The effects of phosphatidylserine (PS) supplementation on performance during and recovery following prolonged intermittent exercise.

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Masters of Philosophy

August 2005

Declaration

I certify that this thesis is the result of my own investigations, except where otherwise stated. All other sources of information have been acknowledged, both in the text and in the reference section. Moreover, this work has not previously been accepted for any degree, and is not being concurrently submitted in candidature for any degree. I agree that, if accepted, this thesis may be available for photocopying and for inter-library loan, and that the title and abstract be made available to outside organisiations.

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Contents

Abstract

Prolonged intermittent exercise, in the guise of simulated soccer match play, has the potential to elevate the production of reactive oxygen species and leave participants susceptible to oxidative stress, subsequent muscle damage (Thompson *et al.*, 2001) and muscle soreness (Wadsworth *et al.*, 2004). Supplementation with phosphatidylserine (PS), a primary constituent of the inner membranes of neurones, has been shown to enhance cognitive function in the elderly (Pepu *et al.*, 1996; Blokland *et al.*, 1999) and inhibit the exercise-induced release of stress hormones (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Fahey and Pearl, 1998). In addition to a myriad of membrane functions, in-vitro studies have demonstrated that PS has the potential to act as an antioxidant (Latorraca *et al.*, 1993; Dacaranhe and Terao, 2001). Consequently, it is plausible that exogenous supplementation with PS may provide additional defence against the oxidative stress caused by exercise; however this action has yet to be explored. Therefore, the aim of the current study was to investigate the effect of chronic soybean-derived PS (S-PS) supplementation on muscle damage, delayed onset muscle soreness, and the body's response to prolonged intermittent exercise.

Sixteen familiarised male subjects were administered with either 750 $mg \cdot day^{-1}$ of S-PS or a glucose placebo, in a double-blind randomised fashion, for 10 days prior to a prolonged intermittent exercise protocol. The protocol was based on the Loughborough intermittent shuttle test (LIST) (Nicholas *et al.*, 2000), but was adapted to specifically simulate soccer match play. Subjects' response to exercise was

assessed by measuring heart rate (HR) throughout exercise, while blood lactate and glucose concentrations, and rate of perceived exertion (RPE) were measured prior to exercise, at half time, and immediately post-exercise. Moreover, perceived muscle soreness and blood concentrations of cortisol, creatine kinase (CK), lipid hydroperoxide (HPO), vitamin C, and vitamin E were measured prior to exercise and after 20 min, 24 hr, and 48 hr of recovery.

The prolonged intermittent exercise protocol used in this study led to exercise-induced stress, as demonstrated by the significant elevation of blood cortisol concentrations during exercise ($P<0.001$). Moreover, the exercise protocol was shown to significantly elevate markers of muscle damage ($P<0.001$), delayed onset muscle soreness (DOMS) ($P<0.001$), and oxidative stress ($P<0.001$). These variables were affected to an equal extent in placebo and S-PS groups, and had returned to pre-exercise levels within 48 hours of recovery. However, those supplemented with S-PS did demonstrate a trend towards enhanced performance ($P=0.082$).

It is plausible that the trend of improved performance of the S-PS group, and consequent elevation in blood cortisol concentration and oxidative stress markers, may have negated any benefits that S-PS supplementation had on these markers. Therefore, future research should attempt to clarify the potential ergogenic effect of S-PS supplementation, and ascertain if such supplementation has any effect on the elevation of blood cortisol and oxidative stress associated with prolonged intermittent exercise.

Acknowledgements

First and foremost, I would like to thank all of the subjects for their time, patience, sweat and blood dedicated to this study; without whom it would not have been possible.

Many thanks to Mr Mike Kingsley for all of your assistance in collecting data, and for your guidance & supervision throughout the study. Thanks also to Rebecca Marson, Mark Miller, Joe Taylor & Steve Sumnal for your help with data collection.

Finally, thanks must go to my family and friends for all of the support they have given me throughout this study. Particular thanks should go to Zoe; without your support & encouragement, this project may never have been finished.

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1 Introduction

Association football (soccer) requires players to work at average exercise intensities of approximately 75% of maximum oxygen uptake (%$\dot{V}O_{2max}$) (Reilly, 1997). However, the intermittent nature of soccer dictates that a player will be required to utilise all sources of energy in order to complete the required concentric, eccentric and isometric muscular actions (Reilly, 1997; Drust *et al.*, 2000).

The aerobic energy system has been calculated to supply more than 90% of the total energy requirements during soccer match play (Bangsbo, 1994a). Reactive Oxygen Species (ROS) are produced as by-products in the electron transport chain during normal aerobic respiration (Kanter, 1994). These ROS have the capability to cause damage to cells by oxidising (removing electrons) components within them. During normal resting respiration, the body's antioxidant defence system can adequately quench the ROS. However, during periods where aerobic metabolism is elevated (for example during soccer) there is a subsequent increase in ROS production (Kanter, 1994). Consequently, the antioxidant defences become saturated and an increase in oxidative stress ensues (Sen, 1995). In addition, the comparatively low antioxidant defences of skeletal muscle (Sen, 1995) make it susceptible to oxidative damage (Armstrong *et al.*, 1991).

The frequent limb accelerations and decelerations during soccer (Reilly and Thomas, 1976; Reilly, 1997) require extensive eccentric muscular activity. Eccentric activity, where the muscle is forcibly extended during activation (Maughan *et al.*, 1989), has

been suggested to cause damage to muscles (Newham *et al.*, 1986) and has been consistently associated with muscle soreness (Maughan *et al.*, 1989; Dekkers *et al.*, 1996; Gulick and Kimura, 1996; Goldfarb, 1999). Consequently, muscle soreness has frequently been used as an indirect marker of muscle damage (for example, Fahey and Pearl, 1998; Thompson *et al.*, 2001). Armstrong *et al.* (1991) hypothesised that initial muscular damage, as caused by eccentric activity, leads to an influx of calcium ions (Ca^{2+}). The resulting loss of Ca^{2+} homeostasis and the subsequent invasion of phagocytic and inflammatory cells are thought to release ROS as by-products (Armstrong *et al.*, 1991). Furthermore, markers of oxidative stress have been demonstrated to increase during eccentric muscular activity (Alessio *et al.*, 1997) and simulated soccer match play (Thompson *et al.*, 2001); consequently, the eccentric component of soccer match play provides an additional foundation for muscle damage during this type of activity.

Exercise protocols designed to simulate soccer match play have been shown to cause increases in blood cortisol concentrations (Thompson *et al.*, 2001), the predominant hormone that regulates normal metabolism and the body's response to stress (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Kelly, 1999). Cortisol has many metabolic functions that include the stimulation of enzymes such as phenylethanolamine *N*-methyltransferase (PNMT), which are responsible for the conversion of noradrenaline to adrenaline within the adrenal gland (Axelrod and Reisine, 1984) and the catabolism of protein through the conversion of protein to glucose by gluconeogenesis (Tortora and Anagnostakos, 1987; McArdle *et al.*, 2000). The increase in blood cortisol concentration during intermittent exercise results in elevated muscle degradation. Furthermore, cortisol exhibits inhibitory effects upon

the inflammatory/immune response (Tsigos and Chrousos, 2002) and consequently any muscle degradation occurring during exercise could, in theory, be left untreated.

Phospholipids are the most predominant component in eukaryotic membranes (Stryer, 1999) and include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) (Vance and Vance, 1996). Phosphatidylserine is found almost exclusively within the inner leaflet of a membrane and is centrally involved in the maintenance of a cell's internal environment by regulating transport across a membrane (Vance and Vance, 1996). Additionally, PS has important roles in signal transduction, secretory vesicle release, cell-to-cell communication, and cell growth regulation (Blokland *et al.*, 1999). Until recently, the prominent source of PS for supplementation was Bovine Cortex (BC-PS); however, the possible transfer of infectious diseases has rendered BC-PS an unsuitable supplement. Soybean-derived PS (S-PS) is an alternative source of PS that has been suggested to produce comparable results to BC-PS (Fahey and Pearl, 1998; Blokland *et al.*, 1999). Furthermore, S-PS has been shown to be a safe nutritional supplement (Jorissen *et al.*, 2002) and is currently commercially available as a memory aid.

Supplementation with BC-PS has been demonstrated to attenuate the loss of neuronal function (such as the cognitive decline of the elderly), and subsequently improve cognitive functions (for example, Villardita *et al.*, 1987; Cenacchi *et al.*, 1993; Blokland *et al.*, 1999). The majority of the available research has focussed on BC-PS supplementation in elderly populations (Palmieri *et al.*, 1987; Villardita *et al.*, 1987; Amaducci, 1988; Crook *et al.*, 1992; Cenacchi *et al.*, 1993); however, more recent

research has suggested that the supplementation of younger subjects with S-PS can reduce stress and enhance mood following exercise (Benton *et al.*, 2001).

In addition, PS supplementation has been shown to attenuate the exercise-induced release of cortisol and its regulatory hormone, adrenocorticotropin hormone (ACTH) (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Fahey and Pearl, 1998). Monteleone and colleagues demonstrated that BC-PS, administered intravenously and orally, blunted the cortisol and ACTH response to intermittent cycling (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992). Oral supplementation with S-PS (800 $mg \cdot day^{-1}$ PS for 14 days) blunted the release of cortisol and ACTH, enhanced perceived well-being, and reduced perceived muscle soreness during a 2 week resistance-training program (Fahey and Pearl, 1998).

Therefore, it is plausible that the stress reducing effects demonstrated by PS may exhibit ergogenic effects on performance during prolonged intermittent activity. Furthermore, recent *in-vitro* investigations have demonstrated a potential antioxidant action of PS (Latorraca *et al.*, 1993; Dacaranhe and Terao, 2001). These findings suggest that PS may have the potential to enhance the *in-vivo* antioxidant defence and, therefore, reduce oxidative stress. However, no published data are available to evaluate this hypothesis.

As previously described, prolonged intermittent running (using a pattern of running designed to simulate soccer match play) has been previously demonstrated to elevate markers of physiological stress, muscle damage, muscle soreness, and oxidative stress. Therefore, the aim of the current study was to investigate the effects of chronic

S-PS (750 $g \cdot day^{-1}$ for 10 days) supplementation on exercise performance and markers of exercise stress, muscle damage, and oxidative stress during and following prolonged intermittent exercise that was designed to simulated soccer match play.

1.1 Null Hypotheses

HO_1 Chronic S-PS supplementation will not affect indicators of muscle damage following prolonged intermittent exercise.

HO_2 Chronic S-PS supplementation will not affect indicators of oxidative stress following prolonged intermittent exercise.

HO_3 Chronic S-PS supplementation will not affect the body's release of cortisol in response to prolonged intermittent exercise.

HO_4 Chronic S-PS supplementation will not affect the body's response to prolonged intermittent exercise.

2 Review of Literature

2.1 Intermittent Exercise (Soccer)

Soccer is a game of immense worldwide popularity; over 200 million players are registered with the game's governing body, the Federation International de Football Association (FIFA, 2003). The financial and commercial opportunities that accompany such worldwide popularity and recognition have led to soccer matches becoming more frequent, with Manchester United Football Club playing in excess of 60 matches in the 2002-2003 season (Manchesteronline, 2003). The intense fixture schedules, coupled with large financial rewards, means that the players are required to produce optimal performances each match, often without sufficient time for the body to fully recover from previous matches.

2.1.1 Physiological requirements of soccer

Many studies have investigated the physiological characteristics of soccer players (Ekblom, 1986; Tumility, 1993; Bangsbo, 1994a; Reilly, 1997) and the energy requirements of soccer matches (Reilly and Thomas, 1976; Bangsbo *et al.*, 1991; Bangsbo, 1994a; Bangsbo, 1994b; Reilly, 1997). Tumility (1993) suggested that soccer players exhibit, in general, a good but not outstanding aerobic capacity. Moreover, Tumility (1993) suggested that $\dot{V}O_{2max}$ values for soccer players average 60 $ml \cdot kg \cdot min^{-1}$, which concurs with work by Bangsbo *et al.* (1991). Additionally, the average work rate during a soccer match has been estimated to be between 70%

(Bangsbo, 1994a) and 75% (Reilly, 1997) of a player's $\dot{V}O_{2max}$. Consequently, it is not surprising that the aerobic energy system is the most prominent source of energy in soccer, appearing to account for more than 90% of total energy consumption (Bangsbo, 1994a).

However, soccer is an intermittent activity; elite players complete approximately 1000 discrete bouts of playing activities during a match (Reilly and Thomas, 1976; Reilly, 1997). Reilly (1997) stated that whilst the majority of exercise in soccer is at submaximal (aerobic) intensity, sprints occur approximately every 90 s, and high-intensity effort occurs approximately once every 30 s. Therefore, a player will utilise anaerobic energy sources, such as phosphocreatine and anaerobic glycolysis, to supply energy for periods of high-intensity, however brief, during soccer match play. Furthermore, such repeated acceleration and deceleration may lead to increased amounts of eccentric muscular contractions, possibly responsible for the increased amount of muscle soreness and damage seen during recovery from simulated soccer matches (Thompson *et al.*, 2001; Wadsworth *et al.*, 2004).

Researchers have used physiological measurements to assess the requirements of soccer match play, with the most frequently used variables being heart rate (HR) and blood lactate concentrations. Reilly (1997) stated an average HR during soccer match play of around 165 beats·min^{-1}, and Ekblom (1986) found a range of 160 – 180 beats·min^{-1}. Bangsbo (1994a) recognised that oxygen uptake ($\dot{V}O_2$) can be directly measured during soccer-related activities; however to directly measure $\dot{V}O_2$ during a soccer match would interfere with normal play (Drust *et al.*, 2000). Subsequently, researchers have used the relationship between HR and $\dot{V}O_2$ to infer the level at

which a player is working during a soccer match. Such estimations have led to a relative exercise intensity of approximately 75% $\dot{V}O_{2max}$ during soccer match play (Ekblom, 1986; Bangsbo, 1994b; Reilly, 1997).

Blood lactate concentration can provide an indication of the amount of anaerobic work that a player performs during a soccer match (Bangsbo, 1994a), although the duration of high-intensity exercise in soccer may be too short to provide a significant increase in blood lactate (Bangsbo, 1994a). Reilly (1997) acknowledged that blood lactate concentrations are not "generally" high, while Ekblom (1986) stated a range of 6 – 10 $mmol \cdot l^{-1}$ for Swedish league players, similar to a range of 7 – 8 $mmol \cdot l^{-1}$ seen in German league players (Gerisch *et al.*, 1988). However, work on Danish league players by Bangsbo *et al.* (1991) found an average blood lactate concentration of 4.4 $mmol \cdot l^{-1}$, and a range of only 2.1 – 6.9 $mmol \cdot l^{-1}$. Moreover, blood lactate concentrations tend to be higher at more intense levels of competition. Ekblom (1986) demonstrated blood lactate levels of Swedish 1st division players to be 9.5 and 7.5 $mmol \cdot l^{-1}$ (for the first and second half respectively), and Swedish 4th division players to be 4.0 and 3.9 $mmol \cdot l^{-1}$. Furthermore, Ekblom (1986) showed 2nd and 3rd division players to exhibit blood lactate concentrations between these groups. Reilly (1997) concluded that the overall anaerobic energy yield during a game is likely to be low and notational analysis work suggests that high-intensity work consists of 8% of total playing time (Bangsbo *et al.*, 1991).

2.1.2 Notational analysis & soccer

Notational analysis studies of soccer matches have used various aspects of the game to quantify a player's work rate. Reilly and Thomas (1976) analysed the distance covered during soccer match play, known as time-distance work. Alternatively, Bangsbo *et al.* (1991) investigated the amount of time spent on different activities, which has been referred to as time-motion analysis. At present, the general consensus within notational analysis is that outfield players cover 8-12 km during a match (Reilly, 1997); however, the distance covered depends upon numerous factors, such as playing position, opposition ability, and team tactics/style of play. The ever-changing tactics and styles of play favoured by coaches, coupled with the contrasting styles of play of different countries and even continents, make it impossible to quantify a solitary value as the average distance covered during a soccer match.

While the distance covered during a match may vary, the amount of high-intensity exercise appears to be more constant (Bangsbo, 1994b). Early work on English League players by Reilly and Thomas (1976) found that, on average, the overall distance covered by outfield players during a match was approximately 10 km, which concurs with more recent work by Reilly (1997). The distance covered in the work of Reilly and Thomas (1976) consisted of 25% walking, 37% jogging, 20% cruising sub-maximally, 11% sprinting and 7% moving backwards. Conducted almost 30 years ago, the work of Reilly and Thomas (1976) is dated, and as such does not, and cannot, reflect the changing styles of soccer match play during the time since its publication. However, this data provides a good example of the activity patterns of English League players, and remains a 'standard' against which other researchers can

compare not only their results but also their procedures (Hughes and Franks, 1997). Subsequently, despite its age, the time-distance work of Reilly and Thomas (1976) should be considered when looking at intermittent exercise.

Time-motion analysis by Bangsbo *et al.* (1991) on Danish League players concentrated on the percentage of total playing time spent on each activity. Bangsbo *et al.* (1991) found that total playing time consisted of 17.1% standing still, 40.4% walking, 35.1% low intensity running (consisting of 16.7% jogging, 17.1% low-speed running and 1.3% backwards running) and 8.1% high intensity running (consisting of 5.3% moderate-speed running, 2.1% high-speed running and 0.7% sprint running). This concurred with the work of Reilly and Thomas (1976) on English league players, as it recognised a significantly greater proportion of time spent on walking and low-intensity running.

Notational work on soccer focuses on categorising a player's movements with regards to walking, jogging, cruising and sprinting, however it should be acknowledged that hidden within these categories are sideways and diagonal movements, as well as skills within the game (such as jumping to win possession of the ball); all of which have energy consuming consequences (Reilly, 1997). Running backwards and sideways lead to elevated energy expenditures in comparison with normal running at the same speeds (Reilly, 1997) and as such should be considered when analysing soccer. Reilly (1997) stated that the added cost of dribbling a ball is 5.2 $kJ \cdot min^{-1}$, however very little distance is actually covered in possession of the ball, leading Reilly (1997) to acknowledge that the majority of activity in soccer match play occurs in the periphery, in the guise of off-the-ball positional movements. Most importantly,

running backwards and sideways leads to increased eccentric muscular activity in numerous different muscle groups, which can in turn lead to elevated soreness and/or damage following exercise.

2.1.3 Simulation of a soccer match

The development of an exercise protocol that accurately simulates the activity patterns and physiological demands of a soccer match is an increasingly prominent area of research (Drust *et al.*, 2000; Nicholas *et al.*, 2000; Wadsworth *et al.*, 2004). However, the simulation of soccer match play remains a relatively new research area, and protocols are therefore not without fault. The random movement patterns during soccer, with the game encompassing in excess of 1000 changes in speed in 90 min of play (Reilly, 1997), makes it difficult for researchers to accurately simulate soccer match play in a controlled environment. Field-based protocols (Nicholas *et al.*, 2000; Wadsworth *et al.*, 2004) and laboratory-based protocols (Drust *et al.*, 2000) do not replicate the game fully, as researchers aim for structure, control and replication within the exercise protocols. The need for structure and control within an exercise protocol can lead to a pay-off in replication, for example neglecting movements such as games skills (e.g. kicking, heading, tackling).

Drust *et al.* (2000) developed a laboratory-based protocol using a motorised treadmill that was designed to represent the work rates observed during soccer match play. The protocol consisted of walking (6 $km \cdot hr^{-1}$), jogging (12 $km \cdot hr^{-1}$), cruising (15 $km \cdot hr^{-1}$) and sprinting (21 $km \cdot hr^{-1}$) for two 22.5-min cycles. Each 22.5-min cycle consisted of 23 discrete bouts of activity: six bouts of walking, six bouts of jogging, three cruises

and eight sprints, arranged in a non-cyclical manner. As the subjects used were university standard soccer players, they can be considered as well familiarised subjects, and such their responses to the protocol can be considered 'normal', with regards to their usual soccer match play, and not 'one-off' responses. The exercise protocol worked players at an average of 68% $\dot{V}O_{2max}$, similar to the 70% $\dot{V}O_{2max}$ stated for soccer match play by Bangsbo (1994a), but was slightly lower than the 75% $\dot{V}O_{2max}$ frequently stated with reference to soccer (Ekblom, 1986; Bangsbo, 1994b; Reilly, 1997). Mean blood lactate concentration of 7.7 ± 0.6 mmol·l^{-1} fell within the range of 6 – 10 mmol·l^{-1} found by Ekblom (1986) and the range of 7 – 8 mmol·l^{-1} suggested by Gerisch *et al.* (1988). However, Bangsbo *et al.* (1991) observed a significantly lower mean blood lactate concentration of 4.4 mmol·l^{-1} (range 2.1-6.9 mmol·l^{-1}). The average HR recorded for the protocol of 168 ± 10 beats·min^{-1} concurred with the average value of 165 beats·min^{-1} stated by Reilly (1997), and fell within the range of 160 – 180 beats·min^{-1} seen during a soccer match by Ekblom (1986). However, the duration (45 min) is only half that of a soccer match (acknowledged by the researchers), and subsequently the physiological demands of a soccer match were not successfully replicated. The duration of the protocol, coupled with the activity being performed on a treadmill, is potentially detrimental to the environmental validity of the protocol, which is concerned with the sum of external influences affecting an individual (Cassell, 1995). With regard to soccer simulation, environmental validity is concerned with how well the whole situation has been replicated, and how realistic the simulation feels to those completing it. Subsequently, it is possible that this laboratory-based protocol sacrifices the degree to which soccer is replicated in favour of structure and control.

The Loughborough Intermittent Shuttle Test (LIST) (Nicholas *et al.*, 2000) is a controlled field test designed to simulate the activity patterns typically seen in soccer match play. The LIST consists of two parts: Part A consisting of 5 x 15-min periods of variable-intensity shuttle running over 20 m, each separated by a 3 min rest period; Part B consisting of continuous running, alternating every 20m between 55% and 95% $\dot{V}O_{2max}$ until volitional exhaustion. Average total distance covered was 12.4 km, similar to an average distance of 12 km stated by Reilly (1997). The average total distance was, however, notably higher than the average values recorded for a soccer match of 10.8 km (Bangsbo *et al.,* 1991) and 10 km (Tumility, 1993). Reilly and Thomas (1976) showed distance covered during a soccer match to vary with position, with values ranging from 8 – 13 km, and Reilly (1997) stated that outfield players cover 8 - 12 km during a match. Nicholas *et al.* (2000) used well-familiarised subjects (University soccer and rugby players) and consequently the exercise protocol can be said to cause 'normal' responses for intermittent exercise. Blood lactate concentration during the LIST reached 7 ± 1.0 mmol·l^{-1} in the 1st 15 min, and maintained this level throughout the test. This blood lactate concentration was similar to those seen during a soccer match (Ekblom, 1986; Gerisch *et al.*, 1988) and below the limit for soccer players of 10 mmol·l^{-1} suggested by Reilly (1997). The blood lactate concentrations observed during the LIST were, however, significantly higher than the average value of 4.4 mmol·l^{-1} (range 2.1-6.9 mmol·l^{-1}) reported by Bangsbo *et al.* (1991). Heart rate (HR) during the LIST was shown to vary between 166 – 176 beats·min^{-1}, similar to the range of 160 – 180 beats·min^{-1} seen during a soccer match by Ekblom (1986) and the average value of around 165 beats·min^{-1} stated by Reilly (1997).

The LIST failed to include a 15-min half-time break, which could decrease the validity of the test with respect to accurate simulation of a soccer match. The half-time break not only allows teams and management a chance to address any tactical shortcomings observed but also presents players with an opportunity to physically and mentally recover. The LIST provides individuals with just a 3-min recovery break after 45 min (half-time), and in doing so denies them the chance to physically recover as they would do in a soccer match, where a 15-min half-time is stated within the rules of the game (FIFA, 2002). During a 15-min half-time recovery period, HR has been shown to return towards baseline values (Wadsworth *et al.*, 2004). Furthermore, the lack of a 15-min half-time has possible negative effects on how realistic the simulation feels to those completing it (the environmental validity).

Wadsworth *et al.* (2004) used a modified version of the LIST protocol (Nicholas *et al.*, 2000) that included sideways running (in the form of a zigzag) and backwards jogging in order to simulate the eccentric component of soccer. The protocol consisted of two parts: Part A consisting of 5 x 15-min periods of variable-intensity shuttle running over 20 m, each separated by a 3 min rest period; Part B consisting of a multi-stage fitness test. Furthermore, this protocol included a 15-min 'half-time' recovery period following 45 min of the protocol, to allow subjects similar recovery time to that of a soccer match, and further simulate the routine of a soccer match. This study used well-familiarised university standard soccer players and consequently the exercise protocol can be said to cause 'normal' responses for intermittent exercise. Mean exercising HR was 152 ± 3 beats·min^{-1}, slightly lower than the range of 160 – 180 beats·min^{-1} seen during a soccer match by Ekblom (1986) and the average value of around 165 beats·min^{-1} stated by Reilly (1997). Average peak blood lactate

concentrations (for all trials) were 5.13 ± 0.33 $mmol \cdot l^{-1}$, slightly below those seen during a soccer match (Ekblom, 1986; Gerisch *et al.*, 1988), and below the limit for soccer players of 10 $mmol \cdot l^{-1}$ suggested by Reilly (1997). The blood lactate concentrations observed were, however, higher than the average value of 4.4 $mmol \cdot l^{-1}$ (range 2.1 - 6.9 $mmol \cdot l^{-1}$) reported by Bangsbo *et al.* (1991). In addition, Wadsworth *et al.* (2004) analysed recovery following exercise, and found the protocol to cause elevated muscle soreness 24 hours post-exercise, and adversely affect sprint performance for 2 days after exercise.

None of these protocols (Drust *et al.*, 2000; Nicholas *et al.*, 2000; Wadsworth *et al.*, 2004) included games skills (e.g. kicking, heading, tackling). This has potential detrimental consequences with regards to environmental validity and replication of the energy demands of soccer match play. However, it is likely that the detrimental effects upon energy demand are negligible, as Reilly (1997) stated that the majority of activity is performed off-the-ball, with the proportion of total distance covered in possession of the ball known to be low, varying between 0.25 and 0.4% (Reilly, 1990). Furthermore, the inclusion of such games skills could make it more difficult to assess the physiological responses during soccer specific exercise under controlled conditions (Drust *et al.*, 2000).

The lack of sideways and backwards movement in both Drust *et al.* (2000) and Nicholas *et al.* (2000) protocols may be much more problematic, limiting how effectively soccer match play is simulated by each protocol, as 16% of the total distance covered during a soccer match is sideways or backwards movement (Reilly, 1990). By leaving such movements out of their respective exercise protocols, Drust *et*

al. (2000) and Nicholas *et al.* (2000) can be said to under-replicate the eccentric component of soccer match play, which is thought to lead to the elevated muscle soreness and adverse performance seen following exercise, such as that exhibited in the 2 days following exercise by Wadsworth *et al.* (2004). Additionally, the exclusion of such movements could compromise the environmental validity of such protocols.

2.2 Acute Responses to Intermittent Exercise

2.2.1 Glucocorticoid responses

Glucocorticoids are a group of hormones that are concerned with normal metabolism and the body's resistance to stress (Tortora and Anagnostakos, 1987; Tsigos and Chrousos, 2002). Cortisol is the most abundant glucocorticoid, and is responsible for approximately 95% of all glucocorticoids' activity (Tortora and Anagnostakos, 1987; McArdle *et al.*, 2000; Tsigos and Chrousos, 2002). Exercise stress has been shown to exert a disruptive influence on normal circadian release of cortisol (Kelly, 1999), while blood cortisol levels have been shown to continually increase until the end of simulated soccer match play, in the form of the LIST (Thompson *et al.*, 2001).

Glucocorticoids participate in the control of whole body homeostasis and regulate the response to exercise stress (Tsigos and Chrousos, 2002), as well as controlling changes in enzymatic activity (Axelrod and Reisine, 1984). Glucocorticoids are catabolic hormones, and during exercise they stimulate protein breakdown (catabolism) to amino acids in all cells except the liver (Kelly, 1999). In the liver, the amino acids may be synthesised into enzymes required for metabolic reactions, such as catecholamine biosynthetic enzymes like phenylethanolamine *N*-methyltransferase (PNMT), responsible for the conversion of noradrenaline to adrenaline within the adrenal gland (Axelrod and Reisine, 1984). Alternatively, the amino acids may be converted to glucose, in a process called gluconeogenesis (Tortora and Anagnostakos, 1987; Kelly, 1999; McArdle *et al.*, 2000), in order to meet the additional metabolic requirements of exercise. Furthermore, glucocorticoids can stimulate the release of

fatty acids from adipose tissue (McArdle *et al.*, 2000), again to meet the additional metabolic requirements of exercise, as well as supporting the gluconeogenic action of hormones such as glucagon and human growth hormone (Kelly, 1999).

The catabolic nature of glucocorticoids means that high concentrations can lead to large amounts of muscle degradation during prolonged exercise, as the need for alternative energy sources increases (Hoffman *et al.*, 1997; Tsigos and Chrousos, 2002). Furthermore, cortisol has inhibitory effects upon virtually all components of the inflammatory/immune response (Tsigos and Chrousos, 2002). Consequently, any muscle degradation occurring during exercise would, in theory, be left untreated. Subsequently, cortisol levels elevated by exercise may result in muscle degradation or damage, which could inhibit a player's performance during a soccer match, and their recovery following match play. The inhibitory effects of cortisol upon the inflammatory/immune response could have far-reaching effects with regards to the elevated production of other potentially damaging reactive oxygen species (ROS), substances that have the potential to damage membranes and other cell components (Hancock, 1999; Urso and Clarkson, 2003). Additionally, cortisol is said to exhibit antireproductive and antigrowth effects (Tsigos and Chrousos, 2002), and as such elevated levels could have negative effects on a player's hormonal balance (homeostasis), and antigrowth effects may inhibit a player's physiological development and recovery from muscle damage.

A variety of stressful events (for example, fright, exercise, temperature extremities, disease, and mental stress) can cause elevated levels of cortisol (Kelly, 1999). However, cortisol response to physical activity can vary depending upon exercise

intensity, duration, participant fitness level and nutritional status (McArdle *et al.*, 2000). Moreover, cortisol output increases directly in relation to exercise intensity (Kelly, 1999). Monteleone *et al.* (1990) showed that cortisol levels increased in 8 familiarised young males during 20 min of intermittent cycling at increasing intensities, and a similar increase was demonstrated in 9 familiarised young males during intermittent cycling of the same protocol (Monteleone *et al.*, 1992). However, 2 weeks of resistive exercise-induced overtraining did not significantly elevate cortisol levels in 11 trained young males (Fahey and Pearl, 1998). In addition, blood cortisol levels of young male subjects have been shown to continually increase until the end of simulated soccer match play, in the form of the LIST (Thompson *et al.*, 2001).

Exercise induced-stress leads to the activation of the hypothalamo-pituitary-adrenal (HPA) axis, which results in elevated levels of cortisol (Axelrod and Reisine, 1984). Exercise induced-stress leads to the hypothalamic secretion of corticotropin-releasing factor (CRF); although, the mechanisms via which stress does this remain unclear (Tsigos and Chrousos, 2002). Corticotropin-releasing factor synthesized by the hypothalamus reaches the pituitary gland by a 'private' portal blood supply, where it stimulates the release of adrenocorticotropic hormone (ACTH) (Axelrod and Reisine, 1984; Tsigos and Chrousos, 2002). This ACTH is then transported in the blood to the adrenal cortex, where the increased concentrations of ACTH subsequently initiate the synthesis and release of cortisol, which consequently initiates its stress-reducing responses (Axelrod and Reisine, 1984; Tsigos and Chrousos, 2002).

Concentrations of ACTH increase with exercise duration if intensity exceeds 25% of $\dot{V}O_{2max}$ (McArdle *et al.*, 2000); however, the release of ACTH is not solely dependant upon CRF (Tsigos and Chrousos, 2002). Multiple factors including CRF, arginine-vasopressin (AVP), catecholamines and possibly other hormones (Axelrod and Reisine, 1984) stimulate the release of ACTH, either individually or interacting in order to achieve optimal results. Tsigos and Chrousos (2002) hypothesised that CRF and AVP have a 'reciprocal positive interaction,' with each neuropeptide stimulating secretion of the other. During acute stress, the amplitude and synchronisation of the CRF and AVP pulsations (in the hypophyseal portal system) increases, resulting in increases of ACTH and cortisol secretory episodes (Tsigos and Chrousos, 2002).

Glucocorticoids not only participate in the control of whole body homeostasis, but also play a key role in the termination of the stress response, by inhibiting the secretion of ACTH (Tsigos and Chrousos, 2002). Glucocorticoids regulate catecholamine biosynthesis (in the adrenal medulla) by releasing amino acids that can then be converted by the liver into catecholamine biosynthetic enzymes such as PNMT, responsible for the conversion of noradrenaline to adrenaline within the adrenal gland (Axelrod and Reisine, 1984). These catecholamines are involved in ACTH regulation, as they stimulate ACTH release (Axelrod and Reisine, 1984). As such, glucocorticoids such as cortisol can inhibit catecholamine synthesis, and subsequently ACTH release (Axelrod and Reisine, 1984); consequently, the HPA axis can be said to have at least some degree of negative feedback control.

The inhibitory glucocorticoid feedback on ACTH acts to limit the duration of total tissue exposure to glucocorticoids. Tsigos and Chrousos (2002) suggest that the acute nature of the HPA axis response to exercise renders its antireproductive, antigrowth, catabolic and immunosuppressive effects temporarily beneficial rather than damaging; however, the immunosuppressive effects of cortisol suggest that any muscle degradation occurring during exercise could be left untreated. This could lead to elevated production of ROS, which may lead to cell membranes being attacked (oxidised), and subsequent muscle damage. This could be inhibitory to a player's performance during a soccer match, and may have negative effects on their recovery post exercise.

2.2.2 Acute heart rate response to exercise

Cardiac output increases in direct proportion to exercise intensity (McArdle *et al.*, 2000), in order to fulfil the aerobic energy requirements of active muscle. To increase cardiac output, the amount of blood ejected with each stroke (stroke volume) and the rate of pumping (heart rate, HR) can both be increased. McArdle *et al.* (2000) stated that a linear relationship exists between HR and oxygen uptake (during increasing intensity exercise) for both endurance athletes & sedentary college students, thus suggesting that such a relationship occurs for most, if not all, individuals. Tsigos and Chrousos (2002) stated that cardiac output is accelerated during stress, and subsequently blood flow is redirected to provide the brain, heart and muscles with fuel. Consequently, an increase in HR is one of the most frequently measured responses to exercise stress.

This increase in HR could be regulated by the HPA axis, as amino acids from proteins broken-down by cortisol may be converted into catecholamine biosynthetic enzymes in the liver. The most important of these with regards to HR is PNMT, which is responsible for the conversion of noradrenaline to adrenaline within the adrenal gland (Axelrod and Reisine, 1984), and as such PNMT regulates the concentrations of adrenaline and noradrenaline. As part of the sympathetic HR control system, elevated levels of adrenaline and noradrenaline cause increased myocardial contractility and accelerate sinoatrial (S-A) node depolarisation, which increases HR (McArdle *et al.*, 2000). Accordingly, an increased level of cortisol, and in turn PNMT and catecholamines, contributes to the increase in HR seen during exercise (Axelrod and Reisine, 1984). Reducing the effect of the sympathetic control system, by suppressing cortisol levels, and in turn PNMT activity, is a method by which HR can be lowered (Tsigos and Chrousos, 2002). However, catecholamines are fast acting hormones, whilst the cortisol response is characterised by a slower increase following the onset of exercise (Tsigos and Chorus, 2002); consequently the benefits of regulating HR in this manner would be limited to the latter stages of exercise, and recovery following exercise.

2.3 Intermittent Exercise and Muscle Damage

2.3.1 Muscle Damage

Eccentric muscular activity, where the muscle is forcibly extended during activation (Maughan *et al.*, 1989), has been suggested to cause damage to muscles (Newham *et al.*, 1986). This damage to muscles is thought to lead to the pain, discomfort and loss of function often experienced after exercise (Newham *et al.*, 1986). Soccer is an intermittent activity, encompassing many discrete bouts of playing activities during a match that involve changing speeds, which requires considerable eccentric muscular activity. Consequently, soccer players are at risk of muscle damage during a game, and simulated soccer match play has been shown to elevate post-exercise markers of muscle damage (for example, Thompson *et al.*, 2001; Wadsworth *et al.*, 2004).

There are four main theories behind the mechanisms of initial muscle damage: (1) torn tissue theory, (2) connective tissue theory, (3) enzyme efflux theory and (4) tissue fluid theory (Gulick and Kimura, 1996). The torn tissue theory suggests that damage occurs at the z-lines, the 'weak link' of the contractile component of muscle (Friden, 1984; Newham *et al.*, 1986). The connective tissue theory focuses on the muscle-tendon junction, as the orientation of the muscle fibres at this point makes these fibres more susceptible to trauma when stretched, as in eccentric activity (Newham *et al.*, 1986). Such muscle trauma disrupts the permeability barrier provided by the sarcolemma, therefore permitting the abnormal diffusion of molecules (for example, calcium ions, Ca^{2+}, and creatine kinase) into or out of the muscle (Armstrong *et al.*, 1991). The enzyme efflux theory suggests that collagen and

protein metabolites, such as creatine phosphokinase, may be released into the extracellular spaces, with the intensity and duration of eccentric activity influencing membrane permeability, and fibre damage. Calcium ions are thought to be released, and subsequently damage the muscle when in direct contact (Gulick and Kimura, 1996). The tissue fluid theory suggests that the accumulation of histamine, kinins and potassium in the interstitial space (between cells and tissue) could disrupt diffusion gradients, thus potentially causing damage, and swelling (oedema) (Gulick and Kimura, 1996).

2.3.2 Oxidative Stress

Oxidative stress refers to an imbalance in the pro- and antioxidant status in favour of the former (Sen, 1995; Urso and Clarkson, 2003). Pro-oxidants are substances that can oxidise (removal of electron(s) from) a substrate. Antioxidants can be defined as any substance that, when present in lower concentrations than the oxidizable substrate, significantly delays or prevents oxidation of the substrate (Sen, 1995). Free radicals are defined as molecules with an unpaired electron in their outer orbital (Kanter, 1994) and, as such, are pro-oxidants. The term reactive oxygen species (ROS) is the collective term for oxygen-based free radicals, such as the hydroxyl radical ($OH\cdot$), and other oxygen derivatives with free radical properties, such as superoxide ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2). All ROS have free radical properties, or serve as precursors for more potent free radicals (Kanter, 1994), and consequently have the capability damage to cells by oxidising components within them. Particularly susceptable are cell membranes (Hancock, 1999), proteins

(enzymes) (Latorraca *et al.*, 1993) and also deoxyribonucleic acid (DNA) (Sen, 1995).

Hydroxyl and $O_2^{\cdot -}$ radicals have the potential to immediately damage whatever they contact (Hancock, 1999). Multiple unsaturation points in polyunsaturated fatty acids (PUFA) make them, and the membranes that they form, highly susceptible to ROS attack and oxidative damage (Sen, 1995). Physical training and acute bouts of exercise are known to decrease phospholipid and PUFA levels in human erythrocyte membranes, and it is likely that this response is due to the lipid peroxidation of the membranes (Sumikawa *et al.*, 1993). Membrane lipid peroxidation occurs when a ROS attacks the lipid membrane of a cell. The by-products of this reaction can be more ROS; H_2O_2, for example, is a by-product of $O_2^{\cdot -}$ oxidising a lipid (Hancock, 1999). In addition, the oxidised lipid then reacts with O_2 to form a lipid peroxy radical, which can react with more lipid molecules, thus propagating a chain reaction that can seriously damage cell membranes and other cell components (Hancock, 1999; Urso and Clarkson, 2003). Lipid hydroperoxide (HPO) is a by-product that is often used as a marker of lipid peroxidation (Urso and Clarkson, 2003).

Armstrong *et al.* (1991) proposed a sequence of damage to recovery hypothesis, suggesting that initial muscular damage disrupts the permeability barrier provided by the sarcolemma, thus permitting abnormal diffusion of molecules (for example, Ca^{2+}, and intramuscular enzymes) down their respective concentration gradients into or out of the cell. The loss of Ca^{2+} homeostasis is prominent, as elevated concentrations of intracellular Ca^{2+} activate numerous proteolytic and phospholipolytic pathways, which degrade structural and contractile proteins and membrane phospholipids

respectively, and subsequently cause further muscle damage. Increased intracellular Ca^{2+} levels inhibit creatine kinase (CK) activity (Armstrong *et al.*, 1991), causing a subsequent rise in extracellular CK; this elevation is associated with, and used as an indirect indicator of, cell membrane damage in muscle (for example, Child *et al.*, 1999; Thompson *et al.*, 2001). Elevated CK activities have been demonstrated following prolonged simulated soccer match play, peaking 24 hours post-exercise in 9 habitually active, familiarised young males (Thompson *et al.*, 2001). Thompson *et al.* (2001) concluded that the simulated soccer protocol increased free-radical production to the extent that antioxidant defences were overwhelmed and damage occurred.

Furthermore, intermittent exercise, such as soccer, elicits a unique HR response, as periods of activity are coupled with periods of inactivity, or recovery periods. Additionally, periods of high-intensity work are often followed by periods of low-intensity work. Consequently, not only does an individual's HR have numerous opportunities to recover (slow-down) but also the constant rise and fall of HR leads to frequent perfusion and reperfusion of muscle cells in particular, and subsequently an inconstant oxygen flux. The inconstant oxygen flux may cause the production of free radicals and ROS (Armstrong *et al.*, 1991), and it could lead to damage to red blood cells, thus releasing the highly reactive free iron ions (FE^{2+} and FE^{3+}) which in turn can lead to more ROS production (Armstrong *et al.*, 1991). Consequently, athletes participating in intermittent exercise could be at risk of damage by ROS, known as oxidative stress (Sen, 1995; Urso and Clarkson, 2003).

2.3.3 Antioxidant defence

It is not only a necessity to stop ROS from engaging in uncontrolled reactions; it is considered a prerequisite for cell survival (Karlsson, 1997). The antioxidant defence system of the body comprisies primarily of scavenger enzymes and carbon-ring (phenol) based compounds such as vitamins A, E, K, beta-carotene and quinol. There are three major antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP). These defend against oxidative stress by reducing the ROS (by donating electrons), and as such are most prevelant in tissues with high oxygen consumption, such as the liver brain and heart (Kanter, 1994). Consequently, antioxidant enzyme concentrations are lower in skeletal muscle, thus making defence systems here more susceptible to be overwhelmed during exercise, and subsequent muscle damage (Kanter, 1994). Phenol-based compounds act in much the same manner, quenching ROS by releasing electrons from the phenol ring, which reduce the ROS. Some antioxidant vitamins (for example, vitamins C and E) have been shown to act together in the form of an antioxidant chain reaction (Goldfarb, 1999).

Despite the 'sequence of damage to recovery' theory for ROS production (Armstrong *et al.*, 1991), the most significant pathway for the production of free radicals (and ROS) within living systems is the one-electron reduction of molecular oxygen (O_2) that occurs in the mitochondrial electron transport system during respiration (Kanter, 1994). When aerobic animals respire, oxygen is reduced to form water (H_2O); during this process adenosine triphosphate (ATP) is produced. Four electrons need to be added to O_2 in order to produce H_2O, but O_2 can only physically receive one electron

at a time. This univalent pathway of O_2 reduction (Kanter, 1994) subsequently leads to the production of ROS.

Under normal respiration, the body's antioxidants can detoxify the ROS created, and thus avoid/limit cellular damage. During exercise, however, there is an elevated metabolism, in order to meet elevated energy requirements, and a subsequent increased production of ROS. In addition, skeletal muscle is highly susceptible to oxidative stress, and subsequent damage, during exercise (Sen, 1995). Simulated soccer match play, in the form of the LIST, has been shown to increase markers of lipid peroxidation in 9 habitually active, familiarised, young males to the extent that antioxidant defences were overwhelmed (Thompson *et al.*, 2001).

In spite of the defence systems of the body, it has frequently been estimated that between 5 and 6% of the total electron flux 'leaks off' to generate ROS (Kanter, 1994; Hancock, 1999). Soccer players consistently work on average at 70% of their $\dot{V}O_{2max}$, and this means that mitochondrial respiration is elevated, to match ATP synthesis to ATP hydrolysis, thus producing more ROS (Armstrong *et al.*, 1991), and hence leading to saturation of the body's defence system. Trained soccer players have demonstrated enhanced antioxidant defences (Brites *et al.*, 1999), yet despite this simulated soccer match play has been shown to elicit oxidative stress (Thompson *et al.*, 2001), suggesting that their enhanced defence is still inadequate during soccer match play.

2.3.4 Delayed Onset Muscle Soreness (DOMS)

Eccentric muscle activity has been consistently shown to cause the greatest post-exercise muscle soreness (Maughan *et al.*, 1989; Dekkers *et al.*, 1996; Gulick and Kimura, 1996; Goldfarb, 1999), a condition known as delayed onset muscle soreness (DOMS). Delayed onset muscle soreness has been shown to peak between 24 and 48 hours after eccentric exercise (Maughan *et al.*, 1989; Gulick and Kimura, 1996) and cease after 5 - 7 days. Intermittent exercise, such as soccer, requires extensive eccentric muscular activity during limb acceleration and deceleration, and soccer has been shown to include up to 1000 changes of activity/speed in a game (Reilly, 1990); as such, soccer players could be at risk of generating DOMS. Furthermore, Wadsworth *et al.* (2004) demonstrated simulated soccer match play to elevate DOMS, measured by perceived muscle soreness, above pre-exercise levels 24 & 48 hours post-exercise in 8 habitually active, familiarised, young males, with levels returning to pre-exercise values after 72 hours.

Delayed onset muscle soreness has been suggested to consist of two phases: (1) muscle destruction caused by excessive force, and (2) secondary cellular damage in response to Ca^{2+} overload induced by tissue trauma (Armstrong *et al.*, 1991; Gulick and Kimura, 1996). Consequently, increased intracellular Ca^{2+} levels, caused by the sequence of damage to recovery hypothesis proposed by Armstrong *et al.* (1991), not only inhibits the activity of intracellular enzymes such as CK but may also be at least partly responsible for the soreness exhibited in young males following prolonged intermittent exercise (Thompson *et al.*, 2001; Wadsworth *et al.*, 2004). Perceived muscle soreness remains the most readily available and accessible method of

assessing DOMS, and has been used to demonstrate DOMS following prolonged intermittent exercise (Thompson *et al.*, 1999; Thompson *et al.*, 2001; Wadsworth *et al.*, 2004). Furthermore, DOMS has been suggested to be a 'manifestation of muscle damage' (Urso and Clarkson, 2003); consequently, perceived soreness is often measured in addition to enzymatic markers of muscle damage such as CK (Thompson *et al.*, 1999; Thompson *et al.*, 2001).

2.4 Phosphatidylserine

Phosphatidylserine (PS) is a glycerol-based phospholipid (a phosphoglyceride), consisting of a glycerol backbone, two fatty acid chains and a phosphorylated alcohol head group (Figure **1**), which in the case of PS is serine. Identified more than 60 years ago by Folch and Schneider (Pepeu *et al.*, 1996), PS is an acidic phospholipid found, in small amounts, in animals, higher plants and micro-organisms (Pepeu *et al.*, 1996). Moreover, PS appears to be essential to the functioning of all mammalian cells (Blokland *et al.*, 1999).

Phosphatidylserine (PS) can be obtained from many sources, and until very recently the predominant source of PS was Bovine Cortex (BC-PS). Studies conducted as recently as 1992 (Monteleone *et al.*, 1992) supplemented with BC-PS; however, the possible transfer of infectious diseases (such as Bovine Spongiform Encephalopathy, BSE, and Creutzfeld-Jakob Disease, CJD) has rendered BC-PS as an unsuitable supplement, especially in humans. Another constraint of BC-PS is that the yield of PS from BC is low (Blokland *et al.*, 1999), although this is of secondary importance to the potential infection of humans. Alternative sources of PS are Soybean-derived PS (S-PS) and Egg-derived PS (E-PS), but these are not identical in structure to BC-PS (Blokland *et al.*, 1999). Phosphatidylserine from these alternative sources has a similar molecular make-up with regards to glycerol moiety and serine head group, but fatty acid composition is different. Soybean-derived PS (S-PS) is virtually devoid of arachidonic and docosa hexenoic acids, whilst BC-PS and E-PS are similarly rich in these fatty acids (Blokland *et al.*, 1999). Despite the different molecular make-up of BC-PS and S-PS, studies have found that supplementing with S-PS produces similar

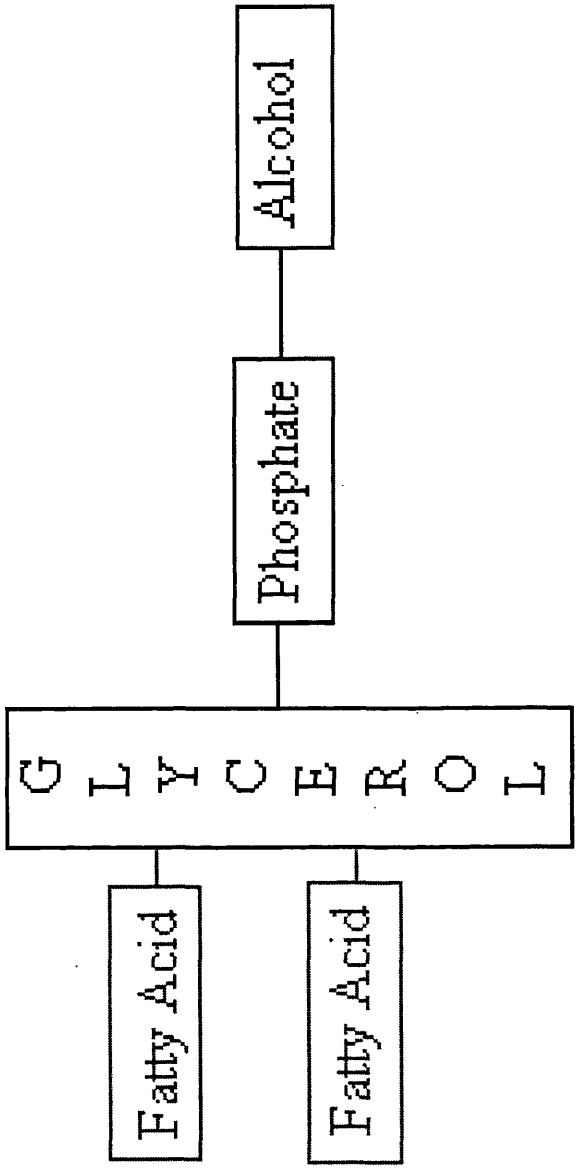

Figure 1. The components of a phosphoglyceride. (adapted from Stryer, 1999)

results as BC-PS with regards to both cognition (Blokland *et al.*, 1999) and recovery after exercise stress (Fahey and Pearl, 1998). Moreover, S-PS has been shown to be a safe nutritional supplement (Jorissen *et al.*, 2002) with a bioavailability that is similar to BC-PS (Blokland *et al.*, 1999).

2.4.1 Phosphatidylserine and cognitive function

Phosphatidylserine is a constituent of the membranes in neurones, and has important roles in their function. Furthermore, PS is involved in the maintenance of a cell's internal environment (by regulating transport across a membrane), signal transduction, secretory vesicle release, cell-to-cell communication, and cell growth regulation (Blokland *et al.*, 1999). Consequently, supplementation with PS is thought to attenuate the loss of neuronal function (such as the cognitive decline of the elderly), and as such improve cognitive functions (Pepeu *et al.*, 1996; Blokland *et al.*, 1999). Numerous studies have investigated the potential cognitive and behavioural benefits of PS; Table **1** summarises the findings of investigations that have used placebo-controlled, double-blind study designs.

Research by Amaducci *et al.* (1988), in which 142 subjects between 40 and 80 years old were treated with 200 $mg \cdot day^{-1}$ BC-PS for 3 months, showed that PS supplementation reduced cognitive decline in the elderly. In addition, memory and verbal ability were significantly improved when compared to placebo, three months after dosing ceased. These findings suggest that PS may have effects after the subject has stopped taking it, possibly as it may become embedded within membranes, and consequently enhance PS concentration within them.

Table 1. Clinical trials investigating the potential cognitive and behavioural benefits of PS.

Study	Subjects (Av. Age) (Years)	Prior Cognitive Decline	Study Design	PS Dosage	Major Inferences
Amaducci *et al.* (1988)	142 (40-80)	Progressive decline.	Double-blind (Placebo-controlled)	200 $mg \cdot day^{-1}$ of BC-PS for 3 months.	Improved memory, verbal ability and daily living. Chronic effects most enhanced.
Benton *et al.* (2001)	17 males (20.6)	None.	Double-blind (Placebo-controlled)	300 $mg \cdot day^{-1}$ of S-PS for 1 month.	Reduced stress and improved mood (possibly due to cortisol inhibition).
Cenacchi *et al.* (1993)	494 (65-93)	Moderate to severe decline.	Double-blind (Placebo-controlled)	300 $mg \cdot day^{-1}$ of BC-PS for 6 months.	Chronic improvements in behaviour, memory, and learning adaptability.
Crook *et al.* (1992)	51 (55-85)	Primary decline.	Double-blind (Placebo-controlled)	300 $mg \cdot day^{-1}$ of BC-PS for 12 weeks.	Mild therapeutic effects, most apparent in subjects with less severe cognitive decline.
Palmieri *et al.* (1987)	87 (73.1)	Moderate decline.	Double-blind (Placebo-controlled)	300 $mg \cdot day^{-1}$ of BC-PS for 60 days.	Positive, chronic effects on memory, learning sociability (behaviour) and daily living.
Villardita *et al.* (1987)	170 (65.7)	Progressive decline.	Double-blind (Placebo-controlled)	300 $mg \cdot day^{-1}$ of BC-PS for 90 days.	Chronic enhancement of memory, learning concentration and verbal ability.

Cenacchi *et al.* (1993) supplemented 425 elderly subjects, all of which suffered from moderate to severe cognitive decline, with 300 $mg \cdot day^{-1}$ BC-PS for 3 months. The Buschke Selective Reminding Test scores of the subjects were significantly improved in favour of PS, indicating an improved memory and learning adaptability. Villardita *et al.* (1987) found similar effects when supplementing 170 subjects, aged 55 – 80 years of age, with 300 $mg \cdot day^{-1}$ BC-PS for 90 days. All subjects suffered from mild to moderate cognitive decline, and supplementation significantly improved memory, learning, concentration and verbal ability. Crook *et al.* (1992) supplemented 51 subjects (55 – 85 years of age) for 12 weeks, and BC-PS was shown to enhance memory and benefit cognitive function.

The work of Cenacchi *et al.* (1993), Crook *et al.* (1992), and Villardita *et al.* (1987) suggested that supplementation with BC-PS may be a feasible treatment to counteract the cognitive decline that accompanies old age, and may also act in the early stages to prevent/limit such decline.

Research by Palmieri *et al.* (1987) replicated the findings of other work (Villardita *et al.*, 1987; Crook *et al.*, 1992; Cenacchi *et al.*, 1993), but did so when supplementing for shorter periods of time. Palmieri *et al.* (1987) supplemented 87 subjects (55 – 80 years of age), all of whom had moderate cognitive deterioration, with 300 $mg \cdot day^{-1}$ of BC-PS for 60 days. Concurrently, BC-PS enhanced memory, and was also found to enhance learning sociability and improve daily living, specifically a lessening of apathy and withdrawal. Consequently, BC-PS may not only be of benefit by reducing cognitive deterioration, but it may also improve the social behaviour of those taking it.

Supplementation with S-PS has been shown to have beneficial effects on younger individuals as well as those exhibited in the elderly. Benton *et al.* (2001) found that supplementing with 300 $mg \cdot day^{-1}$ of S-PS for a month was associated with feeling less stressed and having a better mood in a sub-group of young healthy adults. Fahey and Pearl (1998) showed young males treated with S-PS to display a significantly greater feeling of well being after exercise, measured using a 10 point scale, following administration of 800 $mg \cdot day^{-1}$ of S-PS for two weeks.

2.4.2 Phosphatidylserine and exercise

Physically demanding conditions are known to release stress hormones such as ACTH and cortisol (Kelly, 1999) into the circulation. While this leads to the release of energy that counteracts stress, cortisol has catabolic actions upon protein, which can lead to muscle fibres being broken down (Kidd, 1998). Furthermore, cortisol has also been shown to have inhibitory effects upon the inflammatory/immune response within the body (Tsigos and Chrousos, 2002). Recent research (Table **2**) has shown that PS may attenuate the release of ACTH and cortisol during and following exercise.

Monteleone *et al.* (1990) were among the first researchers to postulate the potential benefits of PS with regards to exercise. Acute supplementation, administered intravenously, with 50 or 75 mg of BC-PS was shown to blunt the stress hormone response to exercise (Monteleone *et al.*, 1990), with cortisol and ACTH being significantly blunted by BC-PS. Chronic oral supplementation of BC-PS (800 $mg \cdot day^{-1}$ BC-PS for 10 days) was also shown to significantly blunt the ACTH and

cortisol responses to physical stress (Monteleone *et al.*, 1992). It is possible that the response achieved with chronic oral supplementation is dose-dependant, as a dose of 400 $mg \cdot day^{-1}$ of BC-PS for 10 days was shown to blunt the cortisol response to physical stress, but this did not reach statistical significance, thus suggesting greater amounts of BC-PS produce greater effects (Monteleone *et al.*, 1992). This research does, however, demonstrate that both single intravenous and chronic oral doses of BC-PS blunt the hormonal response to stress in humans.

Chronic oral supplementation with S-PS has been shown to produce similar effects on the hormonal response to physical stress. Fahey and Pearl (1998) orally administered 11 young males with 800 $mg \cdot day^{-1}$ of S-PS for 14 days, and subjects were required to complete 4 intense resistive training sessions a week. Soybean-derived PS was shown to significantly blunt the rise in cortisol associated with exercise stress (in comparison with placebo), and also blunt ACTH levels. The blunting of the hormonal response to physical stress by S-PS was accompanied with a greater feeling of well being coupled with lower perceived muscle soreness in those treated with S-PS. The greater feeling of well-being exhibited by those treated with S-PS is of particular significance, as it suggests that S-PS blunts the corticosteriod (cortisol) response to exercise stress. Furthermore, it suggests that S-PS does not suppress vital cortisol functions such as the support of gluconeogenesis and suppression of inflammation (Fahey and Pearl, 1998). Subsequently, it can be postulated that S-PS exhibits similar effects to BC-PS with regards to the blunting of the hormonal response to exercise stress.

Table 2. Studies investigating the potential of PS supplementation in attenuating the hormonal response to exercise stress.

Study	Subjects (Av. Age, years)	Study Design	PS Dosage	Exercise type	Major Inferences
Fahey and Pearl (1998)	11 males (22.8)	Double-blind, cross-over	800 mg·day^{-1} of S-PS for 14 days.	Resistance training (2 weeks)	ACTH and cortisol response blunted; Increased feelings of well-being; Reduced muscle soreness.
Monteleone *et al.* (1990)	8 males (32.1)	Double-blind, cross-over	50/75 mg BC-PS (intravenously)	Intermittent cycling (20 min)	Reduced cortisol and ACTH production.
Monteleone *et al.* (1992)	9 males (29.2)	Double-blind, cross-over	400/800 mg·day^{-1} of BC-PS for 10 days.	Intermittent cycling (20 min)	Reduced cortisol and ACTH production at 800 mg·day^{-1} of PS. 400 mg·day^{-1} of PS had no significant effect on cortisol.

2.4.3 Phosphatidylserine and cortisol suppression

Researchers are seemingly in agreement that PS may inhibit the stress-induced activation of the HPA axis (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Pepeu *et al.*, 1996; Fahey and Pearl, 1998). The common consensus is that the mechanism for PS suppression of cortisol is to decrease the concentration of cortisol's principle controlling hormone, ACTH (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Fahey and Pearl, 1998).

It has been hypothesised that PS acts upon CRF receptor interactions, consequently reducing/blunting activation of the HPA axis, and subsequently suppressing ACTH and cortisol levels (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992). It is plausible that PS may alter CRF receptor interactions, and therefore ACTH response to exercise, as exogenous PS appears to interfere with the relative viscosities of lipids within the cell membrane. This could, in turn, affect the position of membrane proteins, and consequently disrupt receptor-ligand interactions (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992). The disruption of such receptor-ligand interactions hypothetically leads to a reduced activation of CRF, and consequently a reduced activation of the HPA axis (Monteleone *et al.*, 1990).

As highlighted by Monteleone *et al.* (1990), it is only possible to hypothesise on the possible mechanisms by which PS blunts the ACTH and cortisol responses to exercise. While the hypothesis that PS blunts cortisol production by altering CRF receptor interactions (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992) remains a plausible one, it is not the sole hypothesis. Other potential mechanisms by which PS

may attenuate cortisol production include the actions of PS upon the neurotransmitter systems involved in the regulation of the HPA axis (Tuomisto and Mannisto, 1985; cited in Monteleone *et al.*, 1992). It has been shown, in rodents, that PS enhances noradrenaline turnover and release (Toffano and Bruni, 1980; cited in Monteleone *et al.*, 1992), which consequently may be responsible for the blunting effect of PS on the hormonal response to exercise stress (Monteleone *et al.*, 1990).

2.4.4 Phosphatidylserine and heart rate

The ability of PS to influence HR recovery after exercise could be of great benefit to intermittent sports such as soccer, which have frequent periods of active and passive recovery; the 15 min half-time break in a soccer match could hypothetically allow for a large degree of recovery in HR. The further an individual's HR can recover during any break, the greater their performance (hypothetically) will be after such a break; as such PS may have ergogenic effects (which may be of greatest benefit in intermittent events).

Investigations on anaesthetized rats have demonstrated that administration of 75 - 150 $mg \cdot kg^{-1}$ of BC-PS (in the form of liposomes) caused a large increase in acetylcholine, the parasympathetic hormone responsible for slowing HR (Casamenti *et al.*, 1979). Consequently, the addition of PS to the body may have direct effects on the central nervous system and HR control, although it is unclear if such an effect is replicated on exercising humans. Furthermore, it is plausible that S-PS supplementation may indirectly affect HR. Phosphatidylserine is known to decrease stress hormone levels during exercise stress (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Fahey and

Pearl, 1998), and this may subsequently reduce the level at which the individual perceives they are working at. Consequently, a reduction in perceived exertion may lead to an autonomic reduction of HR. With no supplementation, no such reduction in perceived exertion would occur, and subsequently no reduction in HR. The effects of S-PS supplementation on an exercising HR are novel, and require further investigation.

2.4.5 Phosphatidylserine, muscle damage and DOMS

PS supplementation has been shown to reduce perceived muscle soreness (Fahey and Pearl, 1998), a frequently used indicator of DOMS. The potential reduction of muscle damage, and subsequent reduction of DOMS, by supplementing with PS has both long and short-term beneficial possibilities. Fahey and Pearl (1998) hypothesised that the suppression of cortisol by S-PS is responsible for the reduction in DOMS, as indicated by a reduced perception of soreness, exhibited in their study. Suppression of cortisol would limit its catabolic effects, which focus particularly upon muscle protein, and subsequently muscle degradation (damage) may be attenuated, or prevented all together (Fahey and Pearl, 1998). In addition, suppression of cortisol would suppress the inhibitory effect of cortisol on the inflammatory/immune response (Tsigos and Chrousos, 2002), theoretically allowing any muscle damage to be treated appropriately.

Moreover, PS may have the potential to prevent/limit muscular breakdown by becoming embedded in the membrane, consequently stabilising it. Such behaviour would also reduce the damage that any ROS may cause by lipid peroxidation of a

membrane, which can propagate a chain reaction that, in turn, can seriously damage cell membranes (Hancock, 1999). Damage to cell membranes may disrupt the permeability barrier provided by the sarcolemma, thus permitting abnormal diffusion of molecules (calcium ions (Ca^{2+}) and intramuscular enzymes) down their respective concentration gradients (Armstrong *et al.*, 1991), something which the stabilising nature of PS might possibly address.

Phosphatidylserine is found predominantly in the inner monolayer of a membrane (Devaux, 1991). The most credible mechanism by which exogenous PS (such as that ingested orally) reaches the inner monolayer is by moving across the bilayer. Carruthers and Melchior (1986; 1988) suggested that lipids (such as PS) can undergo rotational, lateral (along a bilayer), and transverse (across a bilayer) movements. Additionally, Devaux (1991) stated 5 methods for the transverse movement of lipids within biomembranes: (1) simple diffusion, (2) facilitated diffusion, (3) active transport, (4) lipid segregation by pH, and (5) lipid (re)distribution. In the plasma membranes of eukaryotes, the lipid bilayer asymmetry appears to be maintained by active transport of lipids to counterbalance spontaneous lipid diffusion (Devaux, 1991). However, it remains plausible that the transverse movement of PS to the inner monolayer may follow any of the aforementioned methods.

Acidic phospholipids such as S-PS, phosphoglycerol (PG) and phosphatic acid (PA) have the ability to bind free iron, and thus potentially quench ROS (Dacaranhe and Terao, 2001). The polar nature of the serine head group of PS takes up a zwitterion structure, which means that it has separate areas of positive and negative charge (Vance and Vance, 1996). This may be significant in the prevention of muscle

damage during/following exercise where the participant is at risk of oxidative stress, such as a soccer match, as the zwitterionic nature means that S-PS can plausibly act as an antioxidant and thus 'quench' ROS before damage can occur. S-PS could act as an antioxidant because the amide group (NH_3^+), the positively charged section of the serine head group, can feasibly accept free electrons from ROS, thus deactivating them. However, of this group, only S-PS has demonstrated an antioxidant activity in eukaryotic cells (Dacaranhe and Terao, 2001), making it credible that S-PS supplementation may enhance an individual's antioxidant defences. Furthermore, Latorraca *et al.* (1993) have shown exogenous PS to reduce the effects of free radical damage on human cells in in-vitro preparations. Consequently, it is plausible that S-PS may reduce muscle damage in this manner, as shown by reduced muscular soreness (Fahey and Pearl, 1998).

3 Methodology

3.1 Subjects

16 male subjects volunteered to take part in this study, which had Department of Psychology Ethical Committee Approval, University of Wales Swansea (Appendix A). Subjects were all University soccer players, who trained/competed a minimum of 3 times a week. All subjects were non-smokers. The mean ± standard error of the mean (SEM) values for age, height, body mass, and estimated maximal oxygen uptake ($\dot{V}O_{2max}$) of the subjects were 22.12 ± 0.4 years, 1.81 ± 0.02 m, 77.8 ± 1.9 kg, and 53.7 ± 1.3 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively (Table **3**).

All subjects were informed in writing (Appendix B) about the requirements of the study. Furthermore, the demands of the study were fully explained verbally. All subjects subsequently gave written informed consent (Appendix C) and filled in health questionnaires (Appendix D).

3.2 Study Design

Subjects firstly completed a preliminary multi-stage fitness test (MSFT), in order to determine individual $\dot{V}O_{2max}$. Subjects then completed an exercise protocol, designed to simulate soccer match play, on three separate occasions. The first trial was used to familiarise the subjects with the protocol, after which subjects were assigned, in a double blind randomised fashion, to one of two groups (PS or Placebo).

Table 3 Physiological characteristics of the subject groups (mean ± SEM).

	PS group (n = 8)	Placebo group (n = 8)	*P* value
Age (decimal years)	21.7 ± 0.3	22.5 ± 0.7	*P*=0.320
Height (m)	1.82 ± 0.03	1.80 ± 0.04	*P*=0.714
Mass (kg)	81.7 ± 2.4	74.0 ± 2.4	*P*=0.041
Estimated $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	52.8 ± 2.1	54.7 ± 1.7	*P*=0.493

Subjects then completed two main trials (T1 and T2) (Figure **2**), and were administered with S-PS (750 mg·day^{-1}) or a glucose placebo for 10 days prior to T2.

All trials were conducted in the same wooden floored gymnasium. Furthermore, T1 and T2 were completed at a similar time of the morning (within 1 hour), and air temperature and humidity were assessed for all trials using a digital thermometer. In addition, subjects exercised in pairs matched by predetermined $\dot{V}O_{2max}$, consisting of one subject from each group.

3.3 Exercise Protocol

The exercise protocol consisted of two parts, part A and part B. Part A was of a fixed duration (90 min) and consisted of 15 x 4 min bouts of exercise, separated by passive recovery periods. The recovery periods were 1 min in length, with the exception of the 'half time' break, which was 15 min long, and included after 45 min (Figure **3**). Prior to commencing part A, the subjects completed a set warm-up as outlined in Appendix E. Part A of the exercise protocol involved a repeated cycle of exercise (Figure **4**), which consisted of walking, sprinting, zigzag running, jogging (55% $\dot{V}O_{2max}$), backward jogging (55% $\dot{V}O_{2max}$) and cruising (85% $\dot{V}O_{2max}$) for different lengths of time. The running speeds were dictated by timed signals on a CD (audible beeps). The proportion of time and distance for each activity was developed to simulate the physiological demands of a soccer match, thus replicating the amount of time and distance spent on each activity during a game. This was developed with reference to notational analysis work on elite level soccer (Reilly and Thomas, 1976;

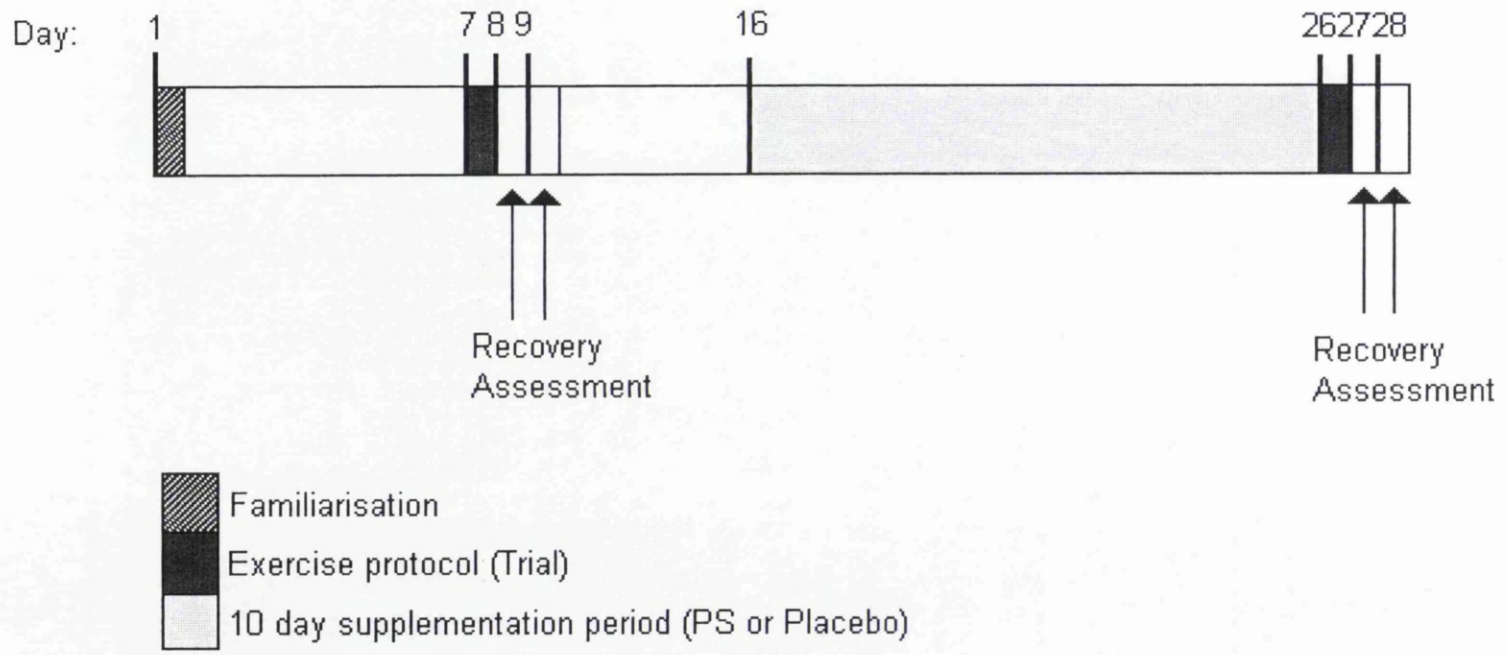

Figure 2 *Schematic time-line of the study.*

Bangsbo *et al.*, 1991; Reilly, 1997) and previous attempts to simulate soccer match play (Nicholas *et al.*, 2000). Part B comprised of the MSFT, a progressive maximal shuttle run test used to assess endurance (Ramsbottom *et al.*, 1988). Part B followed immediately after the 1 min recovery period at the end of part A (on 75 min), as indicated in figure **3**, and was expected to last 10-15 min. Following completion of the protocol, the subjects completed a cool down, which was identical to the warm up (Appendix E).

3.4 Preliminary Measurements

The aerobic fitness of each subject was estimated using the MSFT (Ramsbottom *et al.*, 1988). Subjects ran between two points 20 m apart, and running speeds were dictated by timed signals on a CD (audible beeps). The MSFT continued until volitional exhaustion, or until the subject missed three beeps at the same end. The shuttle level achieved was used to calculate the subject's $\dot{V}O_{2max}$, using linear regression (Ramsbottom *et al.*, 1988). Subjects were assigned to the running speed of exercise protocol closest to their $\dot{V}O_{2max}$, in order to ensure they worked at equivalent relevant exercise intensities.

Immediately prior to familiarisation, subjects' mass (model 712; Seca, Germany) and height (portable stadiometer; Holtain, UK) were measured. Each subject underwent a familiarisation session, in which they completed the exercise protocol, in order to minimise any possible trial-order effect.

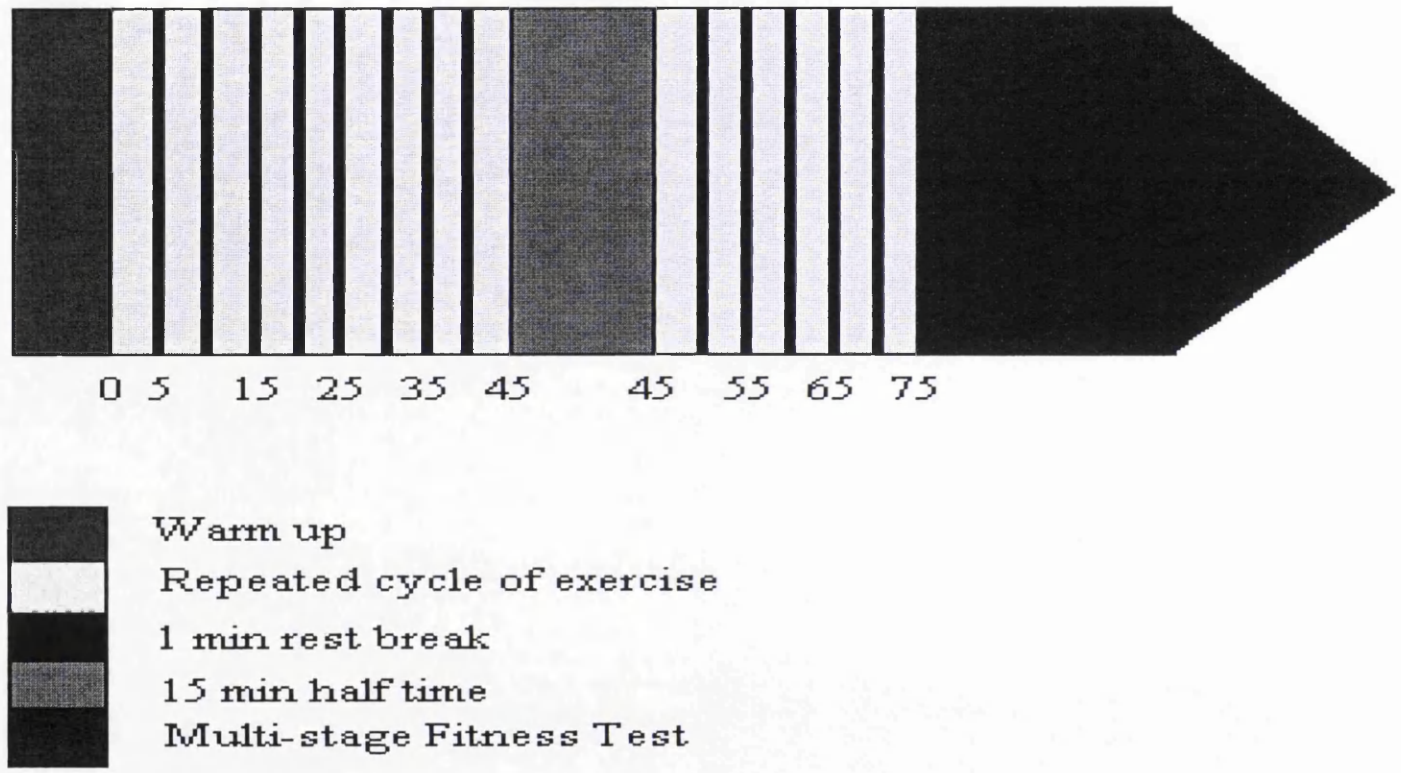

Figure 3 *Diagrammatic representation of exercise protocol.*

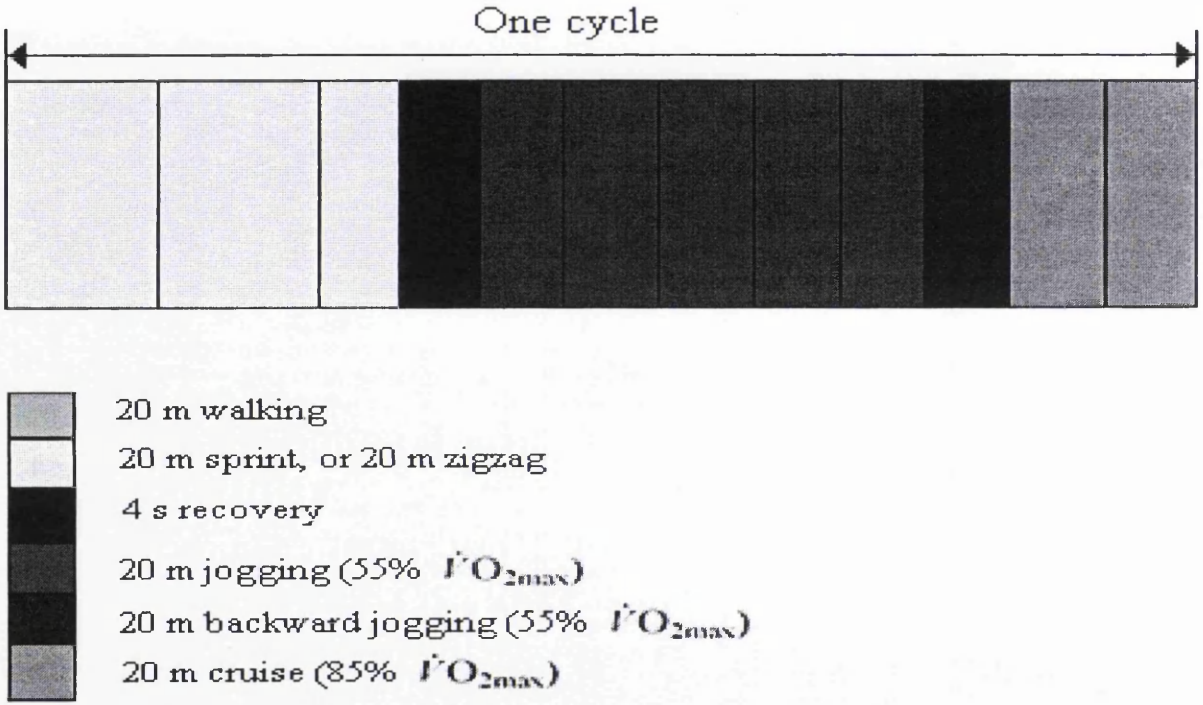

Figure 4 *One cycle of intermittent exercise.*

3.5 Main Trial Procedures

Prior to exercise, the subjects were required to fast for at least 4 hours, and complete a 24-hour dietary record in order to record dietary intake. Subjects were requested to replicate their diet before T1 and T2, and dietary records were undertaken to confirm this. Subjects were also required to abstain from additional exercise and alcohol in the 24 hours prior to exercise, and in the 48 hours post-exercise. For the 10 days prior to T2 subjects were supplemented orally with either 750 $mg \cdot day^{-1}$ of S-PS or a weight matched glucose placebo. The S-PS supplement used in this study was commercially produced by Lucas Meyer, and consisted of a blend of concentrated S-PS and soybean lecithin that gave a final concentration of 20% PS.

Subjects were encouraged to drink water *ad libitum* during their T1. The amount of water consumed in T1 was measured and the subjects given the same amount of water to drink in T2.

Subjects were required to indicate perceived muscle soreness using an 11-point questionnaire (Appendix F) prior to exercise, 20 min after completion, and at 24 and 48 hours after exercise.

Venepuncture blood samples were taken 20 min prior to exercise (PE), and 20 min following completion of exercise (PST). Additional capillary blood samples were collected from a fingertip at half-time (h/t) and immediately following completion of the protocol (Imm PST). Further intravenous blood samples were taken 24 hours (24 Hr) and 48 hours (48 Hr) after exercise (within 1 hour of initial trial time).

During the exercise protocol, heart rate (HR) was recorded every 5 s using short-range telemetry (810s; Polar, Finland). Rate of Perceived Exertion (RPE) was assessed at h/t and upon completion of exercise (Imm PST) using the Borg scale (Appendix G), and ecological validity was assessed upon completion of exercise (Appendix H). Recovery from exercise was assessed 24 Hr and 48 Hr post-exercise, and subjects were instructed to abstain from exercise until this was completed.

3.6 Blood Sampling and Analysis

Venepuncture blood samples were taken by a trained individual, after the subject had been in a seated position for approximately 10 min. Samples were collected from an antecubital vein using a sterile needle (21 gauge precision needle; Becton-Dickinson Ltd, UK) and vacuum tubes, with approximately 30 ml of blood being collected on each occasion.

Approximately 10 ml of blood was collected in an EDTA vacutainer (Becton-Dickinson Ltd, UK). Whole blood was immediately removed and analysed in triplicate for lactate and glucose concentrations, using a YSI 2300 STAT PLUS analyser (YSI inc, Ohio, USA). Triplicate blood haemoglobin concentrations were measured using an automated photometric analyser (Hemocue; UK), whilst triplicate blood haematocrit levels were measured using a haematocrit reader, following 5 minutes of centrifuging (Micro Haematocrit mk IV; Hawksley and Sons Ltd, England). Changes in plasma volume (PV) from pre-exercise values were then calculated according to the method of Dill and Costill (1974).

Approximately 20 ml of blood was collected in Serum Separation Tubes (Becton-Dickinson Ltd, UK), which were left to stand for 30 min. All blood samples (SST and EDTA) were then spun in a centrifuge (Labofuge 200; Kendro, Germany) at 3000 rpm for 15 min. A portion of the blood plasma (100 µl) was added to 900 µl of 10% metaphosphoric acid, mixed, and frozen for vitamin C analysis. Blood serum and plasma samples were then dispensed and immediately frozen at –70°C, prior to further analysis. The process of cell separation was always completed within 1 hour (from the time blood was collected until the time of freezing).

Serum creatine kinase (CK) concentrations were determined using a commercially available reagent (CK-NAC 10 - Ref. A11A00008, ABX Diagnostics, Bedfordshire, UK) and the Cobas MIRA S automated spectrometer (ABX Diagnostics, Bedfordshire, UK). Triplicate measurements were made, and a mean serum CK value calculated for each time point. Total serum cortisol analysis was performed on an AutoDELFIA system (PerkinElmer, Life Sciences), using a time-resolved fluoroimmunoassay. Lipid hydroperoxide (HPO) concentrations were measured using the method of Wolff (1994), as described by McEneny *et al.* (1998). Vitamin C concentrations were determined using a fluorimetric assay, using a centrifugal analyser with fluorescence attachment according to the method of Vuilleumier (1993). Plasma concentrations of vitamin E were measured by liquid chromatography, following the methods of Catignani and Bieri (1983) and Thurnham *et al.* (1988).

Capillary blood samples, taken at half-time (h/t) and immediately post exercise (Imm PST), were collected from a fingertip. The skin on the chosen finger was disinfected

using an alcohol swab, and then pricked using an Accu-check Softclix Pro (Roche Products Ltd, UK) and lancet (Roche Products Ltd, UK). Blood was then collected in a heparinised capillary tube (Hawksley and Sons Ltd, UK), and blood lactate and glucose concentrations were analysed as previously described.

3.7 Statistical Analysis

Statistical analysis was carried out using SPSS (version 11.0; SPSS, IL, USA). All group values are expressed as mean ± standard error of mean (SEM). Significance for all statistical analysis was set at the 5% level ($P<0.05$). Markers of muscle damage, oxidative stress, and cortisol response were compared using a 3-way (trial x time x supplement) mixed model analysis of variance (ANOVA), as were blood lactate and glucose concentrations. If a significant P value was identified for the main effect of time (time of sample), multiple pairwise comparisons were made using Bonferroni confidence interval adjustment. The MSFT performance was compared using a 2-way (trial x supplement) mixed model ANOVA. Independent samples t-tests were used to compare PS and placebo group's characteristics prior to testing. Sprint performance, HR response, and environmental conditions during trials 1 and 2 were also compared using independent samples t-tests. In all cases, a significant interaction effect ($P<0.05$) was taken to signify that the PS group reacted differently to the placebo group.

4 Results

4.1 Environmental Conditions

There were no significant differences in the ambient temperature (trial effect, P=0.79) or humidity (trial effect, P=0.41) between trials, with ambient temperatures being 19.6 ± 0.5 and 21.4 ± 0.4 °C, and humidity 50.5 ± 1.8 and 53.9 ± 1.9%, during trial 1 and 2 respectively. As the subjects ran in pairs of mixed groups (one PS with one placebo) there were no differences between the environmental conditions for supplementation groups.

4.2 Heart Rate Response to Exercise

Figure **5** demonstrates the HR trace for a representative subject throughout the exercise protocol, where the minimum and maximum values were 93 and 204 beats·min^{-1}, respectively. Peak HR occurred upon completion of part B of the protocol for all subjects (Figure **5**). Table **4** displays mean heart rate (HR) values for the supplementation groups. There was no significant difference between the mean HR (of all subjects) in trials 1 and 2 (2 way interaction effect, P=0.687). Peak HR were similar during trials 1 and 2 (trial effect, P=0.805) in both supplementation groups (Table **4**). Furthermore, no significant trial x supplement interaction on peak HR was identified (P=0.626).

Mean h/t HR data were similar during trials 1 and 2 (temporal effect, P=0.961) in both supplementation groups (Table 4); mean h/t HR for all trials being 100 ± 4 beats·min^{-1}. Furthermore, there was no significant trial x supplement interaction on mean h/t HR (P=0.831).

Table 4 Heart Rate (HR) measures for PS and placebo groups.

Supplement Group	Trial	Mean HR ($beats \cdot min^{-1}$)	Peak HR ($beats \cdot min^{-1}$)	Mean h/t HR ($beats \cdot min^{-1}$)
PS	1	143 ± 6	196 ± 4	99 ± 5
PS	2	142 ± 5	196 ± 4	101 ± 5
Placebo	1	144 ± 5	196 ± 2	99 ± 6
Placebo	2	142 ± 7	195 ± 3	99 ± 7

Values represent mean ± SEM (n for PS/placebo = 8)

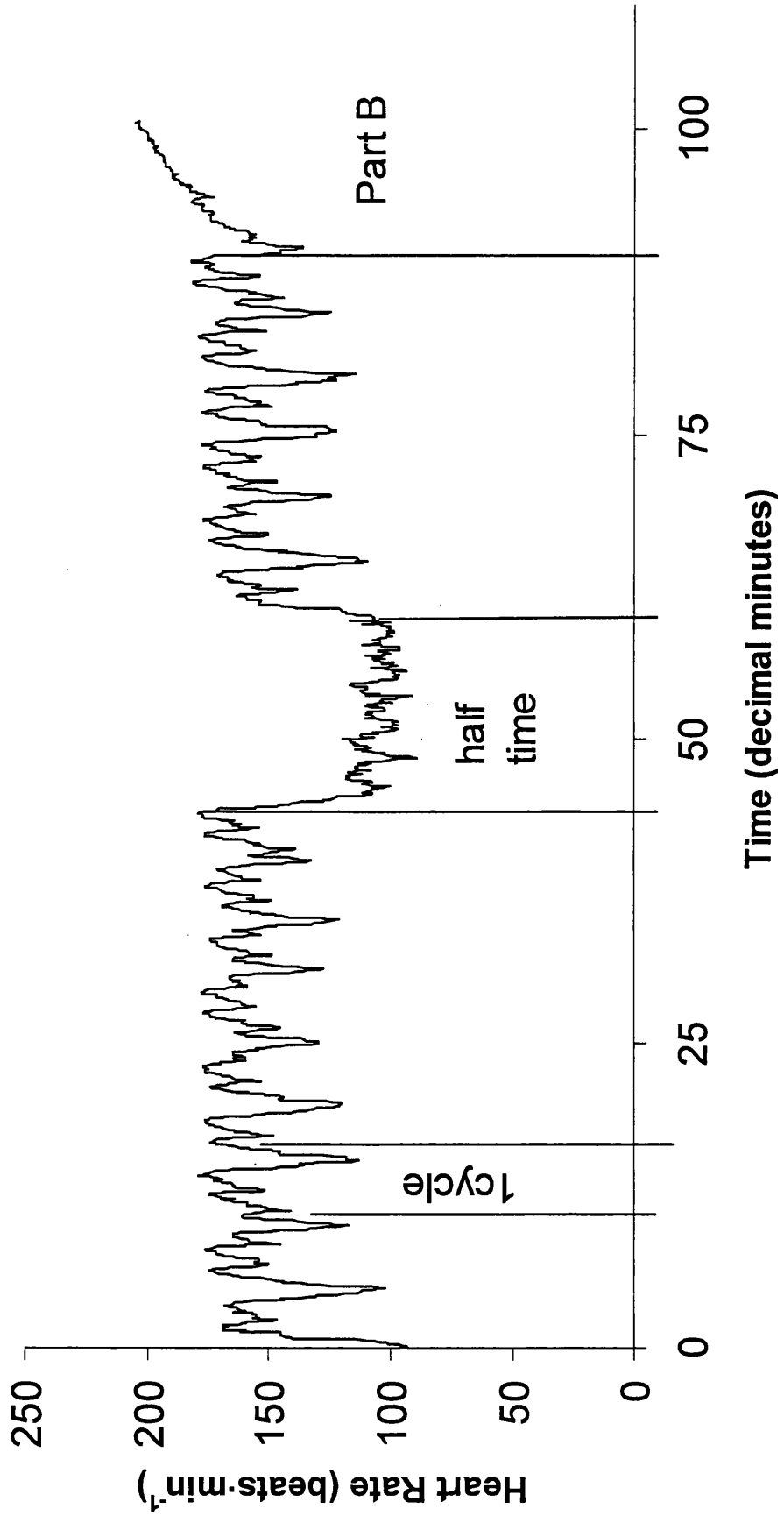

Figure 5 *Example Heart Rate (HR) trace for the intermittent exercise protocol (Subject 7, trial 1). Values presented as 5 s average HR.*

4.3 Exercise Performance

Significant rises in Rate of Perceived Exertion (RPE) were seen from half time to full-time during all trials (temporal effect, $P<0.001$). Furthermore, RPE was highest at full-time for both PS, being 18.0 ± 0.7 and 17.8 ± 0.8 for trials 1 and 2 respectively, and placebo, being 17.4 ± 0.5 and 17.1 ± 1 for trials 1 and 2 respectively. Supplementation with PS had no significant effect upon RPE (trial x time x supplement interaction, $P=0.515$) at any point during or after exercise.

Individuals in the PS group performed (on average for all trials) 16% better than those taking a placebo (Figure **6**), mean MSFT decimal level scores for trial 2 being 11.6 ± 0.7 and 10.9 ± 0.8 respectively. The trial x time x supplement interaction approached significance ($P=0.082$).

The mean sprint speeds and peak sprint speeds for PS and placebo groups during trial 1 and trial 2 are shown in Table **5**. Supplementation with PS had no significant effect upon mean sprint speed (trial x supplement interaction, $P=0.273$) or peak sprint speed (trial x supplement interaction, $P=0.370$).

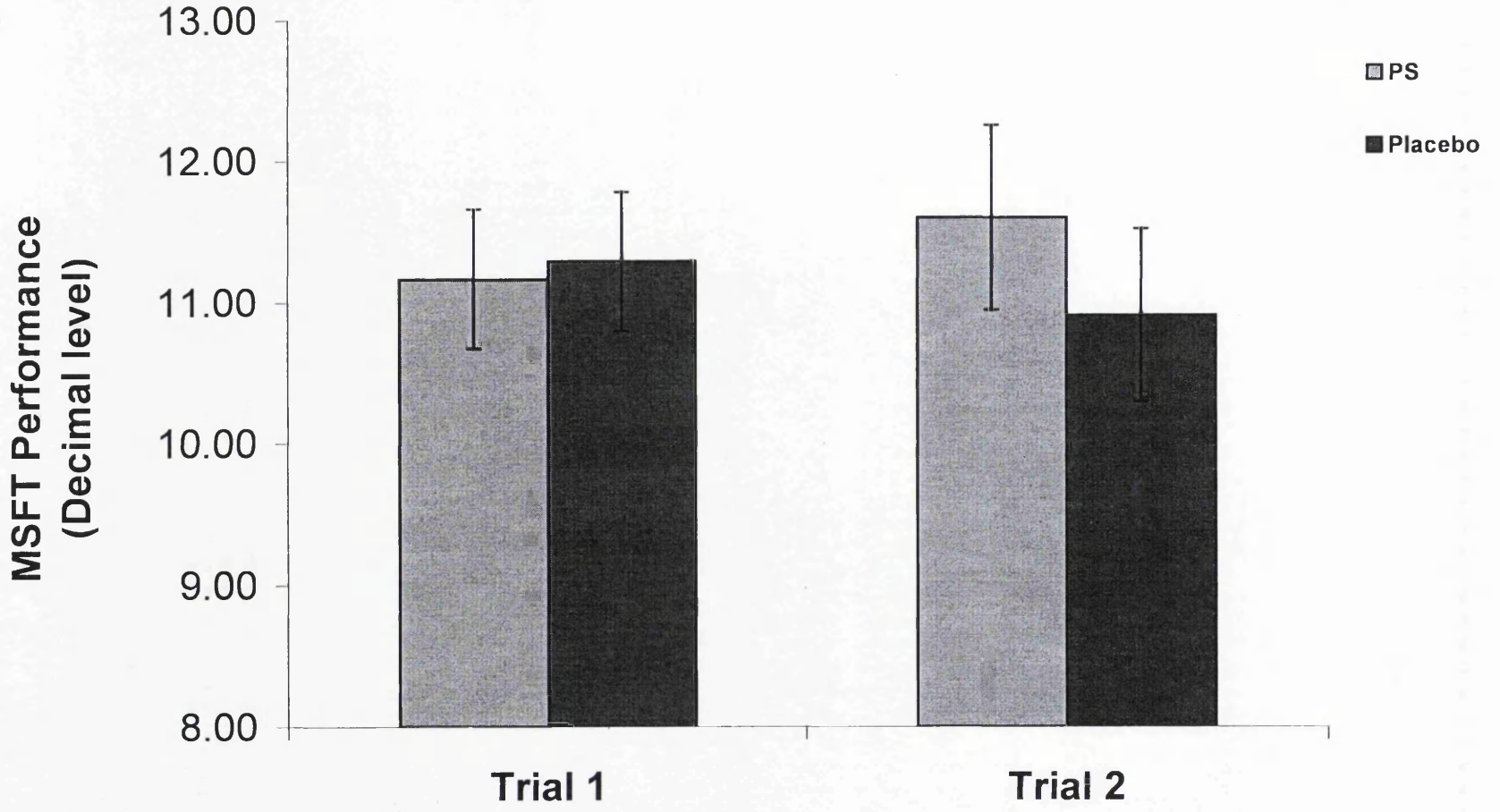

Figure 6 *Multi-stage Fitness Test (MSFT) performance for all trials and all groups. Values represent mean ± SEM (n PS/placebo = 8)*

Table 5 Peak and mean sprint speeds for individual supplement groups.

Supplement Group	Trial	Mean Sprint Speed ($m \cdot s^{-1}$)	Peak Sprint Speed ($m \cdot s^{-1}$)
PS	1	5.66 ± 0.14	5.99 ± 0.20
PS	2	5.83 ± 0.14	6.13 ± 0.17
Placebo	1	5.71 ± 0.14	6.05 ± 0.18
Placebo	2	5.72 ± 0.12	6.20 ± 0.13

Values represent mean ± SEM (n for PS/placebo = 6)

4.4 Blood Analysis

Calculated changes (from pre-exercise trial 1 values) in plasma volume (PV) were similar for both PS and placebo groups (Table **6**), and no significant trial effect (P=0.351) or supplement effect (P=0.651) were identified.

Blood lactate concentrations were significantly elevated by exercise (temporal effect, P<0.001) and peaked immediately post exercise (Figure **7**). Peak values for trial 1 were similar for both groups, being 6.0 ± 0.3 and 6.6 ± 0.6 $mmol \cdot l^{-1}$ for PS and placebo respectively. Additionally, trial 2 peak lactate values were similar for PS and placebo groups, being 6.1 ± 0.4 and 5.7 ± 0.3 $mmol \cdot l^{-1}$, respectively. No significant trial x time x supplement interaction was identified (P=0.209).

Blood glucose was significantly elevated by exercise (temporal effect, P<0.001), and peaked immediately post-exercise (Figure **8**). Peak blood glucose concentrations were 5.4 ± 0.2 and 5.3 ± 0.3 $mmol \cdot l^{-1}$, for PS and placebo respectively, during trial 1, and 5.3 ± 0.3 and 5.1 ± 0.2 $mmol \cdot l^{-1}$, for PS and placebo respectively, during trial 2. Blood glucose concentrations had returned to pre-exercise levels 20 minutes after exercise (Figure **8**). The trial x time x supplement interaction was not significant (P=0.815).

Table 6 Calculated change in plasma volume (from pre-exercise trial 1 values) following prolonged intermittent exercise and recovery.

Supplement Group	Trial	PV PE	PV PST	PV 24 Hr	PV 48 Hr
PS	1	-	2.1 ± 2.6	6.4 ± 3.8	4.1 ± 2.3
PS	2	-3.7 ± 2.1	-2.6 ± 1.5	0.7 ± 1.7	3.9 ± 3.1
Placebo	1	-	5.1 ± 1.4	6.5 ± 1.8	6.5 ± 2.1
Placebo	2	0.8 ± 2.5	3.8 ± 2.0	3.7 ± 2.2	5.4 ± 0.7

Values represent mean± SEM (n for PS/placebo = 8)

PE – Pre Exercise

PST – 20 min Post Exercise

24 Hr – 24 Hr Post Exercise

48 Hr – 48 Hr Post Exercise

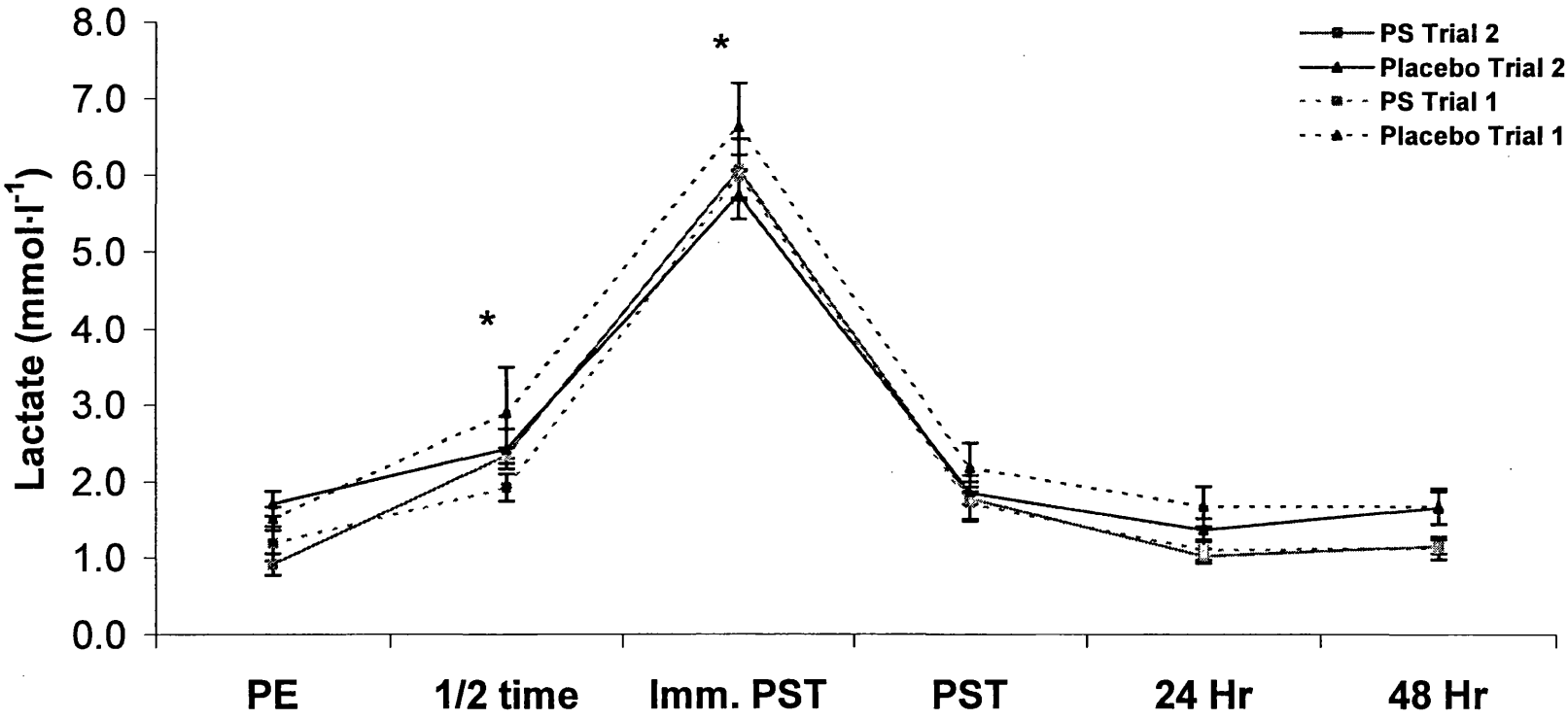

Figure 7 *Blood lactate concentration during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/placebo = 8).* * *All groups significantly different from pre-exercise values (temporal effect, P<0.001).*

Imm. PST – Immediately Post Exercise

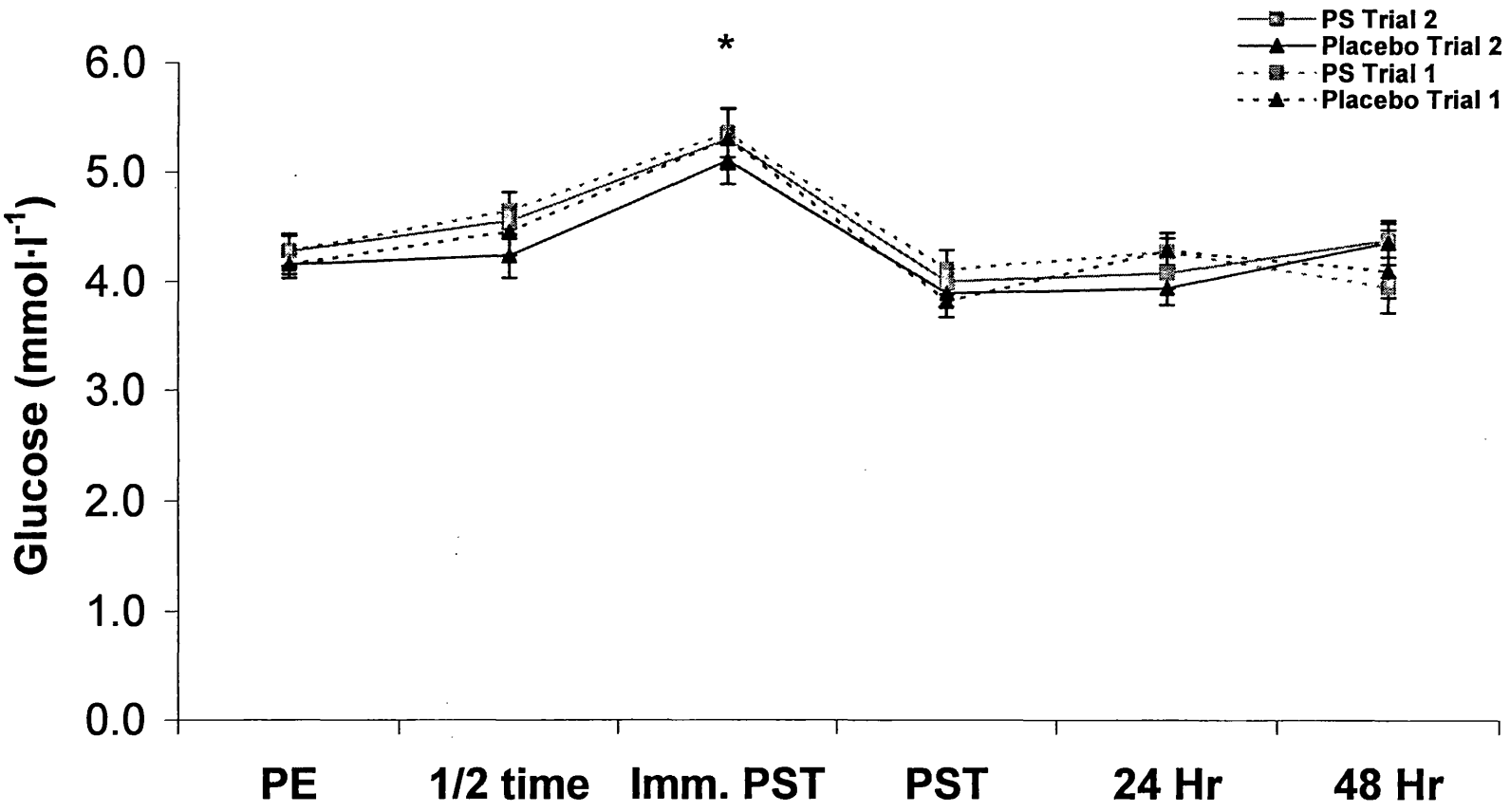

Figure 8 *Blood glucose concentration during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/placebo = 8).* ***** *All groups significantly different from pre-exercise values (temporal effect, P<0.001).*

4.5 Cortisol Response to Exercise

Serum cortisol concentration increased with exercise (temporal effect, $P<0.001$), and peaked 20 minutes post-exercise (Figure **9**). Pre-exercise cortisol levels in trial 1 were 386 ± 25.0 and 397 ± 11.5 $nmol \cdot l^{-1}$, and peak values 489 ± 69.8 and 591 ± 31.9 $nmol \cdot l^{-1}$, for PS and placebo respectively. Pre-exercise cortisol concentrations in trial 2 were 348 ± 38.6 and 470 ± 51.7 $nmol \cdot l^{-1}$, and peak values 470 ± 47.2 and 507 ± 36.2 $nmol \cdot l^{-1}$, for PS and placebo respectively.

Cortisol concentrations were significantly below pre-exercise levels 24 hours after exercise for all subjects. However, individuals supplemented with PS demonstrated a possible enhanced recovery (trial x time x supplement interaction, $P=0.199$). Cortisol concentration decreased on average by 47.2% for the PS group, compared with an average of 32.9% for the placebo group (Figure **9**).

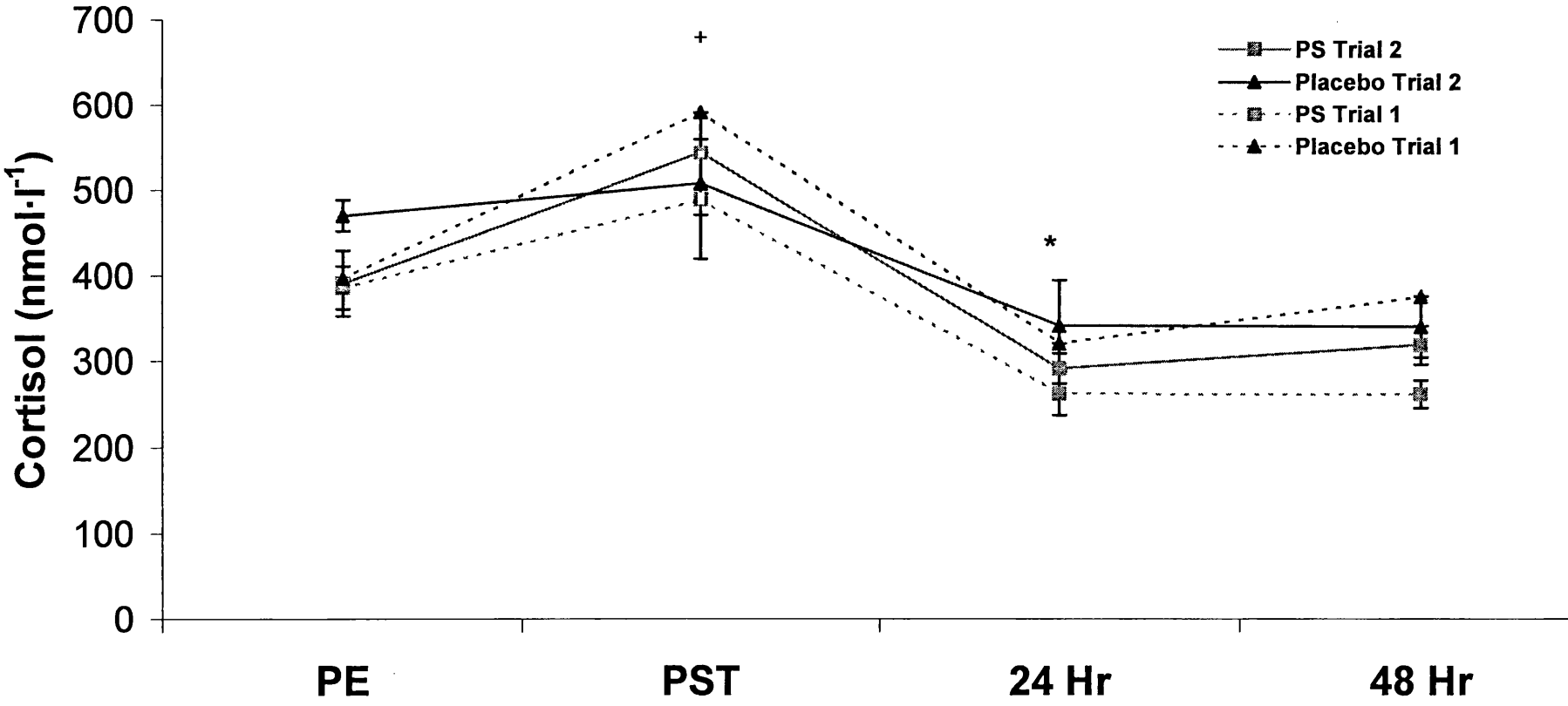

Figure 9 *Blood cortisol concentrations during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/placebo = 8).* ***** *All groups significantly different from pre-exercise/post-exercise values (temporal effect, P<0.001).* $^{+}$ *PS Trial 1 & 2/Placebo Trial 1 significantly different from pre-exercise values (temporal effect, P<0.001).*

4.6 Indicators of Muscle Damage

Serum creatine kinase (CK) activities were significantly elevated (temporal effect, $P<0.001$) 20 minutes after exercise in all trials, and peaked after 24 hours of recovery (Figure **10**). Peak values during trial 1 were 627 ± 122 and 743 ± 142 $U \cdot l^{-1}$ for PS and placebo respectively; peak values during trial 2 were 476 ± 98.3 and 413 ± 53.0 $U \cdot l^{-1}$ for PS and placebo respectively. Serum CK activities had returned to pre-exercise levels 48 hours after exercise in all trials (Figure **10**). No significant trial x time x supplement interaction was identified ($P=0.674$).

Perceived general muscle soreness was significantly elevated by exercise (temporal effect, $P<0.001$) and peaked 20 minutes after exercise (Figure **11**). Peak soreness levels during trial 1 were 3.6 ± 0.6 and 5.1 ± 0.6, for PS and placebo respectively; peak soreness levels during trial 2 were 2.6 ± 0.7 and 3.7 ± 0.6, for PS and placebo respectively. Soreness levels had returned to pre-exercise levels 48 hours after exercise (Figure **11**). Individuals supplemented with PS tended to report lower levels of soreness than those supplemented with a placebo, being on average 9 and 31% lower during trial 2, after 24 and 48 hours of recovery respectively. No significant trial x time x supplement interaction was identified ($P=0.616$).

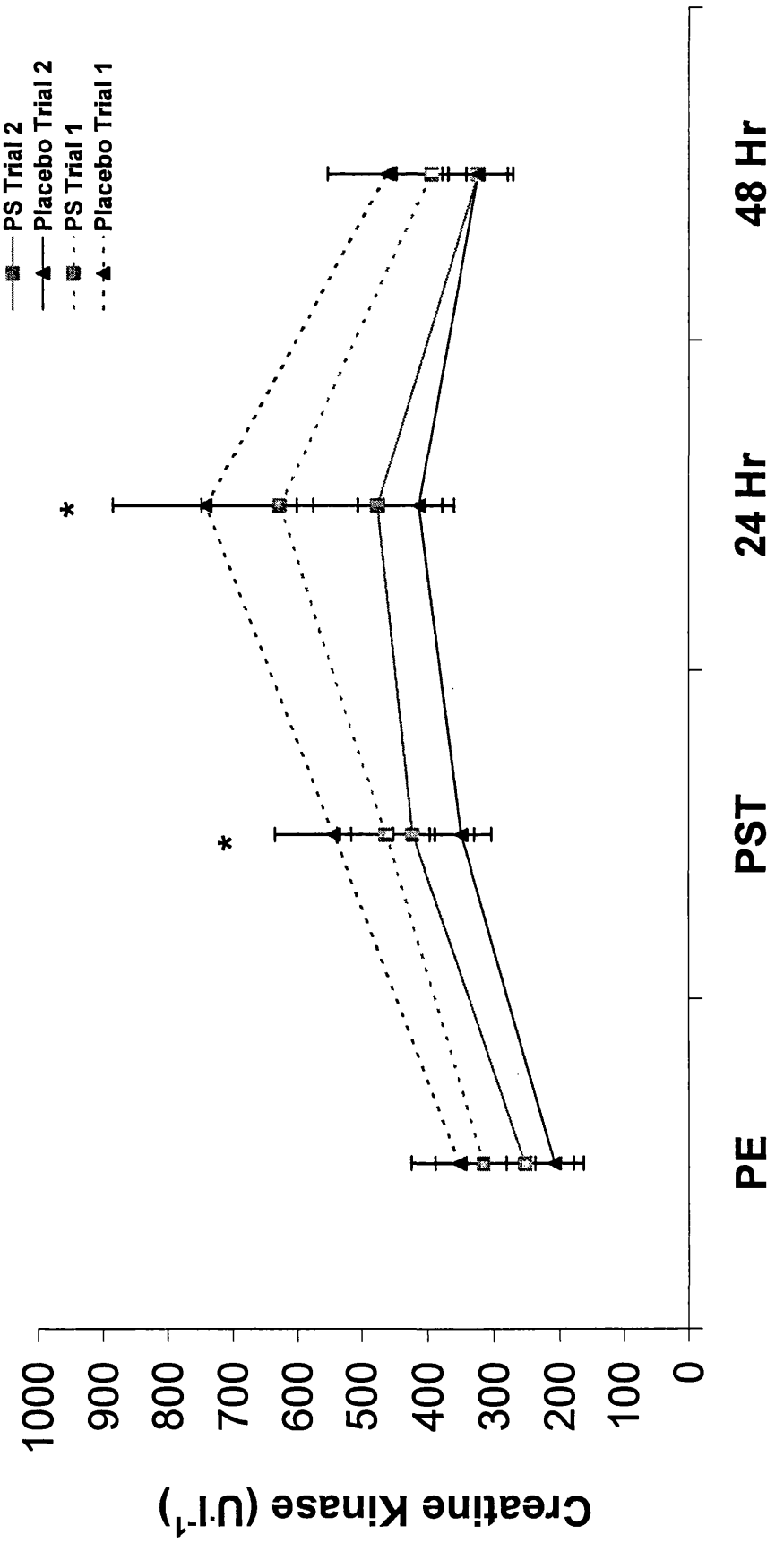

Figure 10 *Serum creatine kinase concentrations during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/placebo = 8).* * *All groups significantly different from pre-exercise values (temporal effect, P<0.001)*

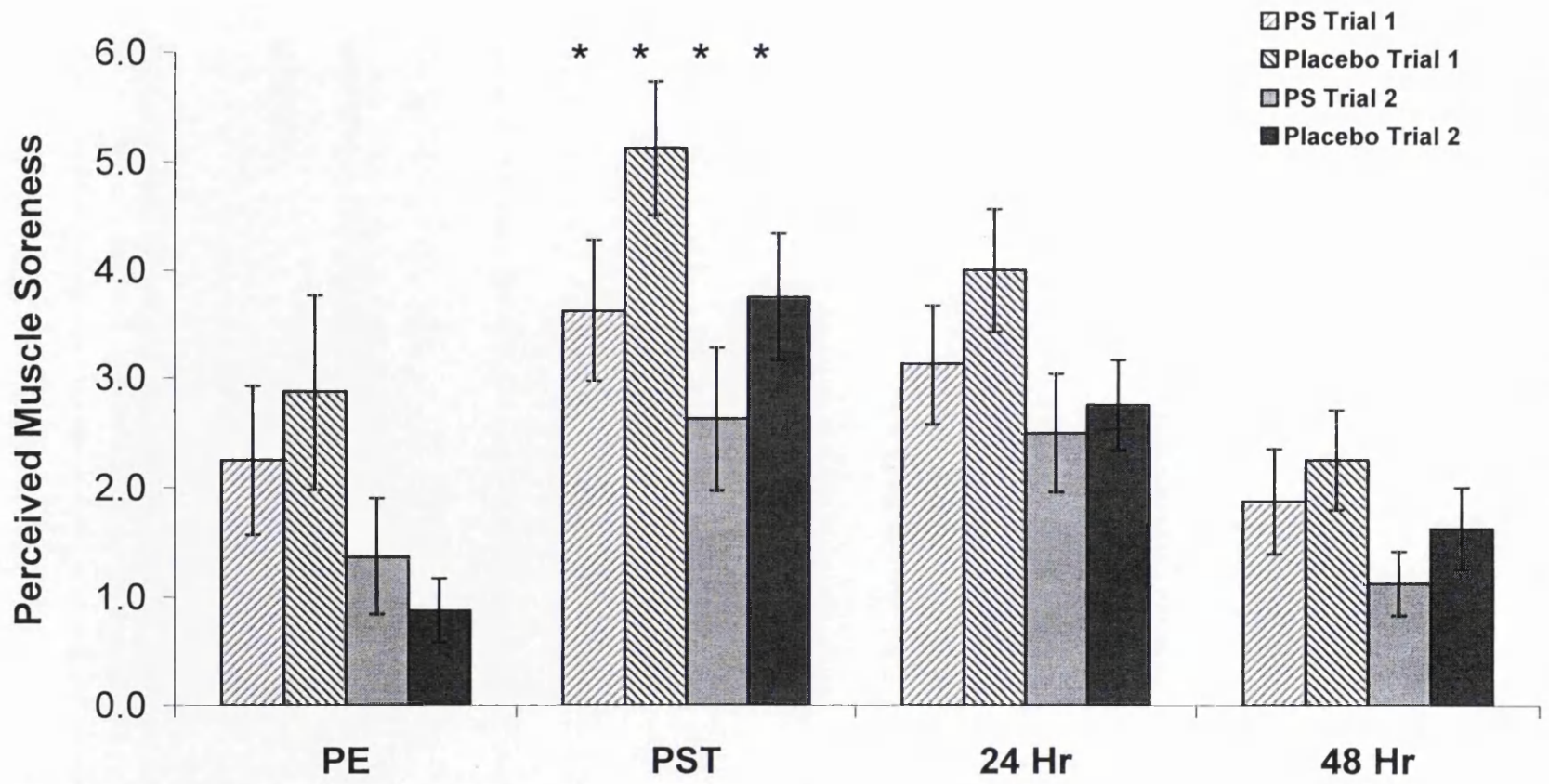

Figure 11 *Perceived general muscle soreness prior to and following prolonged intermittent exercise, and during recovery. Values represent mean ± SEM (n for PS/placebo = 8). * Significantly above pre-exercise values (temporal effect, P<0.001)*

4.7 Indicators of Oxidative Stress

Serum lipid hydroperoxide (HPO) concentrations were significantly elevated by exercise (temporal effect, $P<0.001$), and peaked 20 minutes after exercise (Figure **12**). Peak HPO concentrations during trial 1 were 1.11 ± 0.1 and 0.99 ± 0.1 $\mu mol \cdot l^{-1}$, for PS and placebo respectively; peak HPO concentrations during trial 2 were 1.15 ± 0.1 and 0.98 ± 0.1 $\mu mol \cdot l^{-1}$, for PS and placebo respectively. HPO concentrations had returned to pre-exercise levels within 24 hours of exercise (Figure **12**), and no significant trial x time x supplement interaction was identified ($P=0.495$).

Plasma vitamin C concentrations increased with exercise (temporal effect, $P<0.001$), and were significantly elevated 20 minutes after exercise for all subjects (Figure **13**). Peak plasma vitamin C concentration occurred 24 hours post-exercise for the placebo group, yet did not peak until 48 hours post-exercise in the PS group (Figure **13**). Peak plasma vitamin C concentrations during trial 1 were 45.4 ± 4.9 and 50.9 ± 7.8 $\mu mol \cdot l^{-1}$, for PS and placebo respectively; peak plasma vitamin C concentrations during trial 2 were 42.3 ± 4.5 and 44.8 ± 6.5 $\mu mol \cdot l^{-1}$, for PS and placebo respectively. No significant trial x time x supplement interaction was identified ($P=0.568$).

Plasma vitamin E concentrations were unaffected throughout the trial (Figure **14**), with only PS trial 1 and placebo trial 2 being significantly elevated from pre-exercise values (temporal effect, $P=0.022$). Peak plasma vitamin E concentrations occurred 20 minutes post exercise for all subjects (Figure **14**). Peak plasma vitamin E

concentrations during trial 1 were 28.4 ± 1.8 and 27.9 ± 1.4 $\mu mol \cdot l^{-1}$, for PS and placebo respectively; peak plasma vitamin E concentrations during trial 2 were 29.6 ± 1.5 and 28.5 ± 1.9 $\mu mol \cdot l^{-1}$, for PS and placebo respectively. No significant trial x time x supplement interaction was identified (P=0.600).

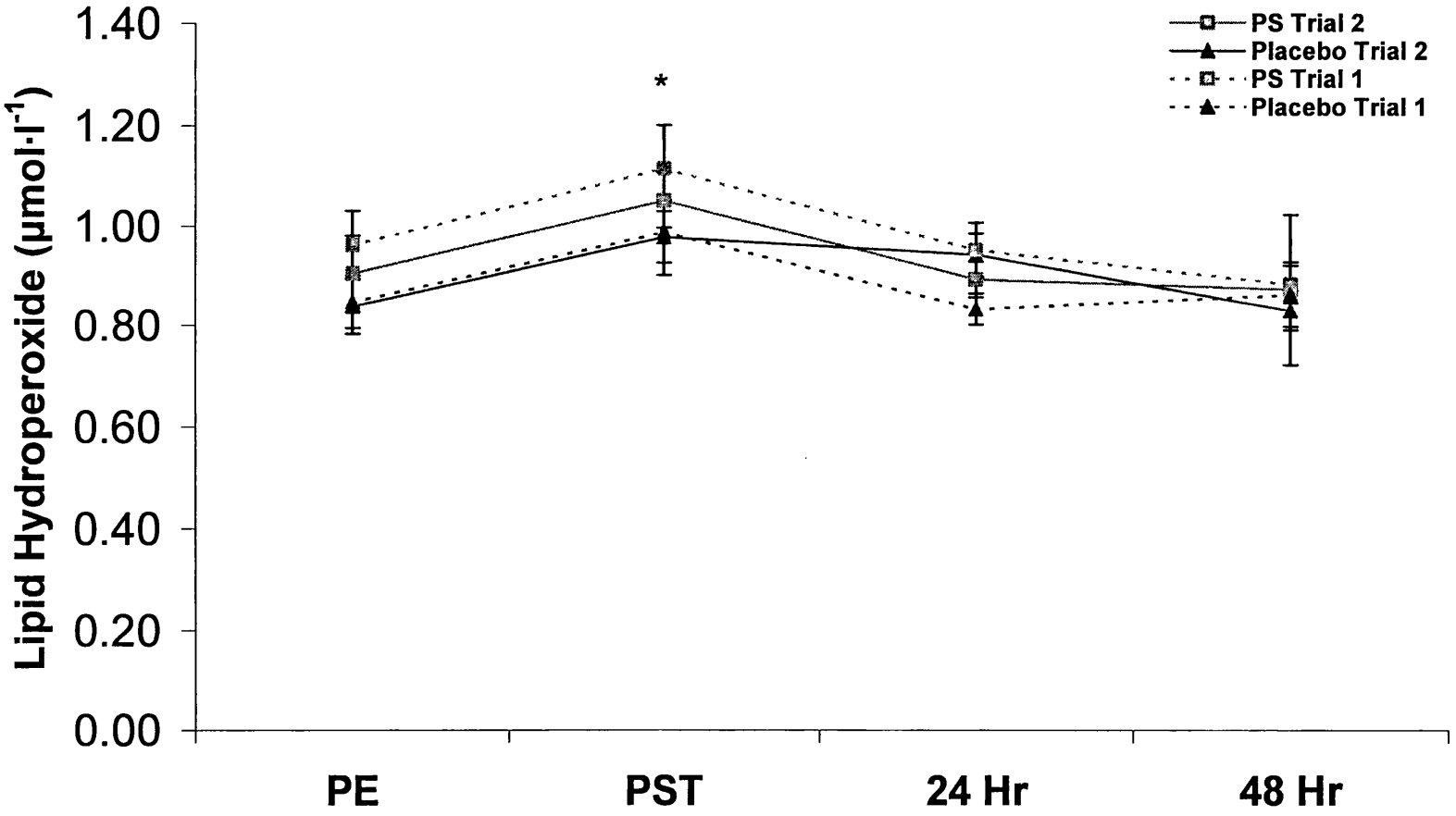

Figure 12 *Lipid Hydroperoxide (HPO) concentrations during prolonged intermittent exercise and recovery.*

Values represent mean ± SEM (n for PS/placebo = 8). ***** *All groups significantly above pre-exercise values (temporal effect, P<0.005)*

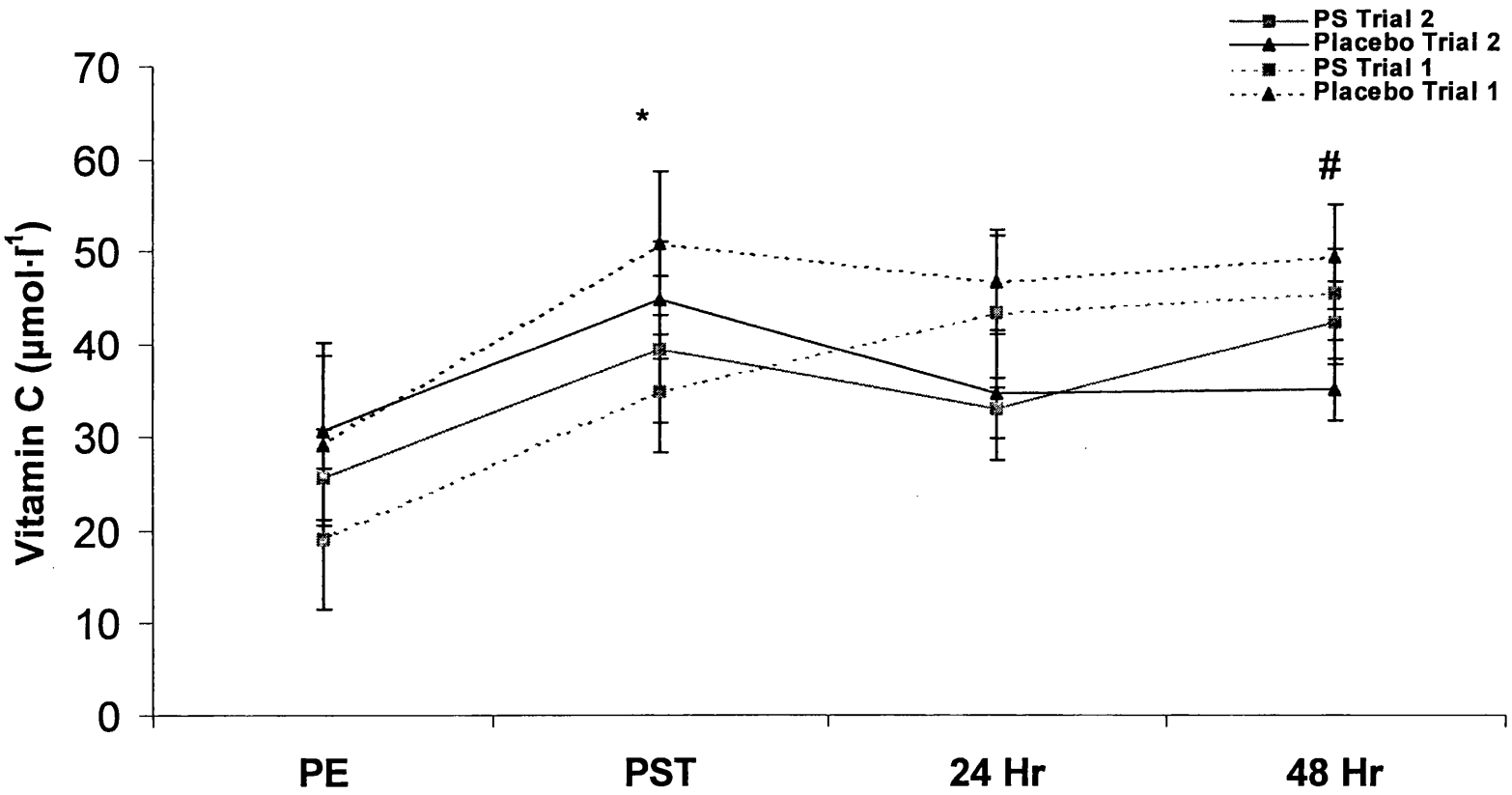

Figure 13 *Vitamin C concentrations during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/Placebo = 8).* * *All groups significantly different from pre-exercise values (temporal effect, P<0.001).* # *Placebo trial 1/PS trial 1 and trial 2 significantly different from pre-exercise values (temporal effect, P<0.001).*

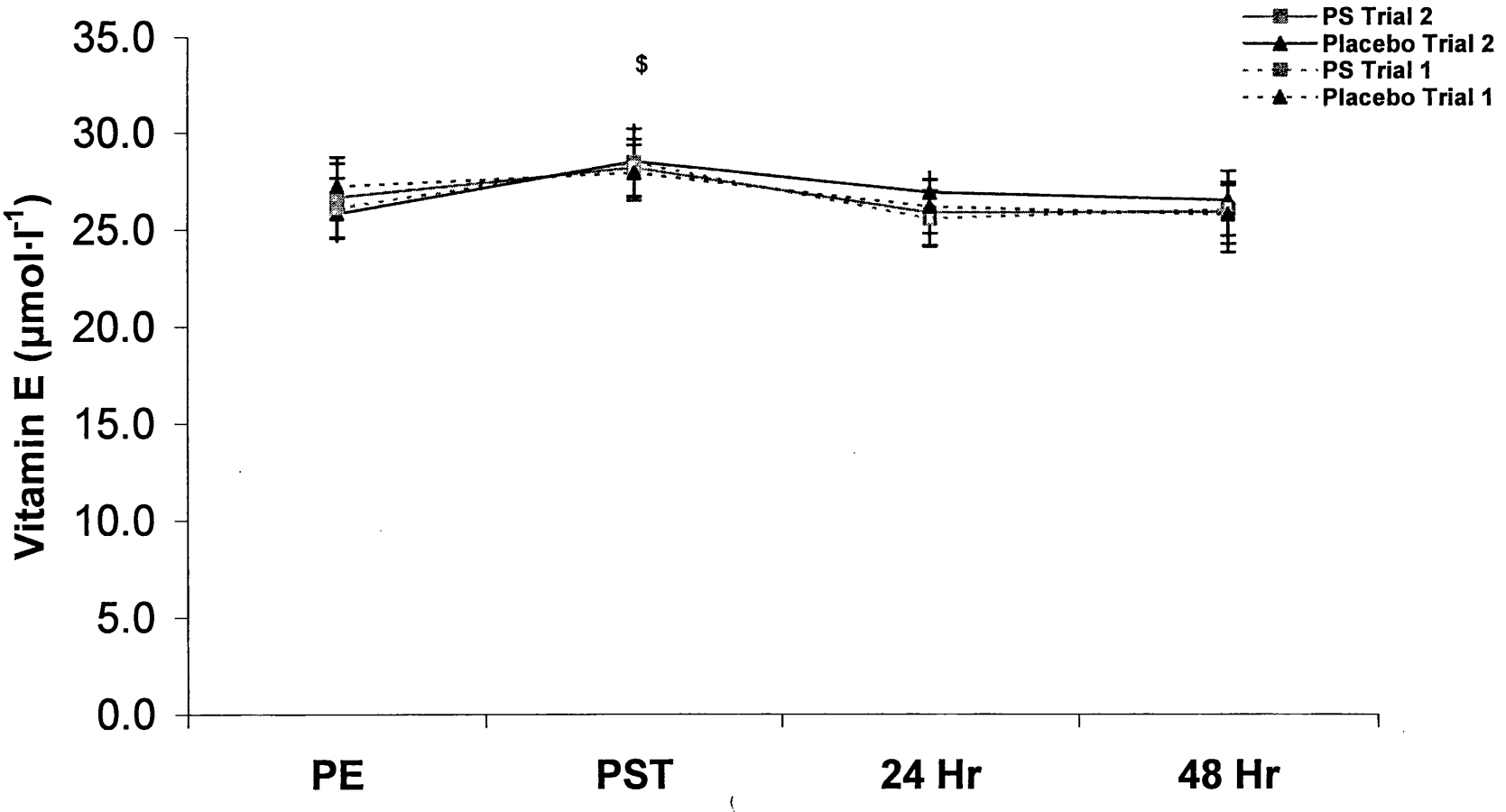

Figure 14 *Vitamin E concentrations during prolonged intermittent exercise and recovery. Values represent mean ± SEM (n for PS/placebo = 8).* $^{\$}$ *PS trial 1/ placebo trial 2 significantly different from pre-exercise values (temporal effect, P<0.005).*

4.8 Ecological Validity

The vast majority (79%) agreed that the protocol provided an excellent replication of soccer match play (Table 7). Furthermore, 41% of all subjects disagreed that the protocol was significantly easier than a soccer match. Subjects were of mixed opinion as to whether the protocol consisted of too frequent rest breaks, but were in agreement that the length of the rest periods was correct.

Table 7 Success of the exercise protocol in simulating the demands of soccer match play (n = 16).

	'Agree'	'Neither Agree nor Disagree'	'Disagree'
Protocol provided an excellent replication of soccer match play	79%	21%	0%
Protocol was significantly easier than a soccer match	12%	47%	41%
Rest breaks were too frequent	22%	44%	34%
Rest breaks were too short	0%	15%	85%

5 Discussion

This study measured the influence of chronic soybean-derived phosphatidylserine (S-PS) supplementation (750 $g \cdot day^{-1}$ for 10 days) on exercise performance and markers of exercise stress, muscle damage and oxidative stress during and following prolonged intermittent exercise that was designed to simulate soccer match play. Supplementation with S-PS had no significant effect upon the elevation of blood cortisol, oxidative stress or muscle damage associated with prolonged intermittent exercise.

The average heart rate (HR) during the protocol (143 ± 3.9 $beats \cdot min^{-1}$) was below the range of 160 – 180 $beats \cdot min^{-1}$ demonstrated by Ekblom (1986) and the average of 165 $beats \cdot min^{-1}$ stated by Reilly (1997). However, the average peak HR of 195.9 ± 2.1 $beats \cdot min^{-1}$ was above these values, suggesting that the subjects' work rate encompassed the values previously reported. The peak blood lactate concentration (5.90 ± 0.25 $mmol \cdot l^{-1}$) was similar to the values that have been previously reported for soccer match play (Ekblom, 1986; Gerisch *et al.*, 1988; Bangsbo *et al.*, 1991), suggesting that the protocol elicited a similar anaerobic response to soccer match play. Furthermore, Ekblom (1986) demonstrated blood lactate concentrations to be higher at more intense levels of competition, and values from this study concurred with values for 3rd and 4th division players (Ekblom, 1986). The individuals used in this study were not professional sportsmen, and therefore do not compete at the highest levels of soccer, offering a plausible explanation for the slightly lower values demonstrated in this study.

The distance covered during exercise varied depending upon part B performance, but a range of 11 – 12 km was recorded, which is concurrent with the range of 8 – 13 km calculated by Reilly and Thomas (1976), although slightly higher than average values of other researchers (Bangsbo *et al.*, 1991; Tumility, 1993). Furthermore, 79% of subjects felt that the protocol provided an excellent replication of soccer match play, as measured by an ecological validity questionnaire. Consequently, the protocol appeared to replicate the physiological and psychological demands of soccer match play.

Serum cortisol concentrations peaked 20 min after exercise and returned to pre-exercise levels following 24 hours of recovery. The time-scale of this response is concurrent with previous research of a similar exercise-type and subject group (Thompson *et al.*, 2001); consequently, the exercise protocol led to an exercise-induced activation of the hypothalamo-pituitary-adrenal (HPA) axis (Axelrod and Reisine, 1984). Exercise stress induces the hypothalamic secretion of a corticotropin-releasing factor (CRF) (Tsigos and Chrousos, 2002), which in turn stimulates the release of adrenocorticotropic hormone (ACTH) by the pituitary gland (Axelrod and Reisine, 1984; Tsigos and Chrousos, 2002). Consequently, ACTH is then transported in the blood to the adrenal cortex, where the increased concentrations of ACTH initiate the synthesis and release of cortisol (Axelrod and Reisine, 1984; Tsigos and Chrousos, 2002). The elevated levels of cortisol within the blood then stimulate the catabolism of proteins in order to synthesise glucose (Tortora and Anagnostakos, 1987; Kelly, 1999; McArdle *et al.*, 2000) and biosynthetic enzymes like phenylethanolamine *N*-methyltransferase (PNMT), which is responsible for the

conversion of noradrenaline to adrenaline within the adrenal gland (Axelrod and Reisine, 1984).

During trial 2 the serum cortisol concentrations of those supplemented with S-PS rose by 26% from pre-exercise to peak cortisol concentrations, in comparison with a rise of 8% exhibited by the placebo group for the same time-span. It is credible that this response in cortisol was a direct result of the exercise performance of the 2 groups; the PS group performed on average 16% better than the placebo group during part B of the protocol, and although no significant supplement effect was identified, this did approach significance (P=0.082). Therefore, previous reports that PS supplementation blunted the cortisol response to physical stress (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Fahey and Pearl, 1998) were not replicated during this study. It is plausible that the prolonged intermittent nature of the exercise was a further reason why previous findings were not replicated in this study; previous research has investigated PS supplementation using either shorter durations (20 min) of intermittent cycling (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992) or 2 weeks of resistance training (Fahey and Pearl, 1998). Consequently, the continued and cumulative increase in cortisol throughout 90 min of intermittent exercise may be too much for S-PS supplementation to attenuate. Additionally, it is possible that the number of subjects for S-PS and placebo groups was not large enough to demonstrate a significant response, although previous research using similar subject numbers has demonstrated the ability of PS to blunt the hormonal response to exercise (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992).

Moreover, previous research has suggested that a potential dose-dependant response to chronic PS supplementation exists (Monteleone *et al.*, 1992), and this presents a possible reason why the supplementation routine used in this study did not attenuate the cortisol response to physical stress. Monteleone *et al.* (1992) showed that chronic oral supplementation with 800 mg·day^{-1} BC-PS for 10 days significantly blunted the ACTH and cortisol responses to exercise, while a dose of 400 mg·day^{-1} of BC-PS for 10 days did not reach statistical significance. The supplementation routine used in this study gave subjects 750 mg·day^{-1} of S-PS for 10 days, and it may be that this amount of S-PS was insufficient to replicate the findings of a similar supplementation routine conducted with BC-PS (Monteleone *et al.*, 1992). Additionally, the supplementation routine used in this study was similar in type (S-PS), but lower in dosage, to that used by Fahey and Pearl (1998), who showed oral supplementation with 800 mg·day^{-1} S-PS for 14 days to successfully attenuate the cortisol response to exercise.

The trend towards enhanced performance demonstrated by those supplemented with S-PS was most prominent during part B of the exercise protocol, where the S-PS group performed on average 16% better (P=0.082). Consequently, this trend towards enhanced performance provides the most credible reasoning as to why ACTH and cortisol responses were not blunted by S-PS supplementation during this study. Since the individuals supplemented with S-PS demonstrated this trend towards enhanced performance, and subsequently enhanced cortisol concentrations, it becomes difficult to quantify if S-PS affected the cortisol response to exercise. Furthermore, it is possible that any effect of S-PS to attenuate the cortisol response to exercise was masked by the (trend towards) improved performance.

The post exercise increases in serum creatine kinase (CK) activity and perceived general muscle soreness indicated that both muscle damage and delayed onset muscle soreness (DOMS) occurred at some point during the exercise protocol. Post-exercise increases in these indicators of muscle damage were in agreement with previous research (Thompson *et al.*, 1999; Thompson *et al.*, 2001; Wadsworth *et al.*, 2004). Moreover, the familiarisation session succeeded in eliminating any trial-order effect, and consequently the exercise protocol led to muscle damage and DOMS despite prior exposure to the test.

Supplementation with S-PS had no significant effect on indicators of muscle damage and DOMS; consequently the findings of previous research (Fahey and Pearl, 1998) were not replicated. Fahey and Pearl (1998) demonstrated that oral supplementation with S-PS (800 $mg \cdot day^{-1}$ PS for 14 days) reduced perceived muscle soreness during a 2 week resistance-training programme. It is possible that the reduced perceived muscle soreness demonstrated by Fahey and Pearl (1998) was associated with the attenuation of the cortisol response to exercise, also demonstrated in the same study. Cortisol is known to have catabolic properties, and inhibit the inflammatory/immune response (Tsigos and Chrousos, 2002). Therefore, any muscle degradation occurring during exercise would, in theory, be left untreated. Moreover, cortisol levels elevated by exercise may in turn emphasise any muscle degradation or damage that occurs. This study did not show S-PS to attenuate the cortisol response to exercise, and consequently the catabolic activities of cortisol might be considered a potential explanation to the elevated levels of muscle damage and DOMS demonstrated.

Individuals supplemented with S-PS demonstrated a tendency towards a lower perception of general muscle soreness, being on average 23% lower, and although no significant supplement effect was identified, this did approach significance (P=0.067). The trend towards a lower perceived general muscle soreness in the PS group suggests that supplementation may have had some effect on the individuals, be it psychological, as proposed by Fahey and Pearl (1998), or physiological as hypothesised by *in-vitro* research (Latorraca *et al.*, 1993; Dacaranhe and Terao, 2001).

The response of serum lipid hydroperoxide (HPO) concentrations suggested that the protocol elicited oxidative stress. Elevated reactive oxygen species (ROS) production occurs as a result of exercise stress (Kanter, 1994). Where antioxidant defences become saturated ROS lead to an increase in oxidative stress (Sen, 1995). Consequently, increased lipid peroxidation occurs as a direct result of the exercise protocol and HPO is released as a by-product of this process (Brites *et al.*, 1999; Child *et al.*, 1999). Supplementation with S-PS had no effect upon serum HPO concentrations, and consequently lipid peroxidation and oxidative stress were unaffected by S-PS supplementation. However, the trend towards enhanced performance, as demonstrated by the S-PS group in part B of the protocol, makes it difficult to quantify if the antioxidant properties of S-PS were insufficient to prevent/reduce oxidative stress, or merely masked by the improved performance (and consequent potential elevation in oxidative stress experienced by this group).

Additionally, the elevated concentrations of plasma vitamin C also suggest that the protocol elicited oxidative stress. Elevated plasma concentrations of vitamin C, an

antioxidant, were probably a result of its mobilization from tissue storage to plasma circulation, in order to counter-act oxidative stress (Alessio *et al.*, 1997; Brites *et al.*, 1999; Thompson *et al.*, 2001; Urso and Clarkson, 2003). It is also plausible that elevated plasma concentrations of vitamin E, another antioxidant, were in part due to the ability of vitamin C to regenerate vitamin E, again in order to counter-act oxidative stress (Brites *et al.*, 1999; Urso and Clarkson, 2003). Therefore, oxidative stress might be at least partially responsible for the muscle damage, and subsequent soreness, demonstrated in this study.

Indicators of oxidative stress and antioxidant markers were all unaffected by S-PS supplementation. Accordingly, the findings of previous *in-vitro* research (Latorraca *et al.*, 1993; Dacaranhe and Terao, 2001) were not supported by this study. Latorraca *et al.* (1993) showed that exogenous PS reduced the effects of free radical damage on human cells in *in-vitro* preparations, whilst Dacaranhe and Terao (2001) found S-PS to be the only acidic phospholipid to demonstrate an antioxidant activity in eukaryotic cells. It is proposed that PS may act as an antioxidant by binding with free iron, and therefore quenching ROS (Dacaranhe and Terao, 2001), and *in-vitro* studies have supported this proposal (Latorraca *et al.*, 1993; Dacaranhe and Terao, 2001). The comparatively low antioxidant defences of skeletal muscle (Sen, 1995) make these tissues particularly susceptible to oxidative damage during exercise stress (Armstrong *et al.*, 1991), when there is a subsequent increase in ROS production (Kanter, 1994). However, the suggestion that those supplemented with S-PS in this study worked harder makes it difficult to assess the effectiveness of S-PS supplementation was effective in attenuating oxidative stress during this study. However, elevated anaerobic work rate, as exhibited in this study by the PS group, is known to lead to

increased ROS production (Sen, 1995; Urso and Clarkson, 2003), subsequent lipid peroxidation and cell damage (Sumikawa *et al.*, 1993). Consequently the response of serum CK activities and HPO concentrations also support the proposal that those supplemented with S-PS worked harder in the second trial.

It is plausible that the potential ergogenic effects of S-PS supplementation suggested in this study are due to the known psychological benefits of S-PS supplementation. Previous research has shown S-PS to significantly enhance feelings of well being (Fahey and Pearl, 1998) during the physical stress brought about by exercise. This could in turn lead to a lower perception of work rate, subsequently leading to the enhanced performance demonstrated by the PS group. The potential for chronic supplementation with S-PS to act as an ergogenic aid is a novel finding, which presents much scope for further investigation.

6 Conclusions

6.1 Conclusions

Evidence exists to suggest that soccer players are at risk of oxidative stress, muscle damage and delayed onset muscle soreness (DOMS) following a soccer match. The current findings confirmed that prolonged intermittent exercise designed to simulate soccer match play led to elevations in markers of oxidative stress, muscle damage and DOMS. Furthermore, these markers were affected to an equal extent in placebo and S-PS groups, and returned to pre-exercise levels within 48 hours.

However, those supplemented with S-PS demonstrated a trend towards enhanced performance. Individuals supplemented with S-PS demonstrated a tendency in trial 2 to perform better in part B of the protocol. Moreover, higher work rates have been consistently shown to elevate markers of stress and muscle damage; during trial 2 the PS group demonstrated a tendancy for higher levels of serum cortisol concentration, lipid hydroperoxide concentration, and serum creatine kinase activity. It is plausible that the trend of improved performance of the PS group, and consequent elevation in serum cortisol concentration and oxidative stress markers, may have negated any benefits that S-PS supplementation had on these markers. Therefore, future research should attempt to clarify the potential ergogenic effect of S-PS supplementation, and ascertain if such supplementation has any effect on the elevation of blood cortisol and oxidative stress associated with prolonged intermittent exercise.

6.2 Limitations and Future Recommendations

The propensity for S-PS supplementation to improve performance presents a novel finding in this study; however, it also presents the biggest limitation of the study. It is plausible that the tendency for improved performance of the S-PS group, and consequent elevation in markers of stress, muscle damage, and oxidative stress, may have negated any benefits that S-PS supplementation had on these markers. Therefore, future research is warranted to clarify if S-PS supplementation does improve performance during, and recovery from, prolonged intermittent exercise. Future research could assess sprint performance in the 48 hours of recovery, as well as during prolonged intermittent exercise; this would allow researchers to quantify the effects of S-PS supplementation on performance during exercise, and performance recovery following it. Furthermore, future research could endeavour to ascertain if the ergogenic effects of S-PS are dose-dependant, by comparing a supplement with varying amounts of S-PS supplementation (for example 1000 $mg \cdot day^{-1}$ and 500 $mg \cdot day^{-1}$ for 10 days prior to exercise).

Moreover, future research is warranted to clarify if S-PS supplementation has any effect on the cortisol response to prolonged intermittent exercise. This may be achieved by experimenting with the supplementation routine, in order to clarify if the ability of S-PS to attenuate cortisol response to prolonged intermittent exercise is dose-dependant. Previous researchers have demonstrated higher doses of S-PS to be more effective in attenuating cortisol response; subsequently future research could focus on supplementing with varying doses of S-PS (for example 1000 $mg \cdot day^{-1}$ and 500 $mg \cdot day^{-1}$ for 10 days prior to exercise). Additionally, future research could

attempt to clarify whether or not S-PS supplementation has any effect on the elevation of oxidative stress associated with prolonged intermittent exercise, as this study was unable to do so. Future research could measure additional indirect markers of oxidative stress (for example malonaldehyde, MDA), or alternatively could use muscle biopsies to directly measure levels of pro-oxidants and antioxidants. Moreover, future research could measure oxidative stress during exercise (for example at half time), as this study only did so 20 min before and after exercise. Future research should attempt to control the amount of exercise across trials, so that the PS and placebo groups undergo the same exercise stress.

This study referred to previous research on soccer, the majority of which used professional soccer players as subjects. Consequently this study may be somewhat limited by the use of semi-professional (University) soccer players instead, as more profound responses to exercise have been demonstrated at higher levels. Therefore, future research may wish to use professional soccer players as subjects, where S-PS supplementation may be of clearest benefit, and any findings would be directly comparable with previous research on the demands of soccer match play. However, such work would require researchers to overcome the constraints posed by long periods of inactivity (for example for a minimum of 48 hours before and after exercise), and it remains to be seen if experimenting in the close season, or using non-first team players, might overcome such constraints.

Previous research has hypothesised upon the mechanisms by which supplementation with S-PS has attenuated the stress response to exercise; the common consensus is that S-PS regulates activation of the hypothalamo-pituitary-adrenal (HPA) axis,

specifically by inhibiting the release of adrenocorticotropic hormone (ACTH) by the pituitary gland (which in turn inhibits cortisol release). However, these hypothesise remain inconclusive, and researchers are unclear how much orally administered S-PS reaches the blood stream and how much is lost in waste products. Consequently, future research could attempt to ascertain how much orally administered S-PS reaches the blood stream, and how quickly. Such research could be conducted independently of any exercise protocol, and could directly measure S-PS concentrations in blood, and waste products (for example urea), and muscle (for example by muscle biopsy).

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Appendix A – Application for Ethical Approval

Application for Ethical Approval,

Department of Psychology, University of Wales Swansea

MPhil Project in Collaboration with the

Department of Sports Science, University of Wales Swansea

All Project students should complete this form in consultation with their project supervisor.
If your supervisor feels that the project involves any potentially controversial procedures (i.e. they feel that the ethical issues raised need to be considered by the Department Ethics Committee) then the form should be placed in the tray in the Resources Centre for consideration by the Departmental Ethics Committee. Further advice will then be given to you by the Ethics Committee via your supervisor. The completed form should be bound along with your project report.

Project Title: The effects of chronic phosphatidylserine (PS) supplementation on recovery following prolonged intermittent exercise.

Students Name: Daniel Wadsworth; BSc (Wales)

Supervisor: Mike Kingsley; MSc (Lough), BPhEd (Otago), PGCE (Wales)

Collaborator: Professor David Benton; PhD

Brief description of the purpose and methods of the project:

Purpose:

Phosphatidylserine (PS), has been shown to reduce blood levels of cortisol in response to resistance training (Fahey et al., 1998) and physical exercise (Monteleone et al., 1992). The elevation of cortisol levels in response to exercise stress leads to the accelerated catabolism of amino acids. As such, elevated cortisol levels can be viewed as a possible precursor to muscle damage. Subsequently, suppression of cortisol levels could theoretically prevent muscle damage, and therefore maintain muscle mass. Supplementation with PS may therefore enhance recovery following exercise. Burke (2000) supports the possible benefits on recovery of PS

supplementation, suggesting that it may minimize muscle fibre damage caused by muscular stress.

Aims:

To investigate the effects of chronic phosphatidylserine (PS) supplementation, on heart rate and cortisol levels, during and immediately following intermittent exercise.

To investigate the effects of chronic phosphatidylserine (PS) supplementation on muscle soreness/damage following intermittent exercise.

No. of participants: 16 - 20

How and where are the participants to be recruited: Undergraduate and postgraduate male (student) volunteers from Swansea University.

Experimental procedure (brief details only): Preliminary testing of subjects will include an estimation of maximal oxygen consumption ($\dot{V}O_{2max}$), by multi-stage fitness test. Subjects will then complete a familiarization trial of a prolonged intermittent exercise protocol, designed to replicate the physiological demands of soccer match play. Subsequently, subjects will complete two further exercise trials, once with no supplement (control) (T1) and once with either a (PS) supplement or a glucose placebo (T2). Chronic supplementation of 900 $mg \cdot day^{-1}$ of PS (or placebo) will take place for 10 days prior to T2. Subjects will be randomly allocated to a placebo controlled double blind procedure. A minimum of 1 week will separate the familiarization and T1 trials, with T2 occurring approximately 4 weeks later.

During T1 and T2, physiological measurement procedures will consist of heart rate monitoring, 2 venous blood samples (< 30 ml per sample), and sprint/endurance performance assessment. Subjects will be required to return for tests 1 and 2 days after exercise to assess recovery, when further blood samples (< 30 ml per sample) will be collected in order to assess their recovery (one sample each day), and muscle soreness assessed. Blood samples will then be analysed, and serum creatine kinase (CK) activity and serum myoglobin concentrations assessed, both of which are markers of muscle damage. Levels of cortisol within the blood will also be measured.

Details of any payment given: None

Consent and Debriefing

Have you prepared a consent form for participants? YES (Appendix A)

Have you prepared an information sheet to debrief participants? YES (Appendix B)

You **must** attach the consent form and information sheet before handing this form to your supervisor. You must also attach a copy of any questionnaire(s) that you intend using:
Appendix C – Environmental Validity Questionnaire;
Appendix D – Perceived Muscle Soreness Questionnaire.

Ethical Considerations

Please read the following declarations carefully and detail below any ways in which your project deviates from them. Then sign where indicated.

1. I have ensured that there will be no active deception of participants.
2. I have ensured that no data will be personally identifiable.
3. I have ensured that no participant should suffer any undue physical or psychological discomfort
4. I certify that there will be no administration of potentially harmful drugs, medicines or foodstuffs. *
5. I will obtain written permission from an appropriate authority before recruiting members of any outside institution as participants.
6. I certify that the participants will not experience any potentially unpleasant stimulation or deprivation?
7. I certify that any ethical considerations raised by this proposal have been discussed in detail with my supervisor.
8. I certify that the above statements are true with the following exception(s):

* (4) - See Appendix E (Safety information)

Student's signature: Date:

In the supervisor's opinion, this project (tick one only):
Does not raise any significant issues.
Raises some ethical issues, but I consider that appropriate steps and precautions have been taken and I have approved the proposal and accept the responsibility for having done so.
Raises ethical issues that need to be considered by the Departmental Ethics Committee.
Raises ethical issues such that it should not be allowed to proceed in its current form.

Supervisor's signature: Date:

(For Ethics Committee use only)

The ethical issues raised by this project have been considered by members of the Departmental Ethics Committee who made the following comments:

.....

.....

.....

.....

.....

.....

.....

Please ensure that you take account of these comments and prepare a revised submission that should be shown to your supervisor/ resubmitted to the Department Ethics Committee (delete as appropriate).

Signed: Date:

(Chair, Departmental Ethics Committee)

PSYCHOLOGY DEPARTMENT

ETHICS COMMITTEE

Memo

To: Professor David Benton

From: Professor David Clark, Chair of Departmental Ethics Committee

Date: 19th March, 2003

Re: **Impact of phosphatidylserine (PS) on recovery following exercise**

Members of the departmental Ethics Committee have now reviewed the above study and agree that it raises no substantive ethical issues, provided the information obtained from the questionnaires is kept absolutely confidential and that no personally identifiable information is entered on computer. You may therefore proceed with your study.

Appendix B – Subject Information Sheet

DEPARTMENT OF SPORTS SCIENCE

SUBJECT INFORMATION SHEET

21st February 2003. Version 1.0

Contact Details:
Daniel Wadsworth, department of Sports Science.
Contact: 07779088153

1. Study title
The effects of chronic phosphatidylserine (PS) supplementation on recovery following prolonged intermittent exercise.

2. Invitation paragraph
You are being asked to volunteer as a subject for this study, which is being conducted for MPhil research.

3. What is the purpose of this study?
This study aims to investigate the effects of chronic supplementation of PS on recovery. The short-term effects (immediately after exercise) of supplementation on heart rate and cortisol levels will be investigated, as will the long-term (up to 48 hours post exercise) effects on muscle soreness and damage.

4. Why have I been chosen?
You have been chosen as a subject for this study as you are an active male, aged 18 – 25 years old. Your participation in this study is entirely voluntary, and you should be aware that you have the right to withdraw at any time without a reason.

5. What will happen to me if I take part?
Preliminary testing will consist of a multi-stage fitness test (the 'bleep test') in order to assess your fitness. After this, you will complete an exercise protocol designed to simulate the activity levels and demands of a soccer match. The protocol consists of 90 minutes of walking, jogging, cruising and sprinting, and also includes frequent rest periods (1 minute breaks) and a 15 minute 'half-time'.

You will need to complete this protocol on **3 separate occasions**, the first trial will familiarize you with the protocol. You will then complete two further trials, once with no supplement (control) (T1) and once with either a (PS) supplement or a glucose placebo (T2). You will be supplemented with either 900 $mg \cdot day^{-1}$ of PS or a glucose placebo for 10 days prior to T2.

During T1 and T2, physiological measurement procedures will consist of heart rate monitoring and sprint/endurance performance assessment **Before and after T1 and T2**

blood will be taken intravenously, (<30 ml per sample). You will be required to return for tests 1 and 2 days after exercise to assess your recovery, when further blood samples will be collected (one each day), and your soreness will be assessed (by questionnaire).

You will be required to control your diet 24 hours prior to T1 and T2, and in the two days following each trial (T1 and T2). You will also be required to do no exercise 24 hours before T1 and T2, and in the two days following each trial.

6. What are the possible disadvantages of taking part?
As the study aims to assess the effects of PS supplementation with regards to muscle soreness, subjects are expected to feel some degree of discomfort on the days following the exercise.

Blood samples are to be taken intravenously by a trained individual, and whilst this is not a painful process, subjects must be comfortable with such collection methods. Even though the volume of blood taken is small (< 30 ml per sample) there exists the possibility that during or immediately following this procedure subjects may feel light headed or faint. There is an extremely small risk that this procedure could result in an air or plastic embolism, but good practice minimises the risk. In addition, every effort will be made to minimise the risks of contaminating the wound by using sterile disposable equipment and standardised procedures for the collection and disposal of biohazard wastes.

7. What are the possible benefits of taking part?
Taking part in this study will allow you to assess your fitness at the time of the study, and also follow your progress throughout the duration of the study. It has been shown that PS improves confidence and composure during exercise, which is clearly of benefit. In addition, PS enhances perceived well-being following exercise, and also reduces perceived soreness following exercise. As such, PS can be said to enhance your recovery from exercise, and therefore has possible benefits on future exercise bouts.

8. Will my taking part in the study be kept confidential?
Whilst the study will be available to all within the University of Wales Swansea, all results from this study will remain anonymous.

Appendix C – Subject Consent Form

DEPARTMENT OF SPORTS SCIENCE
SUBJECT CONSENT FORM

Contact Details:
Daniel Wadsworth, department of Sports Science
Contact: [redacted]

Project Title:
The effects of chronic phosphatidylserine (PS) supplementation on recovery following prolonged intermittent exercise.

Please initial box

1. I confirm that I have read and understood the information sheet dated ...../...../..... (version number .....) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of data obtained may be looked at by responsible individuals from the University of Wales Swansea or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to these records. ☐
4. I agree to take part in the above study.

Name of Subject	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

Appendix D

ACSM Health/fitness preparticipation screening questionnaire

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire.

Name _____
Address _____
Phone number home _____ work _____
Emergency contact name _____
phone _____
Date of birth _____

History ***Assess your health needs by marking all true statements.***

You have had:
☐a heart attack
☐heart surgery
☐cardiac catheterization
☐coronary angioplasty (PTCA)
☐pacemaker/implantable cardiac defibrillator/rhythm disturbance
☐heart valve disease
☐heart failure
☐heart transplantation
☐congenital heart disease

If you marked any of the statements in this section, consult you a health provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Symptoms and other health issues:

☐You experience chest discomfort with exertion.
☐You experience unreasonable breathlessness.
☐You experience dizziness, fainting, blackouts.
☐You take heart medications.
☐You take prescription medication(s).
☐You have musculoskeletal problems.
☐You have concerns about the safety of exercise.
☐You are pregnant.

Cardiovascular risk factors

☐You are a man older than 45 years.
☐You are a woman older than 55 years or you have had a hysterectomy or you are postmenopausal.
☐You smoke.
☐Your blood pressure is greater than 140/90.
☐You don't know your blood pressure.
☐You take blood pressure medication.
☐Your blood cholesterol level is >240 mg/dL.
☐You don't know your cholesterol level.
☐You have a blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
☐You are diabetic or take medicine to control your blood sugar
☐You are physically inactive (i.e., you get less than 30 minutes of physical activity on at least 3 days per week).
☐You are more than 20 pounds overweight.

If you marked two or more of the statements in this section, you should consult your healthcare provider before engaging in exercise. You might benefit by using a facilty with professionally qualified exercise staff to guide your exercise program.

☐**None of the above is true.**
You should be able to exercise safely without consulting your healthcare provider in almost any facility that meets your exercise programme needs.

AHA/ACSM indicates American Heart Association/American College of Sports Medicine.

Appendix E – Set Warm-up

- Jog 6 lengths of the hall (20 m each)
- Sprint 3 lengths of the hall (20 m each)
- Circle each ankle both ways 5 times each
- Calf stretch – 1 leg forward, with feet parallel, put weight on front foot, holding for 8 sec on each leg
- Quadriceps – Hold foot behind body, pulling into buttocks. Push hips forward, holding for 8 sec on each leg
- Hamstrings – 1 leg forward, bend the other knee slightly. Stick behind out, with foot remaining on floor. Do not lift toe, hold for 8 sec on each leg
- Side stretch – Bend towards other side, running arm down leg. Do not lean forwards, hold for 8 sec on each side
- Triceps – Holding arm across chest, hold for 8 sec on each arm
- Back – make a circle in front of the body with the arms (as if hugging a tree!). Curl back, holding for 8 sec
- Arms – Rotate arms at the same time, trying to brush ears. 1 arm forwards, 1 arm backwards, 5 times each way
- Neck – Tilt head to each side (ear to shoulder), do not pull neck. Hold for 8 sec each side

Appendix F - Perceived Muscle Soreness Questionnaire.

Subject: Date:

- **Rate general (overall) muscle soreness on this scale:**

0	1	2	3	4	5	6	7	8	9	10

where:

0 – No soreness
2 – Light soreness
4 – Somewhat sore
6 - Sore
8 – Very sore
10 – Extreme soreness

- **Rate actual areas of soreness (on the scales):**

Neck and Shoulders	0 1 2 3 4 5 6 7 8 9 10
Chest	0 1 2 3 4 5 6 7 8 9 10
Upper limbs	0 1 2 3 4 5 6 7 8 9 10
Trunk (and abdominals)	0 1 2 3 4 5 6 7 8 9 10
Back	0 1 2 3 4 5 6 7 8 9 10
Quadriceps	0 1 2 3 4 5 6 7 8 9 10
Gluteals	0 1 2 3 4 5 6 7 8 9 10
Hamstrings	0 1 2 3 4 5 6 7 8 9 10
Calves	0 1 2 3 4 5 6 7 8 9 10

Appendix G – Borg RPE scale

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Appendix H – Ecological Validity Questionnaire.

Subject: Date:

- **'The exercise protocol provides an excellent replication of soccer match-play.' To what extent do you agree with this statement?**
  - Strongly disagree
  - Disagree
  - Neither agree nor disagree
  - Agree
  - Strongly agree

- **Indicate the extent to which you agree with each of the statements about the protocol listed below.**

Where:

1 - Strongly disagree
2 - Disagree
3 - Neither agree nor disagree
4 - Agree
5 - Strongly agree

'Too much turning'	1	2	3	4	5
'Too much sprinting'	1	2	3	4	5
'Not enough match-skills'	1	2	3	4	5
'The protocol was significantly easier than a soccer match'	1	2	3	4	5
'Too much walking'	1	2	3	4	5
'Too little backwards movement'	1	2	3	4	5
'Too much jogging'	1	2	3	4	5
'Too frequent rest periods/breaks'	1	2	3	4	5
'Rest periods/breaks were too short'	1	2	3	4	5

Appendix I – Raw Data

Appendix I (i) Temperature & Humidity (all trials)

Subject	Temp T1	Temp T2	Humidity T1	Humidity T2
1	21	21	58	64
2	21	21	58	64
3	19	19	51	46
4	19	19	51	46
5	23	22	44	67
6	23	22	44	67
7	16	19	44	53
8	16	19	44	53
9	18	22	42	52
10	18	22	42	52
11	20	22	49	53
12	20	22	49	53
13	20	23	53	45
14	20	24	53	50
15	20	23	63	45
16	20	22	63	52

Appendix I (ii) Subject Data

		RPE		Weight (kg)		Weight loss		dec
Subject	Trial	½ time	PST	PE	PST	(kg)	MSFT	MSFT
1	1	14	19	80.6	79.5	-1.1	8-4	8.37
	2	11	19	80	79	-1	8-8	8.73
2	1	12	19	80.7	80.8	0.1	10-7	10.64
	2	11	20	82.5	82.8	0.3	11-3	11.25
3	1	12	20	79	78.4	-0.6	12-1	12.08
	2	13	20	78.5	78	-0.5	12-4	12.33
4	1	16	18	77.6	77.5	-0.1	9-5	9.45
	2	18	19	79.5	78.6	-0.9	8-2	8.18
5	1	14	18	75	75.1	0.1	12-0	12.00
	2	17	19	75.4	75.5	0.1	13-0	13.00
6	1	17	20	78.2	77	-1.2	14-2	14.15
	2	17	20	78	76.6	-1.4	14-6	14.46
7	1	13	19	67.8	67	-0.8	10-5	10.45
	2	13	19	69	67.6	-1.4	11-0	11.00
8	1	13	18	71	69.8	-1.2	11-0	11.00
	2	14	17	69.6	68.7	-0.9	11-0	11.00
9	1	14	16	88	87	-1	11-2	11.17
	2	13	15	86.7	85.4	-1.3	11-6	11.50
10	1	14	17	60.2	58.1	-2.1	10-3	10.27
	2	14	15	60.2	58.3	-1.9	11-0	11.00
11	1	13	15	88	86.5	-1.5	10-1	10.09
	2	12	15	87.5	85.8	-1.7	10-4	10.36
12	1	15	15	82	81.7	-0.3	10-1	10.09
	2	9	12	81.8	81.2	-0.6	8-10	8.91
13	1	15	18	87.2	87	-0.2	10-0	10.00
	2	15	16	87.3	87.3	0	8-4	8.37
14	1	14	18	74	74	0	15-0	15.00
	2	13	19	76.2	75.4	-0.8	15-0	15.00
15	1	12	17	85	84.5	-0.5	13-0	13.00
	2	13	18	84.4	84.1	-0.3	14-0	14.00
16	1	12	16	71.2	71.5	0.3	11-10	11.83
	2	13	16	71.4	71	-0.4	8-10	8.91

Appendix I (iii) Heart Rate Data

Subject	Trial	Heart Rate (HR)					
		Av	max	h/t av	av (no h/t)	av (no MSFT)	av (no both)
1	1	172.08	215.00	127.87	180.01	169.89	178.29
	2	166.68	213.00	122.11	174.68	164.34	172.78
2	1	141.59	200.00	96.18	149.56	138.00	146.36
	2	132.31	201.00	86.28	140.33	128.42	136.85
3	1	132.04	191.00	88.85	139.67	127.91	135.98
	2	135.90	198.00	95.22	143.48	131.96	139.88
4	1	163.11	198.00	116.92	171.38	160.77	169.59
	2	159.77	195.00	112.84	168.28	158.12	167.08
5	1	145.39	196.00	95.00	154.07	142.11	151.53
	2	142.20	199.00	91.78	150.87	138.45	147.79
6	1	133.23	184.00	96.49	139.42	129.22	135.77
	2	132.17	185.00	95.73	138.29	128.05	134.51
7	1	155.58	205.00	105.18	164.66	151.64	161.25
	2	151.58	205.00	105.77	159.60	148.03	156.49
8	1	146.76	191.00	100.34	154.97	143.49	152.19
	2	140.81	191.00	99.93	150.32	137.90	145.49
9	1	132.62	189.00	90.09	140.29	130.34	138.72
	2	129.72	193.00	82.72	137.91	126.30	135.02
10	1	151.72	203.00	110.63	159.00	148.55	156.14
	2	143.70	201.00	100.42	151.29	140.11	148.05
11	1	156.95	199.00	106.72	165.79	153.86	163.19
	2	154.81	191.00	120.56	160.94	152.91	159.38
12	1	153.70	199.00	107.57	162.43	150.82	160.25
	2	162.10	201.00	125.92	168.50	159.72	166.48
13	1	130.55	186.00	90.56	137.61	127.48	134.87
	2	137.28	182.00	100.00	144.02	135.38	142.45
14	1	110.67	184.00	63.23	118.57	110.67	111.77
	2	100.51	179.00	58.38	107.46	93.31	100.30
15	1	132.79	200.00	88.73	140.30	127.79	135.61
	2	126.04	200.00	82.37	133.42	119.61	127.05
16	1	144.30	200.00	104.37	n/a	135.18	150.28
	2	151.12	194.00	113.18	157.90	148.25	155.26

Appendix I (iv) Blood Haemoglobin & Haematocrit (T1)

Subject	Haemoglobin				Haematocrit			
	PE	PST	rec1	rec2	PE	PST	rec1	rec2
1	151	156	145	147	44	45	42	42
2	164	158	155	153	47	45	45	44
3	141	137	135	136	42	35	41	37
4	146	141	140	140	42	38	41	39
5	152	143	141	146	46	45	42	43
6	147	153	149	145	44	45	45	44
7	123	125	110	127	40	42	30	42
8	142	139	143	140	47	45	43	43
9	150	143	149	142	43	42	43	42
10	159	154	154	152	45	45	45	45
11	141	140	138	143	42	42	42	43
12	151	148	153	150	45	44	45	44
13	155	147	155	148	46	45	47	46
14	149	149	147	142	45	43	43	43
15	162	159	155	154	48	48	46	46
16	156	160	152	163	44	44	42	45

Appendix I (v) Blood Glucose and Lactate (T1)

Subject	Lactate						Glucose					
	PE	1/2 time	Imm. PST	PST	24 Hr	48 Hr	PE	1/2 time	Imm. PST	PST	24 Hr	48 Hr
1	1.005	2.810	6.770	1.497	0.918	1.427	4.117	4.340	4.640	4.130	4.183	4.050
2	1.230	2.950	6.170	1.347	2.683	1.453	3.973	4.420	4.770	3.557	3.950	3.797
3	0.610	1.160	6.805	1.617	0.920	0.932	4.250	4.825	5.725	5.013	4.593	3.417
4	2.123	6.640	8.740	3.320	2.127	2.493	3.920	5.790	5.820	3.293	4.883	4.037
5	1.393	1.900	6.130	2.063	1.803	1.530	3.963	3.710	4.120	3.467	4.093	2.910
6	0.942	1.660	6.060	1.793	1.167	1.817	4.377	4.610	5.910	4.673	4.763	3.997
7	2.693	1.680	5.880	1.810	1.975	1.107	4.553	4.430	6.170	3.860	3.983	4.317
8	2.117	1.040	5.600	3.120	2.510	2.867	4.620	4.260	5.250	4.130	5.140	6.620
9	0.973	1.920	4.860	1.493	0.812	0.905	4.010	4.900	4.930	4.107	4.217	3.773
10	1.440	1.880	3.940	1.383	1.253	1.500	4.203	4.670	5.590	3.663	3.943	3.533
11	1.257	2.200	4.860	1.583	0.912	0.593	4.210	4.850	5.600	3.807	4.103	4.187
12	0.866	2.800	6.250	1.410	0.741	1.060	4.087	4.700	4.820	3.800	4.207	3.827
13	1.257	2.210	5.810	0.950	0.840	0.750	5.143	5.410	4.360	3.493	3.740	3.907
14	1.230	2.540	8.520	1.620	1.483	1.553	3.983	3.920	6.700	4.590	4.060	4.160
15	0.773	1.740	6.730	3.020	1.277	1.520	3.573	3.830	5.530	3.763	4.590	3.880
16	1.723	3.440	7.670	3.197	0.803	0.949	4.503	4.220	5.360	4.017	3.997	3.843

Appendix I (vi) Blood Haemoglobin & Haematocrit (T2)

Subject	Haemoglobin				Haematocrit			
	PE	PST	rec1	rec2	PE	PST	rec1	rec2
1	153	149	152	144	44	44	44	42
2	155	157	161	158	46	46	46	48
3	153	145	138	140	45	43	44	43
4	145	145	149	141	43	43	45	42
5	152	143	149	150	48	46	40	44
6	146	151	147	146	47	46	44	44
7	129	130	129	133	43	40	40	41
8	150	145	145	137	46	45	46	42
9	149	151	145	138	44	43	41	40
10	161	151	158	153	45	45	46	44
11	143	150	142	143	44	44	42	44
12	153	150	144	146	45	45	44	44
13	159	150	157	150	47	47	47	44
14	139	142	148	137	41	40	43	45
15	153	161	152	149	47	47	47	45
16	162	162	150	151	45	44	42	42

Appendix I (vii) Blood Lactate and Glucose (T2)

Subject	Lactate						Glucose					
	PE	1/2 time	Imm. PST	PST	24 Hr	48 Hr	PE	1/2 time	Imm. PST	PST	24 Hr	48 Hr
1	0.670	2.680	6.000	1.407	0.915	1.250	3.88	4.04	4.57	3.68	4.18	5.32
2	1.460	2.320	5.300	2.020	1.393	1.343	3.79	3.71	4.42	3.36	3.85	3.88
3	0.589	1.940	6.400	1.480	0.844	0.851	4.32	4.76	5.79	4.59	4.04	4.47
4	2.600	2.990	5.990	1.857	1.867	1.847	4.72	4.88	5.06	3.49	4.00	3.60
5	1.403	2.820	7.450	2.303	1.740	2.117	4.41	4.00	4.19	3.85	3.38	4.02
6	0.878	2.310	5.840	1.580	1.660	1.400	5.01	5.21	5.64	4.18	3.41	3.91
7	1.747	2.130	6.910	1.820	1.060	1.230	4.45	4.23	5.83	3.82	3.90	3.98
8	1.793	1.210	5.580	2.240	1.927	2.713	4.42	4.49	5.43	4.10	4.19	4.76
9	0.950	2.710	4.900	1.497	0.854	1.337	4.29	5.04	5.01	4.06	4.78	4.53
10	1.590	2.010	4.430	1.333	1.173	1.330	3.99	3.98	5.22	3.95	3.98	4.74
11	0.814	2.300	6.450	1.887	0.980	0.917	4.28	4.65	5.73	4.18	3.79	4.12
12	1.390	3.660	5.450	1.303	0.884	0.883	4.27	5.21	5.30	3.89	4.76	4.51
13	0.463	2.590	4.200	0.850	0.949	0.756	3.65	4.51	3.77	3.57	4.22	4.22
14	2.167	2.170	5.440	1.543	1.053	1.947	3.64	3.71	6.15	4.47	3.83	5.37
15	1.180	2.030	7.830	3.723	0.906	1.450	4.34	3.98	6.04	3.86	4.27	4.46
16	1.290	2.220	6.220	2.200	0.876	1.100	3.99	3.88	5.05	3.99	3.48	3.98

Appendix I (viii) Creatine Kinase T1

Subject	Creatine Kinase (CK)			
	PE	PST	rec1	rec2
1	117	290	656	307
2	395	589	959	682
3	651	760	1339	724
4	144	308	1418	888
5	643	851	1100	698
6	523	666	466	398
7	149	241	224	262
8	166	307	366	345
9	194	375	585	360
10	385	578	387	248
11	127	220	473	275
12	142	319	809	369
13	464	633	867	468
14	637	976	613	281
15	307	525	406	361
16	313	419	288	175

Appendix I (ix) Creatine Kinase T2

Subject	Creatine Kinase (CK)			
	PE	PST	24 Hr	48 Hr
1	160	308	411	193
2	395	650	666	568
3	862	1022	959	516
4	194	302	377	242
5	199	401	509	358
6	286	464	404	397
7	159	275	213	128
8	120	245	516	422
9	204	408	867	518
10	206	311	306	213
11	83	188	318	218
12	202	367	440	224
13	164	467	354	397
14	209	288	198	371
15	104	252	284	226
16	137	239	295	198

Appendix I (x) Cortisol and HPO (all trials)

Subject	Trial	CORTISOL (nmol/l)				HPO (uM)			
		PE	PST	rec1	rec2	PE	PST	rec1	rec2
1	1	470	783	245	250	0.784	0.919	1.149	0.837
	2	387	365	275	258	0.858	1.059	0.893	0.322
2	1	407	647	273	322	0.594	0.746	0.799	0.586
	2	403	559	257	305	0.674	0.724	0.992	0.712
3	1	295	NS	311	325	0.631	0.818	0.763	0.801
	2	368	508	232	202	0.362	0.888	0.834	0.274
4	1	359	739	273	529	1.071	1.244	0.813	1.019
	2	454	391	326	286	0.731	0.862	0.950	0.884
5	1	384	425	290	263	1.004	1.078	0.903	0.922
	2	447	600	589	444	0.717	0.850	0.963	0.610
6	1	355	322	306	292	1.104	1.439	1.028	0.778
	2	431	462	311	263	0.716	1.219	1.061	1.063
7	1	308	455	143	235	1.022	1.100	0.892	0.880
	2	369	454	209	231	0.801	0.990	0.747	1.418
8	1	441	571	307	416	0.894	0.928	0.811	0.778
	2	468	643	NS	238	0.829	0.832	1.021	0.907
9	1	356	365	254	242	0.849	0.842	0.943	1.100
	2	270	467	291	272	0.923	1.131	0.983	1.074
10	1	NS	585	499	518	0.783	0.957	0.832	0.834
	2	489	422	271	536	0.862	0.913	0.762	0.898
11	1	489	568	216	180	0.970	1.163	0.870	0.890
	2	485	701	204	354	0.940	1.251	0.930	0.829
12	1	NS	632	347	314	0.752	1.202	1.007	0.864
	2	583	NS	484	336	0.846	1.098	0.968	0.871
13	1	417	282	243	271	1.221	1.209	0.784	NS
	2	141	244	177	138	1.046	1.314	0.996	1.340
14	1	408	581	361	334	0.884	0.861	0.783	0.706
	2	463	442	233	262	0.860	1.154	1.111	0.940
15	1	397	646	382	296	1.109	1.420	1.176	0.887
	2	265	558	287	279	0.966	1.296	0.983	1.084
16	1	383	545	212	299	0.797	0.876	0.709	1.177
	2	455	492	226	307	1.189	1.376	0.756	0.809

Appendix I (xi) Vitamin C and E (all trials)

Subject	Trial	VITAMIN C (uM)				VITAMIN E (alpha-toc.)			
		PE	PST	rec1	rec2	PE	PST	rec1	rec2
1	1	6.0	27.8	31.0	37.4	26.45	29.55	27.75	26.24
	2	23.6	44.4	33.3	36.9	26.06	27.87	27.93	22.79
2	1	0.0	11.2	13.8	20.0	31.67	32.05	27.74	30.23
	2	NS	11.8	8.1	14.7	25.90	30.57	30.10	31.66
3	1	NS	32.9	63.7	53.8	28.52	30.56	27.45	28.46
	2	44.7	67.8	37.1	39.1	32.80	32.15	31.15	28.89
4	1	73.5	85.2	63.8	68.0	26.05	27.84	23.92	25.14
	2	53.6	72.4	60.0	41.8	26.12	27.20	29.63	28.15
5	1	1.2	65.6	40.8	39.1	22.28	23.15	23.18	22.91
	2	NS	38.5	43.9	40.7	21.00	23.88	22.81	22.46
6	1	6.1	26.3	7.9	45.5	33.33	36.17	32.41	28.97
	2	20.7	53.1	30.7	57.0	31.22	34.50	32.56	32.74
7	1	60.3	71.3	48.9	45.1	19.56	20.18	20.46	19.73
	2	17.1	30.8	30.4	16.2	16.84	22.93	29.19	29.47
8	1	43.0	57.6	42.7	47.5	30.03	27.34	24.25	22.34
	2	49.2	64.4	43.7	43.2	24.00	25.62	24.42	24.33
9	1	3.7	10.0	13.2	30.9	25.77	24.03	25.38	22.42
	2	10.8	17.5	10.9	52.9	24.25	25.55	23.83	19.33
10	1	32.0	49.8	40.8	46.2	28.81	31.33	33.76	26.57
	2	1.8	48.2	1.3	27.4	25.88	31.22	28.87	24.89
11	1	23.4	29.9	51.6	27.1	21.04	27.23	23.20	30.40
	2	7.6	4.7	19.5	49.9	28.97	26.71	17.74	26.81
12	1	54.0	57.2	56.0	51.0	26.63	31.54	25.87	26.67
	2	4.4	41.0	44.8	36.9	27.66	28.89	28.67	29.01
13	1	NS	32.9	63.7	53.8	29.25	32.58	27.48	29.25
	2	44.7	67.8	37.1	39.1	35.98	33.86	31.14	38.50
14	1	29.1	32.9	60.0	54.4	23.11	21.03	21.45	19.45
	2	49.1	42.3	35.7	38.9	21.92	21.59	22.93	23.79
15	1	14.8	46.4	67.3	69.7	25.00	27.22	20.33	22.83
	2	35.5	35.3	34.2	35.9	27.67	30.49	27.84	25.34
16	1	0.2	47.6	56.2	70.0	29.43	29.17	29.16	32.74
	2	26.1	39.6	38.8	35.8	34.07	38.99	27.69	27.70

Appendix I (xii) Sprint Times (all trials)

Subject	Trial	Mean time	mean vel	Peak time	Peak vel
1	1	2.72	5.51	2.59	5.79
	2	2.68	5.61	2.58	5.81
2	1	2.72	5.51	2.55	5.88
	2	2.62	5.72	2.45	6.12
3	1	2.58	5.81	2.51	5.98
	2	2.47	6.10	2.32	6.47
4	1	2.44	6.15	2.36	6.36
	2	2.65	5.65	2.31	6.49
5	1	2.50	5.99	2.34	6.41
	2	2.49	6.02	2.38	6.30
6	1	2.49	6.03	2.39	6.28
	2	2.44	6.15	2.3	6.52
7	1	2.95	5.08	2.84	5.28
	2	2.87	5.24	2.75	5.45
8	1	2.87	5.22	2.75	5.45
	2	2.72	5.52	2.65	5.66
9	1	2.62	5.73	2.52	5.95
	2	-	-	-	-
10	1	3.01	4.98	2.89	5.19
	2	-	-	-	-
11	1	-	-	-	-
	2	-	-	-	-
12	1	-	-	-	-
	2	-	-	-	-
13	1	2.65	5.66	2.3	6.52
	2	2.54	5.91	2.4	6.25
14	1	2.61	5.76	2.32	6.46
	2	2.46	6.10	2.3	6.52
15	1	2.56	5.85	2.48	6.05
	2	2.50	6.01	2.39	6.28
16	1	2.68	5.60	2.62	5.72
	2	2.82	5.32	2.46	6.10

Appendix I (xiii) Perceived General Muscle Soreness (all trials)

Subject	Trial	General Soreness			
		PE	PST	24 Hr	48 Hr
1	1	0	2	4	2
2	1	0	2	4	2
3	1	2	4	3	1
4	1	2	6	6	4
5	1	7	7	2	1
6	1	5	6	4	4
7	1	1	4	2	3
8	1	1	6	3	3
9	1	3	4	2	1
10	1	4	6	2	2
11	1	1	1	3	1
12	1	0	3	4	0
13	1	5	6	6	3
14	1	4	6	6	3
15	1	1	2	1	0
16	1	5	5	5	3
1	2	0	1	2	1
2	2	4	4	2	1
3	2	3	6	3	2
4	2	1	3	4	2
5	2	0	2	0	0
6	2	0	0	2	0
7	2	2	3	5	2
8	2	1	2	2	1
9	2	1	2	2	1
10	2	1	4	4	3
11	2	0	3	4	2
12	2	1	7	2	1
13	2	2	5	3	2
14	2	0	3	1	0
15	2	2	2	2	1
16	2	0	4	4	3