outcome of several activities taking place over minutes, rather than a single activity occurring during match play. Nonetheless, large variations in muscle and blood lactate concentrations are indicative of the intermittent nature of competitive match play; increased lactate concentrations during play suggest that a high rate of glycolysis is required for periods during a match.

2.2.3 Performance Analysis

Performance can be analysed according to locomotion data, whereby all players' movements over the pitch are captured. It is of vital importance to research that the data capture systems used to obtain these data be accurate, reliable, objective and valid.

Reilly and Thomas (1976) performed a motion analysis of English league football players using video recordings of individual players throughout a game. Using predetermined motion categories (walking, backing, jogging, cruising and sprinting), a notational analyst determined the time spent in each motion category with the aid of a stopwatch and video playback. Stride length in each motion category was established by having players perform each activity over a set distance. Total distance covered during the match was then determined using stride frequency and pitch markings. The methods employed by Reilly and Thomas (1976) are susceptible to human error, particularly due to the subjective nature of human movement interpretation. Although within analyst variation can be determined, analyst bias will negatively affect the reliability of this method. Furthermore, this method of analysis is extremely time consuming as it does not allow simultaneous analyses of more than one player.

2.2.3.1. Global Positioning Satellite Systems

Global positioning satellite (GPS) systems are a technology that has been employed to capture locomotion data during games play. This technology consists of a GPS receiver and 27 operational satellites that orbit the earth (Larsson, 2003). The receiver continuously sends and receives signals to and from the satellites at the speed of light. Synchronising its time with the atomic clock of the satellite, the receiver can measure the travel time of each signal from the satellite, which is then converted into distance by multiplying this value by the speed of light. The distance to a minimum of four satellites is used to determine the position of the receiver using trigonometry (Larsson, 2003; Townshend et al., 2008). Originally developed for military use by the US Department of Defence, it was made commercially available in the 1980s, although a deliberate error (selective availability) was incorporated as a safety aspect against hostile forces using the GPS technology (Larsson, 2003). This

triggered an increase in differential GPS (dGPS) systems that increased accuracy using stationary receivers at known locations, which transmit correction factors. Selective availability was reduced in 2000, significantly increasing the accuracy of non-differential GPS technology (Adrados et al. 2002; Witte and Wilson, 2004).

Witte and Wilson (2004) showed that using a non-differential GPS for determination of bicycle speed over ground was accurate and reliable, although errors were encountered during rapid changes of speed and direction. Townshend et al. (2008) also showed a commercially available 1-Hz non-differential GPS receiver to offer accurate estimations of actual speed ($r = 0.99$) and displacement during straight-line human locomotion, with a reduction in accuracy over a curved path. Macleod et al. (2009) assessed the validity of a 1-Hz non-differential GPS system for determining movement patterns during field hockey match play. Participants performed a variety of field-hockey related movements (Macleod et al., 2009) including a T-shaped shuttle drill, a straight-line shuttle, a straight-line sprint shuttle, a zigzag shuttle and 14 circuits of an Astroturf pitch. The 6818 ± 0.0 m course distance was acceptably measured by the GPS unit (6820.5 ± 6.8 m) with a mean difference ± limits of agreement (LoA) of 2.5 ± 5.8 m. Speed during the four shuttle movements with the GPS was strongly correlated ($r = 0.99$) to speed recorded by timing gates, with a mean difference ± LoA of 0.0 ± 0.9 $km \cdot h^{-1}$. Furthermore, Macleod et al. (2009) observed speeds in excess of 20 $km \cdot h^{-1}$ during the shuttle movements and highly favourable mean differences (range: -0.1 ± 0.81 to +0.2 ± 3.9 m) which suggests a 1-Hz GPS system is suitable for obtaining running data during games play.

The horizontal dilution of position (HDOP) represents the error determined from the geometric arrangement of the satellites and reflects the accuracy of the latitude and longitude

of the position fix of the GPS (Witte and Wilson, 2004; Jennings et al., 2010). A value of 1 is desired, meaning a single satellite is overhead with the remaining satellites equally spaced around the horizon (Witte and Wilson, 2004). This value can rise to 50 if the orientations of all the satellites give an unreliable signal. A mean HDOP value of 1.25 ± 0.06 was shown during GPS data capture of simulated team sport running (Jennings et al., 2010). Although a larger error in accuracy could be expected with a larger HDOP, Witte and Wilson (2004) showed no effect on speed accuracy with increasing HDOP up to values of 40. This suggests that, despite changes in satellite orientation that will occur during data capture, the HDOP is unlikely to compromise the validity and reliability of the data.

Randers et al. (2010) showed differences between four motion analysis systems, including a 1-Hz and a 5-Hz GPS unit, in their ability to capture distances covered at various speeds. However, the performance decrements seen during a football game were similar for all four systems; caution should be taken comparing match analysis data between studies using different motion analysis systems. Several studies have shown 1-Hz GPS systems to be appropriate for collecting locomotion data (Witte and Wilson, 2004; Townshend et al., 2008), and importantly, the work of Macleod et al. (2009) has validated the system for in-match running data capture during team games.

2.3 **Mechanisms of Fatigue during High-Intensity Intermittent Exercise**

2.3.1 Introduction

Fatigue is generally considered to be a decline in the maximal force or power capacity of the muscles (Enoka and Duchateau, 2008), which will contribute to a reduced performance and ultimately, the cessation of exercise. Players perform more high-intensity running and cover more distance during the first half of a football match than during the second half (Reilly &

Thomas, 1976; Bangsbo, 1994a; Mohr et al., 2003). Rampinini et al. (2007) observed that the decrement in high-intensity running during the second half was related to the amount of high-intensity activity performed during the first half. This suggests that players fatigue (indicated by significantly less high-intensity running) towards the end of a football game, though Mohr et al. (2003) showed that fatigue also occurred during several periods of a game. High intensity running has been shown to be decreased following the most intense five minute period of a match (Mohr et al., 2003; Krustrup et al., 2006a; Bradley et al., 2009 & 2010), immediately following half time (Mohr et al., 2003) and towards the end of the game (Reilly and Thomas, 1976; Bangsbo et al., 1991; Mohr et al., 2003; Bradley et al., 2009 & 2010). The intermittent nature and prolonged duration of team sports requires players to perform exercise with a large contribution from both aerobic and anaerobic energy systems (Bangsbo et al., 2008); since the development of fatigue occurs during several periods of a game, the underlying mechanism behind this reduction in performance is undoubtedly multifactorial. Therefore, if the mechanisms contributing to fatigue during team sports can be identified, an intervention that can manipulate one of the contributors to the reduction in high-intensity activity during intermittent exercise would be of benefit to games performance.

2.3.2 Phosphorylcreatine Depletion

The requirement for ATP resynthesis during maximal exercise is high and is supplemented by the hydrolysis of PCr and anaerobic glycolysis (Hultman and Sjöholm, 1983). PCr was reduced to 55% of resting levels following a single maximal 6 s cycle sprint (Dawson et al., 1997), and further reduced to 27% following a fifth maximal sprint. During repeated sprint activities such as team sports, the most common recovery time separating subsequent sprints is less than 20 s or in excess of 121 s (Spencer et al., 2005); although some of the recovery time can also require individuals to perform near maximal efforts. The halftime for PCr

resynthesis is approximately 60 s (Bogdanis et al., 1993), which suggests that a decline in energy production may be due to the incomplete resynthesis of PCr stores.

2.3.3 Glycogen Depletion

Depletion of glycogen stores has often been associated with the development of fatigue during prolonged intermittent exercise. Saltin (1973) showed that muscle glycogen in the *m. vastus lateralis* of five football players was 96, 32 and 9 mmol·kg^{-1}wet weight prior to, at half time, and following a friendly football match. Krustrup et al. (2006a) showed that the muscle glycogen content of the *m. vastus lateralis* was 42 ± 6% lower at the end of a match. Differences in the level of glycogen depletion can be attributed to individual variation, standard of football and playing positions. Krustrup et al. (2006a) observed that 36 ± 6% and 11 ± 3% of individual muscle fibres (a 16 µm-thick transverse section) were almost empty or completely empty of glycogen. Furthermore, this depletion following the completion of a 90 minute match was associated with a post-match decrement in sprint performance, suggesting that low muscle glycogen in some individual fibres may contribute to a reduction in repeated sprint performance. Nonetheless, if not all muscle fibres are depleted then it could be suggested that glycogen depletion is not the only contributing factor to reduced high-intensity performance during prolonged intermittent exercise.

2.3.4 Dehydration

Sweat losses from 0.4 to 2% of body mass (equivalent to approximately 0.3 to 1.5 L) have been shown at the end of a football match (Krustrup et al., 2006a), with sweat losses in excess of 3 L shown in hot environments (Mustafa and Mahmoud, 1979). There is a general consensus in the literature that the level of dehydration at which performance is impaired is 2% (Coyle, 2004; Sawka et al, 2007), though Maxwell et al (1999) observed a decrease in

exercise performance (repeated 20 m runs at increasing intensities) with as little as 1.5% dehydration. McGregor et al. (1999) showed that when participants performed the Loughborough Intermittent Shuttle Test (LIST) without fluid ingestion, 15 m sprint times during the final set were longer than when participants were administered drinks (a concentrated lemon drink with no added sugar, diluted 1 : 4 with tap water) throughout the exercise. YoYo Intermittent Recovery Test Level 2 (YoYo IR2) performance was also impaired following a single half of football that elicited 2.4 ± 0.8% (no fluid) and 2.1 ± 0.6% (mouthwash only) dehydration compared to a fluid intake trial that resulted in 0.7 ± 0.4% dehydration. These results suggest that the levels of dehydration experienced by footballers could contribute to reduced performance, though players may not always reach a level of dehydration sufficient to impair performance.

2.3.5 Metabolite Accumulation

High-intensity exercise accounts for approximately 10% of all match activities (Bangsbo et al., 1991), increasing energy demand on the muscle. To meet the increased energy demand there is an increased contribution from anaerobic glycolysis, which results in the accumulation of metabolites such as ADP, inorganic phosphates (Pi), K^+ and H^+ in the skeletal muscle, contributing to fatigue due to a deleterious effect on skeletal muscle function and force generation. A decline in pHi can interfere with several metabolic processes, including a disturbance in the resynthesis of phosphorylcreatine (Harris et al., 1976) and glycolysis (Spriet et al., 1989), which may contribute to a reduction in force production and the onset of fatigue, although not all agree (Bangsbo et al., 1996). Therefore, the increased ability to buffer H^+ might result in an improved capacity to attenuate a decline in repeated sprint performance during high-intensity intermittent exercise. Indeed, an increased RSA has been associated with a greater H^+ buffering capacity in elite female hockey players (Bishop et

al., 2003), recreational team sport females (Bishop and Edge, 2006), untrained females (Bishop et al., 2004b) and professional and amateur male footballers (Rampinini et al., 2009). An intervention designed to increase buffering capacity may thus be of benefit to RSA and team sport performance.

2.4 **Acid-Base Balance**

Acid-base balance in man concerns the regulation of pH homeostasis, maintaining a balance between acids and bases. Acids are substances which liberate H^+ and bases are substances which acquire H^+. Brönsted (1923) discovered that acid-base reactions involve the transfer of H^+ between substances; to maintain homeostasis, there must be a balance between the formation and removal of H^+. This balance is maintained by intracellular and extracellular buffers which can accept or release H^+ to prevent pH changes. In muscle, physicochemical buffers such as organic and inorganic phosphates, bicarbonate anions and histidine containing dipeptides (*e.g.*, carnosine), are the primary mediators of pH homeostasis. H^+ are also actively and passively transported out of the muscle into the blood mediated by transport systems (Juel et al., 2003). H^+ in the blood can be buffered by the circulating anion bicarbonate (HCO_3), which forms carbonic acid, a weak acid ($H^+ + HCO_3 \leftrightarrow H_2CO_3$). Weak acids are relatively stable compared to strong acids, which ionize easily increasing the H^+ content of its surroundings. H^+ are also buffered by the respiratory system through the reversible reaction, $H^+ + HCO_3 \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$, with the resulting carbon dioxide excreted by the lungs. The kidneys also play an important role in maintaining acid-base balance (McNaughton et al., 2008). It is the physicochemical and dynamic buffering systems that are of most importance to high-intensity exercise as they act within seconds of the onset of exercise to maintain pH homeostasis; respiratory buffering occurs within minutes with the kidneys taking days to restore pH balance.

At the onset of exercise, the working muscles increase their energy expenditure dramatically and can reach levels up to one hundred times that seen at rest (Hultman and Sjoholm, 1983). Stores of adenosine 5' triphosphate (ATP) in the muscles are low (25.6 ± 0.7 $mmol \cdot kg^{-1}dm$; Bogdanis et al., 1998), and the aerobic rate of ATP resynthesis is quickly exceeded by the rate of ATP hydrolysis (14.9 ± 2.2 $mmol \cdot kg^{-1}dm \cdot s^{-1}$, Gaitanos et al., 1993) during high-intensity exercise. In order to maintain the energy demands of the muscle, the body relies on resynthesis from PCr, anaerobic glycolysis and anaerobic glycogenolysis, with an increased contribution from aerobic metabolism during prolonged high-intensity exercise bouts (Bogdanis et al., 1996). Glycolysis begins within 5 s of muscle contraction (Hultman and Sjoholm, 1983) and is associated with a large accumulation of metabolites such as ADP, Pi and H^+. In particular, the addition of H^+ poses an increased strain on the body's acid-base balance.

2.4.1 Muscle pH, H^+ and Exercise

Typical resting human pH values of 7.0 are seen in muscle, with arterial and venous blood pH slightly higher at 7.4 and 7.3. An increase of intracellular and subsequently extracellular metabolites during exercise can significantly alter the acid-base balance within the body. Muscle pH may drop to as low as 6.0, with a concomitant drop to ~7.0 seen in both arterial and venous blood during high-intensity exercise (Pan et al., 1991; Bogdanis et al., 1996). Measurements of metabolites in the muscle have shown approximately 94% of H^+ accumulation during exhaustive exercise is a direct result of lactic acid accumulation (Hultman and Sahlin, 1980). Lindinger (1995) showed that 47% of the H^+ accumulation during an all-out 30 s cycle was associated with the increase in lactate concentration, with other major contributing factors including decreased K^+ (32%) and the total concentration of

weak acids and bases (19%). This would suggest that muscle pH can become a limitation to performance and capacity during exercise of a sufficiently high intensity that will result in a large accumulation of lactic acid (and subsequently Lac^- and H^+).

An increase in H^+ and concomitant drop in pHi has been associated with a disturbance in the resynthesis of phosphorylcreatine (Harris et al., 1976), glycolysis (Spriet et al., 1989), oxidative phosphorylation (Jubrias et al., 2003) and can even affect the contractility of the muscle itself (Donaldson and Hermansen, 1978; Fabiato and Fabiato, 1978). Furthermore, individuals can experience an increased perception of effort during high-intensity intermittent exercise due to reduced pHi (Price and Moss, 2007), which may contribute to decreased performance. Conversely, Westerblad et al. (1997) showed that acidification did not directly inhibit force production of the muscle at physiological temperatures, thereby playing an insignificant role in muscle fatigue. Furthermore, Bangsbo et al. (1996) showed that reduced pHi did not inhibit muscle glycolysis, and recent research suggests that increased H^+ accumulation may counter the negative effects of K^+ accumulation in the interstitium (Overgaard et al., 2010). Despite this, increased buffering capacity has been shown to be associated with improved exercise performance (Bishop et al., 2004a and 2004b; Edge et al., 2006; Rampinini et al., 2009) and capacity (Hill et al., 2007), which highlights the potentially deleterious effect of muscle acidification on exercise performance and capacity. It is important to note that supplementation aiming to increase intracellular or extracellular buffering capacity will only be of benefit during exercise limited by reductions in pHi due to the production of H^+.

2.4.2 Intracellular Buffering

Intracellular buffers are the first line of defence of the muscle against increasing H^+ accumulation during intense exercise. These can be classified into physicochemical and metabolic buffering processes. Hultman and Sahlin (1980) determined the contribution of the physicochemical buffers to the buffering process, with Pi, carnosine, bicarbonate anions and protein being the most important. It is only in recent years that high muscle carnosine concentration has been associated with improved high-intensity exercise performance; following the work of Harris et al. (2006) who showed that muscle carnosine concentrations could be increased via supplementation with β-alanine, Hill et al. (2007) were the first to show the subsequent benefit on high-intensity exercise capacity.

2.4.3 Extracellular Buffering

Muscle pH homeostasis is also regulated by active and passive transport of H^+ into the surrounding interstitium where it is buffered by circulating buffers, pulmonary ventilation and the kidneys. The transport of H^+ out of the working cell is mediated by a number of active and passive transporters, the primary at rest being the Na^+/H^+ exchange (Juel et al., 2003). This is further supplemented by the Na^+-dependent and Na^+-independent Cl^-/HCO_3 systems (Juel et al., 2003). The flux of H^+ out of the muscle during exercise is facilitated by MCT1 and MCT4, monocarboxylate transporter proteins that carry monocarboxylates (*ie* lactate) across cell membranes. Due to the increased production of H^+ in the working muscle during exercise, the flux of H^+ out of the muscle and in to the blood will also increase, which will place added strain on the extracellular acid-base balance.

Bicarbonate is a blood buffer that plays an important role in maintaining both extracellular and intracellular pH, despite its inability to permeate the muscle cell membrane (Katz et al.,

1984; Costill et al., 1984). The rate of efflux of H^+ out of the muscle is dependent on the buffer concentration in the surrounding interstitium (Mainwood and Worsley-Brown, 1975). Therefore, if concentrations of bicarbonate in the blood can be increased via supplementation, a theoretical increase in the efflux of H^+ out of the muscle should be observed. An increased removal of H^+ may indirectly enhance exercise performance as muscle fatigue has been linked to the accumulation of intracellular H^+ (Fabiato and Fabiato, 1978; Spriet et al., 1989), although not all agree (Westerblad et al., 1997). Recent research suggests that the mechanism behind an increased exercise tolerance due to metabolic alkalosis may be due to an indirect effect on K^+; Street et al. (2005) showed an association between H^+ and K^+ accumulation with a reduction in both following metabolic alkalosis.

2.5 **Carnosine**

It is more than a century since Gulewitsch and Amiradzhibi (1900) first isolated carnosine; Krimberg (1906, 1908) later classifying it as a histidine containing dipeptide. Carnosine (β-alanyl-L-histidine) is naturally occurring and is found in high concentrations in the skeletal muscle of vertebrates and non-vertebrates (Harris et al., 2006; Hill et al., 2007), and also in the central nervous system. A variety of physiological roles have been attributed to intramuscular carnosine including Ca^{2+} sensitiser (Lamont and Miller, 1992), antioxidant (Boldyrev et al., 1993) and inhibitor against protein glycosylation (Hipkiss et al., 1993 & 1995) and protein cross-linking (Hipkiss, 2000). Its role as pHi buffer is undisputed however, due to its molecular structure and the pKa of its imidazole ring (6.83; Tanokura et al., 1976) (Figure 2.1), making it a suitable buffer over the physiological pH range (Bate-Smith, 1938).

Mannion et al. (1992) reported that carnosine only contributed around 7% to total buffering capacity. These calculations were, however, based upon comparisons of its buffering effect,

derived from its pKa, against calculations of total muscle buffering capacity. Muscle buffering capacity is usually determined by the titration of skeletal muscle homogenates (Harris et al., 1990; Sewell et al., 1991; Mannion et al., 1994; Bishop et al., 2004b). The homogenisation of muscle causes changes to the chemical composition of the intracellular environment, even with the inhibition of glycolysis by iodoacetate (Bueding and Goldfarb, 1941). Included within the homogenised tissue will be intracellular and extracellular pools of pH active compounds from the mitochondria and external membranes, which will contribute to the determination of muscle buffering capacity in the homogenate but would not contribute to physicochemical buffering in the normal cell. In addition, the homogenisation process will also expose lipid-bound phosphate groups, which would also not be involved in intracellular pH control. Furthermore, titration releases bound phosphates contained within phosphorylcreatine, which would contribute to an over estimation of muscle buffering capacity and equally an underestimation of the contribution made by carnosine. In truth, total muscle buffering capacity is constantly changing; being lowest at rest (at which point the relative contribution of carnosine might be calculated to be 3 to 4 times higher than commonly held) and increasing with exercise. The estimate of 7% made by Mannion et al. (1992) represents a minimum estimate and even then in a muscle with a metabolic composition close to that of rigour mortis. However, the findings of Davey (1960) suggest that carnosine can contribute as much as 40% to buffering capacity in the physiological pH range of 6.5 to 7.5.

2.5.1 β-Alanine Supplementation: Effect on Muscle Carnosine

In human blood, carnosine is broken down by carnosinase to its constituent amino acids, β-alanine and histidine (Asatoor et al., 1970), allowing transportation to other organs and tissues, although individuals with a polymorphism in the CNDP1 gene, resulting in decreased

plasma carnosinase activity, have been shown to have detectable plasma carnosine levels 1 h following β-alanine supplementation (Everaert et al., 2012). Importantly, the enzyme carnosinase is not found in muscle; β-alanine and histidine are taken up by the muscle and synthesised to carnosine by carnosine synthase (Figure 2.1). Human carnosine concentrations range from 17.5 ± 4.8 mmol·kg^{-1}dm in females to 21.3 ± 4.2 mmol·kg^{-1}dm in males (Mannion et al., 1992). Higher concentrations have been reported in sprinters, rowers (Parkhouse et al., 1985) and body-builders (Tallon et al., 2005). Furthermore, higher concentrations are found in fast-twitch (type II) compared to slow-twitch (type I) muscle fibres (Dunnet and Harris, 1997; Harris et al., 1998), with human *m. vastus lateralis* carnosine content shown to be 10.5 ± 7.6 mmol·kg^{-1}dm in type I fibres and 23.2 ± 17.8 mmol·kg^{-1}dm in type II fibres (Harris et al., 1998). The higher prevalence of carnosine in type II muscle fibres supports the role of carnosine as an intracellular pH buffer.

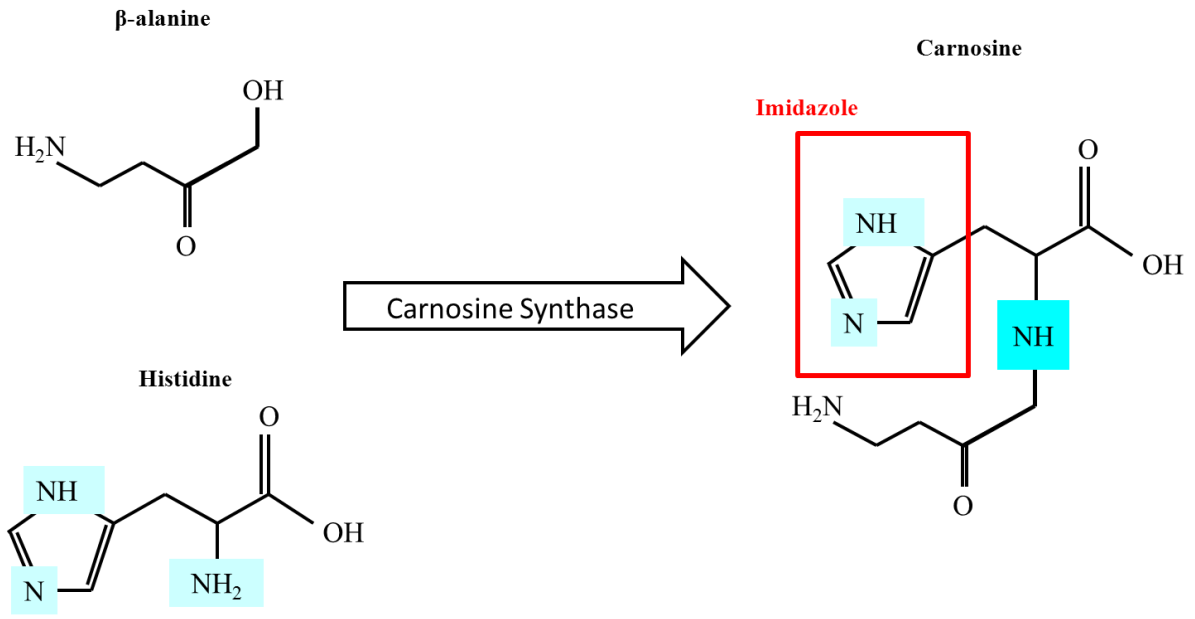

Figure 2.1 The synthesis of carnosine. The imidazole ring, with a pKa of 6.83, is where the H^+ is buffered.

The synthesis of carnosine within the muscle is limited by the availability of β-alanine (Dunnet and Harris, 1999). Harris et al. (2006) were the first to report on the effect of dietary supplementation of β-alanine on skeletal muscle carnosine content over three individual studies. The intention of the first of these studies was to compare the ingestion of β-alanine in free form (0, 10, 20 and 40 mg·kg^{-1}BM) with an equivalent dose (40 mg·kg^{-1}BM) contained within food (in this case a chicken broth). However, upon administration of the higher doses of free β-alanine (from 20 mg·kg^{-1}BM), several participants began to complain of symptoms of flushing (also termed paraesthesia). Symptoms began within 20 minutes of administration and were described as an unpleasant prickly sensation on the skin around the body that lasted up to one hour. This paraesthesia was evident in increasing participant number and intensity with increasing doses (from 20 to 40 mg·kg^{-1}BM of β-alanine; Harris et al., 2006).

Interestingly, no participants complained of these symptoms when ingesting 40 mg·kg^{-1}BM β-alanine in the chicken broth. The peak plasma concentration of β-alanine with the ingestion of 10, 20 and 40 mg·kg^{-1}BM of free β-alanine were 40 ± 26 μmol·L^{-1}, 373 ± 133 μmol·L^{-1} and 833 ± 86 μmol·L^{-1}. The peak concentration of plasma β-alanine following ingestion of the chicken broth was approximately half that of the equivalent 40 mg·kg^{-1}BM β-alanine (428 ± 162 μmol·L^{-1}**)**, but higher than the lower free doses. Time to peak plasma concentration with the ingestion of the chicken broth (90 minutes) was longer than with supplementation in free form (approximately 30 to 40 minutes). Several possible mechanisms exist for the paraesthesia, including β-alanine activated strychnine-sensitive glycine receptor sites, associated with glutamate sensitive N-methyl-D-aspartate receptors in the brain and central nervous system (Mori et al., 2002; Tokutomi et al., 1989; Wang et al., 2003) and the mas-related gene family of G protein coupled receptors, which are triggered by interactions with specific ligands, such as β-alanine (Crozier et al., 2007).

Having confirmed that the peak elevation in plasma β-alanine concentrations, and the time to peak concentration, were unaffected following 2 weeks of supplementation, Harris et al. (2006) examined the effects of 4 weeks of supplementation with either β-alanine or carnosine on muscle carnosine concentrations. In this study, 21 male participants were split into four groups:

Group 1: 5 participants ingested 800 mg of β-alanine four times per day, giving an average daily dose of 3.2 g (89.6 g of β-alanine were ingested over the 4 weeks);

Group 2: 5 participants ingested a total of 145.6 g β-alanine over the 4 weeks supplementation period. However, due to the issue with paraesthesia, participants did not ingest any single dose above 800 mg. This meant that participants were asked to ingest the supplement more frequently to provide the increased dose, with participants ingesting a total of 4.0 $g \cdot d^{-1}$ in week one, increasing to 6.4 $g \cdot d^{-1}$ in week 4;

Group 3: 5 participants ingested L-carnosine using the same dosing strategy as Group 2, with each dose being approximately isomolar with respect to β-alanine. (A total of 364 g of L-carnosine was ingested, equating to 143.3 g of β-alanine);

Group 4: 6 participants ingested placebo capsules (maltodextrin) in the same dosing strategy as employed by Group 2 and Group 3.

All groups showed an increase in muscle carnosine concentrations (Group 1: +7.80 ± 0.36 $mmol \cdot kg^{-1}dm$; Group 2: +11.04 ± 2.68 $mmol \cdot kg^{-1}dm$ and; Group 3: +16.37 ± 3.03 $mmol \cdot kg^{-1}dm$; Group 4: +1.87 ± 1.73 $mmol \cdot kg^{-1}dm$). Thus, the L-carnosine group showed a greater increase in muscle carnosine concentrations than the high and low dose β-alanine groups. However, one participant in the high-dose β-alanine group showed no increase in muscle

carnosine concentrations with supplementation. Interestingly, this participant had the highest pre-supplementation muscle carnosine concentration, which was as high as some of the other participants' post-supplementation carnosine levels. With the exclusion of this participant, based upon the assumption that there was some error in sampling or supplementation adherence, the percentage increases in the high-dose β-alanine group and the L-carnosine group were of a similar magnitude (64.2% and 65.8%).

Hill et al. (2007) confirmed the work of Harris et al. (2006), showing 4 weeks of supplementation with β-alanine (4.0 $g \cdot d^{-1}$ in the first week rising to 6.4 $g \cdot d^{-1}$ in the fourth) increased muscle carnosine in the *m. vastus lateralis* by ~60%. This increased to ~80% when supplementation was continued up to 10 weeks, although the change from 4 to 10 weeks just failed to reach significance ($P = 0.07$). Nonetheless, this suggests that a 4 week period of β-alanine supplementation at 6.4 $g \cdot d^{-1}$ is not sufficient to reach a threshold level for carnosine storage in the skeletal muscle. Stellingwerff et al. (2012) showed a linear dose-response relationship to β-alanine supplementation that is not dependant on baseline muscle carnosine, muscle type, or the daily dose of β-alanine, but is dependent on the total amount of β-alanine consumed. Moreover, 1.6 $g \cdot d^{-1}$ was sufficient to incur significant increases in muscle carnosine after only 2 weeks. The results of Harris et al. (2006), and subsequent supplementation studies, clearly demonstrate that several weeks β-alanine supplementation is sufficient to induce significant increases in muscle carnosine, thereby increasing muscle buffering capacity which may improve exercise performance and capacity limited by the accumulation of H^+.

2.5.2 Carnosine Washout

Baguet et al. (2009) were the first to examine the washout period for carnosine concentrations in the skeletal muscle following β-alanine supplementation. Fifteen participants were supplemented with 4.8 $g \cdot d^{-1}$ β-alanine or a placebo over a 5 – 6 week period. Carnosine content was determined, in the s*oleus*, *tibialis anterior* and *gastrocnemius* using proton magnetic resonance spectroscopy (1H-MRS). Measurements were taken before and after supplementation, as well as after a 3 and 9 week washout period. β-alanine supplementation resulted in an increase in the carnosine content of the *soleus* (39%), *tibialis anterior* (27%) and *gastrocnemius* (23%), which was in line with the previous findings of Derave et al. (2007).

Following the cessation of supplementation, muscle carnosine concentrations declined at a rate of 2 – 4% per week on average (Baguet et al., 2009). Given this, mean muscle carnosine concentrations remained elevated from baseline after 3 weeks of washout, but not following 9 weeks. However, the authors also separated participants into high responders and low responders to β-alanine supplementation. High responders were classified as those participants whose muscle carnosine concentrations increased by over 55% with low responders being those participants whose muscle carnosine concentrations increased by up to 15%. In high-responders, the washout period was increased to 15 weeks, whereas the washout period in the low-responders was 6 weeks. This study indicates that the use of a cross-over design in studies examining the exercise performance effects of β-alanine supplementation is not practical.

2.5.3 β-Alanine Supplementation and Exercise Performance and Capacity

Suzuki et al. (2002) examined the relationship between skeletal muscle carnosine concentrations and exercise performance during 30 s of maximal cycling in 11 healthy males. Muscle biopsies were withdrawn from the *m. vastus lateralis* at rest and were analysed for carnosine concentration. Participants then performed 30 s of maximal sprint cycling, during which the authors calculated mean power output in each of six 5 s periods. The results showed a positive correlation between carnosine concentration and power output during the last two 5 s periods. These results suggest that muscle carnosine concentration could be an important factor in high-intensity exercise performance. However, Bogdanis et al. (1998) indicated that reduced pH did not affect a single bout of cycling as short as 30 s in duration, with reduced ATP following the initial 10 s a more contributing factor.

Indeed, Hoffman et al. (2008) observed no effect of β-alanine supplementation on fatigue rates in 26 collegiate football players during repeated line drills (3 x ~40 s). However, a trend ($P = 0.07$) was observed for a lower rate of fatigue during a modified Wingate power test (lasting 60 s), which was coupled with lower feelings of fatigue (using a 7 point scale) communicated by players supplemented with β-alanine. Similarly, Derave et al. (2007) supplemented sprint trained athletes up to 5 weeks with β-alanine and showed no effect on 400 m running performance (lasting ~52 s). These results suggest that β-alanine supplementation may not be of benefit to single bout high-intensity exercise less than 60 s in duration, a fact confirmed by Hobson et al. (2012) using a meta-analysis of the available literature. Conversely, Baguet et al. (2010) showed a non-significant ($P = 0.07$) effect of β-alanine supplementation on 2000 m rowing performance (typically lasting ~400 s), although there was an absolute improvement in performance that was correlated ($r = 0.498$) with increases in muscle carnosine concentration. Therefore, the optimum exercise duration to be

affected by β-alanine supplementation may be somewhere between 60 and 400 s as this appears to be a period when anaerobic energy sources can contribute between 20 – 60% of the total energy requirement (Maughan et al., 1997) resulting in a large accumulation of H^+. Indeed, Hobson et al. (2012) showed that β-alanine supplementation is most effective on exercise lasting between 60 and 240 s, with exercise over 240 s in duration also improved, though to a lesser extent.

2.5.3.1. Single Bout High-Intensity Exercise

Hill et al. (2007) investigated the effect of β-alanine supplementation on a cycling capacity test at 110% of previously determined Powermax ($CCT_{110\%}$), designed to last between 120 and 240 s. The $CCT_{110\%}$ is a high intensity exercise protocol designed to induce a large accumulation of H^+ and a resultant drop in pHi. Therefore, this type of exercise test would directly focus on the ability of carnosine to improve exercise capacity as a result of an increased muscle buffering capacity.

Twenty-four participants completed the study, with 13 participants being supplemented with β-alanine and 11 with a maltodextrin placebo. Participants were supplemented with an incremental dosage scheme from 4.0 $g \cdot d^{-1}$ in the first week to 6.4 $g \cdot d^{-1}$ in the fourth week, which then continued for a further 6 weeks, meaning that participants were supplemented for 10 weeks in total. There were no differences in total work done (TWD) during the $CCT_{110\%}$ between groups prior to supplementation. The authors showed that TWD during the $CCT_{110\%}$ was increased by 13.0% alongside a 58.8% increase in muscle carnosine following four weeks of β-alanine supplementation. When supplementation was extended to ten weeks, carnosine was increased by 80.1% and total work done by 16.2%. There was no significant change in TWD or muscle carnosine in the placebo group following 4 and 10 weeks of

supplementation. These results provide some support for the notion that increased muscle carnosine content and, as a consequence, increased muscle buffering capacity, allows an increase in high-intensity cycling capacity through a reduction in the impact of H^+ accumulation on muscle function and fatigue.

As the direct result of increased intracellular buffering, following an elevation in skeletal muscle carnosine content, there might also be a delay in CO_2 by-production due to a reduced requirement for extracellular buffering. As such, it could be hypothesised that β-alanine supplementation would have an impact upon the ventilatory threshold in man. Indeed, Zoeller et al. (2007) investigated both the individual and combined effects of 4 weeks of β-alanine and creatine monohydrate supplementation on indices of endurance performance, including markers of the ventilatory threshold and lactate threshold. Participants were allocated to one of four supplementation groups; placebo, creatine monohydrate (21 $g \cdot d^{-1}$ for 6 d and then 10.5 $g \cdot d^{-1}$ for 22 d), β-alanine (6.4 $g \cdot d^{-1}$ for 6 d and then 3.2 $g \cdot d^{-1}$ for 22 d) or a combination of β-alanine and creatine (creatine: 21 $g \cdot d^{-1}$ for 6 d and then 10.5 $g \cdot d^{-1}$ for 22 d plus β-alanine: 6.4 $g \cdot d^{-1}$ for 6 d and then 3.2 $g \cdot d^{-1}$ for 22 d). No between-group differences were observed, indicating that there was no effect of β-alanine or β-alanine plus creatine monohydrate on ventilatory (Orr et al., 1982) and lactate (Weltman et al., 1990) thresholds. However, the authors did report some within group differences following combined β-alanine plus creatine monohydrate supplementation, indicating increases in VO_2 (+5.7%) and power output (+9%) at the lactate threshold and VO_2 (+7.9%), $\%VO_{2peak}$ (+7.9%) and power output (+10.9%) at the ventilatory threshold. However, the true significance of these within-group differences is questionable given the lack of any significant between group effects and the fact that some significant within group changes were also observed in the control group, possibly suggesting that participants lacked familiarisation with the exercise protocols.

Stout and colleagues observed a positive effect of β-alanine supplementation on neuromuscular fatigue in men (Stout et al., 2006), in women (Stout et al., 2007) and in the elderly (Stout et al., 2008). Exercise consisted of incremental cycle stages until exhaustion, during which electromyography was used to determine the onset of neuromuscular fatigue using the physical working capacity at the fatigue threshold (PWC_{FT}). This protocol was developed by deVries et al. (1987) and utilises the relationship between electromyography amplitude and fatigue in order to identify the power output that corresponds to the onset of neuromuscular fatigue. Stout et al. (2006) suggested that the accumulation of metabolic by-products in muscle, including Lac^- and H^+, was a potential mechanism for increased electromyography amplitude during exhaustive exercise. Stout et al. (2006) used the same participants and supplementation protocol as Zoeller et al. (2007). PWC_{FT} improved by 14.5% following 28 days of β-alanine supplementation, while combined β-alanine plus creatine monohydrate supplementation resulted in an observed 11% increase. No effect of creatine monohydrate supplementation was shown, suggesting that the changes that were shown were due to an effect of β-alanine supplementation. Similar to the findings previously shown in men, Stout et al. (2007) showed a 12.6% increase in women in the PWC_{FT} following β-alanine supplementation. This improvement increased to 28.6% when the β-alanine supplemented population were elderly (55 – 92 y) (Stout et al., 2008). The likely cause of the lengthened time to neuromuscular fatigue is an improved intramuscular buffering of H^+, owing to increased carnosine concentration by means of β-alanine supplementation (Harris et al., 2006). However, the precise physiological mechanisms by which improved H^+ regulation would affect neuromuscular fatigue are as yet unclear (Stout et al., 2006).

High-intensity interval training (HIIT) results in a large accumulation of metabolites, which may contribute to fatigue (Robergs et al., 2004). The metabolic response to this increase in

metabolites is suggested to be the mechanism for adaptation, including the ability to delay acidosis (Weston et al., 1997). Therefore, combining HIIT and β-alanine supplementation may result in additive gains. A number of studies have investigated the combined effects of six weeks HIIT, with or without β-alanine supplementation. Smith et al. (2009a) used electromyography to assess fatigue and efficiency of electrical activity, which quantifies the functional state of the muscle (deVries 1968), during a graded exercise cycle. HIIT improved absolute values of fatigue and efficiency of electrical activity following three and six weeks in both the β-alanine and placebo groups, although there was no difference between groups. Similarly, Walter et al. (2010) showed no added benefit of β-alanine supplementation to HIIT on VO_{2peak} during a graded exercise cycle. The training protocol used in these studies may have been a superior stimulus to the untrained population, rendering any changes in muscle carnosine ineffective. Smith et al. (2009b) showed VO_{2peak} and time to exhaustion were improved during graded exercise cycles at three weeks in both supplementation groups, although a further increase from three to six weeks was only observed in those participants also supplemented with β-alanine. In addition, there was an improvement in total work done during a 110% VO_{2peak} test from pre- to mid-training and from mid- to post-training in both groups. However, there was no effect of β-alanine supplementation or training on ventilatory threshold. These results suggest some potential for β-alanine supplementation to further enhance the benefits of high-intensity interval training.

Van Thienen et al. (2009) showed that an 8 week β-alanine supplementation program (2 – 4 $g \cdot d^{-1}$) could enhance sprint power output at the end of a simulated endurance cycle race. Twenty-one participants performed a 110 minute intermittent endurance exercise protocol, varying between 50% and 90% (10 minute stages) of their previously estimated maximal lactate steady state. A time trial with the initial workload set at 100% maximal lactate steady

state (this could be increased or decreased every minute according to the individual's perception of fatigue) proceeded immediately afterwards, followed by a 5 minute active recovery period at 50% of maximal lactate steady state. Exercise was concluded with an all-out 30 s sprint with peak, mean and final power output measured. Following supplementation, participants on β-alanine improved their peak (+11.2%), mean (+4.9%) and final (+10.9%) power output during the 30s sprint. The high blood lactate concentrations observed (~7 $mmol \cdot L^{-1}$) highlight the anaerobic nature of the sprint exercise performed, and suggests that improved performance may have been due to increased H^+ buffering during high intensity anaerobic exercise.

The above studies highlight the ergogenic ability of increased muscle buffering capacity, through supplementation with β-alanine, on single bout high-intensity exercise performance and capacity. However, the nature of team sports is such that players are continually required to repeat short duration high-intensity bouts with recovery periods of varying duration and intensity. Therefore, investigating the effects of β-alanine supplementation on repeated bouts of high-intensity exercise is of more ecological validity to games players.

2.5.3.2. Repeated Bout High-Intensity Exercise

Hoffman et al. (2008) were the first authors to investigate the effect of β-alanine supplementation on repeated sprint performance, supplementing collegiate football players for 30 days with either 4.5 $g \cdot d^{-1}$ β-alanine or matching placebo. Players performed three sets of repeated line drills (200 yards), separated by two minutes rest; sprint times were recorded and used to determine fatigue rate. No significant differences were shown between groups in sprint times or fatigue rate, suggesting no effect of β-alanine supplementation. However, the authors did not take any baseline measurements prior to supplementation; players only

performed the line drills on a single occasion, several weeks into supplementation. Therefore, had baseline measurements been taken, a potential improvement from pre to post supplementation in the β-alanine group cannot be dismissed. Although Hoffman et al. (2008) reported no effect of β-alanine supplementation on repeated line drills, an inappropriate testing strategy may have masked any effects of increased muscle carnosine.

Sweeney et al. (2010) investigated the effect of 5 weeks of β-alanine supplementation (4 $g \cdot d^{-1}$ for 1 week followed by 6 $g \cdot d^{-1}$ for 4 weeks) on 5 x 5 s repeated sprints with 45 s passive recovery. Participants performed two sets of running sprints with a 2 minute active recovery between sets. The authors showed no effect of β-alanine supplementation on horizontal power or performance decrement (%fatigue), although mean power was lower in both groups following supplementation. This was attributed to a change in pacing strategy in both groups, suggesting the participants were not fully familiarised with the protocol.

Table 2.1 Effect of β-alanine supplementation on muscle carnosine concentration and exercise performance and capacity.

Author(s)	Year	Participants	Dose and Timescale	Carnosine Concentrations	Exercise	Exercise Performance & Capacity	Control
Kern & Robinson	2011	22 wrestlers and 15 footballers	4 $g \cdot d^{-1}$ for 60 days with HIIT, RST & resistance training	-	300 y shuttles, 90° flexed arm hang	300 y: FB: ↑1.1% (vs 0.4%), WR: ↑1.6% (vs 1.3%); 90°: FB: ↑3.0% (vs 0.39%), WR: ↑6.5% (vs 5%)	Dextrose
Baguet et al	2010	18 elite rowers	5 $g \cdot d^{-1}$ for 7 weeks	↑45.3% *soleus* ↑28.2% *gastrocnemius*	2000 m rowing	↑Performance trend (P = 0.07)	Maltodextrin
Jordan et al	2010	17 males	6 $g \cdot d^{-1}$ for 4 weeks	-	VO_{2max} treadmill test	Delayed onset of blood lactate accumulation	Maltodextrin
Walter et al	2010	44 females (14, 19, 11 control)	6 $g \cdot d^{-1}$ for 3 weeks, then 3 $g \cdot d^{-1}$ for 3 weeks & 6 weeks HIIT	-	Graded exercise cycle	No added benefit of β-alanine to HIIT improvements	Dextrose
Smith et al	2009a	46 males (18, 18, 10 control)	6 $g \cdot d^{-1}$ for 3 weeks, then 3 $g \cdot d^{-1}$ for 3 weeks & 6 weeks HIIT	-	2 minute cycling work bouts	No added benefit of β-alanine to HIIT improvements	Dextrose
Smith et al	2009b	46 males (18, 18, 10 control)	6 $g \cdot d^{-1}$ for 3 weeks, then 3 $g \cdot d^{-1}$ for 3 weeks & 6 weeks HIIT	-	Constant load to exhaustion and graded cycle tests	↑TWD (not sig. vs. Pla) ↑VO_{2peak} and VO_{2TTE} vs. Pla (3 – 6 weeks)	Dextrose

Sweeney et al	2010	19 males	4 $g \cdot d^{-1}$ for 1 week, then 6 $g \cdot d^{-1}$ for 4 weeks	-	2 sets of 5 x 5 s running sprints	No effect of β-alanine supplementation	Rice flour
van Thienen et al	2009	17 male cyclists	2 $g \cdot d^{-1}$ for 2 weeks, 3 $g \cdot d^{-1}$ for 2 weeks, then 4 $g \cdot d^{-1}$ for 4 weeks	-	110 min intermittent cycle, followed by 10 min TT then 30 s sprint	↑MPO (5.0%) & PPO in sprint (11.4%)	Not stated
Hoffman et al.	2008	8 resistance trained males	4.8 $g \cdot d^{-1}$ for 4 weeks	-	1RM squat test	↑Volume of training	Not stated
Stout et al	2008	9 males and 17 females (55 -92 years)	2.4 $g \cdot d^{-1}$ for 90 days	-	Discontinuous incremental exercise protocol	↑12.6% PWC_{FT}	Cellulose
Kendrick et al.	2008	26 males	6.4 $g \cdot d^{-1}$ for 4 weeks with 4 weeks resistance training of one leg	↑12.8% *vastus lateralis*	WBS, BS, BP, DL. Isokinetic knee extensions (IFP)	↑WBS and IFP (not between supplement groups),	Maltodextrin
Hoffman et al	2008	26 strength & power athletes	4.5 $g \cdot d^{-1}$ for 30 days	-	60 s wingate, line drills and training	Trends for ↑ training volume (P = 0.09) and ↓fatigue rates (P = 0.07)	Maltodextrin

Derave et al	2007	15 sprint trained male athletes	2.4 $g \cdot d^{-1}$ for 4 days, 3.6 $g \cdot d^{-1}$ for 4 days, then 4.8 $g \cdot d^{-1}$ up to 4-5 weeks	↑47% *soleus* ↑37% *gastrocnemius*	5 bouts of max knee extensions, isometric contractions & 400m running	↑Maximal voluntary extensions during bouts 4 & 5	Maltodextrin
Stout et al	2007	22 females	3.2 $g \cdot d^{-1}$ in week 1, 6.4 $g \cdot d^{-1}$ weeks 2 - 4	-	Graded Exercise Test	↑ 12.6% PWC_{FT} ↑13.9% VT ↑ 2.5% TTE	Not stated
Hill et al	2007	13 males (4 weeks) and 8 males (10 weeks)	4.0 $g \cdot d^{-1}$ in week 1 rising to 6.4 $g \cdot d^{-1}$ by week 4 till week 10	↑ 58.8% (4 weeks) and ↑ 80.1% (10 weeks) in *vastus lateralis*	Cycle capacity test at 110% of maximum power	↑ 13.0% (4 weeks) ↑ 16.2% (10 weeks)	Maltodextrin
Zoeller et al	2007	51 males	BA, BACrM, CrM, Pla for 4 weeks, BA = 6.4 $g \cdot d^{-1}$	-	Graded Exercise Test	↑Performance in 5 of 8 parameters with BACrM	Dextrose
Stout et al	2006	51 males	BA, BACrM, CrM, Pla for 4 weeks, BA = 6.4 $g \cdot d^{-1}$	-	Graded Exercise Test	↑ PWC_{FT} in BA and BACrM	Dextrose

Previous studies have not shown a significant effect of β-alanine supplementation on repeated sprint performance (Hoffman et al., 2008; Sweeney et al., 2011), although any effects of supplementation may have been masked by inappropriate testing strategies and insufficient familiarisation of the protocols. Furthermore, these studies did not determine repeated sprint performance during simulated or actual games play and, thus, did not consider the implications of the additional metabolic demand of the entire activity. Further research is warranted employing protocols that simulate games play to determine if β-alanine supplementation is beneficial to team sports performance.

2.6 **Bicarbonate**

2.6.1 Sodium Bicarbonate Supplementation: Effect on Blood Bicarbonate

Sodium bicarbonate supplementation has consistently been shown to cause blood alkalosis (Inbar et al., 1983; Costill et al., 1984; Gaitanos et al., 1991), indicated by an increase in blood bicarbonate concentrations and pH (for reviews see Matson and Tran, 1993 and Carr et al., 2011). Under normal resting conditions, circulating concentrations of bicarbonate range approximately between 23.0 to 27.0 $mmol \cdot L^{-1}$ (Matson and Tran, 1993). Studies investigating sodium bicarbonate supplementation to increase blood bicarbonate levels have used doses relative to body mass (BM), ranging from as little as 0.1 $g \cdot kg^{-1}$BM increasing to as much as 0.5 $g \cdot kg^{-1}$BM (McNaughton, 1992). A dose of 0.1 $g \cdot kg^{-1}$BM may not be sufficient to increase blood bicarbonate, while doses above 0.3 $g \cdot kg^{-1}$BM result in increased gastrointestinal disturbance in all participants (McNaughton, 1992), which may deter participants from ingesting higher doses. Furthermore, doses of 0.4 and 0.5 $g \cdot kg^{-1}$BM have not shown increases in circulating levels of bicarbonate above that of 0.3 $g \cdot kg^{-1}$BM (McNaughton, 1992), suggesting this to be the optimal dose.

Interestingly, Matson and Tran (1993) reported a relatively weak relationship (r = 0.42) between dose and degree of blood alkalosis following sodium bicarbonate supplementation from a meta-analysis of the literature. It was hypothesised that this was due to the large variability in individual pH and bicarbonate responses to supplementation. This would suggest that since acute supplementation with sodium bicarbonate may not result in a similar degree of alkalosis in those ingesting it, any true ergogenic effects might be masked by this individual variability. Therefore, when investigating the effects of sodium bicarbonate on exercise performance, it would seem appropriate to employ a larger sample population to account for the variability in physiological responses. Furthermore, splitting participants into those who benefited from sodium bicarbonate supplementation, and those who did not, would allow analysis into why, physiologically, participants did or did not benefit from supplementation.

2.6.2 Sodium Bicarbonate Supplementation and Exercise Performance and Capacity

The effects of sodium bicarbonate supplementation on exercise performance and capacity have been well researched (for review see McNaughton et al., 2008), although the reported effects are equivocal. Inconsistencies in the performance outcomes of sodium bicarbonate supplementation studies (Table 2.2) can be partly attributed to differing dosing regimens, gastrointestinal discomfort experienced by some participants, exercise models insufficient to be limited by H^+ accumulation and individual variation in the response to supplementation. Despite this, there is a wide range of evidence to support the use of sodium bicarbonate supplementation as an ergogenic aid.

Table 2.2 Effect of sodium bicarbonate supplementation on single bout high-intensity exercise performance and capacity

Author(s)	Year	Participants	Dose ($g \cdot kg^{-1}$ BM)	Loading Time Before Exercise	Exercise Mode and Protocol	Reported Ergogenic Effect	Control
			Exercise Duration ≤ 60 s				
McNaughton	1992	9 males	0.1 0.2 0.3 – 0.5	60 min	1 min cycle sprint	No difference in MPO ↑ MPO ↑ MPO and ↑ PPO	Control and $CaCO_3$
McNaughton et al.	1991	8 males	0.4	60 min	1 min maximal cycle	↑TWD	$CaCO_3$
Inbar et al.	1983	13 males	10 g dose	150 min	30 s cycling Wingate test	↑ MPO (~1.3%) No difference in PPO	NaCl
			60 s < Exercise Duration ≤ 240 s				
Siegler et al.	2008	9 males	0.3	~60-75 min	Cycle to fatigue at 120% MPO (~120 s)	No effect on TTE	$CaCO_3$
Robergs et al.	2005	12 trained cyclists	0.2 $NaHCO_3$ & 0.2 Sodium citrate	~75 min	Cycle to exhaustion at 110% of workload at VO_{2max} (~150 s)	No difference in TTE	$CaCO_3$

van Montfoort et al.	2004	15 male endurance runners	0.3	90-180 min	Running sprint to exhaustion (60 – 120 s)	96, 92 and 66% chance of a substantial improvement with sodium bicarbonate versus citrate, lactate & chloride	Sodium citrate, lactate & chloride
Tiryaki & Atterbom	1995	11 female athletes	0.3	150 min	600 m running test (~120 s)	No difference in running times	Sugarless Kool-Aid
Horswill et al.	1988	9 males	0.1 - 0.2	60 min	2 min maximal cycle sprint	No difference in TWD	NaCl
Katz et al.	1984	8 males	0.2	60 min	Cycle to exhaustion at 125% VO_{2max} (~100 s)	No difference in TTE	NaCl
Exercise Duration > 240 s							
McNaughton & Cedaro	1991	5 trained males	0.3	90 min	6 min maximal row on an ergometer	↑ Rowing distance	$CaCO_3$

2.6.2.1. Single Bout High-Intensity Exercise

McNaughton et al. (1992) showed that total work done during a single bout of high-intensity exercise (60 s cycle sprint) could be improved with sodium bicarbonate supplementation ranging in dose from 0.2 – 0.5 $g \cdot kg^{-1}$BM. McNaughton et al. (1991) and Inbar et al. (1983) also showed improvements in total work done and power output during single cycle sprints no longer than one minute in duration (Table 2.2). In a meta-analysis of the literature, Carr et al. (2011) showed that sodium bicarbonate supplementation prior to a 60 s sprint improved performance by 1.7 ± 2.0%. Katz et al. (1984) employed a cycling capacity test to exhaustion lasting in excess of one minute (~100 s) but showed no benefit from sodium bicarbonate supplementation, and suggested that sodium bicarbonate supplementation may be of more benefit during repeated bout exercise.

2.6.2.2. Repeated Bout High-Intensity Exercise

Sodium bicarbonate supplementation (in doses between 0.2 to 0.3 $g \cdot kg^{-1}$BM) has been shown to increase work output (McKenzie et al., 1986; Bishop et al., 2004a), mean power output during repeated sprint activity (Lavender and Bird, 1989), and delay fatigue in high-intensity cycling to exhaustion following repeated sprints (Costill et al., 1984; McKenzie et al., 1986) (Table 2.3). Lavender and Bird (1989) showed a greater power output in eight out of ten 10 s sprints separated by 50 s recovery following sodium bicarbonate supplementation. Similarly, Bishop et al. (2004a) showed sodium bicarbonate increased total work done (16.5 ± 3.1 vs. 15.7 ± 3.0 kJ) and peak power output during 5 x 6 s repeated cycle sprints with 24 s passive rest. Gaitanos et al. (1991) had participants perform ten 6 s running sprints with 30 s passive recovery. Although mean power output in the sodium bicarbonate trial was 2% higher than in the placebo trial, there was no significant effect. Repeated sprint activity is important to team sport activity, however, cycling protocols do not fully represent the physical demands placed

on a player during competitive match play as they only incorporate lower limb activity. Furthermore, these repeated sprint tests do not incorporate the full metabolic demand placed upon players during prolonged match play.

2.6.2.3. Prolonged High-Intensity Intermittent Exercise

Price et al. (2003) investigated the effect of sodium bicarbonate supplementation on an intermittent protocol based on football notional analysis (Reilly and Thomas, 1976), although this was performed on a cycle ergometer. The protocol incorporated ten repeated 3 minute blocks of intermittent exercise, each consisting of 90 s at 40% of an individual's VO_{2max}, 60 s at 60% VO_{2max}, 14 s maximal sprinting and 16 s rest. Although total work done and peak power output during the sprints were not different between conditions, there was a main effect between trials in peak power output relative to the first sprint. A similar protocol based on intermittent team sport exercise was adopted by Bishop and Claudius (2005) who showed a trend ($P = 0.08$) towards increased total work done with sodium bicarbonate during the second of two halves of 36 minutes of intermittent cycling, although only seven of eighteen second half sprints were improved with sodium bicarbonate. Whether sodium bicarbonate supplementation could benefit team sport athletes who are required to perform repeated bouts of sprinting and high-intensity exercise during competitive match play remains unanswered; although these studies incorporate intermittent exercise activity, their external validity to team sports is questionable. Both studies performed exercise on cycle ergometers, which only incorporates lower limb rather than whole body activity. The duration of the entire protocol used by Price et al. (2003) is much shorter than a competitive team sport match and sprint duration is much longer than the average 2 – 3 s sprint (Spencer et al., 2005) observed in team sports. Therefore, more research needs to be performed on specific exercise protocols

that simulate team sport activity to identify whether sodium bicarbonate supplementation can improve performance during high-intensity intermittent exercise.

To date, studies investigating the effect of sodium bicarbonate supplementation on exercise capacity and performance have reported contrasting results. These conflicting results can be attributed to a variety of factors, including differing doses, different exercise protocols, GI disturbance and individual variation in blood responses to supplementation. Nonetheless, there are numerous studies that have shown sodium bicarbonate to be of benefit during exercise modalities likely to be limited by the accumulation of intramuscular H^+. Furthermore, some evidence suggests that sodium bicarbonate might be of benefit to high-intensity intermittent exercise performance, although the protocols previously used have lacked ecological validity.

Table 2.3 Effect of sodium bicarbonate supplementation on repeated-sprint and high-intensity exercise performance and capacity.

Author(s)	Year	Participants	Dose ($g \cdot kg^{-1}BM$)	Loading Time Before Exercise	Exercise Mode and Protocol	Reported Ergogenic Effect	Control
Zinner et al.	2011	11 males	0.3	90 min	4 x 30 s maximal cycle sprint separate by 5 min passive recovery	↑MPO during sprints 3 and 4	$CaCO_3$
Price & Simons	2010	8 males	0.3	60 min	20 x 24 s runs at 100% VO_{2max} followed by a run to exhaustion at 120% VO_{2max}	No difference in TTE	NaCl
Siegler et al.	2010	9 males	0.3	~60-75 min	3 x 30 s maximal running sprints separated by 3 min of active or passive recovery	↑Average speed in the final bout for placebo and active recovery	NaCl
Siegler and Gleadall-Siddal	2010	6 male & 8 female swimmers	0.3	150 min	8 x 25 m swims separated by 5 s	Improved swim time (2%)	NaCl
Bishop & Claudius	2005	9 female team sport players	2 x 0.2	90 min & 20 min	2 x 36 min intermittent cycling; Repeated 2 min blocks of 4 s sprint, 100 s at 35% VO_{2peak}, and 20 s rest	Trend towards ↑TWD during the 2nd half (P = 0.08)	NaCl
Bishop et al.	2004a	10 females	0.3	90 min	5 x 6 s cycling test	↑TWD and ↑PPO during sprints 3, 4 and 5	NaCl
Price et al.	2003	8 males	0.3	60 min	10 x 3 minute blocks of intermittent cycling	↑ PPO relative to sprint 1	NaCl

Webster et al.	1993	6 males	0.3	105 min	4 x 12 repetitions at 70% of 1RM on a universal leg press machine, followed by a fifth set to exhaustion	No difference in repetitions performed in the fifth set	Flour
Gaitanos et al.	1991	7 males	0.3	150 min	10 x 6 s running sprints	No difference in MPO No difference in PPO	NaCl
Lavender & Bird	1989	15 males 8 females	0.3	60 – 120 min	10 x 10 s cycle sprints	↑ MPO in 8 of 10 sprints	NaCl
McKenzie et al.	1986	6 males	0.15 0.3	60 min	5 x 60 s cycling at 125% VO_{2max} with 60 s recovery, followed by a 6^{th} bout to exhaustion	↑TTE & ↑TWD	
Costill et al.	1984	10 males 1 female	0.2	60 min	4 x 1 min cycling at 100% VO_{2max} followed by a cycle to exhaustion at 100% VO_{2max}	↑ TTE (42%)	NaCl
Inbar et al.	1983	13 males	10 g dose	150 min	30 s cycling Wingate test	↑ MPO (~1.3%) No difference in PPO	NaCl
Sutton et al.	1981	5 males	0.3	15 – 180 min	20 min cycles at 33% and 66% of VO_{2max} followed by a cycle to exhaustion at 95% VO_{2max}	↑ TTE	$CaCO_3$ or NH_4Cl

2.7 **Summary**

High-intensity exercise results in the accumulation of H^+, which increases the strain on acid-base balance in the body; when buffering capacity is exceeded, a resultant drop in intracellular and extracellular pH can negatively affect several metabolic processes, contributing to the early onset of fatigue (Spriet et al., 1989). Consequently, a decline in exercise performance and capacity may be attenuated in individuals who can buffer the reduction in pH more effectively. Therefore, an intervention designed to increase buffering capacity might be of benefit to individuals involved in high-intensity exercise, whether of a continuous or intermittent nature.

Buffering agents are widely used by athletes to augment buffering capacity and potentially improve exercise performance and capacity. Harris et al. (2006) showed that β-alanine supplementation could increase muscle carnosine concentration, with subsequent studies showing concomitant improvements in high-intensity exercise performance and capacity (for reviews see Sale et al., 2010 and Hobson et al. 2012). The most likely explanation for any ergogenic benefit is due to an increased intracellular buffering capacity, resulting in a delay in the decrease of pHi during exercise. Acute sodium bicarbonate supplementation, which results in alkalosis of the blood (Inbar et al. 1983; Costill et al. 1984; Katz et al. 1984; Gaitanos et al. 1991), has long been regarded as an ergogenic aid for high-intensity exercise performance and capacity (for review see McNaughton et al. 2008), most likely due to an increased efflux of H^+ out of the working muscles. Available literature suggests that increased H^+ buffering capacity can be beneficial to exercise performance and capacity, although there remain several unanswered questions requiring investigation.

This thesis reports on eight studies that extend the previous body of research into buffering agents and their ergogenic effect on high-intensity exercise performance and capacity. Despite the fact that studies have shown significant improvements in exercise performance and capacity following supplementation with β-alanine and sodium bicarbonate separately, no study to date has investigated the effects of co-supplementation, thereby increasing both intracellular and extracellular buffering capacity. Furthermore, despite several studies investigating increased buffering capacity on repeated sprint exercise, no study has employed a protocol designed to simulate actual match play, thereby incorporating the full metabolic demand of the entire activity.

Chapter 3.0 General Methods

3.1 **Participants**

Male games players of varying standards volunteered for the studies reported in this thesis. All participants were fully informed of any risks and discomforts associated with their study before completing a health screen and providing informed consent. The health screening procedure was repeated prior to each laboratory visit to ensure the health status of the participants had not changed. Participants had not taken any supplement in the 3 months prior to their study, and had not taken β-alanine for at least 6 months prior to their study due to the long washout period for muscle carnosine (Baguet et al., 2009). Participants were also requested to maintain similar levels of physical activity and dietary intake for the duration of their study and compliance with this request was verbally confirmed with participants prior to commencement of the study. Dietary intake was monitored in the 24 h prior to main trials using a food diary, and repeated prior to any subsequent main trial. None of the participants were vegetarian and therefore would have encountered small amounts of β-alanine in their diet from the hydrolysis of carnosine and methyl derivatives of this in meat; typically 50 to 400 mg per day. All studies were approved by the Nottingham Trent University's Ethical Advisory Committee.

3.2 **Supplementation Protocols**

3.2.1 β-Alanine

Participants were supplemented with either β-alanine (CarnoSyn^{TM}; NAI, USA) or placebo (maltodextrin; NAI, USA), provided in the form of 800 mg sustained-release tablets, in order to minimise the incidence of paraesthesia which is associated with the time to peak plasma concentration (Harris et al., 2006). All administration of supplementation was double-blind, and occurrence of paraesthesia would compromise the blinding of the study, resulting in exclusion of the affected participant. All supplements were tested by HFL Sports Science

prior to use to ensure no contamination with steroids or stimulants according to ISO 17025 accredited tests.

3.2.2 Sodium Bicarbonate

Participants were acutely supplemented with either sodium bicarbonate (SIS, UK) or maltodextrin (SIS, UK) prior to exercise in several chapters reported in this thesis, and ingested a total of 0.3 $g \cdot kg^{-1}$BM of sodium bicarbonate in gelatine capsules made up individually for each participant. This total dose was based upon that used in other studies (McNaughton et al., 2008). The total dose of the maltodextrin placebo was ingested in the same number of opaque gelatine capsules. Following an overnight fast, participants arrived at the laboratory 4 h before the exercise protocol. Participants ingested 0.2 $g \cdot kg^{-1}$BM of sodium bicarbonate or matching placebo alongside a standardised breakfast and a final 0.1 $g \cdot kg^{-1}$BM was ingested 2 h after the standardised breakfast, 2 h prior to commencement of exercise. Each dose of sodium bicarbonate or maltodextrin was ingested with 500 ml of plain water. A split dose strategy for sodium bicarbonate ingestion was employed in order to minimise the gastrointestinal discomfort often associated with supplementation at this level.

3.3 **Experimental Protocols**

3.3.1 Powermax (W_{max}) Test

Participants performed a graded cycle capacity test to exhaustion on a cycle ergometer (Lode Excalibur, Lode B.V., Germany) to determine individual W_{max}. Exercise commenced at a self-selected power between 100 and 150 W, and was increased by 6 W every 15 s (ramp rate of 24 $W \cdot min^{-1}$) until participants reached volitional exhaustion. Participants pedalled at a constant, self-selected pedal cadence and were given verbal encouragement throughout. Volitional exhaustion was deemed to have occurred when participants dropped 20 $rev \cdot min^{-1}$

below their self-selected pedal cadence, at which point they were instructed to stop pedalling. The maximum power output averaged over the final two stages was defined as an individual's W_{max}.

3.3.2 Cycling Capacity Test ($CCT_{110\%}$)

All trials of the cycling capacity test at 110% of W_{max} were performed on a cycle ergometer (Lode Excalibur, Lode B.V., Germany). Individual set up of the cycle ergometer (saddle and handlebar height and length) was determined prior to the initial W_{max} trial and was maintained for all subsequent $CCT_{110\%}$ trials. A 5 min cycling warm up was performed at 100 W followed by a 2 min period of stretching. Each participant's $CCT_{110\%}$ was incremented over the first 30 s which corresponded to 80% W_{max} during the first 15 s, 95% W_{max} over the second 15 s followed by 110% W_{max} until volitional exhaustion. Participants pedalled at a constant, self-selected pedal cadence and were given verbal encouragement throughout. Volitional exhaustion was deemed to have occurred when participants dropped 20 $rev \cdot min^{-1}$ below their self-selected pedal cadence, at which point they were instructed to stop pedalling. Time to exhaustion (TTE, s) and total work done (TWD, kJ) were recorded as the outcome measures for all tests.

3.3.3 Speed Lactate and Maximal Oxygen Uptake (VO_{2max}) Test

Participants began an incremental running speed lactate test at a self-selected starting speed (range: 6 – 10 $km \cdot h^{-1}$) on a motorised treadmill (Pulsar, h/p/cosmos, Germany); initial speed was of a low intensity so as not to illicit an increase in lactate in excess of 1 $mmol \cdot L^{-1}$ above resting concentration. Exercise intensity was increased by 1 $km \cdot h^{-1}$ every 3 min until an increase of lactate above 4 $mmol \cdot L^{-1}$ was reached (lactate threshold). Fingerprick blood samples were obtained at the end of every stage and analysed for lactate using an automated

glucose and lactate analyser (YSI 2300 Stat, YSI Incorporated, USA). Participants rested for 10 min before performing a VO_{2max} test to volitional exhaustion (Jones and Doust, 1996). Treadmill speed was equivalent to that of the individual's lactate threshold, and began at a 1% incline. Inclination was increased by 1% every minute until the participant indicated they had only one minute remaining. A Douglas bag sample was obtained during the final minute and analysed for gas concentration and volume using a calibrated Servomex gas analyser (Servomex 1440, Servomex, UK) and dry gas meter (Harvard, UK).

3.3.4 Football Specific Intermittent Treadmill Protocol (FSINT)

All trials took place in an environmental chamber (Design Environmental Ltd, UK) at a simulated altitude of 2500 m, with desired environmental conditions of 15.5% oxygen, temperature of 18.0°C and a relative humidity of 50.0%. Participants completed a laboratory based intermittent treadmill protocol (Greig et al, 2006) designed to replicate the demands of soccer. The protocol consisted of two 45 min halves (FSINT1 and FSINT2) separated by a 15 min half time period; within each half, the protocol comprised of three 15 min activity bouts consisting of eight different exercise intensities (Table 3.1). All activity was performed on a motorised treadmill (Pulsar, h/p/cosmos, Germany) set at a 1% gradient. Total distance covered during the FSINT was 9.70 km.

Table 3.1 Movement activities performed every 15 min cycle of the FSINT.

Activity	Speed ($km \cdot h^{-1}$)	Number of activities	Mean duration (s)
Standing	0	20	7.8
Walking	4	55	6.7
Jogging	8	42	3.5
Low Speed	12	46	3.5
Moderate Speed	16	20	2.5
High Speed	21	9	2.1
Sprint	25	3	2.0

3.3.5 Repeated Sprints (5 x 6 s)

The repeated sprints comprised of five maximal sprints, each 6 s in duration, with 24 s active recovery, performed on a non-motorised treadmill (Desmo-Force, Woodway, USA), which was adapted in the laboratory. Participants were required to wear a belt around their waist which was attached to a force transducer placed directly behind the treadmill. All data were recorded using a modified version of Spike2 (V5.09, CED, Cambridge). Mean power output (MPO) and peak power output (PPO) of every sprint were recorded.

3.3.6 Multistage Fitness Test

All tests were conducted in a sports hall with an ambient temperature of 19.1 ± 0.8°C and relative humidity of 43.7 ± 6.2%. A 5 min standardised warm-up was performed, consisting of light jogging and running, followed by 5 min of self-selected stretching. Participants were then required to run between markers set 20 m apart at increasing speeds dictated by an audio signal. The test was ended if the participant failed to reach the designated line within the given time frame on two consecutive occasions or at volitional exhaustion. The final level attained by the participant was used to estimate maximal oxygen uptake (Ramsbottom et al., 1988).

3.3.7 Loughborough Intermittent Shuttle Test (LIST)

All tests were conducted in a sports hall with an ambient temperature of 18.8 ± 0.9°C and relative humidity of 42.8 ± 4.7%. A 5 min standardised warm-up was performed, consisting of light jogging and running, followed by 5 min of self-selected stretching. The LIST requires participants to run between markers set 20 m apart at varying speeds dictated by an audio signal (Nicholas et al., 2000). The test consisted of six exercise sets approximately 15 min long separated by periods of 3 min rest (Figure 3.1). Within each set was an exercise pattern (Figure 3.1) repeated 11 times, incorporating walking, recovery, sprinting (over 15 m), cruising and jogging; cruising and jogging were defined as 95% and 55% of an individual's estimated VO_{2max}. These corresponding running speeds were calculated using tables for predicted VO_{2max} values (Ramsbottom et al, 1988). Individual sprint times over 15 m were recorded (Brower Timing Systems IRD-T173, USA), totalling 66 sprints.

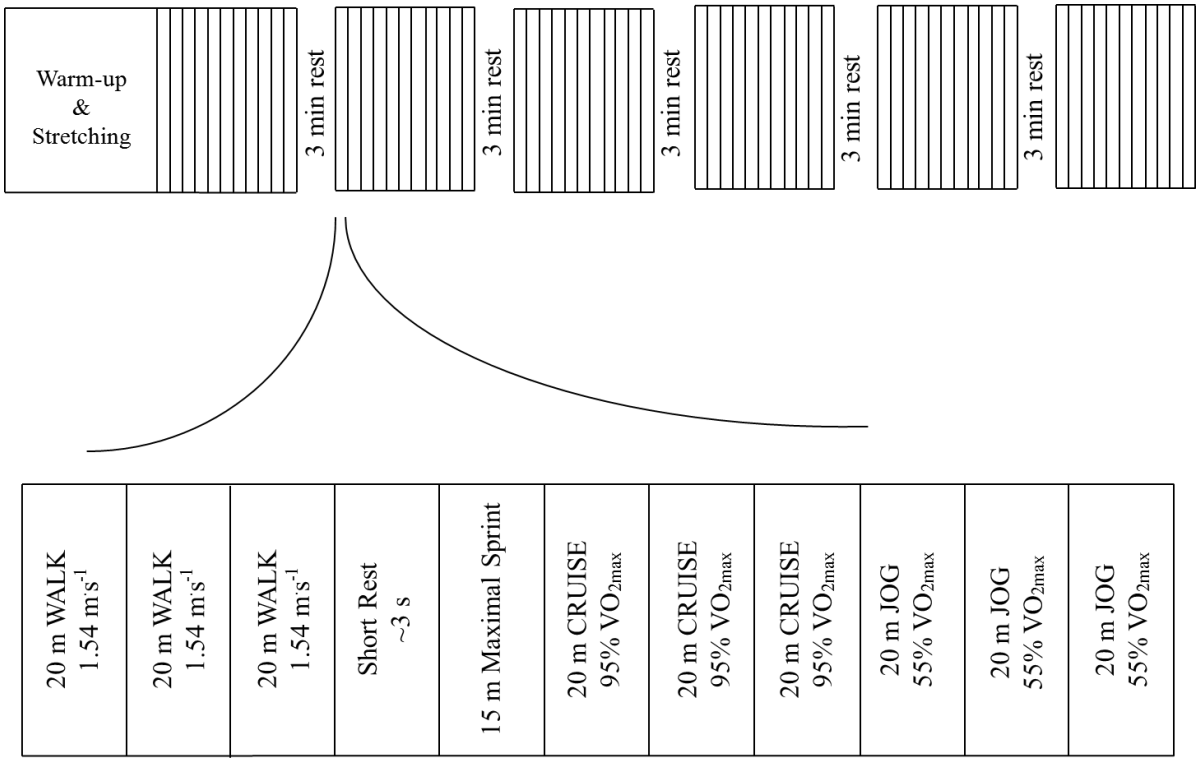

Figure 3.1 The Loughborough Intermittent Shuttle Test.

3.3.8 YoYo Intermittent Recovery Test Level 2 (YoYo IR2)

The YoYo IR2 consisted of repeated 40 m (2 x 20 m) runs at progressively increasing speeds dictated by an audio signal (Bangsbo, 1994b). Participants perform 10 s of active recovery between each running bout, consisting of a 10 m (2 x 5 m) walk. The test was ended if the player failed to reach the finish line within the given time frame on two consecutive occasions or if the player felt unable to continue (volitional exhaustion). The total number of levels was recorded and used to determine total distance covered (m) during the test.

3.3.9 Match Analysis

In match running data were collected during competitive football match play using Global Positioning Satellite technology (GPSports, Australia). Players wore an individual GPS unit contained within a custom made harness (Figure 3.2), which positioned the unit along the spinal column between the scapulae.

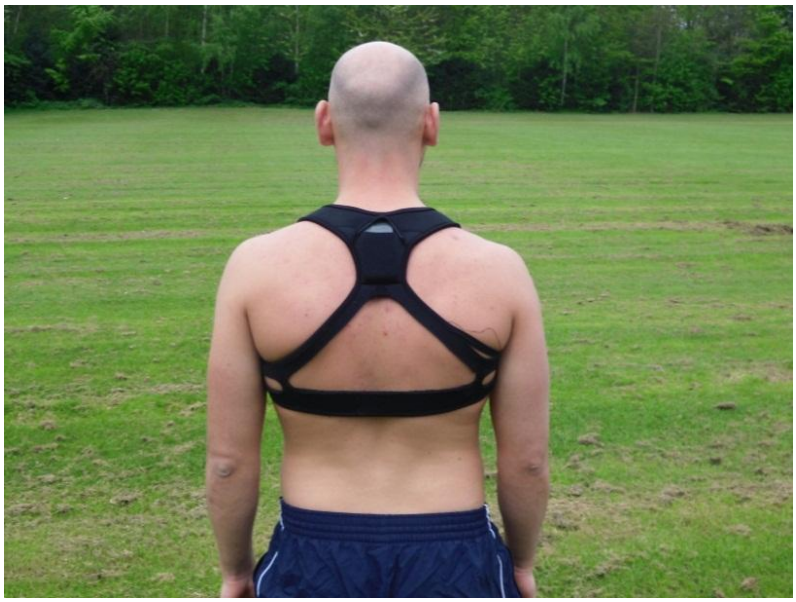

Figure 3.2 The GPS unit contained within the custom made harness worn by players during competitive match play.

Players were tracked for the duration of competitive match play using a 1-Hz GPS athlete tracking device (SPI EliteTM, GPSports, Australia) as validated by Macleod et al. (2009). All match data were downloaded and analysed using Team AMS (V2.1.0.5, GPSports, Australia) software. Throughout all matches, 8 ± 1 (range: 6 – 13) satellites were available for transmission which has been shown to be optimal for the capture of human locomotion (Jennings et al., 2010).

3.4 **Measurements**

3.4.1 Height and Body Mass

Height and body mass were recorded upon arrival to the first session of every study. Height was measured using a stadiometer (Seca, UK) while body mass was recorded using calibrated digital scales (Seca, UK), accurate to the nearest 0.1 kg.

3.4.2 Blood Sampling

Blood samples were obtained using the finger-prick capillary technique, which involved puncturing the skin using a spring propelled lancet (Unistik3, Owen Mumford, UK).

3.4.2.1. Blood Lactate

Blood samples reported in Chapters 4A, 4B, 4C and 6 were analysed for lactate using a hand-held analyser (Lactate Pro, Arkray, Japan). Those reported in Chapter 5A and 5B, 50 µL of blood was collected using microvette blood tubes (Microvette, Germany) and analysed for lactate using an automated glucose and lactate analyser (YSI 2300 Stat, YSI Incorporated, USA). The coefficient of variation for blood lactate, taken from a single bolus of blood, was 6.5% for the Lactate Pro, and 5.6% for the YSI (Table 3.2).

3.4.2.2. Blood Gases

80 µL of blood was obtained using heparin coated glass clinitubes (Radiometer Ltd, UK) and analysed for pH, haemoglobin (Hb) and blood gases using a blood gas analyser (Radiometer ABL 400, UK). Blood bicarbonate was calculated from PCO_2 and pH values according to the Henderson-Hasselbalch equation ($pH = pK_a + \log([A^-]/[HA])$). Base excess, a measure of how alkalotic or acidotic a substance is, was calculated according to ((1 – 0.014[Hb]) x ($[HCO_3^-]$ – 24 + (1.43[Hb] + 7.7) (pH – 7.4))) (Andersen et al., 1960; Andersen and Engel, 1960). This equation takes into account the relative contributions of pH, Hb and bicarbonate to the acid-base balance of the blood. The coefficient of variation for blood gases, taken from a single bolus of blood, was 0.2%, 2.6% and 1.7% for pH, PCO_2 and Hb. (Table 3.2).

Table 3.2 Coefficient of Variation for blood lactate, pH, PCO_2 and Hb

Metabolite		N	Mean	CV (%)
Lactate ($mmol \cdot L^{-1}$)	Lactate Pro	10	1.0 ± 0.1	6.5
	YSI	10	1.06 ± 0.06	5.6
pH		10	7.414 ± 0.017	0.2
PCO_2 (mmHg)		10	36.66 ± 0.97	2.6
Hb ($g \cdot dL^{-1}$)		10	15.6 ± 0.3	1.7

3.4.3 Heart Rate

Heart rate was recorded every 5 s throughout the exercise tests reported in Chapters 5A, 5B, and 6 using heart rate monitors (Polar Team, Polar Electro Oy, Finland) and downloaded using appropriate software (Polar Precision Performance V4.03.040, Polar Electro Oy, Finland).

3.4.4 Ratings of Perceived Exertion (RPE)

Participants were asked to rate their perceived exertion of the LIST reported in Chapter 6 by pointing to a number on a 15 point scale from 6 to 20 (Borg, 1973) during the final walking stage of every set.

3.4.5 Intensity of Stomach ache, Sickness and Headache Scales

In the studies reported in Chapters 5A and 5B, participants were asked to report their intensity of stomach ache, sickness and headache by pointing to an 11 point scale from 0 to 10. This was done on four occasions during each trial; 240, 120 and 0 min prior to exercise, and immediately post-exercise. The scales (Appendix 1) contained descriptors at 0, 3, 6, 9 and 10. The descriptors for stomach ache were *none at all, dull ache on and off, moderate continuous, severe continuous,* and *severe doubled up*; those for sickness were *not at all, slightly, quite, very,* and *throwing up;* and those for headache were *none at all, dull ache on and off, moderate continuous, severe continuous,* and *searing pain*.

3.4.6 Saturated Oxygen (SaO_2)

Exercise reported in Chapter 5A and 5B took place in an environmental chamber at a simulated altitude of 2500 m, equivalent to 15.5% O_2. Participants were monitored for their SaO_2 levels using a portable pulse oximeter (WristOx 3100, Nonin Medical Inc, USA) worn on their index finger, attached to a monitor strapped to their wrist. SaO_2 was monitored continuously throughout exercise at 5 s intervals and downloaded using appropriate software (nVision V5.1, Nonin Medical Inc, USA).

3.4.7 Dehydration

Dehydration levels as a percentage of pre exercise body mass was determined in the studies reported in Chapter 5A, 5B and 6. Body mass was recorded with no shoes and minimal clothing immediately prior to, and following, exercise.

Chapter 4.0 A) Reliability of a high-intensity cycling capacity test

4A.1 **Introduction**

Buffering agents are commonly used to enhance exercise performance and capacity (for reviews see McNaughton et al., 2008 and Sale et al., 2010). Few studies have reported on the reliability of the exercise test employed while investigating the effects of nutritional supplementation on increased buffering capacity, which may have contributed to equivocal results. An appropriate exercise test to investigate the effects of increased buffering capacity should be of sufficient intensity to result in a large accumulation of H^+, and therefore be limited by increasing muscle acidosis. Furthermore, it is of vital importance that a test is reliable in order to interpret the meaningfulness of the data (Atkinson and Nevill, 1998).

Investigations into the effect of buffering agents on exercise performance and capacity require multiple repetitions of a protocol, with and without supplementation, in order to determine any improvements following supplementation. Therefore, the exercise test employed should be reliable to determine whether differences are due to the nutritional intervention or are simply due to the natural variation of the test. The reliability of a protocol is a reflection of the consistency of the data when the measurements are taken on multiple occasions under identical conditions (Vincent, 1994). However, there will always be a degree of measurement error due to a variety of factors including circadian variation, instrumentation failure, and participant and experimenter error (Weir, 2005). Atkinson and Nevill (1998) have suggested, therefore, that reliability is the amount of measurement error deemed acceptable for the effective practical use of an analysis system.

The $CCT_{110\%}$ is a high-intensity cycling capacity test performed at 110% of previously determined Powermax (W_{max}), designed (Hill et al., 2007) to last between 120 and 240 s, an exercise duration when anaerobic energy sources can contribute up to 60% of the total energy

requirement (Maughan et al., 1997). Therefore, the high-intensity nature of the test would be expected to incur a large accumulation of intracellular and subsequently extracellular H^+, and may lead to an early cessation of exercise due to increasing acidosis. Indeed, Hill et al. (2007) showed that TWD during the $CCT_{110\%}$ was increased by 13.0% alongside a 58.8% increase in muscle carnosine following four weeks of β-alanine supplementation; when supplementation was extended to ten weeks, carnosine was increased by 80.1% and TWD by 16.2%. The results of Hill et al. (2007) suggest the $CCT_{110\%}$ to be an appropriate tool for the investigation of dietary interventions designed to manipulate changes in pH during exercise.

The aim of this study was to examine the reliability of the $CCT_{110\%}$ as a high-intensity cycling capacity test. Furthermore, due to the high association between lactate and H^+ production (Hultman and Sahlin, 1980), the reliability of blood lactate concentration was measured alongside several other blood markers. It was hypothesised that the $CCT_{110\%}$ would be a highly repeatable and appropriate model that can be utilised to examine the effects of dietary interventions designed to manipulate changes in pH during exercise.

4A.2 **Methods**

4A.2.1 Participants

Twenty seven recreationally active males (age 23 ± 4 y, height 1.79 ± 0.06 m, body mass 78.0 ± 8.8 kg, W_{max} 306 ± 49 W) volunteered and gave their written informed consent to participate in this study (Chapter 3.1).

4A.2.2 Experimental Design

Participants attended the laboratory on four separate occasions at the same time of day to ensure results were not affected by circadian variation. The first trial consisted of an

incremental cycling test to exhaustion to determine individual Powermax (W_{max}; Chapter 3.3.1). The remaining three sessions (one habituation and two main trials) were for the completion of the main $CCT_{110\%}$ trials (Chapter 3.3.2). All trials were separated by 48 h. Prior to the main trials, participants abstained from alcohol and caffeine and completed a food record for the 24 h period prior to the initial trial. They adopted the same diet and abstained from strenuous exercise for 24 h prior to each subsequent trial. Arterialised finger-prick blood samples were taken immediately pre-, immediately post- and 5-min post-exercise. Blood samples were analysed for lactate (Chapter 3.4.2.1), pH, Hb and blood gases (Chapter 3.4.2.2).

4A.2.3 Statistical Analyses

All data are presented as mean ± 1SD, unless stated otherwise. Exercise capacity data were analysed using intra-class correlations (ICC, 2 way fixed, repeated measures, absolute model; Weir, 2005), systematic bias ratio, ratio limits of agreement (LoA; Bland and Altman, 1986), coefficient of variation (CV) and t-tests. Blood variables were analysed using repeated measures ANOVA and Tukey tests were used for post-hoc analyses. Effect sizes were calculated using Cohen's d (Cohen, 1988). Statistical significance was accepted at $P \leq 0.05$.

4A.3 **Results**

4A.3.1 $CCT_{110\%}$

TTE (P = 0.75; 134 ± 20 s and 135 ± 20 s, d = 0.05) and TWD (P = 0.97; 42.2 ± 10.3 kJ and 42.2 ± 9.8 kJ, d = 0.00) were not different between trials (Table 4A.1). Following confirmation of heteroscedasticity, ratio systematic bias and LoA were determined and are presented in Table 4A.1. The intra-class correlation between trials was $r = 0.88$ for TTE and $r = 0.94$ for TWD, with the CV being 4.43% for TTE and 4.94% for TWD.

Table 4A.1 Absolute and relative reliability measures of the $CCT_{110\%}$.

	TTE	TWD
Trial 1	134 ± 20 s	42.2 ± 10.3 kJ
Trial 2	135 ± 20 s	42.2 ± 9.8 kJ
Trial 1 (ln)	4.89 ± 0.15	3.71 ± 0.27
Trial 2 (ln)	4.90 ± 0.15	3.71 ± 0.26
Systematic Bias	1.005	1.003
×/÷ Ratio LoA	1.156	1.176
CV (%)	4.43	4.94
ICC (CI)	0.884 (0.761 - 0.945)	0.939 (0.931 – 0.986)
t-test	P = 0.745	P = 0.970
Variation LoA	135: 117, 157	42.2: 36.0, 49.8
Variation CV	135: 129.0, 141.0	42.2: 40.1, 44.3

4A.3.2 Blood Analyses

Baseline blood pH, bicarbonate and base excess were similar between both trials (Table 4A.2). In both trials, pH, bicarbonate and base excess were significantly reduced from baseline immediately post exercise and following 5 minutes of recovery ($P \leq 0.001$). Only immediately post-exercise pH was significantly different between trials ($P \leq 0.001$; Table 4A.2).

Blood lactate was not significantly different between trials at baseline, and was significantly increased from baseline immediately post exercise and following 5 minutes of recovery in all trials ($P \leq 0.001$), with no between trial differences.

Table 4A.2 pH, bicarbonate, base excess and lactate in trial 1 and trial 2. Data are mean ± 1SD. $^{*}P \leq 0.001$ from Trial 1 at the equivalent time point. $^{\wedge}P \leq 0.001$ from baseline.

	Baseline	Post-Exercise	Post-Exercise + 5 min
pH			
Trial 1	7.416 ± 0.019	7.246 ± 0.041$^{\wedge}$	7.238 ± 0.044$^{\wedge}$
Trial 2	7.412 ± 0.023	7.269 ± 0.064$^{*\wedge}$	7.247 ± 0.055$^{\wedge}$
Bicarbonate (mmol·L^{-1})			
Trial 1	24.02 ± 1.76	15.06 ± 1.83$^{\wedge}$	12.78 ± 1.90$^{\wedge}$
Trial 2	24.01 ± 1.82	15.71 ± 2.66$^{\wedge}$	13.29 ± 2.20$^{\wedge}$
Base Excess (mmol·L^{-1})			
Trial 1	0.2 ± 1.8	-10.1 ± 2.1$^{\wedge}$	-12.4 ± 2.2$^{\wedge}$
Trial 2	0.2 ± 1.9	-9.2 ± 3.2$^{\wedge}$	-11.7 ± 2.7$^{\wedge}$
Lactate (mmol·L^{-1})			
Trial 1	1.1 ± 0.4	12.1 ± 2.0$^{\wedge}$	12.0 ± 1.8$^{\wedge}$
Trial 2	1.2 ± 0.5	12.1 ± 2.1$^{\wedge}$	11.6 ± 1.9$^{\wedge}$

4A.4 **Discussion**

The aim of this study was to determine the reliability of a high-intensity cycling capacity test. The $CCT_{110\%}$ was designed to provide a high-intensity cycling test with an expected TTE between 120 and 240 s. Main trial times of 134 ± 20 s and 135 ± 20 s lie within the expected timeframe of the test, with no significant differences between trials. The reliability of the $CCT_{110\%}$ was demonstrated by the ratio bias for TTE and TWD being close to 1, narrow agreement ratios and moderate to high intra-class correlations. Furthermore, blood markers were generally consistent across the two trials. Consequently, the $CCT_{110\%}$ can be considered a reliable exercise protocol that can be employed to assess ergogenic benefits of increased buffering capacity.

Relative and absolute reliability for TTE and TWD during the $CCT_{110\%}$ was demonstrated by ratio bias values close to 1, narrow agreement ratios and ICCs just below and above 0.9. CVs of 4.43 and 4.94% for TTE and TWD compare favourably with other cycling capacity tests performed to exhaustion that have shown CVs in excess of 5 and 10% (Coggan and Costill, 1984; Graham and McLellan, 1989; McLellan et al., 1995). The CVs shown in the current study are encouraging considering the results of previous investigations employing the $CCT_{110\%}$. Hill et al. (2007) showed TWD was increased by 13.0 and 16.2% following 4 and 10 weeks supplementation with β-alanine, which are above the expected variation of the test shown in the present study. This provides further support for the conclusion that the exercise capacity improvements shown were due to the intervention employed. Considering β-alanine increases intracellular buffering capacity (Harris et al., 2006) and sodium bicarbonate increases extracellular buffering capacity (McNaughton et al., 2008), it can be suggested that the $CCT_{110\%}$ is limited by increasing muscle acidosis, although muscle pH was not directly measured in this study.

In addition to providing reliable performance data, it is important that blood responses are also similar between trials. Furthermore, if the $CCT_{110\%}$ is to be used as a tool for investigating nutritional based interventions designed to manipulate pH, it must be limited, in part, by increasing acidosis. Although muscle pH was not directly measured in this study, blood pH was significantly reduced from baseline immediately post-exercise, as were bicarbonate and base excess, while lactate concentrations were significantly elevated. Lactate has been associated with up to 94% of the concomitant accumulation of H^+ during high-intensity exercise (Hultman and Sahlin, 1980). Furthermore, lactate concentrations are similar to those shown following high-intensity exercise resulting in low muscle pH (Bogdanis et al., 1996), which can interfere with several metabolic processes and may contribute to the early

onset of fatigue (Spriet et al., 1989), which suggests that decreased muscle pH may have contributed to the cessation of exercise during the $CCT_{110\%}$.

Blood bicarbonate, base excess and lactate were similar at all corresponding time points between trials. pH was similar between trials at pre-exercise and 5 minutes post-exercise; immediately post-exercise blood pH was significantly different between trials, which suggests that blood pH immediately post-exercise may not be sufficiently reliable. Despite this, the absolute difference is much smaller than those expected to be seen using nutritional interventions intended to alter extracellular pH changes during exercise. In a meta-analysis of the literature, Carr et al. (2011) showed that sodium bicarbonate increased blood pH by 0.069 ± 0.018 compared to placebo, which is more than double the between trial difference shown in the present investigation (0.023). Therefore, despite immediately post-exercise blood pH being significantly different between trials, blood responses to the $CCT_{110\%}$ can be considered sufficiently reliable and sensitive to detect changes when investigating nutritional supplements designed to manipulate blood pH, bicarbonate, base excess and lactate.

4A.5 **Conclusions**

There are numerous studies investigating the effects of intracellular and extracellular pH manipulation on exercise performance and capacity. Equivocal results may be a reflection of the variety of exercise protocols employed, many of which have not been shown to be either valid or reliable. This study showed that the $CCT_{110\%}$ is a reliable test for the determination of high-intensity cycling capacity.

Chapter 4.0 B) Effect of sodium bicarbonate supplementation on high-intensity cycling capacity

4B.1 **Introduction**

In the previous section, the $CCT_{110\%}$ was shown to be a reliable test to determine high-intensity cycling capacity and, due to the high levels of lactate, and concomitant H^+ accumulation, is an appropriate model for examining the effects of dietary interventions designed to manipulate intramuscular changes in pH during exercise. A range of exercise tests have been used in previous studies investigating the ergogenic effects of sodium bicarbonate supplementation, which could explain some of the inconsistencies in findings (for review see McNaughton et al. 2008). Few studies have used exercise durations that could theoretically be limited by H^+ accumulation, and those that have used low sodium bicarbonate doses (Katz et al., 1984; Horswill et al., 1988).

Matson and Tran (1993) reported a relatively weak relationship (r = 0.42) between dose and degree of blood alkalosis following sodium bicarbonate supplementation using a meta-analysis of the literature. It was hypothesised that this was due to the large variability in individual pH and bicarbonate responses to supplementation. This would suggest that the purported mechanism underlying a potential ergogenic effect of sodium bicarbonate supplementation might not have been present in all individuals. Nonetheless, in a more recent meta-analysis, Carr et al. (2011) showed that sodium bicarbonate was effective at improving a 1 min all out sprint by 1.7 ± 2.0% when ingested at a dose of 0.3 $g \cdot kg^{-1}$BM prior to exercise.

One potential moderator of the effect of sodium bicarbonate supplementation on exercise capacity and performance is the gastrointestinal (GI) discomfort experienced by some participants. Price and Simons (2010) suggested that the need to individualise supplementation with sodium bicarbonate was related to the individuals' susceptibility to GI discomfort, although GI discomfort was not correlated with performance decrements in their

study. van Montfoort et al. (2004) measured the intensity of sickness and stomach ache prior to, and following high-intensity exercise, but reported little or no GI symptoms following supplementation with 0.3 $g \cdot kg^{-1}$BM sodium bicarbonate. McNaughton (1992) reported increased GI disturbance in all participants consuming doses above 0.3 $g \cdot kg^{-1}$BM which may also explain the lack of a further increase in cycling capacity in these participants.

To date, studies investigating the effect of sodium bicarbonate supplementation on high-intensity cycling capacity and performance have reported contrasting results. These conflicting results can be attributed to a variety of factors, including differing sodium bicarbonate doses and exercise protocols, GI disturbance and individual variation in blood responses to supplementation. Data were analysed for the trial effect of sodium bicarbonate supplementation in all participants and only in those not experiencing GI discomfort. The individual response to supplementation was also explored by separating participants into those who improved their cycling capacity (responders) and those who did not (non-responders). It was hypothesised that exercise capacity would be improved following supplementation with sodium bicarbonate, though not in all participants due to individual variation.

4B.2 **Methods**

4B.2.1 Participants

Twenty-one recreationally active males (age 25 ± 5 y, height 1.79 ± 0.06 m, body mass 80.7 ± 10.6 kg, W_{max} 316 ± 45 W) volunteered and gave their written informed consent to participate in this study (Chapter 3.1).

4B.2.2 Experimental Design

Participants were required to attend the laboratory on four separate occasions over a fourteen day period. All trials were performed at the same time of day to ensure results were not affected by circadian variation. There were two preliminary trials, which comprised of an incremental cycle to exhaustion to determine W_{max} (Chapter 3.3.1), followed by an habituation of the $CCT_{110\%}$ (Chapter 3.3.2). Participants then completed two repeated measures, counterbalanced and double-blind trials following the ingestion of 0.3 $g \cdot kg^{-1}$BM of either sodium bicarbonate (SB) or maltodextrin (P). In the twenty-four hours prior to the main trials, participants refrained from alcohol, caffeine and any strenuous exercise, and reported food intake using a food diary which was used to replicate the diet prior to the second main trial. Following an overnight fast, participants arrived at the laboratory 4 h before the $CCT_{110\%}$. Baseline finger-prick blood samples were taken before consuming a standardised breakfast of 3 slices of toast and jam at 09:00; further blood samples were taken immediately pre-, immediately post- and 5-min post-exercise. Blood samples were analysed and used to determine blood lactate (Chapter 3.4.2.1), pH, bicarbonate and base excess (Chapter 3.4.2.2).

Participants ingested 0.2 $g \cdot kg^{-1}$BM of sodium bicarbonate (Chapter 3.2.2) or maltodextrin alongside the breakfast. A final 0.1 $g \cdot kg^{-1}$BM was ingested 2 h after the standardised breakfast (11:00), 2 h prior to commencement of the $CCT_{110\%}$ (13:00). All supplements were administered in opaque gelatine capsules and participants were supervised during the ingestion of sodium bicarbonate and maltodextrin supplements to ensure 100% compliance. Participants were instructed to report any gastrointestinal or other symptoms experienced during the four hours prior to exercise. They were requested to note down the time, type (*e.g.*,

stomach cramps, bloating, headaches) and the severity (mild, moderate or severe) of symptoms.

4B.2.3 Statistical Analyses

All data were analysed using Statistica 9 (Statsoft, USA) and are presented as mean ± 1SD. Paired samples t-tests were used to determine any differences in performance measures between supplementation trials. A two-way ANOVA (Trial x Time) with repeated measures was used to determine any difference in blood pH, lactate, bicarbonate and base excess levels. Mauchly's test of Sphericity was used to check the data for sphericity, and where it was violated, a Greenhouse-Geisser correction was applied. A post-hoc Bonferroni correction factor was used to test any differences indicated by the ANOVA. Effect sizes were calculated using Cohen's d (1988). Pearson's correlations were used to determine any association between exercise and blood variables. Statistical significance was accepted at the $P \leq 0.05$ level.

4B.3 **Results**

Data were analysed for the trial effect of sodium bicarbonate supplementation in all participants (N = 21). The data were then analysed following the exclusion of participants experiencing GI discomfort (N = 17). In addition, the complete data set was split into two groups, categorising participants as responders (N = 9), in whom exercise capacity was improved, and non-responders (N = 12), in whom exercise capacity was not improved.

4B.3.1 All Participants (N = 21)

TWD was 45.6 ± 8.4 kJ and 46.8 ± 9.1 kJ for P and SB, with no significant difference between conditions (P = 0.16, d = 0.14) (Table 4B.1).

Table 4B.1 TWD for all participants (N = 21), excluding those who experienced gastrointestinal discomfort (N = 17) and for participants who improved exercise capacity (Responders) and participants who did not improve exercise capacity (Non-Responders). *P ≤ 0.01 from placebo trial.

	TWD (kJ)
N = 21	
Placebo	45.6 ± 8.4
$NaHCO_3^-$	46.8 ± 9.1
N = 17	
Placebo	46.2 ± 9.2
$NaHCO_3^-$	48.4 ±9.3*
Responders (N = 9)	
Placebo	43.1 ± 7.3
$NaHCO_3^-$	47.5 ± 8.1*
Non-Responders (N = 12)	
Placebo	47.5 ± 9.0
$NaHCO_3^-$	46.2 ± 10.1*

There was no significant difference in baseline pH, bicarbonate, base excess or lactate between trials (Table 4B.2). Supplementation with SB, but not P, significantly increased pre-exercise pH, bicarbonate and base excess levels from baseline ($P \leq 0.001$). Blood pH, bicarbonate and base excess measured immediately post-exercise and 5 minutes post-exercise (Table 4B.2) were significantly decreased from baseline in both P and SB ($P \leq 0.001$); with values being significantly higher in SB ($P \leq 0.001$). Blood lactate (Table 4B.2) was significantly increased from baseline following exercise in both trials ($P \leq 0.001$), with significantly higher post-exercise concentrations shown following SB ($P \leq 0.001$).

TWD was not correlated with pre-exercise pH ($r = -0.05$), bicarbonate ($r = 0.03$) or base excess ($r = 0.01$), nor with the changes from baseline to pre-exercise. However, TWD was significantly correlated with the changes in pH ($r = -0.43$, $P = 0.004$), bicarbonate ($r = -0.41$, $P = 0.008$) and base excess ($r = -0.45$, $P = 0.003$) from pre- to post-exercise, although there

was no significant correlation with the change in lactate. The difference in TWD between trials was not significantly correlated to the differences between trials in any of the blood markers at pre-exercise.

4B.3.2 Participants Not Experiencing GI Discomfort (N = 17)

Four participants complained of GI discomfort following the ingestion of SB, with the most frequently reported symptoms being mild to severe stomach cramps and diarrhoea. All participants who complained of GI symptoms demonstrated a decline in exercise capacity. When data were analysed without those participants experiencing GI discomfort, TWD was significantly increased ($P = 0.01$, $d = 0.25$) in SB compared with P (Table 4B.2).

Blood responses to supplementation and exercise were similar to the whole group blood responses (Table 4B.2). In addition, the removal of participants who experienced GI discomfort from the analyses did not influence the significance of any of the correlations that were performed on the full data-set.

Table 4B.2 pH, bicarbonate, base excess and lactate for all participants (N = 21) and excluding those who experienced gastrointestinal discomfort (N = 17). Data are mean ± SD. *P ≤ 0.01 from baseline; ^P ≤ 0.01 from placebo trial at the equivalent time point.

	Baseline	Pre-exercise	Post-exercise	Post-ex +5 min
N = 21 (All Participants)				
pH				
Placebo	7.407 ± 0.021	7.402 ± 0.024	7.236 ± 0.044*	7.229 ± 0.056*
$NaHCO_3^-$	7.401 ± 0.015	7.461 ± 0.020$^{*\wedge}$	7.292 ± 0.054$^{*\wedge}$	7.283 ± 0.054$^{*\wedge}$
Bicarbonate (mmol·L^{-1})				
Placebo	24.79 ± 1.14	24.96 ± 0.99	14.43 ± 1.89*	12.82 ± 2.10*
$NaHCO_3^-$	24.66 ± 1.44	30.40 ± 1.01$^{*\wedge}$	18.39 ± 2.52$^{*\wedge}$	15.26 ± 2.78$^{*\wedge}$
Base excess (mmol·L^{-1})				
Placebo	0.78 ± 0.98	0.82 ± 0.78	-10.48 ± 2.06*	-12.69 ± 2.80*
$NaHCO_3^-$	0.54 ± 1.28	6.49 ± 1.03$^{*\wedge}$	-6.89 ± 3.11$^{*\wedge}$	-9.60 ± 3.38$^{*\wedge}$
Lactate (mmol·L^{-1})				
Placebo	1.2 ± 0.4	1.2 ± 0.5	12.6 ± 2.4*	12.4 ± 2.0*
$NaHCO_3^-$	1.1 ± 0.4	1.2 ± 0.3	14.4 ± 3.4$^{*\wedge}$	14.5 ± 2.9$^{*\wedge}$
N = 17 (Participants Not Experiencing GI Discomfort)				
pH				
Placebo	7.407±0.023	7.398±0.024	7.226±0.039*	7.215±0.048*
$NaHCO_3^-$	7.400±0.017	7.459±0.020$^{*\wedge}$	7.276±0.036$^{*\wedge}$	7.268±0.041$^{*\wedge}$
Bicarbonate (mmol·L^{-1})				
Placebo	24.79 ± 1.24	24.87 ± 1.07	15.16±1.78*	12.32±1.84*
$NaHCO_3^-$	24.51 ± 1.41	30.33 ± 1.08$^{*\wedge}$	17.52±1.68$^{*\wedge}$	14.41±2.07$^{*\wedge}$
Base excess (mmol·L^{-1})				
Placebo	0.70 ± 1.08	0.66 ± 0.75	-10.90 ± 1.77*	-13.40 ± 2.40*
$NaHCO_3^-$	0.39 ± 1.27	6.39 ± 1.05$^{*\wedge}$	-7.94 ± 1.98$^{*\wedge}$	-10.61 ± 2.53$^{*\wedge}$
Lactate (mmol·L^{-1})				
Placebo	1.2 ± 0.3	1.3 ± 0.5	13.0 ± 2.4*	12.9 ± 1.4*
$NaHCO_3^-$	1.2 ± 0.4	1.2 ± 0.3	15.5 ± 2.6$^{*\wedge}$	15.5 ± 1.8$^{*\wedge}$

4B.3.3 Responders (N = 9) and Non-Responders (N = 12)

There was a degree of individual variability in exercise capacity between P and SB for all participants, with the difference in TWD between trials ranging from -12 to +19% (Figure 4B.1). In SB, nine of the twelve participants who improved did so above the 4.94% test retest

variability for TWD in the $CCT_{110\%}$ (Chapter 4A), and these were classified as responders. The remaining individuals who did not improve above the CV of the $CCT_{110\%}$ were allocated to the non-responders group.

Blood pH, bicarbonate and base excess levels were significantly increased in both responders and non-responders, from baseline to pre-exercise in SB only (Table 4B.3). In responders, the reduction in pH, bicarbonate and base excess from pre- to post-exercise was greater in SB than in P ($P \leq 0.01$). In non-responders, there was no difference in the reduction in pH, bicarbonate or base excess from pre- to post-exercise between trials (all $P > 0.05$). Immediately-post exercise blood lactate concentrations were significantly higher in SB for the responders ($P = 0.003$) but not for the non-responders ($P = 0.35$).

TWD was not correlated with any pre-exercise blood marker for responders or non-responders, or with their changes from baseline to pre-exercise. TWD was not significantly correlated with any blood changes from pre- to post-exercise in the responders, but was correlated to the change in pH, bicarbonate and base excess in the non-responders (all $P \leq 0.05$).

Table 4B.3 Changes in pH, bicarbonate, base excess and lactate from baseline to pre-exercise and pre-exercise to post-exercise for participants who improved exercise capacity (Responders) and participants who did not improve exercise capacity (Non-Responders) in SB. ($^{*}P \leq 0.001$ from placebo trial; $^{\wedge}P \leq 0.01$ from placebo trial).

	Δ Baseline to Pre-Ex	Δ Pre-Ex to Post-Ex
pH		
Responders		
Placebo	- 0.014 ± 0.036	- 0.158 ± 0.029
$NaHCO_3^-$	+ 0.060 ± 0.020*	- 0.184 ± 0.031$^{\wedge}$
Non-Responders		
Placebo	+ 0.002 ± 0.020	- 0.173 ± 0.047
$NaHCO_3^-$	+ 0.060 ± 0.015*	- 0.158 ± 0.056
Bicarbonate (mmol·L^{-1})		
Responders		
Placebo	+ 0.41 ± 0.83	- 9.19 ± 1.42
$NaHCO_3^-$	+ 5.94 ± 0.90*	- 12.79 ± 1.84*
Non-Responders		
Placebo	+ 0.00 ± 0.35	- 9.78 ± 2.10
$NaHCO_3^-$	+ 5.58 ± 1.53*	- 11.43 ± 2.46
Base Excess (mmol·L^{-1})		
Responders		
Placebo	+ 0.01 ± 0.70	-10.89 ± 1.45
$NaHCO_3^-$	+ 6.10 ± 0.72*	- 14.36 ± 1.97*
Non-Responders		
Placebo	+ 0.08 ± 0.51	-11.60 ± 2.38
$NaHCO_3^-$	+ 5.84 ± 1.35*	- 12.65 ± 3.03
Lactate (mmol·L^{-1})		
Responders		
Placebo	+ 0.1 ± 0.5	+ 11.0 ± 2.4
$NaHCO_3^-$	+ 0.1 ± 0.4	+ 14.0 ± 3.5*
Non-Responders		
Placebo	+ 0.0 ± 0.5	+ 11.8 ± 2.7
$NaHCO_3^-$	+ 0.1 ± 0.4	+ 12.6 ± 3.5

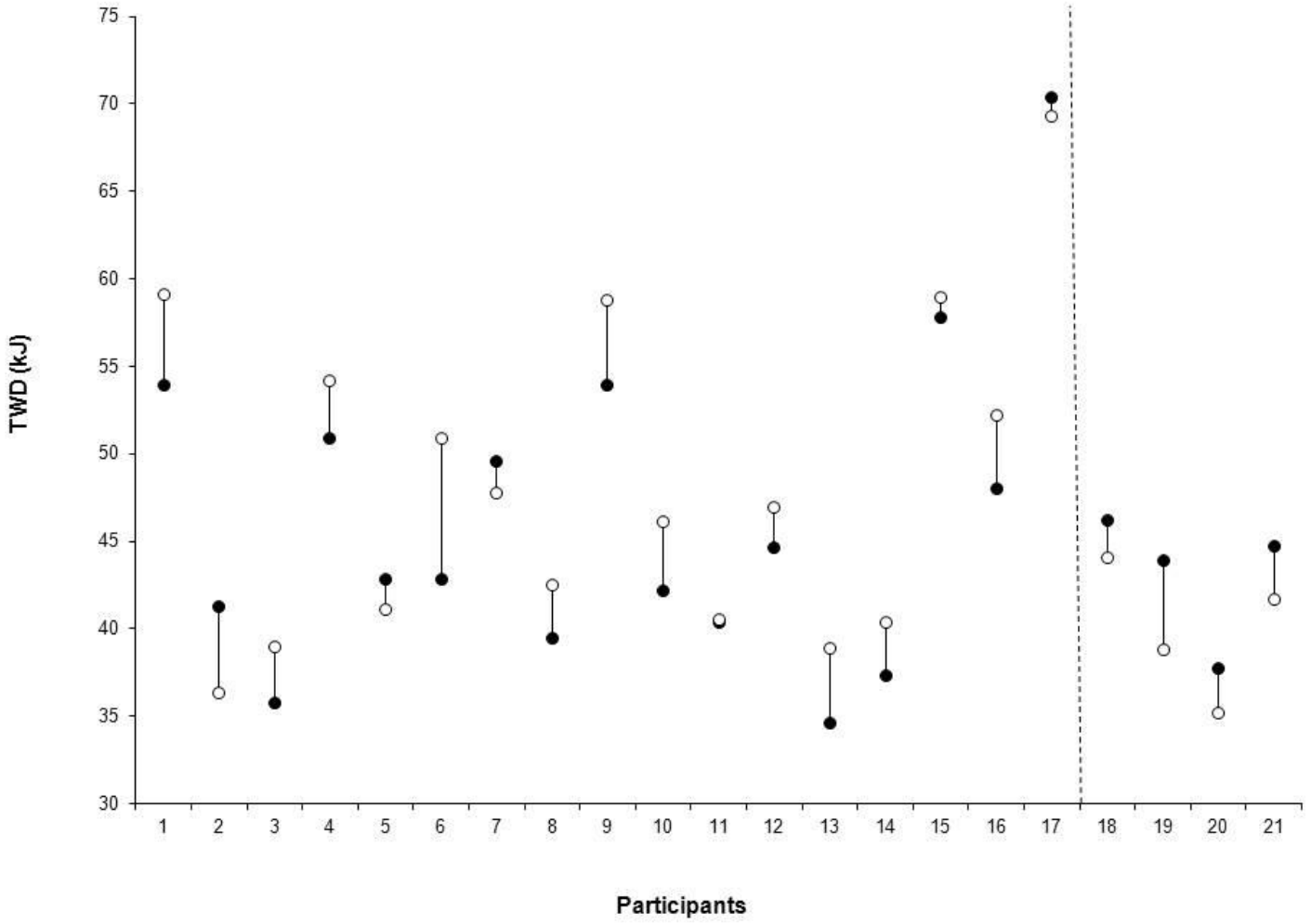

Figure 4B.1 Individual TWD (kJ) in the $CCT_{110\%}$ in both P (black) and SB (white). Participants 18 – 21 are the participants who experienced gastrointestinal symptoms.

4B.4 **Discussion**

The main finding of this study is that TWD during the $CCT_{110\%}$ was unaffected by sodium bicarbonate supplementation in all participants, despite resulting in alkalaemia prior to exercise. This is in contrast to Hill et al. (2007) who showed significant increases in TWD following β-alanine supplementation using the same exercise test. β-alanine supplementation increases muscle carnosine concentration (Harris et al., 2006), thereby directly increasing intracellular buffering capacity. Therefore, the results of Hill et al. (2007) suggest the $CCT_{110\%}$ to be limited by increasing muscle acidosis. Sodium bicarbonate supplementation increases circulating levels of bicarbonate, increasing extracellular buffering capacity and the active transport of H^+ out of the muscle (Mainwood and Worsley-Brown, 1975). Consequently, an increased exercise capacity would be expected during the $CCT_{110\%}$ following supplementation with sodium bicarbonate. Cycling capacity was increased but only when participants reporting GI disturbances were removed from the analyses. However, any contrast in findings between β-alanine and sodium bicarbonate supplementation may be due to a more direct influence of carnosine upon pHi.

Price and Simons (2010) reported no effect of sodium bicarbonate supplementation on high intensity running performance lasting around 75 s. The authors suggested that GI discomfort or individual differences in the blood responses to sodium bicarbonate supplementation might explain the negative findings. None of the four participants reporting GI discomfort showed an increased exercise capacity, meaning that a significant improvement in high intensity exercise capacity was shown when group data were analysed following the exclusion of these participants. GI discomfort only partially explained the lack of an improvement in exercise capacity, however, since twelve participants did not show any improvements in exercise

capacity with sodium bicarbonate supplementation, suggesting that some other physiological differences between participants might also help to explain the individual capacity response.

Increases in blood bicarbonate concentration and subsequently blood alkalosis were shown in all participants prior to exercise following supplementation with sodium bicarbonate. Pre-exercise blood bicarbonate concentrations compare favourably to those reported previously using different supplementation strategies but an identical dose (Price et al., 2003; van Montfoort et al., 2004). However, only twelve participants showed an improved exercise capacity with sodium bicarbonate ingestion, nine of which were increased above the CV of the test. Blood data were also analysed according to the nine participants who showed an improved exercise capacity following sodium bicarbonate supplementation, and the twelve who did not. The change in blood bicarbonate, pH and base excess between baseline and pre-exercise following sodium bicarbonate ingestion were similar between these responders and non-responders. This suggests that the underlying mechanism for an ergogenic effect of sodium bicarbonate supplementation was attained in all participants and thus was not an explanation for the non-response. Further confirmation is provided by the fact exercise capacity was not correlated to either the absolute concentration of, or the change in (from baseline to pre-exercise), any blood marker for all participants, suggesting that the degree of individual blood alkalosis prior to exercise did not influence the individual response in exercise capacity.

Whilst there were no differences between responders and non-responders in the ability of sodium bicarbonate ingestion to promote blood alkalosis, the reduction in blood pH, bicarbonate and base excess from pre- to post-exercise was significantly greater in the sodium bicarbonate trial for the responders but not for the non-responders. This might

suggest that promoting blood alkalosis concentration through sodium bicarbonate supplementation does not necessarily increase blood bicarbonate buffering in all individuals during high-intensity exercise. In the present study, the magnitude of the reductions in blood pH, bicarbonate and base excess from pre- to post-exercise were correlated with exercise capacity. As such, a potential difference exists in the ability of responders and non-responders to make full use of the induced blood alkalosis, which might explain the individual exercise capacity responses to sodium bicarbonate. However, it must be noted that these differences might simply be explained by differences in exercise duration between placebo and sodium bicarbonate in responders and in non-responders.

Ibanez et al. (1995) reviewed the association between changes in peak blood lactate and exercise performance changes across 19 studies examining the potential ergogenic effects of alkalinising treatments. They suggested that a difference in blood lactate concentration of 2 $mmol \cdot L^{-1}$ between treatments was required to show a performance effect. In the present study, there was a difference of +3.0 $mmol \cdot L^{-1}$ in peak blood lactate concentration immediately post-exercise in responders, whereas there was a difference of only +0.8 $mmol \cdot L^{-1}$ in non-responders. As such, we provide some evidence to support the assertions of Ibanez et al. (1995); immediately post-exercise blood lactate concentrations were significantly elevated with sodium bicarbonate ingestion for responders but not for non-responders compared to placebo.

4B.5 **Conclusions**

Sodium bicarbonate supplementation improved exercise capacity during a cycling test shown to be limited by increasing muscle acidosis, but only when the data from participants reporting GI discomfort were removed from the analyses. However, GI discomfort could only

explain a reduced exercise capacity in four out of the eight participants who performed worse following sodium bicarbonate ingestion, with a further participant showing identical performances in both trials. The degree of blood alkalosis induced by sodium bicarbonate ingestion prior to exercise could not explain the individual differences in exercise capacity, although the effect of exercise on pH, bicarbonate and base excess was different in those who showed an increased exercise capacity following sodium bicarbonate compared with those who did not. Variability in exercise capacity and some blood responses between trials suggests that sodium bicarbonate supplementation is beneficial to some, but not all individuals.

Chapter 4.0 C) Effect of β-alanine supplementation, with and without sodium bicarbonate, on high-intensity cycling capacity

4C.1 **Introduction**

In the study reported in Chapter 4B, high-intensity cycling capacity could be improved with sodium bicarbonate supplementation if it did not result in GI discomfort. Furthermore, there was a degree of individual variation in response to supplementation which suggests that supplementation with sodium bicarbonate may not be beneficial to all individuals. This is in contrast to the findings of Harris et al. (2007) who showed an increased TWD during the $CCT_{110\%}$ following 4 and 10 weeks β-alanine supplementation. The differences in findings could be due to the mechanisms by which β-alanine and sodium bicarbonate supplementation work. β-alanine supplementation increases muscle carnosine concentration, thereby directly increasing intracellular buffer capacity, whereas sodium bicarbonate supplementation increases circulating levels of bicarbonate, increasing extracellular buffer capacity. Therefore, any contrast in findings between β-alanine and sodium bicarbonate supplementation may be due to carnosine's more direct influence upon pHi, although increased extracellular buffering has been shown to improve high-intensity exercise performance (for review see McNaughton et al. 2008).

Despite the fact that studies have shown significant improvements in exercise capacity and performance following supplementation with β-alanine and sodium bicarbonate separately, no study has yet examined the effects of co-supplementation on high-intensity exercise capacity. Therefore, the aim of this investigation was to examine the effect of β-alanine supplementation, with and without sodium bicarbonate supplementation, on high-intensity cycling capacity. It was hypothesised that an increase in intracellular pH buffering action of carnosine through β-alanine supplementation, and the increased extracellular buffering action of bicarbonate through sodium bicarbonate supplementation would be additive, thereby resulting in an increased protection against the acidosis produced during high intensity

cycling and contributing to a further improvement in high-intensity cycling capacity above that shown following either sodium bicarbonate supplementation or β-alanine supplementation alone.

4C.2 **Methods**

4C.2.1 Participants

Twenty physically active males (age 24 ± 5 y, height 1.79 ± 0.06 m, body mass 80.0 ± 10.3 kg), who regularly participate in high-intensity exercise, volunteered for the study and were split into a β-alanine group (W_{max} 304 ± 48 W) and a placebo group (W_{max} 323 ± 42 W), matched for W_{max}. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

4C.2.2 Experimental Design

Participants attended the laboratory on five separate occasions. The first two visits were for the determination of each participant's W_{max} (Chapter 3.3.1) and habituation to the $CCT_{110\%}$. The remaining visits were for the completion of the main $CCT_{110\%}$ trials (Chapter 3.3.2). One main trial was completed before and two main trials after a 4 week double-blind supplementation period of either β-alanine or placebo (Figure 4C.1). Participants were supplemented with either 6.4 $g \cdot d^{-1}$ of β-alanine or placebo in tablet form over a 4 week period, ingesting two 800 mg tablets four times per day at 3 – 4 h intervals (Chapter 3.2.1). Participants completed a supplementation log to verify compliance, with the degree of compliance being reported at 95 ± 4% in the β-alanine group (total of 170.9 ± 7.7 g β-alanine), and 98 ± 2% in the placebo group (total of 175.0 ± 3.7 g maltodextrin). For the pre-supplementation trial, participants ingested maltodextrin and after the 4 week

supplementation period, participants ingested either sodium bicarbonate or maltodextrin in a crossover design (Figure 4C.1). Participants ingested 0.2 $g \cdot kg^{-1}$BM of sodium bicarbonate (Chapter 3.2.2) or matching placebo alongside a standardised breakfast of 3 slices of toast and jam. A final 0.1 $g \cdot kg^{-1}$BM was ingested 2 h prior to commencement of exercise. All supplements were administered in opaque gelatine capsules and participants were supervised during the ingestion of sodium bicarbonate and maltodextrin supplements to ensure 100% compliance. Participants were instructed to report any gastrointestinal or other symptoms experienced during the four hours prior to exercise. They were requested to note down the time, type (e.g., stomach cramps, bloating, headaches) and the severity (mild, moderate or severe) of symptoms. Of the participants, 15 participants reported no gastrointestinal discomfort following sodium bicarbonate ingestion. However, 2 participants reported mild gastrointestinal or other symptoms (one a light headache and the other bloating) and 3 participants reported severe symptoms (including stomach cramps, headaches and diarrhoea) with the ingestion of sodium bicarbonate only. The study comprised four experimental conditions: placebo + maltodextrin (PMD), placebo + sodium bicarbonate (PSB), β-alanine + maltodextrin (BAMD) and β-alanine + sodium bicarbonate (BASB).

Arterialised finger-prick blood samples were taken at baseline (prior to breakfast), immediately before, immediately after and 5-min after the $CCT_{110\%}$. Finger-prick blood samples were collected into lithium-heparin coated collection tubes (Radiometer, UK). Blood samples were analysed and used to determine blood lactate (Chapter 3.4.2.1), pH, bicarbonate and base excess (Chapter 3.4.2.2).

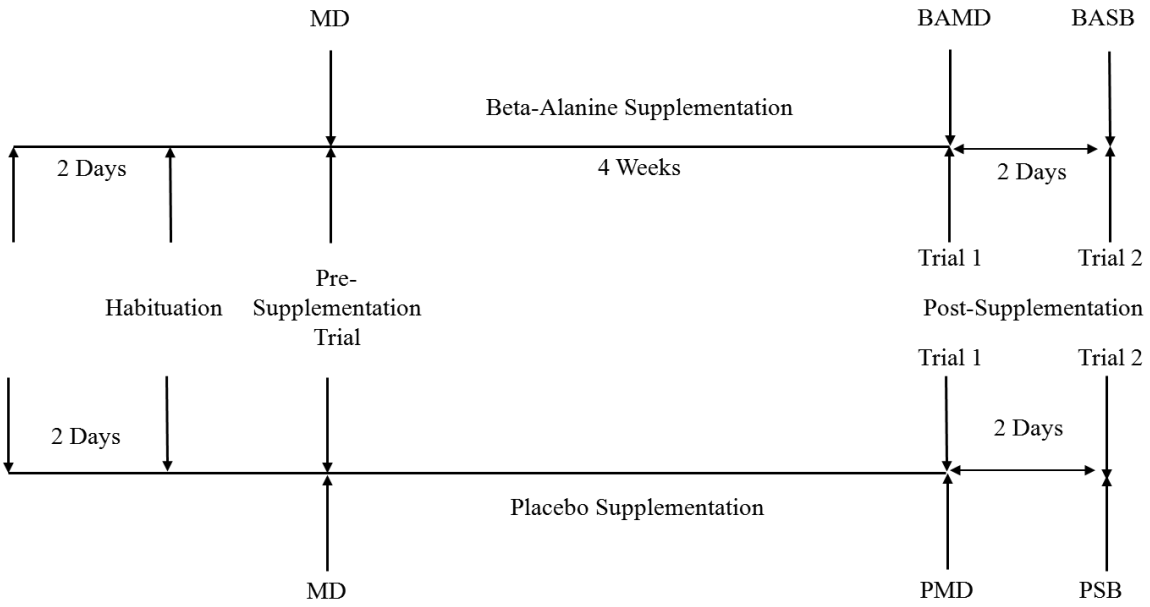

Figure 4C.1 Study Design.

4C.2.3 Statistical Analyses

All data are presented as mean ± 1SD for 10 participants in each group, with the exception of the blood data, which are presented for 9 participants in each group due to blood analyser malfunction. Performance data were analysed using a two way ANOVA (Group x Trial) and blood data were analysed using a three way ANOVA (Group x Trial x Time). Tukey tests were used for post-hoc analyses and effect sizes were calculated using Cohen's d (Cohen 1988). In addition, magnitude based inferences (Batterham and Hopkins, 2006) were used to determine the practical significance of BASB on the $CCT_{110\%}$ using a spread sheet to establish the likelihood of a meaningful effect on exercise capacity. The smallest worthwhile improvement in TTE and TWD was 3.56 s and 1.27 kJ which was equivalent to half the unbiased typical error associated with each measurement. Statistical significance was accepted at the $P \leq 0.05$ level.

4C.3 **Results**

4C.3.1 $CCT_{110\%}$

There was no significant difference in TTE (P = 0.54; placebo: 137.8 ± 23.2 and β-alanine: 143.1 ± 13.4 s, d = 0.29) and TWD (P = 0.69; placebo: 45.5 ± 9.8 and β-alanine: 44.0 ± 7.1 kJ, d = 0.18) between the placebo and β-alanine groups prior to the supplementation period. There was no significant improvement from baseline in TTE (Figure 4C.2) and TWD following PMD or PSB. Following BAMD, TTE (+17.2 ± 14.0 s; Group x Trial P = 0.03, *post hoc* P ≤ 0.01, d = 1.1) (Figure 4C.2) and TWD (+5.8 ± 5.0 kJ; Group x Trial P = 0.03, *post hoc* P ≤ 0.01, d = 0.9) significantly increased from baseline. BASB supplementation resulted in a significantly increased TTE (+23.3 ± 18.2 s; *post hoc* P ≤ 0.001, d = 1.2) (Figure 4C.2) and TWD (+8.1 ± 6.2 kJ; *post hoc* P ≤ 0.01, d = 1.0) from baseline. With co-ingestion of β-alanine and sodium bicarbonate (BASB), 6 out of 10 showed a further increase in TTE and TWD (with a 7^{th} unchanged) compared with BAMD. However, in neither case did the results reach significance (TTE: +6.1 ± 15.3 s, d = 0.4; TWD: +2.3 ± 5.4 kJ, d = 0.4).

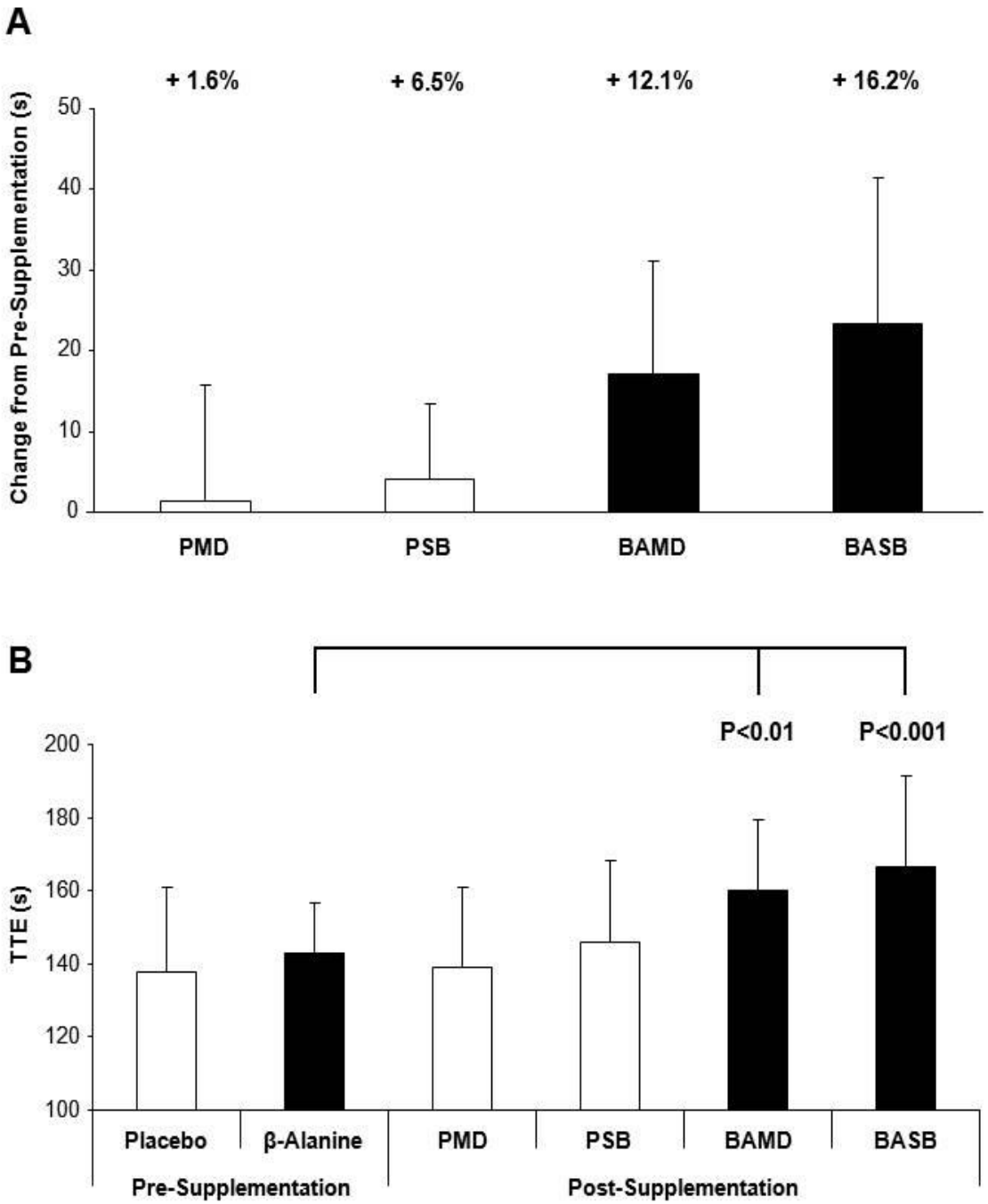

Figure 4C.2 Panel A shows the change in TTE from pre-supplementation following MD and SB ingestion in the placebo (white bars) and β-alanine (black bars) groups. Panel B shows TTE in the $CCT_{110\%}$ in MD and SB in the placebo (white bars) and β-alanine (black bars) groups.

4C.3.2 Blood Analyses

Baseline blood pH, bicarbonate and base excess were similar between all trials (Table 4C.1). There were significant increases in blood pH, bicarbonate and base excess between baseline and pre-exercise in both trials where sodium bicarbonate was consumed (PSB and BASB) (Trial x Time $P \leq 0.001$, *post hoc* $P \leq 0.001$). Increases were shown in all participants with sodium bicarbonate ingestion. In the two conditions where sodium bicarbonate was not consumed (PMD and BAMD), there were no significant alterations to blood pH, bicarbonate or base excess from baseline to pre-exercise (Table 4C.1).

In all trials, pH, bicarbonate and base excess were significantly reduced from baseline immediately post exercise and following 5 minutes of recovery ($P \leq 0.001$). In trials where sodium bicarbonate was ingested (PSB and BASB), blood pH, bicarbonate and base excess were significantly higher than in the trials where maltodextrin was ingested (PMD and BAMD) (Trial, $P \leq 0.001$).

Blood lactate was not significantly different between trials at baseline or pre-exercise (Table 4C.1). Blood lactate was significantly increased from baseline immediately post exercise and following 5 minutes of recovery in all trials ($P \leq 0.001$) and was significantly higher following sodium bicarbonate ingestion (Trial x Time $P \leq 0.001$; Table 4C.1). There was no difference in lactate response between the β-alanine and placebo groups ($P = 0.4$).

Table 4C.1 pH, lactate, bicarbonate and base excess at baseline (Base), pre-exercise (Pre-ex), immediately post-exercise (Post-ex) and 5 minutes post-exercise (+5 min) in the placebo and β-alanine groups pre supplementation (Pre), post-supplementation following maltodextrin (Post-MD) and post-supplementation following sodium bicarbonate (Post-SB). There was a significant time main effect for all blood responses (P<0.0001), with ǂ denoting a significant difference from Post-MD and Pre at P<0.001 and * denoting a significant post-hoc difference from Base at P<0.001.

	Placebo				β-alanine			
	Base	Pre-ex	Post-ex	+5 min	Base	Pre-ex	Post-ex	+5 min
pH								
Pre	7.402 ± 0.016	7.410 ± 0.014	7.246 ± 0.046	7.245 ± 0.054	7.407 ± 0.026	7.394 ± 0.032	7.223 ± 0.043	7.206 ± 0.057
Post-MD	7.416 ± 0.018	7.400 ± 0.015	7.252 ± 0.051	7.243 ± 0.053	7.397 ± 0.012	7.399 ± 0.028	7.226 ± 0.041	7.202 ± 0.055
Post-SBǂ	7.412 ± 0.019	7.467 ± 0.015*	7.303 ± 0.041	7.295 ± 0.051	7.409 ± 0.013	7.456 ± 0.021*	7.259 ± 0.037	7.243 ± 0.046
Lactate ($mmol \cdot L^{-1}$)								
Pre	1.3 ± 0.5	1.3 ± 0.5	13.0±2.3	12.5±2.0	1.1±0.3	1.2±0.5	12.8±2.6	12.6±2.2
Post-MD	1.1 ± 0.3	1.3 ± 0.8	12.9±2.7	12.0±2.1	1.0±0.4	1.4±0.7	14.2±3.2	14.0±2.2
Post-SBǂ	1.1 ± 0.2	1.2 ± 0.3	14.9±3.1	13.9±3.0	1.3±0.5	1.3±0.4	15.5±1.7	15.2±1.9
Bicarbonate ($mmol \cdot L^{-1}$)								
Pre	24.97 ± 1.05	24.97 ± 0.54	15.39 ± 1.92	13.44 ± 2.01	24.57 ± 1.13	24.96 ± 1.23	15.33 ± 1.73	11.99 ± 2.13
Post-MD	25.11 ± 1.31	25.35 ± 1.30	15.51 ± 2.12	13.49 ± 2.46	24.41 ± 1.10	24.80 ± 1.25	14.78 ± 2.60	11.72 ± 2.30
Post-SBǂ	25.04 ± 1.28	31.54 ± 0.95*	19.44 ± 2.19	16.47 ± 2.91	24.31 ± 1.06	29.76 ± 1.32*	16.95 ± 1.99	13.24 ± 1.93
Base excess ($mmol \cdot L^{-1}$)								
Pre	0.82 ± 0.89	0.98 ± 0.66	-10.35 ± 2.24	-11.89 ± 2.78	0.62 ± 0.89	0.62 ± 0.81	-10.90 ± 1.91	-13.92 ± 2.93
Post-MD	1.25 ± 1.34	1.04 ± 1.11	-10.11 ± 2.70	-11.88 ± 3.11	0.25 ± 0.90	0.61 ± 0.90	-11.27 ± 2.84	-14.23 ± 3.00
Post-SBǂ	1.11 ± 1.08	7.52 ± 0.77*	-5.84 ± 2.58	-8.37 ± 3.40	0.46 ± 0.80	5.85 ± 1.28*	-8.81 ± 2.26	-12.09 ± 2.49

4C.4 **Discussion**

The main findings from this study were that there was a significant increase in cycling capacity following β-alanine supplementation but co-supplementation with β-alanine and sodium bicarbonate did not confer any further statistically significant benefit.

The $CCT_{110\%}$ has previously been used by Hill et al. (2007) who showed that TWD was increased by 13.0% alongside a 58.8% increase in muscle carnosine following four weeks of β-alanine supplementation. When supplementation was extended to ten weeks, carnosine was increased by 80.1% and TWD by 16.2%. In the present study we showed a 14.6% increase in TWD during the $CCT_{110\%}$, although carnosine concentrations were not directly measured. However, several studies have shown significant increases in muscle carnosine concentrations following four weeks of supplementation despite employing lower doses than in the current study (Harris et al., 2006; Hill et al., 2007). Therefore, it can be hypothesised that an elevated dose of 6.4 $g \cdot d^{-1}$ for four weeks will have resulted in substantial increases in muscle carnosine.

Previous studies have indicated that intracellular H^+ accumulation with high-intensity exercise can affect metabolism, contributing to fatigue (Spriet et al., 1989). In particular, the accumulation of H^+ in the skeletal muscle might disrupt the resynthesis of phosphorylcreatine (Harris et al., 1976), inhibit glycolysis (Trivedi and Danforth, 1966) or interfere with the contractile machinery directly (Donaldson and Hermansen, 1978; Fabiato and Fabiato, 1978). In support of an effect of H^+ accumulation on the development of fatigue, numerous studies have shown an association between an increase in muscle buffering capacity and an improvement in high-intensity exercise performance and capacity (Weston et al., 1997; Bishop et al., 2004b; Edge et al., 2006), although not all agree (Westerblad et al., 1997). This

appears to be the most likely explanation of the exercise capacity improvements seen in the present investigation, with the increased skeletal muscle carnosine content resulting in an attenuation of the reduction in intracellular pH during high-intensity exercise. However, Hill et al. (2007) reported that they could not exclude the possibility that the increase in high-intensity cycling capacity observed with β-alanine supplementation was caused by some of the other purported physiological effects of elevated muscle carnosine concentrations.

Contrary to the effect of β-alanine on high-intensity cycling capacity, sodium bicarbonate ingestion alone did not significantly increase TWD during the $CCT_{110\%}$. Blood analyses confirmed that sodium bicarbonate ingestion was successful in significantly increasing pH, bicarbonate and base excess in line with previous studies of sodium bicarbonate ingestion (Price et al. 2003; van Montfoort et al. 2004; Robergs et al., 2005; Siegler et al., 2008). This study was based upon the premise that co-ingestion of β-alanine with sodium bicarbonate would result in an increased skeletal muscle carnosine concentration and increased circulating bicarbonate concentration. As such, the intracellular pH buffering action of carnosine and the extracellular buffering action of bicarbonate were hypothesised to be additive, resulting in an increased protection against the acidosis produced during high intensity cycling, as suggested by the results of Hill et al. (2007). It was hypothesised that this would result in a further improvement in high-intensity cycling capacity above that shown following either sodium bicarbonate supplementation or β-alanine supplementation alone. The results of this investigation provide support for an increased exercise capacity following co-ingestion of β-alanine and sodium bicarbonate above that of bicarbonate alone but not compared to β-alanine alone, despite a mean increase of 6 s in TTE and a 2 kJ increase in TWD. Whilst these differences were not significant, it is possible that, in performance terms, a further increase of 6 s in TTE might be important. Calculation of the magnitude-based

inferences (Batterham and Hopkins, 2006) showed that there was a 69% and 71% probability that the magnitude of the differences in TTE and TWD between β-alanine and β-alanine plus sodium bicarbonate were meaningful. This suggests a potentially meaningful increase in high-intensity cycling capacity when combining β-alanine and sodium bicarbonate supplementation over the ingestion of β-alanine alone.

There was a degree of individual variability in the exercise capacities of participants following β-alanine and β-alanine plus sodium bicarbonate ingestion, which might explain the lack of a significant finding. Indeed, 3 participants improved more on β-alanine alone than on the combination of β-alanine plus sodium bicarbonate. Each of these participants responded to sodium bicarbonate ingestion with an increase in both blood pH and bicarbonate concentrations. Furthermore, despite the fact that some participants experienced mild (N = 2) and severe (N = 3) gastrointestinal symptoms, these did not occur in those participants who showed the greatest improvement with just β-alanine.

4C.5 **Conclusions**

This study has confirmed the work of Hill et al. (2007) that four weeks of β-alanine supplementation can improve high-intensity cycling capacity at 110% of W_{max}. Improvements can be attributed to an increase in muscle buffering capacity due to increased muscle carnosine concentration. Although co-ingestion of β-alanine and sodium bicarbonate did not confer any further significant benefit to exercise capacity despite a further 6 s (~4%) increase in TTE, magnitude based inferences suggested a ~70% probability of a meaningful positive difference. This suggests an additive effect through co-supplementation of β-alanine and sodium bicarbonate should not be dismissed.

Chapter 5.0 A) Effect of sodium bicarbonate supplementation on repeated sprint performance during intermittent exercise performed at 2500 m simulated altitude

5A.1 **Introduction**

The results reported in the previous chapters showed that high-intensity cycling capacity could be improved with sodium bicarbonate supplementation if it did not result in GI discomfort and that individuals respond differently to supplementation. The $CCT_{110\%}$ is a single bout exercise test to exhaustion designed to last between 120 and 240 s, likely to be limited by increasing muscle acidosis. Team sports consist of high-intensity intermittent exercise, which is more prolonged and requires players to perform periods of high-intensity running, interspersed with periods of low intensity running. Match play requires players to continually reproduce maximal and near maximal sprints, 2 – 3 s in duration, with short periods of recovery over an extended period of time; this fitness component is termed RSA (Dawson et al., 1993; Bishop et al., 2001).

RSA has been used as an indicator of the ability of top level professional football players to perform high-intensity running during competitive match play (Rampinini et al., 2007). The RSA test used by Rampinini et al. (2007 & 2009) consists of six repeated 40 m (2 x 20 m) sprints, separated by 20 s of passive recovery, each sprint lasting approximately 7 s. Bishop et al. (2001) showed that the performance decrement during a modified RSA (5 x 6 s cycle sprints with 24 s of self-selected active or passive recovery) test was significantly correlated to the 15 m sprint performance decrement during an intermittent exercise protocol replicating the movement patterns of team sports. This suggests that a 5 x 6 s repeated sprint protocol may be a suitable performance measure when investigating interventions on team sport performance.

Increased RSA has been associated with a greater H^+ buffering capacity in elite female hockey players (Bishop et al., 2003), recreational team sport females (Bishop and Edge,

2006), untrained females (Bishop et al., 2004b) and professional and amateur male footballers (Rampinini et al., 2009). An intervention designed to increase intracellular buffering capacity may thus be of benefit to RSA and team sport performance. Indeed, Bishop et al. (2004a) showed that sodium bicarbonate supplementation could improve total work done and work and power output during the final three sprints of a 5 x 6 s sprint protocol, although, these were performed on a cycle ergometer. Furthermore, they did not determine repeated sprint performance during simulated games play or at altitude and, thus, did not consider the implications of these additional metabolic demands. When exercise is performed at altitude, there is an earlier reliance on anaerobic glycolysis due to the higher relative intensity for an absolute level of work (Levine et al., 2008), which leads to an increase in both muscle and blood lactate accumulation (Bueding and Goldfarb, 1941; Wolfel et al., 1991), resulting in a concomitant rise in H^+, which would place an increased reliance on the buffering systems of the body to maintain performance.

The aim of this investigation was to examine the effects of sodium bicarbonate supplementation on repeated sprint performance during a football specific treadmill protocol at simulated altitude. It was hypothesised that repeated sprint performance would be improved following supplementation with sodium bicarbonate.

5A.2 **Methods**

5A.2.1 Participants

Twenty recreationally active games players (age 22 ± 4 y, height 1.78 ± 0.07 m, body mass 75.4 ± 9.3 kg, VO_{2max} 54.3 ± 8.5 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in the study. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

5A.2.2 Experimental Design

Participants attended the laboratory on four separate occasions. The first session comprised of a running speed lactate and VO_{2max} test (Chapter 3.3.3). The remaining three sessions were for the completion of an habituation of the main protocol, and two main trials, which comprised of a football specific intermittent treadmill protocol (FSINT; Greig et al., 2006; Chapter 3.3.4), performed one week apart. The final three sessions were performed in an environmental chamber set at conditions designed to simulate 2500 m altitude ($\%O_2$ 15.5 ± 0.1%; temperature 18.0 ± 0.1^{o}C; relative humidity 52.7 ± 4.0%). Prior to the main trials, participants ingested either maltodextrin or sodium bicarbonate in a double-blind crossover design. Sodium bicarbonate and placebo were ingested in opaque gelatine capsules individually prepared for each participant, totalling 0.3 g·kg^{-1}BM. Participants ingested 0.2 g·kg^{-1}BM of sodium bicarbonate or matching placebo alongside a standardised breakfast of 3 slices of toast and jam. A final 0.1 g·kg^{-1}BM was ingested alongside a snack consisting of a banana and a cereal bar, 2 h prior to commencement of exercise. Participants were supervised during the ingestion of sodium bicarbonate and maltodextrin supplements to ensure 100% compliance. Participants rated their intensity of stomach ache, headache and sickness on an eleven point scale (Chapter 3.4.5) at breakfast, prior to the final dose, prior to exercise and immediately post-exercise.

The 5 x 6 s repeated sprint protocol (Chapter 3.3.5) was performed on three occasions; following a 5 min warm up (Set 1), immediately following FSINT1 (Set 2) and immediately following the FSINT2 (Set 3). Mean power output (MPO) and peak power output (PPO) of every sprint were recorded; MPO was determined as the highest average power output over 6 s for each sprint. Percentage fatigue for MPO and PPO during each set of the sprint protocol was calculated using recommendations made by Glaister et al. (2008) who showed the

performance decrement score devised by Fitzsimons et al. (1993) to be the most suitable formula for determining fatigue during repeated sprint exercise:

$$\% \text{ fatigue} = 100 - ([\text{total power output}/\text{ideal power output}] \times 100),$$

where total power output represents the sum of the power output values for all sprints during the set, and ideal power output represents the number of sprints performed multiplied by the highest power output of all sprints in the set. Participants were instructed to perform each sprint maximally, and were given strong verbal encouragement for the duration of every sprint.

In order to determine the reliability of the repeated sprint test, a further test-retest study was conducted on 16 participants (age 25 ± 3 y, height 1.79 ± 0.05 m, body mass 74.9 ± 8.9 kg) who completed two sets of 5 x 6 s sprints on two occasions separated by 2 days; the two sets were performed 45 minutes apart during which time the participants rested. Both tests were completed in a fasted state (12 h), with no caffeine or alcohol having been consumed for the previous 24 h. All sessions were performed in an environmental chamber set at conditions designed to simulate 2500 m altitude ($\%O_2$ 15.5 ± 0.1%; temperature 18.0 ± 0.1^{o}C; relative humidity 51.4 ± 5.4%). There was no significant difference in MPO or PPO between sets 1 and 2 ($P \geq 0.05$). The intra-class correlation for between sets 1 and 2 was $r = 0.93$ for MPO and $r = 0.87$ for PPO, with a coefficient of variation being 2.11% for MPO and 2.34% for PPO. There was no significant difference in overall MPO or PPO between trials 1 and 2 ($P \geq 0.05$). The intra-class correlation for between trial 1 and 2 was $r = 0.77$ for MPO and $r = 0.75$ for PPO, with a coefficient of variation being 3.99% for MPO and 4.35% for PPO.

Fingerprick blood samples were taken immediately prior to and immediately following every sprint bout, resulting in six individual samples (Pre Set 1, Post Set 1, Post FSINT1, Post Set 2,

Post FSINT2, Post Set 3). Blood samples were analysed and used to determine blood lactate (Chapter 3.4.2.1), pH, bicarbonate and base excess (Chapter 3.4.2.2). Heart rate (Chapter 3.4.3) and SaO_2 (Chapter 3.4.6) were recorded throughout exercise. Participants were allowed to drink water *ad libitum* throughout.

5A.2.3 Statistical Analyses

All data were analysed using Statistica 9 (Statsoft, USA) and are presented as mean ± 1SD. P plots and Cochran's Q were used to confirm normality and homogeneity of variance of the data. A three-way factorial ANOVA (Supplement x Set x Sprint) was used to determine any difference in power output and blood measurements. A two-way factorial ANOVA (Supplement x Set) was used to determine any effect on percentage fatigue for MPO and PPO. Fisher LSD tests were used for post-hoc analyses where appropriate and statistical significance was accepted at the $P \leq 0.05$ level.

5A.3 **Results**

5A.3.1 5 x 6 s Sprint Performance

5A.3.1.1 MPO

There was a main effect of sprints (Sprint, $P \leq 0.001$) and sets (Set, $P \leq 0.001$; Figure 5A.1), with a decline in MPO shown with increasing numbers of sprints and sets performed. There was no interaction effect of supplementation on sprint performance throughout the exercise (Supplementation x Set x Sprint, $P = 0.99$), or when taking into consideration all sets (Supplementation x Set, $P = 0.94$) or sprints (Supplementation x Sprint, $P = 0.99$; Table 5A.1). This indicates that supplementation did not have an effect on sprint performance across sets or sprints. MPO was different between trials (Supplement, $P = 0.02$), with overall lower values shown following sodium bicarbonate supplementation versus placebo (539.4 ±

84.5 vs. 554.0 ± 84.6 W), although there were no between trial differences at any corresponding time points (Table 5A.1). There was no effect of supplementation on %fatigue across sets (Supplementation x Set, P = 0.99), though any effect of supplementation may have been masked by the fact that performance decrements were not significantly different across sets (Set, P = 0.45).

5A.3.1.2 PPO

There was a main effect of sets (Set, $P \leq 0.001$) and sprints (Sprint, $P \leq 0.001$) on PPO, indicating that PPO achieved decreased as the number of sets and sprints performed increased. There was no interaction effect of supplementation on PPO throughout the exercise (Supplementation x Set x Sprint, P = 0.99), or when taking into consideration all sets (Supplementation x Set, P = 0.87) or sprints (Supplementation x Sprint, P = 0.98; Table 5A.1). There was no between trial differences in PPO (Supplement, P = 0.09). There was no effect of supplementation on %fatigue of PPO across sets (Supplementation x Set, P = 0.79), although there was a main effect of sets upon performance decrements (Set, P = 0.02), indicating a greater decline in PPO with increasing sets performed.

5A.3.2 Measurements

Pre-exercise pH, bicarbonate and base excess were different between trials ($P \leq 0.001$), with increased values shown following sodium bicarbonate supplementation. There was no interaction effect of supplementation throughout the exercise on pH (Supplement x Time, P = 0.92), bicarbonate (Supplement x Time, P = 0.12) or base excess (Supplement x Time, P = 0.30), although corresponding values were higher at every time point following supplementation with sodium bicarbonate (Supplement, $P \leq 0.001$; Table 5A.2). pH, bicarbonate and base excess values immediately following FSINT 1 and FSINT 2 were not

significantly different from pre-exercise values with the exception of pH following FSINT 2 ($P = 0.01$; Table 5A.2). pH, bicarbonate and base excess were all reduced immediately following every sprint bout from their corresponding pre-sprint value (Table 5A.2).

Lactate concentrations were similar at pre-exercise in both trials ($P = 0.56$; placebo: 1.9 ± 0.7 $mmol \cdot L^{-1}$ and sodium bicarbonate: 2.2 ± 0.7 $mmol \cdot L^{-1}$). Supplementation did not affect lactate throughout the trials (Supplement x Time, $P = 0.55$), although overall lactate concentrations were higher in the sodium bicarbonate trial (Supplement, $P \leq 0.001$). Lactate was not increased above pre-exercise concentrations following FSINT 1 or FSINT 2 in either trial ($P \geq 0.05$). There was a main effect of time on lactate concentration (Time, $P \leq 0.001$), with significantly higher concentrations shown in the sodium bicarbonate trial following the first and second set of sprints ($P \leq 0.01$; Table 5A.2).

Mean heart rate was similar (Half, $P = 0.62$) between the first half (Placebo: 144 ± 17 $b \cdot min^{-1}$; Sodium bicarbonate: 145 ± 15 $b \cdot min^{-1}$) and second half (Placebo: 146 ± 16 $b \cdot min^{-1}$; Sodium bicarbonate: 147 ± 15 $b \cdot min^{-1}$), with no differences between trials (Supplement, $P = 0.79$) and no interaction effect (Supplement x Half, $P = 0.94$). The level of dehydration was well controlled in the placebo (-0.7 ± 0.6%) and sodium bicarbonate (-0.6 ± 0.9%) trials, with no significant differences between trials ($P = 0.35$).

Table 5A.1 MPO and PPO for the placebo and sodium bicarbonate ($NaHCO_3^-$) trials.

	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Mean
MPO (W)						
Set 1						
Placebo	630.1 ± 83.8	610.8 ± 82.5	557.1 ± 68.9	534.8 ± 52.5	523.4 ± 64.3	571.2 ± 81.7
$NaHCO_3^-$	623.5 ± 87.7	590.3 ± 79.1	546.5 ± 69.4	522.2 ± 63.6	511.1 ± 57.6	558.7 ± 82.6
Set 2						
Placebo	622.7 = 83.1	579.8 ± 80.3	533.1 ± 57.4	530.3 ± 92.5	522.8 ± 67.0	557.7 ± 84.7
$NaHCO_3^-$	608.0 ± 83.8	568.5 ± 71.8	528.0 ± 73.4	507.2 ± 65.8	508.0 ± 78.4	543.9 ± 83.2
Set 3						
Placebo	605.3 ± 90.9	554.0 ± 77.7	507.2 ± 66.9	488.8 ± 61.3	509.4 ± 69.5	533.0 ± 83.9
$NaHCO_3^-$	584.0 ± 93.0	527.2 ± 83.3	492.0 ± 76.6	474.7 ± 52.4	499.6 ± 62.5	515.5 ± 82.9
PPO (W)						
Set 1						
Placebo	752.5 ± 130.4	739.0 ± 127.4	686.9 ± 112.7	666.1 ± 103.5	663.0 ± 117.2	701.5 ± 122.1
$NaHCO_3^-$	745.1 ± 122.5	721.2 ± 113.4	682.6 ± 112.2	663.2 ± 105.4	652.3 ± 107.0	692.9 ± 115.5
Set 2						
Placebo	758.4 ± 113.8	715.4 ± 117.0	671.9 ± 101.7	631.8 ± 97.8	661.2 ± 110.3	687.7 ± 115.1
$NaHCO_3^-$	744.0 ± 120.2	696.8 ± 106.3	642.4± 106.5	630.6 ± 106.3	628.3 ± 113.7	668.4 ± 117.6
Set 3						
Placebo	747.9 ± 130.4	681.2 ± 110.2	642.1 ± 113.6	613.1 ± 90.5	648.1 ± 121.5	666.5 ± 120.9
$NaHCO_3^-$	722.0 ± 142.8	651.1 ± 125.6	620.2 ± 105.2	606.6 ± 96.3	637.7 ± 94.1	647.5 ± 119.1

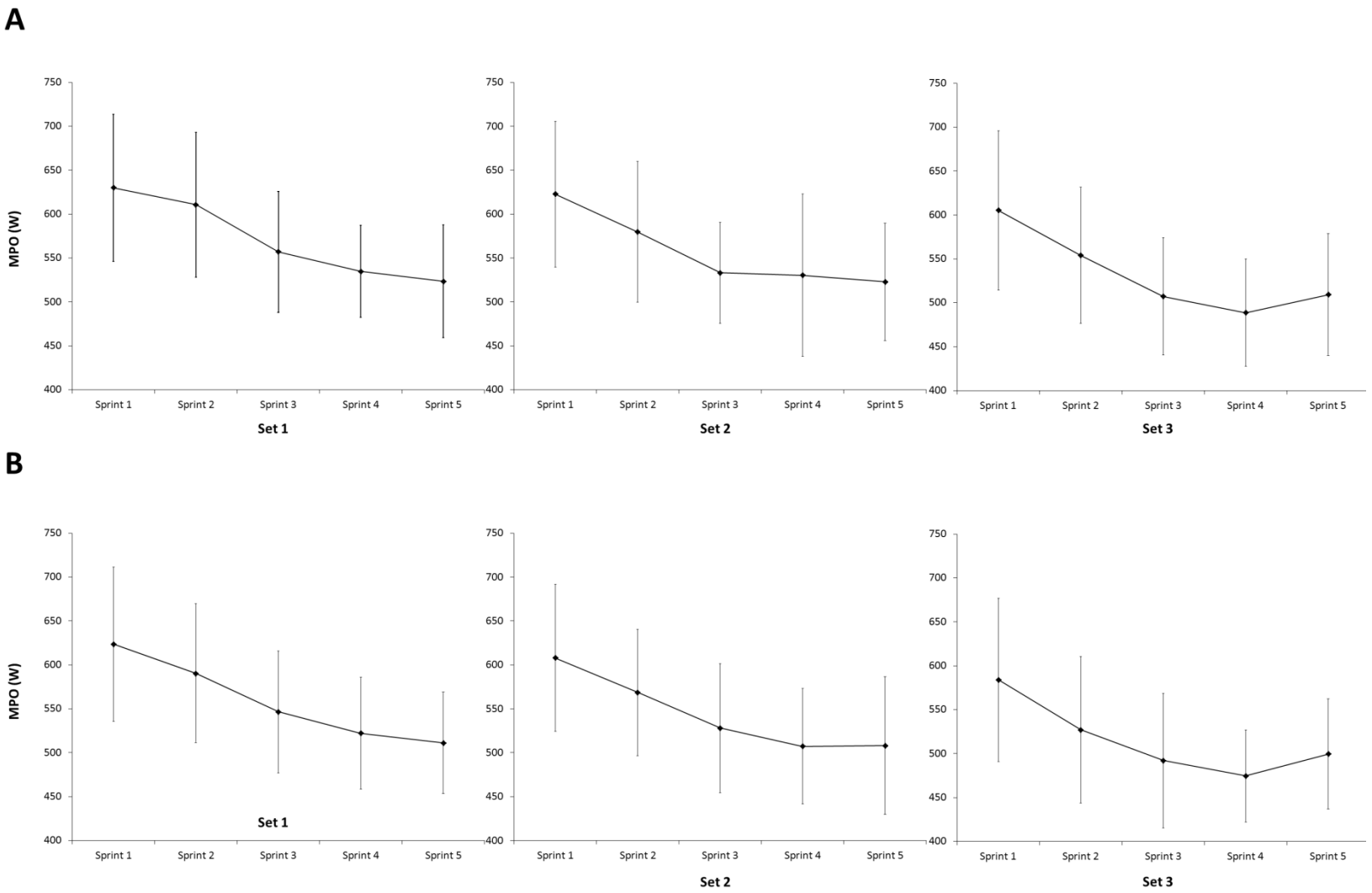

Figure 5A.1 MPO across all sets in the placebo (Panel A) and sodium bicarbonate (Panel B) trials

Table 5A.2 Blood pH, bicarbonate, base excess and lactate during the placebo and sodium bicarbonate trials. ^P ≤ 0.01 from placebo trial at the equivalent time point.

	Pre-Exercise	Post Sprint 1	Post FSINT 1	Post Sprint 2	Post FSINT 2	Post Sprint 3
pH						
Placebo	7.412 ± 0.022	7.243 ± 0.047	7.425 ± 0.018	7.309 ± 0.038	7.441 ± 0.018	7.349 ± 0.040
$NaHCO_3^-$	7.464 ± 0.022^	7.303 ± 0.048^	7.472 ± 0.016^	7.357 ± 0.043^	7.483 ± 0.020^	7.396 ± 0.047^
Bicarbonate ($mmol \cdot L^{-1}$)						
Placebo	23.9 ± 1.1	15.2 ± 1.9	23.7 ± 1.2	17.0 ± 1.6	23.5 ± 1.7	17.9 ± 2.2
$NaHCO_3^-$	28.6 ± 1.6^	18.5 ± 2.8^	28.4 ± 1.7^	19.6 ± 3.2^	27.9 ± 1.6^	21.4 ± 2.0^
Base Excess ($mmol \cdot L^{-1}$)						
Placebo	0.2 ± 1.1	-10.4 ± 2.4	0.3 ± 1.2	-7.6 ± 1.9	0.5 ± 1.6	-6.0 ± 2.5
$NaHCO_3^-$	5.1 ± 1.3^	-6.5 ± 3.2^	5.1 ± 1.5^	-4.7 ± 3.4^	4.9 ± 1.4^	-2.4 ± 2.4^
Lactate ($mmol \cdot L^{-1}$)						
Placebo	1.9 ± 0.7	8.5 ± 1.6	2.6 ± 0.8	7.0 ± 1.6	2.4 ± 1.0	6.2 ± 1.6
$NaHCO_3^-$	2.2 ± 0.7	9.6 ± 2.3^	3.1 ± 1.1	8.1 ± 1.8^	2.9 ± 1.3	6.7 ± 1.3

SaO_2 was not different between trials but was affected by time ($P \leq 0.001$), with significantly lower values during exercise than pre exercise and during half-time (Table 5A.3). There was no effect of time on intensity of stomach ache ($P = 0.09$), sickness ($P = 0.12$) or headache ($P = 0.27$) during any trial. Intensity of stomach ache and sickness were not different between trials ($P > 0.05$), but intensity of headache was higher in the sodium bicarbonate trial ($P = 0.05$).

Table 5A.3 SaO_2 during the placebo and sodium bicarbonate trials. $^{*}P \leq 0.001$ from Pre-Exercise. $^{\wedge}P \leq 0.001$ from Half-Time.

SaO_2 (%)	Pre Exercise	FSINT1	Half-Time	FSINT2
Placebo	98 ± 1	88 ± 4$^{*\wedge}$	93 ± 3	86 ± 3$^{*\wedge}$
$NaHCO_3^-$	99 ± 1	86 ± 5$^{*\wedge}$	93 ± 2	84 ± 4$^{*\wedge}$

5A.4 **Discussion**

This study showed that, despite a decrease in sprint performance across sets and sprints, sprint performance was unaffected by sodium bicarbonate supplementation. This is in contrast to Bishop et al. (2004a) who showed an increase in TWD during 5 x 6 s cycle sprints following sodium bicarbonate supplementation. In the current study there were no differences in MPO or PPO at any corresponding time points between trials. Furthermore, exercise was performed at simulated altitude, likely to induce an earlier reliance on anaerobic glycolysis and consequent increase in H^+ accumulation, theoretically increasing the reliance on buffering capacity. Despite this, sodium bicarbonate did not affect repeated sprint performance throughout prolonged games play activity performed at simulated altitude.

Sodium bicarbonate supplementation resulted in pre-exercise alkalaemia in all participants, with increased blood bicarbonate compared to the placebo condition ($+4.7 \pm 1.2$ mmol·L^{-1}),

which is higher than the average increases (+3.9 ± 0.9 mmol·L^{-1}) shown in a meta-analysis by Carr et al. (2011). However, the results discussed in Chapter 4B showed that alkalosis was not correlated to exercise capacity, which suggests that the degree of individual alkalosis does not directly influence the individual response to exercise. Furthermore, GI discomfort could account for some, but not all, of the decline in cycling capacity shown following sodium bicarbonate supplementation. In the present study, participants were asked to rate their intensity of stomach ache, sickness and headache in order to account for these issues; although the intensity of headache reported was higher during the sodium bicarbonate trial (likely skewed by one participant reporting significant headache prior to exercise), participants reported almost no symptoms during either trial at any point, suggesting that none of these factors contributed to the lack of an ergogenic effect with sodium bicarbonate.

The effect of sodium bicarbonate on repeated sprint exercise has been well researched, with supplementation effective in some (Costill et al., 1984; Lavender and Bird, 1989; Bishop et al., 2004a; Siegler et al., 2010; Zinner et al., 2011) but not all (Gaitanos et al., 1991; Webster et al., 1993; Price and Simons, 2010) studies. Few studies have investigated the effect of sodium bicarbonate on repeated sprints of durations similar to that seen in team sports (6 – 10 s; Lavender and Bird, 1989; Gaitanos et al., 1991; Bishop et al., 2004a), and only Gaitanos et al. (1991) employed a protocol with an exercise modality that represents team sports. Interestingly, this is the only study of the three that did not show a beneficial effect of sodium bicarbonate on exercise performance. It has previously been suggested that running repeated sprint protocols require an increased number of preliminary sessions to allow for full familiarisation of the activity required (Sweeney et al., 2011); however, reliability data of the protocol used in the present investigation suggests that one familiarisation session was

sufficient to allow for consistent results for both MPO and PPO across trials. Therefore, a lack of familiarisation of the protocol cannot explain the results in the current investigation.

Blood lactate was elevated following every set of sprints, with increased concentrations in the sodium bicarbonate trial following the first two sets, but not following the final set. Bishop et al. (2004a) showed no difference in post-exercise muscle pH between trials, despite increased muscle and blood lactate concentrations in the sodium bicarbonate trial, suggesting that there was an increased efflux of lactate and H^+ out of the working muscle, delaying the decline in pHi allowing a better maintenance of performance. In the present investigation, lactate concentrations following the first set of sprints were only 15% higher in the sodium bicarbonate trial, while Bishop et al. (2004a) showed lactate concentrations 28% higher following supplementation with sodium bicarbonate which may account for some of the differences in results. Furthermore, Ibanez et al. (1995) suggested that a difference in blood lactate concentration of 2 $mmol \cdot L^{-1}$ between treatments was required to show a performance effect with sodium bicarbonate. Differences of 1.1 ± 1.9, 1.2 ± 1.5 and 0.5 ± 1.3 $mmol \cdot L^{-1}$ were shown following the first, second and third sets of sprints, which may have contributed to the lack of an effect of sodium bicarbonate supplementation.

Anaerobic glycolysis has been shown to be considerable during 6 s cycle sprints, contributing up to 44% towards total ATP production (Gaitanos et al., 1993), resulting in a large accumulation of H^+. Decreased oxygen saturation may mean that increased anaerobic glycolysis will lead to a higher, and earlier, increase in H^+ within the working muscle. Although H^+ was not directly measured here, due to the high association between lactate and H^+ production (Hultman and Sahlin, 1980), blood lactate concentration can be used as an indicator of H^+ production. It was hypothesised that there would be an increased reliance on

the body's buffering systems as a result of the exercise being performed at simulated altitude (Brooks et al., 1991; Wolfel et al., 1991). Despite this, the highest average lactate concentrations (Post Sprint 1: 8.5 ± 1.6 and 9.6 ± 2.3 $mmol \cdot L^{-1}$ for placebo and sodium bicarbonate) are not in excess of those shown by Bishop et al. (2004a) under normoxic conditions (Placebo: ~8 $mmol \cdot L^{-1}$ and sodium bicarbonate: ~12 $mmol \cdot L^{-1}$). However, differences may be attributed to the exercise modalities employed, as Bishop et al. (2004a) had participants perform cycling sprints on an ergometer. Furthermore, although exercise took place in hypoxic conditions, likely inducing a larger accumulation of H^+, there may have been a saturation in the rate of removal of lactate and H^+, which is near maximal during running sprints (Gaitanos et al., 1991). Jorfeldt et al. (1978) showed a linear relationship between the increase in muscle lactate production and efflux up to a concentration of 20 $mmol \cdot L^{-1}$, where after no increase in efflux was shown with increased lactate production. Therefore, increased levels of circulating bicarbonate may not have increased the already maximal rate of H^+ efflux out of the working muscle, explaining the lack of a performance benefit.

5A.5 **Conclusions**

Sodium bicarbonate supplementation did not affect 5 x 6 s repeated sprint performance during the FSINT at simulated altitude. The lack of a significant finding may be due to a lack of a difference in lactate and H^+ production between trials, or a saturation in H^+ efflux out of the working muscle due to the exercise performed, rendering any increases in blood buffering capacity insignificant.

Chapter 5.0 B) Effect of β-alanine supplementation, with and without sodium bicarbonate, on repeated sprint performance during intermittent exercise performed at 2500 m simulated altitude

5B.1 **Introduction**

In Chapter 5A, it was reported that repeated sprint performance throughout team sport specific exercise at simulated altitude was unaffected by sodium bicarbonate supplementation. The lack of an effect may be attributed to the lack of a difference in lactate production between trials (Ibanez et al., 1995), or could be due to a saturation in the efflux of H^+ out of the working muscle (Jorfeldt et al., 1978). Despite this, increased extracellular buffering capacity has previously been shown to improve repeated sprint performance (Lavender and Bird, 1989; Bishop et al., 2004a). Furthermore, increasing intracellular buffering capacity may be of more benefit to exercise performance and capacity due to a more direct influence upon pHi.

Although Hoffman et al. (2008) showed no effect of β-alanine on fatigue rates during repeated line drills (200 yards) following 30 days of supplementation, no baseline measurements were taken prior to the supplementation period. Therefore, any gains in performance due to increased muscle buffering capacity may have been overlooked due to an inappropriate testing strategy. Similarly, two sets of 5 x 5 s repeated sprints were unaffected following five weeks β-alanine supplementation (Sweeney et al., 2010), although a lack of familiarisation to the protocol may have contributed to a lack of an ergogenic effect. Both of these studies had limitations which may have masked any effect of β-alanine supplementation on repeated sprint performance. Furthermore, participants were not required to perform the sprints during sport specific exercise, which would place an increased metabolic demand on the individuals. Krustrup et al. (2006a) showed that repeated sprint performance was reduced following actual match play, and following the most intense five minutes in the first and second halves. Although decreased sprint performance was not directly correlated to reduced muscle pH in this study, previous research has shown an association between repeated sprint

performance and H^+ buffering capacity (Bishop et al., 2003; Bishop et al., 2004b; Bishop and Edge, 2006; Rampinini et al., 2009).

In the study reported in Chapter 4C, participants were co-supplemented with β-alanine and sodium bicarbonate, on the premise that increases in intracellular and extracellular buffering would be additive. Although co-ingestion of β-alanine and sodium bicarbonate did not confer any further significant benefit to exercise capacity, magnitude based inferences suggested a ~70% probability of a meaningful positive difference, which suggests an additive effect should not be dismissed. When exercise is performed at altitude, there is an earlier reliance on anaerobic glycolysis due to the higher relative intensity for an absolute level of work (Levine et al., 2008), leading to an increase in both muscle and blood lactate accumulation (Brooks et al., 1991; Wolfel et al., 1991). The concomitant rise in H^+ concentration reduces intracellular and extracellular pH, which may contribute to the onset of fatigue. An intervention designed to attenuate the decrease in muscle pH, via increases in intracellular (β-alanine supplementation; Sale et al., 2010) or extracellular (sodium bicarbonate supplementation; McNaughton et al., 2008) buffering capacity, may individually, or additively, improve exercise performance at altitude.

The effect of sodium bicarbonate on repeated sprints is equivocal (Lavender and Bird, 1989; Gaitanos et al., 1991; Bishop et al., 2004a), while repeated sprint performance has been shown to be unaffected by β-alanine supplementation (Hoffman et al., 2008; Sweeney et al., 2011). However, differences in results may be attributed to a number of factors, and the ergogenic effect of increased buffering capacity on repeated sprint performance should not be dismissed. No study to date has considered the implications of the additional metabolic demands of performing sprints throughout simulated match play. Furthermore, co-

supplementation of β-alanine with sodium bicarbonate may provide an additive effect to supplementation with β-alanine alone, particularly during exercise performed at simulated altitude. This study investigated the effects of 4 – 5 weeks β-alanine supplementation, with and without acute sodium bicarbonate supplementation, on repeated sprint performance during a football specific treadmill protocol at a simulated altitude of 2500 m. It was hypothesised that repeated sprint performance would be improved following supplementation with β-alanine, and that performance benefits would be additive when co-supplemented with sodium bicarbonate due to an increased protection against intracellular and extracellular acidosis.

5B.2 **Methods**

5B.2.1 Participants

Twenty physically active games player participated in the study. Participants were split into β-alanine and placebo groups, matched for PPO. Four participants withdrew from the study due to injury, leaving eight participants in each supplementation group (Table 5B.1). Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

Table 5B.1 Participant characteristics.

	Placebo (N = 8)	β-alanine (N = 8)
Age (y)	23 ± 4	22 ± 3
Height (m)	1.7 ± 0.07	1.83 ± 0.05
Body Mass (kg)	72.3 ± 9.1	78.5 ± 10.9
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	55.7 ± 7.1	57.1 ± 9.7
Compliance (%)	99 ± 3	96 ± 8
Total supplement consumed (g)	188.2 ± 5.6	182.3 ± 14.7

5B.2.2 Experimental Design

Participants attended the laboratory on five separate occasions. The first session comprised of a running speed lactate and VO_{2max} test (Chapter 3.3.3). The remaining four sessions were for the completion of an habituation of the main protocol, and three main trials, which comprised of the FSINT (Chapter 3.3.4). The final four sessions were performed in an environmental chamber set at conditions designed to simulate 2500 m altitude ($\%O_2$ 15.5 ± 0.1%; temperature 18.0 ± 0.1^{o}C; relative humidity 52.7 ± 4.0%). One of the main trials was completed before a 5 week supplementation period of either β-alanine or placebo. Participants were supplemented with 6.4 g·d^{-1} β-alanine or placebo for 4 weeks, and 3.2 g·d^{-1} for a further 1 week. The dosing regimen consisted of two 800 mg β-alanine or placebo tablets ingested four times per day at 3 – 4 h intervals during the initial 4 weeks, followed by one 800 mg β-alanine or placebo tablet ingested four times per day at 3 – 4 h intervals for the final week. Compliance was monitored using supplementation logs, with a high degree of compliance being reported in both groups (Table 5B.1).

The final two main trials were performed one week apart following 4 and 5 weeks of supplementation. Prior to the first main trial, participants' ingested maltodextrin, and following the supplementation period, participants ingested either sodium bicarbonate or maltodextrin in a crossover design. Sodium bicarbonate and placebo were ingested in opaque gelatine capsules individually prepared for each participant, totalling 0.3 g·kg^{-1}BM. Participants ingested 0.2 g·kg^{-1}BM of sodium bicarbonate or maltodextrin alongside a standardised breakfast of 3 slices of toast and jam. A final 0.1 g·kg^{-1}BM was ingested alongside a snack consisting of a banana and a cereal bar, 2 h prior to commencement of exercise. To ensure 100% compliance, participants were supervised during the ingestion of sodium bicarbonate and maltodextrin supplements. The study design comprised four

experimental conditions: placebo + maltodextrin (PMD), placebo + sodium bicarbonate (PSB), β-alanine + maltodextrin (BAMD) and β-alanine + sodium bicarbonate (BASB) (Figure 5B.1).

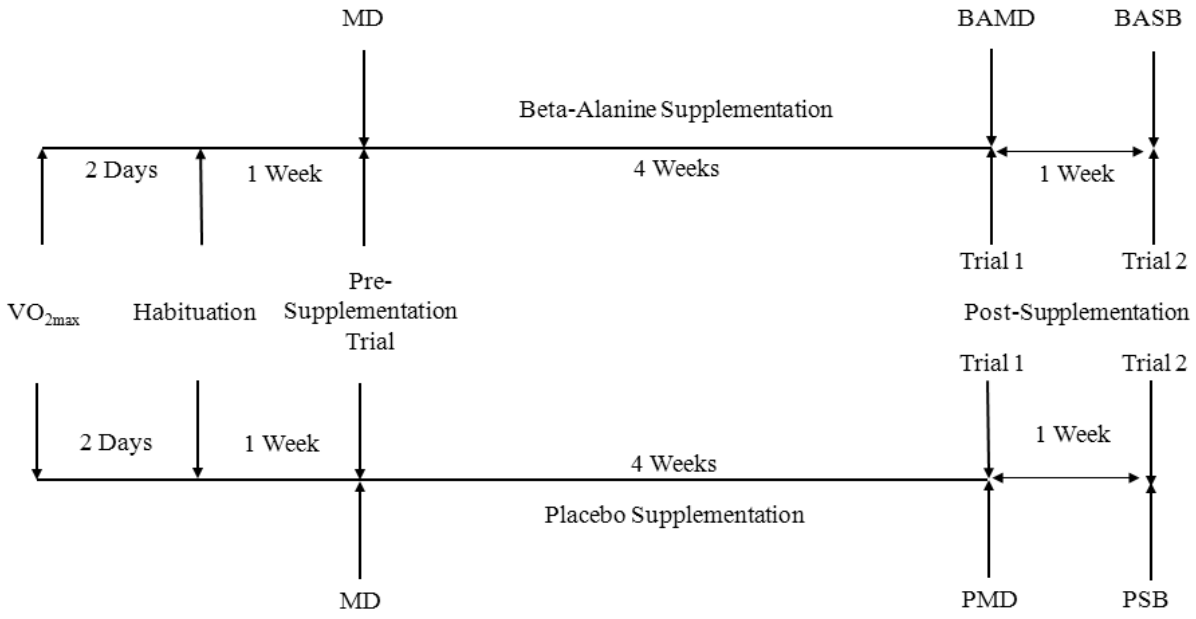

Figure 5B.1 Study Design.

Fingerprick blood samples were taken immediately prior to and immediately following every sprint bout, resulting in six individual samples (Pre-exercise, Post Set 1, Post FSINT 1, Post Set 2, Post FSINT 2, Post Set 3). Blood samples were analysed and used to determine blood lactate (Chapter 3.4.2.1), pH, bicarbonate and base excess (Chapter 3.4.2.2). Heart rate (Chapter 3.4.3) and SaO_2 (Chapter 3.4.6) were recorded every 5 s throughout exercise. Participants rated their intensity of stomach ache, headache and sickness on an eleven point scale (Chapter 3.4.5) at breakfast, prior to the final dose, prior to exercise and immediately post-exercise. Participants were allowed to drink water *ad libitum* throughout.

5B.2.3 Statistical Analyses

All data were analysed using Statistica 9 (Statsoft, USA) and are presented as mean ± 1SD. P plots and Cochran's Q were used to confirm normality and homogeneity of variance of the data. A four-way factorial ANOVA (Group x Trial x Set x Sprint) was used to determine any difference in sprint times and blood measurements. A three-way factorial ANOVA (Group x Trial x Set) was used to determine any effect on percentage fatigue for MPO and PPO. Fisher LSD tests were used for post-hoc analyses where appropriate and statistical significance was accepted at the $P \leq 0.05$ level.

5B.3 **Results**

5B.3.1 5 x 6 s Sprint Performance

5B.3.1.1 MPO

Sprint performance decreased as the number of sprints (Sprint, $P \leq 0.001$) and sets (Set, $P \leq 0.001$) increased. There were no interaction effects for MPO between supplementation and any other variable (all $P > 0.05$). Furthermore, there was no effect of β-alanine or sodium bicarbonate alone on sprint performance (all $P > 0.05$). There was a trend towards higher MPO values in the β-alanine group (Group, $P = 0.07$), although there were no differences between groups for any trial (Group x Trial, $P = 0.43$, *post hoc* all $P > 0.05$; Table 5B.2).

There was no difference in %fatigue across sets (Set, $P = 0.68$), indicating that performance decrements did not worsen as the number of sets performed increased. There were no interaction effects between supplements and any other variables (all $P > 0.05$).

5B.3.1.2 PPO

PPO decreased as the number of sprints (Sprint, $P \leq 0.001$) and sets (Set, $P \leq 0.001$) increased. There were no interaction effects between supplementation and any of the other variables (all $P > 0.05$), nor was there an effect of β-alanine or sodium bicarbonate alone on PPO (all $P > 0.05$). PPO was higher overall in the β-alanine (Group, 723.5 vs. 706.0 W; $P = 0.04$), although PPO was only different between groups during the sodium bicarbonate trial (*post hoc* $P = 0.02$; Table 5B.2).

Performance decrements were not significantly different across sets (Set, $P = 0.28$), which suggests that the deterioration in PPO achieved during each set did not worsen as the number of sets performed increased. There was no interaction effect of supplementation and trial on %fatigue across sets (Group x Trial x Set, $P = 0.43$). Similarly, there was no effect of supplementation on fatigue rates across trials (Group x Trial, $P = 0.64$).

Table 5B.2 Trial MPO and PPO for the β-alanine and placebo groups. *P ≤ 0.05 from placebo group.

	Pre Supplementation	Post Supplementation MD	Post Supplementation SB
MPO (W)			
β-alanine	567.4 ± 89.9	560.5 ± 82.9	580.5 ± 87.7
Placebo	548.5 ± 94.0	560.2 ± 81.8	566.8 ± 85.2
PPO (W)			
β-alanine	719.7 ± 128.0	711.2 ± 122.0	739.7 ± 127.7*
Placebo	697.1 ± 106.0	715.9 ± 113.5	704.9 ± 93.4

5B.3.2 Measurements

There was no interaction effect of supplementation across trials throughout the exercise on pH (Group x Trial x Time, P = 0.99), bicarbonate (Group x Trial x Time, P = 0.99) or base excess (Group x Trial x Time, P = 0.98). Supplementation did not affect trial pH, bicarbonate and base excess (Group x Trial, all P > 0.05), although overall trial blood values were higher following supplementation with sodium bicarbonate (*post hoc* all P ≤ 0.001; Table 5B.3) in both supplementation groups. There was an effect of time (Time, all P ≤ 0.001) on pH, bicarbonate and base excess values; immediately following FSINT 1 and FSINT 2, values were not significantly different from pre-exercise (*post hoc* P > 0.05) but were significantly reduced following every set of sprints (*post hoc*, all P ≤ 0.001).

There was a main effect of time on lactate concentration (Time, P ≤ 0.001), with concentrations elevated above pre-exercise levels shown following every set of sprints (*post hoc*, P ≤ 0.001) but not FSINT 1 or FSINT 2. Lactate concentrations were higher in the sodium bicarbonate trial than both the pre and post supplementation maltodextrin trials (Trial, P = 0.001, *post hoc* P ≤ 0.01). There was an interaction effect of supplementation and trial, although this was due to between trial differences shown in the placebo, not the β-alanine, group (Table 5B.3). Supplementation did not affect lactate across trials and time (Group x Trial x Time, P = 0.57) or across time (Group x Time, P = 0.36).

Mean heart rate was similar between groups (Group, P = 0.32; Placebo: 136 ± 17 b·min^{-1}; β-alanine: 138 ± 11 b·min^{-1}) and was not difference between halves during any trial (Half, P = 0.46). There were no interaction effects of mean heart rate with any variable (all P > 0.05). SaO_2 was not different between groups at any time point (Group, P = 0.49; *post hoc* all P > 0.05), but was affected by time (P ≤ 0.001), with significantly lower values during exercise

(85 ± 9%) than pre exercise (98 ± 2%) and during half-time (94 ± 5%) for both groups during all trials.

Table 5B.3 Blood measurements averaged over the trials for both the placebo and β-alanine groups. *P ≤ 0.01 from placebo group during the same trial. $^{\wedge}$P ≤ 0.05 from Pre-Supplementation. $^{\sim}$P ≤ 0.05 from Post Supplementation MD.

	Pre Supplementation	Post Supplementation MD	Post Supplementation SB
pH			
Placebo	7.364 ± 0.077	7.365 ± 0.071	7.415 ± 0.072
β-alanine	7.372 ± 0.072	7.376 ± 0.069	7.421 ± 0.061*
Bicarbonate (mmol·L^{-1})			
Placebo	20.1 ± 3.8	19.8 ± 4.5	23.6 ± 4.5
β-alanine	20.4 ± 4.0	20.2 ± 3.8	24.4 ± 4.7*
Base Excess (mmol·L^{-1})			
Placebo	-3.8 ± 4.4	-4.2 ± 5.1	-0.1 ± 5.1
β-alanine	-3.4 ± 4.8	-3.5 ± 4.6	1.1 ± 5.0*
Lactate (mmol·L^{-1})			
Placebo	4.8 ± 2.8	5.3 ± 3.2$^{\wedge}$	6.0 ± 3.7$^{\wedge\sim}$
β-alanine	4.7 ± 3.0	4.3 ± 2.8*	4.8 ± 2.9*

5B.4 **Discussion**

The main findings from this study were that repeated sprint performance was unaffected by β-alanine supplementation alone, or co-supplementation of β-alanine with sodium bicarbonate.

Reported in the previous chapter, sodium bicarbonate supplementation was ineffective at improving repeated sprint performance during an identical exercise protocol. However, due to a more direct influence of increased carnosine levels on muscle pH, it was hypothesised that β-alanine supplementation would result in an improved performance. Despite this, β-alanine supplementation did not have an effect on sprint performance. The results of the

present investigation are in accordance with Sweeney et al. (2010), who showed no effect of 5 weeks β-alanine supplementation on horizontal power or performance decrement during 5 x 5 s repeated sprints with 45 s passive recovery, performed twice with a 2 minute active recovery between sets. A lack of an effect was attributed to a change in pacing strategy in both groups since MPO was lower in both groups following supplementation, suggesting the participants were not fully familiarised with the protocol. The results in the current investigation showed that overall MPO was not different between any trial for either group, which suggests that participants did not adopt a pacing strategy and performed maximally during the trials, and cannot explain the lack of an effect.

Further to the results reported in Chapter 4C, which indicated a ~70% likelihood of a meaningful improvement in exercise capacity when β-alanine was co-supplemented with sodium bicarbonate, it was hypothesised that co-supplementation would infer further improvements above any shown with β-alanine alone. Despite this, co-supplementation did not confer any benefits in MPO or PPO above that of bicarbonate or β-alanine alone. Indeed, power output was not improved from pre supplementation levels, suggesting that increased buffering capacity, both intracellular and extracellular, is ineffective at influencing 5 x 6 s repeated treadmill sprints. During maximal exercise, the requirement for ATP resynthesis is high and is supplemented by the hydrolysis of PCr and anaerobic glycolysis (Hultman and Sjöholm, 1983). PCr was reduced to 55% of resting levels following a single maximal 6 s cycle sprint (Dawson et al., 1997), and further reduced to 27% following a fifth maximal sprint. Bogdanis et al. (1993) showed that the halftime for PCr resynthesis was 57 seconds, which is more than double the recovery period between sprints in this study. Therefore, it could be hypothesised that the sprint protocol used in the current investigation did not allow for sufficient recovery of PCr levels, which was a greater contributor to fatigue than H^+

accumulation and reduced muscle pH. This would render any increases in buffering capacity insignificant, and explain the lack of an effect of supplementation with β-alanine and sodium bicarbonate.

Due to the decline in energy production from PCr because of incomplete resynthesis of stores, it has been proposed that an increased demand will be placed on anaerobic glycolysis with increasing sprints, although the reverse of this has been shown (Gaitanos et al., 1993). However, the decline in power output with repeated sprints has been shown to be disproportionately smaller than the reduction in anaerobic energy contribution (Bogdanis et al., 1996), with an increasing contribution in oxygen uptake contributing to this difference. With this type of exercise, H^+ accumulation and muscle lactate concentrations are still likely to be high, but the increasing involvement of aerobic metabolism will not increase the acidosis in the muscle further. However, the low oxygen content of the hypoxic conditions will result in an earlier reliance on anaerobic glycolysis, resulting in an earlier accumulation of H^+, which would theoretically be positively influenced by increased intracellular and extracellular buffering capacity. Therefore, other pathways related to H^+ buffering, such as delaying the fatigue induced increase in ventilation rate (Stout et al., 2007), may be the mechanism behind an ergogenic effect. Nonetheless, no effects of increased intracellular and extracellular buffering capacity were shown, suggesting the contribution of anaerobic energy sources are not sufficient to induce performance limiting accumulations of H^+ during this type of exercise.

Despite this, Bishop et al. (2003) showed a correlation between the change in plasma H^+ and the power decrement during 5 x 6 s cycle sprints. Furthermore, improved RSA has been associated with an increased ability to buffer H^+ (Bishop and Spencer, 2004; Bishop and

Edge, 2006; Edge et al., 2006; Rampinini et al., 2009), which would suggest that increasing intracellular and extracellular buffering capacity would result in improvements in sprint performance. However, neither β-alanine nor sodium bicarbonate supplementation alone or in combination improved sprint performance in the current study. Since supplementation with these buffering agents would increase both intracellular and extracellular buffering capacity, it appears that repeated sprints of this nature are not influenced by the ability to maintain muscle pH. The studies that report on an association between RSA and H^+ buffering capacity determined muscle buffering capacity by the titration of skeletal muscle homogenates, which may have affected results. The homogenisation of muscle causes changes to the chemical composition of the intracellular environment, even with the inhibition of glycolysis by iodoacetate (Bueding and Goldfarb, 1941), most notably the hydrolysis of PCr and ATP resulting in increases in inorganic phosphate (pKa 6.8) and hexose monophosphates (pKa 6.1), which would contribute to an over estimation of muscle buffering capacity. Furthermore, considering that carnosine can contribute as much as 40% to buffering capacity in the physiological range, it would appear that H^+ buffering capacity does not affect 5 x 6 s repeated treadmill sprints, explaining the lack of an effect shown following β-alanine and sodium bicarbonate supplementation.

5B.5 **Conclusions**

β-alanine supplementation did not affect 5 x 6 s repeated sprint performance throughout team sport specific exercise at simulated 2500 m altitude, nor did co-supplementation with sodium bicarbonate. Despite previous research suggesting an association between increased buffering capacity and improved RSA, the current investigation provides evidence that increased intracellular and extracellular buffering capacity does not improve repeated sprint performance.

Chapter 6.0 Effect of β-alanine supplementation on repeated sprint performance during the Loughborough Intermittent Shuttle Test

6.1 **Introduction**

Reported in the previous chapter, repeated 5 x 6 s sprints were unaffected by β-alanine supplementation, or co-supplementation with β-alanine and sodium bicarbonate. This is similar to the findings of Hoffman et al. (2008) and Sweeney et al. (2010) on RSA, despite research showing an association between H^+ buffering capacity and RSA (Bishop et al., 2004b; Rampinini et al., 2009), though this is based on the titration of muscle homogenates. Although the 5 x 6 s repeated sprint protocol was used due to its association with the most intense period of running during a game, team sport players rarely perform sprints of this duration. However, Hobson et al. (2012) showed that β-alanine supplementation was ineffective at improving high-intensity exercise less than 60 s in duration. It has been suggested that an exercise duration of less than 60 s may not be sufficient to induce reductions in pHi that will limit exercise (Sale et al., 2010), although repeated short duration exercise may increase the sensitivity to reduced pHi (Katz et al., 1984).

Previous studies have not shown a significant effect of β-alanine supplementation on repeated sprint performance (Hoffman et al., 2008; Sweeney et al., 2011). However, these studies did not determine repeated sprint performance during simulated or actual games play and, thus, did not consider the implications of the additional metabolic demand of the entire activity. The Loughborough Intermittent Shuttle Test was designed as an exercise test that could be performed indoors and replicates that activity profile of multiple sprint sports such as football (Nicholas et al., 2000). The protocol incorporates sixty-six maximal 15 m sprints, which represents the mean distance and duration of sprints during team sports which are between 10 – 20 m and 2 – 3 s (Spencer et al., 2005). Blood lactate concentrations in excess of 6 $mmol \cdot L^{-1}$ have been shown during the LIST (Nicholas et al., 2000), with the concomitant increase in H^+ potentially contributing to a decline in sprint performance shown as the number of sprints

performed increases (Sunderland and Nevill, 2005; Phillips et al., 2010). This suggests the LIST may be an appropriate exercise model to investigate the effects of increased muscle buffering capacity on prolonged high-intensity intermittent exercise performance.

The aim of this investigation was to examine the effects of four weeks β-alanine supplementation on multiple sprint performance during the LIST. Furthermore, to determine any differences of β-alanine supplementation on games players of varying standard, both elite and non-elite games players were recruited to the study, since reports have suggested improved buffering capacities in trained compared with recreational athletes (Sahlin and Henriksson, 1984; Parkhouse et al., 1985; Edge et al., 2006). It was hypothesised that an increased H^+ buffering capacity due to higher muscle carnosine concentrations would result in an improvement in sprint performance during the LIST.

6.2 **Methods**

6.2.1 Participants

Twenty elite and twenty recreationally active male games players volunteered for the study and were allocated into β-alanine and placebo groups, matched for estimated VO_{2max} (Table 6.1). The elite population consisted of national hockey players, all of whom had represented their country at U18, U21 or full international level. The non-elite population were recreationally active individuals who engaged in team sports (football and hockey) 1 – 2 times per week. Four elite players withdrew from the study due to injury, meaning that 16 elite players were included in the final data set. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

6.2.2 Experimental Design

Participants attended a sports hall on four separate occasions. Main trials comprised of the Loughborough Intermittent Shuttle Test (Chapter 3.3.7). Prior to the first main LIST trial, participants completed the multistage fitness test (Chapter 3.3.6), a progressive shuttle run test to volitional exhaustion (Ramsbottom et al., 1988), and an habituation of the LIST. Participants maintained a food diary in the 24 h period before the first main trial, and this was subsequently used to replicate diet prior to the second main trial. Main trials were separated by four weeks of supplementation with either 6.4 $g \cdot d^{-1}$ β-alanine or placebo. The dosing regimen consisted of two 800 mg β-alanine or maltodextrin tablets ingested four times per day at 3 – 4 h intervals. Participants completed a supplementation log to verify compliance, which was high in all groups (Table 6.1).

Table 6.1 Participant characteristics.

	Elite		**Non-Elite**	
	Placebo (N = 8)	β-alanine (N = 8)	Placebo (N = 10)	β-alanine (N = 10)
Age (y)	19 ± 2	20 ± 1	22 ± 3	22 ± 2
Height (m)	1.77 ± 0.05	1.80 ± 0.06	1.81 ± 0.07	1.79 ± 0.08
Body Mass (kg)	72.1 ± 7.1	75.0 ± 11.0	84.9 ± 10.9	81.0 ± 11.5
Estimated VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	59.4 ± 2.6	58.6 ± 2.4	50.7 ± 5.0	50.5 ± 4.4
Compliance (%)	94 ± 5	87 ± 10	96 ± 4	96 ± 6
Total supplement consumed (g)	169.1 ± 8.8	155.2 ± 17.0	171.2 ± 7.2	171.0 ± 11.6

Fingerprick blood samples were taken immediately upon arrival to the sports hall, and during the 3 minute rest periods between sets; these were analysed for blood lactate concentration using portable lactate monitors (Chapter 3.4.2.1). All 66 individual sprint times over 15 m during the LIST were recorded (Brower Timing Systems IRD-T173, Utah, USA). Participants indicated their overall ratings of perceived exertion (RPE) during the last walking stage of each set on a 15 point scale (Chapter 3.4.4). Pre- and post-exercise body mass was measured (Chapter 3.4.1). Participants were allowed to drink water ad libitum throughout; total fluid ingested during the exercise protocol was recorded.

6.2.3 Statistical Analyses

All data were analysed using Statistica 9 (Statsoft, USA) and are presented as mean ± 1SD for 8 participants in the two elite supplementation groups and 10 participants in the non-elite groups except for heart rate data, which are presented for 8 participants in both groups due to heart rate monitor malfunction. Sprint data were filtered every set to remove any non-maximal sprint times; any sprint time more than two SD outside of the mean of the corresponding set were removed from the data. P plots and Cochran's Q were used to confirm normality and homogeneity of variance of the data. A three-way factorial ANOVA (Supplement x Trial x Time) was used to determine any difference in sprint times, blood lactate, heart rate and RPE. Fisher LSD tests were used for post-hoc analyses where appropriate and statistical significance was accepted at the $P \leq 0.05$ level.

6.3 **Results**

6.3.1 Elites

6.3.1.1. Sprint Times

There was no effect of supplementation on sprint performance across each set of the LIST (Supplement x Trial x Time, P = 0.99), or when taking into consideration all sprints (Supplement x Trial, P = 0.63). There was no main effect of time (P = 0.92) indicating that sprint times did not change significantly as the number of sprints performed increased (Figure 6.1).

6.3.1.2. Measurements

Blood lactate concentration and RPE were increased over time ($P \leq 0.001$), but not heart rate (P = 0.76) (Table 6.2), with no effect of supplementation on any of these variables. Body mass, as a percentage of pre-exercise body mass, was well maintained following exercise in both supplementation groups pre (Placebo: -0.8 ± 0.8%, β-alanine: -0.6 ± 0.8%) and post supplementation (Placebo: -0.9 ± 0.8%, β-alanine: -0.3 ± 0.5%) with no significant differences between trials (P = 0.49) or groups (P = 0.37).

6.3.2 Non-Elites

6.3.2.1. Sprint Times

There was no effect of supplementation on sprint times across each set of the LIST (Supplement x Trial x Time, P = 0.99) or when taking into consideration all sprints (Supplementation x Trial, P = 0.58). There was no main effect of time in the non-elites (P = 0.12) indicating that sprint times did not become significantly slower as the number of sprints performed increased (Figure 6.1).

6.3.2.2. Measurements

Lactate was increased from baseline following every set of the LIST ($P \leq 0.001$) (Table 6.2) in both groups prior to supplementation, with no effect of supplementation. Heart rate remained stable over the duration of the LIST with no effect of supplementation ($P = 0.19$) (Table 6.2). There was a time effect on RPE ($P \leq 0.001$), increasing throughout the LIST, although there was no effect of supplementation in either group. The level of dehydration was well controlled in both supplementation groups pre (Placebo: -1.0 ± 0.4%, β-alanine: -1.0 ± 0.6%) and post supplementation (Placebo: -1.3 ± 0.5%, β-alanine: -1.2 ± 0.6%) with no significant differences between trials ($P = 0.06$) or groups ($P = 0.8$).

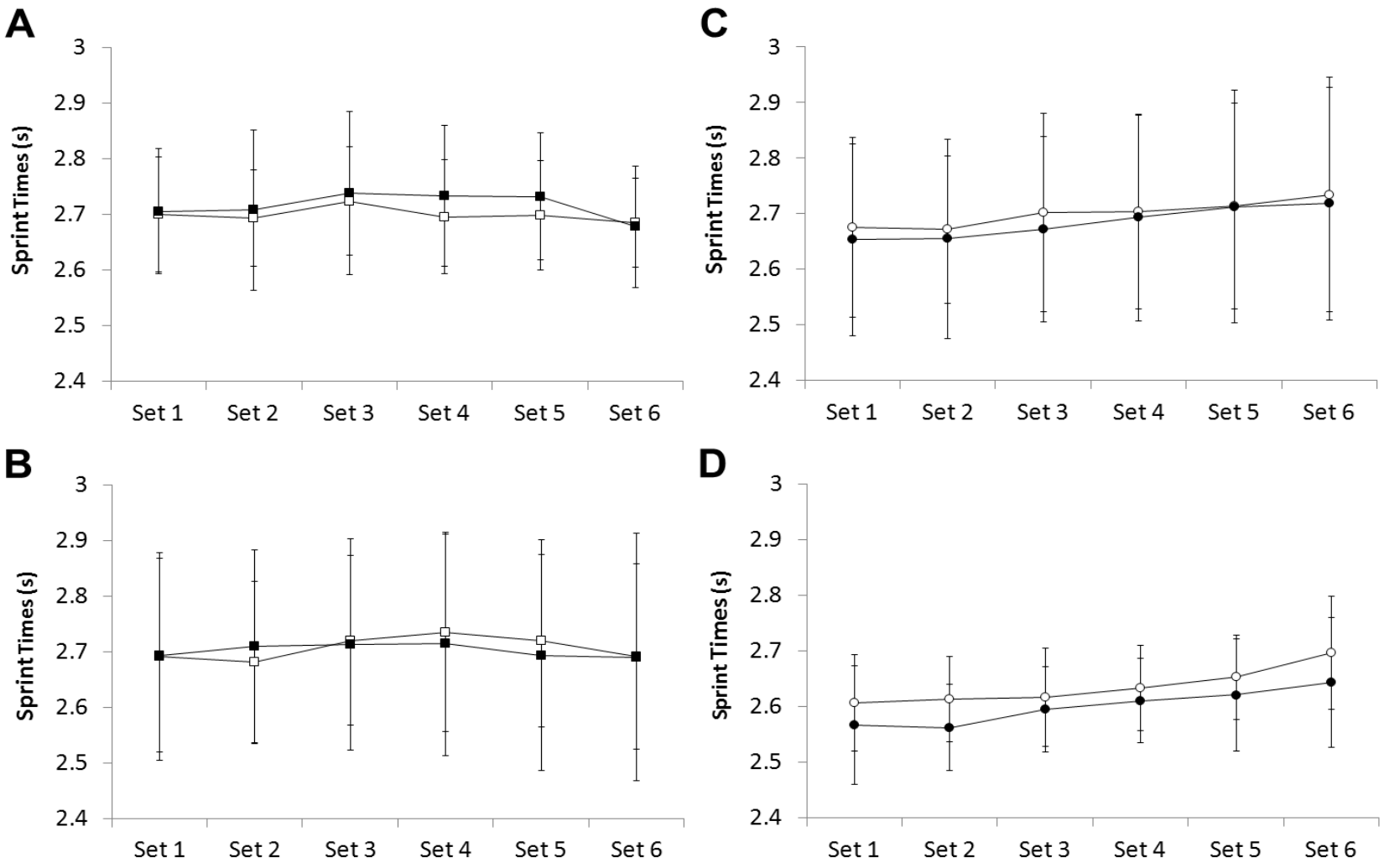

Figure 6.1 Sprint times during the LIST. Panels A and B display sprint times during the LIST for elite participants in the placebo (Panel A) and β-alanine (Panel B) groups both pre (white) and post (black) supplementation. Panels C and D display sprint times during the LIST for non-elite participants in the placebo (Panel C) and β-alanine (Panel D) groups both pre (white) and post (black) supplementation.

Table 6.2 Blood lactate, heart rate and RPE during the LIST for elite and non-elite participants. Pre-MD refers to Pre-supplementation, and Post-MD refers to Post-supplementation in the maltodextrin group. Pre-BA refers to Pre-supplementation, and Post-BA refers to Post-supplementation in the β-alanine group. $^{*}P \leq 0.05$ from baseline.

	Elite							Non-Elite						
	Baseline	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Baseline	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Lactate ($mmol \cdot L^{-1}$)														
Pre-MD	1.4 ± 0.5	2.7 ± 0.9*	2.5 ± 0.7*	2.7± 1.1*	2.7 ± 0.8*	3.2 ± 1.1*	2.9 ± 0.9*	1.8 ± 0.4	5.8 ± 2.2*	7.4 ± 3.4*	5.9 ± 2.6*	6.2 ± 2.7*	6.6 ± 2.7*	7.0 ± 3.0*
Post-MD	2.0 ± 1.0	3.0 ± 1.4	2.7 ± 1.0	3.0 ± 1.5	2.9 ± 1.2	2.8 ± 1.0	3.6 ± 1.3*	1.5 ± 0.4	5.3 ± 2.3*	6.1 ± 2.6*	6.1 ± 2.8*	5.8 ± 2.7*	6.2 ± 3.5*	6.2 ± 3.4*
Pre-BA	1.6 ± 0.7	3.3 ± 1.6*	2.7 ± 1.3	2.9 ± 1.7*	3.7 ± 1.3*	2.9 ± 0.7*	3.5 ± 1.5*	1.6 ± 0.6	6.2 ± 1.7*	5.7 ± 2.2*	5.1 ± 2.1*	5.8 ± 3.0*	4.5 ± 1.8*	4.9 ± 2.0*
Post-BA	1.4 ± 0.5	2.9 ± 1.1*	3.2 ± 1.6*	4.0 ± 1.7*	2.6 ± 0.9*	2.1 ± 0.7	2.5 ± 0.9	1.6 ± 0.5	6.1 ± 2.7*	5.3 ± 3.8*	4.5 ±3.0*	4.5 ± 2.1*	3.9 ± 2.1*	4.5 ± 2.6*
Heart Rate ($b \cdot min^{-1}$)														
Pre-MD	-	159 ± 8	163 ± 10	162 ± 11	161 ± 10	159 ± 8	159 ± 7	-	161 ± 11	166 ± 10	168 ± 9	170 ± 10	170 ± 10	170 ± 10
Post-MD	-	156 ± 10	160 ± 12	160 ± 13	158 ± 12	158 ± 13	160 ± 14	-	161 ± 11	166 ± 12	168 ± 9	168 ± 10	167 ± 10	169 ± 10
Pre-BA	-	155 ± 5	159 ± 6	157 ± 5	156 ± 5	157 ± 5	160 ± 5	-	167 ± 12	171 ± 13	172 ± 12	172 ± 13	171 ± 14	170 ± 10
Post-BA	-	152 ± 10	156 ± 11	155 ± 10	154 ± 10	154 ± 10	154 ± 10	-	161 ± 11	166 ± 12	166 ± 12	166 ± 11	167 ± 12	167 ± 10
RPE														
Pre-MD	-	11 ± 2	12 ± 1	14 ± 2*	15 ± 1*	16 ± 1*	17 ± 1*	-	14 ± 2	15 ± 1	16 ± 2*	17 ± 2*	18 ± 1*	19 ± 1*
Post-MD	-	10 ± 1	13 ± 1*	14 ± 2*	14 ± 2*	15 ± 2*	16 ± 2*	-	14 ± 2	15 ± 2	16 ± 2	16 ± 2*	17 ± 2*	18 ± 2*
Pre-BA	-	11 ± 2	12 ± 1	13 ± 1*	14 ± 1*	15 ± 1*	15 ± 1*	-	13 ± 1	14 ± 2*	15 ± 1*	16 ± 2*	16 ± 2*	18 ± 2*
Post-BA	-	11 ± 1	12 ± 2	13 ± 2	13 ± 3*	14 ± 2*	15 ± 2*	-	12 ± 2	13 ± 2	15 ± 2*	15 ± 2*	17 ± 2*	17 ± 2*

6.4 **Discussion**

This is the first study to investigate the effects of β-alanine supplementation on repeated sprint performance during prolonged intermittent activity simulating team sport games play. Contrary to the hypothesis, β-alanine did not have an effect on sprint performance during the LIST. The lack of a deterioration in sprint times during the LIST, in either group prior to supplementation might, however, have masked any effects of an increase in muscle buffering capacity brought about by elevated muscle carnosine content.

Balsom et al. (1992a) showed that 15 m sprint performance could be maintained over forty exercise bouts separated by 30 s rest, suggesting that changes in the intracellular environment for this type of exercise were not sufficient to induce fatigue. An increase in blood lactate concentration following the second sprint was, however, reported by Balsom et al. (1992a) suggesting that anaerobic glycolysis contributed to every sprint. In the present study, participants completed a total of 66 sprints, albeit with a different recovery profile than Balsom et al. (1992a), in addition to the increased demand of the additional intermittent activity between sprints. For this reason it was hypothesised that a greater decrement in performance would occur during our protocol as the result of H^+ accumulation and that, as a result, β-alanine supplementation would attenuate any decline in sprint performance. However, no significant decrement in sprint performance was observed in either group, and the blood lactate concentrations in the current investigation are similar to those reported by Balsom et al. (1992a). They are also lower (3 – 6 $mmol \cdot L^{-1}$) than previous repeated sprint activity studies that have shown a correlation to H^+ buffering capacity (>8 $mmol \cdot L^{-1}$; Bishop et al., 2003; 2004b). As such, the intensity of the LIST and the duration of the sprints may not

have been sufficient to induce reductions in performance due to a reduced muscle pH, which would then subsequently have been influenced by β-alanine supplementation.

In contrast to the present study, other studies using the LIST have shown a deterioration in sprint performance as the number of sprints performed increased in trained (Sunderland and Nevill, 2005) and youth (Phillips et al., 2010) games players. The decline in sprint times in those studies (>0.09 s) are higher than the maximum shown by the elites (+0.02 s) and non-elites (+0.09 s), which may explain some of the differences. McGregor et al. (1999) showed semi-professional footballers could maintain sprint performance throughout the LIST when fluid was administered, although sprint times worsened when fluid was restricted in the same population. In the present study, the elite population demonstrated inconsistent sprint times across the test including the ability to improve performance in the final set. Sprint times in the non-elites showed a more consistent, but still non-significant, decline in sprint times across the LIST, and, as such, may be a truer reflection of their inclination to perform each sprint maximally. The elite athletes may have adopted a pacing strategy to delay fatigue and optimise performance (Foster et al., 1994), thereby subconsciously affecting the performance outcome of the study.

The ability to perform repeated sprints has been associated with H^+ buffering capacity (Bishop et al., 2003 & 2004b; Bishop and Edge, 2006; Rampinini et al., 2009) as a large accumulation in intramuscular H^+ can negatively impact upon muscle function. Male and female games players of a high standard have increased H^+ buffering capacity compared to games players of a lower standard (Edge et al., 2006; Rampinini et al., 2009) and untrained females (Edge et al., 2006). In addition, higher levels of muscle carnosine have been shown

in runners, rowers (Parkhouse et al., 1985) and bodybuilders (Tallon et al., 2005) than in their endurance trained or untrained counterparts. Although muscle carnosine concentration was not directly determined in this study, it can be hypothesised that baseline carnosine concentration was higher in the elite versus the non-elite population. Nonetheless, β-alanine supplementation has been shown to significantly increase muscle carnosine concentrations in trained sprinters (Derave et al., 2007). The dose of β-alanine used in the present study was higher than that employed by Derave et al. (2007) and has consistently been shown to increase muscle carnosine, with all individuals showing a response to supplementation (for a review see Sale et al., 2010). Therefore, a lack of response to β-alanine supplementation in terms of elevated muscle carnosine concentrations is unlikely to explain the lack of an effect on sprint performance in this study.

Suzuki et al. (2002) showed a positive correlation between muscle carnosine content and power output during the last two 5 s periods of a 30 s maximal cycling bout, although the area occupied by type II muscle fibres is likely to have been of more importance in their study. Indeed, Hill et al. (2007, unpublished thesis) showed no effect of β-alanine supplementation on mean power output, peak power output or fatigue index during three repeated 30 s maximal sprint cycles. It has been suggested that muscle buffer capacity does not affect performance during exercise less than 60 s in duration (Bogdanis et al., 1998) and would therefore be unaffected by increased levels of muscle carnosine brought about by β-alanine supplementation, a suggestion supported by a meta-analysis of the literature by Hobson et al. (2012). Nonetheless, an increased RSA, consisting of repeated 6 s maximal bouts every 30 s, has been shown to be positively correlated to H^+ buffering capacity (Bishop

et al., 2003 & 2004b), which suggests that repeated short duration exercise bouts could well be affected by β-alanine supplementation.

Previous studies have examined the effect of β-alanine supplementation on isolated repeated sprint performance (Hoffman et al, 2008; Sweeney et al., 2011), although not as part of simulated game activity as in the present study. Hoffman et al. (2008) showed no effect of β-alanine supplementation on repeated 200 yard line drills in collegiate football players, although they only compared differences between groups since no baseline measurements were taken. Similarly, Sweeney et al. (2011) showed no effect of β-alanine supplementation on two sets of repeated 5 x 5 s sprints, although the short recovery period (45 s) between sprints may not have been enough to restore PCr to initial levels (Bogdanis et al., 1996), and may have contributed more to fatigue than reduced pHi. Due to the increased period between sprints and additional metabolic demand of the active recovery, it was hypothesised that the LIST would be a more suitable repeated sprint protocol likely affected by large accumulations of H^+. Furthermore, the ecological validity of the LIST (Nicholas et al., 2000) makes it a more suitable protocol to investigate the effect of β-alanine supplementation on team sport performance. Similar to previous studies however, β-alanine supplementation did not have an effect on performance lasting less than 60 s in duration.

6.5 **Conclusions**

The ingestion of 6.4 $g \cdot d^{-1}$ β-alanine over 4 weeks did not improve repeated sprint performance during simulated games play. The lack of a significant finding may be due to the lack of deterioration in performance, in both groups, prior to supplementation, which might have masked any effect of increased muscle carnosine content.

Chapter 7.0 Effect of β-alanine supplementation on YoYo Intermittent Recovery Test Level 2 performance

7.1 **Introduction**

Reported in Chapter 6, 15 m repeated sprint performance during the LIST was unaffected by four weeks of β-alanine supplementation. The lack of a deterioration in sprint times, in either group, prior to supplementation might, however, have masked any effects of an increase in muscle buffering capacity brought about by elevated muscle carnosine content. Furthermore, repeated sprint performance was unaffected during a football-specific treadmill protocol performed at simulated altitude. However, these laboratory based protocols measure high-intensity exercise performance less than 60 s in duration; in a meta-analysis of the literature, Hobson et al. (2012) showed that β-alanine was most effective in improving exercise capacity during exercise lasting in excess of 60 s. Therefore, β-alanine supplementation may be more effective in increasing sport specific high-intensity intermittent exercise capacity.

The YoYo Intermittent Recovery Tests (Level 1 [YoYo IR1] and 2 [YoYo IR2]) were designed (Bangsbo, 1994b) to evaluate the ability of an individual to repeatedly perform and recover from intense exercise, and is applicable to team sports players due to the specificity of the exercise undertaken (Bangsbo et al., 2008). These tests have been shown to be sensitive to training adaptations (Krustrup et al., 2006b; Mohr et al., 2007), seasonal variation (Krustrup et al., 2003 & 2006b) and differences in playing position and playing standard (Mohr et al., 2003; Krustrup et al., 2006b). Furthermore, YoYo IR Test performance is closely related to football match performance. YoYo IR1 performance was correlated to high intensity running and total distance covered during a football match for both top class referees (Krustrup and Bangsbo, 2001) and footballers (Krustrup et al., 2003). The highest distance covered in a 5 min period during a game was associated with YoYo IR2 performance (Bangsbo et al., 2008). These findings suggest that the YoYo IR Tests are

appropriate models for examining the effects of interventions designed to manipulate changes in individual performance during team sport exercise.

Football is a sport that requires players to perform substantial high-intensity running with a large contribution from both aerobic and anaerobic energy pathways. The YoYo IR2 best evaluates an individual's capacity to perform repeated high-intensity exercise while simultaneously stimulating both aerobic and anaerobic energy systems (Krustrup et al., 2006b). At volitional exhaustion, muscle lactate and glycogen utilisation are higher, and muscle pH is lower following the YoYo IR2 compared to the YoYo IR1 test (Bangsbo et al., 2008), suggesting a larger activation of the anaerobic energy system towards the end of the YoYo IR2. Interestingly, muscle pH was significantly decreased (and muscle lactate increased) at exhaustion compared with at 85% exhaustion time, while muscle phosphorylcreatine and glycogen were not (Krustrup et al., 2006b). This indicates that decreased muscle pH may be a significant contributing factor to fatigue during the YoYo IR2, suggesting that the YoYo IR2 is a suitable model to investigate the effect of increased muscle buffering capacity on team sport specific fitness.

No study has examined the effects of supplementation on team sport specific exercise capacity. Therefore, the aim of this investigation was to examine the effect of β-alanine supplementation on YoYo IR2 performance in well-trained amateur footballers throughout a competitive season. It was hypothesised that β-alanine would significantly improve the distance covered during the test due to an increase in intracellular pH buffering as the result of muscle carnosine elevation.

7.2 **Methods**

7.2.1 Participants

Seventeen amateur male footballers (age 22 ± 4 y, height 1.83 ± 0.06 m, body mass 76.9 ± 6.6 kg) from the same club competing in the lower divisions of the English football pyramid volunteered for the study and were randomly allocated to either a placebo (PLA) or β-alanine (BA) group. All players were members of the same team and were engaged in an identical team sport specific training regime over the season. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

7.2.2 Experimental Design

All tests were performed indoor on an artificial running track in ambient conditions (temperature 21.0 ± 0.7ºC, relative humidity 52.4 ± 0.8%). Every participant had performed the YoYo IR2 on a minimum of two previous occasions, and were aware of the requirements of the protocol. Participants were requested to attend the sports hall to perform the YoYo IR2 (Chapter 3.3.8) on two separate occasions during the season, separated by 12 weeks of supplementation. Participants maintained a food diary in the 24 h period before the first main trial, and this was subsequently used to replicate the diet prior to the second main trial.

Participants were randomly allocated to a supplementation group, and were supplemented with either 3.2 $g \cdot d^{-1}$ of β-alanine (CarnoSyn^{TM}, NAI, USA) or placebo (maltodextrin; NAI, USA) in tablet form over a 12 week period. Players were supplemented from early to mid-season (PLA: N = 5; BA: N = 6) or mid- to the end of the season (PLA: N = 3; BA: N = 3). The dosing regimen consisted of one 800 mg β-alanine or placebo tablet ingested four times

per day at 3 – 4 h intervals. Compliance with the supplementation regimen was monitored using supplementation logs, with a high degree of compliance being reported in both groups (PLA: 89%, total of 238.0 ± 21.4 g maltodextrin; BA: 95%, total of 243.2 ± 13.7 g β-alanine). There were no reports of symptoms of paraesthesia from any of the participants in either group.

7.2.3 Statistical Analyses

All data were analysed using Statistica 9 (Statsoft, USA) and are presented as mean ± 1SD. A two factor ANOVA (Group x Trial) was used to determine any differences in YoYo performance. Tukey tests were used for post-hoc analyses and effect sizes were calculated using Cohen's d. Statistical significance was accepted at the $P \leq 0.05$ level.

7.3 **Results**

There was no significant difference in distance covered during the YoYo IR2 ($P = 0.83$; PLA: 1185 ± 216 m and BA: 1093 ± 148 m, $d = 0.54$) between PLA and BA prior to the supplementation periods. There was a significant interaction effect (Group x Trial, $P \leq 0.001$), with no difference for PLA (-7.6 ± 16.2%; *post hoc* $P = 0.62$, $d = 0.43$) and a significant improvement for BA (+34.3 ± 22.5%; *post hoc* $P \leq 0.001$, $d = 1.83$) following supplementation (Figure 7.1).

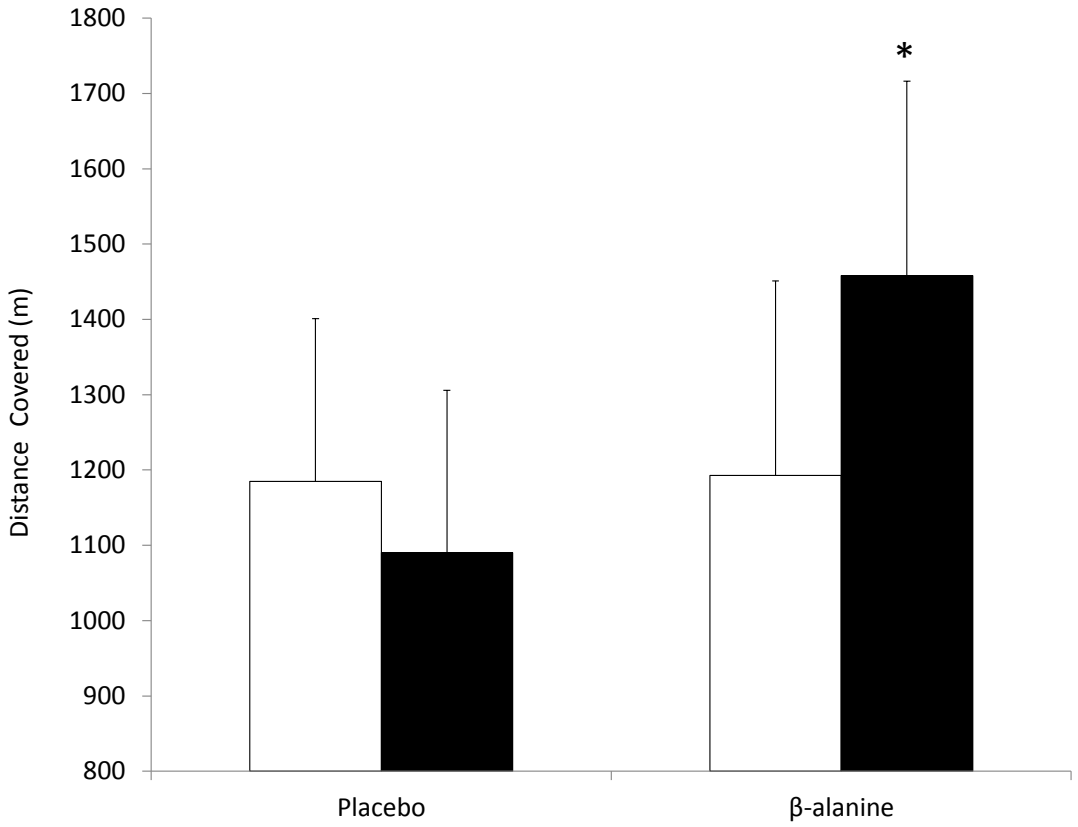

Figure 7.1 Distance covered during the YoYo IR2 for both supplementation groups pre (white bars) and post (black bars) supplementation. *P ≤ 0.001 from pre supplementation.

Performance changes ranged from -37.5 to + 14.7% in PLA, and +0.0 to +72.7% in BA. In total, 2 of the 8 players in PLA showed an improvement in performance, with the remaining participants having a reduction in performance from -40 to -480 m. In comparison, 8 out of 9 players showed improvement in BA (+160 to +640 m), with the remaining player in BA unchanged (Figure 7.2). Subject 17 in the BA group showed an unusually high increase in YoYo IR2 performance (+72.7%) given that the response usually shown in response to pre-season training is 42%. Due to this, participant 17 was removed and the data reanalysed, which did not change any of the study outcomes (Group x Trial, P = 0.001; BA: +29.4 ± 18.4%, *post hoc* P = 0.003).

In the group of players supplemented from early to mid-season, 2 out of 5 in PLA and 6 out of 6 in BA group improved YoYo IR2 performance. Of the remaining players supplemented from mid until the end of season, no one in PLA showed an improvement while 2 out of 3 in BA improved their distance covered.

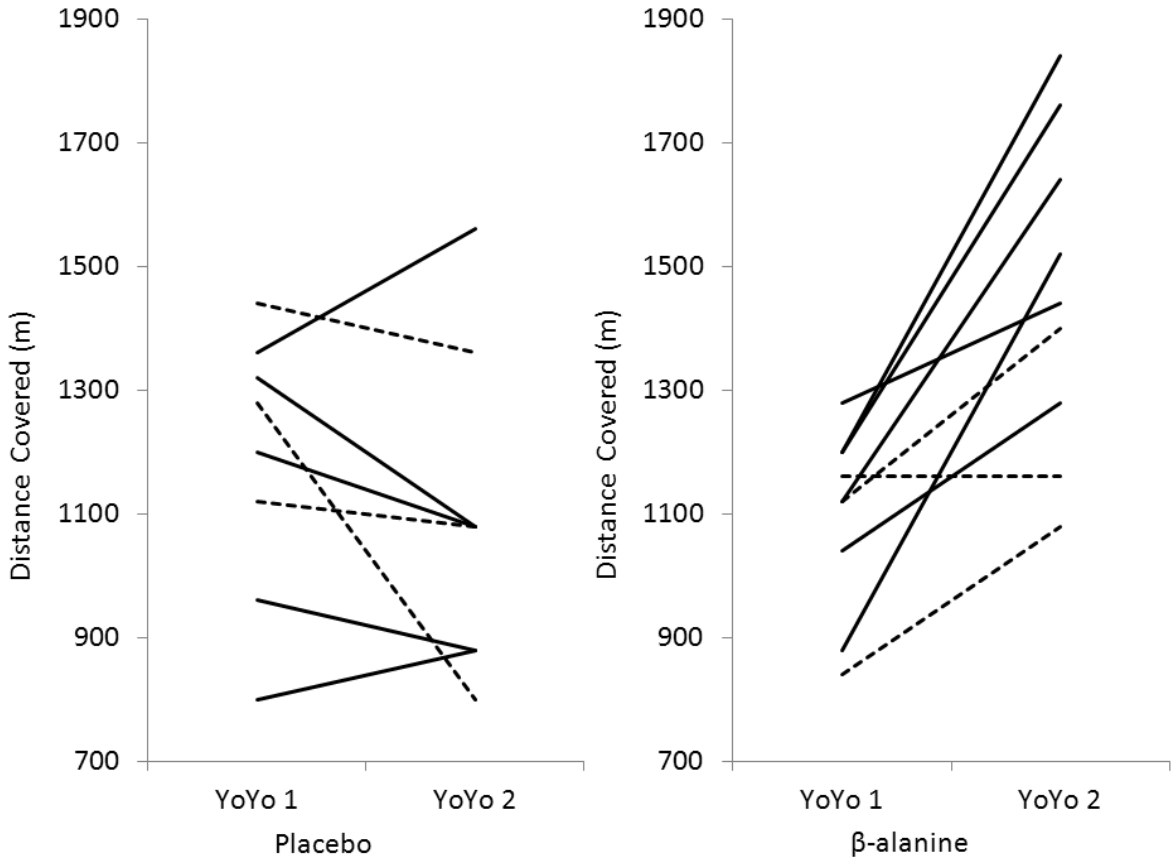

Figure 7.2 Individual response to supplementation in the placebo and β-alanine groups pre (YoYo 1) and post (YoYo 2) supplementation. Players supplemented from early to mid-season are indicated by a solid line and players supplemented from mid- to the end of the season are indicated by a dotted line.

7.4 **Discussion**

There was a clear effect of 12 weeks of β-alanine supplementation on the distance covered during the YoYo IR2 test. This is in contrast to previous research that has shown no effect of β-alanine on repeated sprint exercise (Hoffman et al, 2008; Sweeney et al., 2011; data

reported in Chapter 6), although these studies used exercise protocols consisting of performance tests incorporating periods of high-intensity and sprint activity of less than 60 s in duration, which are suggested to be unaffected by β-alanine supplementation (Hobson et al., 2012). The YoYo IR2 is an exercise capacity test designed to last between 5 and 15 minutes and aims to evaluate an individual's ability to perform repeated bouts of high-intensity exercise with a large contribution from anaerobic energy sources. Furthermore, distance covered during the YoYo IR2 has been associated with high-intensity running performed during competitive games play (Krustrup et al., 2003; Bansgbo et al., 2008). Therefore, the results of the present investigation suggest that β-alanine supplementation is effective at improving team sport specific exercise capacity.

Blood measures were not taken in the current investigation, although others have reported lactate concentrations in excess of 10 $mmol \cdot L^{-1}$ at exhaustion (Krustrup et al., 2006b), which is higher than the concentrations shown in repeated sprint activity studies that have shown a correlation to H^+ buffering capacity (~8 $mmol \cdot L^{-1}$; Bishop et al., 2003; 2004b). Although the rate of muscle phosphorylcreatine and glycogen utilisation are high during the YoYo IR2 (Krustrup et al., 2006b), there is no difference in muscle concentrations of these substrates between 85% and 100% of exhaustion time, indicating that depletion of these substrates is not a main contributing factor to fatigue. Interestingly, muscle pH was significantly lower at exhaustion compared with at 85% of exhaustion time (Krustrup et al., 2006b), which suggests increasing muscle acidity is a limiting factor to YoYo IR2 performance. Although muscle carnosine concentrations were not directly determined in this study, Stellingwerff et al. (2012) showed that as little as two weeks of β-alanine supplementation at half the dose used in the current study was sufficient to increase muscle carnosine by 11.8 ± 7.4% in the *tibialis*

anterior. Therefore, it can be hypothesised that 12 weeks of β-alanine supplementation at 3.2 $g \cdot d^{-1}$ significantly increased muscle carnosine concentrations in the current population. As such, since one of the undisputed roles of muscle carnosine is in muscle buffering, the most likely explanation for the improvement in YoYo IR2 performance is due to an increase in intracellular buffering capacity, resulting in an attenuation of the reduction in intracellular pH during high-intensity exercise.

The YoYo IR2 has been shown to be a highly reproducible capacity test, with a CV of ~10% for two tests performed within a one week period (Krustrup et al., 2006b). In addition, the test is sensitive to detect training adaptions, with performance improvements of approximately 42% shown following pre-season training. In the present investigation, players in the placebo group showed a ~7% decline in performance while β-alanine supplementation improved YoYo IR2 performance by ~34%, which compares favourably with the effects of pre-season training, and exceeds the expected CV of the test (Krustrup et al., 2006b). Furthermore, all 8 of the players who improved with β-alanine did so above this expected CV, while the placebo group showed more variation with 3 players exceeding the CV (1 improved and 2 decreased their performance), which suggests that performance improvements in the β-alanine group can be attributed to the nutritional intervention employed in the current investigation. In addition, 4 of the players in the β-alanine group improved above the highest improvements shown following a 6 – 8 week training period (+45%; Bangsbo et al., 2008). Since all players were involved in an identical training structure throughout the supplementation period, the further increases in these participants could be attributed to an increased ability to train due to increased muscle buffering capacity (Hoffman et al., 2008), providing an additive effect over supplementation alone.

Footballers were supplemented during a competitive season as the YoYo has been shown to be sensitive to seasonal variation (CV: 14%; Krustrup et al., 2006b) with scores, on average, lower during the season than at the start. Although mid-season scores were not different from the start of the season for First Division Scandinavian footballers, YoYo IR2 performance was decreased at the end of the season compared to the start of the season in another group of First and Second division players (Krustrup et al., 2006b). Furthermore, only 4 out of 15 players improved their YoYo IR2 performance during the season, while a further 9 showed a performance decrement (Krustrup et al., 2006b). In the present investigation, performance for players in the placebo group supplemented from early to midseason followed a similar pattern to this, and all 3 supplemented from the middle until the end of the season showed a decline in performance. In contrast, all players supplemented with β-alanine from early- to mid-season improved their YoYo scores, while 2 of the 3 supplemented from mid-season until the end of the season showed a performance improvement, with the remaining player unchanged. These data provide evidence to suggest that β-alanine supplementation can not only halt the decline in YoYo IR2 performance shown during a competitive season (Krustrup et al., 2006b), but may even improve them above typical levels.

7.5 **Conclusions**

The ingestion of 3.2 $g \cdot d^{-1}$ β-alanine over 12 weeks improved YoYo IR2 performance in amateur footballers during a competitive season. Improvements can be attributed to an increase in muscle buffering capacity due to increased muscle carnosine concentration, attenuating the decline in pHi during repeated high-intensity exercise bouts.

Chapter 8.0 High-intensity and sprint activity during competitive football match play: Effect of β-alanine supplementation

8.1 **Introduction**

Reported in the previous section, YoYo IR2 performance was improved during a competitive season with β-alanine supplementation, which suggests that football specific exercise capacity can be improved with prolonged β-alanine supplementation throughout a competitive season. Since performance in the YoYo IR tests has been positively associated with distance covered at high-intensity during competitive match play (Krustrup et al., 2003; Bangsbo et al., 2008), it can be hypothesised that this match variable may also be positively influenced by supplementation with β-alanine.

Team sports such as football are comprised of periods of high-intensity running (>15 $km \cdot h^{-1}$; Abt and Lovell, 2009) interspersed with low intensity activity and rest. The total distance covered at high-intensity during match play has been shown to be higher in high-standard players and successful teams in comparison to comparatively lower standard players and unsuccessful teams (Mohr et al., 2003; Bradley et al., 2009), which suggests that high-intensity activity is an appropriate measure of match performance. It also appears to be an appropriate measure of fatigue during a match since high-intensity running has been shown to be decreased in the second half compared to the first half of matches (Bradley et al., 2009). Therefore, high-intensity activity during match play may be a suitable measure to determine any changes in team sport performance.

This thesis has reported on the effect of β-alanine supplementation on repeated sprint performance during high-intensity intermittent exercise protocols designed to replicate the demands of competitive match play. Laboratory based studies, while providing a controllable environment to obtain physiological and performance data, lack external validity as they fail

to incorporate several factors including match-to-match variability, competition level and player position. Therefore, the aim of this investigation was to examine the effect of β-alanine supplementation on high-intensity and sprint activity during match play in amateur footballers throughout a competitive season. It was hypothesised that players would perform more high-intensity and sprint activity, and show less of a decline in distance covered in these locomotion categories from the 1st to 2nd half, during games play when supplemented with β-alanine.

8.2 **Methods**

8.2.1 Participants

Twenty-three football players playing for the same amateur football team volunteered for the study. Due to several dropouts and transfers across the season, only data from seventeen players (age 21 ± 1 y, height 1.82 ± 0.05 m, body mass 76.5 ± 4.2 kg) was considered suitable for analysis. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent (Chapter 3.1).

8.2.2 Experimental Design

Match analysis was carried out during 52 competitive matches throughout the 2009 – 2010 campaign, and participants were monitored for high-intensity and sprint activity using individual portable GPS systems (Chapter 3.3.9). Only full match (90 min) data files were analysed; the decision to start and substitute players were made at the discretion of the team's manager and were not influenced by the investigator, resulting in 236 individual player match analyses. The team played a 4-4-2 formation, with playing positions for each match

categorised as centre backs (CB), full backs (FB), central midfielders (CM), wingers (W) and forwards (F). High-intensity was defined as >15 $km \cdot h^{-1}$ (Abt and Lovell, 2009) and sprinting as >21 $km \cdot h^{-1}$.

The season was split into three sections and participants were randomly allocated into one of three supplementation groups (Table 8.1). Following an initial baseline period, participants were supplemented with either 3.2 $g \cdot d^{-1}$ of β-alanine or placebo in tablet form over the remainder of the football season. The dosing regimen consisted of one 800 mg β-alanine or placebo tablet ingested four times per day at 3 – 4 h intervals.

Table 8.1 Supplementation design.

	Baseline (Week 1 - 16)	**Supplementation 1 (Week 16-28)**	**Supplementation 2 (Week 28 – 39)**
Group 1 (N = 6)	-	Placebo	Placebo
Group 2 (N = 5)	-	Placebo	β-Alanine
Group 3 (N = 6)	-	β-Alanine	β-Alanine

8.2.3 Statistical Analyses

All data were analysed using MLwiN (v. 2.25, Bristol, UK) and are presented as mean ± 1SD. To explain the variation in match activities, an additive multilevel model (Goldstein et al., 1994) was used to examine potential contributing factors including number of games played, player position and supplementation. All parameters were fixed except the constant, which was allowed to vary randomly at level 1 (repeated measures) and level 2 (individual).

8.3 **Results**

8.3.1 High-Intensity Running

Players covered a total of 1631.1 ± 497.0 m (range: 670.4 – 2739.0 m) at high-intensity speeds during match play, covering 818.1 ± 261.8 m in the 1st half and 813.0 ± 282.2 m in the 2nd half. Multilevel analysis revealed that the variance in distance covered at high-intensity was significantly influenced by position, but not any other explanatory variable including group, supplement or days on active supplement (Table 8.2). The decline in distance covered from the 1st to 2nd half (-5.2 ± 222.0 m) was not influenced by any explanatory variable.

Table 8.2 Multilevel analysis of total distance covered at high-intensity (m) during competitive match play throughout a season. *P ≤ 0.05.

Fixed Explanatory Variables		**Parameter Estimate (SE)**
Constant		1319.4 (96.4)
Position (vs. CB)	FB	+ 411.2 (81.6)*
	CM	+ 292.7 (98.7)*
	W	+ 773.2 (117.7)*
	F	+ 487.9 (113.8)*
β-alanine (vs. placebo)		- 2.9 (85.7)
Days on active supplement		+ 0.4 (1.0)
Random Variance		
Level 2 (Individual)		68775.8 (26113.8)
Level 1 (Repeated Measures)		82436.5 (7895.3)
-2*loglikelihood (IGLS Deviance)		3368.3

8.3.2 Sprinting

Players covered a total of 306.1 ± 164.4 m (range: 35.2 – 776.8 m) sprinting during match play, covering 148.1 ± 95.8 m in the 1st half and 158.0 ± 87.8 in the 2nd half. Multilevel analysis revealed that the variance in distance covered while sprinting was significantly influenced by position, but not any other explanatory variable, including group, supplement or days on active supplement (Table 8.3). Similarly, the variance in the decline in distance covered sprinting from the 1st to 2nd half was influenced by position, but no other variable.

Table 8.3 Multilevel analysis of total distance covered sprinting (m) during competitive match play throughout a season. *P ≤ 0.05.

Fixed Explanatory Variables		**Parameter Estimate (SE)**
Constant		238.4 (30.7)
Position (vs. CB)	FB	+ 100.2 (29.0)*
	CM	- 10.3 (32.4)
	W	+ 242.7 (37.3)*
	F	+ 180.8 (37.2)*
β-alanine (vs. placebo)		+ 21.6 (24.3)
Days on active supplement		- 0.1 (0.1)
Random Variance		
Level 2 (Individual)		4042.4 (1721.4)
Level 1 (Repeated Measures)		11235.0 (1075.7)
-2*loglikelihood (IGLS Deviance)		2887.7

8.4 **Discussion**

This study investigated the effect of β-alanine supplementation on high-intensity and sprint activity during competitive match play during a season in amateur footballers. The variability in distance covered in these locomotion categories were not influenced by β-alanine supplementation, although positional differences were a significant contributing factor.

Although high-intensity exercise accounts for approximately 10% of all match activities (Bangsbo et al., 1991), it has been shown to be a suitable measure of team sport performance (Mohr et al., 2003; Bradley et al., 2009). Furthermore, activity of a high-intensity will result in the largest accumulations of H^+, and will likely be most affected by increases in muscle buffering capacity. Gregson et al. (2010) showed that total distance covered at high-intensity varied on average by ~18% and total sprint distance by ~31%, which suggests that large sample sizes are required to detect systematic changes in performance characteristics. However, the data analysed by Gregson et al. (2010) comprised match observations from players from different teams over a number of seasons, which may have contributed to the large variation in results due to differences in team formations and tactics. The current study used players from a solitary team that employed the same formation (4-4-2) over the entire duration of one season with the aim of minimising any variation due to these factors. Despite this, variability between individuals and repeated measures variability in high-intensity and sprint activity was high, and may have been too large to detect any changes with β-alanine. Although high-intensity and sprint activity was not shown to be influenced by β-alanine supplementation, variability in these performance measures may have been too high to detect any changes due to supplementation.

This study aimed to investigate the effects of β-alanine in an actual performance setting, monitoring amateur footballers during competitive match play over the duration of a season. Total distance covered at high-intensity has been shown to be dependent on player position (DiSalvo et al., 2009), with wide and attacking players covering more distance at these high speeds than their central playing counterparts. Indeed, in the current study, player position was shown to be a significant contributing factor towards the variation in high-intensity running and sprint activity. Laboratory based protocols that simulate games play (Nicholas et al., 2000; Greig et al., 2006) standardise the amount of running performed at all intensities, thereby eliminating the bias of positional differences. However, due to the applied nature of this study, control is lost over several factors including player position, with a number of players playing in multiple positions throughout the season, which will have influenced the amount of high-intensity running and sprinting performed from match-to-match. Although no effect of supplementation was shown when positional differences were accounted for, the lack of an effect due to positional variation of individuals throughout the season cannot be dismissed.

Large variations in muscle and blood lactate concentration during games play are indicative of the intermittent nature of competitive match play, with increased lactate concentrations suggesting a high rate of glycolysis is required for periods during a match. Concomitantly, muscle H^+ can double during the most intense periods of match play (Krustrup et al., 2006a), with muscle pH dropping to as low as 6.96, which could impair muscle function and contribute to fatigue (Fabiato and Fabiato, 1978; Spriet et al., 1989). Players supplemented with β-alanine would likely be able to better maintain muscle pH due to increased carnosine concentrations, potentially allowing them to perform more high-intensity activity, though this

was not shown in the present study. Krustrup et al. (2006a) showed that, despite the decline in muscle pH, this was not correlated to the decrement in repeated sprints during actual match play, which suggests low muscle pH may not contribute to fatigue during games play. However, Krustrup et al. (2006a) only monitored eleven players across three games, which may not have been sufficient to show an effect due to the variability of match play. Furthermore, in the current study, the footballers used were of an amateur standard, who have been shown to perform less high-intensity running and sprinting than their comparatively higher standard counterparts (Mohr et al., 2003). Subsequently, a larger increase in H^+ due to a higher volume of high-intensity activity can be expected in professional and elite footballers, perhaps making them more susceptible to improvements with increased muscle buffering capacity due to β-alanine supplementation.

The conception of this study developed from the lack of research into the prolonged supplementation of β-alanine, and supplementation on an applied exercise measure. Only one study has supplemented participants for a period of more than 10 weeks (Hoffman et al., 2008), but this was performed on elderly participants at a low dose (2.4 $g \cdot d^{-1}$ for 90 days). Several studies exist on the effect of β-alanine on applied exercise (Derave et al., 2007; van Thienen et al., 2009; Baguet et al., 2010), although none of these investigated team sports performance. Although this study aimed to fill the void in the current literature, there remain several limitations which should be considered in future investigations that will serve to enhance the quality of the research undertaken. Twenty-three players were allocated into three supplementation groups in order to identify the effect of β-alanine, although a maximum of six players in any group remained. Furthermore, although two weeks has been shown to be sufficient to increase muscle carnosine (Stellingwerff et al., 2012), the

corresponding gains in buffering capacity, and their contribution to improved performance, have not yet been quantified. Although the number of days on active supplement was accounted for in the analysis, the loading phase of supplementation with β-alanine means that a limited number of matches remained that took place after a period of time sufficient to have increased muscle buffering capacity to theoretically ergogenic levels. Therefore, larger sample sizes are needed which, in the current study, could have been done by eliminating Group 2, leaving only a β-alanine and a placebo group.

8.5 **Conclusions**

High-intensity and sprint activity performed by amateur footballers during competitive match play was unaffected by β-alanine supplementation. The lack of an effect may have been due to the large variability in these performance characteristics, which is likely to be greater than any potential increases in performance due to β-alanine supplementation.

Chapter 9.0 General Discussion

9.1 **Overview of the Key Findings**

- The $CCT_{110\%}$ was shown to be a reliable test for the determination of high-intensity cycling capacity.
- High intensity cycling capacity was only improved with sodium bicarbonate when participants experiencing GI discomfort were removed from the data.
- β-alanine supplementation improved high-intensity cycling capacity during the $CCT_{110\%}$. Co-ingestion of β-alanine and sodium bicarbonate did not confer any further significant benefit to exercise capacity, although magnitude based inferences suggested a ~70% probability that the additive effect was meaningful.
- 5 x 6 s repeated sprint performance throughout team sport specific exercise at simulated 2500 m altitude was unaffected by sodium bicarbonate, β-alanine or co-supplementation of the two.
- 15 m sprint performance throughout the LIST was unaffected by β-alanine supplementation for both elite and non-elite games players.
- YoYo IR2 was improved following 12 weeks β-alanine supplementation during a competitive football season.
- The amount of high-intensity and sprint activity performed during competitive match play was not influenced by β-alanine supplementation, although variability of the data may have been too large to detect any changes.

9.2 **Reliability and Validity**

This aim of this thesis was to investigate the separate and combined effects of two buffering agents on high-intensity exercise performance and capacity using various exercise modalities, progressing from single bout high-intensity exercise to high-intensity intermittent exercise.

An exercise test should be both reliable and valid in order to interpret the meaningfulness of the data (Atkinson and Nevill, 1998); reliability allows the determination of whether differences are due to the intervention or are simply due to the natural variation of the test, and the validity reflects the ability of the test to measure exactly what it is required to measure.

The $CCT_{110\%}$ was chosen as a suitable single bout high-intensity cycling capacity test to investigate the effects of β-alanine and sodium bicarbonate supplementation, both separately and in combination, due to its association with increasing acidosis (Hill et al., 2007). The results reported in Chapter 4A showed the $CCT_{110\%}$ to be a highly reliable protocol, with CVs less than 5% for both TTE and TWD, which allows the improvements in exercise capacity to be contextualised with regard to the natural variation of the test.

The remaining exercise tests were chosen due to their relevance to team sports. Greig et al. (2006) based their intermittent treadmill protocol on notional match analysis from Bangsbo (1994a), and the 5 x 6 s repeated sprint protocol has previously been associated with the most intense period during a team sports game (Dawson et al., 1997) and the decrement in 15 m sprint time during a simulated game (Bishop et al., 2001). A reliability study reported in Chapter 5A showed the sprint protocol to be a highly repeatable test (CV: ~4%) when performed ~45 min and 48 h apart. The LIST is a prolonged intermittent running protocol designed to simulate the demands of match play, and has been shown to be a valid and reliable protocol (Nicholas et al., 2000). Furthermore, the 15 m sprints performed during the LIST represents the length of a typical sprint performed during actual games play (Spencer et al., 2005), giving further credence to the test as a tool to investigate the effects of an

intervention on team sport performance. The YoYo IR2 is a reliable (Krustrup et al., 2006b) test of team sport fitness and is applicable to team sports due to the specificity of the exercise undertaken (Bangsbo et al., 2008).

Despite the high reliability and validity of the protocols used in this thesis, there is no substitution for the implementation of an intervention strategy in an applied setting, where the true effect of the supplement can be monitored. Therefore, players were monitored for high-intensity and sprint activity during actual competitive match play over an entire season with the use of individual GPS units worn by outfield players. The validity of these units for monitoring the types of speeds and movements performed by team sports players was assessed by MacLeod et al. (2009), who showed a 1-Hz non-differential GPS system is suitable for obtaining running data during games play.

The protocols and techniques used to assess changes in performance and capacity due to dietary interventions reported in this thesis are unique in that they have all been shown to have a degree of reliability and validity within their own respective areas. The effects of the two buffering agents, β-alanine and sodium bicarbonate, on exercise performance and capacity are equivocal (for reviews see Sale et al., 2010 and McNaughton et al., 2008), though contrasting results may be due to exercise protocols not limited by increasing acidosis. The $CCT_{110\%}$ had previously been associated with increasing acidosis (Hill et al., 2007), and was therefore considered an appropriate model to investigate the true effect of buffering agents once reliability of the test was determined. Following confirmation of the true effects of β-alanine and sodium bicarbonate supplementation using the $CCT_{110\%}$, it was then possible

to investigate their effects on different exercise modalities, where increasing acidosis might not be the only contributing factor towards fatigue.

9.3 **Supplementation Protocols**

Harris et al. (2006) were the first to report on the symptoms of paraesthesia associated with β-alanine supplementation, with participants describing an unpleasant prickly sensation on the skin around the body with increasing doses from 20 to 40 $mg \cdot kg^{-1}$BM of β-alanine. The likely mechanism for this sensation is the mas-related gene family of G protein coupled receptors, which are triggered by interactions with specific ligands, such as β-alanine (Crozier et al., 2007). Consequently, several studies have reported symptoms of paraesthesia within their sample population (Hill et al., 2007; Sweeney et al., 2010), which would compromise the double-blinded nature of the investigation. Early β-alanine supplementation studies used a maximum single dose of 800 mg administered up to 8 times a day to give a total dose of 6.4 $g \cdot d^{-1}$ (Harris et al., 2006; Hill et al., 2007) in order to prevent symptoms of paraesthesia. This thesis reports on the first β-alanine supplementation studies to employ a sustained release formulation (CarnoSynTM SR, from Natural Alternatives International, San Marcos, California, USA) enabling two 800 mg SR tablets to be given simultaneously without symptoms of paraesthesia. Significantly, no participant in any of the experimental chapters discussed in this thesis reported any symptoms of paraesthesia (N = 56), meaning the integrity of the blinding in the investigations was maintained and would not have influenced the results.

Similar to β-alanine, acute sodium bicarbonate supplementation is associated with side effects that can compromise the blinding of the supplement. Due to the acute nature of

sodium bicarbonate supplementation, associated discomfort may also contribute to a lack of an ergogenic effect. McNaughton (1992) reported increased GI disturbance in all participants consuming doses of 0.4 and 0.5 $g \cdot kg^{-1}$BM, despite no further increases in circulating levels of bicarbonate above that of 0.3 $g \cdot kg^{-1}$BM, suggesting this to be the optimal dose. In an attempt to maintain blinding of the supplement and to minimise the discomfort associated with supplementation at this level, the studies in this thesis reporting on sodium bicarbonate supplementation incorporated a total dose of 0.3 $g \cdot kg^{-1}$BM in opaque gelatine capsules using a split dose strategy (0.2 $g \cdot kg^{-1}$BM and 0.1 $g \cdot kg^{-1}$BM ingested 4 and 2 h prior to exercise). Increases in blood bicarbonate were shown in all participants prior to exercise and concentrations compare favourably to those reported previously using different supplementation strategies but an identical dose (Price et al., 2003; van Montfoort et al., 2004).

Several participants reported symptoms of GI discomfort, despite the split dose strategy, which may have contributed to the uncertainties surrounding the results of the sodium bicarbonate studies reported in this thesis. In the studies reported in chapters 4B and 4C, immediately prior to exercise, participants were asked to report any feelings of GI discomfort, rating them as mild or severe. Despite the split dose ingestion protocol, four and five participants reported symptoms of GI discomfort, which suggests it is not always possible to reduce symptoms of discomfort. Nonetheless, this prompted a different approach in subsequent studies to quantify the amount of discomfort experienced by each individual. In the studies reported in chapters 5A and 5B, participants were asked to rate their intensity of stomach ache, sickness and headache on a 10 point scale from 0 to 10 on four occasions during each main trial. These were based on the scales used by van Montfoort et al. (2004),

and reports of these symptoms were low across both studies, with only the incidence of headache higher during the sodium bicarbonate trial reported in Chapter 5C.

The ingestion protocols reported in this thesis are a unique approach to the conventional procedures associated with β-alanine and sodium bicarbonate supplementation, and were designed to avoid any discomfort experienced by the participants with supplementation of this nature, and to maintain full blinding of the supplements. This was maintained with β-alanine, and research has shown that the slow release formulation improves whole body retention of β-alanine (Décombaz et al., 2012), which suggests that carnosine levels will have been elevated in all participants (Harris et al., 2006; Stellingwerff et al., 2012). There were several incidences of GI discomfort with sodium bicarbonate, which may have contributed to the contrasting results. Nonetheless, blood markers suggest that the dosing strategy was successful in inducing alkalosis in all participants prior to exercise. Therefore, these two supplementation strategies can be used in future research into the effects of β-alanine and sodium bicarbonate supplementation.

9.4 **β-alanine Supplementation and High-Intensity Exercise Capacity and Performance**

Chapter 4C reported on the effect of β-alanine supplementation on high-intensity cycling capacity using an exercise test designed by Hill et al. (2007), who had previously shown TWD to be improved by 13.0% following 4 weeks supplementation with β-alanine. TWD was improved by 14.6%, likely due to an increased muscle buffering capacity due to increases in muscle carnosine, delaying the decrement in muscle pH. The slightly increased improvement in TWD seen in the study reported in this thesis could be attributed to a slightly higher dose of β-alanine over the initial four week period, although muscle carnosine

concentrations were not directly measured here. However, the results shown in this study are in contrast to those of Bellinger et al. (2012) who showed no effect of β-alanine on average power output during a four minute cycling time trial in highly-trained cyclists, and magnitude based inferences demonstrated that there was only a 37% likelihood of a meaningful increase in power output with β-alanine supplementation. The differences in results between the two studies could be attributed to the differing exercise protocols, as Hobson et al. (2012) showed that exercise capacity is more likely to be improved following β-alanine supplementation than exercise performance. Furthermore, it is possible that the trained cyclists adopted a pacing strategy, similar to the elite games players reported in Chapter 6, which may have masked any true effect of increased muscle carnosine.

Despite the hypothesis that β-alanine supplementation would improve repeated sprint performance, the studies reported in this thesis were unanimous in showing that there was no effect on repeated sprints of a short duration (2 – 6 s). Previous research has suggested that the ability to perform repeated sprints is associated with the ability to effectively buffer H^+ (Bishop et al., 2003; Bishop et al., 2004; Bishop and Edge, 2006; Rampinini et al., 2009), suggesting that increased intracellular buffering capacity would result in an increased RSA. Furthermore, the repeated sprint protocols in the current thesis were performed during simulated games play, which would theoretically increase the metabolic demands on the muscle and result in an increased reliance on the buffering systems of the body. However, this thesis has contributed to the increasing body of evidence to suggest that high-intensity exercise less than 60 s in duration is unaffected by β-alanine supplementation (Hoffman et al., 2006; Sweeney et al., 2010; Kern and Robinson, 2011). The cause of fatigue during this type of exercise is unlikely to be an extreme acidosis and more likely to be due to the gradual

decline in anaerobic ATP production and/or an increase in ADP accumulation, and therefore the increased ability of the muscle to buffer H^+ is unlikely to be fully utilised.

Hobson et al. (2012) showed that exercise capacity was improved with β-alanine supplementation, as was exercise of durations in excess of 60 s due to the large accumulation of H^+ associated with high-intensity exercise of this duration. However, the studies analysed in this meta-analysis all incorporated single bout exercise capacity tests which are not representative of the intermittent nature of the exercise undertaken by team sports players. The YoYo IR 2, a protocol designed to assess an individual's ability to repeatedly perform high-intensity exercise, was improved with β-alanine supplementation, suggesting intermittent exercise is affected by the accumulation of H^+ within the muscle. Indeed, muscle pH has been shown to be significantly lower at exhaustion compared with at 85% of exhaustion time, which suggests increasing muscle acidity is a limiting factor to YoYo IR2 performance. One of the undisputed roles of muscle carnosine is as a muscle buffer, contributing to the attenuation of the reduction in intracellular pH during exercise; increased carnosine concentrations due to β-alanine supplementation would increase the buffering capacity of the muscle (Harris et al., 2006). Therefore, the results of this study contribute to the growing evidence that the ergogenic effects of β-alanine supplementation are due to an increased muscle buffering capacity of the working muscles.

An increasing number of studies have looked to investigate the effect of β-alanine supplementation on applied exercise performance, with no effect shown on 400 m running (Derave et al., 2007) and 2000 m rowing (Baguet et al., 2010) performance, although 30 s sprint performance was improved following a simulated endurance cycle race (van Thienen et

al., 2009). The CV for match-to-match performance in team sports is much more variable than these single-bout events (Gregson et al., 2009), meaning significantly more measurements are required to meaningfully interpret any changes in performance. Although multiple measures were taken throughout the season, the median improvement from β-alanine compared with placebo (+2.85%; Hobson et al., 2012) is far less than the individual (~40%) and repeated measures (~35%) variability of match-to-match performance. The results of this study have further contributed to the lack of an effect of β-alanine shown on applied exercise performance, although any changes in performance due to supplementation may have been masked by the large variation in performance measures.

9.5 **Sodium Bicarbonate and High-Intensity Exercise Capacity and Performance**

The effects of sodium bicarbonate on exercise performance and capacity are equivocal; contrasting results can be attributed to unsuitable exercise protocols not limited by increasing acidosis, GI disturbance and individual variation in the response to supplementation. These contributing factors were assessed using the $CCT_{110\%}$, with exercise capacity only shown to be improved if participants did not experience GI discomfort, although variation in the blood response to exercise may also have contributed to a lack of an effect in some participants. Bellinger et al. (2012) showed improved four minute time trial cycling performance in trained cyclists when supplemented with sodium bicarbonate. The exercise duration used by Bellinger et al. (2012) is longer than the duration of the $CCT_{110\%}$ reported in Chapter 4B (~145 s), and may be more likely to be influenced by the efflux of H^+ out of the working muscles, while the $CCT_{110\%}$ was more directly influenced by intracellular buffering (Chapter 4C).

Sodium bicarbonate has previously been shown to improve 5 x 6 s cycle sprints (Bishop et al., 2004), which suggests that running sprints of an identical duration may also benefit from increased extracellular buffering capacity. However, the results described in this thesis showed no effect on three bouts performed during simulated team sports treadmill running. The suggested mechanism by which performance was improved in Bishop et al. (2004) is the maintenance of muscle pH despite an increased anaerobic energy contribution, due to an increased efflux of H^+ out of the working muscle. Although muscle pH and lactate were not directly measured in the studies reported in this thesis, blood lactate was used as an indirect measure of muscle metabolism due to its association with H^+ production (Hultman and Sahlin, 1980). Despite this study taking place in a simulated altitude environment, blood lactate concentration during the sodium bicarbonate trial was lower than shown by Bishop et al. (2004) and only ~15% higher than the placebo trial, suggesting that there was a minimal increased contribution from anaerobic energy sources. The length of these sprints may not have been of sufficient duration to be affected by an increased efflux of H^+ out of the working muscle.

9.6 **Co-Supplementation of β-alanine and Sodium Bicarbonate and High-Intensity Exercise Capacity and Performance**

Numerous studies exist investigating the separate effects of β-alanine and sodium bicarbonate on exercise performance and capacity, though no study had examined the effect of co-supplementation of these buffering agents. Two studies reported within this thesis investigated the effect of co-supplementation of β-alanine and sodium bicarbonate, thereby increasing both intracellular and extracellular buffering capacity, on exercise capacity and performance. The first of these looked at the effect on high-intensity cycling capacity and

showed no further benefit of co-supplementation of β-alanine and sodium bicarbonate above that of β-alanine alone, although magnitude based inferences suggested a ~70% likelihood of a meaningful difference, likely due to the additive effect of increased intracellular and extracellular H^+ buffering capacity. Bellinger et al. (2012) showed TWD during a four minute cycling time trial was improved when participants supplemented with β-alanine were co-supplemented with sodium bicarbonate (+3.2 ± 3.1%, P = 0.04), although the beneficial effects were similar to those seen with sodium bicarbonate (+3.0 ± 2.2%, P = 0.04) and were not statistically improved over β-alanine alone (P = 0.13), which suggests the performance benefits were due to acute sodium bicarbonate supplementation. Despite this, Bellinger et al. (2012) report that 6 of the 7 participants on β-alanine showed a further improvement with sodium bicarbonate supplementation yet only performed magnitude based inferences in comparison with the pre supplementation trial. Similar to the results reported in Chapter 4C, Bellinger et al. (2012) perhaps should have investigated the further benefit of co-supplementation over supplementation with a single buffering agent to discover its true beneficial effect.

The results reported in Chapter 4C suggest that an additive effect through co-supplementation of β-alanine and sodium bicarbonate should not be dismissed, and current research from this this group suggest an additive effect in rowing (Hobson et al., Unpublished data) and swimming (Painelli et al., Unpublished data). Further to this, the effect of co-supplementation of β-alanine and sodium bicarbonate on repeated sprint performance was investigated. Despite previous research showing an association between increased H^+ buffering capacity and improved RSA (Bishop and Spencer, 2004; Bishop and Edge, 2006; Edge et al., 2006; Rampinini et al., 2009), co-supplementation did not improve 5 x 6 s repeated sprint

performance from baseline, or above supplementation with β-alanine or sodium bicarbonate alone. Since both intracellular and extracellular buffering capacity would have been increased, it is surprising that there was no subsequent effect on performance and provides strong evidence to suggest that, despite the claims of several studies, H^+ buffering capacity is not a contributing factor to RSA.

9.7 **Conclusions**

The results in this thesis showed that β-alanine was effective at improving exercise capacity ($CCT_{110\%}$; YoYo IR 2) but not exercise performance (5 x 6 s sprints; LIST) (Table 9.1). Furthermore, exercise less than 60 s in duration was unaffected, but in excess of 60 s was positively influenced by β-alanine supplementation which supports the work of Hobson et al. (2012).

The effects of sodium bicarbonate were equivocal, with a large variation in the response to exercise contributing to contrasting results. Furthermore, GI discomfort is likely to contribute to a lack of an effect with supplementation, which further contributes to the growing amount of data that suggests that sodium bicarbonate supplementation is only beneficial to some, and not all, individuals.

Co-supplementation of β-alanine and sodium bicarbonate did not have a statistically significant benefit on single-bout high-intensity exercise performance and capacity over β-alanine alone, although benefits to exercise capacity may be meaningful in an applied setting. Therefore, an additive effect through co-supplementation of β-alanine and sodium bicarbonate should not be dismissed and warrants further investigation.

Short duration repeated sprints were unaffected by β-alanine, sodium bicarbonate and co-supplementation of the two which suggests that H^+ buffering capacity might not influence exercise of this nature.

Table 9.1 Percentage change in exercise performance and capacity following supplementation reported in this thesis. PLA refers to placebo, SB refers to sodium bicarbonate, BA refers to β-alanine and BA + SB refers to β-alanine plus sodium bicarbonate. *P ≤ 0.01 from pre supplementation.

			PLA	SB	BA	BA + SB
Chapter 4B	$CCT_{110\%}$	TTE	-	+2.5%	-	-
		TWD	-	+2.6%	-	-
Chapter 4C	$CCT_{110\%}$	TTE	+1.6%	+6.5%	+12.1%*	+16.2%*
		TWD	+1.7%	+6.9%	+14.6%*	+18.8%*
Chapter 5A	5 x 6 s	MPO	-	-2.6%	-	-
	Sprints	PPO	-	-2.3%	-	-
Chapter 5B	5 x 6 s	MPO	+2.1%	+3.3%	-1.2%	+2.3%
	Sprints	PPO	+2.7%	+1.1%	-1.2%	+2.8%
Chapter 6	LIST	Elites	-0.6%	-	+0.1%	-
		Non-Elites	+0.6%	-	+1.4%	-
Chapter 7	YoYo		-7.6%	-	+34.3%*	-
Chapter 8	Games Play	HI Running	-	-	+6.1%	-
		Sprinting	-	-	+0.1%	-

9.8 **Future Investigation**

- **β-alanine:** There is now extensive evidence to support the ergogenic effects of β-alanine on exercise limited by increasing acidosis, therefore future investigation should focus on the potential ergogenic effects on applied performance in events of a duration theoretically limited by increasing muscle acidosis (Table 9.2).

  Further research is warranted on prolonged intermittent exercise capacity such as the YoYo IR1 and Part B of the LIST (Nicholas et al., 2000), performed following five sets of the LIST, and intended to exhaust participants within ten minutes. A capacity test of this sort may be more sensitive to changes in muscle buffering capacity than the performance measures in the main part of the LIST.

  The effect of β-alanine combined with training would be of interest considering the results shown in Chapter 7; supplementation improved intermittent exercise capacity to levels similar to those seen following pre-season training and a 6 to 8 week training program. A study to this effect could determine whether team sport specific training, in combination with β-alanine, has an additive effect.

  Further to the study reported in Chapter 8, it would be of interest to supplement elite games players over a season; players of a higher standard perform more high-intensity activity during competitive match play and may, therefore, be more susceptible to improvements with increased muscle buffering capacity.

  In addition, more long term supplementation studies are merited to determine the upper limits to muscle carnosine concentration and concomitant exercise improvements, and if these are influenced by certain factors including body mass, age and gender. Subsequent effects on the washout period could be investigated, as well as long term health implications.

- **Sodium bicarbonate:** Future studies should employ the $CCT_{110\%}$ and have participants perform exercise following acute sodium bicarbonate supplementation on a repeat number of occasions to determine the variability in the response to supplementation and exercise. This would allow for detailed analysis into the reasons for these large individual differences and whether the ergogenic effect, or lack thereof, is consistent for individuals.

  YoYo IR2 was improved with β-alanine supplementation, and is of sufficient duration to theoretically be improved by sodium bicarbonate supplementation. Furthermore, the effect of sodium bicarbonate supplementation on YoYo IR1 would also be of interest.

  Sporting events of an intensity and duration that could theoretically be benefited by the increased efflux of H^+ out of the working muscle should be investigated to determine if sodium bicarbonate supplementation can improve performance (Table 9.2).

- **β-alanine and sodium bicarbonate:** To determine the true benefits of increased intracellular and extracellular buffering capacity due to co-supplementation of β-alanine and sodium bicarbonate, future investigation should again use the $CCT_{110\%}$ and incorporate large sample sizes and repeated trials to account for individual variation in the response to sodium bicarbonate supplementation and exercise. In addition to this, the contribution of increased intracellular and extracellular buffering to increased exercise capacity could be determined by taking multiple muscle biopsies and blood samples.

YoYo IR 2 performance was shown to be improved with β-alanine alone, therefore investigation into the additional benefit of co-supplementation with sodium bicarbonate is warranted. Additionally, research into co-supplementation of these buffering agents on other high-intensity intermittent exercise capacity tests (YoYo IR1, LIST Part B) is of interest.

Furthermore, sporting events that could theoretically be enhanced by a single buffering agent may be further benefited by co-supplementation (Table 9.2).

Long term β-alanine supplementation, resulting in the highest attainable muscle carnosine concentrations, could potentially minimise the contribution of increased extracellular buffering capacity. To determine this, investigation into the effect of prolonged β-alanine supplementation should also incorporate acute supplementation with sodium bicarbonate.

Table 9.2 Olympic sporting events of a duration which theoretically may evoke an ergogenic benefit from buffering agents.

Olympic Sporting Event		Recent Summer and Winter Olympics Gold Medal Time	
		Men	**Women**
Running	400 m Hurdles	47:63	52:70
	800 m	1:40:91	1:56:19
	1500 m	3:34:08	4:10:23
Swimming	100 m freestyle	47:52	53:00
	200 m freestyle	1:43:14	1:53:61
	400 m freestyle	3:40:14	4:01:45
Flat-Water	500 m K1	N/A	1:51:46
Kayaking	1000 m K1	3:26:46	N/A
Cycling	Track team pursuit	3:51:66	3:14:05
Rowing	2000 m 8+	5:48:75	6:10:59
	Double Sculls	6:31:67	6:55:82
Speed skating	1500 m	1:45:57	1:56:89
Alpine skiing	Downhill	1:54:31	1:44:19

Chapter 10.0 References

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Appendix 1

Intensity of Sickness

0	Not at all
1	
2	
3	Slightly
4	
5	
6	Quite
7	
8	
9	Very
10	Throwing up

Intensity of Stomach ache

0	None at all
1	
2	
3	Dull ache on and off
4	
5	
6	Moderate continuous
7	
8	
9	Severe continuous
10	Severe doubled up

Intensity of Headache

0	None at all
1	
2	
3	Dull ache on and off
4	
5	
6	Moderate continuous
7	
8	
9	Severe continuous
10	Searing pain