Effect of Sodium Phosphate Loading on Endurance and Sprint Performance in Trained Females

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Executive Summary

Nutritional ergogenic supplements have long been used by athletes in an attempt to improve exercise performance. One nutritional supplement that has shown some positive benefits for sporting performance is sodium phosphate (SP). Specifically, SP is supplemented orally in capsule form, commonly at a dose of 3-5 g or 50 mg·kg⁻¹ of fat free mass (FFM) a day for between 3-6 days to benefit aerobic and endurance performance. Numerous mechanisms have been proposed to explain the performance improvements seen following SP ingestion. These include: enhanced 2,3-diphosphoglycerate (2,3-DPG) concentration in red blood cells, which allows for a greater unloading of oxygen to the peripheral tissues; improved buffering capacity of hydrogen ions due to increased hydrogen phosphate concentrations; improved myocardial efficiency, which results in more efficient oxygenation of the exercising muscle; potentially greater adenosine triphosphate/phosphocreatine (ATP/PCr) resynthesis due to increased availability of extracellular and intracellular phosphate; and an enhanced rate of glycolysis, due to the positive effects of phosphate on the glycolytic energy pathway.

Notably, no studies to date have assessed the effects of SP supplementation on repeated-sprint ability (RSA) as performed in a team-sport game and only one study has investigated the effect of SP loading in females (using an aerobic capacity test). Further research is needed here, as it is possible that females may respond differently to SP loading than males, due to several gender related physiological differences that may affect the aforementioned mechanisms associated with SP supplementation. Specifically, compared with males, females have a decreased affinity of oxygen with haemoglobin, which may dampen the potential positive effect of any increases in 2,3-DPG concentration. Furthermore, females
have higher natural levels of (and fluctuations in) oestrogen, which plays a role in renal phosphate reabsorption within the body.

In addition, it is common for two (or more) nutritional supplements to be ingested together to further enhance exercise performance, however no studies have assessed the effect of SP loading in combination with other common ergogenic aids. Notably, caffeine and nitrate represent two legal, beneficial supplements that if ingested in combination with SP may further improve exercise performance than if taken alone, as each of these supplements is proposed to enhance exercise via completely separate mechanisms.

Similar to SP, nitrate in the form of beetroot juice (BJ) has been found to improve both exercise tolerance (by 15-25%) during high-intensity, incremental cycling tests and cycling time-trial (TT) performance (by 2.7% for 4 km, 1.3% for 10 km and 2.8% for 16.1 km) following 5–11.2 mmol of nitrate (BJ or sodium nitrate) ingestion, as well as exercise efficiency, based on reductions in submaximal oxygen uptake ($\bar{V}O_2$). Mechanisms proposed to account for these effects include more efficient mitochondrial respiration, due to reduced proton leakage through the inner mitochondrial membrane and more efficient ATP metabolism during muscle force production, due to lower levels of calcium ATPase and actomyosin ATPase activity. However, similar to SP, no studies have assessed the effects of BJ supplementation on RSA as it relates to team-sports performance.

Similarly, numerous studies have reported that consuming 3-6 mg·kg$^{-1}$ of body mass of caffeine 60 min before exercise resulted in significant improvements in endurance performance. More recently, the effects of caffeine on RSA, as performed during team-sports, have been investigated, with some studies finding benefit while others have not.
Improvement in exercise performance following caffeine ingestion has primarily been attributed to adenosine receptor antagonism, which is proposed to result in reduced sensations of effort and pain, increased alertness, improved neural firing rates and enhanced motor unit recruitment and frequency of activation. Notably, all studies that have assessed the effects of caffeine on RSA have used male participants.

Consequently, the purpose of this thesis was to investigate the effect of SP, BJ and caffeine supplementation, either alone or in combination, on endurance and RSA in female athletes with sprints performed prior to, midway and at the end of a simulated team-sport game.

Specifically, study one investigated the effect of six days of SP supplementation on 500 kJ (simulated 20-km) cycling TT performance. A secondary aim of this study was to determine the optimal SP loading dose for females, as it may differ to that used in males. Consequently, three different dosing protocols were compared (25, 50 and 75 mg·kg\(^{-1}\) of FFM doses). Overall, results indicated that none of these SP doses improved 500 kJ TT performance in female cyclists when compared with placebo.

The second study investigated the effect of SP loading (50 mg·kg\(^{-1}\) of FFM per day for 6 days) on RSA (3 sets of 6 x 20 m sprints) performed at the start (set 1), during the half-time break (set 2) and at the end (set 3) of a 60 min simulated team game circuit. This study also aimed to determine if RSA performance could be further improved by combining SP with caffeine (SP+C) supplementation. It was observed that both SP and SP+C resulted in some improvement to RSA for various sets compared with placebo and caffeine, but caffeine alone had minimal effect on RSA.
Similarly, study three, using a similar exercise protocol to study two, aimed to determine if RSA performance improvements could be further enhanced in females by combining SP with BJ (SP+BJ). Here, when compared with BJ and placebo, SP resulted in improved RSA (as determined by qualitative and quantitative analysis) for all sprints in every set, with some improvement also found when compared with SP+BJ. Further, compared with placebo and BJ, SP+BJ resulted in some improvement in RSA for all sets apart from total sprint time in set 3. However, when supplemented alone, BJ had little effect on RSA compared with placebo.

In summary, based on study one results, it was concluded that SP loading did not influence endurance performance in female cyclists. However, based on the results of study two and three, it was concluded that SP is likely to be beneficial to RSA performance in female athletes, with combined supplementation (SP+C and SP+BJ) also demonstrating some improvements, but not to any greater degree than SP in isolation.
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List of Abbreviations

1,25-D, hydroxyvitamin D3 (1,25-D3)

2,3-diphosphoglycerate (2,3-DPG)

$^{31}$phosphate-magnetic resonance spectroscopy (P-MRS)

Adenosine triphosphate (ATP)

Approximately (~)

Beats per minute (BPM)

Beetroot juice (BJ)

Best Sprint (BS)

Central nervous system (CNS)

Coefficient of variation (CV)

Correlation (r)

Degrees Celsius (°C)

Dihydrogen phosphate (H$_2$PO$_4^-$)

Effect Size (d)

Fat free mass (FFM)

First sprint (FS)

Grams (g)

Grams per day (g d$^{-1}$)

Greater than (>)

Heart Rate (HR)

Haemoglobin (Hb)
Hours (h)
Hydrogen phosphate (HPO_4^-)
Kilocalories (kcal)
Kilogram (km)
Kilojoule (kJ)
Kilometre (km)
Less than (<)
Maximal oxygen uptake ( \( \dot{V}O_{2\text{max}} \) )
Metres (m)
Microlitre (µL)
Micromolar (µM)
Milligrams (mg)
Milligrams per haemoglobin (mg/Hb)
Millilitre (mL)
Millilitres per kilogram per-minute (mL·kg\(^{-1}\)·min\(^{-1}\))
Minute (min)
Millimole (mmol)
Millimole per litre (mmol·L\(^{-1}\))
Nanomolar (nM)
Nanomolar per millilitre (nmol·mL\(^{-1}\))
Nitrate (NO_3^-)
Nitric oxide (NO)
Nitrite (NO₂⁻)
Parathyroid hormone (PTH)
Peak maximal oxygen uptake (VO₂peak)
Phosphocreatine (PCr)
Plus (+)
Plus or-minus (±)
Oxygen uptake (VO₂)
Rating of perceived exertion (RPE)
Red blood cells (RBC)
Repeated-sprint ability (RSA)
Seconds (s)
Simulated team game circuit (STGC)
Sodium phosphate (SP)
Sodium phosphate and beetroot juice (SP+BJ)
Sodium phosphate and caffeine (SP+C)
Standard deviation (SD)
Stroke volume (SV)
Time to exhaustion (TTE)
Time-trial (TT)
Total sprint (TS)
Watts (W)
Years (y)
Statement of Candidate Contribution

The work involved in designing and conducting the studies described in this thesis has been conducted primarily by Christopher Buck (PhD Candidate). The thesis outline and experimental design of the studies were developed and planned in consultation with Professor Karen Wallman, Associate Professor Kym Guelfi and Winthrop Professor Brian Dawson (the candidate's supervisors). All participant recruitment and management was carried out entirely by the candidate, along with the organisation, implementation and collection of data for the studies. In addition, the candidate was responsible for all data analysis and original drafting of the thesis and the peer-reviewed publications. Professor Karen Wallman, Associate Professor Kym Guelfi and Winthrop Professor Brian Dawson have provided feedback for further drafts and editing of the thesis and manuscripts.

This thesis contains published work and work prepared for publication, some of which has been co-authored. Permission has been granted by Professor Karen Wallman, Associate Professor Kym Guelfi and Winthrop Professor Brian Dawson for the published work listed on the following page to be included in this thesis.

Signed: ____________________    ____________________

Christopher Buck          Professor Karen Wallman
(PhD Candidate)            (Coordinating Supervisor)
Publications Arising From This Thesis


Chapter One

Introduction
**Background**

Legal nutritional ergogenic aids offer athletes an additional avenue to enhance their performance beyond what they can achieve through training alone. Consequently, the efficacy of new nutritional ergogenic aids is constantly being tested. One nutritional supplement that has shown some positive benefits for both aerobic and endurance performance is SP. For ergogenic purposes, SP is supplemented orally in capsule form, at a dose of 3-5 g or 50 mg·kg⁻¹ of FFM a day for a period of between 3-6 days (Czuba, Zajac, Poprzecki, & Cholewa, 2008; Czuba, Zajac, Poprzecki, Cholewa, & Woska, 2009; Folland, Stern, & Brickley, 2008; Kreider, Miller, Williams, Somma, & Nasser, 1990; Kreider et al., 1992). Several exercise performance enhancements have been reported to occur with SP supplementation, including improvements in aerobic capacity, peak power output, anaerobic threshold, myocardial and cardiovascular exercise responses (Kreider, 1999). A variety of mechanisms have been proposed to account for these ergogenic effects. These include enhancements in 2,3-DPG concentrations (Cade et al., 1984; Kreider et al., 1992), myocardial efficiency (Kreider et al., 1992), buffering capacity (Kreider, 1999), activation of glycolysis (Chasiotis, 1988) and ATP/PCr resynthesis (Bredle, Stager, Brechue, & Farber, 1988).

Importantly, no studies to date have assessed the effects of SP loading on RSA as typically performed in a team-sport game and only one study has investigated the effect of SP loading in females (using an aerobic capacity test). Further research is required here, as females may potentially respond differently to SP supplementation than males (Fukuda, Smith, Kendall, & Stout, 2010), due to a number of gender related physiological
differences that may affect the previously mentioned mechanisms associated with SP supplementation.

Furthermore, it is common for two (or more) nutritional supplements to be ingested together to further enhance exercise performance, however no studies to date have examined the effect of SP loading on exercise performance in combination with other ergogenic aids. Caffeine and nitrate supplementation through BJ ingestion represent two legal nutritional supplements that have been shown to improve exercise performance. Notably, it is possible that when combined with SP, these ergogenic aids may enhance RSA as performed during a team-game even more than when either supplement is ingested alone.

**Statement of the Problem**

The overall aim of this thesis was to investigate the effects of six days of SP supplementation on endurance exercise performance and RSA in female athletes. A further aim was to determine the optimal SP dosing protocol for females and to examine whether the combination of SP with caffeine or BJ would result in any further benefit to exercise performance.

**Specific Aims of the Studies**

*Chapter Three: Sodium phosphate supplementation and time-trial performance in female cyclists*

This study aimed to determine the effects of SP supplementation on 500 kJ (~20 km) cycling TT performance in trained females, as well as the optimal FFM corrected SP
loading protocol for females. Participants completed a familiarisation session (500 kJ TT), followed by one placebo and three SP trials (25, 50 or 75 mg·kg\(^{-1}\) of FFM a day for six days) in a randomised, double-blind, Latin-square design, each separated by a washout period of ~21 days (based on menstrual cycle).

**Chapter Four: Effects of sodium phosphate and caffeine loading on repeated-sprint ability**

This study investigated the effects of 50 mg·kg\(^{-1}\) of FFM of SP (per day for six days) and 6 mg·kg\(^{-1}\) of body mass of caffeine supplementation (60 min pre-exercise), alone and combined, on RSA during a simulated team game circuit (STGC) protocol in female, team-sport players. Participants completed a familiarisation trial (consisting of the STGC and three sets of repeated-sprints), followed by a placebo and three supplement trials (SP and caffeine combined, SP or caffeine alone) in a randomised, double-blind, Latin-square design, each separated by approximately a 21-day washout period (based on menstrual cycle).

**Chapter Five: Effects of sodium phosphate and beetroot juice supplementation on repeated-sprint ability in females**

This study examined the effects of 50 mg·kg\(^{-1}\) of FFM of SP (per day for six days) and 70 mL of BJ (containing 6 mmol of nitrate) supplementation (taken 1 h pre-exercise), alone and combined, on RSA during a STGC protocol in female team sport players. Participants completed a familiarisation session (consisting of the STGC and three sets of repeated-sprints), followed by one placebo and three supplement trials (SP and BJ combined, SP or
BJ alone) in a randomised, double-blind, Latin-square design, each separated by approximately a 21-day washout period (based on menstrual cycle).

**Contributions of this Research**

The findings of this thesis can improve the body of knowledge available to sport scientists, coaches and athletes relating to the effects of SP in female cyclists and team-sport players. Importantly, this research will contribute to the scientific knowledge in the literature regarding the optimal SP dosing protocol for females, as well as the efficacy of SP supplementation for improving endurance and RSA performance in female athletes. Furthermore, this research may determine whether improvement in exercise performance can be obtained by combining SP with either caffeine or BJ.
References


Chapter Two

Literature Review

Forward

Large portions of the sodium phosphate section of the following review forms the basis of a review paper published in Sports Medicine:

Introduction

Nutritional ergogenic aids refer to dietary supplements ingested with the aim of enhancing exercise performance, typically by altering energy metabolism or central nervous system (CNS) responses (Benardot, 2006; Maughan, 1999). The use of nutritional ergogenic aids in sports is widespread (Maughan, 1999), with much research over the past 30 years devoted to identifying medically safe supplements. One promising nutritional and legal ergogenic aid may be sodium phosphate (SP) (Fukuda, Smith, Kendall, & Stout, 2010), which has been reported to improve endurance exercise performance (Kreider et al., 1992). However, due to limited research, many unanswered questions remain regarding the use of SP as an ergogenic aid. Therefore, the primary purpose of this review is to critically examine the use of SP as an ergogenic aid, with a focus on identifying relevant further research.

Another ergogenic aid that has been reported to enhance exercise performance is caffeine (Graham, 2001). This well-established ergogenic aid has been shown to result in improved exercise performance, both aerobically and anaerobically (Tarnopolsky, 2010). Due to differing mechanisms of action in the body, it is possible that the combined use of SP and caffeine supplementation may augment exercise performance more so than if either supplement is taken alone. Consequently, this review will also briefly consider the use of caffeine. In addition, the physiological mechanisms and pharmacokinetics of phosphate and caffeine will be discussed. Finally, this review will also consider the effect of dietary nitrate (NO₃⁻) in the form of beetroot juice (BJ) on exercise performance. Similar to caffeine, BJ has demonstrated ergogenic effects for aerobic exercise via mechanisms thought to be different to SP. However, BJ has only recently been researched as an
ergogenic aid and consequently there are still many unanswered questions relating to its use.

**Phosphate in the Body**

*What is phosphate?*

Phosphorus is a non-metallic essential nutrient that is second only to calcium in abundance in the human body as an inorganic element (Gaasbeek & Meinders, 2005; Groff, Smith, & Gropper, 2000). On average, about 11-14 g of phosphorus per kg of fat free mass (FFM) is stored in the human body (Kreider, 1999), of which approximately 85% is stored in the skeletal system (Groff et al., 2000; Kreider, 1999). A further 14% is associated with organic compounds in the soft tissue (i.e., muscle; Kreider, 1999), while 1% is located in the blood and body fluids (Groff et al., 2000). The majority of phosphorus exists as either free phosphate or phosphate bound with sodium, calcium and magnesium in a mineral salt form (Fukuda et al., 2010; Kreider, 1999). In addition, some proteins and amines are bound to phosphorus (Kreider, 1999).

**Sources of phosphate**

Phosphate is consumed in the daily dietary intake (Fukuda et al., 2010) and is found in a variety of foods such as red meat, poultry, fish, eggs and milk products (Groff et al., 2000; Kreider, 1999; Tremblay, Galloway, & Sexsmith, 1994). Cereals, grains, nuts and legumes also contain phosphate (Kreider, 1999). However, because some of the phosphate in grains is not easily absorbed, animal products are considered to be a superior source of phosphate (Groff et al., 2000; Kreider, 1999). In addition to food sources, many commercially available supplements containing phosphate exist (Groff et al., 2000). The recommended
minimum dietary allowance for phosphate in adults is 700 mg/day (Kreider et al., 2010), while the average daily intake of phosphate in the developed world is approximately 1000 mg/day (Markowitz & Perazella, 2009). Due to the wide availability of phosphate, deficiencies are rare in the general population (Kreider, 1999).

**Regulation of phosphate levels**

Systemic regulation of phosphate homeostasis is a complex process, which is normally maintained by finite coordination between the intestine, the kidney and a range of hormones (Ohnishi & Razzaque, 2010). Under normal conditions, 60 to 70% of dietary phosphate is absorbed in the small intestine (Kreider, 1999; Markowitz & Perazella, 2009), with the resultant circulating phosphate readily absorbed into the plasma (Ohnishi & Razzaque, 2010; Tremblay et al., 1994). Once absorbed, phosphate enters the bone matrix protein, cells or the kidney (Ohnishi & Razzaque, 2010). The kidney is the main organ regulator of phosphate homeostasis (Burnett et al., 2006) and because phosphate acts as a threshold substance, it is thought that serum phosphate levels are regulated in part by an overflow mechanism (Kreider, 1999). For example, when serum phosphate levels are low, additional phosphate is absorbed from the proximal tubules of the nephron and/or from the small intestine (Burnett et al., 2006; Czuba, Zajac, Poprzecki, Cholewa, & Woska, 2009; Kreider, 1999). In contrast, when serum phosphate levels are high, extra phosphate is excreted by the kidney (Kreider, 1999).

Essential to maintaining this overflow mechanism and phosphate homeostasis is the hormonal regulation of parathyroid hormone (PTH) and 1,25-D,hydroxyvitamin D3 (1,25-D3) (Burnett et al., 2006; Kreider, 1999). Parathyroid hormone levels increase in response
to high serum phosphate levels to increase the excretion of phosphate from the kidneys into urine (Burnett et al., 2006; Czuba et al., 2009; Kreider, 1999). Conversely, with low serum phosphate levels, the concentration of 1,25-D3 increases to promote the reabsorption of phosphate from the gut and the release of phosphate from bone as a means to increase phosphate levels (Burnett et al., 2006). Cortisol and oestrogen may also play a part in the hormonal regulation of phosphate levels (Kreider, 1999), however, the influence of these two hormones is less clearly defined. Under normal circumstances, serum phosphate concentrations range between 0.75 to 1.35 mmol·L⁻¹ (Kreider, 1999; Tremblay et al., 1994), with approximately 175 to 300 mmol of phosphate excreted in the urine each day (Avioli, 1988; Guyton, 1987).

**Role of phosphate in the body**

Phosphates are involved in a number of important processes in the body. Phosphate has been identified as an essential component in bone mineralisation (Burnett et al., 2006), protein modification (Burnett et al., 2006), encoding of genetic material and nucleic acid synthesis (Burnett et al., 2006; Fukuda et al., 2010; Kreider, 1999; Ohnishi & Razzaque, 2010) and cell signaling (Burnett et al., 2006; Kreider, 1999; Ohnishi & Razzaque, 2010). In addition, phosphates play an important role in the acid-base balance of blood plasma, as well as within the muscle cells (Czuba et al., 2009; Kreider, 1999). The phosphate buffer is very important in the intracellular fluids where its concentration is high, but is relatively weak in the extracellular fluids where its concentration is lower (Kreider, 1999). Phosphate also helps to regulate oxygen release by haemoglobin (Hb) (Benesch & Benesch, 1969; Czuba et al., 2009) and may influence the contractile properties of the heart muscle (Kreider et al., 1992).
However, one of the most important roles of phosphate in the body is related to energy metabolism. Phosphate is essential for the formation and hydrolysis of adenosine triphosphate (ATP; Berner & Shike, 1988), which is the body’s primary energy substrate (Fukuda et al., 2010). Phosphate is also involved in the regulation of energy metabolism in a variety of ways (Kreider, 1999). For example, phosphate is vital in the metabolism of glucose, with glucose phosphorylated upon entry into the muscle cell (Kreider, 1999). Further, phosphate is an important component of phosphocreatine (PCr), which is central to the phosphagen energy system and the provision of anaerobic energy during intense exercise (Fukuda et al., 2010; Kreider, 1999).

Phosphate Supplementation as an Ergogenic Aid to Athletic Performance

Based on the important role of phosphate in energy metabolism, there has been increasing research on this compound as a potential ergogenic aid to athletic performance. For ergogenic purposes, extra phosphate has been supplemented orally, in capsule form, at a dose of 3-5 g a day for a period of between 3-6 days (Fukuda et al., 2010; Tremblay et al., 1994). Possible performance enhancing alterations associated with phosphate loading include an increased aerobic capacity (Cade et al., 1984; Czuba et al., 2009; Kreider et al., 1992), increased peak power (Czuba et al., 2009), increased anaerobic threshold (Cade et al., 1984; Kreider et al., 1992; Kreider, Miller, Williams, Somma, & Nasser, 1990) and improved myocardial and cardiovascular responses to exercise (Kreider et al., 1992).

Mechanisms behind the ergogenic effects of phosphate supplementation

A number of mechanisms have been posited to account for the ergogenic effects of phosphate supplementation. These include enhancements in 2,3-diphosphoglycerate (2,3-
DPG) concentrations, myocardial efficiency, buffering capacity and ATP/PCr resynthesis (Czuba et al., 2009; Kreider et al., 1992; Tremblay et al., 1994). These mechanisms have been proposed based on the notion that phosphate supplementation increases serum phosphate levels, which allows processes normally limited by phosphate availability to operate at an enhanced level (Tremblay et al., 1994).

Enhanced 2,3-DPG concentration

One prominent explanation for the ergogenic effects associated with phosphate supplementation proposes that phosphate loading increases the concentration of 2,3-DPG precursors (i.e. 1,3-diphosphoglycerate), resulting in enhanced 2,3-DPG synthesis and greater 2,3-DPG concentrations in the red blood cell (RBC) (Bremner, Bubb, Kemp, Trenell, & Thompson, 2002). Importantly, 2,3-DPG binds to Hb and reduces the affinity of oxygen with Hb. Thus, increased 2,3-DPG allows for a greater unloading of oxygen to the peripheral tissues/muscle (Benesch & Benesch, 1969; Duhm, 1971; Goss et al., 2001). This process is reflected by a rightward shift in the oxygen-Hb dissociation curve with increased 2,3-DPG concentrations (Bremner et al., 2002; Duhm, 1971; Fukuda et al., 2010; Goss et al., 2001).

Oxygen release at the peripheral tissues is considered to be a major influence on maximal oxygen uptake (\(\dot{V}O_{2\text{max}}\)) (Bremner et al., 2002), which is the highest rate at which oxygen can be taken up and utilised by the body during intense exercise (Goran, Fields, Hunter, Herd, & Weinsier, 2000; McArdle, Katch, & Katch, 2006). Thus, increased 2,3-DPG concentrations could promote increases in \(\dot{V}O_{2\text{max}}\) (Fukuda et al., 2010; Goss et al., 2001). Of relevance, an increased \(\dot{V}O_{2\text{max}}\) could translate into exercise performance benefits for
endurance athletes, as it would allow them to exercise at a greater work rate for longer due to enhanced oxygen supply during exercise (Bremner et al., 2002).

Support for enhanced 2,3-DPG concentrations with phosphate loading has come primarily from two studies, which have successfully demonstrated increases in serum phosphate and 2,3-DPG concentrations in response to phosphate supplementation (Cade et al., 1984; Czuba et al., 2009). Specifically, Cade et al., (1984) reported a significant increase in resting 2,3-DPG concentration from 13.00 to 13.92 mg/Hb after oral consumption of 4 g of SP per day, over a three day period, when compared with a placebo. Furthermore, Czuba et al., (2009) identified that serum phosphate levels were significantly correlated to resting 2,3-DPG concentration and that after participants consumed 50 mg·kg\(^{-1}\) of FFM of tri-SP a day (split into four equal doses over the day) for a total period of six days, both serum phosphate and 2,3-DPG concentrations increased significantly (30 and 25% increase, respectively). This led the authors to suggest that an increase in 2,3-DPG concentration with phosphate loading was likely to reflect an increase in serum phosphate levels (Czuba et al., 2009). Subsequent to the increases in 2,3-DPG concentrations seen with phosphate loading, Cade et al., (1984) and Czuba et al., (2009) both reported increases in \(\dot{V}O_{2\max}\).

Importantly, it should be noted that some studies on phosphate supplementation have not measured 2,3-DPG concentrations (Folland, Stern, & Brickley, 2008; Kreider et al., 1990), while others have found ergogenic benefits associated with phosphate loading without a significant change in 2,3-DPG concentrations (Stewart, McNaughton, Davies, & Tristram, 1990). This raises the possibility that changes in 2,3-DPG concentration may not have to be statistically significant to result in ergogenic benefits. Consequently, it is important for future research to evaluate 2,3-DPG levels when investigating phosphate loading.
Nonetheless, it is not clear whether this mechanism is completely, or even partially, responsible for the ergogenic effects associated with phosphate supplementation.

*Enhanced myocardial efficiency*

Another mechanism which has been proposed to account for the ergogenic effects associated with phosphate loading is enhanced myocardial efficiency (Czuba, Zajac, Poprzecki, & Cholewa, 2008; Fukuda et al., 2010). Specifically, it has been suggested that phosphate supplementation may contribute to enhanced myocardial contractility, resulting in improved myocardial efficiency. Improving myocardial efficiency would theoretically provide ergogenic benefits to athletes engaged in endurance exercise by allowing an increase in stroke volume (SV), which could result in a greater cardiac output for the same heart rate (HR) during exercise (Czuba et al., 2009) and consequently more efficient oxygenation of the exercising muscles.

The basis for this explanation comes from work on hypophosphatemia (Rubin & Narins, 1990; Fuller, Nichols, Brenner, & Peterson, 1978), which is a condition characterised by abnormally low blood concentrations of inorganic phosphates (Gaasbeek & Meinders, 2005; Newman, Neff, & Ziporin, 1977). During hypophosphatemia, the contractile properties of the heart are reduced and as a result SV also decreases (O’Connor, Wheeler, & Bethune, 1977). As phosphate deficiencies have been linked to decreased synthesis of ATP (Fuller et al., 1978; Lichtman, Miller, Cohen, & Waterhouse, 1971), it is thought that the reduced contractility of the heart associated with hypophosphatemia is most likely related to very low levels of cardiac cell ATP (Gaasbeek & Meinders, 2005; O’Connor et al., 1977). Research on the reversal of hypophosphatemia through phosphate loading has
reported that hyperphosphatemia (high blood concentrations of inorganic phosphates) may increase the concentration of cardiac cell ATP, which in turn could increase the contractility of the heart muscle and improve myocardial efficiency (O’Connor et al., 1977; Stoff, 1982; Zazzo, Troche, Ruel, & Maintenant, 1995). In agreement with this, O’Connor et al., (1977) observed that mean left ventricular SV increased after phosphate administration in critically ill, hypophosphatemic patients.

Improved myocardial efficiency with phosphate supplementation is also supported by studies in athletes that have demonstrated increases in SV (Kreider et al., 1992) and decreases in HR both at rest and during exercise following a period of phosphate supplementation (Czuba et al., 2009; Farber, Sullivan, Fineberg, Carlone, & Manfredi, 1984; Lunne, Zauner, Cade, Wright, & Conte, 1980). For example, Czuba et al., (2009) reported a significant decrease in resting (9.6%) and maximal exercise HR (2.7%) after supplementation with 50 mg·kg⁻¹ of FFM of SP per day for a total of six days. The authors suggested that the decreased HR observed with phosphate loading could be due to enhanced contractility of the heart causing an increase in SV. This notion of enhanced myocardial efficiency with phosphate loading is supported by a study by Kreider et al., (1992), who employed cardiac ultrasound and colour flow Doppler technology to examine the effects of phosphate loading on myocardial responses to exercise. In this study, six elite male cyclists and/or triathletes consumed either 1 g of tribasic SP or a glucose placebo four times a day for a total of five days. On the fourth day of supplementation, participants performed either an incremental maximal cycling test or a simulated 40 km cycling time trial (TT). The opposite exercise test was then performed on the fifth day of supplementation. A 17 day washout period was observed between trials. Results from the
myocardial analysis revealed that SP loading significantly increased mean maximal left ventricular end diastolic diameter by 4%, ejection fraction by 5% and SV by 4% during the incremental maximal exercise test. Furthermore, an 8% reduction in HR was observed during the 40 km TT. These results are indicative of enhanced myocardial efficiency. However, it is important to note that although increases in SV and a decrease in HR are likely to be strong indicators that the heart is functioning more efficiently, they are not direct measures of myocardial efficiency or heart contractility (Czuba et al., 2008; O’Connor et al., 1977). Consequently, in order to fully support this proposed mechanism, evidence of changes to cardiac cell ATP and cardiac contractility with phosphate loading are required (Bredle, Stager, Brechue, & Farber, 1988). Of relevance, no research to date has been conducted to directly assess cardiac cell ATP and heart contractility with phosphate supplementation due to the extremely invasive protocols that would be required (Wu, Zhang, Zhang, Bache, & Beard 2008). However, advancements in $^{31}$phosphate-magnetic resonance spectroscopy (P-MRS) technology may allow such research to be undertaken in the future (Wu et al., 2008).

Enhanced buffering capacity

It has also been suggested that the ergogenic benefits associated with phosphate loading may be attributable to an enhanced buffering capacity (Czuba et al., 2008; Kreider, 1999). Phosphates are involved in the body’s physiological buffering systems and consequently it has been hypothesised that phosphate loading may enhance the capacity of the phosphate buffering system in the intracellular fluid where the concentration of phosphate is greatest (Czuba et al., 2009; Kreider, 1999). Specifically, it has been proposed that phosphate loading may delay the decrease in pH associated with the production of lactic acid during
intense exercise (Kreider, 1999) and consequently improve exercise performance by increasing the anaerobic threshold (Czuba et al., 2009). This is based on the notion that phosphate loading may increase hydrogen phosphate (HPO$_4$\(^-\)) concentrations, which could enhance the ability of the body to buffer hydrogen ions produced during intense exercise (Kreider, 1999). Hydrogen phosphate is a weak base and is one of the main components of the phosphate buffering system, with the other main component being the weak acid dihydrogen phosphate (H$_2$PO$_4$\(^-\)) (Kreider, 1999; Vander, Sherman, & Luciano, 1994). When a strong acid (e.g. lactic acid) is added to these two substances, the hydrogen from the acid can be accepted by HPO$_4$\(^-\) and converted to H$_2$PO$_4$\(^-\) to minimise the decrease in pH (Vander et al., 1994). Therefore, an augmented HPO$_4$\(^-\) concentration resulting from phosphate loading, may allow for more hydrogen ions to be buffered during exercise, which would attenuate the decrease in pH associated with intense exercise (Kreider, 1999). This could improve both aerobic and anaerobic exercise performance by allowing athletes to work harder for longer before reaching their anaerobic threshold (Czuba et al., 2008) and by allowing individuals to exercise with higher lactate levels during high intensity exercise efforts (Czuba et al., 2009; Kreider, 1999; Kreider et al., 1992; Stewart et al., 1990). Thus, phosphate loading is thought to enhance buffering capacity and improve exercise performance in a similar manner to sodium bicarbonate supplementation (Kreider, 1999).

Whilst there is strong theoretical support for enhanced hydrogen ion removal during maximal exercise efforts with phosphate loading, there is limited significant experimental evidence to support this notion. The majority of the evidence supporting an enhanced buffering capacity with phosphate supplementation is based upon studies that have demonstrated a shift in the anaerobic threshold to higher workloads with phosphate loading.
(Czuba et al., 2009; Kreider et al., 1992; Kreider et al., 1990). For example, Kreider et al., (1992) reported that during a 40 km cycling TT, participants supplemented with SP exercised at 86% of \( \dot{V}O_{2\text{max}} \) compared with a placebo trial where participants exercised at 80% of \( \dot{V}O_{2\text{max}} \). Exercising at a higher percentage of \( \dot{V}O_{2\text{max}} \) during a prolonged TT could be a reflection of an improved buffering system extending the capacity of the body to exercise at a greater intensity before excessive hydrogen ion accumulation occurs (i.e. increase the anaerobic threshold). Furthermore, Czuba et al., (2009) demonstrated that during a graded maximal exercise test, the anaerobic threshold occurred at a significantly higher workload after phosphate supplementation [295 ± 19 watts (W)] when compared with baseline performance (280 ± 26 W). These previous studies (Czuba et al., 2009; Kreider et al., 1992) both showed increases in serum phosphate concentrations with phosphate loading, suggesting that the observed changes in the anaerobic threshold may be phosphate-stimulated. Whilst these studies support the notion that phosphate loading may enhance the body’s buffering capacity, due to a lack of studies directly assessing the effects of phosphate loading on intracellular phosphate buffering, further research is needed to support this mechanism.

Enhanced ATP/PCr resynthesis

Another mechanism proposed to explain the ergogenic effects of phosphate supplementation is enhanced ATP resynthesis and oxidative energy metabolism via increased availability of extracellular and intracellular phosphate (Bredle et al., 1988; Fukuda et al., 2010; Kreider, 1999; Kreider et al., 1992). Enhanced ATP resynthesis could provide ergogenic benefits by providing a larger energy (phosphate) pool (Fukuda et al., 2010; Galloway, Tremblay, Sexsmith, & Roberts, 1996; Kreider, 1999; Kreider et al., 2009; Kreider et al., 1992).
Three main mechanisms have been identified by which phosphate supplementation may enhance oxidative energy metabolism and ATP resynthesis. Firstly, phosphate supplementation may increase the amount of cellular phosphate available for glucose phosphorylation (Kreider, 1999). This could promote more efficient entry of glucose into the oxidative pathway and thus enhance ATP resynthesis (Chasiotis, 1988). Secondly, there is evidence that phosphate loading may stimulate the activity of various enzymes involved in oxidative metabolism, such as phosphofructokinase and glyceraldehyde 3-phosphate (Brazy, Mandel, Gullans, & Soltoff, 1984; Lichtman et al., 1971; Passonneau & Lowry, 1962). As these enzymes are involved in the regulation of oxidative metabolism, greater stimulation is likely to promote increased and more efficient oxidative metabolism (Brazy, Gullans, Mandel, & Dennis, 1982). Thirdly, phosphate loading may lead to increased availability of phosphate in the electron transport chain, which could promote increased ATP production (Chasiotis, 1988). Support for this mechanism comes from studies showing increased mitochondrial oxidative capacity after increasing extracellular and intracellular phosphate levels (Brazy et al., 1982; Brazy et al., 1984; Brazy & Mandel, 1986). However, these studies may have limited relevance to humans as they were performed in-vitro and involved animals (Brazy et al., 1982; Brazy et al., 1984). Furthermore, it has been suggested that serum phosphate concentrations may not accurately reflect the effects of phosphate supplementation on oxidative metabolism and intracellular phosphate levels (Kreider et al., 1992). Consequently, more research is needed on the effect of phosphate loading on ATP resynthesis.

In addition to the potential influence of phosphate supplementation on oxidative metabolism and ATP resynthesis, it has been hypothesised that increased cellular
concentrations of phosphate may allow for more rapid restoration of ATP and PCr in the muscles during and following intense anaerobic exercise (Kreider, 1999). This is thought to consequently improve exercise performance by allowing more efficient production and recovery of energy stores during exercise (Kreider, 1999). Whilst this explanation has a strong theoretical basis (Kreider et al., 1992; Kreider, 1999), there is a paucity of research investigating PCr resynthesis following phosphate loading. As a result, it is unclear whether phosphate supplementation influences PCr resynthesis, suggesting that further research is required, potentially utilizing Magnetic Resonance Spectroscopy.

**Research Investigating the Ergogenic Effects of Phosphate Supplementation**

Studies investigating the ergogenic value of phosphate supplementation date back to the 1920’s (Emden, Grafe, & Schmitz, 1921). Two of the initial studies suggested that phosphate supplementation may increase physical working capacity and prevent fatigue in adult men (Emden et al., 1921; Riabuschinsky, 1930), however other early reports were equivocal (Flinn, 1926; Johnson & Black, 1953). Some later results supported the hypothesis that alterations in the availability of extracellular and intracellular phosphate salts may influence endurance exercise performance (Cade et al., 1984; Keller & Kraut, 1959). This led to a number of more recent studies investigating the ergogenic effects of sodium and/or calcium phosphate supplementation on endurance exercise (Bredle et al., 1988; Czuba et al., 2008; Kreider et al., 1992). From these studies, varied results have been observed, depending on whether sodium or calcium phosphate was used for supplementation.
Contemporary studies investigating the ergogenic effects of phosphate supplementation on endurance exercise have demonstrated ergogenic benefits with SP but not with calcium phosphate (Bredle et al., 1988; Cade et al., 1984; Czuba et al., 2008; Czuba et al., 2009; Folland et al., 2008; Galloway et al., 1996; Kreider et al., 1992; Kreider et al., 1990; Mannix, Stager, Harris, & Farber, 1990; Stewart et al., 1990). For example, Bredle et al., (1988) observed no change in $\dot{V}O_{2\text{max}}$ in male runners after four days of 5.7 g a day of calcium phosphate loading. Likewise, Galloway et al., (1996) reported no change in time to exhaustion (TTE) or $\dot{V}O_{2\text{max}}$ after an acute (90 min pre exercise) dose of 22.2 g of calcium phosphate, in trained and untrained male cyclists. This is in contrast to studies employing SP loading, where increases in $\dot{V}O_{2\text{max}}$ ranging between 5-12% (Cade et al., 1984; Czuba et al., 2008; Czuba et al., 2009) and improvements in endurance TT performance (Kreider et al., 1992; Schenck et al., 1991) have been reported following 3-6 days of loading. However, it is important to acknowledge that not all studies utilising SP have demonstrated ergogenic effects (Brennan & Connolly, 2001; West, Ayton, Wallman, & Guelfi, 2012).

The superiority of SP over calcium phosphate is surprising as many initial investigations hypothesised that calcium phosphate would be advantageous over SP, based on early reports suggesting that calcium phosphate would result in larger plasma phosphate increases than SP (Bredle et al., 1988; Tremblay et al., 1994). This notion was supported by multiple studies reporting higher resting serum phosphate concentrations after calcium phosphate loading, compared with those studies utilising SP (Bredle et al., 1988; Fukuda et al., 2010; Tremblay et al., 1994). This raises the possibility that SP may influence
intracellular phosphate levels and endurance exercise in a unique way, which is not seen with calcium phosphate supplementation (Bredle et al., 1988).

The exact nature of SP’s influence on intracellular phosphate levels and endurance exercise has yet to be identified. However, an explanation may come from research showing that intracellular concentrations of phosphate are, at least in part, sodium dependent (Chobanian, Anderson, & Brazy, 1995; Galloway et al., 1996). Such research has demonstrated a sodium-phosphate, co-transportation interaction in controlling phosphate levels in the canine kidney. Specifically, this SP co-transportation mechanism is thought to be an active process needed to maintain cellular phosphate levels above equilibrium (Chobanian et al., 1995). Consequently, for extra phosphate ingestion to allow intracellular phosphate concentrations to increase and thereby result in ergogenic effects, some form of enhanced sodium-phosphate co-transportation could be required (Galloway et al., 1996). This may explain why only studies using SP have reported ergogenic benefits. However, this explanation is only speculative as the above co-transportation mechanism has not yet been researched in humans. Regardless of the mechanism responsible, due to the divergent results seen with sodium and calcium phosphate, it has been suggested that only SP should be viewed as a nutritional ergogenic aid to improve endurance exercise (Tremblay et al., 1994). Consequently, the following sub-sections will only review the recent studies which have evaluated the effects of SP loading on exercise performance.
Sodium phosphate loading and endurance exercise

Aerobic capacity (\(\dot{V}O_{2\text{max}}\))

The majority of studies investigating the ergogenic effects of SP loading have focused on aerobic capacity. In one of the first studies to systematically evaluate the ergogenic effects of phosphate loading, Cade et al., (1984) examined the influence of SP supplementation on \(\dot{V}O_{2\text{max}}\), serum phosphate and RBC 2,3-DPG levels in ten trained male runners (\(\dot{V}O_{2\text{max}} 56.2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)). A cross-over and counterbalanced design was employed in which participants performed an incremental exercise test to exhaustion on a treadmill, following three days of consumption of either 1 g of SP or 0.1 g of sodium citrate (placebo) four times a day. The SP protocol was performed twice. Each loading protocol was separated by a four day washout period. Results demonstrated that SP loading significantly increased resting serum phosphate (1.17 to 1.22 mmol·L\(^{-1}\)) and RBC 2,3-DPG levels (13.00 to 13.92 mg/Hb). Furthermore, a 6-12% increase in \(\dot{V}O_{2\text{max}}\) was observed with phosphate supplementation.

Further research ensued, with other studies showing similar effects on \(\dot{V}O_{2\text{max}}\) (Czuba et al., 2008; Czuba et al., 2009; Fukuda et al., 2010; Kreider et al., 1992; Kreider et al., 1990; Stewart et al., 1990). For example, Stewart et al., (1990) investigated the effects of SP loading on \(\dot{V}O_{2\text{max}}\), serum phosphate and RBC 2,3-DPG concentration in eight trained male cyclists (\(\dot{V}O_{2\text{max}} 48.5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)). Specifically, participants consumed either a placebo or 3.6 g of SP a day, for three days, followed by a maximal cycle ergometry test. A four day washout period separated each supplementation trial. Despite no change in either resting serum phosphate or 2,3-DPG concentration, an 11% increase in \(\dot{V}O_{2\text{max}}\) and a
20% increase in TTE were observed following SP supplementation compared with baseline.

Additional support for the ergogenic effects of SP has come from Kreider et al., (1990), who reported a 9% increase in $\dot{V}O_{2\text{max}}$ and a 12% increase in the ventilatory anaerobic threshold during a treadmill exercise test following SP supplementation. Specifically, seven highly trained male runners ($\dot{V}O_{2\text{max}} 73.9 \pm 6.3 \text{ mL·kg}^{-1}·\text{min}^{-1}$) ingested either 4 g of glucose (placebo) or SP per day for six days. Participants were required to perform either an incremental treadmill exercise TTE or a five mile TT on the third day of loading. This was then followed by the opposite exercise test on the sixth day of loading. A 14 day washout period was undertaken between each loading protocol. The results showed that, in addition to the increase in $\dot{V}O_{2\text{max}}$ and ventilatory anaerobic threshold seen in the maximal exercise test, a 17% increase in resting serum phosphate levels also occurred after SP supplementation. Red blood cell 2,3-DPG concentrations were not examined in this study.

Following on from their previous work, Kreider et al., (1992) recruited six elite male cyclists and/or triathletes (40 km time trial completion time of 56.7 ± 2.5 min) who, in a counterbalanced and crossover design, consumed either 1 g of tribasic SP or a glucose placebo four times a day, for a total of five days. Participants performed either an incremental maximal cycling TTE or a simulated 40 km cycling TT on the fourth day of supplementation. The opposite exercise test was then performed after an additional day of supplementation. A 17 day washout period was employed between trials. It was observed that phosphate supplementation significantly increased oxygen uptake ($\dot{V}O_2$) and power output at the anaerobic threshold by 10% and 9% respectively, time to anaerobic threshold
by 10% and $\dot{V}O_{2\text{max}}$ by 9% during the maximal exercise test. Furthermore, resting serum phosphate levels were 17% greater with phosphate supplementation, but no significant differences were seen between the placebo and the SP trial for red cell 2,3-DPG concentration (Kreider et al., 1992).

Research conducted by Czuba et al., (2008; 2009) also supports the use of SP as an ergogenic aid. Specifically, Czuba et al., (2009) examined the effect of both short-term and long-term SP supplementation on $\dot{V}O_{2\text{max}}$ in 19 well-trained cyclists (mean $\dot{V}O_{2\text{max}} \sim 73 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Their SP loading protocol consisted of two phases. In the initial phase, participants consumed 50 mg·kg$^{-1}$ of FFM of SP a day, split into four equal doses over the day for six days. Following the six days of supplementation, an incremental, maximal cycling test was performed. In the second phase of phosphate supplementation, which continued on from the first phase, participants consumed 25 mg·kg$^{-1}$ of FFM of SP a day for a further three weeks. After phase two of supplementation, another incremental, maximal cycling test was performed. Following the initial six days of loading, these researchers reported a significant 5% increase in $\dot{V}O_{2\text{max}}$ and a 5% increase in the ventilatory (anaerobic) threshold compared with baseline. Continued supplementation in phase two did not result in a further significant increase in $\dot{V}O_{2\text{max}}$ or the ventilatory threshold compared with the initial six days. However, after phase two, $\dot{V}O_{2\text{max}}$ was still significantly higher compared with baseline values. It was also observed that resting serum phosphate and 2,3-DPG concentration increased after phase one, whilst both resting and exercising HR significantly decreased. No significant changes were reported in regards to any lactate variables following phosphate supplementation. The phosphate loading protocol used by Czuba and colleagues in this study, as well as in an earlier study (50
mg·kg\(^{-1}\) of FFM, for six days; Czuba et al., 2008) are particularly important to note because unlike previous research on phosphate loading, both these studies supplemented SP relative to body composition or size, by using a FFM corrected dose.

Finally, a recent study conducted by Brewer, Dawson, Wallman and Guelfi, (2013) adopted the initial loading protocol of Czuba et al., (2009) and investigated the effects of repeated SP loading (50 mg·kg\(^{-1}\) of FFM per day over six days) on aerobic capacity using an incremental cycle protocol to exhaustion. After the initial supplementation phase and exercise testing, a 14 day washout period was undertaken, followed by a secondary supplementation phase and retesting. These researchers found a significant increase in peak maximal oxygen uptake (\(\dot{V}O_{2\text{peak}}\)) following the first SP loading phase compared with baseline and placebo (3.5 and 4.3\%, respectively), with a further significant increase found after the second phase of SP loading (7.1 and 7.7\%) compared with baseline and placebo.

Whilst the majority of studies investigating SP loading and \(\dot{V}O_{2\text{max}}\) have demonstrated ergogenic effects, it must be noted that some studies have shown no effect. For example, research by Brennan and Connolly (2001) was unable to show ergogenic benefits with SP supplementation. Specifically, they reported that 4 g a day of sodium diphosphate supplementation for four days resulted in no significant changes to \(\dot{V}O_{2\text{max}}\) in a group of 12 trained male cyclists (\(\dot{V}O_{2\text{max}}\) 60.6 ± 4.4 mL·kg\(^{-1}\)·min\(^{-1}\)). However, the authors did note an increase in 2,3-DPG concentration with phosphate supplementation. Likewise, West et al., (2012) observed no change in \(\dot{V}O_{2\text{peak}}\) following six days of SP supplementation (50 mg·kg\(^{-1}\) of FFM) in moderately trained males and females. The reason for this inconsistency between studies is not clear. West et al., (2012) suggested that it was
possible that only certain individuals responded positively to SP supplementation since the relationship between the change in serum phosphate and the change in $\dot{V}O_{2\text{peak}}$ approached significance in their study. The factors influencing this potential variable response may relate to baseline/pre-loading phosphate levels or other unknown factors that require investigation.

*Endurance performance*

The previously mentioned improvements in aerobic capacity as a result of SP loading suggest that SP may also have ergogenic effects in relation to endurance performance. However, to date, only a limited number of studies have assessed the effects of SP supplementation on actual measures of endurance performance. In the previously mentioned study by Kreider et al., (1990), the influence of SP loading was examined on both $\dot{V}O_{2\text{max}}$ and five mile running TT performance. Whilst the results from the TT showed only slight, non-significant reductions in performance time (11.8 s decrease in time to completion) and average mile split time (2.5 s decrease), $\dot{V}O_2$ during the run was significantly lower. This decreased mean $\dot{V}O_2$ may indicate enhanced physiological efficiency resulting from phosphate supplementation, which would theoretically be of ergogenic value during endurance exercise (Kreider 1999). However, this was not reflected in significantly improved performance time in this study (Kreider et al. 1990).

Another study by Kreider et al., (1992) (described earlier) further investigated an ergogenic effect of phosphate loading on endurance performance. Here, in addition to a $\dot{V}O_{2\text{max}}$ test, participants were required to complete a simulated 40 km cycling TT after either SP or placebo supplementation. Results from the 40 km cycling TT revealed that SP loading
resulted in an increased cycling power output by 17%, \( \dot{V}_\text{O}_2 \) by 18%, ventilation by 15% and an 8% reduction in HR, which were all thought to contribute to an 8% (3.5 min) reduction in completion time when compared with the placebo trial. A similar study conducted by Schenck et al., (1991) also demonstrated improvements in 40 km cycling TT performance with SP supplementation. Six trained male cyclists (mean \( \dot{V}_\text{O}_2\text{max} \) 69.3 mL·kg\(^{-1}\)·min\(^{-1}\)) were required to ingest either 4 g of SP or a placebo, for 3-4 days prior to performing a simulated 40 km cycling TT. Each loading protocol was separated by a 14 day washout period. Their results showed an increase in \( \dot{V}_\text{O}_2 \) and cycling power output by 17% and 16% respectively, during the phosphate supplemented trial, as well as a reduced 40 km cycling TT completion time when compared to the placebo trial (46.14 to 42.25 min).

Folland et al., (2008) also investigated endurance performance following SP supplementation, by examining the effect of SP loading on 16.1 km (10 mile) cycling TT performance in seven trained male cyclists. Specifically, prior to completing a 16.1 km cycling TT, participants consumed either a placebo (alpha lactate monohydrate) or 1 g of SP four times a day for six days. In the SP trial, mean cycling power output was significantly higher (9.8%) and TT completion time 3% faster when compared with the placebo trial. The improved performance following SP supplementation was associated with similar blood lactates and HR to that observed during the placebo trial.

In the only other study to examine the effect of SP loading on endurance performance, Brewer et al., (2013) investigated the effect of repeated SP loading on 1000 kJ (~40 km) cycling TT performance. Here, nine trained male cyclists were supplemented with 50 mg·kg\(^{-1}\) of FFM of tribasic SP or a placebo for six days. The SP loading protocol was
undertaken by each participant twice. Results demonstrated that although the 40 km TT completion times were faster after SP supplementation compared with placebo and baseline, these changes were not statistically significant or supported by smallest worthwhile change or effect size analysis. However, mean power output was significantly higher compared with placebo at the 250 kJ and 500 kJ time points of the TT test following the second SP loading phase. Reasons for differences in results between studies on endurance performance following SP supplementation are still unclear.

Research Considerations for Sodium Phosphate Supplementation

The limited amount of research investigating endurance performance is not the only issue associated with studies that have assessed the ergogenic value of SP loading. A further complicating issue relates to a lack of consistent methodology used (Kreider et al., 1992; Tremblay et al., 1994). A critical analysis of the studies examining the ergogenic effects of SP loading demonstrates that many of these studies differ in terms of the administered dose and dosing protocol, the length of the supplementation and washout period employed, and the fitness level of the participants used (Galloway et al., 1996; Kreider et al., 1992; Tremblay et al., 1994). Of relevance, these particular variables are thought to influence the ergogenic effects of phosphate loading (Tremblay et al., 1994) as outlined below.

Another important methodological consideration regarding phosphate loading is the effect of gender, which has received very little attention in the literature. To date, males have primarily been studied with respect to the ergogenic effects of phosphate loading on aerobic capacity and endurance performance (Fukuda et al., 2010), with only one study investigating females (West et al., 2012). However, due to a number of physiological
differences between males and females (O'Brien, 1985), gender is likely to have an influence on the ergogenic effects of phosphate loading (Fukuda et al., 2010).

An additional critical area of research that has not yet been investigated is the effect of SP loading on anaerobic exercise performance and aspects of team sport performance such as repeated-sprint ability (RSA). Many of the mechanisms proposed to explain the ergogenic effect of SP loading on endurance performance also indicate a potential benefit for anaerobic exercise.

Furthermore, the side-effects associated with SP loading are also an important factor to consider when investigating SP supplementation. The following sections will systematically evaluate the current literature on SP loading with respect to the above experimental considerations, in order to provide some clarity on the ergogenic value of SP supplementation, as well as to suggest future research directions.

**Dose and dosing protocol**

The administered dosage of SP, as well as the dosing strategy used has varied between studies (Fukuda et al., 2010; Kreider, 1999; Tremblay et al., 1994). Furthermore, there is no conclusive evidence in the literature indicating an optimal SP dosing protocol. Most studies investigating SP loading have administered doses of phosphate ranging between 3-5 g per day, for a period of 3-6 days (Fukuda et al., 2010; Kreider, 1999; Tremblay et al., 1994). The majority of studies using doses within this range have reported significant increases in both $\dot{V}O_{2\text{max}}$ and resting serum phosphate levels post supplementation (Cade et al., 1984; Czuba et al., 2009; Fukuda et al., 2010; Kreider et al., 1992; Stewart et al., 1990). Doses greater than 6 g have typically been avoided as they are thought to be associated with
a PTH-mediated down regulation in serum phosphate levels (Burnett et al., 2006; Galloway et al., 1996), while doses below 3 g are generally not used as this dose is thought to be too small to significantly raise serum phosphate levels (Tremblay et al., 1994). Whilst this effective 3-5 g spectrum has been identified, no studies to date have conducted systematic research to determine an optimal dose within this range, although a 4 g dose has most consistently shown ergogenic effects, as well as significant increases in serum phosphate levels (Fukuda et al., 2010; Tremblay et al., 1994).

When ingesting phosphate, the dosing strategy commonly requires the SP to be taken orally, in capsule form, either as a single dose each day (Bredle et al., 1988; Brennan & Connolly, 2001; Galloway et al., 1996; Goss et al., 2001; Mannix et al., 1990; Schenck et al., 1991) or split into 3-4 equal doses over the day (Cade et al., 1984; Czuba et al., 2008; Czuba et al., 2009; Folland et al., 2008). Of these two protocols, recent research has supported using multiple doses over the day as the preferred method of consumption (Fukuda et al., 2010). Whilst both protocols have been effective in increasing serum phosphate levels (Tremblay et al., 1994), multiple smaller doses are preferred, as it is thought to avoid the PTH response associated with a single, larger dose of phosphate (Tremblay et al., 1994). Based on this premise, it has been recommended that for improvements in aerobic capacity and endurance performance, the administered dose should consist of 1 g of SP, ingested four times a day (Czuba et al., 2009; Fukuda et al., 2010). However, this recommendation does not account for differences in body size and body composition between individuals. It has been suggested that it may be important to use a dose relative to body composition when loading SP, as it would help to ensure that participants of differing body sizes are receiving a similar proportion of exposure (Casais et
al., 2009). Support for this notion comes from studies using oral SP as a bowel purgative, which note that the response to a phosphate dose is likely to vary with differing body sizes (Casais et al., 2009; Deepak & Ehrenpreis, 2011; Markowitz & Perazella, 2009). To date, the only exercise studies to use a relative dose of phosphate when loading are those by Czuba et al., (2008; 2009), West et al., (2012) and Brewer et al., (2013) in which 50 mg·kg$^{-1}$ of FFM of SP was ingested each day, divided into four equal doses over the day, for six days. Czuba et al., (2008, 2009) demonstrated that this dosing protocol was effective in increasing $\dot{V}O_{2\text{max}}$ and serum phosphate levels. However, Brewer et al., (2013) saw an increase in $\dot{V}O_{2\text{max}}$ without a change in serum phosphate, while West et al., (2012) saw no change in either variable. Consequently, it is currently unclear as to what the optimal relative dose for SP loading is to achieve an ergogenic effect in respect to endurance performance. Therefore, further research is required in this area.

**Length of loading period**

The duration of loading employed in studies investigating SP supplementation have typically ranged from 3-6 days, and more recently up to three weeks (Czuba et al., 2009; Fukuda et al., 2010; Goss et al., 2001). Studies by Cade et al., (1984) and Czuba et al., (2008; 2009) have all shown significant increases in serum phosphate levels after 3-6 days of SP loading. From these studies, it has been identified that phosphate loading periods of 3-6 days may effectively improve endurance performance (Folland et al., 2008; Kreider et al., 1992; Schenck et al., 1991) and also increase $\dot{V}O_{2\text{max}}$ by between 5-12% (Cade et al., 1984; Czuba et al., 2008; Czuba et al., 2009). Furthermore, based on these studies it has been proposed that at least three days of loading may be required for serum phosphate levels to increase to concentrations sufficient to provide ergogenic benefits (Bremner et al.,
2002; Goss et al., 2001; Kreider et al., 1992), while serum phosphate levels are likely to have peaked by six days, with no additional benefits gained from further supplementation (Cade et al., 1984). However, it is important to note that no studies have systematically measured the response of serum phosphate to different durations/days of loading.

**Washout period**

There is a lack of consistency between studies on phosphate loading with respect to the length of the washout period used (Tremblay et al., 1994). Generally, studies investigating the ergogenic effects of SP supplementation have employed washout periods ranging between 7-14 days (Cade et al., 1984; Kreider et al., 1990; Kreider et al., 1992; Schenck et al., 1991; Stewart et al., 1990; Tremblay et al., 1994). However, in some instances, shorter washout periods such as four days (Cade et al., 1984; Stewart et al., 1990) and longer washout periods such as 17 days (Kreider et al., 1992) have been used. Of importance, it has been identified in the previously reviewed study by Cade et al., (1984), that after three days of SP loading, it took nearly two weeks for RBC 2,3-DPG concentration to return to near baseline levels (Tremblay et al., 1994). Based on this, Cade et al., (1984) advocated that a minimum 14 day washout period was required between supplementation trials in order to remove any carryover effects from phosphate supplementation. Consequently, studies employing brief washout periods (i.e. less than 14 days) are unlikely to give an accurate representation of the influence of phosphate loading (Kreider, 1999). Therefore, in order to accurately assess the ergogenic value of SP loading, future research should aim to employ washout periods of no less than 14 days.
**Fitness level of participants**

It has been suggested that the fitness levels of study participants may influence the ergogenic properties of SP loading (Galloway et al., 1996; Tremblay et al., 1994). Specifically, it has been argued that participants with higher fitness levels (represented by $\dot{V}O_{2\text{max}}$) may be less likely to receive ergogenic benefits from SP loading when compared with participants with lower fitness levels (Galloway et al., 1996; Tremblay et al., 1994). However, no studies have systematically examined this issue to date. Of relevance, a number of longitudinal and cross-sectional studies have demonstrated that participants of varying aerobic fitness levels have different RBC 2,3-DPG concentrations (Brodthagen, Norregaard Hansen, & Bjerre Knudsen, 1985; Galloway et al., 1996; Hespel et al., 1988; Mairbaurl, Humpeler, Schwabager, & Pessenhofer, 1983; Remes, Vuopio, & Harkonen, 1979). Specifically, some endurance trained participants have been reported to have higher 2,3-DPG concentrations when compared with untrained participants (Remes et al., 1979), with these higher levels being attributed to an increase in red cell turnover (Brodthagen, et al., 1985). This process is thought to be an adaptive response to endurance training (Tremblay et al., 1994). Consequently, it has been hypothesised, that due to an existing higher 2,3-DPG concentration, trained endurance athletes may be less responsive to the possible ergogenic effects of phosphate supplementation when compared with untrained participants (Galloway et al., 1996; Tremblay et al., 1994). Nonetheless, research has demonstrated that trained participants can still receive ergogenic benefits from SP supplementation (Cade et al., 1984; Czuba et al., 2008; Kreider et al., 1992; Stewart et al., 1990). Importantly, there has been very little research conducted on the effect of SP
loading in untrained participants. Thus, further investigation is needed to determine whether fitness levels influence the effect of SP loading on endurance performance.

**Gender**

Gender is an important methodological consideration when investigating phosphate loading (Fukuda et al., 2010) because a number of physiological differences between the genders may cause females to respond differently to phosphate supplementation when compared with males (Fukuda et al., 2010). For example, it has been reported that females have a decreased oxygen-Hb affinity when compared with males (Humpeler & Amor, 1973; Samaja et al., 1990). This has been identified through studies demonstrating higher $P_{50}$ values (oxygen tension at 50% oxygen saturation) (Humpeler & Amor, 1973) and increased 2,3-DPG concentrations in females compared with males (Bonner, Tate, & Buffington, 1975), with the exact reason for this difference being unknown (Humpeler & Amor, 1973). However, it has been hypothesised that this difference may be due to a compensatory response to the lower Hb concentration and red cell mass seen in females (Bonner, Tate, & Buffington, 1975; Humpeler & Amor, 1973; Samaja et al., 1990). A potential effect of this lower oxygen affinity is that it may cause females to be unresponsive to the ergogenic effects of SP supplementation. One of the main mechanisms by which phosphate loading is thought to be ergogenic is through an increase in 2,3-DPG concentration leading to greater peripheral oxygen delivery and an increased $\dot{V}O_{2max}$ (Fukuda et al., 2010; Kreider, 1999). However, as females already have an increased 2,3-DPG concentration (Bonner et al., 1975), there may be less opportunity for a phosphate stimulated increase in 2,3-DPG concentration to further decrease oxygen affinity. This could mean that females may not receive ergogenic benefits at the same magnitude as males.
Females also have both higher and greater fluctuations in oestrogen levels when compared with males (Samaja et al., 1990). This raises the possibility that females may respond differently to phosphate loading, as it is known that oestrogen is involved in the hormonal regulation of phosphate levels in the body (Avioli, 1988; Dick, Devine, Beilby, & Prince, 2004). Notably, it has been identified that oestrogen acts to decrease renal phosphate reabsorption (Dick et al., 2004). Thus, if phosphate is loaded during the ovulatory phase of the menstrual cycle when oestrogen levels are at their highest, it is possible that less phosphate may be reabsorbed, resulting in smaller increases in serum phosphate levels (Birch, 2000). Conversely, oestrogen levels are low in males and are therefore unlikely to have a major influence on phosphate reabsorption.

It is also possible that females may respond differently to SP loading because they tend to have smaller hearts, SV, left ventricular masses and a reduced Hb concentration and red cell mass when compared with males (O'Brien, 1985). This is of relevance given that phosphate loading has been proposed to improve myocardial efficiency, resulting in an increased SV and decreased HR in males (Czuba et al., 2009; Kreider et al., 1992), which is thought to lead to a better oxygen supply to the muscles and improved exercise performance (Kreider, 1999). Consequently, it is not clear whether phosphate loading would be able to alter myocardial efficiency to a level sufficient to increase SV in females. Furthermore, the reduced Hb concentration and red cell mass in females (O'Brien, 1985) may limit the magnitude of any oxygen transport benefits from enhanced myocardial efficiency when compared with males.

Furthermore, females may respond differently to phosphate loading when compared with males as a result of differences in body composition and size. Females are typically smaller
than males (Markowitz & Perazella, 2009) and have a greater percent of body fat and less FFM (O'Brien, 1985). Consequently, when ingesting an absolute dose of phosphate, it is likely that females are actually consuming a larger (relative to body mass) dose of phosphate than males. This would mean that females are more likely to induce a PTH-mediated down-regulation in phosphate levels than males, when consuming the same absolute dose of phosphate. This difference in body composition between males and females highlights the importance of using a FFM relative dose when loading phosphate. Of interest is whether a higher dose (FFM) of SP in females would be needed for an ergogenic effect to occur, (based on gender differences previously discussed), or conversely would a higher dose result in a PTH down regulation in phosphate and hence provide no benefit to exercise performance. Consequently, research should investigate varying FFM corrected doses of SP, in order to identify the ideal dose of SP for both males and females. Importantly, only one published study to date has examined the effects of SP loading in females (West et al., 2012), with this study focusing on aerobic capacity only, with no studies having investigated the effect of SP loading on endurance performance in females (Fukuda et al., 2010).

**Sodium phosphate and anaerobic exercise**

Whilst there have been a number of studies that have investigated the effect of SP on aerobic and endurance exercise, there have been only two studies that have investigated the effect of SP loading on anaerobic exercise. In a study conducted by Tourville, Brennan, and Connolly (2001), no benefit to peak or mean power output was seen following SP supplementation during a single 30 s Wingate sprint test in trained male cyclists. However, these results should be interpreted with caution as the primary focus of this study was on
aerobic capacity and it is possible that the study was underpowered in respect to anaerobic performance.

In another study, Brewer, Dawson, Wallman and Guelfi (2014) investigated the effect of SP loading (50 mg·kg⁻¹ of FFM of SP per day for six days) on repeated-sprint (4 sets of 6 x 15 s) and TT (2 x 5 min) cycling efforts in seven trained male cyclists on day 1 and 4 post loading. Compared with baseline, the SP group recorded several significantly improved work and mean power output values for overall sprint and TT efforts, as well as for individual sets on both days 1 and 4 post supplementation (Brewer et al., 2014). The authors suggested that these improvements in performance may be related to mechanisms previously proposed to explain the ergogenic effects of SP loading on aerobic exercise such as an increased 2,3-DPG concentration, enhanced myocardial efficiency and an improved buffering capacity (Kreider et al., 1992).

Although Brewer et al., (2014) demonstrated improved performance during high intensity repeated cycling efforts, no studies have investigated the effect of SP on sprint activities more closely related to team-sport performance. Importantly, many of the previously reviewed mechanisms that are proposed to explain the ergogenic effects of SP loading on endurance exercise may also influence repeated-sprint efforts as undertaken in a team-sport setting. For example, the improved buffering capacity that has been associated with phosphate loading and endurance exercise would also be beneficial to repeated-sprint performance, as it may help to delay the onset of fatigue associated with increased hydrogen ions produced during short duration, high-intensity exercise bouts. This could consequently allow athletes to work harder for longer before the effects of fatigue set in. In addition, the potential increase in ATP/PCr resynthesis that has been suggested to occur
with phosphate loading would also be beneficial to repeated-sprint efforts. An increase in ATP/PCr resynthesis could allow athletes involved in repeated-sprints to perform better by increasing the amount of energy available to the exercising muscles. Given the supporting theoretical evidence for an effect of SP loading on repeated-sprint performance it is important for future research to investigate the effect of phosphate loading on RSA and other types of anaerobic exercise.

**Side effects**

An important aspect of any potential ergogenic aid relates to the possible side effects associated with its use. The overwhelming consensus in the literature is that there are minimal side effects associated with phosphate supplementation when used as an ergogenic aid (Fukuda et al., 2010; Kreider et al., 2010). Furthermore, a recent review article on a wide range of nutritional supplements by Kreider et al., (2010) categorised SP as an apparently effective and generally safe supplement. Nonetheless, it should be noted that it has been suggested that regular prolonged use of phosphate may upset the balance of phosphate and other minerals in the body, leading to gastrointestinal distress (i.e. diarrhoea and constipation) (Folland et al., 2008; Tremblay et al., 1994). However, there is very little evidence to support this, with only two studies to date reporting cases of gastrointestinal distress (Folland et al., 2008; West et al., 2012). It is also possible that SP consumption can lead to vomiting, although this side effect has been largely eliminated by ingesting the phosphate dose with fluids (Kreider et al., 1992). Despite the minimal side effects associated with phosphate supplementation, it is still recommended that individuals with kidney diseases avoid phosphate loading protocols (Folland et al., 2008).
Sodium Phosphate and Caffeine

It is not uncommon to combine different nutritional supplements in order to further enhance exercise performance (Burke, 2008). Thus, it may be beneficial to evaluate the effects of SP combined with other nutritional ergogenic aids in order to see if an augmented effect is possible. Of relevance, caffeine ingestion alone has been demonstrated to improve exercise performance (Bell, McLellan, & Sabiston, 2002; Graham & Spriet, 1991; Sasaki, Maeda, Usui, & Ishiko, 1987; Spriet et al., 1992), with the mechanisms postulated to support the ergogenic effects of caffeine being markedly different to those of SP (described later). Of relevance, no studies to date have examined the combined effect of SP and caffeine supplementation on exercise performance, nor have any studies assessed the effect of caffeine on RSA as performed in team-game sports in females.

Pharmacokinetics of Caffeine

Orally ingested caffeine is rapidly absorbed from the gastrointestinal tract (Sinclair & Geiger, 2000) and begins to appear in the blood within 5 min of consumption (George, 2000). The time to peak plasma concentrations of caffeine ranges between 15-120 min (Armstrong, 2002; Bonati, Latini, Tognoni, Young, & Garattini, 1984), with 99% absorbed after approximately 45 min (Fredholm, Battig, Holmen, Nehlig, & Zwartau, 1999). Following acute ingestion, the principal pharmacological actions of caffeine are stimulation of the cardiovascular, respiratory and central nervous systems, as well as effects on skeletal and smooth muscle (Armstrong, 2002; Falk et al., 1990).
Caffeine Supplementation as an Ergogenic Aid to Athletic Performance

As early as the 1970’s, numerous studies and reviews had reported that consuming 3-6 mg·kg$^{-1}$ of body mass of caffeine, in capsule form, 60 min before exercise could result in significant improvements in endurance performance (Bell & McLellan, 2002; Ganio, Klau, Casa, Armstrong, & Maresh, 2009; Tarnopolsky, 2010). Initial research indicated that caffeine improved endurance TTE in prolonged events (Armstrong, 2002; Bell & McLellan, 2002; Costill, Dalsky, & Fink, 1978; Kovacs, Stegen, & Brouns, 1998), whilst other studies found a beneficial effect of caffeine on endurance TT completion times (Bridge & Jones, 2006; MacIntosh & Wright, 1995) and perception of effort during exercise (Cox et al., 2002; Denadai & Denadai, 1998). More recently, research has investigated the effect of caffeine supplementation on anaerobic exercise tasks as well as RSA, with results being equivocal (Anselme, Collomp, Mercier, Ahmaidi, & Prefaut, 1992; Bruce et al., 2000; Bell, Jacobs, & Ellerington, 2001; Carr, Dawson, Schneiker, Goodman, & Lay, 2008; Glaister et al., 2008; Schneiker, Bishop, Dawson, & Hackett 2006; Stuart, Hopkins, Cook, & Cairns, 2005).

Mechanisms behind the ergogenic effects of caffeine

Extensive research has been conducted in determining the mechanisms through which caffeine may provide its ergogenic effects (Sinclair & Geiger, 2000). While several propositions have been made, the general consensus in recent years is that the ergogenic effects of caffeine are related to adenosine receptor antagonism (Tarnopolsky, 2010). Adenosine is a normal cellular constituent that acts as a CNS depressant by blocking neuronal transmission (Fredholm et al., 1999), with receptors widely present in human
tissues, such as the brain, skeletal muscle and adipose tissue (Graham, 2001; Ribeiro & Sebastiao, 2010; Tarnopolsky 2010). The effects of adenosine include reduced alertness and arousal, lowered firing of neurons and reduced neurotransmitter release (Fredholm et al. 1999; Ribeiro & Sebastiao, 2010). Of relevance, caffeine is very similar in chemical structure to adenosine (Ribeiro & Sebastiao, 2010) and can bind to adenosine cell membrane receptors, consequently blocking the inhibitory actions of adenosine as described above (Fredholm et al., 1999). As a result of adenosine receptor antagonism, caffeine increases neuronal activity by increasing neurotransmitter release and lowers the threshold for neuronal activation (Fredholm et al., 1999). In skeletal muscle, increased neuronal activity may facilitate the recruitment of additional motor units and/or increase their frequency of activation (Fredholm et al., 1999), leading to sustained or increased power output in exercising muscles (Williams, 1991). Additionally, caffeine is able to cross the blood-brain barrier, acting as a powerful antagonist of adenosine receptors in the CNS (Fredholm et al., 1999). Blocking the depressant effects of adenosine on the CNS, combined with the reported analgesic properties of caffeine (Kalmar, 2005) is proposed to result in enhanced mental arousal (Ribeiro & Sebastiao, 2010), a decreased feeling of pain (Fredholm et al., 1999) and a lower level of perceived exertion (and hence fatigue), during exercise (Ganio et al., 2009). Together, these effects are thought to be responsible for the improved aerobic and anaerobic performance observed with caffeine supplementation (Doherty & Smith, 2005).

**Research investigating the ergogenic effects of caffeine**

To date, the majority of studies that have investigated the effects of caffeine on exercise performance have used endurance performance tests (Denadai & Denadai, 1998; Greer,
Friars, & Graham, 2000; Jackman, Wendling, Friars, & Graham, 1996; Pasman, Van Baak, Jeukendrup, & De Haan, 1995). Typically, these studies have reported significantly increased TTE when running or cycling at 70-85% peak $\dot{V}O_2$, after ingesting caffeine doses ranging between 3-13 mg·kg$^{-1}$ of body mass, consumed approximately 0-90 min before exercise (Bell & McMellan, 2002; Costill, et al., 1978; Denadai & Denadai, 1998; Greer et al., 2000; Jackman et al., 1996; Pasman et al., 1995; Spriet et al., 1992). Other studies have reported improved TT performance after caffeine ingestion (Bridge & Jones, 2006; Cox et al., 2002; Ganio et al., 2009; MacIntosh & Wright, 1995). For example, Bridge and Jones (2006) demonstrated a significant 1.2% (23.8 s) reduction in 8 km running time to completion (compared with a control and placebo trial) in eight trained male runners, who ingested 3 mg·kg$^{-1}$ of body mass of caffeine, 60 min prior to exercising. Furthermore, a meta-analysis by Ganio et al., (2009) identified 19 separate experimental trials involving pre-exercise caffeine supplementation and TT performance and reported a mean performance improvement of 2.3 ± 3.2%. In addition to improvements in completion time and TTE tests, studies have also demonstrated a decreased rating of perceived exertion following caffeine supplementation compared with placebo at similar exercise intensities (Bell & McLellan, 2002; Cox et al., 2002; Denadai & Denadai, 1998; Ivy, Costill, Fink, & Lower, 1979).

A number of studies have also investigated the effect of caffeine supplementation on short, intense, anaerobic exercise lasting between 6 s to 4 min and have reported improved performance (Bell et al., 2002, Bruce et al., 2000, Jackman et al., 1996, Anselme et al., 1992). For example, Anselme et al., (1992) demonstrated an increase in maximal anaerobic power following the ingestion of 250 mg of caffeine in 14 participants (males and females),
who performed 6 s sprints against increasing resistance until exhaustion. Similarly, Bell et al., (2001) reported increases in TTE and oxygen deficit during a 30 s Wingate cycling test in 16 untrained men who ingested 5 mg·kg⁻¹ of body mass of caffeine 1.5 h before exercising.

Given the benefits of caffeine supplementation for short sprint performance, recent research has focused on investigating the effect of caffeine supplementation on RSA in team-sport athletes using either cycling sprint bouts (Crowe, Leicht, & Spinks, 2006; Schneiker et al., 2006), simulated team-sport game protocols (Stuart et al., 2005) or repeated running sprint bouts (Carr et al., 2008; Paton, Hopkins, & Vollebregt, 2001; Pontifex, Wallman, Dawson, & Goodman, 2010; Glaister et al., 2008), while one study recruited trained swimmers who swam 2 x 100 m freestyle at maximal speed (Collomp, Ahmaidi, Chatard, Audran, & Prefaut, 1992). Many of these studies reported that caffeine supplementation was beneficial in respect to repeated-sprint performance (Glaister et al., 2008; Stuart et al., 2005; Carr et al., 2008; Pontifex et al., 2010). Specifically, Glaister et al., (2008) found a 1.4% reduction in sprint times and a 1.2% decrease in fatigue during a 12 x 30 m sprint protocol performed by 21 physically active men who consumed 5 mg·kg⁻¹ of body mass of caffeine 60 min prior to exercise. Additionally, Carr et al., (2008) reported a significant reduction in total sprint time during 5 sets of 6 x 20 m in 10 team-sport male athletes following ingestion of 6 mg·kg⁻¹ of body mass of caffeine. Similarly, Stuart et al., (2005) demonstrated a 0.5 – 2.9% reduction in sprint times in nine competitive male rugby players following ingestion of 6 mg·kg⁻¹ of body mass of caffeine supplementation during a testing protocol aimed at simulating the demands of a rugby union match. Furthermore, Pontifex et al., (2010) investigated the effect of a 6 mg·kg⁻¹ dose of caffeine on 5 sets of 6 x 20 m
sprints in 10 moderately trained males. These authors reported that caffeine ingestion significantly improved combined total sprint time, best sprint time and percentage decrement across sprint bouts compared with placebo (Pontifex et al., 2010).

Although a number of studies have shown improved performance follow caffeine ingestion, it is important to note that others have not. Specifically, Paton et al., (2001) and Crowe et al., (2006) did not find improved repeated 20-m sprint or maximal 60 s cycling performance, respectively, following the ingestion of 6 mg·kg\(^{-1}\) of body mass of caffeine. This lack of an effect may be explained by the testing protocols used as the sustained sprint efforts with short recovery periods may have led to excessive accumulation of metabolic waste products (Paton et al., 2001).

Whilst a number of studies have shown positive effects of caffeine on anaerobic exercise and RSA, only three studies to date have assessed the effect of caffeine on RSA similar to that performed in team-sport games (Carr et al., 2008; Pontifex et al., 2010; Stuart et al., 2005), with none of these studies recruiting females. Consequently, further research is warranted here.

**Sodium Phosphate and Nitrate**

Another ergogenic aid that has been proposed to improve exercise performance is NO\(_3^-\), which is commonly ingested through BJ supplementation. Similar to caffeine, BJ may potentially have an additive effect when combined with SP supplementation as both ergogenic aids are thought to act via different mechanisms. However, only in the last decade has BJ received attention as an ergogenic aid for sporting performance and as a result there are a number of unanswered questions relating to its use.
Pharmacokinetics of Nitrate

Nitrate can be consumed in the diet, via green, leafy vegetables such as lettuce, spinach, rocket, celery and beetroot (Jones, 2013). Specifically, when foods rich in NO$_3^-$ are consumed, they are broken down by the digestive system, with NO$_3^-$ being absorbed into the blood plasma similar to other nutrients (Weitzberg & Lundberg, 2013). A portion of this absorbed NO$_3^-$ (up to ~25%) is then directed to the salivary glands (Cingi, Cingi, & Cingi, 1992; Spiegelhalder, Eisenbrand, & Preussmann, 1976), where it is concentrated tenfold before being excreted back onto the tongue within the saliva. Bacterial anaerobes on the tongue then begin to convert the NO$_3^-$ to nitrite (NO$_2^-$) (Cingi et al., 1992; Tannenbaum, Weisman, & Fett, 1976), which is then swallowed a second time and partially reabsorbed into the blood. Following this, some of the NO$_2^-$ circulating in the blood plasma can be converted to the metabolically active nitric oxide (NO) (Weitzberg & Lundberg, 2013).

Nitrate Supplementation as an Ergogenic Aid to Athletic Performance

Mechanisms behind the ergogenic effects of nitrate

Initial studies investigating the effect of NO$_3^-$ supplementation on exercise performance reported improved aerobic efficiency during submaximal exercise (Bailey et al., 2009), which led to a number of mechanisms being proposed to account for this ergogenic effect. The two primary mechanisms thought to contribute to the ergogenic effects of NO$_3^-$ supplementation are an increased efficiency of mitochondrial respiration due to reduced proton leakage through the inner mitochondrial membrane (Bailey et al., 2009; Clerc, Rigoulet, Leverve, & Fontaine, 2007; Larsen et al., 2011) and more efficient use of ATP.
during muscle force production via lower levels of calcium ATPase and actomyosin ATPase activity (Bailey et al., 2009; Bailey et al., 2010; Lansley et al., 2011). Support for an increased efficiency of mitochondrial respiration has come from Bailey et al., (2010), who used P-MRS and pulmonary $\dot{V}O_2$ to investigate muscle metabolism, while Larsen, Weitzberg, Lundberg and Ekblom (2010) provided support for more efficient ATP use through the investigation of $\dot{V}O_2$ during maximal exercise.

Research investigating the ergogenic effects of nitrate supplementation on aerobic and endurance exercise performance

One of the earliest studies to investigate the effect of NO$_3^-$ as an ergogenic aid for exercise performance was performed by Larsen, Weitzberg, Lundberg and Ekblom (2007). In this study, nine well trained male athletes ($\dot{V}O_{2\text{peak}}$ 55 ± 3.7 mL·kg$^{-1}$·min$^{-1}$) performed both a maximal and submaximal cycle ergometer exercise test following three days of supplementation with 0.1 mmol·kg$^{-1}$ per day of sodium NO$_3^-$ (or a sodium chloride placebo). These authors reported that sub-maximal $\dot{V}O_2$ decreased from 2.98 ± 0.57 to 2.82 ± 0.58 L·min$^{-1}$ after NO$_3^-$ supplementation. In addition, resting plasma NO$_3^-$ levels were significantly elevated in response to the NO$_3^-$ trials compared with placebo (27 µm vs 182 µm, respectively). Larsen et al., (2007) concluded that the reduction in submaximal $\dot{V}O_2$ was likely related to the in vivo reduction of NO$_3^-$ to NO$_2^-$ and NO.

These findings were later supported by Bailey et al., (2009), who supplemented eight, recreationally active males ($\dot{V}O_{2\text{max}}$ 49 ± 5 mL·kg$^{-1}$·min$^{-1}$) with 500 mL of either a NO$_3^-$ rich BJ (containing ~11.2 mmol of NO$_3^-$) or a placebo over a six day period. On the last three days of supplementation participants were required to complete submaximal and
maximal intensity cycling exercise. The BJ condition resulted in a significantly greater plasma NO\textsubscript{2}\textsuperscript{−} concentration following supplementation (273 ± 44 nM) compared with the placebo (123 ± 50 nM) and this coincided with a ~20% reduction in the oxygen cost of a given increment in work rate, as well as a ~16% increase in the time to task failure during maximal exercise.

In a recent study, Wylie et al., (2013) supplemented 14 male recreational team-sport participants with 490 mL of either a NO\textsubscript{3}\textsuperscript{−} rich BJ concentrate, or a NO\textsubscript{3}\textsuperscript{−} depleted placebo juice 30 h prior to the completion of a Yo-Yo intermittent running test. A 4.2% improvement in performance was reported following consumption of the NO\textsubscript{3}\textsuperscript{−} rich juice, compared with placebo and this increase in performance was coupled with a 400% increase in plasma NO\textsubscript{2}\textsuperscript{−} concentration.

To date, only a few studies have investigated the effect of NO\textsubscript{3}\textsuperscript{−} supplementation on endurance performance (Lansley et al., 2011; Wilkerson et al., 2012). Lansley et al., (2011) recruited nine trained male cyclists (\dot{V}\textsubscript{O}\textsubscript{2}\textsubscript{peak} 56.0 ± 5.7 mL\cdot kg\textsuperscript{−1}\cdot min\textsuperscript{−1}) and supplemented them with a NO\textsubscript{3}\textsuperscript{−} rich BJ (~6.2 mmol of NO\textsubscript{3}\textsuperscript{−}), or a NO\textsubscript{3}\textsuperscript{−} depleted placebo 2.5 h before completing 4 and 16.1 km TT. Performance was increased by 2.8% (4 km) and 2.7% (16.1 km) after the NO\textsubscript{3}\textsuperscript{−} rich BJ supplementation, which was also associated with significantly higher mean power output during the TT compared with placebo. Although these results were not statistically significant, they were supported by a 99.8 and a 99.9% (respectively) ‘almost certain’ chance of having a positive meaningful effect, based on smallest worthwhile change analysis as described by Batterham and Hopkins (2005).
Counter to the previously mentioned study by Lansley et al., (2011), Wilkerson et al., (2012) showed no beneficial effect of dietary NO₃⁻ supplementation on TT performance. In this study, eight well trained male cyclists (VO₂max 63 ± 8 mL·kg⁻¹·min⁻¹) consumed 500 mL of a NO₃⁻ rich BJ or a NO₃⁻ depleted BJ 2.5 h before performing a 50 mile cycling TT (Wilkerson et al., 2012). Despite a significant elevation in plasma NO₂⁻ levels in the BJ trial compared with placebo (472 vs 379 nM, respectively), TT performance was not significantly improved (BJ; 136.7 min vs placebo; 137.9 min). The authors noted that this lack of improvement seen following NO₃⁻ supplementation may have been related to the training status of the participants recruited. Specifically, well trained endurance athletes are thought to have higher resting NO₃⁻ concentrations compared with lesser trained individuals due to greater training-related NO synthase activity (McAllister & Laughlin, 2006, McConell et al., 2007). In support of this notion, the participants recruited by Wilkerson et al., (2012) had ~33% higher baseline plasma NO₂⁻ levels compared with those of Lansley et al., (2011).

**Research investigating the ergogenic effects of nitrate supplementation on anaerobic exercise**

Whilst the effect of NO₃⁻ supplementation on submaximal aerobic exercise, maximal aerobic capacity, TTE, TT and endurance exercise has been researched frequently, only a small number of studies have recently investigated the effect of nitrate supplementation on short duration, intense anaerobic exercise.

Bond, Morton and Braakhuis (2012) investigated the effect of NO₃⁻ supplementation on 6 x 500 m maximal rowing ergometer repetitions in 14 well trained male junior rowers. In this
study, participants consumed either 500 mL of BJ (5.5 mmol per day of NO₃⁻) or a placebo daily for six days in a randomised, crossover design. The authors reported a “likely” benefit for average repetition time in the BJ condition compared with placebo, with a 0.4% improvement in performance over all repetitions. Furthermore, the authors reported that the benefit appeared greater in the later repetitions (4-6) where performance was improved by 1.7%. The authors explained that this improvement in performance occurring in the later sets may be due to the NO₃⁻-NO₂⁻-NO pathway being activated under the high metabolic demand and consequently hypoxic and acidic conditions (Lundberg, Weitzberg, & Gladwin, 2008). However, the authors noted that the underlying mechanisms for the differences in performance were unknown as power output and metabolic mechanisms were not measured. However, it is likely that the previously mentioned mechanisms of a reduced ATP cost for muscle force production (Bailey et al., 2009) could potentially explain the benefits seen in RSA. Furthermore, it has been demonstrated that NO₂⁻ reduction to NO is facilitated when O₂ availability is low (Lundberg & Weitzberg, 2010; Lundberg, Carlstrom, Larsen, & Weitzberg, 2011), which often occurs during high intensity exercise. This suggests that BJ supplementation may be especially helpful in intense exercise requiring significant ATP contribution from both anaerobic glycolysis and aerobic metabolism exercise.

Based on the above, it is possible that BJ supplementation could be beneficial for prolonged RSA as performed during a team-sport game, where there is a greater reliance on anaerobic glycolysis and aerobic metabolism as exercise progresses. However, no studies to date have assessed the effects of BJ supplementation on this type of exercise performance. Consequently, further research is warranted here.
Research Considerations for Nitrate Supplementation

A wide variety of different dosing and loading modalities have been employed in studies investigating the effects of NO$_3^-$ on exercise performance. For example, some studies have used supplementation with sodium NO$_3^-$ (either three days of supplementation, or acutely; Bescos et al., 2011; Larsen et al., 2007), while others have used fresh BJ (typically about 500 mL) (Bailey et al., 2010; Vanhatalo et al., 2011). However, generally it is recognised that a dose of between 5.2 – 11.2 mmol (Bailey et al., 2009; Bailey et al., 2010; Lassila, 2001; Vanhatalo et al., 2011) of NO$_3^-$ regardless of the delivery technique, results in a significant increase in plasma NO$_2^-$ and associated physiological benefits. Previous research has demonstrated that plasma NO$_2^-$ concentrations peak typically 3 h following NO$_3^-$ ingestion and stay elevated for between 6 to 8 h, returning to baseline within 24 h (Webb et al., 2008). Consequently, an acute dose taken 2.5 – 3 h prior to exercise performance may be the optimal timing for NO$_3^-$/BJ ingestion.

Conclusion

In summary, a review of the literature evaluating the ergogenic benefits of phosphate loading on exercise performance suggests that SP is a likely ergogenic aid, which can improve aerobic capacity in males (Fukuda et al., 2010). In addition, there have been some positive results related to the ergogenic effects of SP on endurance performance in males (Kreider et al., 1992). However, research regarding the use of SP as an ergogenic aid is complicated by a number of key methodological considerations. Specifically, the administered dose and dosing protocol, the length of the loading and washout period and the fitness level of the participants used are all thought to influence the ergogenic effect of
SP loading (Fukuda et al., 2010, Tremblay et al., 1994). A review of these key methodological considerations indicates that SP is most likely to be ergogenic when ingested for 3-6 days, at a dose of 3-5 g per day, split into multiple doses over the day (Fukuda et al., 2010). However, this notion has not yet been assessed in females. As physiological differences between males and females may cause females to respond differently to phosphate loading, it is important for future research to examine the effect of SP supplementation on exercise performance in this population. Of further consideration is the investigation of a range of different FFM relative doses of SP on endurance performance to determine the optimal dose of SP in females. Furthermore, only one study to date has investigated the effect of SP loading on RSA, with this related to cycling sprint bouts only. Of relevance, a number of the mechanisms proposed to explain the ergogenic effects of SP, such as enhanced buffering capacity and an increased energy (phosphate) pool may benefit this form of exercise. Consequently, further research is needed here.

Furthermore, caffeine is an ergogenic aid that has been shown to improve both aerobic and anaerobic exercise performance (Bell & McKellan, 2002; Ganio et al., 2009), with some research also showing a beneficial effect for RSA in males (Pontifex et al., 2010; Carr et al., 2008). Of relevance, the mechanisms proposed to explain these improvements in performance following caffeine supplementation are different to those of SP. Therefore, it is possible that a combined dose of SP and caffeine may result in enhanced exercise performance compared with the ingestion of SP or caffeine alone. This has yet to be investigated.

Likewise, $\text{NO}_3^-$ in the form of BJ is another ergogenic aid that has been reported to improve exercise performance (Cermak, Gibala, & Van Loon, 2012; Lansley et al., 2011) using
mechanisms markedly different to those associated with SP. To date, no studies have investigated the effect of BJ on RSA as it relates to team sport performance. Nor have any studies assessed the effect of BJ in females. Furthermore, no studies have assessed the combined effect of SP and BJ on exercise performance. Therefore, future research should investigate the effect of a combined SP and BJ supplementation protocol on RSA in order to further enhance our understanding of these ergogenic aids.
References


Spiegelhalder, B., Eisenbrand, G., Preussmann, R. (1976). Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to in vivo formation of N-nitroso compounds. *Food and Cosmetics Toxicology, 14*(6), 545-548.


Chapter Three

Study One

SODIUM PHOSPHATE SUPPLEMENTATION AND TIME-TRIAL PERFORMANCE IN FEMALE CYCLISTS

Presented here in journal format

Based on:

Abstract:

This study investigated the effects of three doses of sodium phosphate (SP) supplementation on cycling 500 kJ (119.5 kcal) time-trial (TT) performance in female cyclists. Thirteen cyclists participated in a randomised, Latin-square design study where they completed four separate trials after ingesting either a placebo, or one of three different doses (25, 50 or 75 mg·kg⁻¹ fat free mass: FFM) of trisodium phosphate dodecahydrate which was split into four equal doses a day for six days. On the day after the loading phase, the TT was performed on a cycle ergometer. Serum phosphate blood samples were taken at rest both before and after each loading protocol, while a ~21 day washout period separated each loading phase. No significant differences in TT performance were observed between any of the supplementation protocols (p = 0.73) with average completion times for the 25, 50 or 75 mg·kg⁻¹ FFM being, 42:21 ± 07:53, 40:55 ± 07:33 and 40:38 ± 07:20 min respectively, and 40:39 ± 07:51 min for the placebo. Likewise, average and peak power output did not significantly differ between trials (p = 0.06 and p = 0.46, respectively). Consequently, 500 kJ cycling TT performance was not different in any of the supplementation protocols in female cyclists.

Key words: ergogenics, endurance performance, 2,3-DPG
Introduction

Sodium phosphate (SP) is a nutritional supplement, which has been reported to provide significant ergogenic benefits (5-12% improvement) for aerobic capacity (Brewer et al., 2013; Cade et al., 1984; Czuba et al., 2008; 2009; Kreider et al., 1990; 1992). Furthermore it has also been shown to be effective in improving endurance performance (Folland et al., 2008; Kreider et al., 1992). A number of mechanisms have been proposed to provide benefit following SP ingestion which include an enhanced 2,3-diphosphoglycerate (2,3-DPG) concentration, which allows for greater unloading of oxygen to the peripheral tissues/muscle (Buck et al., 2013; Benesch and Benesch, 1969; Duhm, 1971) and an improved buffering capacity due to increased hydrogen phosphate concentration, which could buffer hydrogen ions produced during intense exercise (Kreider, 1999). Additionally, it is also thought that an improved myocardial efficiency, which is proposed to result in increased stroke volume, a larger cardiac output and consequently greater and more efficient oxygenation of the exercising muscles (Czuba et al., 2009), and greater adenosine triphosphate/phosphocreatine resynthesis due to increased availability of extracellular and intracellular phosphate, thus providing a larger energy pool (Bredle et al., 1988; Kreider, 1999), may also contribute to the benefits seen with SP loading. These proposed mechanisms underlie the notion that phosphate supplementation increases serum phosphate levels, thereby allowing processes normally limited by phosphate availability to operate at an enhanced level (Tremblay et al., 1994).

Of the few studies that have assessed the effects of SP supplementation on endurance performance (Brewer et al., 2013; Folland et al., 2008; Kreider et al., 1990; 1992), only male participants were recruited, suggesting that future research should be performed in
females. Importantly, due to a number of physiological differences, it is possible that females may respond differently to doses of SP that have been reported to be beneficial in males (Buck et al., 2013; Fukuda et al., 2010; O’Brien, 1985). Females, for example, have a decreased oxygen-hemoglobin affinity compared with males (Humpeler and Amor, 1973; Samaja et al., 1990). Specifically, Samaja et al., (1990) have reported higher P50 values (oxygen tension at 50% oxygen saturation) and increased 2,3-DPG concentrations in females, which may result in females being less responsive to SP supplementation compared with males (Fukuda et al., 2010). Females also have greater and higher fluctuations in estrogen levels compared with males (Janse de Jonge, 2003; Remes et al., 1979), with estrogen acting to decrease renal phosphate reabsorption (Dick et al., 2005). Furthermore, females tend to have smaller hearts, left ventricular masses, stroke volume and reduced red cell mass compared with males (O’Brien, 1985). Related to this difference between the genders is that SP loading has been proposed to improve myocardial efficiency (Czuba et al., 2009; Kreider, 1999), which is thought to lead to a better oxygen supply to the muscles and hence improved exercise performance (Kreider, 1999). However, reduced red cell mass in females (O’Brien, 1985) may limit the magnitude of any oxygen transport benefits gained from enhanced myocardial efficiency.

Therefore, the aim of this study was to assess the effects of a variety of doses of SP supplementation (25, 50 and 75 mg·kg⁻¹ of free fat-mass; FFM) on 500 kJ (119.5 kcal) cycling time-trial (TT) performance in female cyclists. A 50 mg·kg⁻¹ of FFM dose was chosen as this dose has been shown to benefit exercise performance in males (Czuba et al., 2009), while a 75 mg·kg⁻¹ of FFM dose was selected as this overall dose is similar to the absolute dose of SP (i.e. ~4 g per day) also reported to benefit exercise performance in
males (Folland et al., 2006; Kreider et al., 1990; 1992). The 25 mg·kg⁻¹ of FFM dose was chosen as this dose is similar to the recommended daily dietary intake of phosphate. We hypothesised that ingestion of a moderate dose of SP (50 mg·kg⁻¹ of FFM) would improve 500 kJ TT performance more than a higher (75 mg·kg⁻¹) or lower (25 mg·kg⁻¹) dose in trained female cyclists, as a high dose may cause a parathyroid hormone mediated down regulation in phosphate levels, while a low dose may be insufficient to change phosphate levels in the body.

Methods

Participants

Thirteen females who cycled on a regular basis (3.6 h ± 2.1 per week) were recruited to this study based on a G-power analysis (Faul et al., 2009) that determined that this number of participants were needed to detect changes that occurred at an alpha level of 0.05. The physical characteristics of the participants were: \( \dot{V}O_{2peak} 49.8 \pm 7.4 \text{ mL·kg}^{-1}·\text{min}^{-1} \), age 25.5 ± 4.4 y, body-mass 62.8 ± 5.5 kg, height 1.65 ± 0.05 m, fat-mass 15.0 ± 3.6 kg, FFM 44.9 ± 5.1 kg. During the study, six participants were taking Levlen ED for birth control, with the remaining participants took no oral contraceptives. Participants took no nutritional supplements for at least two months prior to or during this study. The Human Research Ethics Committee of the University of Western Australia approved the study and all participants provided written informed consent prior to participation.

Experimental design

Participants completed a familiarisation session and four different supplementation protocols over an 18 week period. During the familiarisation session, body composition
was determined using dual energy x-ray absorptiometry (Lunar Prodigy, GE Medical Systems, Madison, USA) to determine each participant’s FFM. Additionally, full familiarisation with a 500 kJ (119.5 kcal) cycling TT protocol was undertaken. On a separate day, participants returned to the laboratory in order to complete a graded exercise test to measure their $\dot{V}O_2$peak. This test was performed on a wind-braked cycle ergometer (Evolution Pty. Ltd., Adelaide, Australia) and consisted of cycling for 3 min intervals, with a 1 min rest period between each interval, until volitional exhaustion was reached. The test began at 150 watts (W), with workload increasing by 50 W for every subsequent interval (Brewer et al., 2013). During this test, expired air was assessed using a gas analysis system consisting of a ventilometer (Universal ventilation meter, VacuMed, Ventura, California, USA) and oxygen and carbon dioxide analysers (Ametek Applied Electrochemistry S-3A/1 and CD-3A, AEI Technologies, Pittsburgh). Before, and after each test, the gas analysers were calibrated using gases of known concentration (BOC gases, Chatswood, Australia) and gas analysers were verified immediately after testing to check for drift. If the post-test calibration demonstrated that drift had occurred, then a correction factor (based on the magnitude of the drift) was applied to test data.

Participants were then assigned, in a randomised, Latin-square design, to three SP and one placebo loading protocol. To control for phase of menstrual cycle, the first supplementation protocol began ~3-5 days post the first menstruation (follicular phase) after completing the familiarisation session. All subsequent testing was performed during this same phase, with ~21 days between trials. Notably, 17 days has been proposed to be the minimum washout period for SP (Brewer et al., 2013; Kreider et al., 1992).
The SP trials consisted of the consumption of a 25, 50 or 75 mg·kg$^{-1}$ of FFM dose of trisodium phosphate dodecahydrate (Challenge Chemicals Australia, Western Australia), split into four equal doses a day, consumed for six consecutive days. Each dose was placed into an opaque capsule by a blinded researcher. In order to prevent gastrointestinal upset, each capsule was emptied into a glass and consumed with 15 g of Powerade powder (Coca-Cola Amatil, Australia) that had been dissolved in ~300 mL of water (Brewer et al., 2013; West et al., 2012). Importantly, no side effects were reported. Participants were instructed to separate the ingestion of each capsule by ~4 h. The placebo protocol followed the same loading strategy with a 4 g dose of glucose each day. On the day after the end of each loading protocol, participants returned to the laboratory and completed the same 500 kJ cycling TT. Finally, participants attended the laboratory one day prior to the commencement of each supplementation protocol so that a venous blood sample could be taken to assess serum phosphate, while a second sample was taken at the end of each supplementation protocol, prior to performing the 500 kJ (119.5 kcal) TT. The timing of each venous blood sample was standardised between supplementation phases.

Participants were instructed to follow their normal training diet and fluid intake during each supplementation protocol and were also required to complete a detailed dietary record for the 24 h period prior to each TT. A copy of the food diary from the first TT was provided to participants with the instructions to replicate this eating pattern before each subsequent TT. Dietary analysis of each participant’s self-reported caloric intake was undertaken on completion of the study using FoodWorks software package (FoodWorks v 4.2.0, Xyris Software, Qld, Australia). Participants were required to maintain a consistent training volume throughout the study and completed a six day physical activity diary during each
supplementation period. Participants were requested to replicate their physical activity patterns using this activity diary during each phase of the study.

**Cycling time-trial**

The simulated 500 kJ (119.5 kcal) cycling TT was performed on a wind-braked cycle ergometer (Evolution Pty. Ltd., Adelaide, Australia), modified with clip-on pedals and racing handle bars. Exercise was self-paced in order to replicate actual TT situations, with participants instructed to complete the 500 kJ (119.5 kcal) of work in the fastest time possible. A 500 kJ (119.5 kcal) workload has been shown in previous research to approximate to a 20 km TT distance in trained male cyclists (Peeling et al., 2005). A test-retest of the 500 kJ (119.5 kcal) TT in 10 female cyclists resulted in a coefficient of variance of 2.9%.

Participants determined optimal moderate warm-up intensity during the familiarisation session, with this warm-up intensity replicated for all experimental sessions. Participants refrained from exercise for 24 h prior to each experimental session, with all TT completed at the same time of the day to minimise any circadian rhythm effects. No food and/or caffeine intake was permitted for 2 h prior to each TT. Participants consumed 200 mL of water prior to commencing each TT, with no further fluid ingestion allowed until completion of the trial.

During each TT, participants were blinded to their power output and duration of their performance, but had a visual display of the accumulated kJ of work completed. The performance variables recorded during the 500 kJ (119.5 kcal) TT were time to completion and peak and average power output, which were recorded by a customised computer.
program (Cyclemax; University of Western Australia). These variables were recorded automatically at 125 kJ split times and heart rate (Polar Heart Rate Monitors, Kempele, Finland) was recorded at each 125 kJ (29 kcal) split. Rating of perceived exertion (RPE - Borg’s 6-20 point scale; Borg, 1982) and blood lactate were measured at the start and end of the 500 kJ (119.5 kcal) TT. To determine blood lactate, a capillary blood sample was collected in a 35 µL heparinised glass capillary tube from the earlobe. The sample was then immediately analysed for plasma lactate concentration using a blood-gas analyser (ABL 725, Radiometer, Copenhagen, Denmark).

**Venepuncture**

Before and after loading, resting venous blood samples for serum phosphate determination were collected via venepuncture of an antecubital vein in the forearm. A total of 8.5 mL of blood was collected for each sample, which were then left to clot at room temperature for 60 min prior to being centrifuged at 1000 g at 4°C for 15 min. The serum obtained was stored at -80°C for later analysis, with serum phosphate determined using an Abbott Architect c16000 analyser, employing specified Abbott reagents (Abbott Laboratories, Abbott Park, IL 60065, USA). Observed coefficients of variation were 4.2% at a level of 0.95 mmol·L⁻¹ and 2.0% at a level of 2.95 mmol·L⁻¹.

**Statistical analyses**

Statistics Package for the Social Sciences Version 16.0 for Windows (SPSS, Inc., Chicago, IL) was used to perform one-way repeated-measures ANOVAs to test for significant differences between the varying doses of SP for each dependent variable. Bonferroni post-hoc tests were applied to determine the location of significant differences. In addition,
Cohen’s $d$ effect sizes (Cohen, 1988) ($d < 0.2$, small; $0.5 - 0.79$, moderate; $\geq 0.8$, strong) were calculated to assess the magnitude of difference between experimental trials. Further analysis identified the smallest worthwhile change in performance scores between trials using the method outlined by Batterham and Hopkins (2005). The smallest worthwhile value of change was set at a Cohen’s unit of 0.2, representing the hypothetical, smallest change in physiological measures that would benefit the athlete. Where the chance of benefit and harm were both calculated to be $> 5\%$, the true effect was deemed unclear. When clear interpretation was possible, a qualitative descriptor was assigned to the following quantitative chances of benefit: 25-74%, benefit possible; 75-94%, benefit likely; 95-98%, benefit very likely; $> 99\%$, benefit almost certain (Batterham and Hopkins, 2005).

In order to include only pertinent information in this manuscript, only quantitative chances $> 25\%$ and moderate to large effect sizes ($d \geq 0.5$) are reported. Pearson correlations were also performed to assess for any relationship between serum phosphate levels and main performance variables. All results are presented as mean ± SD.

**Results**

**500 kJ (119.5 kcal) time-trial**

There were no significant differences observed between trials for time to completion ($p = 0.73$; Table 1). Further, there were no ‘possible’ or ‘likely’ benefits, or moderate or large effect sizes found between trials for this variable. The 75 mg·kg$^{-1}$ of FFM trial resulted in the highest average power and peak power output but this was not significantly different to any other trial ($p = 0.06$ and $p = 0.46$, respectively), nor were there any ‘possible’ or ‘likely’ benefits or moderate to large effect sizes associated with the results (Table 1).
Notably, four participants recorded their best time following the 50 mg·kg⁻¹ of FFM trial, with an additional four participants performing better on this dose than the placebo (Table 2). In addition, five participants recorded their best TT following the 75 mg·kg⁻¹ of FFM trial, with an additional participant recording a faster time following this dose compared with the placebo (Table 2). Time to completion was slowest and average power output lowest following the 25 mg·kg⁻¹ of FFM trial compared with all other trials, with these results supported by ‘very likely’ detriments in performance when compared with the placebo and the 75 mg·kg⁻¹ of FFM trials (Table 1).

Due to the large differences in TT completion times (28 min 46 s vs 57 min 32 s), a separate analysis of results was performed for those who completed the four trials in ≤ 45 min (n = 8, average $\bar{V}O_{2peak} = 53.1$ mL·kg⁻¹·min⁻¹) and those who completed the trials ≥ 45 min (n = 5, average $\bar{V}O_{2peak} = 45$ mL·kg⁻¹·min⁻¹) (Table 2), with 45 min representing approximately the half-way point between the fastest and slowest trials. This analysis resulted in no moderate or large effect sizes, no beneficial or detrimental effects or significant differences between the four trials for either the faster group (p = 0.19) or the slower group (p = 0.37).

In addition, analysis of post exercise blood lactate concentrations and ratings of perceived exertion values demonstrated no significant differences between any trials (p = 0.16 and p = 0.72, respectively). It was observed that post TT heart rate was different between trials (p = 0.03), with post hoc analysis showing that heart rate was lower in the 25 mg·kg⁻¹ trial than both the 50 mg·kg⁻¹ of FFM and the placebo trial (p = 0.04 and p = 0.01, respectively).
Table 1. Performance variables for 500 kJ cycling time-trial following placebo and sodium phosphate supplementation (n = 13). Data are means (±SD). FFM = fat free mass.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>25 mg·kg(^{-1}) of FFM</th>
<th>50 mg·kg(^{-1}) of FFM</th>
<th>75 mg·kg(^{-1}) of FFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to completion (min:s)</td>
<td>40:39.8 ± 07:51</td>
<td>42:21.6 ± 07:53(^a)</td>
<td>40:55.2 ± 07:33</td>
<td>40:38.4 ± 07:20</td>
</tr>
<tr>
<td>Average power output (W)</td>
<td>212 ± 40</td>
<td>203 ± 40(^a)</td>
<td>211 ± 42</td>
<td>213 ± 40</td>
</tr>
<tr>
<td>Peak power output (W)</td>
<td>330 ± 77</td>
<td>328 ± 102</td>
<td>349 ± 70</td>
<td>335 ± 74</td>
</tr>
<tr>
<td>Post exercise RPE</td>
<td>17.5 ± 1.3</td>
<td>17.5 ± 1.2</td>
<td>17.8 ± 1.2</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Post exercise lactate (mmol·L(^{-1}))</td>
<td>6.4 ± 1.2</td>
<td>6.2 ± 1.6</td>
<td>7.1 ± 2.4</td>
<td>8.5 ± 2.4</td>
</tr>
<tr>
<td>Post exercise heart rate (bpm)</td>
<td>170 ± 7</td>
<td>165 ± 7</td>
<td>169 ± 5</td>
<td>169 ± 8</td>
</tr>
</tbody>
</table>

\(^a\) = ‘very likely’ to be detrimental compared with 75 mg·kg\(^{-1}\) of FFM trial and the placebo trial
Table 2. Participant time to completion for 500 kJ time-trial following placebo and sodium phosphate supplementation (n = 13). FFM = free fat mass; * = average time for faster participants (n = 8); † = average time for slower participants (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>25 mg·kg⁻¹ FFM</th>
<th>50 mg·kg⁻¹ FFM</th>
<th>75 mg·kg⁻¹ FFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min:s</td>
<td>min:s</td>
<td>min:s</td>
<td>min:s</td>
</tr>
<tr>
<td>Participant 1*</td>
<td>40:20.2</td>
<td>40:11.8</td>
<td>40:16.8</td>
<td>41:16.6</td>
</tr>
<tr>
<td>Participant 2†</td>
<td>44:25.2</td>
<td>47:25.2</td>
<td>42:45.0</td>
<td>46:50.6</td>
</tr>
<tr>
<td>Participant 3†</td>
<td>43:54.4</td>
<td>42:24.0</td>
<td>45:24.6</td>
<td>41:57.4</td>
</tr>
<tr>
<td>Participant 4*</td>
<td>38:36.4</td>
<td>41:25.8</td>
<td>34:48.0</td>
<td>40:14.6</td>
</tr>
<tr>
<td>Participant 5*</td>
<td>38:52.0</td>
<td>40:19.0</td>
<td>38:33.0</td>
<td>37:53.2</td>
</tr>
<tr>
<td>Participant 6†</td>
<td>56:14.0</td>
<td>57:11.0</td>
<td>54:22.2</td>
<td>52:04.6</td>
</tr>
<tr>
<td>Participant 7†</td>
<td>44:54.4</td>
<td>49:28.6</td>
<td>50:56.8</td>
<td>49:01.4</td>
</tr>
<tr>
<td>Participant 8*</td>
<td>32:07.0</td>
<td>30:25.6</td>
<td>33:51.8</td>
<td>32:10.6</td>
</tr>
<tr>
<td>Participant 9*</td>
<td>36:25.6</td>
<td>40:56.2</td>
<td>38:06.4</td>
<td>36:07.0</td>
</tr>
<tr>
<td>Participant 10*</td>
<td>30:45.4</td>
<td>32:43.6</td>
<td>28:46.8</td>
<td>29:26.2</td>
</tr>
<tr>
<td>Participant 11*</td>
<td>31:34.2</td>
<td>33:44.0</td>
<td>31:27.0</td>
<td>33:45.6</td>
</tr>
<tr>
<td>Participant 12†</td>
<td>56:02.6</td>
<td>57:32.0</td>
<td>54:25.0</td>
<td>51:19.2</td>
</tr>
<tr>
<td>Participant 13*</td>
<td>34:26.0</td>
<td>36:50.0</td>
<td>38:16.0</td>
<td>36:19.2</td>
</tr>
</tbody>
</table>

Average time benefit compared to placebo for those that benefited

<table>
<thead>
<tr>
<th></th>
<th>-</th>
<th>1 min 7 s</th>
<th>1 min 25 s</th>
<th>2 min 14s</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Average time for faster participants (n = 8)</td>
<td>35:23.0</td>
<td>37:04.0</td>
<td>35:53.0</td>
<td>35:23.4</td>
</tr>
<tr>
<td>†Average time for slower participants (n = 5)</td>
<td>50:48.2</td>
<td>49:34.7</td>
<td>48:14.0</td>
<td>49:05.0</td>
</tr>
</tbody>
</table>

**Serum phosphate**

There were no significant differences between trials for post-loading serum phosphate levels (p = 0.47) or between pre and post-loading values (p = 0.63). Pre and post-loading serum phosphate levels for the 25, 50 and 75 mg·kg⁻¹ of FFM trials were 1.35 ± 0.13, 1.36 ± 0.15, 1.28 ± 0.18, 1.31 ± 0.09, 1.41 ± 0.09 and 1.37 ± 0.1 mmol·L⁻¹ respectively. The pre and post-loading serum phosphate levels for the placebo trial were 1.38 ± 0.10 and 1.37 ± 0.15 mmol·L⁻¹.
There were no correlations between serum phosphate concentrations and any of the performance variables for the total group or in those participants who had improved their TT completion times following the 50 and 75 mg·kg⁻¹ of FFM dosing protocols. Additionally, when changes in serum phosphate were correlated with changes in TT completion times between the phosphate and placebo trials, no significant relationships were found (r = -0.448 to -0.202 range).

**Food diaries**

There was no significant differences (p = 0.16) in energy intake for the 24 h prior to the 500 kJ TT for the 25 mg·kg⁻¹ of FFM, 50 mg·kg⁻¹ of FFM, and 75 mg·kg⁻¹ of FFM of SP trials, or the placebo trial (6680 ± 2052 kJ (1629 ± 500 kcal), 7419 ± 1637 kJ (1809 ± 399 kcal), 7453 ± 2032 kJ (1817 ± 495 kcal) and 7949 ± 1796 kJ (1938 ± 438 kcal), respectively).

**Discussion**

This is the first study to investigate the effects of SP loading on endurance exercise in female cyclists, as well as to assess the effects of a number of SP loading doses on cycling TT performance. Overall, results showed that while the 75 mg·kg⁻¹ of FFM trial resulted in the fastest completion time and highest average and peak power, compared with all other trials, these results were not significant. Similarly, when TT scores were separated into fastest times (TT completion of ≤ 45 min) and slowest times (TT completion ≥ 45 min), there were also no significant differences in results between trials for either sub-group. This suggests that either a 25, 50 or 75 mg·kg⁻¹ of FFM dose of SP ingested for six days has no significant effect on endurance performance in females cyclists, regardless of
whether participants were of higher (mean $\dot{V}O_{2\text{peak}} = 53.1 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or lower fitness (mean $\dot{V}O_{2\text{peak}} = 45 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Previous studies have reported improved exercise performance in male participants after a relative dose of 50 mg·kg$^{-1}$ of FFM of SP. Czuba et al. (2008; 2009) reported significant increases (5–5.6%) in cycling $\dot{V}O_{2\text{max}}$ after 6 and 21 days of SP supplementation, while Brewer et al., (2013) reported significant increases in $\dot{V}O_{2\text{max}}$ (3.4% and 7.1%) after an initial and follow-up loading phase using this relative dose of SP, respectively. However, while Brewer et al. (2013) also reported significant improvement in cycling power output 250 kJ (59.75 kcal) and 500 kJ (119.5 kcal) split times after SP loading during the second phase of a two phase loading protocol in male cyclists performing a 1000 kJ TT, completion times were not significantly different between trials. Brewer et al. (2013) suggested that the overall dose used in their study, which equated to ~3.4 g/day over 6 days, may not have been high enough to elicit an ergogenic effect on overall exercise performance. This comment was based on studies by Kreider et al. (1992) and Folland et al. (2006) who reported an 8% and 3% improvement in 40 and 16.1 km cycling TT performance, respectively, following the ingestion of 4 g/day of SP over 3-4 day. Of relevance, the 75 mg·kg$^{-1}$ of FFM dose of SP used in the current study was similar to the higher absolute dose used by both Kreider et al. (1992) and Folland et al. (2006) (i.e. ~4 g/day), yet no benefit was found for endurance performance here.

Differences in results between the current study and those that reported benefit of SP on endurance performance may be related to gender. As noted earlier, females differ to males in respect to oxygen affinity, hormonal concentrations and heart function (O’Brien, 1985), with these factors all associated with mechanisms proposed to result in ergogenic effects.
following SP loading. These differences either separately or as a whole, could have reduced the magnitude of benefit received by the participants in this study, resulting in no overall benefit of SP supplementation on 500 kJ (119.5 kcal) cycle performance. It is possible that a higher relative dose may be needed in females for an ergogenic effect to occur in respect to endurance performance.

While SP loading resulted in no improvement in TT performance in the current study, 8 of the 13 participants recorded faster times following the 50 mg·kg⁻¹ of FFM trial compared with the placebo trial (1 min 25 s ± 1 min 15 s faster on average), with four of these participants recording their fastest time overall. Furthermore, six participants recorded faster times following the 75 mg·kg⁻¹ of FFM trial, compared with the placebo trial, with five of these participants recording their fastest time overall. Differences in TT results between this highest dose trial and the placebo trial for these participants was 2 min 14 s ± 1 min and 43 s faster on average. These results allude to the possibility of responders and non-responders to SP supplementation, which has been previously described by West et al. (2013), who noted that changes in serum phosphate levels were correlated with changes in aerobic capacity between a SP and placebo trial and suggested that possibly only certain individuals responded positively to SP supplementation. Individuals should therefore test for the effectiveness of this supplement prior to its use in competition. Furthermore, other studies have also referred to the concept of responders and non-responders in respect to the effects of proposed ergogenic aids on exercise performance. Specifically, when investigating the effects of 5-6 weeks of 4.8 g·d⁻¹ of beta-alanine supplementation on carnosine levels, Baguet et al. (2009) categorised individuals as either high or low responders to beta-alanine supplementation. While the results of the current study support
the possibility of responders and non-responders to SP supplementation, correlation analysis of the change in serum phosphate pre and post loading with the change in TT completion trials between the SP and placebo trials did not show significance. However, serum phosphate levels may not be the best indicator for assessing the effect of SP loading on exercise performance (Kreider, 1999). A better measure may be 2,3-DPG concentration, where increases in this substrate are proposed to improve endurance performance. However, due to financial constraints, 2,3-DPG was unable to be measured in the current study; this variable should be considered in future studies.

An unexpected outcome of the current study was that TT performance and average power output for the 25 mg·kg⁻¹ of FFM trial were ‘likely to be detrimental’ when compared with the placebo and the 75 mg·kg⁻¹ of FFM trials, as determined by qualitative statistical analysis. These results were unexpected because theoretically the explanation of the mechanisms on which the ergogenic benefits of SP are based implies that any increase in phosphate levels should enhance these mechanisms and consequently improve exercise performance (Kreider, 1992). While the 25 mg·kg⁻¹ of FFM dose of SP was much lower than doses that have previously shown an ergogenic effect on exercise performance (Czuba et al, 2009), this still does not explain the impairment found in exercise performance compared with the placebo and the 75 mg·kg⁻¹ of FFM trials. Furthermore, similar results for pre and post exercise variables relating to serum phosphate and blood lactate concentrations and RPE between the placebo, the 75 and the 25 mg·kg⁻¹ of FFM loading trials do not provide any further evidence to explain this result. Finally, it is unlikely that these results are a consequence of a small cohort due to this study meeting the required number of participants determined by power analysis. Consequently, it is unclear as to why
this result occurred. Additionally, it must be noted that a limitation of the current study is that the effectiveness of the blinding procedure was not recorded.

Conclusion

In summary, the present study found no benefit of a 25, 50 or 75 mg·kg\(^{-1}\) of FFM dose of SP on 500 kJ (119.5 kcal) TT cycle performance in females of varying fitness levels. However, due to the possibility of individual responders to either the 50 or 75 mg·kg\(^{-1}\) of FFM loading protocols, competitive cyclists should trial these doses prior to competition.

Acknowledgements

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References


Chapter Four

Study Two

EFFECTS OF SODIUM PHOSPHATE AND CAFFEINE LOADING ON REPEATED-SPRINT ABILITY

Presented here in journal format

Based on:

Abstract

The effects of sodium phosphate and caffeine supplementation were assessed on repeated-sprint ability. Using a randomised, double-blind, Latin-square design, 12 female, team-sport players participated in four trials: (1) sodium phosphate and caffeine, (2) sodium phosphate and placebo (for caffeine), (3) caffeine and placebo (for sodium phosphate) and (4) placebo (for sodium phosphate and caffeine), with ~21 days separating each trial. After each trial, participants performed a simulated team-game circuit (4 x 15 min quarters) with 6 x 20-m repeated-sprints performed once before (set 1), at half-time (set 2), and at the end (set 3). Total sprint times were faster after sodium phosphate and caffeine compared with placebo (set 1: \(P=0.003\); set 2: \(d=-0.51\); set 3: \(P<0.001\); overall: \(P=0.020\)), caffeine (set 3: \(P=0.004\); overall: \(P=0.033\)) and sodium phosphate (set 3: \(d=-0.67\)). Furthermore, total sprint times were faster after sodium phosphate compared with placebo (set 1: \(d=-0.52\); set 3: \(d=-0.58\)). Best sprint results were faster after sodium phosphate and caffeine compared with placebo (set 3: \(P=0.007\), \(d=-0.90\)) and caffeine (set 3: \(P=0.024\), \(d=-0.73\)). Best sprint times were also faster after sodium phosphate compared with placebo (\(d=-0.54 \sim 0.61\) for all sets). Sodium phosphate and combined sodium phosphate and caffeine loading improved repeated sprint ability.

Keywords: ergogenic aids, anaerobic exercise, aerobic exercise, 20 metre sprints
Introduction

Nutritional supplements have long been used in team-sports in an attempt to improve exercise performance. One such supplement that has received significant research attention over the years is caffeine. To date, numerous studies have reported that consuming 3-6 mg·kg\(^{-1}\) body-mass of caffeine, 60 min before exercise, resulted in significant improvement in endurance performance (Ganio, Klau, Casa, Armstrong, & Maresh, 2009). More recently, the effects of caffeine have been investigated in respect to repeated-sprint ability as performed during team-sports, with some studies finding benefit (Carr, Dawson, Schneiker, Goodman, & Lay, 2008; Pontifex, Wallman, Dawson, & Goodman, 2010), while others have not (Paton, Hopkins, & Vollebregt, 2001). Reasons for these differences may relate to the differences in training status of participants between studies (Greer, McLean, & Graham, 1998) and the use of protocols that employed sustained sprint efforts and/or short recovery periods, resulting in an excessive accumulation of metabolic waste products (Schneiker, Bishop, Dawson, & Hackett, 2006). Improvement in exercise performance following caffeine ingestion have been attributed to adenosine receptor antagonism, which is proposed to result in reduced sensations of effort and pain, increased alertness, improved neural firing rates and enhanced motor unit recruitment and frequency of activation (Tarnopolsky, 2008). Notably, all studies that have assessed the effects of caffeine on repeated-sprint ability have recruited men.

Sodium phosphate is another legal, nutritional supplement, where the ingestion of ~4 g or 50 mg·kg\(^{-1}\) fat free mass (FFM) per day for 3-6 days has been reported to improve endurance exercise performance (Brewer, Dawson, Wallman, & Guelfi, 2013; Kreider et al., 1992; Kreider, Miller, Williams, Somma, & Nasser, 1990) and aerobic capacity (Brewer, Dawson, Wallman, & Guelfi, 2014; Cade et al., 1984; Czuba, Zajac,
Poprzecki, Cholewa, & Woska, 2009; Kreider et al., 1992), with other studies finding no benefit (Buck, Wallman, Dawson, McNaughton, & Guelfi, 2014; West, Ayton, Wallman, & Guelfi, 2012). Numerous mechanisms have been proposed to provide benefit to exercise performance following sodium phosphate loading, with these including: enhanced 2,3-diphosphoglycerate (2,3-DPG) concentration in red blood cells, which allows for a greater unloading of oxygen to the peripheral tissues (Benesch & Benesch, 1969); improved buffering capacity of hydrogen ions due to increased hydrogen phosphate concentrations (Kreider, 1999); improved myocardial efficiency, which results in more efficient oxygenation of exercising muscles (Kreider et al., 1992); greater adenosine triphosphate/phosphocreatine (ATP/PCr) resynthesis due to increased availability of extracellular and intracellular phosphate (Bredle, Stager, Brechue, & Farber, 1988; Kreider, 1999); and enhanced activation of rate-limiting constituents of the glycolytic and Krebs cycles (Kreider et al., 1992). Theoretically, a number of these mechanisms could benefit repeated-sprint ability, as performed during team-sport match play, however no studies to date have assessed this aspect of sodium phosphate supplementation.

Notably, only one study to date has assessed the effect of sodium phosphate supplementation on exercise performance in females (Buck et al., 2014), with investigators finding no benefit of varying sodium phosphate dosing strategies (25, 50 or 75 mg·kg⁻¹ FFM per day for 6 days) on 500 kJ time-trial cycle performance. Additionally, no studies have investigated the effects of combined sodium phosphate and caffeine supplementation on repeated-sprint ability. As the mechanisms proposed to improve exercise performance after sodium phosphate and caffeine ingestion theoretically work independently of each other, it is possible that these mechanisms
combined could result in greater improvement in exercise performance than when either supplement is ingested alone.

Therefore, the aim of this study was to investigate the effect of sodium phosphate and caffeine supplementation, either alone or in combination, on repeated-sprint ability performed before (while fresh), at half-time and after a simulated, team-game circuit (i.e., while fatiguing) in female team-sport players. We hypothesised that combined sodium phosphate and caffeine ingestion would result in the greatest improvement in repeated-sprint ability and that sodium phosphate and caffeine ingestion alone would result in faster repeated-sprint ability when compared with placebo.

Methods

Participants

Twelve female (age 25.5±1.9 years, body-mass 61.1±7.1 kg, height 1.66±0.05 m, fat mass 16.6±4.5 kg, FFM 42.4±3.9 kg) who played amateur team-sports (netball, n=5; basketball, n=4; soccer, n=3; training/competition involvement per week of 183 ± 72 min) were recruited to this study. This number was determined using a G-power analysis (Faul, Erdfekder, Buchner, & Lang, 2009), based on an effect size of 1.1 (Goss et al., 2001), with an alpha level of 0.05 and 85% power. Participants were not taking any nutritional supplements for at least two months prior to or during this study. During the study, nine participants were taking Levlen ED for birth control, while the remaining participants took no oral contraceptives. The Human Research Ethics Committee of the University of Western Australia approved the study and all participants provided written informed consent prior to participation.
**Experimental design**

Participants completed a practice session and four different supplementation protocols over an 18 week period. All testing occurred during the competitive season of each participant’s sport. During this practice session, body composition was assessed using dual energy x-ray absorptiometry (Lunar Prodigy, GE Medical Systems, Madison, USA) so to determine each participant’s FFM. Additionally, full practice of the simulated team-game circuit and the repeated-sprint ability sets was undertaken.

The four experimental trials were performed in a randomised, double-blind, Latin-square design. These trials consisted of: sodium phosphate, sodium phosphate and caffeine; caffeine and placebo (for sodium phosphate); sodium phosphate and placebo (for caffeine); and placebo (for both sodium phosphate and caffeine) loading protocols. To control for the phase of menstrual cycle, the first supplementation protocol began ~3 days post the first menstruation (follicular phase) after completing the practice session. All subsequent testing was performed during this phase, with ~21 days between trials. Notably, 17 days has been proposed to be the minimum washout period for sodium phosphate (Kreider et al., 1992).

The sodium phosphate trials consisted of the consumption of 50 mg·kg\(^{-1}\) FFM of trisodium phosphate dodecahydrate (Challenge Chemicals, Western Australia), split into four equal doses a day, consumed for six consecutive days. Each dose was placed into an opaque capsule by a blinded researcher. In order to prevent gastrointestinal upset, each capsule was emptied into a glass and consumed with 15 g of Powerade powder (Coca-Cola Amatil, Australia) that had been dissolved in ~300 mL of water (Brewer et al., 2013). Importantly, no side effects were reported. In addition to sodium
phosphate ingestion, participants also ingested either caffeine or placebo as detailed below.

The caffeine supplementation trial involved participants consuming 6 mg·kg$^{-1}$ body-mass of caffeine (No Doz; Key Pharmaceuticals, Rhodes, NSW, Australia) that was placed in an opaque capsule and ingested 1 h prior to performing the first repeated-sprint ability set. The caffeine placebo consisted of 1 g of glucose that was ingested 1 h before performing the first repeated-sprint ability set, while the sodium phosphate placebo consisted of 1 g of Splenda (Splenda, Johnson & Johnson Pacific Pty Ltd, Ultimo, NSW, Australia) and 0.5 g of table salt (to match the slightly salty taste of sodium phosphate) ingested following the same protocol described for the sodium phosphate protocol. Both these protocols were replicated during the placebo loading phase. On the day after the end of each loading protocol, participants returned to the lab and completed the repeated-sprint ability sets and the simulated team-game circuit.

Participants were instructed to follow their normal training diet and fluid intake (except for the ingestion of caffeine) during each supplementation protocol and were also required to complete a detailed dietary record for the 24 h period prior to each simulated team-game circuit. They were advised to abstain from consuming caffeine (in fluids and food) in the 48 h prior to each trial. Participants were instructed to replicate this eating pattern before each subsequent simulated team-game circuit. Dietary analysis of each participant’s self-reported caloric intake was undertaken on completion of the study (FoodWorks v 4.2.0, Xyris Software, Qld, Australia). Participants were also required to maintain a consistent training volume throughout the study and completed a six day physical activity diary during each supplementation period. Participants were requested to replicate their physical activity patterns using this diary during each
supplementation phase of the study. Compliance was confirmed upon arrival to the laboratory for each time-trial after inspection of the training diaries by the investigator.

**Exercise protocol**

Before performing the initial repeated-sprint ability trial, participants performed a 10 min standardised warm-up in an indoor gymnasium on a sprung wooden surface. All exercise testing was performed here. The warm-up replicated that typically undertaken before a team-sport game and included light jogging (5 min), run throughs (3 x 20 m at a light, medium and fast pace) and stretching. Participants then rested for 3 min before commencing the first repeated-sprint ability trial. One set of 6 x 20-m sprints was performed immediately before the simulated team-game circuit, as well as before the half-time break and immediately after the simulated team-game circuit. Repeated-sprints were timed using two pairs of electronic, single-beamed, infrared timing gates (20 m: 0.001 s; Fitness Technologies, Adelaide, Australia), which were positioned 20 m apart. A slow jog return was performed after each sprint and 25 s separated the start of each sprint. Participants adopted a standing sprint start position, with the front foot ~10 cm behind the start line. Strong verbal encouragement was provided for each sprint to ensure maximal effort. The typical error and coefficient of variance for one set of sprints and best 20-m time have been determined to be 0.060 s, 1.8% and 0.19 s, 1.1% respectively (Sim, Dawson, Guelfi, Wallman, & Young, 2009).

Immediately following the completion of the first repeated-sprint ability set, participants commenced the simulated team-game circuit. This 60 min exercise circuit was designed to replicate the typical intermittent exercise demands and movement patterns observed in team-sports (Bishop, Spencer, Duffield, & Lawrence, 2001). Each 15-min quarter consisted of 15 x 1 lap repetitions, with a lap beginning each min. Each lap
involved three maximal sprints (2 x 10-m, 1 x 20-m), a 12-m agility (change of direction) section, one 30-m striding effort, two periods of jogging and three periods of walking. The total distance per lap repetition was ~122 m, equating to a total distance of 7320 m over the 60 min period. The time taken to complete each lap was ~45 s. A 4-min rest separated the first and second quarter and the third and fourth quarter, with a 10-min recovery period at half-time. Circuit times were recorded for every lap using a digital stopwatch (Hart Sport, Virginia BC, QLD, Australia; 0.01 s) in order to assess for consistency between trials.

Heart rate (Polar, Kempele, Finland) and ratings of perceived exertion (Borg’s 6-20 point scale; Borg, 1982) were recorded at the end of each quarter of the simulated team-game circuit. Blood lactate concentration was measured at baseline and after the 2nd and 3rd set of repeated sprints. To determine this, a capillary blood sample was collected in a 35 µL heparinised capillary tube from the earlobe. The sample was then analysed by an assistant using a blood-gas analyser (ABL 725, Radiometer, Copenhagen, Denmark), which was situated nearby.

**Determination of phosphate, 2,3-DPG and caffeine**

Resting venous blood samples (8.5 mL) were collected pre- and post-supplementation (at a standardised time of day) for the determination of phosphate, 2,3-DPG and caffeine concentrations. Post-loading venous blood samples were taken 60 min following ingestion of the caffeine/caffeine placebo. Samples for phosphate and caffeine were then left to clot at room temperature for 60 min prior to being centrifuged at 1000 g at 4°C for 15 min. The serum obtained was stored at -80°C for later analysis; with serum phosphate determined using an Abbott Architect c16000 analyser, employing specified Abbott reagents (Abbott Laboratories, Abbott Park, IL 60065,
USA). Observed coefficient of variation for serum phosphate analysis was 2.0% for 2.95 mmol·L⁻¹. A further 2 mL of blood was collected in heparinised tubes for analysis of 2,3-DPG concentrations using a Roche diagnostic kit (Cat. No. 148 334; Roche Diagnostics, Indianapolis, Indiana). Analysis was performed on a Cobas Mira Plus chemistry analyser (Roche Diagnostics, Indianapolis, Indiana) at the Centre for Metabolomics, University of Western Australia. Immediately upon collection, the blood was treated using perchloric acid and centrifuged at 1000 g at 4°C for 5 min. The sample was then treated with potassium carbonate solution and left on ice for 30 min before again being centrifuged at 1000 g at 4°C for 5 min. One mL of the resulting sample was then stored at -80°C for later analysis. The observed coefficient of variation for 2,3-DPG analysis was 1.45% for 2.38 mmol·L⁻¹. Caffeine was quantified from venous blood samples using a high performance liquid chromatography reverse phase separation process against a 4-point calibration curve. Samples were introduced from an autosampler (Waters Corporation, Milford, Massachusetts) onto a reverse phase C18 column at 40°C in a 10% acetonitrile mobile phase. The eluted caffeine was detected at 267 nm using 340 nm as the reference wavelength to remove interfering absorbing compounds. Retention on the column is a function of polarity, where 7 – (b-hydroxyethyl) theophylline (Sigma Chemicals, Perth, Western Australia) is eluted before caffeine. The Empower software (Version 2, Waters Corporation, Milford, Massachusetts) controls the injection, data acquisition and result processing of the samples. The observed coefficient of variation for serum caffeine was 10.04% for 22.20 mmol·L⁻¹. Due to financial constraints, blood samples were taken from nine participants.
Statistics

Statistical Package for the Social Sciences Version 16.0 for Windows (SPSS, Inc., Chicago, IL) was used to perform two-way (4 trials by 3 time points), repeated-measures, ANOVAs for the primary variables, which consisted of first sprint and best sprint times for each set and total sprint times for the six sprints in each set and overall. Significance was determined by $P < 0.05$. This statistical analysis was based on that by Duvnjak-Zaknich, Dawson, Wallman, and Henry (2011), who used a similar exercise protocol to that of the current study. Two-way, repeated-measures ANOVAs were also used to assess differences in lap times, environmental conditions, food intake, serum phosphate, 2,3-DPG and caffeine concentrations. Bonferroni post-hoc tests were applied to determine the location of significant differences if applicable. In addition, Cohen’s $d$ effect sizes (Cohen, 1988) ($d \leq 0.2$, small; $0.5-0.79$, moderate; $\geq 0.8$, strong) were calculated to assess the magnitude of difference between trials. Only moderate and large $d$ are reported. Pearson correlations were also performed to assess for any relationship between serum phosphate and primary variables.

Results

All results are presented as mean±SD. Ambient temperature in the gym during the trials was 21±4°C, while relative humidity was 62±5% (model QuestTemp 32; Quest Technologies, Oconomowoc, WI, USA) with these not being significantly different between trials ($P=0.721$; $P=0.423$, respectively). Furthermore, the average completion times for the 15 laps of each quarter were not different between trials ($P=0.071$) and within trials ($P=0.671$). Analysis of the total training load for each 6 day loading period demonstrated no significant differences between trials ($P=0.781$).
**Total, first and best sprint times**

An interaction effect was found for total sprint times ($P<0.001$) with *post hoc* analyses finding that these were faster after sodium phosphate and caffeine compared with placebo for set 1 ($P=0.003$, $d=-0.70$), set 2 ($d=-0.51$), set 3 ($P<0.001$, $d=-1.22$) and overall ($P=0.020$, $d=-0.74$; Table 1). Total sprint times were also faster after sodium phosphate and caffeine compared with caffeine (set 3: $P=0.004$, $d=-0.76$; overall: $P=0.033$, $d=-0.53$) and compared with sodium phosphate (set 3: $d=-0.67$). Additionally, total sprint times were faster after sodium phosphate compared with placebo (set 1, $d=-0.52$ and set 3, $d=-0.58$).

There was also an interaction effect for first sprint times ($P=0.001$; Table 1) with *post hoc* analyses finding faster first sprint times in the sodium phosphate trial for sets 1 and 2 compared with set 3 ($P=0.002$ and $P=0.012$, respectively).

An interaction effect was also found for best sprint times ($P=0.014$; Table 1), with *post hoc* analyses finding that best sprint times were faster after sodium phosphate and caffeine compared with placebo for all sets combined, with this supported by a faster time for set 3 ($P=0.007$, $d=-0.90$). The sodium phosphate and caffeine trial also resulted in a faster best sprint time for set 3 when compared with caffeine ($P=0.024$, $d=-0.73$). Additionally, best sprint times were faster after sodium phosphate compared with placebo, with this supported by moderate $d$ (set 1, $d=-0.54$; set 2, $d=-0.61$; set 3 $d=-0.55$).

**Heart rate, ratings of perceived exertion and food intake**

Only main effects for time were found for heart rate and ratings of perceived exertion ($P=0.041$; $P<0.001$, respectively). Scores for ratings of perceived exertion were higher for all quarters compared with baseline, while heart rate was higher for quarters 2, 3,
and 4 compared with 1 (Table 2). These results were also supported by numerous moderate to large $d$ (Table 2). Additionally, there was a main effect for time for blood lactate ($P < 0.001$) with values being higher midway and at the end of the simulated team-game circuit compared with baseline (Table 2), with this supported by moderate to large $d$. Quantitative analysis of food diaries found no differences ($P=0.17$) in total caloric intake for the 24 h prior to the sodium phosphate, caffeine, sodium phosphate and caffeine and placebo trials (7210±1953 kJ, 7289±1732 kJ, 7433±2101 kJ and 7642±1899 kJ, respectively).

**Serum phosphate, red blood cell 2,3-DPG and caffeine concentrations**

Pre-loading serum phosphate levels for the placebo, sodium phosphate, sodium phosphate and caffeine and caffeine trials (n=9) were 1.30±0.21, 1.29±0.11, 1.39±0.13 and 1.35±0.19 mmol·L$^{-1}$ respectively, whilst post-loading values were 1.35±0.14, 1.33±0.12, 1.30±0.07 and 1.29±0.11 mmol·L$^{-1}$ respectively. There were no moderate or large $d$ or differences between trials for post-loading serum phosphate levels ($P=0.44$) or between pre- and post-loading values ($P=0.36$). Further, there were no significant correlations between serum phosphate concentrations and any primary variable. Additionally, when the change in serum phosphate levels pre- and post-loading were correlated with changes in first sprint, best sprint and total sprint between the sodium phosphate and caffeine, sodium phosphate, caffeine and placebo trials, no significant relationships were found ($r=-0.54$ to 0.43 range, $P>0.05$).

Pre-loading serum caffeine levels for the placebo, sodium phosphate, sodium phosphate and caffeine and caffeine trials (n=9) were 2.0±0.95, 1.83±0.57, 2.33±0.88 and 2.0±0.73 mmol·L$^{-1}$ respectively, whilst post-loading values were 2.0±1.04, 2.0±0.73, 2.16±0.71 and 2.75±0.96 mmol·L$^{-1}$ respectively.
Table 1. Repeated-sprint ability times for the four conditions: placebo (PLA), caffeine (CAFF), sodium phosphate (SP) and combined sodium phosphate and caffeine (SP+C), n=12. Each set = 6 x 20-m sprints.

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>CAFF</th>
<th>SP</th>
<th>SP+C</th>
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<tr>
<td><strong>Mean ± SD</strong></td>
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<tr>
<td><strong>First sprint time (s)</strong></td>
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<tr>
<td>Set 1</td>
<td>3.70 ± 0.27</td>
<td>3.73 ± 0.33</td>
<td>3.62 ± 0.21</td>
<td>3.71 ± 0.25</td>
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<tr>
<td>Set 2</td>
<td>3.80 ± 0.38</td>
<td>3.84 ± 0.42</td>
<td>3.69 ± 0.27</td>
<td>3.73 ± 0.25</td>
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<tr>
<td>Set 3</td>
<td>3.82 ± 0.26</td>
<td>3.84 ± 0.34</td>
<td>3.91 ± 0.23</td>
<td>3.79 ± 0.28</td>
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<tr>
<td><strong>Best 20-m time (s)</strong></td>
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<tr>
<td>Set 1</td>
<td>3.71 ± 0.24</td>
<td>3.59 ± 0.28</td>
<td>3.58 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.61 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Set 2</td>
<td>3.78 ± 0.27</td>
<td>3.69 ± 0.32</td>
<td>3.61 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.66 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Set 3</td>
<td>3.78 ± 0.25</td>
<td>3.73 ± 0.28</td>
<td>3.63 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.54 ± 0.24&lt;sup&gt;abcd&lt;/sup&gt;</td>
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<tr>
<td><strong>Total sprint time (s)</strong></td>
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<tr>
<td>Set 1</td>
<td>23.11 ± 1.47</td>
<td>22.56 ± 1.72</td>
<td>22.39 ± 1.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.15 ± 1.32&lt;sup&gt;bcde&lt;/sup&gt;</td>
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<tr>
<td>Set 2</td>
<td>23.07 ± 1.87</td>
<td>22.97 ± 1.82</td>
<td>22.63 ± 1.58</td>
<td>22.28 ± 1.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Set 3</td>
<td>23.70 ± 1.65</td>
<td>23.09 ± 1.85</td>
<td>22.80 ± 1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.85 ± 1.38&lt;sup&gt;bced&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall</td>
<td>69.83 ± 4.14</td>
<td>69.39 ± 5.54</td>
<td>67.81 ± 4.13</td>
<td>66.83 ± 4.01&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> significantly different from PLA (p < 0.05)
<sup>b</sup> significantly different from CAFF (p < 0.01)
<sup>c</sup> moderate to large effect sizes (d = -0.51 to -1.22) when compared with PLA
<sup>d</sup> moderate effect sizes (d = -0.53 to -0.76) when compared with CAFF
<sup>e</sup> moderate effect size (d = -0.67) when compared with SP
Table 2. Mean ±standard deviation blood lactate, rating of perceived exertion (RPE) and heart rate (HR) values for placebo (PLA), caffeine (CAFF), sodium phosphate (SP) and combined SP and caffeine (SP+C) trials during a simulated team-game circuit. n=12.

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>CAFF</th>
<th>SP</th>
<th>SP+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Lactate (mmol·L⁻¹) Baseline</td>
<td>1.6 ± 0.78</td>
<td>1.6 ± 0.63</td>
<td>1.4 ± 0.76</td>
<td>1.5 ± 0.44</td>
</tr>
<tr>
<td>Blood Lactate (mmol·L⁻¹) Quarter 2</td>
<td>6.0 ± 1.52ᵃ</td>
<td>5.2 ± 1.79ᵇᶜ</td>
<td>5.4 ± 1.47ᵃ</td>
<td>5.6 ± 2.06ᵃ</td>
</tr>
<tr>
<td>Blood Lactate (mmol·L⁻¹) Quarter 4</td>
<td>6.0 ± 1.57ᵃ</td>
<td>6.4 ± 1.27ᵇᶜ</td>
<td>6.1 ± 1.22ᵇᶜ</td>
<td>6.4 ± 1.45ᵃ</td>
</tr>
<tr>
<td>RPE Baseline</td>
<td>8 ± 1.0</td>
<td>8 ± 0.7</td>
<td>8 ± 0.5</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td>RPE Quarter 1</td>
<td>15 ± 1.4ᵃ</td>
<td>14 ± 2.0ᵃ</td>
<td>14 ± 1.1ᵃ</td>
<td>14 ± 2.1ᵃ</td>
</tr>
<tr>
<td>RPE Quarter 2</td>
<td>13 ± 1.2ᵇⁱ</td>
<td>14 ± 1.9ᵃ</td>
<td>13 ± 1.8ᵍ</td>
<td>15 ± 1.8ᵇᵉ</td>
</tr>
<tr>
<td>RPE Quarter 3</td>
<td>14 ± 1.0ᵉᶠ</td>
<td>14 ± 1.7ᵃ</td>
<td>14 ± 1.6ᵇᵉ</td>
<td>15 ± 1.7ᶠᵉ</td>
</tr>
<tr>
<td>RPE Quarter 4</td>
<td>14 ± 1.5ᵃᵈ</td>
<td>15 ± 1.3ᵇᵃᵈ</td>
<td>15 ± 1.8ᵇᵃᵈ</td>
<td>15 ± 1.6ᵃᵈ</td>
</tr>
<tr>
<td>HR Baseline (bpm)</td>
<td>66 ± 8</td>
<td>65 ± 6</td>
<td>67 ± 8</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>HR Quarter 1 (bpm)</td>
<td>170 ± 12</td>
<td>161 ± 17</td>
<td>165 ± 15</td>
<td>162 ± 24</td>
</tr>
<tr>
<td>HR Quarter 2 (bpm)</td>
<td>175 ± 13</td>
<td>169 ± 25</td>
<td>165 ± 16</td>
<td>172 ± 14ᵍ</td>
</tr>
<tr>
<td>HR Quarter 3 (bpm)</td>
<td>174 ± 14</td>
<td>168 ± 23</td>
<td>169 ± 17</td>
<td>172 ± 15ᶠ</td>
</tr>
<tr>
<td>HR Quarter 4 (bpm)</td>
<td>178 ± 11ᵈ</td>
<td>170 ± 28</td>
<td>164 ± 24</td>
<td>170 ± 23</td>
</tr>
</tbody>
</table>

ᵃ Higher than baseline (Effect size range: d = 0.77–4.79)
ᵇ Quarter 4 higher than quarter 3 (Effect size range: d = 0.59-0.66)
ᶜ Quarter 4 higher than quarter 2 (Effect size range: d = 0.61-1.11)
ᵈ Quarter 4 higher than quarter 1 (Effect size range : d =0.54 – 0.69)
ᵉ Quarter 3 higher than quarter 2 (Effect size range: d = 0.59 – 0.91)
ᶠ Quarter 3 higher than quarter 1 (Effect size range d = 0.50 – 0.82)
ᵍ Quarter 2 higher than quarter 1 (Effect size range: d = 0.51-1.53)
There were no differences between trials for post-loading serum caffeine levels \((P=0.116)\) or between pre and post-loading values \((P=0.073)\). However, a large \(d\) was found between post- and pre-loading serum caffeine levels in the caffeine trial \((d=0.87)\), while moderate and large \(d\) were found between post-loading levels in the placebo, sodium phosphate and sodium phosphate and caffeine trials compared with the caffeine trial \((d=-0.75, -0.87 \text{ and } -0.69, \text{ respectively})\).

Pre-loading 2,3-DPG concentrations for the placebo, SP, SP+BJ and BJ trials \((n=13)\) were 1.70±0.37, 1.93±0.26, 1.82±0.34 and 1.99±0.32 mmol/L respectively, whilst post-loading values were 1.85±0.53, 1.82±0.33, 1.76±0.45 and 1.88±0.37 mmol/L respectively. The concentration of 2,3-DPG did not differ between trials \((P=0.563)\) or between loading phases \((P=0.871)\).

**Discussion**

This is the first study (to our knowledge) to assess the effects of sodium phosphate loading, either alone or in combination with caffeine, on repeated-sprint ability performed before, during and after a simulated team-game circuit. This is also the first study to assess the effect of sodium phosphate loading and caffeine ingestion on repeated-sprint ability in women. Overall, results showed that sodium phosphate and caffeine and sodium phosphate improved repeated-sprint ability for numerous sprints performed while fresh (set 1) and fatiguing (sets 2 and 3) when compared with placebo, with some benefit also found when sodium phosphate and caffeine was compared with caffeine. Notably, caffeine alone resulted in no improvement to repeated-sprint ability.

**Effects of sodium phosphate and caffeine on repeated-sprint ability while fresh**

No benefit of caffeine for the initial repeated-sprint ability set, when participants were fresh, is supported by Pontifex et al. (2010), who used a similar protocol to the current
study and found no significant improvement in initial total sprint or best sprint times following caffeine ingestion compared with placebo. Possibly, the number of sprints performed during the first sprint bout here were too few and/or the duration too short for caffeine to provide an ergogenic benefit in respect to improved neural firing rates, enhanced motor unit recruitment and frequency of activation (Tarnopolsky, 2008).

The current study also found that sodium phosphate and caffeine and sodium phosphate resulted in faster total sprint times, while sodium phosphate also improved best sprint time during the first repeated-sprint ability set, compared with placebo. Notably, these early sprints would have initially relied more heavily on the phosphocreatine energy system, with a greater emphasis on anaerobic (and aerobic) glycolysis as sprinting continued (total sprint time; set 1 = 22.15±1.32 s and 22.39±1.27 s for sodium phosphate and caffeine and sodium phosphate, respectively) (Gaitanos, Williams, Boobis, & Brookes, 1993). Mechanisms associated with sodium phosphate loading that may have benefited initial sprint performance here may relate to an increased phosphate energy pool (ATP/PCr resynthesis), with enhanced buffering capacity also possibly providing benefit in respect to total sprint times (Czuba et al., 2009, Kreider et al., 1992). However, the possibility of improved buffering here is speculative as buffering capacity was not assessed at this specific time point. In respect to the existence of a greater energy (phosphate) pool, Kreider et al. (1992) proposed that one of the primary mechanisms of sodium phosphate loading was increased extracellular phosphate availability. This was thought to result in a greater intracellular diffusion of phosphate, which potentially stimulated phosphofructokinase and citric acid cycle intermediates, resulting in enhanced anaerobic and oxidative metabolism. It was also assumed that increased extracellular phosphate availability was reflected by increases in serum phosphate values (Kreider et al., 1992), however, as a number of studies have reported
equivocal results in relation to this measure (Brewer et al., 2013; Kreider et al., 1992; Stewart, McNaughton, Davies, & Tristram, 1990), it has been suggested that this variable may be unresponsive to supplementation (Stewart et al., 1990). Nonetheless, it is possible that increased intra- and extracellular phosphate availability following sodium phosphate loading may have played a role in improving initial repeated-sprint ability in the current study, despite no significant differences found for this variable either pre- or post- sodium phosphate loading.

**Effects of sodium phosphate and caffeine on repeated-sprint ability while fatiguing**

This study also found a greater benefit of sodium phosphate and caffeine and sodium phosphate on repeated-sprint ability performed during the later stages of a simulated team-game circuit (sets 2 and 3, whilst fatiguing), compared with placebo and also for sodium phosphate and caffeine compared with caffeine, with differences between the sodium phosphate and the sodium phosphate and caffeine trials being minimal. The metabolic energy contribution for these later sprints is likely to have seen an increasing usage of both anaerobic and aerobic glycolysis (Gastin, 2001), as PCr stores became further depleted. Evidence for use of anaerobic glycolysis is provided here by significantly higher blood lactate concentrations for repeated-sprint ability in sets 2 and 3 compared with baseline values. Furthermore, the fatiguing nature of the exercise protocol was demonstrated by final ratings of perceived exertion, heart rate and blood lactate values that ranged between 14–15, 164–178 bpm and 6.0–6.4 mmol·L⁻¹, respectively.

Benefits of sodium phosphate supplementation on these latter repeated-sprint ability sets may in part be related to the existence of a larger energy (phosphate) pool and improved buffering (as described earlier), with some support for enhanced buffering
provided here by improved repeated-sprint ability (sets 2 and 3) after sodium phosphate and sodium phosphate and caffeine supplementation that was not associated with any significant increase in blood lactate concentrations between trials. Another factor that may have improved aerobic energy contribution during these latter repeated-sprint ability sets is enhanced RBC 2,3-DPG concentration (Buck, Wallman, Dawson, & Guelfi, 2013; Czuba et al., 2009). To date, a number of studies have reported improved aerobic exercise capacity (\(\dot{V}O_{2\text{max}}\)) following sodium phosphate supplementation associated with an increase in 2,3-DPG concentration (Cade et al., 1984; Czuba et al., 2009; Stewart et al., 1990). Notably, similar to a study by Kreider et al. (1992), improved exercise performance following sodium phosphate loading found in the current study was not associated with any significant differences between pre and post-loading 2,3-DPG values.

Another mechanism associated with sodium phosphate loading that may have improved aerobic metabolism during the latter repeated-sprint ability sets is improved myocardial efficiency, which is proposed to result in greater stroke volume leading to greater oxygenation of the exercising muscles (Buck et al., 2013). Interestingly, maximal heart rate associated with improved \(\dot{V}O_{2\text{max}}\) (Czuba et al., 2009) and 40-km cycle time-trial performance (Kreider et al., 1992) has been found to be both significantly lower (Czuba et al., 2009) and higher (Kreider et al., 1992) after sodium phosphate loading compared with placebo. Notably, heart rate values were similar between all trials in the current study, even though repeated-sprint ability was generally more improved following sodium phosphate and sodium phosphate and caffeine loading. Since heart rate does not appear to be a reliable indicator for enhanced myocardial contractility, it is uncertain whether this mechanism benefited repeated-sprint ability performance here.
Furthermore, no benefit was found for caffeine for these later sprint sets in the current study. This result was surprising as two previous studies that used a similar repeated-sprint ability protocol consisting of 5 sets of 6 x 20 m sprints (4 min recovery between sets) reported improvement ($P<0.05$) for a combination of total sprint time sets after caffeine ingestion (6 mg·kg$^{-1}$ body-mass) compared with placebo (Carr et al., 2008; Pontifex et al., 2010). A possible explanation for this difference may relate to the likelihood that the women in the current study would have had less muscle-mass per kg of body-mass than the men (O’Brien, 1985) assessed in the aforementioned studies, which may have diminished any potential ergogenic effect of caffeine on glycolytic muscle fibres, overall muscle performance and hence repeated-sprint ability. This is despite the likelihood of the women here receiving a larger dose of caffeine per kg of FFM (6 mg·kg$^{-1}$ body-mass). It is possible that women may need a different dose of caffeine than men or perhaps a certain ratio of FFM to body-mass is required for ergogenic effects of caffeine to occur on repeated-sprint ability. Further studies are needed to explore this premise. Additionally, similar ratings of perceived exertion scores reported at the end of the final repeated-sprint ability set for the caffeine trial compared with all other trials in the current study suggests a negligible effect of caffeine on sense of effort or pain. Furthermore, it must be noted that a limitation of the current study is that the effectiveness of the blinding procedure was not recorded.

**Conclusion**

In conclusion, this study found that sodium phosphate and sodium phosphate and caffeine improved numerous sprints performed when fresh (set 1) and while fatiguing (sets 2 and 3). Improved sprint performance is important during a team-game where players typically strive to score first in the early stages of a match, while the ability to maintain repeated-sprint ability, particularly during the final stages of a team-game
when players are more fatigued, could be critical to the outcome of the match, especially if scores are close.

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References


Chapter Five

Study Three

EFFECTS OF SODIUM PHOSPHATE AND BEETROOT JUICE SUPPLEMENTATION ON REPEATED-SPRINT ABILITY IN FEMALES

Presented here in journal format

Based on:

Abstract

**Introduction:** Sodium phosphate (SP) and beetroot juice (BJ) supplementation was assessed on repeated-sprint ability (RSA). **Methods:** Thirteen female team-sport participants completed four trials: (1) SP and BJ (SP+BJ), (2) SP and placebo (for BJ), (3) BJ and placebo (for SP) and (4) placebo (for SP+BJ), with ~21 days separating each trial. After each trial, participants performed a simulated team-game circuit (STGC) consisting of four 15 min quarters, with a 6 x 20-m repeated-sprint set performed at the start, half-time and end. **Results:** Total sprint times were between 0.95-1.30 s and 0.83-1.12 s faster for each RSA set and 3.25 s and 3.12 s faster overall (~5% improvement) after SP compared with placebo and BJ (p=0.02, p=0.02, respectively; Cohen’s effect size: $d=-0.51$ to -0.90 for all sets). Total sprint times were 0.48 s faster after SP+BJ compared with placebo (set 2; p=0.05, ~2% improvement). Furthermore, a main effect for trial (p<0.01) determined that first sprint times were fastest after SP, with these being 0.17-0.23 s and 0.15-0.22 s faster (~6% improvement) after SP compared with placebo and BJ, respectively for all sets ($d=-0.50$ to -0.85). Best sprints were 0.13-0.23 s and 0.15-0.20 s faster (~6% improvement; p<0.01) after SP compared with placebo and BJ, respectively for all sets ($d=-0.54$ to -0.89). **Conclusion:** SP improved RSA in team-sport female athletes when fresh (set 1) and during the later sets of a STGC (sets 2 and 3). Specifically, total, first and best sprints were faster after SP compared with placebo and BJ.

**Keywords:** ergogenic aids, 20-m sprints, anaerobic energy, aerobic energy, team-sports
Introduction

Sodium phosphate (SP) and dietary nitrate in the form of beetroot juice (BJ) represent two supplements that have been shown to be beneficial to exercise performance. While some studies have found no benefit of SP on exercise performance (Brennan and Connolly 2001; Buck et al. 2014; Brewer et al. 2014; West et al. 2012), others have reported improved endurance exercise performance (Folland et al. 2008; Kreider et al. 1992) and aerobic capacity of 3.5-12% (Brewer et al. 2013; Cade et al. 1984; Czuba et al. 2008; Czuba et al. 2009; Kreider et al. 1992; Kreider et al. 1990; Stewart et al. 1990) following 3.6–4 g or 50 mg·kg⁻¹ of fat free mass (FFM) of SP ingested each day over a 3–6 day loading period. Furthermore, Brewer et al. (2014) recently reported improved sprinting and time-trial efforts whilst cycling following SP loading. Despite these positive results, there have been no published studies to our knowledge, that have assessed the effects of SP supplementation on repeated sprint ability (RSA) similar to that performed during team-sport games (Spencer et al. 2005). This is surprising, as in most countries the most popular sports, and those with the highest participation levels, are team-sports, such as football, rugby, soccer and hockey, which require athletes to sprint intermittently throughout the match (Yaicharoren et al. 2012). Faster RSA during a team-game is important where players strive to score first in the early stages of a game (Armatas et al. 2009) and during the later stages of a game, particularly if scores are close (Anderson et al. 2014). Importantly, the mechanisms attributed to improving exercise performance following SP loading relate to both aerobic and anaerobic metabolism (Kreider et al. 1992), suggesting that SP loading may benefit prolonged RSA, as is required during a team-game. Specifically, these mechanisms include: increased 2,3-diphosphoglycerate (2,3-DPG) concentration in red blood cells, resulting in a greater release of oxygen from haemoglobin to the muscle (Benesch and Benesch
enhanced buffering capacity of hydrogen ions (Kreider 1999); improved myocardial efficiency resulting in greater cardiac output and oxygenation of the exercising muscle (Kreider et al. 1992); increased adenosine triphosphate/phosphocreatine (ATP/PCr) resynthesis, due to increased availability of extracellular and intracellular phosphate (Bredle et al. 1988; Kreider et al. 1992); and enhanced activation of glycolysis and oxidative phosphorylation as a result of increased phosphate availability (Chasiotis 1988).

Similarly, BJ has been found to improve exercise performance (Bond et al. 2012; Lansley et al. 2011). Using either a chronic (~5–8 mmol per day over three days; Bailey et al. 2010) or an acute dose of BJ (~5–8 mmol ingested 2.5 h prior to exercise; Lansley et al. 2011), studies have reported a 3-5% reduction in submaximal \( \dot{V}_\text{O}_2 \) for a given workload, indicating increased efficiency of exercise (Bailey et al. 2009; Larsen et al. 2011; Vanhatalo et al. 2010). Other studies have reported improvement in cycling time-trial performance (Cermak et al. 2012; Lansley et al. 2011) following 5–11.2 mmol of acute (2.5 h) and chronic (6 days) nitrate (BJ or sodium nitrate) ingestion. Further, Wylie et al. (2013) found a 4.2% improvement in Yo-Yo intermittent recovery test performance, while Bond et al. (2012) reported a 1.7% improvement in repeated rowing efforts following 490-500 mL of BJ supplementation ingested either acutely or chronically (6 days). However, similar to SP, no studies have assessed the effects of BJ on RSA as it relates to team-sport performance.

Mechanisms proposed to account for the ergogenic benefits seen after nitrate supplementation include more efficient mitochondrial respiration, due to reduced proton leakage through the inner mitochondrial membrane (Bailey et al. 2009; Lansley et al. 2011) and more efficient ATP metabolism during muscle force production, due to lower
levels of calcium ATPase and actomyosin ATPase activity (Bailey et al. 2010; Bailey et al. 2009; Lansley et al. 2011).

Furthermore, to the author’s knowledge, there are no known published studies that have assessed the combined effects of SP and BJ on exercise performance or the effects of SP or BJ on RSA in women. Considering that the mechanisms associated with these supplements theoretically work independently of each other, it is possible that in combination these supplements could result in greater improvement in exercise performance than when either supplement is ingested alone. Additionally, while a number of mechanisms associated with SP and BJ ingestion may result in faster first sprint (FS) performance, of particular interest is the effect of SP loading on the ATP-PCr energy system, which may be more evident here. Potentially, a series of faster FS efforts by players during the opening stages of a team-game (or after being rested) may result in positive scoring outcomes.

Therefore, the aim of this study was to assess the effect of SP and BJ, both alone and combined, on RSA, performed before (while fresh, set 1), midway (set 2) and after (set 3) a simulated team-game circuit (STGC) in women. It was hypothesised that compared with placebo, SP and BJ alone would result in faster total sprint (TS) times for all sets assessed. Furthermore, the combination of SP and BJ would result in faster TS times for all sets than what would occur from either supplement alone. A secondary hypothesis was that compared with placebo, SP and BJ alone would result in faster FS and best sprint (BS) times for each RSA set performed and that the combination of SP and BJ would result in faster FS and BS times than what would occur from either supplement alone.
Methods

Participants

Thirteen females (age 26.4±1.5 y, body-mass 60.1±7.7 kg, height 1.66±0.05 m, fat mass 15.7±4.7 kg, FFM 41.5±2.8 kg) who played amateur team-sports (netball, n=4; basketball, n=2; and soccer, n=7; training/competition involvement per week of 195±42 min) were recruited to this study. Inclusion criteria required participants to be aged between 18 and 30 y, injury free and to be currently playing in a team-sport that required repeated-sprint efforts. Participants were excluded if they had taken any nutritional supplements in the two month period prior to or during this study. Through a G-power analysis (Faul et al. 2009) it was determined that twelve participants were needed based on a study by Bond et al. (2012) and using an effect size of 0.8, at an alpha level of 0.05 with 90% power. During the study, nine participants were taking Levlen ED for birth control, while the remaining participants took no oral contraceptives. The institutional Human Research Ethics Committee approved the study and all participants provided written informed consent prior to participation.

Experimental design

Participants completed a familiarisation session and four different supplementation trials over 18 wk. All testing occurred during the competitive season of each participant’s sport. During the familiarisation session, each participant’s FFM was determined using dual energy x-ray absorptiometry (Lunar Prodigy, GE Medical Systems, Madison, USA). Additionally, full familiarisation with the STGC and the RSA sets was undertaken.
The four experimental trials were performed in a randomised, double-blind, Latin-square design. The trials consisted of supplementation with: 1) both SP and BJ (SP+BJ); 2) BJ alone (with placebo for SP); 3) SP alone (with placebo for BJ); 4) placebo for both SP+BJ. To control for menstrual cycle phase, the first supplementation protocol began ~3 days post onset of menstruation (follicular phase) after completing the familiarisation session. All subsequent testing was performed during this same phase, with ~21 days between trials. Notably, 17 days has been proposed to be the minimum washout period for SP (Kreider et al. 1992).

The SP trials consisted of the consumption of a 50 mg·kg⁻¹ of FFM dose of trisodium phosphate dodecahydrate (Challenge Chemicals Australia, Western Australia), split into four equal doses a day, consumed for six consecutive days. Each dose was placed into an opaque capsule by a blinded researcher. In order to prevent gastrointestinal upset, each capsule was emptied into a glass and consumed with 15 g of Powerade powder (Coca-Cola Amatil, Australia) that had been dissolved in ~300 mL of water (Brewer et al. 2013). Importantly, no gastrointestinal side effects were reported. Participants were instructed to separate the ingestion of each capsule by at least 4 h. In addition to SP ingestion, participants also ingested either BJ or placebo as detailed below.

Nitrate supplementation involved one acute 70 mL shot of ‘Beet It stamina shot’ (James White Drinks Ltd, Ipswich, UK) concentrated BJ containing 6 mmol of nitrate, taken 3 h before exercise. The nitrate placebo consisted of BJ treated using a reverse ion exchange system designed to remove all nitrate, while leaving all other aspects of the juice intact, therefore making it impossible to tell apart from the nitrate rich juice. This process has been reported to be the optimal method of providing a BJ placebo (Lansley et al. 2011). Participants were advised to avoid using any antibacterial mouthwash after ingestion as the rise in plasma nitrite can be reduced by the destruction of anaerobic
bacteria in the mouth (Govoni et al. 2008). On the day after completing the loading protocol, participants returned to the lab and again performed the RSA sets and the STGC.

Participants were instructed to follow their normal training diet and fluid intake prior to each supplementation trial and were also required to complete a detailed dietary record for the 24 h period prior to each STGC. A copy of the food diary from the first STGC was provided to participants with the instructions to replicate this eating pattern before each subsequent STGC. Compliance was confirmed upon arrival to the laboratory for each time trial after inspection of the food diaries by the investigator. Dietary analysis of each participant’s self-reported caloric intake was undertaken on completion of the study using FoodWorks software package (FoodWorks v 4.2.0, Xyris Software, Qld, Australia). In addition, participants were also asked to maintain a consistent diet for each six day loading protocol. Participants were also required to maintain a consistent training volume throughout the study: therefore they completed a daily activity diary during each supplementation period to assist them in replicating their physical activity patterns during each phase of the study.

**Exercise protocol**

Before performing the initial RSA trial, participants performed a 10 min standardised warm-up in an indoor gymnasium on a sprung wooden surface. All exercise testing was performed here. The warm-up replicated that typically undertaken before a team-sport game and included light jogging (5 mins), run throughs (3 x 20 m at a light, medium and fast pace) and stretching (which consisted of performing the same calf, hamstring and quadriceps stretches). Participants then rested for 3 min before commencing the first RSA trial. One set of 6 x 20 m sprints was performed immediately before the
STGC (set 1), before the half-time break (set 2) and at the end of the STGC (set 3). Of relevance, studies that have published time-motion analysis during competition in general have reported the mean distance and duration of sprints during field-based sports to be between 10-20 m and 2 - 3 s, respectively (Spencer et al. 2005). Repeated sprints were timed using two pairs of electronic, single-beamed, infrared timing gates (Fitness Technologies, Adelaide, Australia), which were positioned 20 m apart. A slow jog return was performed after each sprint and 25 s separated the start of one sprint and the next. Participants adopted a standing sprint start position, with the front foot ~10 cm behind the start line. Strong verbal encouragement was provided for each sprint to ensure maximal effort. The typical error and coefficient of variation (CV) for one set of sprints and best 20-m time have been determined to be 0.060 s, 1.8% and 0.19 s, 1.1% respectively (Sim et al. 2009).

Immediately following the completion of the first RSA set, participants commenced the STGC. This 60 min exercise circuit was designed to replicate the typical intermittent exercise demands and movement patterns observed in team-sports (Bishop et al. 2001). Each 15-min quarter consisted of 15 x 1 lap repetitions, with a lap beginning each min. Each lap involved three maximal sprints (2 x 10 m, 1 x 20 m), a 12 m agility (change of direction) section, one 30 m striding effort, two periods of jogging and three periods of walking. The total distance per lap repetition was ~122 m, equating to a total distance of 7320 m over the 60 min period (60 laps). The time taken to complete each lap was ~44-45 s. A 4-min rest period separated the first and second quarter and the third and fourth quarter, with a 10-min half-time recovery. Circuit times were recorded for every lap using a digital stopwatch (Hart Sport, Virginia BC, QLD, Australia; 0.01 s) in order to assess for consistency between trials. Prior to beginning the circuit, 200 mL of water
was ingested, as well as 100 mL at each quarter time break, with 200 mL of water ingested during the half-time break in an attempt to reduce dehydration.

Heart rate (HR: Polar Heart Rate Monitors, Kempele, Finland) and ratings of perceived exertion (RPE) using Borg’s 6-20 point scale (Borg 1982) were recorded at baseline and at the end of each quarter of the circuit protocol. Blood lactate concentrations were measured at baseline and immediately after each set of RSA. To determine blood lactate, a capillary blood sample was collected in a 35 µL heparinised glass capillary tube from the earlobe and immediately analysed using a blood-gas analyser (ABL 725, Radiometer, Copenhagen, Denmark).

**Blood analysis**

Resting venous blood samples (8.5 mL) were collected pre- and post-supplementation (at a standardised time of day) for the determination of serum phosphate, 2,3-DPG and plasma nitrate concentrations. Samples for phosphate were then left to clot at room temperature for 60 min prior to being centrifuged at 1000 g at 4°C for 15 min. Serum phosphate was determined using an Abbott Architect c16000 analyser, employing specified Abbott reagents (Abbott Laboratories, Abbott Park, IL 60065, USA). The CV for serum phosphate analysis was 2.0% at 2.95 mmol·L⁻¹. A further 2 mL of blood was collected in heparinised tubes for analysis of 2,3-DPG concentrations using a Roche diagnostic kit (Cat. No. 148 334; Roche Diagnostics, Indianapolis, Indiana). Analysis was performed on a Cobas Mira Plus chemistry analyser (Roche Diagnostics, Indianapolis, Indiana) at the Centre for Metabolomics, University of Western Australia. Immediately upon collection, the blood was treated using perchloric acid and centrifuged at 1000 g at 4°C for 5 min. The sample was then treated with potassium carbonate solution and left on ice for 30 min before again being centrifuged at 1000 g at
4°C for 5 min. One mL of the resulting sample was then stored at -80°C for later analysis. The CV for 2,3-DPG analysis was 1.45% at a concentration of 2.38 mmol·L⁻¹. Nitrate concentration was measured in EDTA-treated plasma and measured using a recently published gas chromatography-mass spectrometry method (Yang et al. 2013). The CV for nitrate analysis was 9.4% at a concentration of 0.65 µM. Only nitrate samples from six participants were analysed due to financial constraints.

**Statistics**

Statistical Package for the Social Sciences Version 16.0 for Windows (SPSS, Inc., Chicago, IL) was used to perform 4 (trial) x 3 (time) two-way repeated measures analysis of variance (ANOVA) for the primary variables which consisted of; FS and BS times for each set and TS times for the six sprints in each set, plus overall. This statistical analysis was based on that performed by Duvnjak-Zaknich et al. (2011), who used a similar exercise protocol to that of the current study. One-way ANOVAs were also used to assess differences in lap time, environmental conditions, food intake, serum phosphate, 2,3-DPG and plasma nitrate concentrations. Bonferroni post-hoc tests were applied to determine the location of significant differences. In addition, Cohen’s $d$ effect sizes ($d \leq 0.2$, small; 0.5 - 0.79, moderate; $\geq 0.8$, large) (Cohen 1988) were calculated to assess the magnitude of difference between experimental trials. Further analysis identified the smallest worthwhile change in performance scores between trials using the method outlined by Batterham and Hopkins (2005). The smallest worthwhile value of change for a 20-m sprint has been calculated to be 0.8% (Paton et al. 2001). Where the chance of benefit and harm were both calculated to be $> 5\%$, the true effect was deemed unclear. When clear interpretation was possible, a descriptor was assigned to the following quantitative chances of benefit: 25-75%, benefit possible; 76-95%, benefit likely; 96-99%, benefit very likely; $> 99\%$, benefit almost certain (Batterham
and Hopkins 2005). Pearson correlations were also performed to assess the relationship between serum phosphate and main performance variables. All results are mean ± SD.

**Results**

Analysis of participant’s food diaries revealed no significant differences (p = 0.40) in total caloric intake for the 24 h prior to the SP, BJ, SP+C and placebo trials (8106±856 kJ, 7876±679 kJ, 7956±659 kJ and 7494±679 kJ, respectively). Ambient temperature in the gym during the trials was 22±1°C, and relative humidity 59±8% (model QuestTemp 32; Quest Technologies, Oconomowoc, WI, USA), with there being no significant differences between trials for these two measures (p=0.25; p=0.51, respectively). The average completion times for the 15 laps of each quarter were also not significantly different between (p=0.32) and within trials (p=0.88).

**Total sprint time**

Significant main effects for trial, time and interaction were found for TS times (p<0.01 for all, Table 1). The main effect for time found that across all trials, TS time for set 1 was significantly faster than set 2 and 3 for the placebo, SP+BJ and BJ trial (p<0.01, p=0.03, p=0.02, p=0.03, p<0.01 and p=0.04, respectively), while in SP, set 1 was significantly faster than set 3 (p<0.01). Further, TS times were faster after SP compared with placebo for set 1 (p=0.02, moderate \( d \), ‘almost certain’ benefit), set 2 (p=0.02, large \( d \), ‘very likely’ benefit), set 3 (moderate \( d \), ‘very likely’ benefit), and overall (p=0.02, moderate \( d \) and ‘very likely’ benefit; ~5% improvement). Further, TS times were faster after SP compared with BJ for sets 1, 2 and overall (p=0.02, p=0.02, p=0.02, respectively), with moderate \( d \) and ‘very likely’ or ‘almost certain’ benefits for all sets and overall. Total sprint times were also faster after SP compared with the SP+BJ trial for sets 1 and 2 (moderate \( d \), ‘likely’ benefits), and set 3 and overall (‘likely’
benefit). Furthermore, the SP+BJ trial was faster than the BJ trial for sets 1, 2 and overall (‘likely’ benefit), and the placebo trial for set 2 (p=0.05), with a ‘likely’ benefit found for all sets and overall.

**First sprint time**

There was no significant interaction effect for FS times (p=0.84), although a main effect was observed for both time (p<0.01) and trial (p<0.01; Table 1). The main effect for time indicated that generally across all trials, FS times were faster for set 1 than for sets 2 and 3, while the main effect for trial determined that FS times were fastest with the SP protocol, compared with all other trials. Further, first sprint times were 0.17-0.23 s and 0.15-0.22 s faster (~6% improvement) after SP compared with placebo and BJ, respectively for all sets, with this supported by moderate or large $d$ (all sets) and ‘likely’, ‘very likely’ or ‘almost certain’ chances of benefit (all sets). Furthermore, FS times were faster after SP compared with SP+BJ (‘likely’ chance of benefit for sets 1 and 2). Additionally, SP+BJ resulted in ‘likely’ benefit for all sets compared with BJ, as well as a ‘likely’ benefit (set 1), an ‘almost certain’ benefit (set 2) and a ‘very likely’ benefit (set 3) when compared with placebo.

**Best sprint time**

No significant interaction effect was found for BS time (p=0.39), however, a main effect was seen for both time (p<0.01) and trial (p<0.01; Table 1), indicating that overall BS times in set 1 were faster than sets 2 and 3, with the fastest BS times recorded following SP compared with all other trials. Furthermore, BS times were faster after SP compared with placebo (moderate to large $d$ and ‘likely’ or ‘very likely’ chances of benefit; ~6% improvement) for all sets, and with BJ (moderate $d$ and ‘very likely’ benefit; ~6% improvement) for all sets. Additionally, BS times were faster after SP
compared with SP+BJ for sets 1 and 2 (moderate $d$ and ‘likely’ chances of benefit). Best sprint times were also faster after SP+BJ compared with placebo for all sets (‘likely’ benefits), and when compared with BJ for set 1 and 2 (‘likely’ benefits), and set 3 (‘almost certain’ benefit).

**Heart rate, ratings of perceived exertion and blood lactate**

Only significant main effects for time were found for HR and RPE ($p<0.01$; $p<0.01$, respectively), with both these variables being significantly higher overall by the end of the fourth quarter compared with baseline and the first quarter (Table 2). These results were also supported by numerous moderate to large $d$.

Baseline blood lactate values were 1.8±0.7, 1.6±0.6, 1.6±0.6 and 1.8±0.6 mmol·L$^{-1}$ for the placebo, BJ, SP and SP+BJ trials, respectively. Blood lactate values following the first RSA set were 4.6±1.6, 4.3±1.1, 4.6±1.2, 4.5±1.1 mmol·L$^{-1}$ for the placebo, BJ, SP and SP+BJ trials, respectively, while values following the second RSA set were 5.5±1.7, 5.4±1.8, 6.0±1.8 and 5.7±1.6 mmol·L$^{-1}$ for the placebo, BJ, SP and SP+BJ trials, respectively. Following the third RSA set, blood lactate values were 6.0±1.7, 5.7±1.6, 6.1±2.1 and 6.0±1.9 mmol·L$^{-1}$ for the placebo, BJ, SP and SP+BJ trials, respectively. A main effect for time for blood lactate was observed ($p<0.01$), with increases in this variable after the first set, the half-way point and at the end of the STGC compared with baseline and increases after the half-way point and at the end of the STGC when compared with the first set, which were supported by moderate to large $d$ when compared with the first set values and baseline values ($d$ range: 0.43 to -3.09).

**Serum phosphate, nitrate and red blood cell 2,3-DPG concentrations**

There were no significant differences between trials for post-loading serum phosphate levels ($p=0.66$) or between pre- and post-loading values ($p=0.12$) (Table 3).
Additionally, when the change in serum phosphate levels pre- and post-loading were correlated with changes in FS, BS and TS between the SP+BJ, SP, BJ and placebo trials, no significant relationships were found (r=-0.34 to 0.57 range). The concentration of 2,3-DPG did not differ between trials (p=0.56) or between loading phases (p=0.87) (Table 3). An interaction effect (p<0.01) and a main effect for time (p <0.01) and trial (p<0.01) were observed for nitrate concentration with post hoc analysis demonstrating that pre- and post-loading nitrate concentrations were significantly different in the BJ trial (p<0.01), and likewise for the SP+BJ trial (p<0.01) (table 3). Furthermore, post hoc analysis also revealed a significant difference between post-loading nitrate concentration in the BJ trial compared with the post-loading concentration in the placebo (p<0.01) and SP (p<0.01) trials and for the SP+BJ trial compared with the post loading concentration in the placebo (p<0.01) and SP (p<0.01) trial.

**Discussion**

This is the first study that has assessed the effects of SP loading in combination with BJ on RSA when participants were fresh (RSA set 1) and midway and at the end of a STGC (RSA sets 2 and 3). This is also the first study to assess the effects of BJ supplementation on RSA in women. Overall, results showed that SP loading resulted in faster TS times for all sets assessed and overall, as well as faster FS and BS times for each set assessed when compared with placebo and BJ. Additionally, some benefit was found for SP+BJ compared with placebo and BJ, while BJ alone resulted in no improvement in RSA compared with placebo.
Table 1. Mean ± SD repeated-sprint ability times (first sprint time, best sprint time, total sprint time and overall sprint time) for the four conditions: placebo (PLA), beet root juice (BJ), sodium phosphate (SP) and combined sodium phosphate and beetroot juice (SP+BJ), n=13.

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>BJ</th>
<th>SP</th>
<th>SP+BJ</th>
<th>SP+BJ vs PLA</th>
<th>SP+BJ vs BJ</th>
<th>SP+BJ vs SP</th>
<th>SP vs PLA</th>
<th>SP vs BJ</th>
<th>BJ vs PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total sprint time (s)</strong></td>
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<td></td>
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</tr>
<tr>
<td>Set 1</td>
<td>22.95 ± 1.45</td>
<td>22.95 ± 1.61</td>
<td>21.94 ± 1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.66 ± 1.60</td>
<td>-0.19/88 (11/1)</td>
<td>-0.18/83 (16/1)</td>
<td>0.51/2 (6/92)</td>
<td>-0.75/99 (1/0)</td>
<td>-0.71/98 (1/1)</td>
<td>0/40 (41/19)</td>
</tr>
<tr>
<td>Set 2</td>
<td>23.55 ± 1.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.37 ± 1.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.25 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.07 ± 1.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.30/93 (6/1)</td>
<td>-0.17/81 (17/2)</td>
<td>0.52/4 (7/89)</td>
<td>-0.90/98 (1/1)</td>
<td>-0.70/98 (1/1)</td>
<td>-0.11/52 (35/13)</td>
</tr>
<tr>
<td>Set 3</td>
<td>23.48 ± 1.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.36 ± 1.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.53 ± 1.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.11 ± 1.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.23/91 (8/1)</td>
<td>-0.14/63 (28/9)</td>
<td>0.36/7 (11/82)</td>
<td>-0.66/97 (2/1)</td>
<td>-0.51/95 (4/1)</td>
<td>-0.07/62 (28/10)</td>
</tr>
<tr>
<td>Overall</td>
<td>69.97 ± 4.17</td>
<td>69.84 ± 4.94</td>
<td>66.72 ± 4.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.91 ± 5.14</td>
<td>-0.23/89 (9/2)</td>
<td>-0.18/86 (13/1)</td>
<td>0.42/3 (7/90)</td>
<td>-0.79/98 (1/1)</td>
<td>-0.69/99 (1/0)</td>
<td>-0.03/43 (43/14)</td>
</tr>
<tr>
<td><strong>First sprint time (s)</strong></td>
<td></td>
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<tr>
<td>Set 1</td>
<td>3.75 ± 0.32</td>
<td>3.72 ± 0.28</td>
<td>3.54 ± 0.22</td>
<td>3.66 ± 0.28</td>
<td>-0.31/91 (6/3)</td>
<td>-0.19/90 (9/1)</td>
<td>0.47/2 (7/91)</td>
<td>-0.78/99 (1/0)</td>
<td>-0.50/96 (3/1)</td>
<td>-0.13/68 (20/12)</td>
</tr>
<tr>
<td>Set 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.86 ± 0.28</td>
<td>3.85 ± 0.33</td>
<td>3.63 ± 0.26</td>
<td>3.74 ± 0.29</td>
<td>-0.40/99 (1/0)</td>
<td>-0.36/92 (6/2)</td>
<td>0.42/5 (9/86)</td>
<td>-0.85/99 (1/0)</td>
<td>-0.81/98 (1/1)</td>
<td>-0.01/40 (32/28)</td>
</tr>
<tr>
<td>Set 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.86 ± 0.30</td>
<td>3.84 ± 0.34</td>
<td>3.69 ± 0.28</td>
<td>3.79 ± 0.41</td>
<td>-0.19/96 (3/1)</td>
<td>-0.13/86 (11/3)</td>
<td>0.31/17 (14/69)</td>
<td>-0.62/97 (2/1)</td>
<td>-0.52/95 (3/2)</td>
<td>-0.06/69 (20/11)</td>
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<tr>
<td><strong>Best sprint time (s)</strong></td>
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</tr>
<tr>
<td>Set 1</td>
<td>3.69 ± 0.25</td>
<td>3.68 ± 0.26</td>
<td>3.53 ± 0.21</td>
<td>3.64 ± 0.26</td>
<td>-0.19/85 (13/2)</td>
<td>-0.14/89 (11/0)</td>
<td>0.50/3 (9/88)</td>
<td>-0.73/97 (2/1)</td>
<td>-0.66/97 (2/1)</td>
<td>-0.05/46 (39/15)</td>
</tr>
<tr>
<td>Set 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.80 ± 0.25</td>
<td>3.77 ± 0.32</td>
<td>3.57 ± 0.27</td>
<td>3.73 ± 0.28</td>
<td>-0.27/95 (4/1)</td>
<td>-0.15/85 (13/2)</td>
<td>0.57/4 (6/90)</td>
<td>-0.89/97 (2/1)</td>
<td>-0.69/97 (2/1)</td>
<td>-0.09/52 (30/18)</td>
</tr>
<tr>
<td>Set 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.77 ± 0.25</td>
<td>3.81 ± 0.32</td>
<td>3.64 ± 0.26</td>
<td>3.72 ± 0.30</td>
<td>0.21/92 (7/1)</td>
<td>-0.31/99 (1/0)</td>
<td>0.29/11 (12/77)</td>
<td>-0.54/95 (3/2)</td>
<td>-0.61/98 (1/1)</td>
<td>0.13/20 (38/42)</td>
</tr>
</tbody>
</table>

*25-74%, benefit possible; 75-94%, benefit likely; 95-99%, benefit very likely; > 99%, benefit almost certain.

a Significantly different to PLA (p < 0.05)
b Significantly different to BJ (p < 0.05)
c Significantly different to set 1 within trial (p < 0.05)
d Significantly different to all other trials for both first and best sprint (p < 0.05)
Table 2. Mean ± SD rating of perceived exertion (RPE) and heart rate (HR) for placebo (PLA), beetroot juice (BJ), sodium phosphate (SP) and combined SP and beetroot juice (SP+BJ) trials during a simulated team-game circuit, n=13.

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>BJ</th>
<th>SP</th>
<th>SP+BJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE Baseline</td>
<td>7 ± 1.1</td>
<td>7 ± 1.0</td>
<td>7 ± 1.0</td>
<td>7 ± 0.7</td>
</tr>
</tbody>
</table>
| RPE Quarter 1
                  | 14 ± 1.5 | 14 ± 1.6 | 14 ± 2.1 | 14 ± 1.9 |
| RPE Quarter 2
                  | 15 ± 2.6 | 16 ± 2.0 | 16 ± 2.0 | 16 ± 1.9 |
| RPE Quarter 3
                  | 15 ± 2.4 | 16 ± 1.3 | 16 ± 1.7 | 16 ± 1.6 |
| RPE Quarter 4
                  | 17 ± 1.9 | 17 ± 1.8 | 16 ± 1.7 | 16 ± 1.8 |
| Baseline HR (bpm)| 69 ± 7  | 64 ± 7  | 67 ± 6  | 68 ± 6  |
| HR Quarter 1 (bpm)
                  | 171 ± 13 | 174 ± 14 | 173 ± 14 | 179 ± 10 |
| HR Quarter 2 (bpm)
                  | 179 ± 13 | 181 ± 13 | 179 ± 13 | 181 ± 11 |
| HR Quarter 3 (bpm)
                  | 168 ± 13 | 171 ± 11 | 168 ± 13 | 171 ± 10 |
| HR Quarter 4 (bpm)
                  | 179 ± 14 | 181 ± 12 | 180 ± 10 | 182 ± 10 |

* Significantly different to Baseline (p < 0.05)

* Significantly different to Quarter 1 (p < 0.05)

* Significantly different to Quarter 2 (p < 0.05)

* Significantly different to Quarter 3 (p < 0.05)
Table 3. Serum phosphate (n=13), 2,3-Diphosphoglycerate (n=13) and nitrate (n=6) concentrations recorded pre- and post-loading for the sodium phosphate (SP), beetroot juice (BJ), SP+BJ and placebo trials.

<table>
<thead>
<tr>
<th></th>
<th>SP Trial</th>
<th>BJ Trial</th>
<th>SP+BJ Trial</th>
<th>Placebo Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phosphate (mmol·L⁻¹)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-loading</td>
<td>1.27 ± 0.12</td>
<td>1.31 ± 0.16</td>
<td>1.36 ± 0.18</td>
<td>1.24 ± 0.18</td>
</tr>
<tr>
<td>Post-loading</td>
<td>1.22 ± 0.14</td>
<td>1.27 ± 0.14</td>
<td>1.34 ± 0.15</td>
<td>1.29 ± 0.19</td>
</tr>
<tr>
<td><strong>2,3-DPG (mmol·L⁻¹)</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Pre-loading</td>
<td>1.93 ± 0.26</td>
<td>1.99 ± 0.32</td>
<td>1.82 ± 0.34</td>
<td>1.70 ± 0.37</td>
</tr>
<tr>
<td>Post-loading</td>
<td>1.82 ± 0.33</td>
<td>1.88 ± 0.37</td>
<td>1.76 ± 0.45</td>
<td>1.85 ± 0.53</td>
</tr>
<tr>
<td><strong>Nitrate (nmol·mL⁻¹)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre-loading</td>
<td>31.25 ± 7.76</td>
<td>24.51 ± 8.85</td>
<td>23.28 ± 6.44</td>
<td>26.67 ± 4.56</td>
</tr>
<tr>
<td>Post-loading</td>
<td>25.37 ± 9.75⁺</td>
<td>270.67 ± 32.73⁺</td>
<td>204.95 ± 96.65⁺</td>
<td>27.30 ± 18.80⁺</td>
</tr>
</tbody>
</table>

* Significantly different to pre-loading BJ (p < 0.01)
⁺ Significantly different to pre-loading SP+BJ (p < 0.01)
⁻ Significantly different to post-loading BJ and BJ+SP trial (p < 0.01)

**Effects of sodium phosphate and beetroot juice on repeated-sprint ability while fresh – set 1**

One of the primary findings from this study was that SP and SP+BJ loading improved all sprints assessed during the first RSA set (when participants were fresh) compared with the placebo and BJ (as supported by qualitative and quantitative results). This was not surprising as the main energy systems used for this first set were likely to be influenced by a number of the ergogenic mechanisms proposed to be associated with SP loading. Specifically, these early sprints would have initially relied more heavily on the PCr energy system (Gastin 2001), with a greater emphasis on anaerobic (and aerobic) glycolysis as sprinting continued (TS time range for set 1: 22.15±1.32 s – 23.11±1.47 s) (Gaitanos et al. 1993). It is therefore possible, that SP loading resulted in an improvement in this first sprint set due to an increased phosphate energy pool (ATP/PCr resynthesis) and enhanced buffering capacity, particularly in respect to improved TS performance (Czuba et al. 2009; Kreider et al. 1992). Some support for improved buffering here is provided by similar blood lactate concentrations between trials for this first sprint set, despite faster sprinting following SP supplementation. In respect to the
possibility of a greater phosphate energy pool, it has previously been thought that SP supplementation increased extracellular phosphate availability, which then resulted in a greater intracellular diffusion of phosphate, leading to greater stimulation of phosphofructokinase and citric acid cycle intermediates, resulting in enhanced anaerobic and oxidative metabolism (Kreider et al. 1992). Interestingly, the current study found no difference in pre- and post-loading serum phosphate concentrations after SP loading. Notably, other studies have reported improved exercise performance after SP supplementation that was not associated with changes in pre- and post-loading serum phosphate concentrations (Brewer et al. 2013; Stewart et al. 1990), suggesting that this variable may not be a reliable indicator of increased phosphate availability.

This study also found that BJ did not improve initial sprint ability, suggesting that none of the mechanisms proposed to improve exercise performance following ingestion of this supplement had a positive influence here. Perhaps these initial sprints were too few and/or too short in duration for benefit of BJ to occur. Further studies are needed to explore this issue.

**Effects of sodium phosphate and beetroot juice on repeated-sprint ability - sets 2 and 3**

When compared with the placebo and BJ, SP alone resulted in faster RSA for overall TS times as well as for all other sprints assessed during the later stages of the STGC (sets 2 and 3), with some benefit also found for SP+BJ. The fatiguing nature of the STGC is demonstrated here by higher final RPE, HR and blood lactate values in the last quarter [17, 180 beats per min (bpm) and 5.9 mmol·L⁻¹, respectively] compared with the first quarter (14, 174 bpm, 4.5 mmol·L⁻¹, respectively). Of relevance, it is likely that these later sprints would have relied more heavily on both anaerobic and aerobic
glycolysis (Gastin 2001), as PCr stores become further depleted. Evidence for greater use of anaerobic glycolysis here is provided in part by higher blood lactate concentrations at the end of the second and third RSA sets compared with values for the first set. Given that anaerobic glycolysis was likely to have played an important energy role in these later sprints, it is possible that improved sprinting following SP loading may, in part, be related to enhanced buffering capacity (as described earlier), with some support for this provided by improved sprint ability (sets 2 and 3) following SP loading that was not associated with significant increases in blood lactate concentrations compared with the other trials. Additionally, an increased (phosphate) energy pool as a result of SP loading (as described earlier) may have also contributed to improved (later) sprint ability here. Further, due to the likelihood of an increased reliance on aerobic metabolism during these later sprints, it is possible that an enhanced 2,3-DPG concentration may have also contributed to improved sprinting performance. To date, a number of studies have reported improved exercise performance of 5-12% (maximal oxygen uptake; \( \bar{V}O_{2\text{max}} \)) following SP supplementation associated with an increase in 2,3-DPG concentration (Cade et al. 1984; Czuba et al. 2009; Stewart et al. 1990). However, the current study found no significant differences between pre- and post-loading 2,3-DPG concentrations, supporting an earlier finding by Kreider et al. (1992), who also reported improved exercise performance (40 km cycling time-trial) following SP loading without a significant difference in pre- and post-loading 2,3-DPG concentrations.

Additionally, improved myocardial efficiency is another mechanism of SP loading that may have played a role in improving the later sprint bouts performed in the current study. Previously, Czuba et al. (2009) proposed that a significant 5.3% increase in \( \bar{V}O_{2\text{max}} \) observed after SP supplementation, compared with placebo, could be attributed to
improved stroke volume, and hence myocardial efficiency, as evidenced by significant
decreases in resting and maximal exercise HR in the SP trial. Of relevance, similar HR
values between all trials at the end of the final RSA set, despite faster sprints associated
with SP loading, lends some support for this mechanism. However, this is speculative
as not all studies have reported lower final HR values associated with improved exercise
performance after SP loading (Kreider et al. 1992).

Notably, the current study found no benefit of BJ on RSA for these later sets, despite a
significant increase in nitrate concentrations pre- to post-BJ loading and compared with
the placebo trial. Based on the previously mentioned ergogenic mechanisms relating to
mitochondrial respiration and ATP use during muscle force production, it was thought
that BJ would have some positive effect, particularly in the later sprint sets when there
was an increasing reliance on aerobic metabolism. Reasons for these results are
unknown, with the possibility that the study may have been underpowered in respect to
BJ supplementation as our calculations could not be based on studies that used the same
exercise protocol as that used here. Of relevance, a study by Wilkerson et al. (2012)
suggested that nitrate supplementation may not be as effective in well-trained
participants; however this is unlikely to be the case here as our participants were only
amateur athletes with training/competition approximating ~3 h per week. Nonetheless,
this represents a limitation to our study with more research needed in this respect.

Furthermore, the combination of SP+BJ did not result in the same benefit to RSA as SP
alone. While it was initially thought that mechanisms for improved exercise
performance associated with both these supplements worked independently of each
other, it is possible that some interplay may have occurred that ultimately dampened the
ergogenic effect of SP. Similarly, Ducker et al. (2013) also compared the effects of two
proposed ergogenic supplements on sprint performance (3 sets of 6 x 20-m) and found
that sodium bicarbonate alone resulted in faster sprinting than when combined with beta alanine, with reasons for this being unclear. While more studies are needed here, care should be taken when combining supplements to make sure that the overall effect is safe for the individual. Furthermore, it must be noted that a limitation of the current study is that the effectiveness of the blinding procedure was not recorded.

**Conclusion**

This study found that SP loading improved RSA in women team-sport players when performed prior to, midway and at the end of a STGC. Consequently, women team-sport athletes should consider loading with SP prior to a team-game, as improved RSA is important at the start of a team-game when players strive to score first, as well towards the end of a game, particularly if scores are close.

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References


Chapter Six

Thesis Summary and Future Directions
Thesis Summary

One nutritional supplement that has shown some positive benefits for sporting performance is SP. To date, a number of well-controlled studies have reported some benefit of SP supplementation for endurance exercise (> 20-min) (Folland, Stern, & Brickley, 2008; Kreider et al., 1992) and aerobic capacity (Czuba, Zajac, Poprzecki, & Cholewa, 2008; Czuba, Zajac, Poprzecki, Cholewa, & Woska, 2009). For ergogenic purposes, SP is supplemented orally in capsule form, at a dose of 3-5 g (Cade et al., 1984; Kreider, Miller, Williams, Somma, & Nasser, 1990; Kreider et al., 1992) or 50 mg·kg\(^{-1}\) of FFM (Czuba et al., 2008; Czuba et al., 2009; West, Ayton, Wallman, & Guelfi, 2012) a day, for a period of between 3-6 days. A number of mechanisms have been proposed to explain the ergogenic effect seen with SP supplementation. These include: an enhanced 2,3-DPG concentration, which allows for greater unloading of oxygen to the peripheral tissues/muscle (Benesch & Benesch, 1969; Duhm, 1971); improved buffering capacity due to increased hydrogen phosphate concentration, which could buffer hydrogen ions produced during intense exercise (Kreider, 1999); improved myocardial efficiency, which is proposed to result in a larger cardiac output via an increased stroke volume and consequently greater and more efficient oxygenation of the exercising muscles (Czuba et al., 2009); increased rate of glycolysis and oxidative phosphorylation (Chasiotis, 1983); and greater ATP/PCr resynthesis due to increased availability of extracellular and intracellular phosphate, thus providing a larger phosphate energy pool (Bredle, Stager, Brechue, & Farber, 1988; Kreider, 1999).

Notably, to the author’s knowledge, no studies have previously been published that have assessed the effects of SP supplementation on RSA as performed in a team-sport setting. Furthermore, all studies that have assessed the effects of SP loading on exercise performance have only recruited male participants, apart from one study by West et al.,
(2012) that assessed SP loading on aerobic capacity in a combined sample of males and females. Of relevance, a number of gender differences may alter the effect of some of the proposed mechanisms of SP on exercise performance in females. Specifically, compared with males, females have a decreased affinity of oxygen with haemoglobin (Benesch & Benesch, 1969), which may dampen the potential positive effect of any increases in 2,3-DPG concentration. Furthermore, females have higher natural levels of (and fluctuations in) oestrogen, which plays a role in renal phosphate reabsorption within the body (Remes, Vuopio, & Harkonen, 1979).

In addition, multiple nutritional supplements are often ingested by athletes to improve exercise performance, however no studies to date have assessed the effect of SP loading when combined with other ergogenic aids, such as caffeine and nitrate (in the form of BJ). As caffeine and BJ are proposed to improve exercise performance using different mechanisms to SP, it is possible that if ingested in combination with SP further improvements in exercise performance may be seen than if either supplement was ingested alone. In order to address these gaps in the literature three studies were conducted.

The first study of this thesis aimed to investigate the effect of six days of SP supplementation on 500 kJ (~20-km) cycling TT performance in female cyclists. A secondary aim of this study was to determine the optimal SP loading dose for females, as it may differ to that used in males. As a result, three different daily dosing protocols were used, a low dose 25 mg·kg\(^{-1}\) of FFM, the standard dose previously used in males, which is 50 mg·kg\(^{-1}\) of FFM and a high dose, 75 mg·kg\(^{-1}\) of FFM. No significant differences in TT performance were observed between any of the supplementation protocols (p = 0.73), with average completion times for the 25, 50 and 75 mg·kg\(^{-1}\) of FFM trials being 42:21 ± 7:53, 40:55 ± 7:33 and 40:38 ± 7:20 min respectively, and
40:39 ± 7:51 min for the placebo. Likewise, average and peak power output did not significantly differ between trials (p > 0.05). As a result, it was concluded that 500 kJ cycling TT performance was not improved by SP supplementation in female cyclists, regardless of the dosing protocol used. It was noted however, that 8/13 participants demonstrated their fastest TT performance after the 50 mg·kg\(^{-1}\) of FFM dose.

Given the lack of significant improvement in endurance performance noted in the first study of this thesis, the second study aimed to investigate the effects of SP loading on RSA as typically performed during a team-sport game in trained females. This study also aimed to determine whether RSA could be further improved by combining SP with caffeine supplementation, while the effect of caffeine ingestion alone on RSA was also determined. Twelve female team-sport athletes participated in four trials: (1) SP and caffeine (SP+C), (2) SP and placebo (for caffeine), (3) caffeine and placebo (for SP) and (4) placebo (for SP+C), with ~21 days separating each trial to allow for wash-out and to standardise for menstrual cycle phase. In the SP trials participants consumed 50 mg·kg\(^{-1}\) of FFM of SP for six days, whilst in the caffeine trials 6 mg·kg\(^{-1}\) of body mass of caffeine was consumed 1 h prior to performing a 60 min STGC. One set of sprints (6 x 20-m) was performed prior to (set 1), midway through (set 2) and immediately after (set 3) the STGC. Using statistical significance, effect sizes and smallest worthwhile change values, both first sprint and best sprint times were faster after SP compared with placebo and caffeine for numerous sets. First sprint times were also faster in SP compared with SP+C, although SP+C was faster than caffeine. Likewise, best sprint times were faster after SP+C compared with placebo and caffeine. With respect to total sprint time, SP was faster than placebo and SP+C was faster than placebo, caffeine and SP.
These results suggest that SP and SP+C loading improved RSA, compared with the placebo trial, in female team-sport athletes during a STGC, while both fresh (set 1) and fatiguing (sets 2 and 3), with performance benefits being similar between these two trials. Furthermore, caffeine ingestion alone provided minimal benefit to RSA when compared with placebo.

Based on the positive results associated with SP loading in relation to RSA in the second study of this thesis, the third study aimed to determine whether RSA could also be improved by combining SP with BJ, a relatively new proposed ergogenic aid. Thirteen female team-sport athletes participated in four trials: (1) SP and BJ (SP+BJ), (2) SP and placebo (for BJ), (3) BJ and placebo (for SP) and (4) placebo (for SP+BJ), with ~21 days separating each trial for washout and to standardise for menstrual cycle phase. In the SP trials participants consumed 50 mg·kg⁻¹ of FFM of SP for six days, whilst in the BJ trials 6 mmol of nitrate was consumed in a 70 mL shot of BJ 3 h prior to exercise testing. The RSA sets, STGC protocol and data analysis methods were the same as those performed in study two. First sprints were fastest after SP compared with placebo, BJ, and SP+BJ for numerous sets. Additionally, best sprint times were fastest after SP compared with placebo and BJ and also faster after SP+BJ compared with placebo and BJ. Finally, total sprint times were faster after SP compared with placebo, BJ and SP+BJ, and also faster after SP+BJ compared with placebo and BJ (for all but one set). Interestingly, no benefit was found for BJ alone on RSA when compared with placebo.

**Synthesis of Results**

The primary results from this thesis suggest that six days of SP loading (50 mg·kg⁻¹ of FFM per day) is likely to be beneficial to repeated-sprint performance (simulated team-
sport demands) in trained females. Further, no significant benefit was found for SP loading on 500 kJ cycle (~20 km) TT performance, although 8/13 participants recorded faster cycling TT times following the 50 mg·kg⁻¹ of FFM SP supplementation protocol. On this basis, the 25 and 75 mg·kg⁻¹ of FFM per day doses trialled in study one were not continued with in studies two and three. Consequently, it appears that SP loading (50 mg·kg⁻¹ of FFM per day for six days) may be more effective in exercise tasks that require a large contribution of energy from both aerobic and anaerobic sources. Furthermore, benefit could still be seen when participants were fatiguing during the later RSA sets (sets 2 and 3 performed midway through and after a 60 min STGC), when a growing contribution of energy from aerobic metabolism would be expected (Gastin, 2001).

As prior research had largely not used female participants and in consideration of noted gender differences, it was unknown what SP dose would provide an ergogenic effect (if any) in females. Previous studies that used a SP dosing protocol based on FFM in males reported improved maximal oxygen uptake (VO₂max) (Brewer, Dawson, Wallman, & Guelfi, 2013; Czuba et al., 2008; Czuba et al., 2009) and mean power output at the 250 and 500 kJ time points of a 1000 kJ (~40 km) cycle TT effort (Brewer et al., 2013) after 50 mg·kg⁻¹ of FFM of SP per day, ingested for six days, compared with placebo. Consequently, one aim of this thesis was to explore this issue. Notably, results from study one found no significant difference in cycling TT performance observed between the three dosage (25, 50 and 75 mg·kg⁻¹ of FFM per day for six days) protocols. Of relevance, 62% of participants recorded a faster TT performance (not significant) following the 50 mg·kg⁻¹ of FFM protocol compared with 38% who demonstrated faster times following the 75 mg·kg⁻¹ of FFM dose.
It is possible that a more prolonged effort, requiring a more sustained contribution from aerobic metabolism (over exercise >40 min) may be needed for SP supplementation to be effective in females (particularly in respect to enhanced 2,3-DPG concentration). Furthermore, the variability in time trial performance between participants may be a contributing factor as to why no effect of sodium phosphate was seen in this study. A possible trend for an increasing effectiveness of SP loading in longer duration endurance events in males is suggested by previous research, as Brewer et al., (2013) found no benefit of SP loading on a 250 kJ (10-12 min) cycling TT in trained male cyclists, compared with faster times found for longer duration TT in males by Folland et al., (2009; ~21 min) and Kreider et al., (1992; ~45 min). Support for this premise is provided by studies two and three of this thesis where RSA was improved following SP supplementation after 60 min of participation in a STGC where there was a likely growing contribution of energy from aerobic metabolism (Gaitanos, Williams, Boobis, & Brookes, 1993; Gastin, 2001) due to insufficient time for PCr stores to completely replenish, as well as increasing blood lactate concentrations (~1.6 mmol·L⁻¹ at baseline to >6 mmol·L⁻¹ after RSA for set 3 in studies two and three). Importantly, the prolonged (60 min) nature of this protocol may have ultimately resulted in mechanisms of SP loading related to aerobic metabolism, such as a larger ATP pool, improved myocardial efficiency and in particular, enhanced 2,3-DPG concentration, becoming more effective in the female participants in these studies. Further studies are required to investigate this premise.

The improved sprint performance found in studies two and three of this thesis following SP loading is consistent with a study by Brewer, Dawson, Wallman and Guelfi, (2014) who reported faster sprint performance in a cycling road race simulation consisting of repeated-sprint (4 sets of 6 x 15 s) and TT (TT: 2 x 5 min) efforts following SP loading
(50 mg·kg⁻¹ of FFM per day for six days) compared with placebo. However, this thesis is the first to show such benefits in females.

Of note, the initial RSA set in studies two and three of this thesis would have initially relied more heavily on the PCr energy system (Gaitanos et al., 1993; Gastin, 2001), given the brief duration of each sprint (~4 s), with a likely greater emphasis on anaerobic glycolysis and aerobic metabolism as sprinting continued (Gastin, 2001). This suggests that the improved RSA performance found after SP loading in these studies may have been due to mechanisms proposed to improve both anaerobic metabolism, such as increased ATP/PCr resynthesis, enhanced activation of glycolysis (Chasiotis, 1983) and enhanced buffering of hydrogen ions (Kreider et al., 1992), as well as aerobic metabolism, such as a larger ATP pool, improved myocardial efficiency and enhanced 2,3-DPG concentrations. Some evidence for an increased role for anaerobic glycolysis as sprinting continued is provided by the higher blood lactate concentrations recorded at the end of RSA sets 2 and 3 compared with baseline values found in both studies. Although muscle pH and buffering capacity were not measured in the present thesis, some indirect support for enhanced buffering is in part provided by the faster sprint ability in sets 2 and 3 following SP loading, despite no significant increases in blood lactate concentrations compared with the other trials. Alternatively, it may be that similar blood lactate levels between trials reflect a greater aerobic contribution to overall energy output as a result of SP loading. More studies are needed to investigate muscle pH during sprinting following SP loading, in order to determine any effects of enhanced buffering capacity.

Interestingly, the studies of this thesis found no significant changes in serum phosphate or 2, 3-DPG concentrations in response to SP loading. Other similar SP loading studies in the literature have reported varying results. For example, some studies have reported
positive effects of SP supplementation on exercise performance, but with either no change (Brewer et al., 2013) or significantly higher levels (Cade et al., 1984; Czuba et al., 2008; Czuba et al., 2009) in serum phosphate concentrations. Consequently, it has been suggested that serum phosphate concentration may not be an accurate marker of the effects of SP loading on intracellular phosphate concentrations (Kreider et al., 1992). Alternatively, it is possible that current laboratory techniques for measuring serum phosphate may not be sensitive enough to accurately reflect phosphate concentrations in the body, or perhaps changes in serum phosphate concentrations do not need to be significantly increased for ergogenic benefit. Indeed, in respect to the studies that comprise this thesis, serum phosphate levels increased post-loading (compared with pre-loading) in all SP trials (non-significant), apart from the 75 mg·kg⁻¹ of FFM trial in study one and the SP+BJ trial in study three. Furthermore, it is important to note that the average serum phosphate concentration measured (~1.25 mmol·L⁻¹) in this thesis is similar to previous studies (Brewer et al., 2014; Kreider et al., 1992). Similar to serum phosphate concentration, 2,3-DPG concentrations have also returned varying results in respect to improved exercise performance following SP loading. For example, Kreider et al., (1992) reported improved 40 km TT and \( \dot{\text{V}}_{\text{O}}_{2\text{max}} \) performance together with an associated increase in 2,3-DPG concentrations post-SP loading, while Stewart et al., (1990) reported an improved aerobic capacity despite no change in this measure. However, it must be noted that it is difficult to compare the 2,3-DPG concentrations measured in this thesis to previous studies, due to differences in both measurement techniques and the reporting of values between studies.

Another important finding of this thesis was that the addition of either caffeine or BJ to SP supplementation did not improve exercise performance to a greater extent than when SP was ingested alone. Moreover, caffeine alone resulted in minimal benefit to RSA,
while there was no benefit of BJ alone on RSA when compared with placebo. It is unknown as to why performance was not improved further when caffeine or BJ were added to SP as it was presumed that mechanisms associated with each supplement worked independently of each other. However, this is not the first study to report no additional benefit in respect to combining proposed ergogenic aids. Specifically, Ducker, Dawson and Wallman, (2013) compared the effects of two proposed ergogenic supplements on sprint performance (3 sets of 6 x 20-m) and reported that supplementing with sodium bicarbonate alone resulted in faster sprinting times than when combined with beta alanine, with reasons for this being unclear. This suggests that more research is needed in assessing the effects of combining ergogenic aids, with particular emphasis on the interplay of associated mechanisms. With respect to the effect of caffeine alone on RSA, some studies have found benefit of a 6 mg·kg⁻¹ of body mass dose of caffeine on RSA similar to that performed in team-sport games (Carr, Dawson, Schneiker, Goodman, & Lay, 2008; Pontifex, Wallman, Dawson, & Goodman, 2010), while other studies have reported no benefit (Paton, Hopkins, & Vollebregt, 2001). Notably, none of the studies that reported benefit of caffeine on RSA required sprinting to be performed before, during and after a 60 min STGC, as performed in study two. It is possible that the prolonged nature of the exercise protocol used here may have resulted in a build-up of metabolic waste products, leading to a reduction in caffeine induced activation of Na⁺/K⁺ ATPase (Lindinger, Graham, & Spriet, 1993), with this possibly minimising any benefit of caffeine on RSA. However, this contention is not supported by blood lactate concentration values, which were higher at the end of each protocol in the studies by Carr et al., (2008) and Pontifex et al., (2010) compared with study two here (~9-13 mmol·L⁻¹ vs ~6 mmol·L⁻¹). Of relevance, all previous studies that have assessed the effect of caffeine on RSA similar to that performed in team-sport games
recruited male participants. As females generally have a lower muscle mass than males (O’Brien, 1985) and caffeine is proposed to have positive/ergogenic effects on muscle contraction, this may represent another reason for the results found in study two, suggesting that further research is required here.

In respect to BJ supplementation, few studies have assessed the effects of this proposed ergogenic aid on repeated sprint performance [Yo-Yo intermittent recovery test; Wylie et al., (2013) and 5 x 6 200-m rowing efforts; Bond, Morton, & Braakhuis, (2012)], with none of these protocols being similar to that used in study three of this thesis. Nonetheless, a lack of effect of BJ supplementation on RSA when consumed alone was surprising as it has been suggested that the nitrate-nitrite-nitric oxide pathway is activated under high metabolic demands and hypoxic and acidic conditions (Lunderberg, Weitzberg, & Gladwin, 2008). Indeed, final lactate concentrations after RSA set 3 in studies two and three (~6 mmol·L⁻¹) were similar to those reported by Wylie et al., (2013) at the end of the Yo-Yo protocol. Consequently, reasons for the contrasting results found here are unclear. Possibly, the study may have been underpowered in respect to BJ supplementation, as our calculations could not be based on studies that used the same exercise protocol as that used here.

In conclusion, trained females demonstrated faster RSA following SP supplementation, with this benefit found for initial sprint performance when participants were fresh, as well as for later sprint performance when participants were fatiguing (RSA sets 2 and 3). However, no significant benefit was found for cycling TT performance (~20 km) following SP loading. Consequently, it would appear that SP loading has an ergogenic effect on activities that require considerable contribution from both aerobic and anaerobic metabolism, especially when exercise is prolonged (> 60 min as performed in studies two and three). Furthermore, in trained females, it would appear that there is no
further benefit to RSA by adding caffeine or BJ to SP loading and that BJ and caffeine ingested alone provide no or very minimal benefit to RSA respectively, compared with placebo.

**Practical Applications**

The following practical recommendations can be made based on the findings of this thesis:

- A dose of 50 mg·kg⁻¹ of FFM per day of tribasic SP dodecahydrate taken over a period of six days as four split doses per day and mixed with 15 g of Powerade® powder and ~300 mL of water is well tolerated in females, with no side effects reported.

- Due to the mixed results of SP supplementation on TT performances in trained females, SP should be individually trialled before use in endurance competition.

- The effects of SP supplementation on cycling TT performance appears to be similar regardless of whether or not a medium (50 mg·kg⁻¹ of FFM per day) or high (75 mg·kg⁻¹ of FFM per day) dose is ingested in females.

- Supplementation with a dose of 50 mg·kg⁻¹ of FFM per day of SP taken over a period of six days is likely to be effective in improving initial (when fresh) RSA, as well as later RSA (whilst fatiguing), in trained females.

- Combining SP with either caffeine or BJ does not improve RSA more than when SP is ingested alone.

- Caffeine or BJ supplementation appears to have negligible ergogenic effects on RSA in trained females.
Future Research Directions

Future research may be directed in the following areas:

- To investigate the effect of SP supplementation in combination with other common ergogenic aids (i.e. sodium bicarbonate, creatine) on RSA and team sport performance. However, it must be noted that a limitation of the current study is that the effectiveness of the blinding procedure was not recorded.
- To investigate the effect of SP supplementation on longer duration endurance performance lasting over 60 min.
- To further investigate the proposed ergogenic mechanisms of SP loading which may assist exercise performance, by specifically focusing on buffer capacity and ATP/PCr resynthesis in repeated-sprint efforts.
- To investigate the effectiveness of blinding protocols used in the employed.
References


Appendices

APPENDIX A
Participant Information Sheets and Informed Consent

APPENDIX B
Ethics Approval

APPENDIX C
Dual Energy X-ray Absorptiometry / Radiation Approval Form

APPENDIX D
Raw Data Studies, One, Two and Three
APPENDIX A

PARTICIPANT INFORMATION SHEETS AND INFORMED CONSENT

PARTICIPANT INFORMATION SHEET – STUDY ONE
INFORMED CONSENT FORM – STUDY ONE
PARTICIPANT INFORMATION SHEET – STUDY TWO
INFORMED CONSENT FORM – STUDY TWO
PARTICIPANT INFORMATION SHEET – STUDY THREE
INFORMED CONSENT FORM – STUDY THREE
The Effect of Sodium Phosphate Supplementation on 500 kJ Cycling Time-Trial Performance in Trained Female Cyclists

- Participant Information Sheet -

PURPOSE

We wish to investigate the effects of different doses of sodium phosphate (25, 50 and 75 mg·kg\(^{-1}\) of fat free mass) loading on 500 kJ cycling time-trial performance in trained female cyclists and triathletes. This study will be done in an attempt to determine whether or not females actually receive any ergogenic benefits from sodium phosphate supplementation. This study will also aim to identify an optimal female specific sodium phosphate loading dose.

PROCEDURES

- You will be required to attend the lab on nine different occasions.
- The first visit involves a familiarisation session with a 500 kJ cycling time-trial protocol. In addition, a body composition scan will be conducted.
- The simulated 500 kJ cycling time trial will be performed on a cycle ergometer, where you will be required to complete 500 kJ of work in the fastest time possible. A 500 kJ workload represents a distance of approximately 20 km, which should take you about 40 to 50 minutes to complete.
- The body composition scan will require you to lie quietly on a scanning bed for approximately 5 minutes. During this time a scanning arm will pass over your body and a report of your body’s fat, muscle and bone mass will be determined.
- You will then be required to complete four different supplementation protocols, which will be assigned in a randomised manner. The first supplementation protocol will begin approximately 3-5 days post the first menstruation that occurs after completing the familiarisation session.
- The supplementation protocols consist of consumption of a 25, 50 or 75 mg·kg\(^{-1}\) of fat free mass dose of trisodium phosphate dodecahydrate or 4 g of glucose, each consumed for six consecutive days, split into four equal doses over each day. After the sixth day of each loading protocol you will return to the lab to complete a 500 kJ cycling time trial on a cycle ergometer.
- You will also be required to attend the lab one day prior to the commencement of each supplementation protocol so that a venous blood sample can be taken. A venous blood sample will also be taken at the end of each six day supplementation protocol, prior to
performing the 500 kJ time-trial. The venous blood samples will be obtained from your forearm by a qualified professional and will require the use of a needle to collect the sample.

- A 21 day washout (break) period will separate each supplementation protocol.
- All testing will take place at the University of Western Australia School of Sport Science, Exercise and Health.

RISKS

Adverse affects resulting from sodium phosphate loading may include intestinal discomfort. However, this reaction is very rare, with only one reported case in previous research on sodium phosphate loading.

The body composition scan involves the use of a low dose x-ray about equal to one thousandth of the background radiation you would receive in one year living in Perth.

INCONVENIENCES

It may be difficult or inconvenient for you to adhere to the six day loading protocols required by the study.

TIME REQUIREMENTS

You will be required to attend the lab on nine occasions over four months. Total time spent at the lab will be approximately seven hours.

DISCOMFORTS

You may find the cycling time trial unpleasant, although the purpose of these trials is to simulate a race, which you should be familiarised with. You are able to withdraw from the cycling time trials at any stage.

The experimental protocol requires that venous blood samples are taken at various stages of testing. However, no pressure will be placed on you if you do not want to have blood samples taken.

BENEFITS

By participating in this study, you may find that the supplement (sodium phosphate) used in this study may allow you to perform better in training and in competition.

Furthermore, you will receive personal information on physiological markers such as heart rate during exercise and on performance measures such as peak and average power output whilst cycling. These variables are useful in prescribing exercise programs. You will also receive a free body composition scan that will determine body fat percentage and muscle mass levels and receive information on blood markers such as hematocrit and haemoglobin concentration.

In addition, upon completing the study you will have the option to undertake a free gold standard physiological assessment of aerobic capacity ($\dot{V}O_{2\text{max}}$).
This project will fill a large knowledge gap in the literature relating to sodium phosphate supplementation and at the same time, assess the effects and validity of using sodium phosphate loading to enhance endurance performance in females.

CONFIDENTIALITY

The confidentiality regarding your details will be strictly maintained at all times. Data will be kept and viewed only by authorised personnel.

PARTICIPANT RIGHTS

You may withdraw from the study at any time without prejudice. If you withdraw, we may wish to retain the data that we have recorded from you, but only if you agree, otherwise your records will be destroyed. If you have any questions concerning the research, please feel free to contact one of the researchers (contact details listed above).

The committee for Human Rights at the University of Western Australia requires that all participants are informed that if they have any complaint regarding the manner in which a research project is conducted, it may be given to the researcher or alternatively to the Secretary, Committee for Human Rights, Registrar’s Office, University of Western Australia, Crawley, WA, 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.
The Effect of Sodium Phosphate Supplementation on 500 kJ Time-Trial Performance in Trained Female Cyclists

- Participant Consent Form -

As a participant, you are free to withdraw your consent to participate at any time without prejudice. The researchers will answer any questions you may have in regard to the study at any time.

I _________________ (participant’s name) acknowledge that I have read the above statement and information sheet, which explains the nature, purpose and risks of the investigation and that any questions I have asked have been answered to my satisfaction. I agree to participate in this study realising that I may withdraw at any time without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to do so by law.

I agree that research data gathered for the study may be published provided my name or other identifying information, such as photographs including my face, is not used.

I have been advised as to what data is being collected, the purpose for collecting the data, and what will be done with the data upon completion of the research.

Participant     Date
I am willing for the experimenter to photograph me during the trials so that images can be used in his final presentation.

Participant Date

The committee for Human Rights at the University of Western Australia requires that all participants are informed that if they have any complaint regarding the manner in which a research project is conducted, it may be given to the researcher or alternatively to the Secretary, Committee for Human Rights, Registrar’s Office, University of Western Australia, Crawley, WA, 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.
The Combined Effect of Sodium Phosphate and Caffeine Supplementation on Team Sport Performance in Female Team Sport Athletes

- Participant Information Sheet -

PURPOSE

We wish to determine whether combined sodium phosphate and caffeine supplementation enhances team sport performance greater than when sodium phosphate and caffeine are ingested alone in female team sport athletes. This will be done in an attempt to identify possible ways to further increase team sport performance through new, combined supplementation procedures.

PROCEDURES

- You will be required to attend the lab on nine different occasions. Your first visit will consist of a familiarisation session with a circuit running protocol. In addition, a body composition scan will be conducted.

- The circuit will be performed inside the Exercise and Sports Science gymnasium.

- The exercise protocol consists of performing six 20 m sprints followed by 15 minutes of continuous running on the circuit protocol (outlined on the last page). After the 15 minutes a four minute break will be taken. This will then be followed by another 15 minutes of running on the circuit protocol and a further six 20 metre sprints. This will be followed by a 10 minute break. After the 10 minute break you will run the circuit for another 15 minutes followed by a four minute break. The exercise protocol will then finish with 15 minutes on the circuit protocol followed by six 20 m sprints.

- The body composition scan will require you to lie quietly on a scanning bed for approximately 5 minutes. During this time a scanning arm will pass over your body and a report of your body’s fat, muscle and bone mass will be determined.

- You will then be required to complete four different supplementation protocols, which will be assigned in a randomised manner. The first supplementation protocol will begin approximately 3-5 days post the first menstruation that occurs after completing the familiarisation session.

- The supplementation protocols consist of consumption of either 50 mg·kg⁻¹ of fat free mass of trisodium phosphate dodecahydrate or 4 g of glucose, split into four equal doses a day, for six consecutive days. After the sixth day of each supplementation protocol (day 7), the circuit protocol will be performed with consumption of either glucose or
caffeine at a dosage 6 mg·kg⁻¹, which will be ingested 60 minutes prior to performing the circuit.

- One day prior to the commencement of each supplementation protocol, you will be required to attend the lab so that a venous blood sample can be taken. A venous blood sample will also be taken at the end of each supplementation protocol immediately before performing the circuit. The venous blood sample will be obtained from your forearm by a qualified professional and will require the use of a needle to collect the sample.
- A 21 day washout (break) period will separate each supplementation protocol.
- All testing will take place at the University of Western Australia School of Sport Science, Exercise and Health.

RISKS

Adverse effects resulting from sodium phosphate loading may include intestinal discomfort. However, this reaction is very rare, with only one reported case in previous research on sodium phosphate loading.

The body composition scan involves the use of a low dose x-ray about equal to one thousandth of the background radiation you would receive in one year living in Perth.

INCONVENIENCES

It may be difficult or inconvenient for you to adhere to the six day loading protocols required.

TIME REQUIREMENTS

You will be required to attend the lab on nine occasions. Total time spent at the lab/oval will be approximately seven hours.

DISCOMFORTS

You may find the circuit protocol unpleasant, although the purpose of these trials is to simulate a team sport match, which you should be familiarised with. You are able to withdraw from the circuit protocol at any stage.

The experimental protocol requires that venous blood samples are taken at various stages of testing. However, no pressure will be placed on you if you do not want to have blood samples taken.

BENEFITS

By participating in this study, you may find that the supplements (sodium phosphate and caffeine) used in this study may allow you to perform better in training and in competition.

You will also receive a free body composition scan before and after the study that will determine body fat percentage and muscle mass levels and upon completing the study you will have the option to undertake a free gold standard physiological assessment of aerobic capacity ($\bar{V}O_{2\text{max}}$).
This project will fill a large knowledge gap in the literature relating to sodium phosphate supplementation and at the same time, assess the effects and validity of using sodium phosphate loading to enhance team sport performance in females.

In addition, this study will also enhance our overall understanding of supplementation procedures relating to sodium phosphate and caffeine and may identify possible ways to further increase team sport performance through new, combined supplementation procedures.

CONFIDENTIALITY

The confidentiality regarding your details will be strictly maintained at all times. Data will be kept and viewed only by authorised personnel.

PARTICIPANT RIGHTS

You may withdraw from the study at any time without prejudice. If you withdraw, we may wish to retain the data that we have recorded from you, but only if you agree, otherwise your records will be destroyed. If you have any questions concerning the research, please feel free to contact one of the researchers (contact details listed above).
The Combined Effect of Sodium Phosphate and Caffeine Supplementation on Team Sport Performance in Female Athletes

As a participant, you are free to withdraw your consent to participate at any time without prejudice. The researchers will answer any questions you may have in regard to the study at any time.

I ________________ (participant’s name) acknowledge that I have read the above statement and information sheet, which explains the nature, purpose and risks of the investigation and that any questions I have asked have been answered to my satisfaction. I agree to participate in this study realising that I may withdraw at any time without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to do so by law.

I agree that research data gathered for the study may be published provided my name or other identifying information, such as photographs including my face, is not used.

I have been advised as to what data is being collected, the purpose for collecting the data, and what will be done with the data upon completion of the research.

Participant     Date

177
I am willing for the experimenter to photograph me during the trials so that images can be used in his final presentation.

Participant     Date

The committee for Human Rights at the University of Western Australia requires that all participants are informed that if they have any complaint regarding the manner in which a research project is conducted, it may be given to the researcher or alternatively to the Secretary, Committee for Human Rights, Registrar’s Office, University of Western Australia, Crawley, WA, 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.
The Combined Effect of Sodium Phosphate and Beetroot Juice Supplementation on Repeated Sprint Performance in Female Athletes

- Participant Information Sheet -

PURPOSE

We wish to determine whether combined sodium phosphate and beetroot juice supplementation enhances team sport performance performance greater than when sodium phosphate and beetroot juice are ingested alone in female team sport athletes. This will be done in an attempt to identify possible ways to further increase team sport performance through new, combined supplementation procedures.

PROCEDURES

• You will be required to attend the lab on nine different occasions. Your first visit will consist of a familiarisation session with a circuit running protocol. In addition, a body composition scan will be conducted.

• The circuit will be performed inside the Exercise and Sports Science Gymnasium. The circuit protocol consists of performing five 20 m sprints followed by 30 minutes of continuous running on circuit (outlined on the last page). After the 30 minutes another five 20 metre sprints will be performed. This will be followed by a 10 min break and another five 20 metre sprints. The protocol will then finish with a further 30 minutes of continuous running on the same circuit performed earlier.

• The body composition scan will require you to lie quietly on a scanning bed for approximately 5 minutes. During this time a scanning arm will pass over your body and a report of your body’s fat, muscle and bone mass will be determined.

• You will then be required to complete four different supplementation protocols, which will be assigned in a randomised manner. The first supplementation protocol will begin approximately 3-5 days post the first menstruation that occurs after completing the familiarisation session.

• The supplementation protocols consist of consumption of:

  (1) sodium phosphate (50 mg·kg⁻¹ of fat free mass ingested each day over a 6 day period) and a beetroot placebo (70 ml acute dose of beetroot juice on the trial day, where the nitrate content has been removed),
(2) beetroot juice (70 ml acute dose of beetroot juice on the trial day) and a sodium phosphate placebo (50 mg·kg$^{-1}$ of fat free mass of glucose per day ingested in capsule form over six days), (3) sodium phosphate and beetroot juice combined (using doses and loading procedure described above), or (4) placebo for sodium phosphate and beetroot juice using doses and loading protocol described for each placebo above.

- One day prior to the commencement of the first supplementation protocol, you will be required to attend the lab so that a venous blood sample can be taken. A venous blood sample will also be taken at the end of the first supplementation protocol, both 60 minutes prior to performing the circuit and again immediately before performing the circuit. For the remaining three supplementation protocols a venous blood sample will only be taken immediately prior to performing the circuit. The venous blood sample will be obtained from your forearm by a qualified professional and will require the use of a needle to collect the sample.
- A 21 day washout (break) period will separate each supplementation protocol.
- All testing will take place at the University of Western Australia School of Sport Science, Exercise and Health.

**Risks**

Adverse affects resulting from sodium phosphate loading may include intestinal discomfort. However, this reaction is very rare, with only one reported case in previous research on sodium phosphate loading.

The body composition scan involves the use of a low dose x-ray about equal to one thousandth of the background radiation you would receive in one year living in Perth.

Finally, the consumption of beetroot juice (with or without nitrate) over a 3 day period may result in a red discolouration of your urine and bowel movements. This discolouration comes as a result of the beetroot colour, and is quite normal when consuming large amounts of this vegetable.

**Inconveniences**

It may be difficult or inconvenient for you to adhere to the six day loading protocols required.

**Time Requirements**

You will be required to attend the lab on nine occasions. Total time spent at the lab/oval will be approximately seven hours.

**Discomforts**

You may find the circuit protocol unpleasant, although the purpose of these trials is to simulate a team sport match, which you should be familiarised with. You are able to withdraw from the circuit protocol at any stage.
The experimental protocol requires that venous blood samples are taken at various stages of testing. However, no pressure will be placed on you if you do not want to have blood samples taken.

**BENEFITS**

By participating in this study, you may find that the supplements (sodium phosphate and beetroot juice) used in this study may allow you to perform better in training and in competition. You will also receive a free body composition scan before and after the study that will determine body fat percentage and muscle mass levels and upon completing the study you will have the option to undertake a free gold standard physiological assessment of aerobic capacity ($\dot{V}O_{2\text{max}}$).

This project will fill a large knowledge gap in the literature relating to sodium phosphate supplementation and at the same time, assess the effects and validity of using sodium phosphate loading to enhance team sport performance in females.

In addition, this study will also enhance our overall understanding of supplementation procedures relating to sodium phosphate and beetroot juice and may identify possible ways to further increase team sport performance through new, combined supplementation procedures.

**CONFIDENTIALITY**

The confidentiality regarding your details will be strictly maintained at all times. Data will be kept and viewed only by authorised personnel.

**PARTICIPANT RIGHTS**

You may withdraw from the study at any time without prejudice. If you withdraw, we may wish to retain the data that we have recorded from you, but only if you agree, otherwise your records will be destroyed. If you have any questions concerning the research, please feel free to contact one of the researchers (contact details listed above).

The committee for Human Rights at the University of Western Australia requires that all participants are informed that if they have any complaint regarding the manner in which a research project is conducted, it may be given to the researcher or alternatively to the Secretary, Committee for Human Rights, Registrar’s Office, University of Western Australia, Crawley, WA, 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.
The Combined Effect of Sodium Phosphate and Beetroot Juice Supplementation on Repeated Sprint Performance in Female Athletes

- Participant Consent Form -

As a participant, you are free to withdraw your consent to participate at any time without prejudice. The researchers will answer any questions you may have in regard to the study at any time.

I ____________________ (participant’s name) acknowledge that I have read the above statement and information sheet, which explains the nature, purpose and risks of the investigation and that any questions I have asked have been answered to my satisfaction. I agree to participate in this study realising that I may withdraw at any time without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to do so by law.

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I have been advised as to what data is being collected, the purpose for collecting the data, and what will be done with the data upon completion of the research.

________________________________________
Participant                                      Date

I am willing for the experimenter to photograph me during the trials so that images can be used in his final presentation.

________________________________________
Participant                                      Date
The committee for Human Rights at the University of Western Australia requires that all participants are informed that if they have any complaint regarding the manner in which a research project is conducted, it may be given to the researcher or alternatively to the Secretary, Committee for Human Rights, Registrar’s Office, University of Western Australia, Crawley, WA, 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the information sheet and consent form for their personal records.
APPENDIX B

ETHICS APPROVAL
Our Ref: RA/4/1/4940

16 September 2011

Associate Professor Karen Wallman
Sport Science, Exercise & Health (School of)
MBDP: M408

Dear Professor Wallman

HUMAN RESEARCH ETHICS APPROVAL - THE UNIVERSITY OF WESTERN AUSTRALIA

The Effect of Sodium Phosphate Supplementation on Endurance Performance in Female Cyclists

Student(s): Christopher Buck - PhD - 2024326

Ethics approval for the above project has been granted from 16 September 2011 to 01 October 2012 in accordance with the requirements of the National Statement on Ethical Conduct in Human Research (National Statement) and the policies and procedures of The University of Western Australia.

You are reminded of the following requirements:

1. The application and all supporting documentation form the basis of the ethics approval and you must not depart from the research protocol that has been approved.
2. The Human Research Ethics Office must be approached for approval in advance for any requested amendments to the approved research protocol.
3. The Chief Investigator is required to report immediately to the Human Research Ethics Office any adverse or unexpected event or any other event that may impact on the ethics approval for the project.
4. The Chief Investigator must inform the Human Research Ethics Office as soon as practicable if a research project is discontinued before the expected date of completion, providing reasons.

Any conditions of ethics approval that have been imposed are listed below:

Special Conditions

None specified

The University of Western Australia is bound by the National Statement to monitor the progress of all approved projects until completion to ensure continued compliance with ethical standards and requirements.

Please note that the maximum period of ethics approval for this project is five (5) years from the date of this notification. However, ethics approval is conditional upon satisfactory progress reports being received by the designated renewal date for continuation of ethics approval.

The Human Research Ethics Office will forward a request for a Progress Report approximately 60 days before the due date. A further reminder will be forwarded approximately 30 days before the due date.

If your progress report is not received by the due date for renewal of ethics approval, your ethics approval will expire, requiring that all research activities involving human participants cease immediately.

If you have any queries please do not hesitate to contact the Human Research Ethics Office (HREO) at hreo-research@uwa.edu.au on (08) 6488 3763.

Please ensure that you quote the file reference – RA/4/1/4940 – and the associated project title in all future correspondence.

Yours sincerely

[Signature]

Peter Johnstone
Manager
Human Research Ethics Committee
APPENDIX C

DUAL ENERGY X-RAY ABSORPTIOMETRY / RADIATION APPROVAL FORM
Estimated Effective Doses for Radiographic Procedures

School: UWA School of Sport Science, Exercise and Health
Project Title: The effect of sodium phosphate supplementation on endurance performance in female cyclists
Chief Investigators: Christopher Buck, Winthrop Professor Brian Dawson, Associate Professor Karen Wallman, Assistant Professor Kym Guelfi

X-ray procedures:

Total body scan – 1 scan per participant at the start of the intervention programme so to determine lean body mass. The scan will consist of a full body composition scan.

Forty two adult female participants (aged 18 – 35) will be recruited to this project. Start date: June, 2011 or later depending on ethics approval being granted.

- Scanning will be carried out once ethics approval has been received and will continue until approximately June, 2013.
- All study participants will be volunteers.
- All patients will be healthy female athletes.
- Pregnant women will not be scanned. A pregnancy test will be performed prior to scanning to determine this.
- Informed consent will be obtained from all participants prior to scanning.

Radiation License details:

Licensee Name: Prof Tim Ackland License No. LX337/2004 14265
School of Sport Science, Exercise and Health
The University of Western Australia

Only trained operators will have permission to use the x-ray machine. A software password system is available on the machine to prevent unauthorized use. The training course is a half-day regulator approved course and trained operators have received certification for operation of the machine.
Machine data from manufacturer’s literature

From: Operator’s Guide
X-ray machine: Lunarprodigy
X-ray beam: 76kV, 2.9mmAl at 70kVp, focal spot 0.5 mm.
Max 76 kV, 3 mA
Focal spot to image receptor distance 67 cm
Table attenuation 0.7 mm Al

Summary:

A total body (thick) scan has a calculated effective dose of 0.8 µSv. Total dose is 1 x 0.8 µSv = 0.8 µSv.

The patient consent form will state the estimated effective dose and place the radiation dose into a context understandable by all parties. The effective dose may be compared to that which is received from natural background sources eg.

“The tests involve the use of a low dose x-rays about equal to one thousandth of the background radiation you would receive in one year living in Perth. The total background radiation in Western Australia is about 2mSv per year. The radiation dose from cosmic rays from flying in a jet from Perth to London is approximately 0.1 mSv.”

The hypothetical risk of developing cancer from the radiation exposure may be calculated using the ICRP 103 risk factor of 5%/Sv (Table 1, p.53). For participation in the complete procedure the fatal cancer risk is (0.8 x 10^-6) x (5 x 10^-2) = 4 x 10^-8 = 4 in one hundred million.

Since the estimated effective dose to patients will be much less than 5 mSv in a year, a submission to the West Australian Radiological Council for approval is not required.

Yours faithfully

Jonathon Thwaites
Radiation and Safety Officer

Cc: Prof Tim Ackland

References:

ICRP 103. 2007. Recommendations of the international commission on radiological protection.
APPENDIX D

Sodium Phosphate as an Ergogenic Aid

(Review paper published in Sports Medicine)
Sodium Phosphate as an Ergogenic Aid

Christopher L. Buck · Karen E. Wallman · Brian Dawson · Kym J. Guelfi

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Abstract Legal nutritional ergogenic aids can offer athletes an additional avenue to enhance their performance beyond what they can achieve through training. Consequently, the investigation of new nutritional ergogenic aids is constantly being undertaken. One emerging nutritional supplement that has shown some positive benefits for sporting performance is sodium phosphate. For ergogenic purposes, sodium phosphate is supplemented orally in capsule form, at a dose of 3–5 g/day for a period of between 3 and 6 days. A number of exercise performance-enhancing alterations have been reported to occur with sodium phosphate supplementation, which include an increased aerobic capacity, increased peak power output, increased anaerobic threshold and improved myocardial and cardiovascular responses to exercise. A range of mechanisms have been posited to account for these ergogenic effects. These include enhancements in 2,3-Diphosphoglycerate (2,3-DPG) concentrations, myocardial efficiency, buffering capacity and adenosine triphosphate/phosphocreatine synthesis. Whilst there is evidence to support the ergogenic benefits of sodium phosphate, many studies researching this substance differ in terms of the administered dose and dosing protocol, the washout period employed and the fitness level of the participants recruited. Additionally, the effect of gender has received very little attention in the literature. Therefore, the purpose of this review is to critically examine the use of sodium phosphate as an ergogenic aid, with a focus on identifying relevant further research.
1 Introduction

Nutritional ergogenics refers to dietary supplements ingested with the aim of enhancing exercise performance by altering energy metabolism or central nervous system (CNS) responses.\textsuperscript{[1-2]} The use of nutritional ergogenic aids in sports is widespread,\textsuperscript{[2]} with much research over the past 30 years devoted to identifying medically safe supplements. One promising nutritional and legal ergogenic aid may be sodium phosphate,\textsuperscript{[3]} which has been reported to improve aerobic capacity and endurance exercise performance.\textsuperscript{[4]} However, due to limited research, many unanswered questions remain regarding the use of sodium phosphate as an ergogenic aid. Therefore, the purpose of this review is to critically examine the use of sodium phosphate as an ergogenic aid, with a focus on identifying relevant further research.

Between May and July 2011, articles were identified by searching, proQuest, Sports Discus, MEDLINE, Scopus and Ovid journal databases using the following search terms in varying combinations: phosphate, sodium, endurance, performance, exercise, 2,3-diphosphoglycerate, ergogenic aids, nutritional supplements, aerobic capacity, females, physiology, oxidative metabolism and phosphorus. Only articles published in peer-reviewed journals were utilized.

2 Phosphate in the body

2.1 What is phosphate?

Phosphorus is a non-metallic essential nutrient,\textsuperscript{[5-6]} with about 11-14 g of phosphorus per kg of fat free mass (FFM) stored in the human body,\textsuperscript{[7]} of which approximately 85% is located in the skeletal system.\textsuperscript{[6-7]} A further 14% is associated with organic compounds in the soft tissue,\textsuperscript{[7]} while 1% is stored in the blood and body fluids.\textsuperscript{[6]} The majority of phosphorus exists as either free phosphate or phosphate bound with sodium, calcium and magnesium in a mineral salt form.\textsuperscript{[3,7]}

2.2 Sources of phosphate

Phosphorus is consumed in the daily dietary intake\textsuperscript{[3]} and is found in a variety of foods such as red meat, poultry, fish, eggs and milk products.\textsuperscript{[3,7,8]} Cereals, grains, nuts and legumes also contain phosphorus.\textsuperscript{[7]} However, because some of the phosphorus in grains is not highly absorbable, animal products are considered to be a superior source of phosphorus.\textsuperscript{[6-7]} The recommended minimum dietary allowance for phosphorus in adults is 700 mg/d,\textsuperscript{[4]} while the average daily intake of phosphorus in the developed world is approximately 1000 mg/d.\textsuperscript{[9]} Phosphate deficiencies are rare in the general population.\textsuperscript{[7]}

2.3 Regulation of phosphate levels

Systemic regulation of phosphate homeostasis is maintained by close coordination between the intestine, the kidney and a range of hormones.\textsuperscript{[10]} Under normal conditions, 60 to 70% of dietary phosphate is absorbed in the small intestine,\textsuperscript{[7,9]} with the resultant circulating phosphate readily absorbed into the plasma.\textsuperscript{[8,10]} Once absorbed, phosphate enters the bone matrix protein, cells or the kidney.\textsuperscript{[10]} The kidney is the main organ regulator of phosphate homeostasis\textsuperscript{[11]} and because phosphorus acts as a threshold substance, it is thought that serum phosphate levels are regulated in part by an overflow mechanism.\textsuperscript{[7]}

Essential to this overflow mechanism is the hormonal regulation of parathyroid hormone (PTH) and 1,25-D,hydroxyvitamin D3 (1,25-D3).\textsuperscript{[7,11]} Parathyroid hormone levels rise in response to high serum phosphate levels in order to increase the excretion of phosphate from
the kidneys into urine. Conversely, with low serum phosphate levels, the concentration of 1,25-D3 increases to promote the reabsorption of phosphate from the gut and the release of phosphate from bone as a means to increase phosphate levels. Cortisol and estrogen may also play a part in the hormonal regulation of phosphate levels, however, the influence of these two hormones is less clearly defined. Under normal circumstances, serum phosphate concentrations in the body range between 0.75 to 1.35 mmol·L⁻¹, with approximately 175 to 300 mmol of phosphate excreted in the urine each day.

2.4 Role of phosphate in the body

Phosphate is essential for the formation and hydrolysis of adenosine triphosphate (ATP), which is the body’s primary energy substrate. Phosphate is also involved in the regulation of energy metabolism in a variety of ways. For example, phosphate is vital in oxidative phosphorylation and is an important component of phosphocreatine (PCr), which is intimately involved in the phosphagen energy system and the provision of anaerobic energy during intense exercise.

3 Proposed mechanisms for improved exercise performance after sodium phosphate loading

3.1 Overview

Exercise performance benefits associated with phosphate loading include: an increased aerobic capacity, increased peak power, increased anaerobic threshold and improved myocardial and cardiovascular responses to exercise. A number of mechanisms have been posited to account for these ergogenic effects. These include enhancements in 2,3-Diphosphoglycerate (2,3-DPG) concentrations, myocardial efficiency, buffering capacity and adenosine triphosphate/phosphocreatine (ATP/PCr) synthesis.

3.1.1 Enhanced 2,3-DPG concentration

It has been proposed that phosphate loading affects the glyceraldehyde-3 phosphate dehydrogenase equilibrium, which in turn increases the concentration of 2,3-DPG precursors (i.e. 1,3-diphosphoglycerate), resulting in greater 2,3-DPG concentrations in the red blood cell (RBC). Importantly, 2,3-DPG binds to hemoglobin and reduces the affinity of oxygen with hemoglobin. Thus, increased 2,3-DPG allows for a greater unloading of oxygen to the peripheral tissues/muscle.

Oxygen release at the peripheral tissues is considered to have a major influence on maximal oxygen consumption (\(\dot{V}O_{2\text{max}}\)), which is the highest rate at which oxygen can be taken up and utilized by the body during intense exercise. Maximal oxygen consumption (\(\dot{V}O_{2\text{max}}\)) is typically defined as the point where oxygen consumption levels off (plateaus) or increases only slightly in response to additional increases in exercise intensity. Thus, increased 2,3-DPG concentrations could promote increases in \(\dot{V}O_{2\text{max}}\). Importantly, an increased \(\dot{V}O_{2\text{max}}\) could translate into exercise performance benefits for endurance athletes, as it would allow them to exercise at a greater work rate for longer due to enhanced oxygen supply during exercise.

Support for enhanced 2,3-DPG concentrations with phosphate loading has come from studies which have successfully demonstrated increases in serum phosphate and 2,3-DPG concentrations associated with phosphate supplementation. Cade et al. reported a significant increase in resting 2,3-DPG concentration from 13.00 to 13.92 mg/Hb after ingestion of 4 g of
sodium phosphate per day, over a three day period, when compared to a placebo, with these enhanced levels providing evidence for 2,3-DPG as a possible mechanism for improvement in subsequent exercise performance. Furthermore, a significant correlation between serum phosphate levels and resting 2,3-DPG concentrations was described by Czuba et al.,[12] who found that the ingestion of 50 mg·kg⁻¹ of FFM of trisodium phosphate a day (split into four equal doses a day) over a six day period resulted in a 30% increase in serum phosphate and a 25% increase in 2,3-DPG concentrations. These results indicate a link between phosphate loading and increased 2,3-DPG concentrations. In addition to the increase in 2,3-DPG concentrations seen with phosphate loading, Cade et al.[17] and Czuba et al.[12] both reported increases in VO₂max. Importantly, it should be noted that a number of studies on phosphate supplementation have not measured 2,3-DPG concentrations[18-19,25] while some studies have shown ergogenic benefits associated with phosphate loading without a significant change in 2,3-DPG levels.[26] Of relevance, it should be noted that the 2,3-DPG content of blood changes quickly after sampling and thus differences in analysis protocols may explain divergent results for 2,3-DPG concentrations between studies.[27]

3.1.2 Enhanced myocardial efficiency

Phosphate supplementation may contribute to enhanced myocardial contractility, resulting in improved myocardial efficiency.[28] Myocardial efficiency relates to how effectively the heart can pump blood.[29-30] Improving myocardial efficiency would theoretically impart ergogenic benefits to athletes engaged in endurance exercise by increasing stroke volume (SV), thus providing a greater cardiac output during exercise,[12] resulting in greater and more efficient oxygenation of the exercising muscles.

The basis for this explanation comes from hypophosphatemia,[29-30] which is a condition characterized by abnormally low concentrations of inorganic phosphates in the blood.[5,31] During hypophosphatemia, the contractile properties of the heart are reduced and as a result, SV decreases.[32] As phosphate deficiencies have been linked to decreased synthesis of ATP,[30,33] it is thought that this reduced contractility of the heart is most likely related to very low levels of cardiac cell ATP.[5,32] Research on the reversal of hypophosphatemia through phosphate loading has reported that hyperphosphatemia (high concentrations of inorganic phosphates in the blood) may increase the concentration of cardiac cell ATP, which in turn could increase the contractility of the heart muscle and improve myocardial efficiency.[32,34-35] In agreement with this, O’Connor et al.[32] observed that mean left ventricular SV increased after phosphate administration in critically ill, hypophosphatemic patients.

Improved myocardial efficiency with phosphate supplementation is also supported by studies in athletes that have demonstrated increases in SV[18] and decreases in heart rate both at rest and during exercise following a period of phosphate supplementation.[36-37] For example, Czuba et al.[12] reported a significant decrease in resting and maximal exercise heart rate after supplementation with 50 mg·kg⁻¹ of FFM of sodium phosphate per day for a total of six days. The authors suggested that this decreased heart rate could be due to enhanced contractility of the heart causing an increase in SV.[12] Another study by Kreider et al.,[18] employed cardiac ultrasound and colour flow Doppler technology to examine the effects of phosphate loading on myocardial responses to exercise. In this
study, six elite male cyclists and/or triathletes consumed either 1 g of tribasic sodium phosphate or a glucose placebo four times a day for a total of five days. On the fourth day of supplementation, participants performed either an incremental maximal cycling test or a simulated 40 km cycling time trial. The opposite exercise test was then performed on the fifth day of supplementation. Results from the myocardial analysis revealed that sodium phosphate loading significantly increased mean maximal left ventricular end diastolic diameter, ejection fraction and SV during the incremental maximal exercise test. Furthermore, a reduction in heart rate was observed during the 40 km time trial. While these results are indicative of enhanced myocardial efficiency, SV and heart rate are not direct measures of improved myocardial contractility.\textsuperscript{28,32} Consequently, in order to fully support this proposed mechanism, direct evidence of changes to cardiac cell ATP and cardiac contractility with phosphate loading may be required.\textsuperscript{38} To date, no research has been conducted to directly assess cardiac cell ATP and heart contractility with phosphate supplementation due to the extremely invasive protocols that would be required.\textsuperscript{39} However, advancements in \textsuperscript{31}phosphate-magnetic resonance spectroscopy technology may allow such research to be undertaken in the future.\textsuperscript{39}

3.1.3 Enhanced buffering capacity

Ergogenic benefits associated with phosphate loading may also be attributable to an enhanced buffering capacity.\textsuperscript{7,28} Phosphates are involved in the body’s physiological buffering systems and consequently it has been hypothesised that phosphate loading may enhance the capacity of the phosphate buffering system in the intracellular fluid where the concentration of phosphate is greatest.\textsuperscript{7,12} Specifically, phosphate loading may increase hydrogen phosphate (HPO$_4^{-}$) concentrations, which could enhance the ability of the body to buffer hydrogen ions produced during intense exercise.\textsuperscript{7} Hydrogen phosphate is a weak base and is one of the main components of the phosphate buffering system, with the other main component being the weak acid dihydrogen phosphate (H$_2$PO$_4^{-}$).\textsuperscript{7,40} When a strong acid (e.g. lactic acid) is added to these two substances, the hydrogen from the acid can be accepted by HPO$_4^{-}$ and converted to H$_2$PO$_4^{-}$ to minimize the decrease in pH.\textsuperscript{40} Therefore, an augmented HPO$_4^{-}$ concentration resulting from phosphate loading, may allow for more hydrogen ions to be buffered during exercise, which would slow the decrease in pH associated with intense exercise.\textsuperscript{7} This could improve both aerobic and anaerobic exercise performance by allowing athletes to work harder for longer before reaching their anaerobic threshold.\textsuperscript{28}

The majority of the evidence supporting an enhanced buffering capacity with phosphate supplementation is based upon studies that have demonstrated a shift in the anaerobic threshold to higher workloads with phosphate loading.\textsuperscript{12,18-19} For example, Kreider et al.\textsuperscript{18} reported that during a 40 km cycling time trial, participants supplemented with sodium phosphate exercised at 86% of VO$_{2\text{max}}$ compared to 80% of VO$_{2\text{max}}$ associated with a placebo trial. Furthermore, Czuba et al.\textsuperscript{12} demonstrated that during a graded maximal exercise test, the anaerobic threshold occurred at a significantly higher workload after phosphate supplementation compared to baseline performance (details related to these two studies can be found in 4.1.1). In addition, these studies\textsuperscript{12,18} both reported increases in serum phosphate levels with phosphate loading, which suggests that the observed changes in the anaerobic threshold may be phosphate-stimulated. Even though these studies support the notion that
phosphate loading may enhance the body’s buffering capacity, there is currently a lack of studies that have directly assessed the effects of phosphate loading on intracellular phosphate buffering, suggesting that further research is needed in this area.

3.1.4 Enhanced ATP/PCr synthesis

Another mechanism proposed to explain the ergogenic effects of phosphate supplementation is enhanced ATP synthesis and oxidative energy metabolism via increased availability of extracellular and intracellular phosphate.\textsuperscript{3,7,18,38} Enhanced ATP synthesis could provide ergogenic benefits by providing a larger energy pool.\textsuperscript{3,7,18,41} Three main mechanisms have been identified by which phosphate supplementation may enhance oxidative energy metabolism and ATP synthesis. Firstly, phosphate supplementation may increase the amount of cellular phosphate available for glucose phosphorylation.\textsuperscript{7} This could promote more efficient entry of glucose into the oxidative pathway and thus enhance ATP synthesis.\textsuperscript{42} Secondly, there is evidence that phosphate loading may stimulate the activity of various enzymes involved in oxidative metabolism, such as phosphofructokinase and glyceraldehyde 3-phosphate.\textsuperscript{33,43-44} As these enzymes are involved in the regulation of oxidative metabolism, greater stimulation is likely to promote increased and more efficient oxidative metabolism.\textsuperscript{45} Thirdly, phosphate loading may lead to increased availability of phosphate in the electron transport chain, which could promote increased ATP production.\textsuperscript{42} Support for this mechanism comes from studies showing increased mitochondrial oxidative capacity after increasing extracellular and intracellular phosphate levels.\textsuperscript{43,45-46} However, these studies may have limited relevance to humans as they were performed in-vitro and involved animals.\textsuperscript{43,45} Importantly, it has been suggested that serum phosphate concentrations may not accurately reflect the effects of phosphate supplementation on oxidative metabolism and intracellular phosphate levels.\textsuperscript{18} Consequently, more research is needed on the effect of phosphate loading on ATP synthesis.

It has also been hypothesised that increased cellular concentrations of phosphate may allow for more rapid restoration of ATP and PCr in the muscles during and following intense exercise.\textsuperscript{7} This is thought to consequently improve exercise performance by allowing more efficient production and recovery of energy stores during exercise.\textsuperscript{7} However, due to limited research, it is unclear whether phosphate supplementation influences PCr synthesis, suggesting that further investigation is required.

4 Research investigating the ergogenic effects of phosphate supplementation

Studies investigating the ergogenic value of phosphate supplementation date back to the 1920’s, with results being equivocal.\textsuperscript{47-50} It is possible that this lack of certainty regarding the ergogenic effects of sodium phosphate may be the reason for phosphate receiving little attention until the late 1980’s. However, some later results supported the hypothesis that alterations in the availability of extracellular and intracellular phosphate salts may influence endurance exercise.\textsuperscript{17,51} This led to a number of more recent studies investigating the ergogenic effects of sodium and/or calcium phosphate supplementation on aerobic capacity and endurance exercise performance.\textsuperscript{18,28,38,52-54}

4.1 Sodium vs calcium phosphate

Contemporary studies investigating the ergogenic effects of phosphate supplementation on aerobic capacity and endurance exercise have demonstrated
ergogenic benefits with sodium phosphate but not with calcium phosphate.\[^{12,17-19,25-26,38,41,52}\] For example, Bredle et al.\[^{38}\] observed no change in \( \dot{V}O_{2\text{max}} \) in male runners after four days of 5.7 g a day of calcium phosphate loading. Likewise, Galloway et al.\[^{41}\] reported no change in time to exhaustion (TTE) or \( \dot{V}O_{2\text{max}} \) after an acute (90 minute pre exercise) dose of 22.2 g of calcium phosphate, in trained and untrained male cyclists. This is in contrast to studies employing sodium phosphate loading, where increases in \( \dot{V}O_{2\text{max}} \) ranging between 5-12%\[^{12,17,28}\] and improvements in endurance time trial performance\[^{18,53}\] have been reported following 3-6 days of loading. This raises the possibility that sodium phosphate may influence intracellular phosphate levels and endurance exercise in a unique way, which is not seen with calcium phosphate supplementation.\[^{38}\]

The exact nature of sodium phosphate’s influence on intracellular phosphate levels and exercise performance has yet to be identified. However, an explanation may come from research showing that intracellular concentrations of phosphate are, at least in part, sodium dependent.\[^{41,55}\] Such research has demonstrated a sodium-phosphate, co-transportation interaction in controlling phosphate levels in the canine kidney.\[^{55}\] Specifically, this sodium phosphate co-transportation mechanism is thought to be an active process needed to maintain cellular phosphate levels above equilibrium.\[^{55}\] Consequently, for intracellular phosphate concentrations to increase and thereby result in ergogenic effects, some form of enhanced sodium-phosphate co-transportation could be required.\[^{41}\] This would explain why only studies using sodium phosphate have reported ergogenic benefits. However, this explanation is only speculative as the above co-transportation mechanism has not yet been researched in humans. Regardless of the mechanism responsible, it has been suggested that only sodium phosphate should be viewed as a nutritional ergogenic aid to improve endurance exercise.\[^{6}\]

4.2 Sodium phosphate loading, aerobic capacity and endurance exercise

4.2.1 Aerobic capacity (\( \dot{V}O_{2\text{max}} \))

In one of the first studies to systematically evaluate phosphate loading, Cade et al.\[^{17}\] examined the influence of sodium phosphate supplementation on \( \dot{V}O_{2\text{max}} \), serum phosphate and RBC 2,3-DPG levels in ten trained male runners (\( \dot{V}O_{2\text{max}} 56.2 \text{ ml·kg}^{-1}·\text{min}^{-1} \)). A cross-over and counterbalanced design was employed where participants performed an incremental, maximal, exercise test on a treadmill, following three days consumption of either 1 g of sodium phosphate or 0.1 g of sodium citrate (placebo) ingested four times a day. Each loading protocol was separated by a four day washout period. Results demonstrated that sodium phosphate loading significantly increased resting serum phosphate (1.17 to 1.22 mmol·L\(^{-1}\); \( p < 0.05 \)) and RBC 2,3-DPG levels (13.00 to 13.92 mg/Hb; \( p < 0.05 \)). Furthermore, a 6-12% increase in \( \dot{V}O_{2\text{max}} \) was observed with phosphate supplementation.

Other studies have shown similar effects on \( \dot{V}O_{2\text{max}} \).\[^{3,12,18-19,26,28}\] For example, Stewart et al.\[^{20}\] investigated the effects of sodium phosphate loading on \( \dot{V}O_{2\text{max}} \), serum phosphate and RBC 2,3-DPG concentration in eight trained male cyclists (\( \dot{V}O_{2\text{max}} 48.5 \text{ ml·kg}^{-1}·\text{min}^{-1} \)). Specifically, participants consumed either a placebo or 3.6 g of sodium phosphate a day, for three days, followed by a maximal cycle ergometry test. A four day washout period separated each trial. Despite no change in either resting serum phosphate or 2,3-DPG concentration, an 11% increase in \( \dot{V}O_{2\text{max}} \) and a 20% increase in TTE were observed.
following sodium phosphate supplementation.

Additional support has come from Kreider et al.,\[19\] who reported a 9% increase in $\dot{V}O_{2\text{max}}$ and a 12% increase in the ventilatory anaerobic threshold during a treadmill exercise test following sodium phosphate supplementation. In this study, seven highly trained male runners ($\dot{V}O_{2\text{max}} 73.9 \pm 6.3 \text{ml·kg}^{-1}·\text{min}^{-1}$) ingested either 4 g of glucose (placebo) or sodium phosphate per day for six days. Participants were required to perform either an incremental treadmill exercise test to exhaustion or a five mile time trial on the third day of loading. This was then followed by the opposite exercise test on the sixth day of loading. A 14 day washout period was undertaken between each protocol. The results showed that in addition to the increase in $\dot{V}O_{2\text{max}}$ and ventilatory anaerobic threshold seen in the maximal exercise test, that a 17% increase in resting serum phosphate levels also occurred after sodium phosphate supplementation. Red blood cell 2,3-DPG concentrations were not examined in this study.

Following on from their previous work, Kreider et al.\[18\] performed one of the most comprehensive studies to investigate the ergogenic benefits of sodium phosphate supplementation on aerobic capacity and endurance exercise. They recruited six elite male cyclists and/or triathletes ($\dot{V}O_{2\text{max}} 69.3 \pm 0.12 \text{ml·kg}^{-1}·\text{min}^{-1}$) who, in a counterbalanced and crossover design, consumed either 1 g of tribasic sodium phosphate or a glucose placebo four times a day, for a total of four days. Participants performed either an incremental maximal cycling test or a simulated 40 km cycling time trial on the fourth day of supplementation. The opposite exercise test was then performed after an additional day of supplementation. A 17 day washout period was employed between trials.\[18\] It was observed that phosphate supplementation significantly increased oxygen uptake at the anaerobic threshold by 10%, time to anaerobic threshold by 10%, power output at the anaerobic threshold by 9% and $\dot{V}O_{2\text{max}}$ by 9% during the maximal exercise test. Furthermore, resting serum phosphate levels were 17% higher after phosphate supplementation, but as in their previous study,\[19\] RBC 2,3-DPG concentration was not assessed.\[18\]

Recent research conducted by Czuba et al.\[12,28\] also supports the use of sodium phosphate as an ergogenic aid. Specifically, Czuba et al.\[12\] examined the effect of both short-term and long-term sodium phosphate supplementation on $\dot{V}O_{2\text{max}}$ in 19 elite cyclists ($\dot{V}O_{2\text{max}} 73 \text{ml·kg}^{-1}·\text{min}^{-1}$). The sodium phosphate loading protocol used by Czuba et al.\[12\] consisted of two phases. In the initial phase, participants consumed 50 mg·kg$^{-1}$ of FFM of tri-sodium phosphate a day, split into four equal doses over the day for six days. Following this, an incremental, maximal cycling test was performed. In the second phase of phosphate supplementation, which continued on from the first phase, participants consumed 25 mg·kg$^{-1}$ of FFM of sodium phosphate a day for a further three weeks. After phase two of supplementation, another incremental, maximal cycling test was performed. Following the initial six days of loading, these researchers reported a significant 5% increase in $\dot{V}O_{2\text{max}}$ and a 5% increase in the ventilatory (anaerobic) threshold compared to baseline. Continued supplementation in phase two did not result in a further significant increase in $\dot{V}O_{2\text{max}}$ or the ventilatory threshold compared to the initial six days. However, after phase two, $\dot{V}O_{2\text{max}}$ was still significantly (p<0.05) higher (5.6%) compared with baseline values. It was also observed that resting serum phosphate and 2,3-DPG concentration increased after phase one, even though both
resting and exercising heart rate significantly decreased (9.6% and 2.7% respectively). No significant changes were reported in regards to any lactate variables following phosphate supplementation. The phosphate loading protocol used by Czuba and colleagues in this study, as well as in an earlier study (50 mg·kg⁻¹ of FFM, for six days[28]) is particularly important because unlike previous research on phosphate loading, both these studies supplemented sodium phosphate relative to body composition or size, by using a FFM corrected dose.

Whilst the majority of studies investigating sodium phosphate loading and \( \dot{V}O_{2\text{max}} \) have demonstrated ergogenic effects, it must be noted that some studies have shown no effect. For example, Brennan and Connolly[54] reported that 4 g a day of sodium diphosphate supplementation for four days resulted in no significant changes to \( \dot{V}O_{2\text{max}} \) in a group of 12 well trained male cyclists (\( \dot{V}O_{2\text{max}} 60.6 \pm 4.4 \text{ ml·kg}^{-1·\text{min}^{-1}} \)). Likewise, West et al.[56] observed no change in \( \dot{V}O_{2\text{peak}} \) (\( \dot{V}O_{2\text{peak}} \) being the highest value for oxygen consumption during a graded exercise test to exhaustion, where oxygen values do not meet the strict criteria needed for a \( \dot{V}O_{2\text{max}} \) to be reported[23-24]) following 6 days of sodium phosphate supplementation (50 mg·kg⁻¹ of FFM) in moderately trained men and women. The reason for this inconsistency between studies is not clear. West et al.[56] suggested that it was possible that only certain individuals responded positively to phosphate supplementation since the relationship between the change in serum phosphate and the change in \( \dot{V}O_{2\text{peak}} \) approached significance in their study. The factors influencing this potential variable response may relate to pre-loading phosphate levels or other unknown factors that require investigation. Regardless, this highlights that the effectiveness of sodium phosphate should be tested and confirmed for each athlete prior to use.

4.2.2 Endurance performance

To date, only a limited number of studies have assessed the effects of sodium phosphate supplementation on actual measures of endurance performance. In the previously mentioned study by Kreider et al.[19] the influence of sodium phosphate loading was examined on both \( \dot{V}O_{2\text{max}} \) and five mile running time trial performance. Results from the five mile time trial showed only slight, non-significant reductions in mean performance time (11.8 s decrease in time to completion) and average mile split time (2.52 s decrease), while mean oxygen uptake during the run was significantly lower. Decreased mean oxygen uptake may have been due to enhanced physiological efficiency resulting from phosphate supplementation (with efficiency defined here as the amount of energy/oxygen required to perform a task compared to the actual work accomplished).[24] This would theoretically be of ergogenic value during endurance exercise,[7] even though this was not reflected by a significant improvement in performance time.[19] Of relevance, a reduction in performance time does not always need to be statistically significant to provide a performance benefit for athletes.

Kreider et al.[18] (study details in 4.2.1) further investigated the ergogenic effect of phosphate loading on endurance performance. Here, in addition to a \( \dot{V}O_{2\text{max}} \) test, participants were required to complete a simulated 40 km cycling time trial after either sodium phosphate or placebo supplementation. Results from the 40 km cycling time trial revealed that sodium phosphate increased mean power output by 17%, oxygen uptake by 18%, ventilation by 15% and reduced heart rate by 8%, which together may have contributed to an average 8% (3.5 minute) reduction in
completion time when compared to the placebo trial.

5 Research considerations for sodium phosphate supplementation

Many studies on sodium phosphate loading differ in terms of the administered dose and dosing protocol used, the length of the supplementation and washout period employed, and the fitness level of the participants recruited.\textsuperscript{[8,18,28,41]} Furthermore, predominantly male participants have been studied with respect to the ergogenic effects of phosphate loading on aerobic capacity and endurance performance.\textsuperscript{[3]} Due to a number of physiological differences between males and females,\textsuperscript{[57]} gender is likely to have an influence on the ergogenic effects of phosphate loading.\textsuperscript{[3]} Additionally, the side-effects associated with sodium phosphate loading represent important research considerations.

5.1 Dose and dosing protocol

Most studies investigating sodium phosphate loading have administered doses of phosphate ranging between 3-5 g per day, for a period of 3-6 days.\textsuperscript{[5,7-8]} The majority of studies using doses within this range have reported significant increases in both $\dot{V}O_{2\text{max}}$ and resting serum phosphate levels post supplementation.\textsuperscript{[3,12,17-18,26]} Doses greater than 6 g have typically been avoided as they are associated with a PTH-mediated down regulation in serum phosphate levels,\textsuperscript{[11,41]} while doses below 3 g are generally considered too small to significantly raise serum phosphate levels.\textsuperscript{[8]} Even though this effective 3-5 g spectrum has been identified, no studies have attempted to determine an optimal dose within this range.

Phosphate dosing strategies commonly require sodium phosphate to be taken orally, in capsule form, either as a single dose each day\textsuperscript{[22,38,41,52-54]} or split into 3-4 equal doses over the day.\textsuperscript{[12,17,25,28]} While both protocols have been effective in increasing serum phosphate levels,\textsuperscript{[8]} multiple smaller doses are thought to avoid the PTH response associated with a single, larger dose of phosphate.\textsuperscript{[8]} Based on this premise, it has been recommended that for improvements in aerobic capacity and endurance performance to occur, the administered dose should consist of 1 g of sodium phosphate, ingested four times a day.\textsuperscript{[3,12]} However, this recommendation does not account for differences in body composition between individuals. It has been suggested that it may be important to use a dose relative to body composition when loading sodium phosphate, as it would help to ensure that participants of differing body sizes are receiving a similar proportion of exposure.\textsuperscript{[58]} To date, the only exercise studies to use a relative dose of phosphate when loading are those by Czuba et al.,\textsuperscript{[12,28]} and West et al.,\textsuperscript{[56]} in which 50 mg·kg$^{-1}$ of FFM of sodium phosphate (divided into four equal doses) was ingested each day, over a six day period. While Czuba demonstrated that this dosing protocol was effective in increasing $\dot{V}O_{2\text{max}}$ and serum phosphate levels, the study by West et al.,\textsuperscript{[56]} did not. Therefore, it is currently unclear as to the optimal relative dose for sodium phosphate loading required to achieve an ergogenic effect.

5.2 Length of loading period

Studies by Cade et al.\textsuperscript{[17]} and Czuba et al.\textsuperscript{[12,28]} have all shown significant increases in serum phosphate levels after 3-6 days of sodium phosphate loading. From these studies, it has been identified that phosphate loading periods of this duration may effectively improve endurance performance,\textsuperscript{[17,25,53]} as well as increase $\dot{V}O_{2\text{max}}$ by 5-12%.\textsuperscript{[12,17,28]} Importantly, Czuba et al.\textsuperscript{[28]} reported further improvements in $\dot{V}O_{2\text{max}}$ after sodium phosphate was loaded for a further 21 days (25 mg·kg$^{-1}$ of FFM
dose per day) following an initial 6 day (50 mg·kg$^{-1}$ of FFM dose per day) loading protocol. Based on these studies, it has been proposed that at least three days of loading is required for serum phosphate levels to increase to concentrations sufficient to provide ergogenic benefits, with these benefits improving further when loading is continued for a further 24 days, using the dosing protocols described above.$^{[18,20,22,28]}$ Further studies are needed to determine the maintenance dose of sodium phosphate in respect to ergogenic effects, as well as the maximum safe time of supplementation.

5.3 Washout period

Generally, studies investigating the ergogenic effects of sodium phosphate supplementation have employed washout periods ranging between 7-14 days.$^{[8,17-19,26,53]}$ Of importance, Cade et al.$^{[17]}$ showed that after three days of sodium phosphate loading, it took nearly two weeks for RBC 2,3-DPG concentration to return to near baseline levels. Based on this, Cade et al.$^{[17]}$ advocated that a minimum 14 day washout period was required between supplementation trials in order to remove any carryover effects from phosphate supplementation. Consequently, studies employing brief washout periods (i.e. less than 14 days) are unlikely to give an accurate representation of the influence of phosphate loading.$^{[7]}$

5.4 Fitness level of participants

A number of longitudinal and cross-sectional studies have demonstrated that participants of varying aerobic fitness levels have different RBC 2,3-DPG concentrations.$^{[41,59-62]}$ For example, some endurance trained participants have been reported to have higher 2,3-DPG concentrations when compared with untrained participants,$^{[62]}$ with these higher levels being attributed to an increase in RBC turnover.$^{[59]}$ This process is thought to be an adaptive response to endurance training.$^{[8]}$ Consequently, it has been hypothesised, that due to their higher 2,3-DPG concentration, trained endurance athletes may be less responsive to the possible ergogenic effects of phosphate supplementation when compared to untrained participants.$^{[8,40]}$ Nonetheless, research has demonstrated that trained participants can still receive ergogenic benefits from sodium phosphate supplementation.$^{[17-18,26,28]}$ Importantly, there has been no research conducted on the effect of sodium phosphate loading in untrained participants, warranting further studies in this area.

5.5 Gender

A number of physiological differences between the sexes may cause females to respond differently to phosphate supplementation.$^{[3]}$ For example, it has been reported that females have a decreased oxygen-hemoglobin affinity, compared to males.$^{[63-64]}$ This has been identified through studies demonstrating higher $P_{50}$ values (oxygen tension at 50% oxygen saturation)$^{[63]}$ and increased 2,3-DPG concentrations in females compared to males, with the exact reason for this difference unknown.$^{[63]}$ A potential effect of this lower oxygen affinity is that females may be unresponsive to the ergogenic effects of sodium phosphate supplementation, as one of the main mechanisms by which phosphate loading is thought to be ergogenic is through an increase in 2,3-DPG concentration leading to greater peripheral oxygen delivery and an increased $\dot{VO}_{2max}$. As females already have an increased 2,3-DPG concentration,$^{[65]}$ there may be less opportunity for a phosphate stimulated increase in 2,3-DPG concentration to further decrease oxygen affinity.
Females may also respond differently to phosphate loading due to higher and greater fluctuations in estrogen levels compared to males, particularly as estrogen is involved in the hormonal regulation of phosphate levels in the body. Notably, it has been identified that estrogen acts to decrease renal phosphate reabsorption. Therefore, if phosphate is loaded during the ovulatory phase of the menstrual cycle when estrogen levels are at their highest, it is possible that less phosphate may be reabsorbed resulting in smaller increases in serum phosphate levels. Conversely, estrogen levels are low in males and are unlikely to have a major influence on phosphate reabsorption.

Furthermore, females tend to have smaller hearts, SV, left ventricular masses and a reduced red cell mass compared with males. Related to this physiological difference between genders is that phosphate loading has been proposed to improve myocardial efficiency, resulting in an increased SV and decreased heart rate, which is thought to lead to a better oxygen supply to the muscles and hence improved exercise performance. Currently, it is not clear whether phosphate loading in females would be able to alter myocardial efficiency to a level sufficient to increase SV. Furthermore, reduced red cell mass in females may limit the magnitude of any oxygen transport benefits from enhanced myocardial efficiency.

In addition, differences in body composition between genders, in that females are typically smaller and have a greater percent of body fat and less FFM than males, may affect how females respond to sodium phosphate supplementation. Consequently, when ingesting an absolute dose of phosphate, it is likely that females are actually consuming a larger (relative to body mass) dose of phosphate than males. This would mean that females are more likely to induce a PTH mediated down regulation in phosphate levels. This difference in body composition between genders highlights the importance of using a FFM relative dose when loading phosphate. Future research should investigate varying FFM corrected doses of sodium phosphate, in order to identify the ideal dose of sodium phosphate for both males and females.

5.6 Side effects

The consensus in the literature is that there are minimal side effects linked to phosphate supplementation and that it is generally safe when used as an ergogenic aid. Nonetheless, it has been suggested that regular prolonged use of phosphate may upset the balance of phosphate and other minerals in the body leading to gastrointestinal distress (i.e. diarrhea and constipation). It is also possible that sodium phosphate consumption can lead to vomiting, however this side effect has been eliminated by ingesting the phosphate dose with fluids. Despite minimal side effects, it is still recommended that individuals with kidney diseases avoid phosphate loading.

6 Conclusion

Sodium phosphate has been shown to improve aerobic capacity, and in some cases endurance performance, in male participants. However, support for the ergogenic effects of sodium phosphate is complicated by a number of key methodological considerations. Specifically, the administered dose and dosing protocol, the length of the loading and washout period and the fitness level of the participants are all thought to influence the ergogenic effect of sodium phosphate loading. A review of these methodological considerations indicates that sodium phosphate is likely to be ergogenic when ingested for 3-6 days, at a
dose of 3-5 g per day, split into multiple doses over the day. However, this notion is yet to be confirmed in female participants, highlighting this as an area for future investigation. Furthermore, additional research investigating the effect of fitness levels on phosphate loading is also needed. It is also recommended that future research should focus on investigating a range of different FFM relative doses of sodium phosphate on endurance performance, while the ergogenic effect of sodium phosphate on anaerobic performance should also be investigated.

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8 Reference list


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<table>
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<tr>
<th>Study, year</th>
<th>Supplement</th>
<th>Dose</th>
<th>Washout period (days)</th>
<th>Participants</th>
<th>Exercise Test(s)</th>
<th>Ergogenic Effect</th>
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<tr>
<td>Cade et al. [17], 1984</td>
<td>Sodium phosphate</td>
<td>1 g, 4 9/day, for 3 days</td>
<td>4</td>
<td>10 trained M runners</td>
<td>Treadmill $\dot{V}O_2$</td>
<td>6–12 % increase in $\dot{V}O_2$ ($p &lt; 0.05$)</td>
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<td>Sodium phosphate</td>
<td>3.6 g/day, for 3 days</td>
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<td>8 trained M cyclists</td>
<td>Cycle ergometer $\dot{V}O_2$</td>
<td>11 % increase in $\dot{V}O_2$, ($p &lt; 0.05$); 20 % increase in total time to exhaustion ($p &lt; 0.05$)</td>
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<td>Kreider et al. [19], 1990</td>
<td>Tribasic sodium phosphate</td>
<td>1 g, 4 9/day for 3 and 6 days</td>
<td>14</td>
<td>7 highly trained M runners</td>
<td>Treadmill $\dot{V}O_2$, test 5-mile time trial</td>
<td>9 % increase in $\dot{V}O_2$; ($p &lt; 0.05$). No effect on 5-mile time trial ($p = 0.14$)</td>
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<td>Kreider et al. [18], 1992</td>
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<td>1 g, 4 9/day, for 4 days</td>
<td>17</td>
<td>6 elite M cyclists/ triathletes</td>
<td>Cycle ergometer $\dot{V}O_2$ test 40-km time trial</td>
<td>9 % increase in $\dot{V}O_2$ ($p &lt; 0.01$) and mean power (17 %; $p = 0.08$) in 40-km time trial</td>
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<td>Folland et al. [25], 2008</td>
<td>Tribasic dodecahydrate sodium phosphate</td>
<td>1 g, 4 9/day, 6 for days</td>
<td>14</td>
<td>6 well trained M cyclists</td>
<td>16.1-km cycle ergometer time trial</td>
<td>3 % decreased time to complete time trial ($p &lt; 0.05$). 9.8 % increase in mean power output ($p &lt; 0.05$)</td>
</tr>
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<td>Czuba et al. [28], 2008</td>
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<td>50 mg·kg$^{-1}$ of lean body mass divided into 4 doses for 6 days</td>
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<td>19 elite M cyclists</td>
<td>Cycle ergometer $\dot{V}O_2$</td>
<td>Improvement in $\dot{V}O_2$ by *5 % ($p &lt; 0.05$)</td>
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<td>Czuba et al. [12], 2009</td>
<td>Tri-sodium phosphate</td>
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<td>19 elite M cyclists</td>
<td>Cycle ergometer $\dot{V}O_2$</td>
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<td>West et al. [56], 2012</td>
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<td>50 mg·kg$^{-1}$ of lean body mass divided into 4 doses for 6 days</td>
<td>14</td>
<td>11 and 9 moderately trained M and F, respectively</td>
<td>Treadmill $\dot{V}O_2$</td>
<td>No significant benefit ($p = 0.483$)</td>
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* Female; M male; N/A not applicable; $\dot{V}O_2$ maximal oxygen consumption
APPENDIX E

RAW DATA STUDIES, ONE, TWO AND THREE
Study one time-trial data for 25 mg·kg\(^{-1}\) of FFM of sodium phosphate dose.

<table>
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<tr>
<th>Participant</th>
<th>Resting lactate (mM)</th>
<th>Post TT lactate (mM)</th>
<th>RPE Pre</th>
<th>PRE Post</th>
<th>HR rest (bpm)</th>
<th>HR post (bpm)</th>
<th>Time to completion (mins)</th>
<th>Peak Power (W)</th>
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<td>9</td>
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<td>10</td>
<td>18</td>
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<td>169</td>
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<td>8</td>
<td>17</td>
<td>79</td>
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<td>19</td>
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<td>12</td>
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<td>64</td>
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<td>57:11.6</td>
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Study one time-trial data for 50 mg·kg\(^{-1}\) of FFM of sodium phosphate dose.

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Study one time-trial data for placebo trial.

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Study one participant characteristics.

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Study one serum phosphate data for pre and post loading for each trial (measured in mmol·L⁻¹).

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Study two data for best sprint (BS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds).

(PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Study two data for first sprint (FS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds).

(PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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<th>FS Set 3 PHOS</th>
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<th>FS Set 3 COMB</th>
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Study two data for total sprint (TS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds). (PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Total sprint times for sets 1, 2 and 3 combined (in seconds)

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Study two participant characteristics.

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<th>Age (y)</th>
<th>Height (m)</th>
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Study two data for lactate (mmol\(\text{L}^{-1}\)) for baseline, set 2 and 3 of repeated sprint ability (RSA) during the simulated team game circuit.  
(PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Study two data for rating of perceived exertion (RPE) for baseline (PRE), quarter (Q) 1, 2, 3 and 4 of the simulated team game circuit.  
(PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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217
Study two data for heart rate (HR; bpm) for baseline (PRE), quarter (Q) 1, 2, 3 and 4 of the simulated team game circuit. (PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

|        | PRE HR PLA | PRE HR PHOS | PRE HR CAF | Q1 HR PLA | Q1 HR PHOS | Q1 HR CAF | Q2 HR PLA | Q2 HR PHOS | Q2 HR CAF | Q3 HR PLA | Q3 HR PHOS | Q3 HR CAF | Q4 HR PLA | Q4 HR PHOS | Q4 HR CAF | Q4 HR COMB |
|--------|------------|-------------|------------|-----------|------------|------------|-----------|------------|------------|-----------|------------|------------|-----------|-----------|------------|------------|------------|
| 1      | 72         | 72          | 69         | 65        | 171        | 170        | 139       | 172        | 180        | 171       | 172        | 179        | 182       | 181       | 192        | 171        | 182        |
| 2      | 72         | 75          | 72         | 72        | 160        | 160        | 176       | 168        | 161        | 180        | 172        | 158        | 165       | 179       | 172        | 158        | 165        |
| 3      | 60         | 66          | 67         | 58        | 144        | 169        | 150       | 162        | 160        | 157        | 155        | 176        | 142       | 139       | 157        | 171        | 171        |
| 4      | 56         | 54          | 54         | 59        | 170        | 165        | 170       | 160        | 170        | 165        | 172        | 185        | 169       | 180       | 179        | 182        | 164        |
| 5      | 75         | 78          | 72         | 71        | 165        | 170        | 163       | 177        | 146        | 175        | 160        | 169        | 160       | 175        | 152        | 172        | 183        |
| 6      | 65         | 65          | 68         | 61        | 160        | 159        | 124       | 98         | 168        | 140        | 172        | 137        | 169        | 147        | 126        | 152        | 161        |
| 7      | 69         | 68          | 64         | 69        | 185        | 190        | 160       | 183        | 141        | 170        | 167        | 190        | 165        | 130        | 172        | 181        | 160        |
| 8      | 67         | 66          | 66         | 62        | 170        | 176        | 181       | 158        | 194        | 190        | 181        | 176        | 193        | 192        | 196        | 176        | 194        |
| 9      | 61         | 58          | 68         | 63        | 177        | 108        | 108       | 185        | 179        | 169        | 183        | 192        | 183        | 158        | 185        | 191        | 182        |
| 10     | 55         | 59          | 68         | 71        | 178        | 153        | 166       | 151        | 180        | 153        | 162        | 164        | 180        | 158        | 159        | 168        | 183        |
| 11     | 61         | 76          | 58         | 71        | 186        | 187        | 188       | 162        | 186        | 186        | 184        | 170        | 188        | 191        | 178        | 192        | 189        |
| 12     | 81         | 72          | 55         | 65        | 175        | 175        | 161       | 180        | 183        | 174        | 177        | 180        | 192        | 176        | 177        | 183        |

Study two serum phosphate data for pre and post loading for each trial (measured in mmol·L⁻¹). (PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Study two 2,3-Diphosphoglycerate data for pre and post loading for each trial (measured in mmol L\(^{-1}\)). (PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Study two serum caffeine data for pre and post loading for each trial (measured in mmol L\(^{-1}\)). (PLA = placebo, PHOS = phosphate, CAF = caffeine, COMB = combined).

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Study three data for best sprint (BS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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<th>BS Set 2 PLA</th>
<th>BS Set 2 PHOS</th>
<th>BS Set 2 BJ</th>
<th>BS Set 2 COMB</th>
<th>BS Set 3 PLA</th>
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Study three data for first sprint (FS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Study three data for total sprint (TS) times for set 1, 2 and 3 of repeated sprint ability during the simulated team game circuit (in seconds). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Total sprint times for sets 1, 2 and 3 combined (in seconds)

Study three participant characteristics.

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Study three data for lactate (mmol\,L\(^{-1}\)) for baseline, set 2 and 3 of repeated sprint ability (RSA) during the simulated team game circuit. (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Study three data for rating of perceived exertion (RPE) for baseline (PRE), quarter (Q) 1, 2, 3 and 4 of the simulated team game circuit. (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

| Participant | PRE RPE PLA | PRE RPE PHOS | PRE RPE BJ | Q1 RPE PLA | Q1 RPE PHOS | Q1 RPE COMB | Q2 RPE PLA | Q2 RPE PHOS | Q2 RPE COMB | Q3 RPE PLA | Q3 RPE PHOS | Q3 RPE BJ | Q4 RPE PLA | Q4 RPE PHOS | Q4 RPE COMB |
|-------------|-------------|-------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1           | 7           | 7           | 6         | 7         | 7         | 15        | 13        | 12        | 15        | 14        | 13        | 15        | 14        | 13        | 14        |
| 2           | 8           | 8           | 8         | 12        | 14        | 14        | 13        | 16        | 16        | 13        | 15        | 16        | 16        | 14        | 16        |
| 3           | 9           | 9           | 8         | 12        | 14        | 13        | 12        | 16        | 15        | 14        | 13        | 16        | 15        | 15        | 16        |
| 4           | 8           | 8           | 7         | 13        | 14        | 16        | 16        | 14        | 16        | 12        | 15        | 17        | 17        | 13        | 17        |
| 5           | 8           | 7           | 7         | 13        | 14        | 14        | 13        | 14        | 16        | 14        | 15        | 16        | 15        | 16        | 14        |
| 6           | 9           | 8           | 7         | 15        | 17        | 14        | 15        | 12        | 15        | 14        | 17        | 16        | 14        | 18        | 15        |
| 7           | 7           | 8           | 8         | 16        | 18        | 14        | 16        | 15        | 15        | 15        | 15        | 14        | 16        | 17        | 16        |
| 8           | 6           | 6           | 6         | 13        | 13        | 15        | 18        | 18        | 19        | 18        | 17        | 16        | 18        | 17        | 19        |
| 9           | 6           | 6           | 6         | 16        | 14        | 10        | 13        | 19        | 18        | 20        | 13        | 17        | 18        | 20        | 15        |
| 10          | 6           | 6           | 6         | 15        | 17        | 15        | 15        | 19        | 17        | 18        | 19        | 17        | 18        | 19        | 19        |
| 11          | 7           | 7           | 6         | 13        | 10        | 14        | 10        | 16        | 15        | 15        | 15        | 17        | 16        | 15        | 16        |
| 12          | 6           | 6           | 6         | 13        | 15        | 11        | 19        | 18        | 17        | 18        | 19        | 17        | 19        | 18        | 18        |
| 13          | 6           | 6           | 6         | 16        | 13        | 14        | 13        | 17        | 16        | 15        | 15        | 16        | 17        | 16        | 18        |
Study three data for heart rate (HR; bpm) for baseline (PRE), quarter (Q) 1, 2, 3 and 4 of the simulated team game circuit. (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Study three serum phosphate data for pre and post loading for each trial (measured in mmolL\(^{-1}\)). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Study three 2,3-Diphosphoglycerate data for pre and post loading for each trial (measured in nmol\textcdot L^{-1}). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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Study three plasma nitrate data for pre and post loading for each trial (measured in mmol\textcdot mL^{-1}). (PLA = placebo, PHOS = phosphate, BJ = beetroot juice, COMB = combined).

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