

THE EFFECT OF DIETARY NITRATE SUPPLEMENTATION ON AGILITY, LINEAR SPRINT AND VERTICAL JUMP PERFORMANCE

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Abstract

Dietary nitrate (NO_3^-) supplementation has been shown to speed reaction time and enhance linear sprint speed and power production in an unfatigued state. It may therefore be suggested that NO_3^- supplementation could be ergogenic during all-out sprint running and reactive agility tasks, as well as during explosive forms of exercise such as vertical jumping. NO_3^- supplementation may also attenuate the decline in such exercise performance following fatiguing exercise. The purpose of this thesis was firstly to determine the reliability of selected exercise tests for measuring running reactive agility (reactive agility test), planned agility (change of direction t-test), 15 m linear sprint and countermovement jump performance, and then to investigate the effect of NO_3^- supplementation on these parameters of team sports performance in an unfatigued state and following fatiguing exercise that mimics the high-intensity intermittent demands of team sport game-play. **Chapter 3:** Examined the reliability of selected exercise protocols for reactive agility, change of direction, sprint and vertical jump performance. The lowest coefficient of variation (COV) and highest intraclass correlation coefficient (ICC) was observed when the fastest 15 m linear sprint out of 5 attempts (COV: 1.0%; ICC: 0.98), and the highest countermovement jump out of 3 attempts (COV: 4.6%; ICC: 0.94) were assessed independently and when the mean of all 6 reactive agility (COV: 2.0%; ICC: 0.96) and all 3 change of direction t-test (COV: 2.9%; ICC: 0.87) attempts were assessed. This information was used to inform statistical analyses within chapter 4. Results from chapter 3 provided confidence in the use of the selected exercise protocols in an intervention study due to the low day-to-day variability in performance. **Chapter 4:** Five days of NO_3^- supplementation did not improve reactive agility (NIT: 2.64 ± 0.21 s vs PLA: 2.65 ± 0.17 s, $P > 0.05$), change of direction t-test (NIT: 7.12 ± 0.71 s vs PLA: 7.10 ± 0.76 , $P > 0.05$), 15 m sprint (NIT: 3.204 ± 0.212 s vs. PLA: 3.215 ± 0.206 s, $P > 0.05$) or countermovement jump (NIT: 36.38 ± 6.58 cm vs PLA: 37.02 ± 6.83 cm, $P > 0.05$) performance in an unfatigued state. In a fatigued state, 15 m sprint (NIT: 3.27 ± 0.25 s vs PLA: 3.27 ± 0.25 , $P > 0.05$) and countermovement jump (NIT: 36.7 ± 7.2 cm vs PLA: 36.5 ± 7.0 cm, $P > 0.05$) performance were also unaltered following NO_3^- supplementation. Performance declined in a fatigued compared to unfatigued state for 15 m sprint performance ($P < 0.05$) but was unchanged for countermovement jump performance ($P >$

0.05). NO₃⁻ supplementation did not attenuate the decline in fatigued 15 m sprint performance ($P > 0.05$). These findings suggest that NO₃⁻ supplementation does not alter agility, linear sprint or vertical jump performance. Overall, these findings provide an important contribution to the literature regarding the limits of the ergogenic effect of NO₃⁻ supplementation for particular determinants of team sports performance, specifically in male team sports players at a dose of 8 mmol · day⁻¹ for 5 days.

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Symbols and Abbreviations

[]	concentration
Δ	change/difference
ANOVA	analysis of variance
ATP	adenosine tri-phosphate
BP	blood pressure
BR	nitrate rich beetroot juice
Ca^{2+}	calcium
CI	confidence interval
CMJ	countermovement jump
COD	change of direction
COV	coefficient of variation
<i>d</i>	Cohen's <i>d</i> statistic
DBP	diastolic blood pressure
eNOS	endothelial nitric oxide synthase
HR	heart rate
ICC	intraclass correlation
iNOS	inducible nitric oxide synthase
IR1	intermittent recovery test, level 1
IST	intermittent sprint test
KNO_3^-	potassium nitrate
MAP	mean arterial pressure
NaNO_3^-	sodium nitrate
NIT	nitrate-rich supplement
nNOS	neuronal nitric oxide synthase

NO	nitric oxide
NO ₂ ⁻	nitrite
NO ₃ ⁻	nitrate
NOS	nitric oxide synthase
O ₂	oxygen
PCr	phosphocreatine
PLA	placebo
RAT	reactive agility test
RNS	reactive nitrogen species
SBP	systolic blood pressure
SD	standard deviation
TT	time trial

Conference Activity and Awards

Conference Activity

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CHAPTER 1: Literature Review and Introduction

Nitric Oxide

Nitric oxide production

The ubiquitous free-radical gas, nitric oxide (NO), also known as nitrogen monoxide, is a signalling molecule known to play an imperative role in many cellular functions within the human body. These include processes such as vasodilation (Moncada & Higgs, 1993), mitochondrial function (Brown & Cooper, 1994; Larsen *et al.*, 2011), glucose uptake (Merry, Lynch & McConell, 2010), calcium (Ca^{2+}) handling (Hart & Dulhunty, 2000; Stamler & Meissner, 2001), neurotransmission (Garthwaite, 2008) and skeletal muscle fatigue (Percival *et al.*, 2010); with the effective production of NO considered essential to facilitate normal physiological function.

Initially, NO was believed to be exclusively synthesised from the oxidation of L-arginine by NO synthase (NOS) enzymes (NOS-dependent pathway) with endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) isoforms having been described, enabling NO production at various locations around the body (Stamler & Meissner, 2001). The production of NO via this pathway involves a complex five electron oxidation of L-arginine to yield L-citrulline and NO (Alderton, Cooper & Knowles, 2001). The oxidation of NOS-derived NO was also known to produce nitrate (NO_3^-) and nitrite (NO_2^-), which were formerly considered inert by-products of NO metabolism (Moncada & Higgs, 1993). However, it is now known that both NO_3^- and NO_2^- can be reduced to NO through the stepwise reduction of NO_3^- to NO_2^- to NO (Benjamin *et al.*, 1994; Lundberg *et al.*, 1994); termed the NO_3^- - NO_2^- -NO (or NOS-independent) pathway. In addition to the endogenous synthesis of NO via the NO_3^- - NO_2^- -NO pathway, exogenous NO_3^- from the diet can also be utilised to increase NO bioavailability. Both pathways for NO production can be summarised in figure 1.1.

Nitrate-Nitrite-Nitric Oxide pathway

Approximately 80% of human dietary NO_3^- intake originates from the ingestion of vegetables (Hord, Tang & Bryan, 2009). Vegetables such as leafy greens

(spinach, rocket and lettuce) and beetroot are especially rich in NO_3^- (Webb *et al.*, 2008) containing up to ~480 mg NO_3^- per 100 g of fresh produce (Alexander *et al.*, 2008). Other sources of NO_3^- in the diet include cured meats (where sodium NO_3^- and related products are added as a preservative) as well as drinking water (Hord *et al.*, 2009).

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Figure 1.1: *The nitric oxide synthase dependent and nitrate-nitrite-nitric oxide pathways for nitric oxide production, and the physiological processes nitric oxide plays a role in (From Bailey et al., 2012). The dashed lines indicate how nitric oxide can be recycled back to nitrite and nitrate within the nitrate-nitrite-nitric oxide pathway.*

Following the consumption of NO_3^- -rich foods, NO_3^- is absorbed into the systemic circulation from the upper gastrointestinal tract (Lundberg & Weitzberg, 2009). The majority of this NO_3^- (~60%) is excreted in the urine while ~25% enters the enterosalivary circulation and is concentrated in the saliva at least 10-fold (Spiegelhalder, Eisenbrand & Preussmann, 1976). Following this, facultative, anaerobic bacteria located on the dorsal surface of the tongue reduce this NO_3^- to NO_2^- , via NO_3^- reductase enzymes (Duncan *et al.*, 1995). This NO_2^- rich saliva is swallowed and the acidic environment of the stomach permits the further reduction of some of this NO_2^- to NO and other reactive nitrogen species (RNS;

Lundberg *et al.*, 2011). However, some of this NO_2^- escapes and is absorbed into the systemic circulation increasing plasma NO_2^- concentration ($[\text{NO}_2^-]$ where square brackets indicate concentration) where it can then undergo a final one electron reduction to NO and other RNS, catalysed by deoxyhaemoglobin (Cosby *et al.*, 2003), deoxymyoglobin (Shiva *et al.*, 2007) and xanthine oxidase (Zhang *et al.*, 1998) to increase NO availability.

Interestingly, the reduction of NO_2^- to NO within the systemic circulation is potentiated in both hypoxic (Castelo *et al.*, 2006) and acidic (Modin *et al.*, 2001) environments, and given that during exercise for example, contracting skeletal muscle becomes more hypoxic and acidic, the production of NO via the NO_3^- - NO_2^- -NO pathway may be an increasingly important source of NO (Lundberg & Weitzberg, 2010). Furthermore, the activity of NOS in such environments is known to be reduced (Lundberg, Weitzberg & Gladwin, 2008), therefore reducing the production of NO via the NOS-dependent pathway. It is also important to note that NO_2^- itself may influence physiological processes, independent of its reduction to NO via inducing post-translational modifications to haem groups by nitrosylation and protein thiols by S-nitrosation (Bryan *et al.*, 2005). To increase NO and NO_2^- availability through the NO_3^- - NO_2^- -NO pathway, dietary supplementation of natural NO_3^- -rich beetroot juice (BR; Webb *et al.*, 2008; Vanhatalo *et al.*, 2010; Shannon *et al.*, 2017b) and pharmacological sodium nitrate (NaNO_3 ; Larsen *et al.*, 2007) and potassium nitrate (KNO_3 ; Kapil *et al.*, 2010) are commonly employed within human experimental studies. This thesis will focus on dietary supplementation with BR.

Dietary nitrate supplementation

Following supplementation with NO_3^- , both plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ increase after their absorption into the systemic circulation. Wylie *et al.* (2013a) investigated the pharmacokinetic relationship of acute BR supplementation (at doses of 4.2, 8.4 and 16.8 mmol NO_3^-) and plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$. Plasma $[\text{NO}_3^-]$ was reported to peak 1 h post ingestion at a dose of 4.2 mmol and 8.4 mmol NO_3^- and 2 h post ingestion of 16.8 mmol NO_3^- (~160 μM , ~269 μM and ~581 μM increase above baseline, respectively). A slightly later peak in plasma $[\text{NO}_2^-]$ was reported at 2 h post ingestion of 4.2 mmol and 8.4 mmol NO_3^- and 4 h post ingestion of 16.8 mmol NO_3^- (~220 nM, ~374 nM and ~653 nM increase

above baseline, respectively; Wylie *et al.*, 2013a). These findings indicate a dose-dependent relationship between BR supplementation and both plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$. Furthermore, the delayed peak in plasma $[\text{NO}_2^-]$ reflects the time required for the stepwise reduction of NO_3^- to NO_2^- in the oral cavity before it enters the systemic circulation. This information is now commonly used to inform human physiology studies that administer NO_3^- supplementation, whereby supplements are generally consumed 2 h prior to physiological assessment of exercise performance to coincide with peak plasma $[\text{NO}_2^-]$.

Established effects of nitrate supplementation

The effect of NO_3^- supplementation on physiological functions in humans has been, and continues to be, extensively researched. This research has investigated the potential of NO_3^- supplementation to act, not only as a therapeutic aid, but also as an ergogenic aid within sports performance.

Reduction in blood pressure

NO_3^- has been shown to significantly reduce resting blood pressure (BP) following both acute (e.g. Webb *et al.*, 2008; Kapil *et al.*, 2010; Vanhatalo *et al.*, 2010) and chronic (between 3-15 days) supplementation (e.g. Larsen *et al.*, 2007; Bailey *et al.*, 2009). A reduction in systolic BP (SBP) of 4-10 mmHg is commonly reported in many (Larsen *et al.*, 2007; Webb *et al.*, 2008; Bailey *et al.*, 2009; Kapil *et al.*, 2010; Vanhatalo *et al.*, 2010) but not all (Larsen *et al.*, 2010; Cermak, Gibala & Van Loon, 2012) previous studies. Such variation in reported BP reductions may be attributed to the differing NO_3^- doses employed within each individual study, as SBP is reduced in a dose-dependent manner following NO_3^- supplementation (Wylie *et al.*, 2013a). Wylie and colleagues (2013a) reported that SBP was reduced in this dose-dependent manner following acute BR supplementation of 4.2 mmol NO_3^- (~5 mmHg) and 8.4 mmol NO_3^- (~10 mmHg) with no additional reduction gained from a higher dose of 16.8 mmol NO_3^- (~9 mmHg). These authors also reported an ~3 mmHg reduction in diastolic BP (DBP) following the acute consumption of 8.4 and 16.8 mmol NO_3^- with no change evident following a 4.2 mmol NO_3^- dose (Wylie *et al.*, 2013a). Similar reductions in DBP following

NO₃⁻ ingestion have also been reported elsewhere in the literature (Webb *et al.*, 2008; Bailey *et al.*, 2009; Sobko *et al.*, 2010; Vanhatalo *et al.*, 2010).

The hypotensive effect of NO₃⁻ supplementation is likely mediated by the stepwise reduction of NO₃⁻ to yield NO (Ignarro *et al.*, 1987). This increase in NO can stimulate smooth muscle relaxation leading to vasodilation and reduced peripheral resistance (Webb *et al.*, 2008), resulting in a reduction in resting BP. It is also known that those with a higher resting BP benefit most from the hypotensive effect of NO₃⁻ supplementation (e.g. Kapil *et al.*, 2010). Importantly, the reduction in BP commonly reported following NO₃⁻ supplementation (> ~4mmHg) is of clinical significance as it is in line with the magnitude of BP reduction expected to lower the incidence of stroke and ischaemic heart disease (Law, Wald & Morris, 2003). NO₃⁻ supplementation therefore has potential to act as an effective non-pharmacological therapeutic aid within the general population to maintain or improve cardiovascular health.

Improved exercise economy, tolerance and performance

The oxygen (O₂) cost of submaximal cycle exercise has been reported to be reduced following 3 days of NaNO₃⁻ supplementation (e.g. Larsen *et al.*, 2007) as well as following BR supplementation (e.g. Bailey *et al.*, 2009), indicating increased exercise economy for a given work rate. Since then, exercise tolerance during constant work rate exercise has been investigated and was reported to improve by ~12-25% following dietary NO₃⁻ supplementation within a range of modalities including cycling (Bailey *et al.*, 2009; Breese *et al.*, 2013; Kelly *et al.*, 2013; Thompson *et al.*, 2014), running (Lansley *et al.*, 2011) and knee extension (Bailey *et al.*, 2010; Vanhatalo *et al.*, 2011) exercise. Such improvements in exercise tolerance would be expected to translate to an improvement in exercise performance, determined by the time taken to complete a set distance (e.g. a time trial; TT). Improvements in exercise performance of ~1-3% have been reported following NO₃⁻ supplementation (Lansley *et al.*, 2011b; Cermak, Gibala & Van Loon, 2012; Murphy *et al.*, 2012; Shannon *et al.*, 2017a), however, this finding does not seem to be universal, with several studies reporting no improvement in performance (Peacock *et al.*, 2012; Muggeridge *et al.*, 2013; Sandbakk *et al.*, 2015; McQuillan *et al.*, 2016). Such equivocal findings may be attributed to differences in participant population (e.g. trained vs. recreationally

active), exercise modality (e.g. cycling vs. running), exercise intensity and duration, as well as the NO_3^- dose and duration of supplementation (e.g. acute vs. chronic) employed within each individual study.

There is a growing body of literature to suggest that NO_3^- supplementation may be less effective at improving exercise tolerance and performance in highly trained and elite endurance athletes (see Jones, 2014 for review). Indeed, no improvements in performance were reported in a highly trained or elite population (Peacock *et al.*, 2012; Muggeridge *et al.*, 2013; Sandbakk *et al.*, 2015); conversely, improvements in performance have been reported when recreationally active participants were recruited (Lansley *et al.*, 2011b; Murphy *et al.*, 2012). It must, however, be acknowledged that these particular studies did not solely differ on the participant population recruited but also differed in supplementation dose/regime, and type/duration of exercise employed. The differing effect of NO_3^- supplementation between highly trained endurance and recreational athletes suggested within the literature (for review see Jones, 2014) may, in part, be attributed to highly trained endurance individuals having elevated baseline NO_3^- and NO_2^- (Vassalle *et al.*, 2003) and elevated NOS activity (Jungersten *et al.*, 1997) as well as having greater skeletal muscle capillarisation (Jensen, Bangsbo & Hellsten, 2004) minimising areas of hypoxia, in which the reduction of NO_2^- to NO is potentiated (Castelo *et al.*, 2006). Highly trained endurance athletes also have a lower proportion of type II muscle fibres compared to their untrained counterparts (Tesch & Karlsson, 1985), with recent research suggesting a targeted effect of NO_3^- supplementation on type II muscle fibres.

Fibre type specific effect

It has been suggested that NO_3^- supplementation may preferentially enhance the physiological responses of type II compared to type I skeletal muscle fibres (Hernández *et al.*, 2012; Ivarsson *et al.*, 2016). This may be attributed, in part, to the differing physiology of these muscle fibres. Type II muscle fibres differ compared to type I fibres in terms of myofibrillar protein content and Ca^{2+} handling as well as having lower mitochondrial and capillary density (Bottinelli & Reggiani, 2000). Together, these differences result in type II fibres having a greater reliance on non-oxidative compared to oxidative pathways for energy production, which

arguably provide a more favourable environment for NO production via the NO_3^- - NO_2^- -NO pathway as the reduction of NO_2^- to NO is potentiated in hypoxic conditions (Castelo *et al.*, 2006).

Hernández and colleagues (2012) were some of the first authors to investigate the fibre type specific effects of NO_3^- supplementation. They supplemented mice with NaNO_3^- in drinking water for 7 days and performed force-frequency assessment of the extensor digitorum longus (EDL) and soleus muscle harvested from these mice. A significantly greater force production, up to a stimulation frequency of 50 Hz, was reported in the EDL muscle but not the soleus muscle of the supplemented mice compared to the non-supplemented controls. The EDL muscle is made up almost exclusively of type II muscle fibres in comparison to the soleus muscle which is made up of predominantly type I muscle fibres. These authors also reported an increased expression of the Ca^{2+} handling proteins calsequestrin-1 and the dihydropyridine receptor in the EDL muscle but not the soleus muscle following NaNO_3^- supplementation (Hernández *et al.*, 2012). However, improvements in force production following NO_3^- supplementation in humans have been suggested to occur independently of changes in Ca^{2+} handling protein content (Whitfield *et al.*, 2017). It has been proposed that changes in cellular redox balance following NO_3^- supplementation (Whitfield *et al.*, 2016) may underpin improvements in force production in humans. However, the exact mechanistic underpinning of improvements in force production following NO_3^- supplementation in humans has yet to be firmly established.

In recent human studies, NO_3^- supplementation has been reported to enhance the contractile properties of skeletal muscle and enhance evoked explosive force production (Haider & Folland, 2014) and power during voluntary exercise (Coggan *et al.*, 2015; Rimer *et al.*, 2016). Coggan and colleagues (2015) reported a significant increase in muscle force production at a high, but not low, angular velocity during knee extensor exercise in healthy untrained participants following an acute 11.2 mmol NO_3^- dose. Similarly, Bailey *et al.* (2015) reported enhanced exercise tolerance in healthy recreationally active males when cycling at high, but not low, pedal cadences following a chronic supplementation period of 9 days (6.2 mmol $\text{NO}_3^- \cdot \text{day}^{-1}$). Together, these findings suggest that NO_3^- supplementation can improve human skeletal muscle force and power

production, specifically during movements at high contraction speeds, with such exercise recruiting a high proportion of type II muscle fibres.

In addition to the influence on contractile function, fibre type specific effects of dietary NO_3^- on skeletal muscle blood flow have also been observed. Indeed, Ferguson and colleagues (2013) reported that, following 5 days of BR supplementation in rats, hind-limb muscle blood flow was significantly increased during submaximal exercise with targeted increases in blood flow to muscles and parts of muscles that were predominantly made up of type II muscle fibres.

Given these preferential effects of NO_3^- supplementation on type II fibres, it may, in part, explain the ergogenic effect of NO_3^- supplementation during short duration (< 30 min), high intensity exercise where type II muscle fibres are preferentially recruited (Krustrup *et al.*, 2004). A number of previous studies (e.g. Bailey *et al.*, 2009; Vanhatalo *et al.*, 2010; Lansley *et al.*, 2011b; Cermak *et al.*, 2012; Shannon *et al.*, 2017a), have reported improvements in exercise tolerance and/or performance during short duration (< 30 min) exercise when sub-elite participants were recruited. In contrast to this, no improvement in performance over short duration running (Peacock *et al.*, 2012; Sandbakk *et al.*, 2015; Boorsma *et al.*, 2016) or cycling (Muggeridge *et al.*, 2014) protocols were reported following the recruitment of highly trained or elite participants. In such participant cohort, it does however appear that NO_3^- supplementation may be ergogenic during upper, rather than lower, body short duration exercise such as kayaking (Peeling *et al.*, 2015) and rowing (Bond *et al.*, 2012; Hoon *et al.*, 2014) exercise. With the upper, compared to the lower, body musculature comprised of a higher portion of type II muscle fibres (e.g. Sanchis-Moysi *et al.*, 2010) further supporting the preferential effect of NO_3^- supplementation on type II muscle fibres.

This provides promising evidence to suggest an ergogenic effect of NO_3^- supplementation in continuous, short duration exercise performance where type II muscle fibres are predominantly recruited. The ergogenic effect of NO_3^- supplementation in other forms of short duration exercise that also recruit a high portion of type II muscle fibres such as intermittent (Krustrup *et al.*, 2006) and sprint (Greenhaff *et al.*, 1994) exercise therefore may also appear promising

Nitrate supplementation and team sports performance

Intermittent exercise performance

High-intensity intermittent exercise is a hallmark of many team sports such as football, rugby union/league and hockey (Bangsbo, 1994). Intermittent exercise is associated with significant type II muscle fibre recruitment due to the nature of such exercise (Bangsbo, Iaia & Krstrup, 2008), transitioning from a low to high metabolic rate in rapid and repeated succession (Krstrup *et al.*, 2006). Typically, intermittent exercise involves repeated short-duration bouts of high intensity interspersed with brief periods of recovery. A valid and reliable intermittent exercise test that mimics the high intensity, intermittent nature of team sports match-play has been developed; the Yo-Yo intermittent recovery level 1 (IR1) test (Bangsbo *et al.*, 2008). A significant improvement in Yo-Yo IR1 test performance of 4.2% was reported by Wylie *et al.* (2013b) following supplementation of ~24.6 mmol NO₃⁻ over a 36 h period prior to exercise testing. This has since been replicated by Thompson *et al.* (2016) and Nyakayiru *et al.* (2017) who reported a similar improvement in Yo-Yo IR1 performance of 3.9% and 3.4% following 5 (6.4 mmol · day⁻¹) and 6 (~12.9 mmol · day⁻¹) days of NO₃⁻ supplementation, respectively.

The ergogenic effect of NO₃⁻ supplementation on intermittent exercise performance, although not found in all studies (Christensen, Nyberg & Bangsbo, 2013; Muggeridge *et al.*, 2013; Martin *et al.*, 2014), has been reported within running (Thompson *et al.*, 2016; Nyakayiru *et al.*, 2017), cycling (Thompson *et al.*, 2015; Wylie *et al.*, 2016) and rowing (Bond, Morton & Braakhuis, 2012) exercise. Improvements in intermittent exercise performance may indicate that NO₃⁻ could be ergogenic for team sports players where intermittent exercise is performed throughout the duration of a game. In support of this, Thompson and colleagues (2015) reported improved sprint cycling performance during an intermittent sprint test (IST), and, interestingly, improved indices of cognitive function during computer based cognitive tasks following chronic BR supplementation (~12.8 mmol NO₃⁻ · day⁻¹ for 7 days). Throughout the IST, which mimicked the metabolic demands of team sports game play, response accuracy was maintained but significant improvements in reaction time were reported (Thompson *et al.*, 2015). Furthermore, improvements in reaction time were most

prominent within the second half of the IST protocol which may suggest that NO_3^- is effective at attenuating some of the decline in cognitive function (e.g. decision making reaction time) that typically occurs during prolonged intermittent exercise (Reilly & Smith, 1986; Fery *et al.*, 1997). Interestingly, Thompson *et al.* (2016) went on to report improvements in reaction time, determined by a faster response time for the same response accuracy, when completing the Stroop test at rest but not at intervals between intermittent running exercise (Thompson *et al.*, 2016). The literature remains equivocal regarding improvements in reaction time following NO_3^- supplementation with some (Gilchrist *et al.*, 2014; Thompson *et al.*, 2015) but not all (Kelly *et al.*, 2013; Thompson *et al.*, 2014) reporting improvements.

Important determinants of team sports performance

Improvements in intermittent exercise performance following NO_3^- supplementation (e.g. Wylie *et al.*, 2013b; Thompson *et al.*, 2015) suggest that such supplementation may be ergogenic for team sports performance where game-play is highly intermittent in nature (Bangsbo, 1994). However, intermittent exercise is not the only hallmark of team sports game-play. Key movement patterns also include sprint running, quick changes of direction and vertical jumping (Wisløff *et al.*, 2004; Little & Williams, 2005; Gabbett, Kelly & Sheppard, 2008; Castagna & Castellini, 2013) with such movements being performed maximally and repeatedly throughout the entire duration of a game. Movement analysis within team sports game-play has highlighted that sprint efforts between 10-20 m are commonly performed (Spencer *et al.*, 2004; 2005) with over 50% of these sprint efforts occurring after >60 s recovery (Spencer *et al.*, 2004). This highlights the importance of single sprint efforts within a game in addition to high intensity intermittent exercise. Alongside this, over 700 changes of direction are made throughout a football game (Bloomfield, Polman & O'Donoghue, 2007) and within a rugby game ~16% of sprints involve at least one change of direction (Duthie *et al.*, 2006). These data highlight the importance of changes of direction, be they planned or unplanned, within team sports game-play.

The effect of NO_3^- supplementation on single sprint performance has been investigated, with a significant improvement in 180 m sprint running performance reported in 9 elite cross-country skiers following acute KNO_3^- supplementation

(~9.9 mmol NO₃⁻; Sandbakk *et al.*, 2015). However, this ergogenic effect on sprint performance has not been confirmed in all studies (Christensen *et al.*, 2013; Muggeridge *et al.*, 2013; Martin *et al.*, 2014). Thompson and colleagues (2016) investigated the effect of 5 days NO₃⁻ supplementation (6.4 mmol · day⁻¹) on 20 m linear sprint running performance in an unfatigued state, in 36 healthy male team sports players. These authors reported a significant improvement of 1.2% in all-out sprint running performance, and when sprint performance was separated into split times, the improvement in performance over the first 10 m was 1.6% and over the first 5 m was 2.3% (Thompson *et al.*, 2016). Taking these findings together with improvements in skeletal muscle contractility (Bailey *et al.*, 2010), explosive force (Haider & Folland, 2014) and maximum power production (Coggan *et al.*, 2015), it may be suggested that the ergogenic effect of NO₃⁻ supplementation might be most pronounced within the initial acceleration phase of all-out sprint running exercise. It may therefore be proposed that NO₃⁻ supplementation could be ergogenic during exercise involving repeated accelerations such as within planned and unplanned change of direction tasks. Moreover, it may also suggest NO₃⁻ supplementation could be ergogenic during explosive movements such as vertical jumping. However, these possibilities have yet to be investigated.

Importantly, sprint movements are also performed under fatigue throughout the duration of a game, where the ability to perform such high intensity explosive exercise is reduced (Mohr *et al.*, 2004). Interestingly, NO₃⁻ supplementation has been suggested to reduce fatigue development and therefore muscle metabolic perturbation through reducing the adenosine triphosphate (ATP) cost of skeletal muscle contraction while sparing the rate of phosphocreatine (PCr) depletion (Bailey *et al.*, 2010). This is important as the rate of PCr depletion is a significant determinant of fatigue development during maximal intensity exercise (Gaitanos *et al.*, 1993; Fulford *et al.*, 2013). NO₃⁻ supplementation has also been suggested to facilitate the O₂ dependent recovery of PCr (Vanhatalo *et al.*, 2011) which may be related, in part, to its ability to improve type II muscle fibre perfusion and oxygenation (Ferguson *et al.*, 2013; 2015). Independent of this, during exercise following NO₃⁻ supplementation, NO availability may be enhanced as the reduction of NO₂⁻ to NO is enhanced in hypoxic and acidic environments (Lundberg & Weitzberg, 2010) such as the environment within contracting skeletal muscle. Increased NO availability may be

advantageous as it may stimulate acute NO-mediated improvements in contractile function (Hernández *et al.*, 2012). Overall, fatigue development may be reduced and NO availability enhanced with NO_3^- supplementation, such that the performance decline expected in subsequent exercise bouts may be attenuated. However, the effect of NO_3^- supplementation on linear sprint or vertical jump performance when fatigued has yet to be investigated.

Understanding the potential ergogenic effect of NO_3^- supplementation upon planned and unplanned agility, vertical jump and sprint performance in an unfatigued state and following fatiguing exercise will build upon the current literature and provide information for its use as a nutritional aid to enhance performance in movements commonly performed within team sports.

Reliability of exercise tests for agility, linear sprint and vertical jump performance

Various exercise protocols have been developed to assess planned and unplanned agility, linear sprint running and vertical jump performance in a controlled, scientific manner rather than within actual game-play (Currell & Jeukendrup, 2008; Singh *et al.*, 2010). This enables team sports performance to be measured, and also enables the effect of an intervention on performance to be assessed in a controlled environment. Before an exercise protocol is employed to assess the efficacy of an intervention, it is important to assess the day-to-day reliability of each protocol to determine whether it would be appropriate to detect small but potentially meaningful changes in performance following an intervention. The reliability of test performance refers to the consistency or reproducibility of performance when the same person performs the same test under the same conditions on a number of occasions, when no intervention is used (Atkinson & Nevill, 1998; Hopkins, 2000).

For planned and unplanned agility, the change of direction (COD) t-test and reactive agility test (RAT) are commonly employed, respectively (Sheppard & Young, 2006; Sporis *et al.*, 2010). Information regarding the reliability of these tests is rarely reported within intervention studies that employ these protocols (Brughelli *et al.*, 2008). When assessed, the reliability of these tests expressed as a coefficient of variation (COV) has been reported to be between ~2.0-3.5% (McBride *et al.*, 2002; Gabbett *et al.*, 2006; 2008; Oliver & Meyers, 2009; Sassi *et al.*, 2009). However, the data used to perform these statistical analyses

appears to vary greatly between studies with reports of the mean of 4/8 attempts, fastest attempt from 2 efforts and fastest 2 attempts from 8 efforts being used, for example. The reliability of linear sprint performance over split times up to 15 m is also limited. When assessed as a COV within a simulated team sport circuit, it was reported to be 3.7% when the mean of 60 sprint performances was assessed, and 2.0% when absolute best sprint performance was assessed independently (Singh *et al.*, 2010). This is important as following test completion, the data handling techniques employed appear to affect the reliability of test performance. In addition to this, the reliability of vertical jump performance appears to be varied within the literature. When assessed as a COV, reliability of vertical jump performance has been reported between ~2.0-5.2% (Gabbett *et al.*, 2006; Cormak *et al.*, 2008; Singh *et al.*, 2010) with studies using the highest jump from a varying number of attempts for analysis.

Prior to conducting an intervention study, it is important that the reliability of the required exercise tests are confirmed. Within studies it is common for multiple attempts of the same protocol to be completed, yet the selection of data for statistical analysis from these attempts is not consistent. However, the reliability of test performance appears to vary depending on the data handling techniques used and therefore it is important to consider the most appropriate selection of data to perform statistical analysis on to ensure the greatest reliability of test performance is achieved. This information will enable the selected exercise protocols' suitability for an intervention study to be considered (Hopkins *et al.*, 1999) where detecting small changes in performance following an intervention is important. For example, following caffeine supplementation agility performance has been reported to improve ~2% (e.g. Stuart *et al.*, 2005; Duvnjak-Zaknich *et al.*, 2011) and following NO₃⁻ supplementation sprint performance has been reported to improve ~1% (Sandbakk *et al.*, 2015; Thompson *et al.*, 2016), showing the small but potentially meaningful improvements in performance (Hopkins *et al.*, 1999) that are required to be detected if present.

Summary

In summary, dietary NO₃⁻ supplementation is known to act not only as a therapeutic aid but also as an ergogenic aid within sports performance. NO₃⁻ supplementation may improve team sports performance through its ability to

improve intermittent exercise performance (Wylie *et al.*, 2013b), as well as all-out sprint running performance (Thompson *et al.*, 2016). However, team sports performance is not limited to these determinants. Movements such as planned and unplanned changes of direction, vertical jumping and sprint running are considered hallmarks of team sports game play. It may be suggested that NO_3^- supplementation could be ergogenic during such movements as highlighted in the above literature review. However, in order to fully understand the efficacy of NO_3^- supplementation for team sports performance, its effect on these determinants of team sports performance must be investigated. Such information would build upon our existing knowledge regarding the ergogenic effects of NO_3^- supplementation and elucidate its efficacy within key determinants of team sports performance and therefore the potential of its use as a nutritional aid for team sports athletes. Prior to assessing the effect of NO_3^- supplementation on parameters of team sports performance, it is important to assess the reliability of the exercise tests that will be employed. The reliability of test performance also appears to vary depending on data handling procedures used, therefore it is important to consider the most appropriate selection of data to perform statistical analysis on to ensure the greatest reliability of test performance can be achieved.

Aims

The aim of this thesis is therefore twofold. Firstly, to assess the reliability of chosen exercise protocols to measure agility, linear sprint and vertical jump performance, and secondly, to investigate the effect of NO₃⁻ supplementation on agility, linear sprint and vertical jump performance in an unfatigued state and linear sprint and vertical jump performance in a fatigued state.

The following research questions will be addressed:

- 1) What is the reliability of the selected exercise tests that measure agility, linear sprint and vertical jump performance?
- 2) What are the performance effects of NO₃⁻ supplementation on key parameters of team sports performance?
 - Can NO₃⁻ supplementation improve agility, linear sprint and vertical jump performance in an unfatigued state?
 - Can NO₃⁻ supplementation attenuate the decline in linear sprint and vertical jump performance expected following fatiguing exercise?

Hypotheses

The following hypotheses will be tested:

- 1) The exercise tests selected to measure agility, linear sprint and vertical jump performance will be reliable and suitable for use within an intervention study
- 2) NO₃⁻ supplementation will improve agility, linear sprint running and vertical jump performance in an unfatigued state, and, following fatiguing exercise, NO₃⁻ supplementation will attenuate the decline in performance in subsequent linear sprint and vertical jump performance

CHAPTER 2: General Methods

General experimental procedures

All experimental testing was approved by the Institutional Ethics Committee prior to the onset of data collection. All participants gave their written, informed consent prior to commencing the study, after the experimental procedures, potential benefits and possible risks associated with participation were explained. Any additional questions or concerns participants had were addressed before volunteers provided written informed consent. Participants who were enrolled onto the study were informed that they were free to withdraw at any time, without reason and with no disadvantage to themselves should they not wish to complete the study.

During all experimental testing, health and safety guidelines established within the department of Sport and Health Sciences were closely followed. The researchers were vigilant throughout to ensure the laboratory and sports hall provided a clean and safe environment for the testing of human participants.

Participants

Volunteers were recruited from the University of Exeter student and staff population. All participants were competitive but non-elite male, team sports players who were non-smokers, free from disease and were not using any dietary supplements at the time of data collection. Participants were instructed to report to the laboratory ≥ 3 hours postprandial and fully hydrated having not consumed caffeine in the 12 hours or alcohol in the 24 hours preceding each experimental visit. Participants were however free to drink water *ad libitum* prior to, and during, experimental visits. All Participants were also instructed to attend the laboratory having not performed strenuous exercise in the 24 hours preceding each experimental visit. Experimental testing was performed at the same time of day (± 2 hours) for each participant. Participants were instructed to replicate their diet in the 24 hours prior to visit 1 in chapter 3 and 48 hours prior to visit 3 in chapter 4, for all subsequent experimental visits.

In chapter 4, participants were asked to refrain from consuming foods rich in NO_3^- (beetroot, lettuce, spinach, rocket and cured meats) for the entire duration of the

study. Participants were also instructed to avoid the use of antibacterial mouthwash for the duration of the study, as it is known to attenuate the reduction of NO_3^- to NO_2^- in the oral cavity (Govoni *et al.*, 2008), thus altering NO_3^- metabolism.

Supplementation procedure

Within chapter 4, dietary NO_3^- supplementation was administered in the form of a NO_3^- -rich beverage (PepsiCo Beet product, PepsiCo, USA). Participants were instructed to consume either a NO_3^- -rich (NIT; 100 mL · day⁻¹; 8 mmol NO_3^-) or NO_3^- -free (PLA; 100 mL · day⁻¹; PepsiCo placebo product, PepsiCo, USA) beverage for 5 days (1 x 100 mL · day⁻¹), in a double-blind randomised cross over design. The beverages were colour matched and provided in identical, code-labelled plastic bottles. They were isocaloric and sugar matched, however, they were not taste matched. In order to ensure full blinding of the supplementation procedure throughout the investigation, participants were deliberately misinformed the aim of the investigation was to compare the effect of two different NO_3^- containing beverages on performance. Follow up interviews with participants confirmed that they were unaware of the actual research hypothesis.

Participants were instructed to consume the supplement (1 x 100mL) at their scheduled visit time for days 1-4 of supplementation, and 2 hours prior to arrival at the laboratory on day 5 of supplementation so that they would consume one bolus every 24 hours. Consequently, experimental testing began 2 hours post supplementation to coincide with expected peak plasma [NO_2^-] (Wylie *et al.*, 2013a). A washout period of at least 5 days separated each supplementation period. Prior to the supplementation period, participants were informed that supplementation may cause temporary and harmless side effects including beeturia (red urine) and red stools.

Measurement procedure

Descriptive data

Prior to experimental testing, each participant's age, height (Seca Stadiometer SEC-225, Seca, Hamburg, Germany) and mass (Seca Digital Column Scale SEC-170, Seca, Hamburg, Germany) was recorded.

Blood pressure

In chapter 4, upon arrival to the laboratory, participants were seated in an isolated room for 3 mins before blood pressure of the brachial artery was measured using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, USA). Four measurements were taken and results for SBP, DBP and mean arterial pressure (MAP) were recorded from the automated sphygmomanometer. The first measurement was discounted and the mean of the final 3 measurements was used for data analysis.

Heart rate

Within chapter 4, heart rate (HR) was measured (Polar M400, Polar Electro, Finland) throughout the Yo-Yo IR1 exercise test performed in visits 2-5.

Exercise testing procedures

All exercise testing was performed indoors, on the same sports hall surface. The floor was swept to remove settled dust prior to each exercise test and participants were instructed to wear the same footwear for all exercise testing to ensure consistency.

In chapters 3 and 4, a standardised warm up was performed prior to exercise testing. Specifically, this consisted of 3 minutes continuous jogging followed by static stretches of the quadriceps, hamstring and gluteus maximus held for 10 s on the left and right side. Participants then performed 3 countermovement jumps (CMJs) followed by 4 change of direction runs (5 m straight line with 45° change of direction, including 2 x left and 2 x right) and 3 straight line 10 m sprints, all performed at 70% maximum effort. Overall, this was completed in ~8 mins.

For chapter 3, participants reported to the laboratory on 3 separate occasions and each time completed the exercise protocol as illustrated in figure 2.1 and described in full below. In brief, participants completed a series of maximal effort tests, including a RAT, COD t-test, 15 m sprint test and maximal CMJ test. For chapter 4, the first visit was for screening, visit 2 was used to complete the Yo-Yo IR1 test to exhaustion, and all subsequent visits followed the

exercise testing protocol illustrated in figure 2.1 and explained in detail below. The full exercise testing procedure comprised of a series of maximal effort tests, including a RAT, COD t-test, 15 m sprint test and maximal CMJ test. Following this, participants completed the Yo-Yo IR1 test to 90% of their predetermined maximum achievable distance, before then repeating the 15 m sprint and CMJ protocols.

For all exercise protocols, participants were instructed to start the attempt from a split stance with their left foot leading. The time taken to complete all exercise protocols was recorded using a timing gate system (Smartspeed, Fusion Sports, Australia) positioned with a 2 m wide running lane. Sprint times were recorded telemetrically to the nearest 0.001 s and all data was transmitted to a personal digital assistant (PDA). Prior to each protocol participants were verbally instructed to complete every exercise attempt with maximum effort.

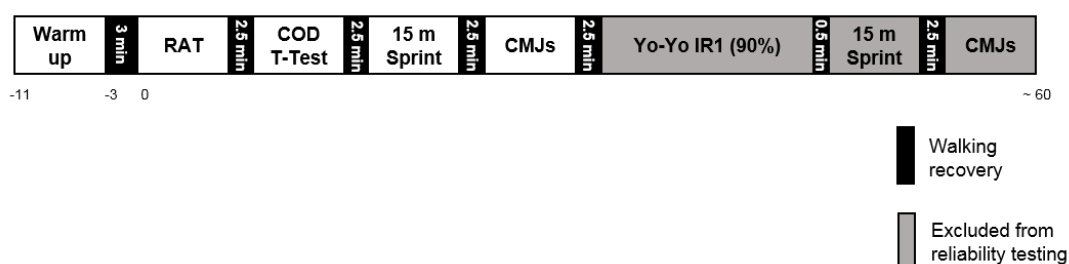


Figure 2.1: Exercise testing protocol for reliability and experimental investigation following nitrate supplementation (Chapters 3 and 4).

Yo-Yo IR1 test

The Yo-Yo IR1 test consists of repeated 20 m shuttle runs at a progressively increasing speed controlled by an audio recording, with each 40 m running bout separated by a 10 s active recovery period (Bangsbo *et al.*, 2008). When an exhaustive test was completed (visit 2, chapter 4), the test was terminated at volitional exhaustion or when participants failed to reach the 20 m marker on two consecutive runs. The distance covered was recorded and this represented the test result. For subsequent exercise visits, participants completed the Yo-Yo IR1 test to 90% of their predetermined maximum achievable distance.

Reactive agility test

Four timing gates (Smartspeed, Fusion Sport, Australia) were set up in a 'Y-Shape' formation (See Fig. 2.2). Participants completed 6 maximal effort attempts of this protocol, 3 to the left and 3 to the right, with all attempts starting from the start line 0.75 m behind the first timing gate. To begin, participants were instructed to sprint through the first two timing gates. Forty milliseconds after breaking the timing gate beam at 5 m, lights on either the left or right exit gate began to flash. Participants were required to react to this light stimuli and sprint through the illuminated gate to complete the test attempt. Participants were deliberately misinformed that the timing gate system would randomly allocate either a left or right attempt and therefore up to 8 attempts may be required to achieve 3 x left and 3 x right attempts. This was to ensure participants were unable to determine test direction for any attempt and a true measure of reactive agility could always be recorded. Total test time was recorded along with 0-5 and 5-10 m split times. Each attempt was separated by a 30 s walking recovery.

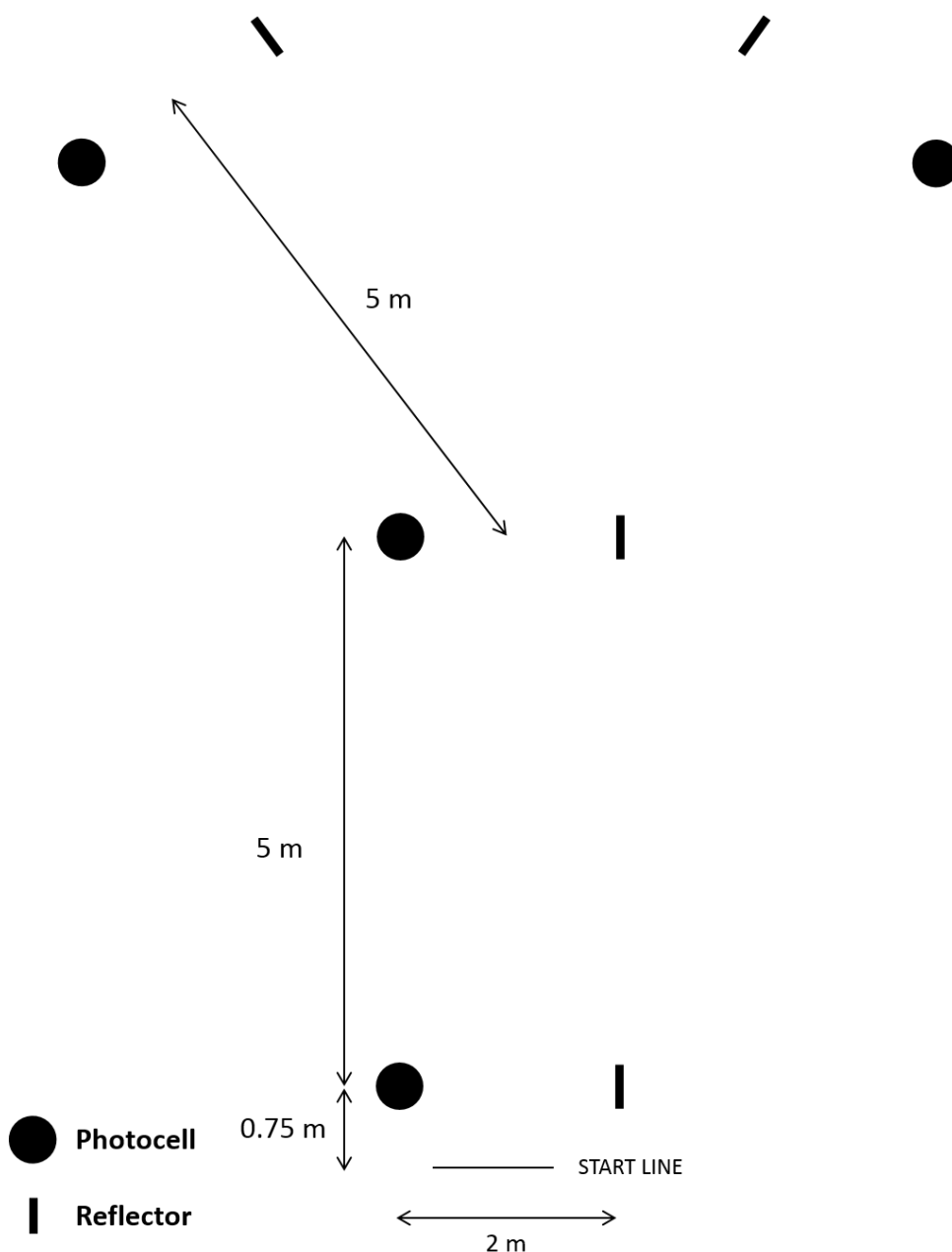


Figure 2.2: Reactive agility test protocol diagram.

Change of direction t-test

An adapted version of the t-test protocol (Semenick, 1990) was used with minor modifications (See Fig. 2.3). Specifically, a modified distance of 5 m was used and bells were used in place of cones (A, B and C). Subjects began the test 0.75 m behind the timing gate (Smartspeed, Fusion Sport, Australia) at the start line. Participants were instructed to sprint forwards and ring bell A by hand. They were then instructed to sidestep to point B and ring bell B, before sidestepping across to point C and ringing bell C, then sidestepping back to point A to ring bell A again. Participants were then instructed to sprint backwards from this point, through the

timing gate to the start line to complete the test. It was made clear to participants that a test attempt would be discounted if they crossed their legs in the sidestep movement or failed to ring the bells by hand at the specific points of the test. Three attempts were performed separated by 60 s walking recovery. Total test time was recorded.

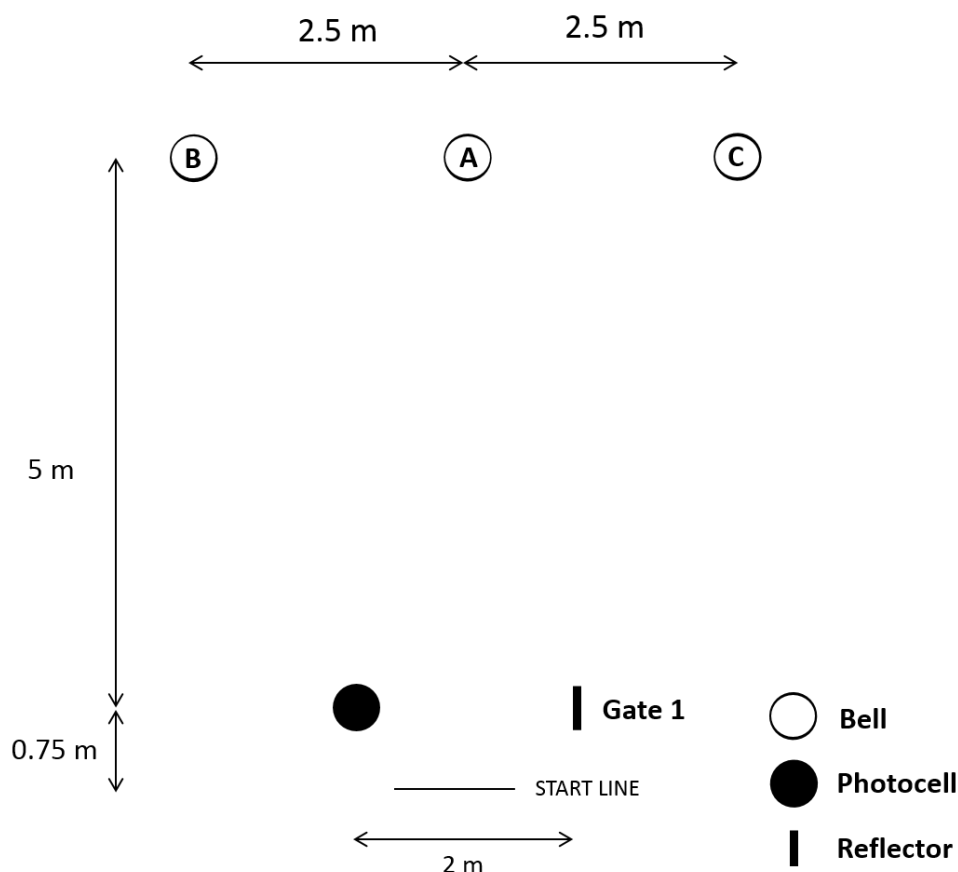


Figure 2.3: Change of direction t-test protocol diagram.

15 m linear sprint

Participants began each sprint with their left foot positioned on a starting jump mat (Smartspeed, Fusion Sports, Australia) as shown in figure 2.4. A timing gate system positioned at 0, 5, 10 and 15 m provided a randomly timed (0.2-2 s) buzzer and light stimuli to start each sprint. Participants were instructed to react to the light and buzzer stimuli and sprint the 15 m distance as quickly as possible. Five attempts were performed separated by a 30 s walking recovery. Total test time was recorded along with reaction time, 5, 10 and 15 m split times.

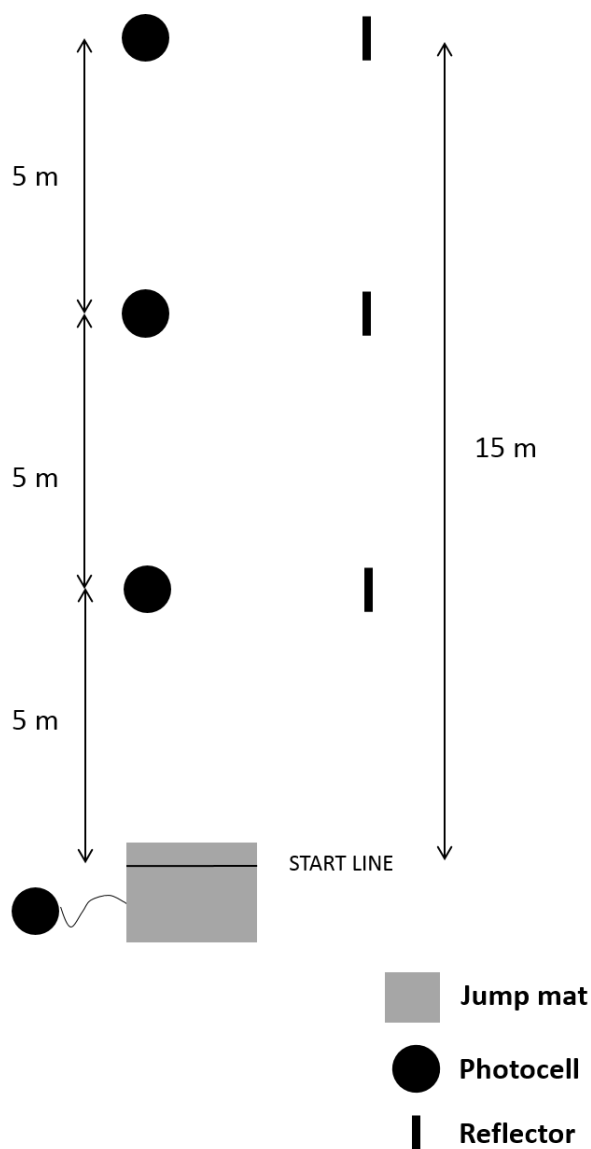


Figure 2.4: Linear sprint protocol diagram.

Countermovement jump

Participants completed 3 maximal CMJs on a jump mat (Smartspeed, Fusion Sport, Australia) each separated by 30 s standing recovery. Participants were instructed to start the movement with their feet shoulder-width apart and keep their hands on their hips throughout the test. In the countermovement phase participants reached a squat position with their upper leg parallel to the ground, they were instructed not to pause in this position but immediately perform a maximal jump. Participants were instructed to maintain extension in the knee and hip joints throughout the time spent in the air to minimise any additional flight time from bending the legs. Maximum jump height was recorded.

Statistical analysis

All statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 23. The specific statistical analysis conducted within each chapter is outlined where appropriate. All data are presented as mean \pm standard deviation (SD) unless otherwise stated. Statistical significance was accepted at $P < 0.05$.

CHAPTER 3: Reliability of exercise tests for agility, linear sprint and vertical jump performance in male team sports players

Abstract

Purpose: This investigation aimed to determine the day-to-day reliability of commonly used exercise protocols that measure agility, linear sprint and vertical jump performance. It also aimed to determine the most appropriate data, taken from multiple attempts of the same protocol, to perform statistical analysis on to yield the greatest reliability of test performance. **Methods:** Six male team sports players (age: 23 ± 1 years; height: 1.80 ± 0.03 m; weight: 80.92 ± 8.21 kg) volunteered to complete 3 identical experimental visits. Within each visit, participants completed a series of maximal effort tests including 6 attempts of a reactive agility test, 3 attempts of a change of direction t-test, 5 attempts of a 15 m linear sprint test and 3 attempts of a countermovement jump test. Reliability was assessed using intraclass correlation coefficients (ICC) and coefficient of variation (COV) between trials 2 and 3 and was performed on varying selections of test data to assess which data handling technique produced the lowest variability. **Results:** Test performances were considered most reliable when the mean of all 6 reactive agility test performances (COV: 2.0%; ICC: 0.96) and all 3 change of direction t-test performances were considered (COV: 2.9%; ICC: 0.87), and when the fastest linear sprint (COV: 1.0%; ICC: 0.98) and the highest countermovement jump performance (COV: 4.6%; ICC: 0.94) was considered independently. **Conclusion:** This study highlights the reliability of the selected exercise tests and the most reliable selection of data for each exercise protocol tested. Such information may be useful for informing data analysis of these particular exercise tests within subsequent intervention studies.

Introduction

Important determinants of performance within team sports game-play include agility, linear sprint and vertical jump performance (Wisløff *et al.*, 2004; Little & Williams, 2005; Gabbett, Kelly & Sheppard, 2008; Castagna & Castellini, 2013), with such movements performed maximally and repeatedly throughout the duration of a game. Various exercise protocols have been developed to assess such exercise performance in a controlled environment rather than within actual game-play (Currell & Jeukendrup, 2008; Singh *et al.*, 2010), which not only enables changes in performance across a season to be monitored, but also enables the effectiveness of an intervention on parameters of team sports performance to be assessed. To date, studies that investigate the effect of an intervention on such exercise performance rarely report the reliability of the exercise tests employed (Brughelli *et al.*, 2008). However, it is important to accurately assess the day-to-day reliability of specific exercise tests before they are selected to assess the efficacy of an intervention to ensure they are suitable to detect small changes in performance that may be meaningful to team sports performance (Hopkins, Hawley & Burke, 1999).

A commonly employed exercise test to measure planned agility performance is the change of direction (COD) t-test (Semenick, 1990). Although the reliability of t-test performance is not commonly reported within intervention studies that have employed this protocol (Brughelli *et al.*, 2008), where assessed, reliability determined by intraclass correlation coefficient (ICC) and coefficient of variation (COV) appears to be relatively consistent with an ICC of 0.82-0.83 and COV of ~2-3% reported (McBride *et al.*, 2002; Gabbett *et al.*, 2006; Hickey *et al.*, 2009; Sassi *et al.*, 2009; Munro & Herrington, 2011). However, the data used to perform this statistical analysis appears to vary greatly between studies with reports of a mean of 4/8 attempts, fastest attempt from 2 efforts and fastest 2 attempts from 8 efforts being used, for example. In addition to planned agility, reactive agility is also important for team sports performance (Gabbett *et al.*, 2008) and is commonly assessed using a reactive agility test (RAT; Farrow, Young & Bruce, 2005; Oliver & Meyers, 2009; Sheppard *et al.*, 2006; Gabbett & Benton, 2009). To our knowledge, Oliver and Meyers (2009) are the only authors to have assessed the reliability of RAT performance in response to a light stimuli, compared to other literature that used sports specific cues (Farrow, Young &

Bruce, 2005; Sheppard *et al.*, 2006; Gabbett & Benton, 2009). Oliver and Meyers (2009) reported a COV of 3.3% for RAT performance when the mean of the fastest 4 attempts (2 x left, 2 x right) was assessed. Due to such limited research regarding the reliability of these exercise protocols, it is important to assess this prior to their use within an intervention study.

Other common movements within team sports game-play include vertical jumping (Little & Williams, 2005; Castanga & Castellini, 2013) and all-out sprint running typically up to 10-20 m (Spencer *et al.*, 2004; 2005). The reliability of vertical jump performance appears to be varied with a COV reported between 2.0-5.2% (Gabbett *et al.*, 2006; Cormak *et al.*, 2008; Singh *et al.*, 2010) with studies using the highest jump from a varied number of attempts for statistical analysis. In addition, the reliability of a reactive start sprint has, to our knowledge, never been reported in the literature. However, the reliability of 5, 10 and 20 m standing start sprint performance has been assessed with a COV of 5.1%, 3.5% and 1.9% reported respectively for these distances (Lockie *et al.*, 2013). This appears to be consistent within previous literature that report the reliability of sprint performance (Cochrane, Legg & Hooker, 2004; Gabbett *et al.*, 2006; Sheppard *et al.*, 2006; Oliver & Meyers, 2009; Singh *et al.*, 2010; Till *et al.*, 2011). Interestingly, when the reliability of sprint performance was assessed as a COV within a simulated team sport circuit, it was reported to be 3.7% when the mean of 60 sprint performances was assessed, compared to 2.0% when absolute best sprint performance was assessed independently (Singh *et al.*, 2010). This is important as following test completion, the data handling techniques employed appear to affect the reliability of test performance. Within studies, it is common for multiple attempts of the same protocol to be completed yet the data selected for statistical analysis appears to be inconsistent. As the reliability of test performance appears to be altered depending on data handling technique, it is important this is considered for future analysis to ensure the lowest day-to-day variability in test performance can be achieved. It is also important to ensure our laboratory can at least match the reliability of these tests reported elsewhere as a reduced reliability of test performance may indicate a source of test error that would need to be eliminated before their use in an intervention study.

The aim of the current study was to examine the reliability of commonly employed exercise protocols to measure performance in key determinants of team sports performance; specifically, the day-to-day reliability of COD t-test,

RAT, 15 m linear sprint and CMJ performance. As researchers often ask participants to complete multiple attempts of the same task, yet the data selected for statistical analysis is variable, a second aim was to consider the most appropriate selection of test performance data for statistical analysis to yield the greatest reliability. This information will enable the selected exercise protocols suitability for use within an intervention study to be considered. It may also inform subsequent data analysis when using these protocols to ensure the greatest reliability of test performance is achieved.

Methods

Participants

Six competitive but non-elite male, team sports players (age: 23 ± 1 years; height: 1.80 ± 0.03 m; weight: 80.92 ± 8.21 kg) volunteered to participate in this study which was approved by the institutional ethics committee. This sample size reflects that employed by Samozino *et al.* (2016) where the reliability of parameters of sprint running performance was assessed. Written informed consent was obtained prior to beginning exercise testing, once the experimental procedures, potential benefits and associated risks were explained in full.

Experimental design

Participants reported to the laboratory on three separate occasions to complete experimental testing. All experimental visits were identical. Each visit was separated by a minimum of 48 hours and was performed at the same time of day (± 2 hours) to account for diurnal variation in high intensity exercise performance (Souissi *et al.*, 2007).

Participants were instructed to arrive at the laboratory ≥ 3 h post-prandial, having avoided strenuous exercise and alcohol consumption in the 24 h and caffeine 12 h prior to each visit. Participants were instructed to record their diet in the 24 h prior to the first visit and to replicate this in the 24 h preceding each subsequent visit.

Exercise protocol

All exercise visits were conducted indoors on the same floor surface and participants were instructed to wear the same footwear for all visits to ensure consistency. Upon arrival at the laboratory, participants completed a standardised warm up prior to commencing exercise testing.

Each exercise protocol was separated by a 2.5 min walking recovery. A timing gate system (Smartspeed, Fusion Sports, Australia) was used for all exercise protocols, arranged to allow a 2 m running lane. Time to complete each protocol was measured to the nearest 0.001 s. Participants were instructed to start every attempt from a split stance with their left foot leading. The exercise testing protocols were conducted as described below and have been illustrated in the general methods chapter of this thesis (see fig. 2.2-2.4).

RAT:

Four timing gates (Smartspeed, Fusion Sport, Australia) were set up in a 'Y-Shape' formation (See Fig. 2.2). Participants completed 6 maximal effort attempts of this protocol, 3 to the left and 3 to the right, with all attempts starting from the start line 0.75 m behind the first timing gate. To begin, participants were instructed to sprint through the first two timing gates. Forty milliseconds after breaking the timing gate beam at 5 m, lights on either the left or right exit gate began to flash. Participants were required to react to this light stimuli and sprint through the illuminated gate to complete the test attempt. Participants were deliberately misinformed that the timing gate system would randomly allocate either a left or right attempt and therefore up to 8 attempts may be required to achieve 3 x left and 3 x right attempts. This was to ensure participants were unable to determine test direction for any attempt and a true measure of reactive agility could always be recorded. Total test time was recorded along with 0-5 and 5-10 m split times. Each attempt was separated by a 30 s walking recovery.

COD t-test:

An adapted version of the t-test protocol (Semenick, 1990) was used with minor modifications (See Fig. 2.3). Specifically, a modified distance of 5 m was used and bells were used in place of cones (A, B and C). Subjects began the test 0.75 m behind the timing gate (Smartspeed, Fusion Sport, Australia) at the start line.

Participants were instructed to sprint forwards and ring bell A by hand. They were then instructed to sidestep to point B and ring bell B, before sidestepping across to point C and ringing bell C, then sidestepping back to point A to ring bell A again. Participants were then instructed to sprint backwards from this point, through the timing gate to the start line to complete the test. It was made clear to participants that a test attempt would be discounted if they crossed their legs in the sidestep movement or failed to ring the bells by hand at the specific points of the test. Three attempts were performed separated by 60 s walking recovery. Total test time was recorded.

15 m sprint:

Participants began each sprint with their left foot positioned on a starting jump mat (Smartspeed, Fusion Sports, Australia) as shown in figure 2.4. A timing gate system positioned at 0, 5, 10 and 15 m provided a randomly timed (0.2-2 s) buzzer and light stimuli to start each sprint. Participants were instructed to react to the light and buzzer stimuli and sprint the 15 m distance as quickly as possible. Five attempts were performed separated by a 30 s walking recovery. Total test time was recorded along with reaction time, 5, 10 and 15 m split times.

CMJ:

Participants completed 3 maximal CMJs on a jump mat (Smartspeed, Fusion Sport, Australia) each separated by 30 s standing recovery. Participants were instructed to start the movement with their feet shoulder-width apart and keep their hands on their hips throughout the test. In the countermovement phase participants reached a squat position with their upper leg parallel to the ground, they were instructed not to pause in this position but immediately perform a maximal jump. Participants were instructed to maintain extension in the knee and hip joints throughout the time spent in the air to minimise any additional flight time from bending the legs. Maximum jump height was recorded.

Statistical analysis

In order to assess the reliability of the exercise protocols above, statistical analysis was conducted between trial 2 and trial 3. This was conducted to reflect the trials that comprise the experimental conditions within the intervention study

of this thesis (Chapter 4) while also enabling trial 1 to act as a familiarisation and reduce the likelihood of systematic error within the data.

Paired sampled t-tests were used to determine whether there were any differences in performance between trials 2 and 3. Statistical significance was accepted at $P < 0.05$. The ICC and 95% confidence interval and COV and 95% confidence interval was calculated using the log-transformed data as proposed by Hopkins (2000). Respective mean and standard deviation (SD) values are reported as non-transformed data.

Results

All exercise visits were separated by a minimum of 48 hours with the mean \pm SD time between each trial 7 ± 4 days.

There was no significant difference in performance between trials 2 and 3 for any exercise test ($P > 0.05$).

RAT:

The COV and ICC between trial 2 and trial 3 for RAT performance are reported in table 3.1 as independent and combined left and right attempts. The absolute COV was lowest when all 6 attempts (3 x left, 3 x right) were considered together (COV [95% CI]: 2.0% [1.3 - 5.1]), illustrated in figure 3.1. RAT split times for all 6 attempts (3 x left, 3 x right) are reported in table 3.2.

COD t-test:

The COV and ICC between trial 2 and trial 3 for change of direction t-test performance are reported in table 3.3. Overall, t-test performance was considered most repeatable when the mean of all 3 attempts was considered (COV [95% CI]: 2.9% [1.8 - 7.1]), illustrated in figure 3.2.

15 m sprint:

The COV and ICC between trial 2 and trial 3 for 15 m sprint performance are reported in table 3.4. Absolute COV was lowest when only the fastest sprint per attempt was considered independently (COV [95% CI]: 1.0 % [0.6 - 2.4]),

illustrated in figure 3.3. Taking the fastest attempt, analysis of sprint split times was conducted and are reported in table 3.5.

CMJ:

The COV and ICC between trial 2 and trial 3 for CMJ performance are reported in table 3.6. The absolute lowest COV was reported when the highest jump performance was analysed independently (COV [95% CI]: 4.6% [2.8 - 11.6]), illustrated in figure 3.4.

Table 3.1: The coefficient of variation (95% confidence interval) and intraclass correlation coefficient (95% confidence interval) between trial 2 and trial 3 for reactive agility performance.

Average of:	Trial 2		Trial 3		COV 95% CI	ICC	ICC 95% CI
	Time (s) Mean ± SD	Time (s) Mean ± SD	COV (%)	COV (%)			
All 6 attempts (3 x L, 3 x R)	2.68 ± 0.17	2.68 ± 0.19	2.0	2.0	1.3 - 5.1	0.96	0.74 - 0.99
Fastest 4 attempts (2 x L, 2 x R)	2.63 ± 0.16	2.65 ± 0.19	2.2	2.2	1.3 - 5.4	0.95	0.70 - 0.99
Fastest 2 attempts (1 x L, 1 x R)	2.60 ± 0.18	2.61 ± 0.18	2.8	2.8	1.7 - 6.9	0.92	0.54 - 0.99
All 3 Left	2.71 ± 0.17	2.67 ± 0.18	2.4	2.4	1.5 - 5.9	0.94	0.64 - 0.99
Fastest 2 Left	2.66 ± 0.18	2.64 ± 0.18	2.9	2.9	1.8 - 7.2	0.90	0.47 - 0.99
Fastest Left	2.62 ± 0.20	2.60 ± 0.18	3.6	3.6	2.3 - 9.2	0.86	0.29 - 0.98
All 3 Right	2.65 ± 0.18	2.68 ± 0.20	2.4	2.4	1.5 - 6.0	0.95	0.68 - 0.99
Fastest 2 Right	2.61 ± 0.16	2.66 ± 0.20	2.4	2.4	1.5 - 6.0	0.95	0.67 - 0.99
Fastest Right	2.58 ± 0.18	2.62 ± 0.19	2.9	2.9	1.8 - 7.3	0.92	0.53 - 0.99

Values are reported as mean time (s) ± SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence interval; L = left, R = right.

Table 3.2: The coefficient of variation (95% confidence interval) and intraclass correlation coefficient (95% confidence interval) between trial 2 and trial 3 for split times of reactive agility performance. Data of all 6 attempts (3 x left and 3 x right) was included.

Average of:	Trial 2		Trial 3		COV (%)	COV 95% CI	ICC	ICC 95% CI
	Time (s)	Mean \pm SD	Time (s)	Mean \pm SD				
0 - 5 m split		1.29 \pm 0.10		1.28 \pm 0.09	3.3	2.0 - 8.3	0.89	0.41 - 0.98
5 - 10 m split		1.39 \pm 0.10		1.40 \pm 0.12	1.9	1.2 - 4.7	0.98	0.84 - 1.00

Values are reported as mean time (s) \pm SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence interval

Table 3.3: The coefficient of variation (95% confidence interval) and intraclass correlation (95% confidence interval) between trial 2 and trial 3 for change of direction t-test performance.

Average of:	Trial 2		Trial 3		COV (%)	COV 95% CI	ICC	ICC 95% CI
	Time (s)	Mean \pm SD	Time (s)	Mean \pm SD				
All 3 attempts		7.07 \pm 0.40		7.01 \pm 0.41	2.9	1.8 - 7.1	0.87	0.34 - 0.98
Fastest 2 attempt		7.01 \pm 0.41		6.98 \pm 0.43	3.1	1.9 - 7.8	0.85	0.27 - 0.98
Fastest attempt		6.94 \pm 0.41		6.97 \pm 0.44	3.4	2.1 - 8.6	0.82	0.16 - 0.97

Values are reported as mean time (s) \pm SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence interval

Table 3.4: The coefficient of variation (95% confidence interval) and intraclass correlation (95% confidence interval) between trial 2 and trial 3 for total 15 m linear sprint performance.

Average of:	Trial 2		Trial 3		COV (%)	COV 95% CI	ICC	ICC 95% CI
	Time (s)	Mean \pm SD	Time (s)	Mean \pm SD				
All 5 attempts		3.34 \pm 0.14		3.29 \pm 0.13	1.1	0.7 - 2.8	0.97	0.80 - 1.00
Fastest 4 attempts		3.33 \pm 0.14		3.28 \pm 0.13	1.1	0.7 - 2.8	0.97	0.81 - 1.00
Fastest 3 attempts		3.32 \pm 0.15		3.28 \pm 0.13	1.3	0.8 - 3.1	0.96	0.76 - 0.99
Fastest 2 attempts		3.30 \pm 0.15		3.27 \pm 0.13	1.2	0.7 - 2.9	0.97	0.80 - 1.00
Fastest attempt		3.29 \pm 0.15		3.26 \pm 0.14	1.0	0.6 - 2.4	0.98	0.87 - 1.00

Values are reported as mean time (s) \pm SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence interval

Table 3.5: The coefficient of variation (95% confidence interval) and intraclass correlation (95% confidence interval) between trial 2 and trial 3 for split times within 15 m linear sprint performance. Split times taken from fastest overall sprint data.

Average of:	Trial 2		Trial 3		COV 95% CI	ICC	ICC 95% CI
	Time (s) Mean ± SD	Time (s) Mean ± SD	COV (%)	COV (%)			
Reaction time	0.35 ± 0.28	0.32 ± 0.32	60.3	60.3	34.3 - 218.3	0.42	-0.49 - 0.89
5 m split	1.73 ± 0.08	1.71 ± 0.07	1.8	1.8	1.1 - 4.5	0.92	0.54 - 0.99
10 m split	2.54 ± 0.12	2.51 ± 0.11	1.7	1.7	1.1 - 4.3	0.92	0.56 - 0.99

Values are reported as mean time (s) ± SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence Interval

Table 3.6: The coefficient of variation (95% confidence interval) and intraclass correlation (95% confidence interval) between trial 2 and trial 3 for countermovement jump performance.

Average of:	Trial 2		Trial 3		COV 95% CI	ICC	ICC 95% CI
	Height (cm) Mean ± SD	Height (cm) Mean ± SD	COV (%)	COV (%)			
All 3 attempts	36.2 ± 4.8	35.2 ± 3.6	6.2	6.2	3.8 - 15.8	0.86	0.29 - 0.98
Highest 2 attempt	37.1 ± 5.0	36.7 ± 4.2	5.2	5.2	3.2 - 13.2	0.92	0.56 - 0.99
Highest attempt	37.6 ± 4.8	37.3 ± 4.2	4.6	4.6	2.8 - 11.6	0.94	0.63 - 0.99

Values are reported as mean height (cm) ± SD. COV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence Interval

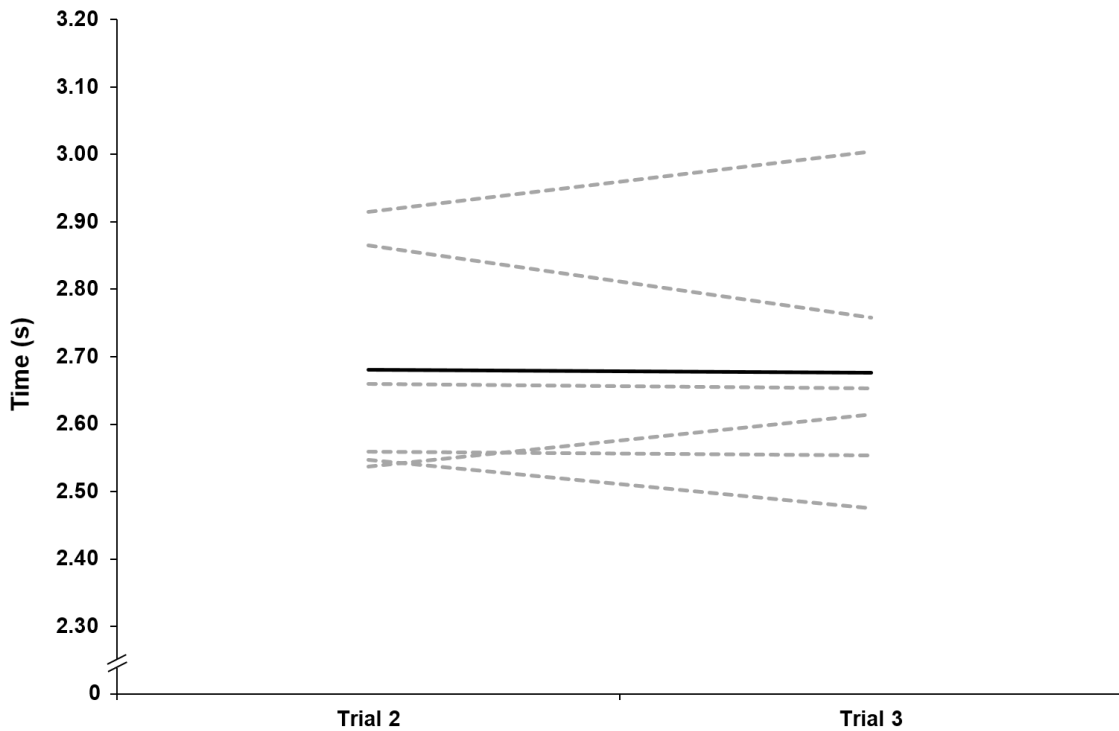


Figure 3.1: Mean reactive agility test performance from all 6 attempts. The dashed lines indicates individual performance times and the solid line indicates the group mean.

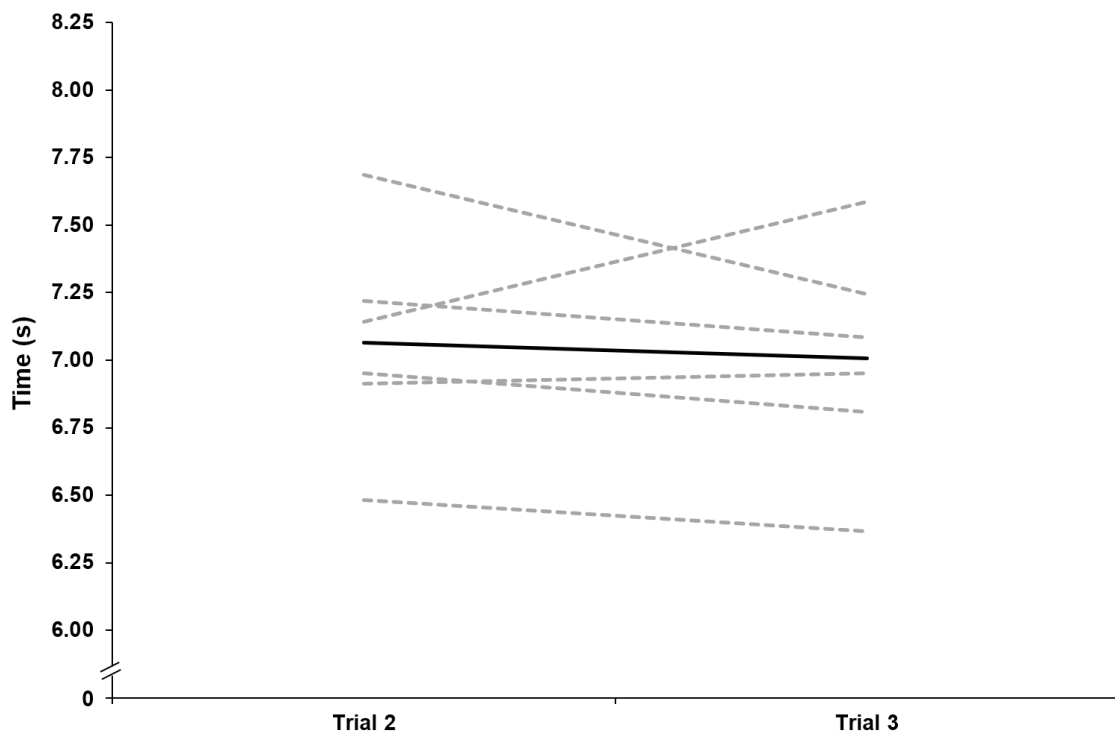


Figure 3.2: Mean change of direction t-test performance from all 3 attempts. The dashed lines indicates individual performance times and the solid line indicates the group mean.

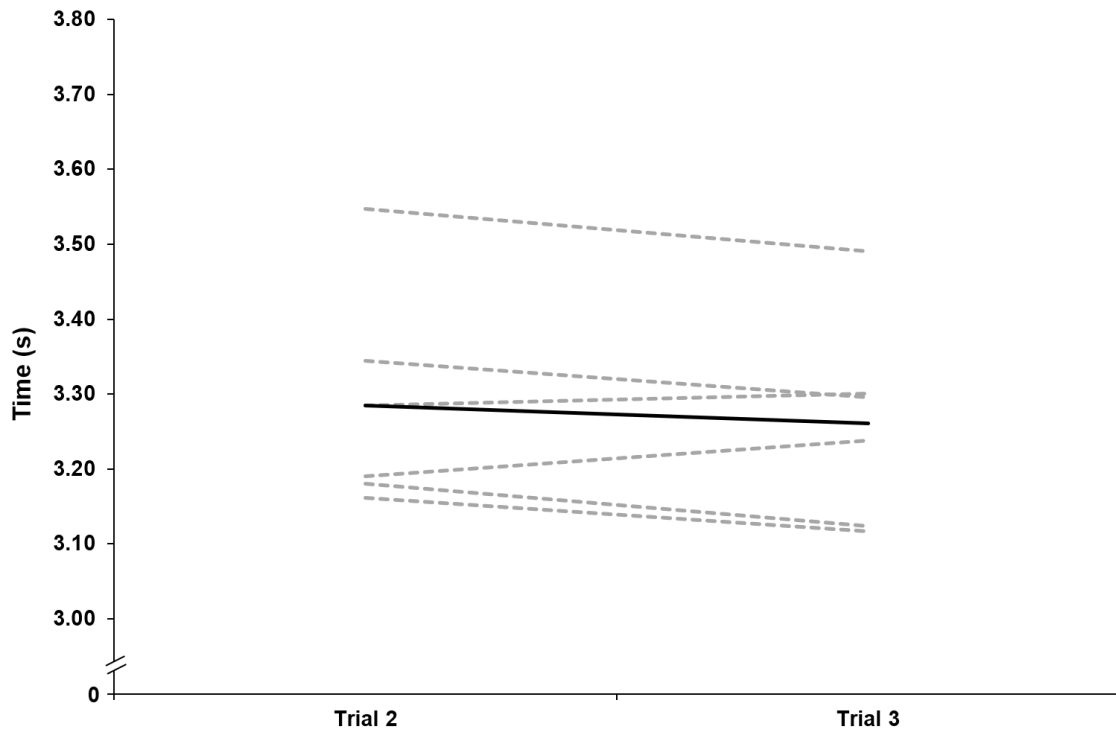


Figure 3.3: Fastest 15 m sprint performance from 5 attempts. The dashed lines indicates individual performance times and the solid line indicates the group mean.

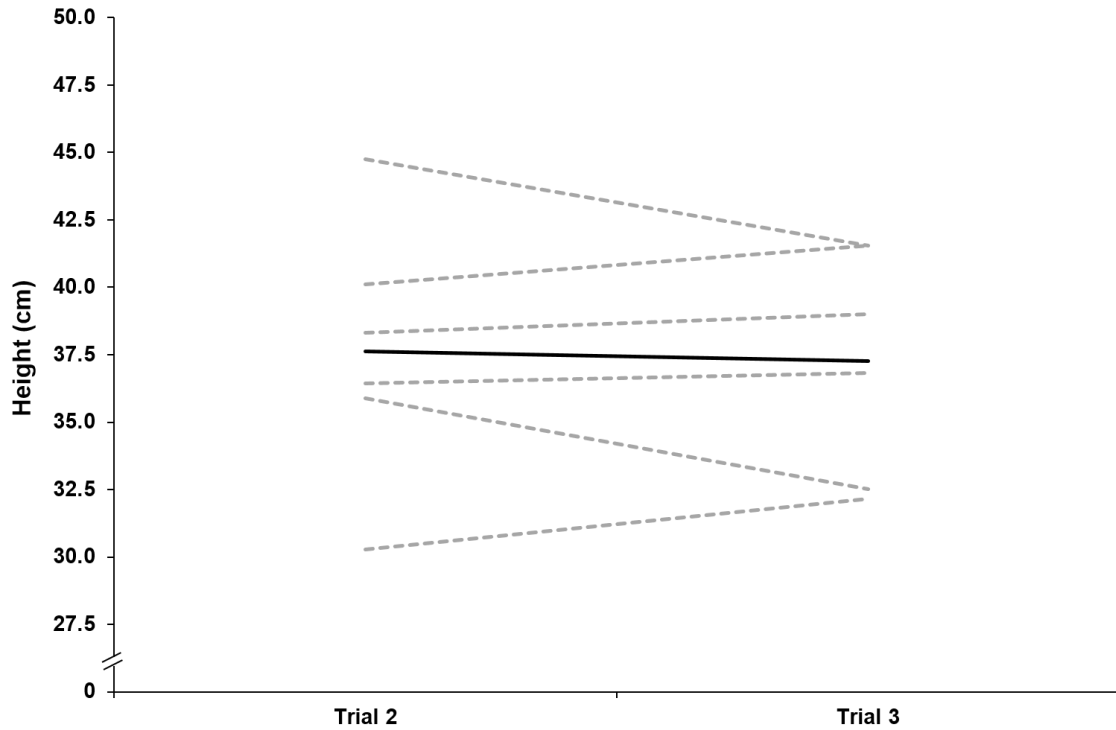


Figure 3.4: Highest countermovement jump performance from 3 attempts. The dashed lines indicates individual performance times and the solid line indicates the group mean.

Discussion:

The aim of the current study was to examine the reliability of commonly employed exercise protocols used to measure performance in key determinants of team sports performance, and, to consider the most appropriate selection of data from multiple test attempts to yield the greatest reliability. All exercise protocols were considered reliable and in line with that reported elsewhere in the literature for the same exercise protocols. Test performances were considered most reliable when the mean of all 6 RAT performances and all 3 COD t-test performances were considered, and when the fastest 15 m linear sprint and the highest CMJ performance was considered independently.

Change of direction t-test

The reliability of t-test performance is not commonly reported within intervention studies that employ this exercise protocol (Brughelli *et al.*, 2008), however, it appears to remain consistent when reported. When reliability was assessed as an ICC it was reported between 0.82-0.83 and as a COV between ~2-3% (McBride *et al.*, 2002; Gabbett *et al.*, 2006; Hickey *et al.*, 2009; Sassi *et al.*, 2009; Munro & Herrington, 2011; Steward, Turner & Miller, 2014). In agreement with that reported elsewhere, reliability assessed as an ICC was 0.87 and as a COV was 2.9% in the present study. Change of direction t-test performance was most reliable when the mean of all three attempts was considered for statistical analysis. As the data handling processes employed within the literature does not appear to be consistent, this is important information to take forward to inform the data handling processes of subsequent studies to obtain the greatest reliability.

Reactive agility test

Several previous studies have assessed the reliability of the RAT, either in response to a sports specific (Farrow *et al.*, 2005; Sheppard *et al.*, 2006) or a light stimuli (Oliver & Meyers, 2009). In agreement with Oliver and Meyers (2009), RAT performance in the present study was considered most reliable when reported as the mean of left and right attempts combined, rather than separating movements to the left and right independently. The COV reported within the present study for the mean of all 6 attempts was 2.0%, compared to the 3.3%

reported previously (Oliver & Meyers, 2009) where the mean of 4 attempts (2 x left, 2 x right) of the same protocol was evaluated. In addition, reliability assessed as an ICC was 0.96 in the present study. This is comparable to the reliability of RAT performance in response to a sports specific stimuli evaluated by Farrow *et al.* (2005) and Sheppard *et al.* (2006) where an ICC of 0.83 and 0.88 were reported, respectively. In conclusion, RAT performance in the present study was considered most reliable when a mean of all 6 attempts was used for statistical analysis.

15 m linear sprint

To our knowledge, this is the first study to investigate the reliability of sprint performance including a reactive start. Fifteen meter sprint performance was considered most reliable when the fastest total sprint time (COV = 1.0%) was used independently for data analysis compared to other data handling techniques assessed such as the mean of all 5 (COV = 1.1%) or a selection of fastest sprint attempts (COV = 1.1-1.3%). Comparisons can be drawn between the reliability of sprint performance in the present study and that reported by Singh *et al.* (2010) who also reported 15 m sprint performance was most reliable when the single fastest sprint was used for analysis compared to the mean of all sprints when completed within a team sports specific circuit. Reliability of sprint performance assessed as a COV was 1.0% in the present study compared to 2.0% reported by Singh *et al.* (2010).

Surprisingly, when sprint split times were assessed, reaction time was highly variable with a COV of 60.3% and ICC of 0.42. However, overall 15 m sprint reliability as a COV (1.0%) was comparable to that reported by Singh *et al.* (2010) when no reactive start was employed (2.0%) indicating that the reactive element did not negatively affect the reliability of the sprint test. Supporting this, split time reliability was also comparable to the literature. When 5 m split time was assessed, reliability was calculated as an ICC of 0.92 and COV of 1.8%, similar to the ICC of 0.89 reported by Cochrane *et al.* (2004) and higher than the ICC of 0.76 and lower than the COV of 5.1% reported by Lockie *et al.* (2013). Similarly, 10 m split time reliability was calculated in the present study to have an ICC of 0.92 and COV of 1.7% which is comparable to the literature where an ICC between 0.85-0.95 and COV of between 1.8-3.5% have been reported (Cochrane

et al., 2004; Gabbett *et al.*, 2006; Lockie *et al.*, 2013). It was therefore considered that the reactive start sprint was a highly reliable test and consequently a suitable protocol to employ in a future intervention study.

Countermovement jump

The reliability of CMJ performance within the present study was greatest when the highest vertical jump was considered independently. When assessed as an ICC, reliability was 0.94 and the COV was 4.6% in the present study. The better reliability using solely the highest jump is in agreement with Singh *et al.* (2010) who reported jump performance reliability to be better when the highest jump was considered alone (COV = 2.7%) compared to the mean of multiple attempts performed (COV = 4.3%). The reliability of CMJ performance in the present study, although showing slightly more variation day-to-day than that reported previously by Gabbett *et al.* (2006; COV = 2.9%, ICC = 0.96) and Singh *et al.* (2008; COV = 2.7%, ICC = 0.99), is comparable to the COV of 5.1% reported by Cormak *et al.* (2008) when performance was measured using a force plate in a similar population of team sports athletes. It is noteworthy that within the literature, different participant populations, jump techniques and measurement devices have been employed, which may account for some of the variation in jump test reliability reported within the literature. Countermovement jump performance reliability in the present study is comparable to that reported elsewhere in the literature, and, was considered most reliable when the highest jump out of 3 efforts was used for statistical analysis alone.

Conclusion

In conclusion, this study highlights the reliability of selected exercise tests for planned and unplanned agility, linear sprint and vertical jump performance and indicates the most reliable selection of data for statistical analysis for each protocol. Specifically, the most reliable data handling procedure was achieved when the single fastest 15 m linear sprint out of 5 attempts, and the highest CMJ out of 3 attempts was considered independently, and when the mean of all 3 COD t-test and all 6 RAT attempts were considered. Such findings may provide important information for informing data analysis within these exercise protocols in subsequent intervention studies to yield the greatest possible reliability.

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CHAPTER 4: No improvement in agility, linear sprint or vertical jump performance following nitrate supplementation

Abstract

Purpose: This investigation tested the hypothesis that nitrate (NO_3^-) supplementation would improve planned and unplanned change of direction, linear sprint and vertical jump performance in an unfatigued state, and secondly, would attenuate the decline in performance following fatiguing exercise that mimicked the high-intensity intermittent exercise demands of team sport game-play. **Methods:** In a double blind, randomised and balanced crossover design, 32 male team sports players received either a NO_3^- -rich (NIT; $100 \text{ mL} \cdot \text{day}^{-1}$; 8 mmol NO_3^-) or NO_3^- -free (PLA; $100 \text{ mL} \cdot \text{day}^{-1}$) beverage for 5 days. On day 5 of supplementation, participants completed a series of maximal effort tests for reactive agility (reactive agility test), planned agility (change of direction t-test), 15 m sprint and countermovement jump performance. The 15 m sprint and countermovement jump protocols were completed at rest and following the Yo-Yo intermittent recovery level 1 test to 90% predetermined maximum achievable distance. **Results:** NO_3^- supplementation did not improve reactive agility (NIT: $2.64 \pm 0.21 \text{ s}$ vs PLA: $2.65 \pm 0.17 \text{ s}$, $P > 0.05$), change of direction t-test (NIT: $7.12 \pm 0.71 \text{ s}$ vs PLA: $7.10 \pm 0.76 \text{ s}$, $P > 0.05$), 15 m sprint (NIT: $3.20 \pm 0.21 \text{ s}$ vs PLA: $3.22 \pm 0.21 \text{ s}$, $P > 0.05$) or countermovement jump (NIT: $36.4 \pm 6.6 \text{ cm}$ vs PLA: $37.0 \pm 6.8 \text{ cm}$, $P > 0.05$) performance in an unfatigued state. In a fatigued state, 15 m sprint (NIT: $3.27 \pm 0.25 \text{ s}$ vs PLA: $3.27 \pm 0.25 \text{ s}$, $P > 0.05$) and countermovement jump (NIT: $36.7 \pm 7.2 \text{ cm}$ vs PLA: $36.5 \pm 7.0 \text{ cm}$, $P > 0.05$) performance were also unaltered following NO_3^- supplementation. Performance declined in a fatigued compared to unfatigued state for 15 m sprint performance ($P < 0.05$) but was unchanged for countermovement jump performance ($P > 0.05$). NO_3^- supplementation did not attenuate the decline in fatigued 15 m sprint performance ($P > 0.05$). **Conclusion:** NO_3^- supplementation did not improve reactive agility, change of direction t-test, linear sprint or vertical jump performance when performed in an unfatigued state. NO_3^- supplementation also did not attenuate the decline in 15 m sprint performance in a fatigued state. Therefore it may be suggested that NO_3^- supplementation is not ergogenic for these particular determinants of team sports performance.

Introduction

Recent studies have reported improvements in intermittent exercise (Wylie *et al.*, 2013b; Thompson *et al.*, 2015) as well as all-out sprint running (Sandbakk *et al.*, 2015; Thompson *et al.*, 2016) performance following nitrate (NO_3^-) supplementation. Since team sports match-play can, in part, be characterised by such exercise performance (Bangsbo, 1994; Spencer *et al.*, 2004), these findings may suggest NO_3^- supplementation could be ergogenic for team sports performance.

Following the ingestion of NO_3^- , it is reduced in a stepwise fashion to nitrite (NO_2^-) and then preferentially to nitric oxide (NO; Benjamin *et al.*, 1994; Lundberg *et al.*, 1994) and it is this increase in NO_2^- and NO that likely mediates the positive physiological responses and enhanced exercise capacity reported following such supplementation (e.g. Bailey *et al.*, 2009; Lansley *et al.*, 2011). Importantly, the production of NO via this pathway is potentiated in hypoxic and acidic environments (Lundberg, Weitzberg & Gladwin, 2008; van Faassen *et al.*, 2009), such as that within contracting skeletal muscle. Therefore, this pathway may be an increasingly important source of NO production during exercise where the production of NO through the oxidation of L-Arginine is reduced (Lundberg & Weitzberg, 2010). Interestingly, NO_3^- supplementation has been suggested to preferentially enhance the physiological responses of type II muscle fibres to exercise (Hernández *et al.*, 2012; Ferguson *et al.*, 2013; 2015; Ivarsson *et al.*, 2016). Improvements in intermittent sprint (Thompson *et al.*, 2015; Wylie *et al.*, 2016) and single sprint (Sandbakk *et al.*, 2015; Thompson *et al.*, 2016) exercise performance following NO_3^- supplementation may therefore be attributed, in part, to the fact such exercise recruits a high portion of type II fibres (Greenhaff *et al.*, 1994; Krusturp *et al.*, 2006). Given the preferential effect of NO_3^- supplementation on type II muscle fibres, it may be particularly well placed to improve exercise performance in other exercise that recruits a high portion of type II fibres such as that requiring high force generation and muscle contraction speeds (Bottinelli *et al.*, 1996) like rapid changing of direction and vertical jumping.

Single effort sprint as well as agility and vertical jump performance are hallmarks of team sports performance (Wisløff *et al.*, 2004; Little & Williams, 2005; Gabbett, Kelly & Sheppard, 2008; Castagna & Castellini, 2013). Multiple, maximal effort sprints of 10-20 m (Spencer *et al.*, 2004; 2005) are commonly

performed throughout the duration of a game (Little & William, 2005; Gabbett *et al.*, 2006) with >50% of sprints occurring after >60 s recovery period (Spencer *et al.*, 2004) and with ~16% of sprints involving at least one change of direction (Duthie *et al.*, 2006). Following NO_3^- supplementation, sprint performance has been reported to be enhanced in some (Sandbakk *et al.*, 2015; Thompson *et al.*, 2016) but not all (Muggeridge *et al.*, 2013; Christensen, Nyberg & Bangsbo, 2013; Martin *et al.*, 2014) previous studies. Thompson *et al.* (2016) reported an improvement in 20 m linear sprint performance of 1.2% in an unfatigued state following 5 days of NO_3^- supplementation ($6.4 \text{ mmol} \cdot \text{day}^{-1}$). When the sprint was separated into split times, performance was enhanced 1.6% over the first 10 m split and 2.3% over the first 5 m split (Thompson *et al.*, 2016), suggesting the ergogenic effect of NO_3^- supplementation is more pronounced in the initial acceleration phase of the sprint. This may be attributed to the enhanced contractile properties of skeletal muscle (Bailey *et al.*, 2010), enhanced evoked explosive force production (Haider and Folland, 2014) and power during voluntary exercise (Coggan *et al.*, 2015; Rimer *et al.*, 2016) reported elsewhere in the literature following NO_3^- supplementation. Taken together, this research provides reason to suggest that NO_3^- may be ergogenic within movements that involve repeated acceleration bouts such as within planned and unplanned change of direction tasks. Additionally, it may also suggest NO_3^- supplementation could be ergogenic within explosive movements such as vertical jumping. However, these possibilities have yet to be investigated.

Within team sports game-play, sprint and jump movements are not only performed in an unfatigued state but also under fatigue, where the ability to perform such high intensity explosive exercise is reduced (Mohr *et al.*, 2004). Interestingly, NO_3^- supplementation has been suggested to reduce fatigue development and therefore muscle metabolic perturbation by reducing the ATP cost of skeletal muscle contraction and sparing the rate of PCr depletion (Bailey *et al.*, 2010). In addition, NO_3^- supplementation has been reported to facilitate the O_2 dependent restoration of PCr (Vanhatalo *et al.*, 2011). This is important as the rate of PCr depletion is a significant determinant of fatigue development during maximal intensity exercise (Gaitanos *et al.*, 1993; Fulford *et al.*, 2013). Overall, fatigue development may be reduced with NO_3^- supplementation and therefore the performance decline expected in subsequent exercise bouts may be attenuated. However, the effect of NO_3^- supplementation on key parameters of

team sports performance, such as linear sprinting and vertical jumping when fatigued, has yet to be investigated.

The purpose of this study was to investigate the effect of NO_3^- supplementation on key determinants of team sports performance, specifically, agility, linear sprint and vertical jump performance in an unfatigued state, and linear sprint and vertical jump performance in a fatigued state to simulate the nature of exercise performed within match-play. It was hypothesised that: 1) dietary NO_3^- supplementation would improve agility, linear sprint and vertical jump performance in an unfatigued state; and 2) attenuate the decline in linear sprint and vertical jump performance when performed in a fatigued state.

Methods

Participants

Thirty two male team-sports players (mean \pm SD; age: 21 ± 3 years, height: 1.79 ± 0.06 m; weight: 81.8 ± 15.2 kg) volunteered to participate in this study which was approved by the institutional research ethics committee. All volunteers were screened to ensure they were non-smokers, free from disease, and were not taking any dietary supplements prior to recruitment. All participants gave their written informed consent to participate once the experimental procedures, associated risks and potential benefits of participation were explained in full.

Experimental design

Participants reported to the laboratory on 5 occasions in total. In the first laboratory visit participants were screened, and once enrolled onto the study reported to the laboratory for visit 2 and completed the Yo-Yo IR1 test to exhaustion. During visit 3, participants completed the full experimental protocol (see full details below) without any prior supplementation which acted as a familiarisation. Then in a double blind, balanced, repeated measures design, participants were allocated to receive either a NO_3^- -rich or a NO_3^- -free beverage to consume for 5 days, with a minimum 5 day washout period separating each supplementation period. On day 5 of supplementation, participants returned to the laboratory to complete the full experimental protocol.

The full experimental protocol completed in visits 3-5 comprised of a resting blood pressure (BP) measurement then a series of maximal effort tests, including a reactive agility test (RAT), change of direction (COD) t-test, 15 m sprint test and maximal countermovement jump (CMJ) test. Following this, participants completed the Yo-Yo IR1 test to 90% of their predetermined maximum achievable distance, before repeating the 15 m sprint and CMJ protocols. A detailed description of each exercise test protocol is provided in the exercise protocol section below.

During the investigation, participants were deliberately misinformed that visit 3 was a control condition rather than a familiarisation and that the aim of the investigation was to compare the effect of two different NO_3^- containing beverages to this control condition. This misinformation was relayed to participants to ensure full blinding of the supplementation procedure was achieved for the duration of the investigation. Follow up interviews with participants confirmed that they were unaware of the actual research hypothesis.

All experimental visits were scheduled at the same time of day (± 2 h). Participants were instructed to arrive at the laboratory ≥ 3 h post-prandial, having avoided strenuous exercise and alcohol consumption in the 24 h and caffeine 12 h prior to each visit. Participants were instructed to record their diet in the 48 h prior to the familiarisation visit and to replicate this in the 48 h preceding each subsequent visit. For the duration of the study participants were instructed to avoid foods rich in NO_3^- (e.g., beetroot, spinach, rocket, kale and cured meats), and to refrain from consuming any other dietary supplements. Participants were also instructed to abstain from the use of antibacterial mouthwash and chewing gum for the entire study duration as this is known to alter the reduction of NO_3^- to NO_2^- in the oral cavity and thus effect NO_3^- metabolism (Govoni *et al.*, 2008).

Supplementation

Following initial screening and familiarisation to the experimental protocol, participants were allocated either a NO_3^- -rich (NIT; 100 mL \cdot day $^{-1}$; 8 mmol NO_3^- ; PepsiCo Beet product, PepsiCo, USA) or NO_3^- -free (PLA; 100 mL \cdot day $^{-1}$; PepsiCo placebo product, PepsiCo, USA) beverage for 5 days in a double-blind randomised cross over design. Participants consumed 1 x 100 mL each day with the final dose consumed 2 h prior to arrival at the laboratory for exercise testing.

Consequently, physiological testing begun 2 h post supplementation to coincide with expected peak plasma [NO₂⁻] (Wylie *et al.*, 2013a). A washout period of at least 5 days separated each supplementation period. Compliance to the supplementation regime was assessed through the completion of a supplementation log during each supplementation period and with questionnaires during each experimental visit.

Measurements

Blood pressure

Upon arrival at the laboratory, participants were seated in an isolated room for 3 min before 4 BP measurements of the brachial artery were taken. (Dinamap Pro, GE Medical Systems, Tampa, USA). The first measurement was discounted and statistical analysis was performed on the final 3 measurements

Exercise protocol

All exercise visits were conducted indoors on the same floor surface and participants were instructed to wear the same footwear for all visits. Before commencing exercise testing, participants completed a standardised warm up. Participants then completed the exercise protocols described below, interspersed with 2.5 min walking recovery. A timing gate system (Smartspeed, Fusion Sports, Australia) was used for all exercise protocols and performance was recorded to the nearest 0.001 s. Timing gates were arranged to allow a 2 m running lane. Participants were instructed to start every attempt from a split stance with their left foot leading. For the duration of the Yo-Yo IR1 test in visits 2-5, heart rate (HR) was measured (Polar M400, Polar Electro, Finland).

RAT:

Four timing gates (Smartspeed, Fusion Sport, Australia) were set up in a 'Y-Shape' formation (See Fig. 2.2). Participants completed 6 maximal effort attempts of this protocol, 3 to the left and 3 to the right, with all attempts starting from the start line 0.75 m behind the first timing gate. To begin, participants were instructed to sprint through the first two timing gates. Forty milliseconds after breaking the timing gate beam at 5 m, lights on either the left or right exit gate began to flash.

Participants were required to react to this light stimuli and sprint through the illuminated gate to complete the test attempt. Participants were deliberately misinformed that the timing gate system would randomly allocate either a left or right attempt and therefore up to 8 attempts may be required to achieve 3 x left and 3 x right attempts. This was to ensure participants were unable to determine test direction for any attempt and a true measure of reactive agility could always be recorded. Total test time was recorded along with 0-5 and 5-10 m split times. Each attempt was separated by a 30 s walking recovery.

COD t-test:

An adapted version of the t-test protocol (Semenick, 1990) was used with minor modifications (See Fig. 2.3). Specifically, a modified distance of 5 m was used and bells were used in place of cones (A, B and C). Subjects began the test 0.75 m behind the timing gate (Smartspeed, Fusion Sport, Australia) at the start line. Participants were instructed to sprint forwards and ring bell A by hand. They were then instructed to sidestep to point B and ring bell B, before sidestepping across to point C and ringing bell C, then sidestepping back to point A to ring bell A again. Participants were then instructed to sprint backwards from this point, through the timing gate to the start line to complete the test. It was made clear to participants that a test attempt would be discounted if they crossed their legs in the sidestep movement or failed to ring the bells by hand at the specific points of the test. Three attempts were performed separated by 60 s walking recovery. Total test time was recorded.

15 m sprint:

Participants began each sprint with their left foot positioned on a starting jump mat (Smartspeed, Fusion Sports, Australia) as shown in figure 2.4. A timing gate system positioned at 0, 5, 10 and 15 m provided a randomly timed (0.2-2 s) buzzer and light stimuli to start each sprint. Participants were instructed to react to the light and buzzer stimuli and sprint the 15 m distance as quickly as possible. Five attempts were performed separated by a 30 s walking recovery. Total test time was recorded along with reaction time, 5, 10 and 15 m split times.

CMJ:

Participants completed 3 maximal CMJs on a jump mat (Smartspeed, Fusion Sport, Australia) each separated by 30 s standing recovery. Participants were instructed to start the movement with their feet shoulder-width apart and keep their hands on their hips throughout the test. In the countermovement phase participants reached a squat position with their upper leg parallel to the ground, they were instructed not to pause in this position but immediately perform a maximal jump. Participants were instructed to maintain extension in the knee and hip joints throughout the time spent in the air to minimise any additional flight time from bending the legs. Maximum jump height was recorded.

Yo-Yo IR1:

The Yo-Yo IR1 test consists of repeated 20 m shuttle runs at a progressively increasing speed controlled by an audio recording, with each 40 m running bout separated by a 10 s active recovery period (Bangsbo, Iaia & Krstrup, 2008). When completed to exhaustion, the test was terminated when participants failed to reach the 20 m marker on two consecutive runs. The distance covered at this time was recorded and represented the test result. For subsequent exercise visits, participants completed the Yo-Yo IR1 test to 90% of their maximum achievable distance.

Subsequent to Yo-Yo IR1:

In experimental visits, 30 s following the Yo-Yo IR1 test to 90% of maximum achieved distance, participants completed the 15 m linear sprint and CMJ exercise protocols for a second time.

Statistical analysis

Statistical analysis for exercise performance was informed by the reliability work conducted in chapter 3 of this thesis to ensure analysis was performed on data that was most repeatable day-to-day. Analysis was conducted on the mean of all 6 RAT attempts, mean of all 3 COD t-test attempts and on the absolute fastest 15 m sprint and the absolute highest CMJ attempt.

A two-way repeated measures ANOVA (supplement x time) was used to assess differences between NIT and PLA in sprint and CMJ performance. Significant main and interaction effects were followed up with Fisher's LSD post

hoc tests. Differences between NIT and PLA for resting BP, RAT and COD t-test performance was analysed using paired samples t-tests. Paired samples t-tests were also employed to assess differences in performance between visit 4 and 5 for all exercise protocols, to assess the effect of testing order on performance. Relationships between change in BP and performance following NIT (vs PLA) compared to PLA was assessed using Pearson product moment correlation coefficients.

Determination of an appropriate sample size for this investigation was informed by data from Thompson *et al.* (2016) where 10 m linear sprint performance was assessed following NO_3^- supplementation. The minimum sample size required, determined from these data, was 30 participants ($1 - \beta = 0.80$; 0.05α -level).

All data are reported as mean \pm SD unless otherwise stated. Statistical significance was accepted at $P < 0.05$.

Results

Both the NIT and PLA treatments were well tolerated by all participants. Each participant fully complied with the supplementation protocol and followed all instructions prior to each exercise visit.

Blood pressure

There was no significant difference between NIT and PLA for systolic BP (SBP), diastolic BP (DBP) or mean arterial pressure (MAP), (SBP: 122 ± 9 mmHg vs 122 ± 11 mmHg; DBP: 67 ± 6 mmHg vs 68 ± 8 mmHg; MAP: 88 ± 6 mmHg vs 88 ± 7 mmHg respectively; $P > 0.05$ for all comparisons). However, the change in BP following NIT was negatively correlated with baseline BP in PLA (SBP: $r = -0.55$, $P < 0.01$; DBP: $r = -0.74$, $P < 0.01$; MAP: $r = -0.64$, $P < 0.01$).

RAT performance

Reactive agility performance was not different between NIT and PLA (2.64 ± 0.21 s vs 2.65 ± 0.17 s respectively; $P > 0.05$; fig. 4.1). Similarly, there was no significant difference between NIT and PLA when attempts were considered independently to the left (2.65 ± 0.22 s vs 2.65 ± 0.19 s respectively; $P > 0.05$) or to the right (2.64 ± 0.20 s vs 2.66 ± 0.18 s respectively; $P > 0.05$). There was no

significant difference between NIT and PLA for 0-5 m or 5-10 m split time when attempts were considered combined or independently to the left or right ($P > 0.05$).

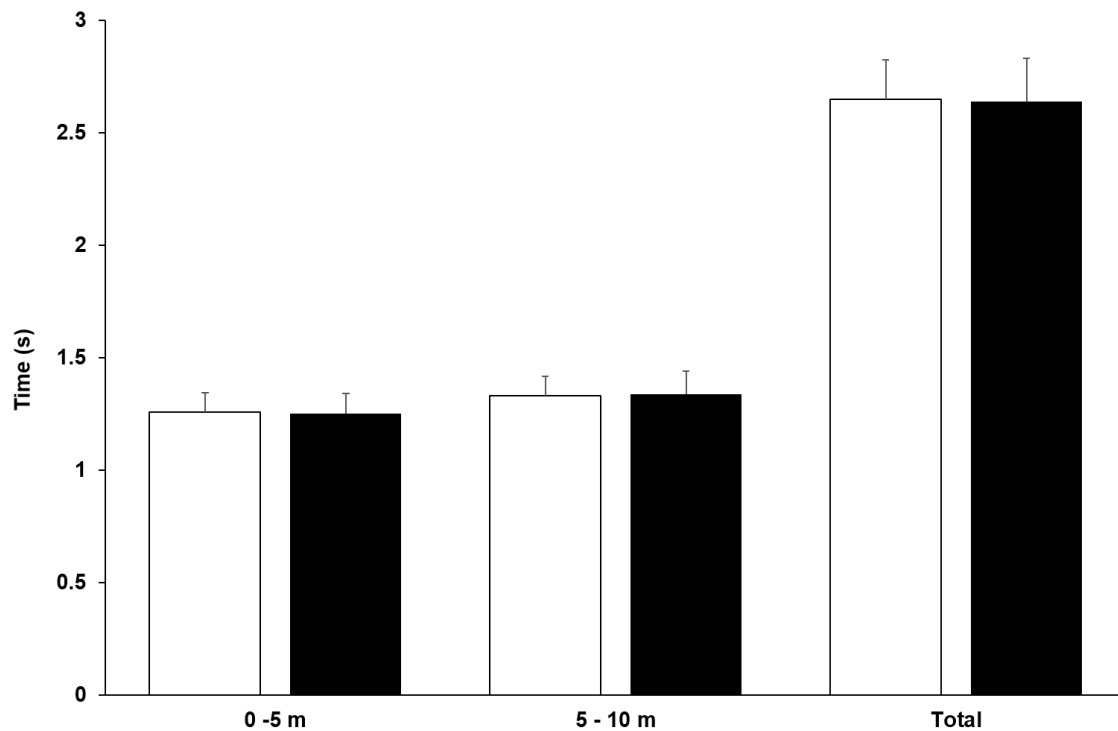


Figure 4.1: Reactive agility test total and split time following PLA (□) and NIT (■) supplementation. Data presented as group mean \pm SD.

Change of direction t-test performance

There was no difference between PLA and NIT for change of direction t-test performance (7.10 ± 0.76 s vs 7.12 ± 0.71 s respectively; $P > 0.05$; Fig. 4.2).

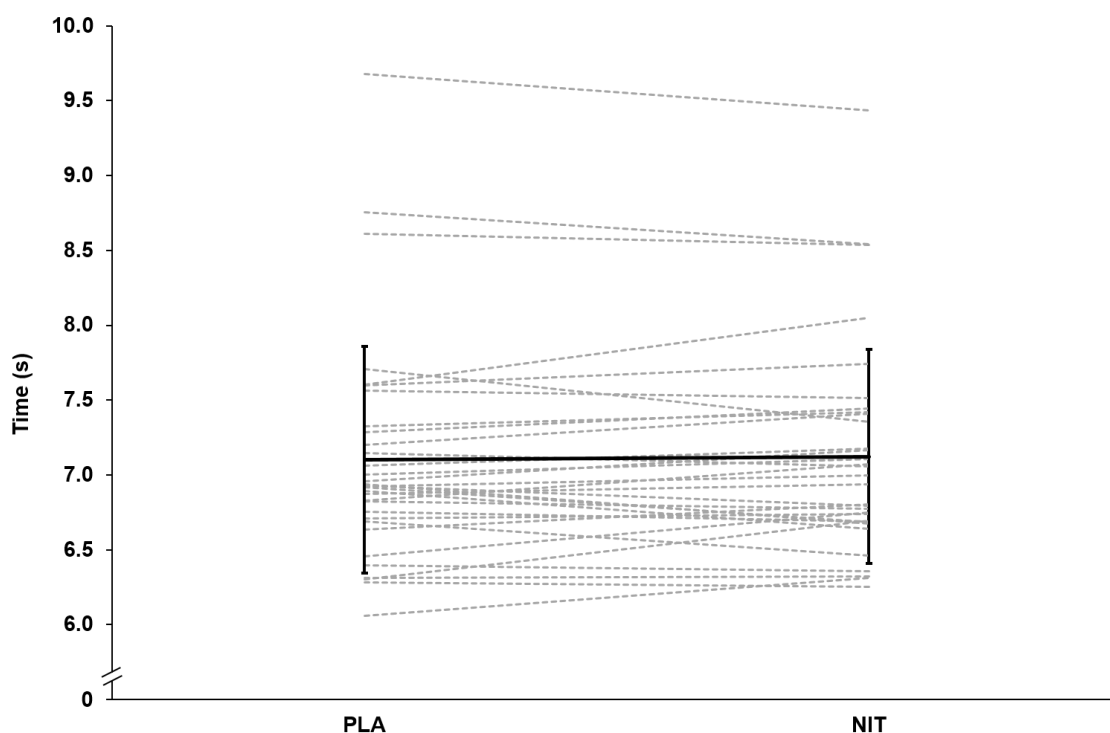


Figure 4.2: Time taken to complete change of direction t-test. The dashed lines indicate individual responses and solid line indicates group mean \pm SD.

Sprint performance

Compared to PLA, there was no effect of NIT on sprint performance for reaction time, 5, 10 or 15 m split time in either an unfatigued or a fatigued state ($P > 0.05$; figure 4.3). There was no supplement \times time interaction effect for reaction time, 5, 10 or 15 m split time ($P > 0.05$; fig 4.3).

Sprint performance was significantly slower in a fatigued state compared to an unfatigued state in 10 m split for NIT (2.53 ± 0.19 s vs 2.49 ± 0.17 s respectively, $P < 0.05$) and for 15 m sprint performance in PLA (3.27 ± 0.25 s vs 3.22 ± 0.21 s respectively) and NIT (3.27 ± 0.25 s vs 3.20 ± 0.21 s respectively, $P < 0.05$ for both comparisons). However, NIT did not attenuate the decline in 15 m sprint performance from an unfatigued to fatigued state ($P > 0.05$). There was a trend for slower reaction time in an unfatigued compared to a fatigued state in PLA (0.38 ± 0.23 s vs 0.32 ± 0.23 s respectively, $P = 0.06$).

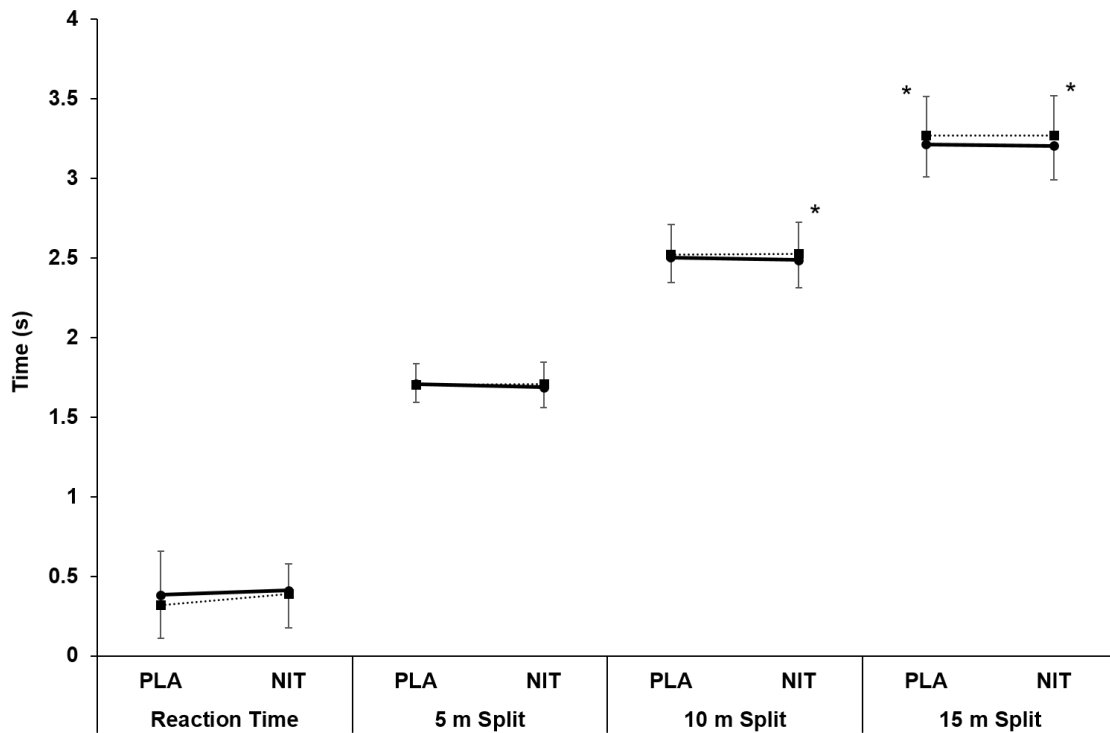


Figure 4.3: Sprint performance following NIT and PLA in an unfatigued (solid line; ●) and fatigued state (dashed line; ■). Data presented as mean \pm SD. * significantly different to performance in unfatigued state within same condition, $P < 0.05$.

CMJ performance

There was no effect of NIT, compared to PLA on CMJ performance in either an unfatigued (36.4 ± 6.6 cm vs 37.0 ± 6.8 cm) or a fatigued (36.7 ± 7.2 cm vs 36.5 ± 7.0 cm, respectively, $P > 0.05$ for both comparisons) state. Additionally, CMJ performance in an unfatigued state was similar to performance in a fatigued state ($P > 0.05$, fig 4.4).

Heart rate

Maximum HR at the end of the exhaustive Yo-Yo IR1 was 191 ± 8 bpm. Heart rate at the end of the Yo-Yo IR1 completed to 90% maximum achievable distance was on average 98% of maximum HR. HR recorded at this point was not different between PLA and NIT (186 ± 9 bpm vs 184 ± 15 bpm, respectively; $P > 0.05$).

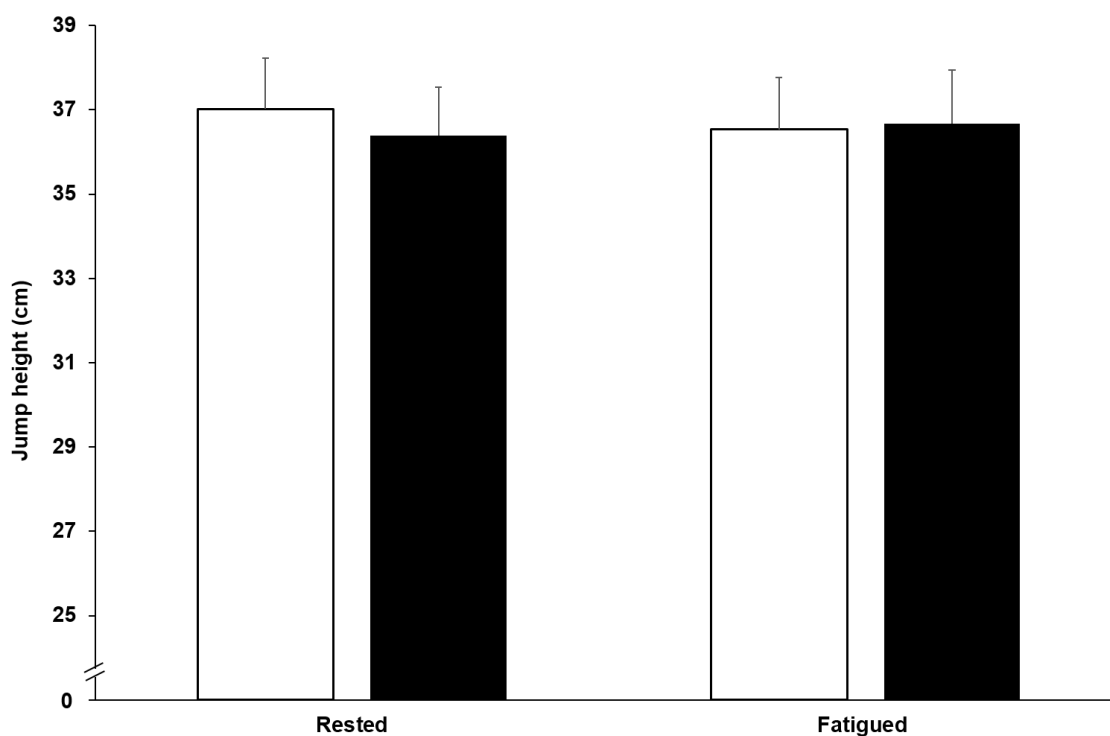


Figure 4.4: Counter-movement jump height following PLA (□) and NIT (■) conditions in an unfatigued and fatigued state. Data presented as mean \pm SE.

There was no effect of testing order on performance in any exercise test ($P > 0.05$).

Discussion

The principal original finding from this study was that short term dietary NO_3^- supplementation did not improve agility, 15 m linear sprint or vertical jump performance when completed in an unfatigued state. NO_3^- supplementation also did not improve 15 m sprint or vertical jump performance when completed following fatiguing exercise. Fifteen meter sprint, but not CMJ performance, was reduced in a fatigued compared to an unfatigued state; however, NO_3^- supplementation did not attenuate this decline in performance. These findings suggest that NO_3^- supplementation may not be ergogenic for these particular determinants of team sports performance.

Effect of NO_3^- supplementation on blood pressure

In the present study, SBP and DBP were unchanged following NO_3^- supplementation in agreement with some (Cermak *et al.*, 2012; Wilkerson *et al.*, 2012; Haider & Folland, 2014) but not all (e.g. Larsen *et al.*, 2007; Webb *et al.*,

2008) previous studies. Although no change in SBP or DBP was present at a group mean level, the change in both SBP and DBP following NIT (vs PLA) was negatively correlated with BP in PLA. Therefore, those with the highest resting BP in PLA had the greatest reduction following NO_3^- supplementation, in agreement with previous literature (e.g. Kapil *et al.*, 2010). The relatively low mean BP (SBP: 122 ± 11 mmHg and DBP: 68 ± 8 mmHg) of the study cohort may have therefore limited the potential for a significant BP reduction to be found at a group mean level.

Effect of NO_3^- supplementation on unfatigued agility, linear sprint and vertical jump performance

NO_3^- supplementation has been suggested to preferentially enhance some of the physiological properties of type II muscle fibres (Hernández *et al.*, 2012; Ferguson *et al.*, 2013; 2015) with improvements in human skeletal muscle contractility (Bailey *et al.*, 2010), force (Haider & Folland, 2014), and power production (Coggan *et al.*, 2015) having been reported. Improvements in repeated sprint cycling power output (Rimer *et al.*, 2016; Wylie *et al.*, 2016) and sprint performance within an intermittent cycling protocol (Thompson *et al.*, 2015) following NO_3^- supplementation may be explained, in part, by such exercise requiring explosive power production and a high recruitment of type II muscle fibres. Single sprint performance has also been reported to be improved following NO_3^- supplementation over both 180 m (Sandbakk *et al.*, 2015) and 20 m (Thompson *et al.*, 2016). In contrast to this and our hypothesis, no improvement in unfatigued 15 m sprint, or 5 and 10 m split time performance, was found in the present study following NO_3^- supplementation. Similar findings have been reported in the literature, where no improvement in 8 s sprint cycling (Martin *et al.*, 2014) and 10 s sprint kayaking (Muggeridge *et al.*, 2013) was reported following an acute ~ 5 mmol NO_3^- dose. Additionally, while improvements in mean power output across 24 x 6 s cycle sprints has been reported (Wylie *et al.*, 2016), when the first sprint was considered independently, which more closely reflects the single unfatigued sprints conducted in the present study, no improvement in performance was present. Improvements in intermittent exercise performance reported previously in the literature (e.g. Wylie *et al.*, 2013b, 2016; Thompson *et al.*, 2015) may have benefited from improved muscle O_2 delivery following NO_3^-

supplementation (Ferguson *et al.*, 2013; 2015) and therefore improved recovery of PCr (Vanhatalo *et al.*, 2011) between repeated bouts, something single sprint efforts would not benefit from. This may suggest an ergogenic effect in repeated, but not single, sprint efforts (Wylie *et al.*, 2016), which has also been reported by Thompson *et al.* (2015). This may suggest that NO₃⁻ supplementation is better placed to improve intermittent, rather than single short distance sprint performance. However, the present study and that conducted by Thompson *et al.* (2016) show conflicting findings regarding the effect of NO₃⁻ supplementation on short distance sprint running performance, which, to our knowledge are the only studies to have investigated this to date. It is unclear why these discrepancies are present; however, it does indicate that further work in this area is required.

A novel aspect of this investigation was to determine the effect of NO₃-supplementation on agility and vertical jump performance. In contrast to our hypothesis, neither planned (COD t-test) nor unplanned (RAT) change of direction or vertical jump performance was improved following NO₃-supplementation. However, considering the supplementation regime employed did not improve 5, 10 or 15 m linear sprint performance, this may be unsurprising. Previous studies have reported improved force and power production (Haider & Folland, 2014; Coggan *et al.*, 2015) as well as improved short distance (5-20 m) running performance, accentuated over the initial acceleration phase (Thompson *et al.*, 2016) following NO₃- supplementation, which may have been expected to translate to improved sprint performance requiring multiple accelerations such as change of direction tasks. However, no improvements were found in the present study. In addition, previous findings could be interpreted to translate to improvements in explosive forms of exercise such as vertical jumping; however, no improvement in CMJ performance was found in the present study. In agreement with this, Fulford *et al.* (2013) reported no improvement in peak or mean skeletal muscle force production during 50 x 6.6 s maximum voluntary contractions following NO₃- supplementation. Interestingly, the effect of NO on skeletal muscle contractility has been suggested to be dependent on the mode, intensity and duration of muscle contraction (Murrant, Frisbee & Barclay, 1997). Therefore, improvements in force and power production during knee extension exercise reported elsewhere (Haider & Folland, 2014; Coggan *et al.*, 2015) may not translate to improved vertical jump performance.

Equivocal findings in the literature regarding the effect of NO_3^- supplementation on sprint performance may be explained, at least in part, by the different NO_3^- -rich supplements employed. A novel NO_3^- -rich supplement was employed in the present study which, although it may contain a similar NO_3^- content to other NO_3^- -rich products that have been shown to elevate plasma [NO_3^-] and [NO_2^-] (e.g. Larsen *et al.*, 2007; Wylie *et al.*, 2013a; Thompson *et al.*, 2016), likely differs in other ingredients such as antioxidant and polyphenol content. Since polyphenols and vitamin C can facilitate the synthesis of NO from NO_2^- in the stomach (Weitzberg & Lundberg, 1998; Rocha *et al.*, 2009; Lundberg *et al.*, 2011), systemic NO availability may differ following the ingestion of different NO_3^- -rich supplements. Plasma [NO_3^-] and [NO_2^-] data was however not measured in the present study and therefore it is not known whether, or to what extent, the product was successful in elevating markers of NO bioavailability. The fibre-type distribution of individuals within the study cohort may also partly explain these equivocal findings, with NO_3^- supplementation known to preferentially enhance some of the physiological responses of type II compared to type I muscle fibres (Hernández *et al.*, 2012; Ferguson *et al.*, 2013). Therefore, individuals with a higher portion of type II fibres may benefit more, and those with a low portion of type II muscle fibres may not be as well placed, to benefit from NO_3^- supplementation. Information regarding individual's fibre-type distribution is not available in the present study to explore this possibility further.

From the 32 participants of the present study, 17 appeared to positively respond to the NO_3^- supplementation regime, indicated by improved unfatigued 15 m sprint performance. However, not all participants responded positively and consequently no improvement in performance was seen at a group mean level. Such individual variability also appears to be present even where a positive group mean response is reported, indicated by individual data highlighted within the literature (Wylie *et al.*, 2013b; Mugeridge *et al.*, 2013; Thompson *et al.*, 2016). For example, Thompson *et al.* (2015 unpublished data) reported 10 out of 16 participants responded to NO_3^- supplementation in terms of exercise performance. The response to NO_3^- supplementation is known to be highly individualised, with responders and non-responders having been suggested (Wilkerson *et al.*, 2012; Christensen *et al.*, 2013). NO_3^- supplementation may be less useful as an ergogenic aid to individuals who have elevated baseline [NO_2^-], such as endurance trained individuals (Vassalle *et al.*, 2003). However, these

individuals have been reported to benefit from NO_3^- supplementation when plasma $[\text{NO}_2^-]$ is elevated successfully (Wilkerson *et al.*, 2012). Plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ data was not available in the present study to determine baseline levels or whether plasma $[\text{NO}_2^-]$ was elevated sufficiently following supplementation. It is therefore possible that a selection of participants had a high baseline $[\text{NO}_2^-]$ and subsequently did not show a suitable elevation in plasma $[\text{NO}_2^-]$ following supplementation to elicit positive performance improvements. Given that the response to NO_3^- supplementation is highly individualised, further research is required to explore the mechanisms that govern this individualised response. This would enable us to understand those individuals who are most likely to benefit from NO_3^- supplementation and provide information to develop strategies to optimise the effects of NO_3^- supplementation.

Effect of NO_3^- supplementation on fatigued linear sprint and vertical jump performance

In the present study, 15 m sprint performance following NO_3^- supplementation was not significantly different compared to PLA when completed following exercise that mimicked the high-intensity intermittent nature of team sports game-play (Bangsbo *et al.*, 2008). Total sprint time was significantly reduced in a fatigued state by 1.7% in PLA and 2.1% in NIT compared to an unfatigued state suggesting the protocol did successfully induce fatigue; however, NO_3^- supplementation did not attenuate this decline in 15 m sprint performance. This may be unsurprising considering the supplementation regime did not improve 15 m sprint performance in an unfatigued state. During exercise, NO_3^- supplementation has been reported to improve perfusion and oxygenation (Ferguson *et al.*, 2013; 2015) in type II muscle fibres as well as to reduce muscle metabolic perturbation by sparing the rate of PCr depletion (Bailey *et al.*, 2010). As the rate of PCr depletion is an important determinant of fatigue development during maximal intensity intermittent exercise (Gaitanos *et al.*, 1993; Fulford *et al.*, 2013) it may be suggested that NO_3^- supplementation could reduce fatigue development during such exercise. In addition, NO availability may be enhanced during such fatiguing exercise as the reduction of NO_2^- to NO is potentiated in hypoxic and acidic environments (Lundberg & Weitzberg, 2010) such as the environment within contracting skeletal muscle. It was therefore hypothesised

that NO_3^- supplementation may reduce fatigue development and increase NO availability and therefore attenuate the decline in performance expected following fatiguing exercise. However, in contrast to our hypothesis, the decline in fatigued 15 m sprint performance was not attenuated following NO_3^- supplementation. The absence of an attenuation in the decline in performance in this fatigued state may indicate that the NO_3^- supplementation procedure employed was unsuccessful at altering the rate of PCr depletion. It should also be noted here that reaction time showed a trend for improvement ($P = 0.06$) in a fatigued state compared to an unfatigued state. However, this should be interpreted with caution as assessing reaction time independently of sprint performance was considered highly variable in this protocol with a coefficient of variation of 60.3% (See Chapter 3). Therefore, this result may be due to the highly variable nature of this outcome measure when considered independently.

Countermovement jump performance was also assessed in a fatigued state, and no improvement in performance was found following NO_3^- supplementation. Fatigued jump height was also not different to unfatigued jump performance. Although the Yo-Yo IR1 test is known to induce fatigue and significantly reduce muscle [PCr] and [glycogen], taxing both the aerobic and anaerobic energy systems (Krustrup *et al.*, 2003), the restoration of ATP stores even after fatiguing exercise is reported to be replenished by ~90-95% within 3 minutes (e.g. Connolly, Brennan & Lauzon, 2003). Therefore the 2.5 min recovery period before the CMJ protocol may have provided adequate time for the recovery of ATP (Singnorile, Tremblay & Ingalls, 1993), the predominant energy source for explosive jump performance lasting <1 s (McArdle, Katch & Katch, 2006).

An explanation for the lack of attenuation in the decline in 15 m linear sprint performance in a fatigued state following NO_3^- supplementation is not clear. It may be related, in part, to differences in baseline as well as supplemented plasma [NO_3^-] and [NO_2^-] following the ingestion of the novel NO_3^- -rich supplement employed in this study, and differences in muscle fibre type distribution; previously discussed in detail above. However, as the supplementation regime was unable to improve unfatigued exercise performance even when highly reliable exercise tests, capable of detecting very small changes in performance, were used to assess such exercise performance (See Chapter 3), it might be

considered unlikely to have attenuated the decline in fatigued exercise performance.

Conclusion

In conclusion, this study contributes to the literature regarding the boundaries of the ergogenic effect of NO_3^- supplementation within team sport specific exercise performance. Specifically, NO_3^- supplementation at a dose of $8 \text{ mmol} \cdot \text{day}^{-1}$ for 5 days did not improve RAT or COD t-test performance in an unfatigued state, nor did it improve 15 m linear sprint or CMJ performance in an unfatigued or fatigued state. It also did not attenuate the decline in 15 m linear sprint performance seen in a fatigued state. NO_3^- supplementation therefore may not be ergogenic for these particular important determinants of team sports performance.

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CHAPTER 5: General Discussion and Conclusion

The aim of the current thesis was to expand our knowledge of the ergogenic effect of NO_3^- supplementation within sports performance, and specifically to elucidate its efficacy within key determinants of team sports performance. In order to achieve this, two studies were undertaken, and they addressed the following research questions:

- 1) Are the exercise tests selected to measure agility, linear sprint and vertical jump performance reliable day-to-day when no intervention is employed?
- 2) What are the performance effects of NO_3^- supplementation on key parameters of team sports performance?

Summary of findings

Reliability of exercise tests for key determinants of team sports performance

In chapter 3, the reliability of commonly employed exercise protocols to measure agility, linear sprint and vertical jump performance were assessed; specifically, the COD t-test, RAT, 15 m reactive start linear sprint test and CMJ tests were examined. The reliability of the tests established within chapter 3 was in line with that reported elsewhere in the literature for similar exercise tests, and the exercise testing protocols were determined suitable for use within an intervention study. Within studies that employ such tests it is common for multiple attempts of the same protocol to be completed. However, the selection of data from these multiple attempts that is used for statistical analysis is not consistent even though the reliability of test performance appears to vary depending on the data handling techniques used. Taking the reliability established as a COV, performance was most reliable for COD t-test performance when the mean of all three attempts was analysed (COV = 2.9%), for RAT when the mean of all 6 (3 x left, 3 x right) attempts was analysed (COV = 2.0%), for CMJ when the highest jump out of 3 was analysed independently (COV = 4.6%) and for 15 m sprint performance when the fastest sprint out of 5 was considered independently (COV = 1.0%). The specific results from this study may provide important information for

informing data analysis within these exercise protocols in subsequent intervention studies to yield the greatest possible reliability.

Influence of nitrate supplementation on agility, linear sprint and vertical jump performance in an unfatigued state

In chapter 4, for the first time, the effect of NO_3^- supplementation on agility and vertical jump performance was investigated. Fifteen meter sprint performance was also assessed following NO_3^- supplementation due to the limited but promising reports of improved 20 m linear sprint speed in the literature (Thompson *et al.*, 2016). Such movements are hallmarks of team sports performance and therefore this investigation enabled us to elucidate the effect of NO_3^- supplementation on key parameters of team sports performance. In contrast to our hypothesis, the supplementation regime employed in chapter 4 (8 mmol \cdot day⁻¹ for 5 days) did not improve performance within the COD t-test, RAT, 15 m linear sprint or CMJ protocols in our participant cohort of 32 male team sports players. These findings suggest that NO_3^- supplementation may not be ergogenic for these particular determinants of team sports performance when performed in an unfatigued state.

Influence of nitrate supplementation on linear sprint and vertical jump performance in a fatigued state

In chapter 4, for the first time, the effect of NO_3^- supplementation on 15 m linear sprint and CMJ performance was investigated under fatigue as such movements are commonly performed under fatigue within game-play. To achieve this fatigued state, participants completed the Yo-Yo IR1 test, which is a valid and reliable test that mimics the high intensity intermittent nature of team sports game-play (Bangsbo *et al.*, 2008), to 90% of their predetermined maximum distance. Following this, 15 m linear sprint and CMJ performance was assessed. Fifteen meter linear sprint performance was significantly reduced by ~2% when completed in this fatigued state compared to an unfatigued state; however, NO_3^- supplementation did not attenuate the decline in performance following such fatiguing exercise. Countermovement jump performance was also unchanged following NO_3^- supplementation and was not different between unfatigued and fatigued tests. These findings suggest that NO_3^- supplementation

is not ergogenic for linear sprint and vertical jump performance when performed under fatigue that mimicked the high-intensity intermittent nature of team sports game-play.

Experimental considerations and future directions

The work completed within the current thesis contributes to the growing literature regarding the putative ergogenic effects of NO_3^- supplementation in sport and exercise. Specifically, this work indicates that NO_3^- supplementation does not enhance the performance of certain key determinants of team sports performance; specifically planned and unplanned agility, linear sprint and vertical jump performance. However, this can only be considered true for the participant population of chapter 4 and for the particular dose and duration of the NO_3^- supplementation procedure investigated. Further work is required to investigate the effect of NO_3^- supplementation on these and other determinants of team sports performance within other participant populations such as female team sports players. Whilst this thesis does provide an important contribution to the literature regarding the boundaries of the ergogenic effect of NO_3^- supplementation, it also highlights further important questions for future research.

The smallest practically meaningful effect of nitrate supplementation within key determinants of team sports performance

It is important to consider whether improvements in performance following supplementation are equal to or above a magnitude eliciting the smallest worthwhile change in performance (Hopkins, 2004). This can be done by calculating the standardised change or difference in performance, expressed as a fraction of the between-subject standard deviation (Cohen's d statistic (d) = Δ mean/SD), where the smallest worthwhile change for sports performance is ~ 0.2 (Hopkins, 2004). In chapter 4, considering the unfatigued exercise tests, no statistically significant improvements in performance were found following NO_3^- supplementation. The effect of NO_3^- supplementation on performance in the linear sprint was considered trivial for 5 m split ($d = 0.16$), 10 m split ($d = 0.09$) and total time ($d = 0.05$). The effect of NO_3^- supplementation on test performance in all other protocols was also trivial ($d < 0.2$ for all tests).

Furthermore, magnitude based inferences using 90% confidence limits can also be used to determine the effect of an intervention on performance (Batterham & Hopkins, 2006). Using this method, for unfatigued 15 m sprint performance, the effect of NO_3^- supplementation on performance was considered “unclear” for total time and 10 m split time. However, for 5 m split time the effect was considered “likely beneficial” (Hopkins, 2002). Although no statistically significant improvement in 5 m sprint performance was reported in chapter 4, this “likely beneficial” effect of NO_3^- supplementation may be important to team sports players where even small improvements in performance can be advantageous within a game. Taking this information, and the equivocal nature of the literature regarding the effect of NO_3^- on sprint performance, with reports of positive effects (Sandbakk *et al.*, 2015; Thompson *et al.*, 2016), no effects (Christensen *et al.*, 2013; Muggeridge *et al.*, 2013) and even one reporting a negative effect (Martin *et al.*, 2014), it is clear that further research is required to elucidate the effect of NO_3^- supplementation on sprint exercise performance.

Conclusion

Scientific investigations into both the therapeutic and ergogenic effect of NO_3^- supplementation are continuing. This thesis aimed to provide a contribution to the literature regarding the ergogenic effects of NO_3^- , specifically with regard to key determinants of team sports performance. Overall, it can be concluded from the investigations conducted within this thesis that NO_3^- supplementation does not enhance planned or unplanned agility, linear sprint or vertical jump performance when performed in an unfatigued state. It also does not improve linear sprint or vertical jump performance when completed in a fatigued state. In addition, when performance is reduced following fatiguing exercise, NO_3^- supplementation does not attenuate this decline in performance.

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