Phosphatidylserine and its effects on intermittent exercise.

Miller, Mark Philip

Department of Sports Science

University of Wales Swansea

Phosphatidylserine and its Effects on Intermittent Exercise

Mark Philip Miller

Masters of Philosophy

June 2005

Contents

Abstract

Phosphatidylserine (PS) is a naturally occurring phospholipid nutrient that is predominantly found in the membrane of cells. The biochemical actions of PS have been demonstrated to include; for example: (1) regulation of calcium uptake (Floreani *et al.*, 1991); (2) stimulation of ATPase activity (Tsakiris and Deliconstantinos, 1984); and (3) activation of different Protein Kinase-C (PKC) isoforms (Kaibuchi *et al.*, 1981). Previous research has shown that supplementation with 800 $mg \cdot day^{-1}$ of bovine cortex-PS (BC-PS) for 10 days attenuated the adrenocorticotrophin and cortisol responses to intermittent exercise (Monteleone *et al.*, 1992). Unfortunately, the possible risk of infectious diseases has rendered BC-PS as an unsuitable supplement; however, PS derived from soybean (S-PS) has emerged as a safe alternative (Blokland *et al.*, 1999). To date, few studies have evaluated the actions of S-PS during exercise; consequently, the current study aimed to investigate the effects of S-PS on exercise capacity during intermittent cycling (for example, Fahey and Pearl, 1998).

Fourteen healthy young male volunteers, who were matched according to body mass and $\dot{V}O_{2max}$ values, completed this placebo-controlled, double blind supplementation study. Following preliminary assessment and familiarisation sessions, each subject completed two main exercise trials separated by 15 ± 1 days. During the ten days prior to undertaking the second main trial the subjects received either 750 $mg \cdot day^{-1}$ S-PS or a glucose placebo. During the main exercise trials the subjects completed three

10-min stages of cycling at approximately 45, 55 and 65% $\dot{V}O_{2max}$ (bouts 1-3, respectively) followed by a final exercise bout at 85% $\dot{V}O_{2max}$ (bout 4) that was continued until volitional exhaustion. The exercise bouts were interspaced with 5-min passive rest periods. Breath-by-breath respiratory data (Jaeger Oxycon Pro; Erich Jaeger GmbH, Germany) and heart rate data (Polar S810; Polar electro, Finland) were continually recorded during both main trials. Venous blood samples and the Exercise Induced Feeling Inventory (EFI) (Gauvin and Rejeski, 1993) were collected before exercise (pre-exercise), after each exercise bout and on the day following the main trial.

The main finding was that exercise capacity, as indicated by time to exhaustion (TTE) during bout 4, was 25 ± 6% longer during trial 2 when compared to trial 1 in PS (*P<0.01*) while TTE were not significantly different between supplementation groups prior to supplementation (*P=0.87*). Supplementation had no significant effect on serum cortisol responses during intermittent exercise in either supplementation group. In addition, feeling states (as indicated on the EFI scale) did not differ following supplementation.

This is the first study that has evaluated the effects of PS supplementation on exercise capacity. The mechanism(s) by which PS supplementation delayed the onset of fatigue remain unclear. However, it is plausible that PS might have: (1) increased ATPase activity in muscle membrane, and hence maintained ionic balance for longer during exercise and; (2) increased calcium availability in the myofibril, prolonged

cross-bridge cycling, therefore delaying fatigue. Further research is warranted to investigate the effects of S-PS supplementation on physiological function and exercise performance.

Acknowledgements

I wish to thank my tutor and now my friend Mike Kingsley for his willingness to help me throughout the completion of this project, without his patient guidance I have no doubt that I would not of completed this.

I am also in debt (by serious amounts of money!) to my father who has always been there no matter what I have thrown at him. For me he has been my rock.

To all my subjects, without their help during their free time I would not have had a study to write up.

I would like to thank Dr Liam Kilduff and Rebecca Dietzig for their continuing help.

To my mum, family and friends (you know who you are).

List of Tables

List of Figures

1 Introduction

Phosphatidylserine (PS) is a naturally occurring phospholipid nutrient that is predominantly found in the membrane of cells (Blokland *et al.*, 1999). It was first isolated and characterised as a constitute of the cephalin fraction of the brain by Folch (1942). Structurally, it consists of 3 different parts: a glycerol backbone, a polar head group (which is hydrophilic in nature and contains phosphorus and serine) and 2 hydrophobic tails, which are fatty acids. The composition of PS in mammalian tissue is relatively small compared to other phospholipids. However, due to the unique atomic and electronic topography of the head piece, the PS molecule is destined for preferential association with membrane 'integral proteins' which include enzymes, receptors and ion channels. It is these PS-protein associations that may be the ultimate key to the proposed effects of PS on a plethora of physiological functions (Pepeu *et al.*, 1996). These functions have been shown to include for example: (1) release of Acetlycholine (ACh) (Davis and Bailey, 1997); (2) activation of Protein Kinase C (Kaibuchi *et al.*, 1981); (3) activation of Tyrosine Hydroxylase (TH) (Raese *et al.*, 1976); (4) activation of the sodium-potassium pump ((Na^+-K^+) pump) (Tsakiris and Deliconstantinos, 1984); and (5) increase in total calcium uptake (Floreani *et al.*, 1991).

Until recently bovine-cortex PS (BC-PS) was the most widely used source of PS and the majority of the studies that investigated the effects of PS has on cognitive function and endocrine responses to exercise have used PS derived from this source. However,

BC-PS is now considered unsuitable for human consumption due to the possibility of transferring infectious diseases such as Spongiform Encephalopathy and Creutzfeldt-Jakob Disease. Alternative sources of PS include soybean derived PS (S-PS) and egg derived PS (E-PS), which do not posses the health risks that have been associated with BC-PS. More recent investigations (for example, Blokland *et al.*, 1999) have used S-PS as it has been demonstrated to produce similar effects on cognition as BC-PS and the yield of PS from soybean lecithin is relatively high. In addition, no serious side effects have been reported in approximately 100 published clinical investigations that have supplemented patients with S-PS and BC-PS. Phopsphatidylserine has been shown to have good bioavailability following intravenous, intraperitoneal and oral supplementation (Toffano *et al.*, 1987) and it has been shown that exogenous PS is efficiently incorporated into the cell membrane of animal cells (Nishijima *et al.*, 1986). However, the biokinetics of PS are not clear and, as yet, the wash-out period of PS in the human body is not known; consequently, the interpretation of cross-over study designs may be difficult.

The effect of PS supplementation on the ageing brain has been extensively researched. The majority of this research has been carried out using BC-PS and the available evidence suggests that PS can positively influence higher brain functions with subjects who are experiencing cognitive decline (for example, Cenacchi *et al.*, 1993) and subjects that were considered to have normal cognitive function (for example, Benton *et al.*, 2001). However, the limited research that has been completed on the effects that S-PS supplementation has on cognitive function has produced

equivocal findings. Monteleone *et al.* (1992) demonstrated that oral supplementation with 800 mg day^{-1} of BC-PS for 10 days significantly attenuated ACTH and cortisol responses to intermittent exercise. In addition, Fahey and Pearl (1998) orally administered 800 mg day^{-1} of S-PS for 14 days to 12 well familiarised subjects during a resistance training programme that was designed to promote overtraining. They reported that S-PS improved subjective ratings of well-being, decreased perceived muscle soreness and reduced cortisol concentrations after training.

Fatigue during high intensity exercise (at exercise intensities above anaerobic threshold) is probably associated with increases in lactate levels leading to a decrease in pH in the blood and the working muscle (Westerblad *et al.*, 2002) and the breakdown of phosphacreatine (PCr) to inorganic phosphate (P_i) (Westerblad *et al.*, 2002). Therefore, the ability of PS to stimulate ACh release, activate the Na^+-K^+ pump and increase total calcium uptake may act to prolong the time to fatigue. In addition, the effects of PS on the endocrine response to exercise may contribute to an increase in exercise capacity and performance by positively increasing the feeling states of subjects. Furthermore, to date, no data have been published to evaluate the effects of PS supplementation during high intensity exercise.

The aims of the study were to examine the effects of supplementation with 750 mg day^{-1} of S-PS for 10 days on the neuroendocrine, cardiorespiratory and psychological responses to intermittent exercise in active young males. Also, the final

bout of exercise, which was continued to exhaustion, was intended to evaluate the effects of S-PS on exercise capacity during high intensity exercise.

2 Review of Literature

2.1 Phosphatidylserine within the Cell Membrane

2.1.1 Organisation and Function of the Cell Membrane

The cell membrane is essential for the function of most if not all-living organisms. Bloom *et al.* (1991) offered a mechanical description of the membrane as "*a material with very small thickness in comparison with its radii of curvature, which separates two adjacent liquid-like domains and supports the stresses created by the embedding medium*". In addition to the many specific functions performed by the cell membrane, Yeagle (1987) identified four main generic functions. These were described as: (1) maintaining transmembrane gradients; this involves creating and maintaining a concentration gradient from one side of the membrane to the other; (2) the organisation of enzymes into a complex; (3) the control of enzyme activity within the cell; and (4) their use as substrates; the components of the membranes may be required for use in a specific reactions.

Located within the cell membrane are lipids. These are a diverse group of heterogeneous biomolecules that are insoluble in water, but soluble in non-polar substances such as chloroform, hydrocarbons or alcohols (McKee and McKee, 1999). The functions of lipids include: (1) energy storage; (2) protective or water proofing;

and (3) the delivery chemical signals. Their composition varies considerably as organelles and membranes having to fulfil a wide variety of functions. The concentrations of lipids in the cell membrane range from 80% in myelin to approximately 25% in mitochondria (Gurr and Harwood, 1991). Lipids can be classified into the following classes: (1) fatty acids; (2) triacylglycerols; (3) wax esters; (4) phospholipids; (5) sphingolipids; and (6) isoprenoids.

2.1.2 Structure of Phospholipids

Phospholipids are the major component found in the cell membrane. In the living organism the primary role of the phospholipid is to maintain the structure in the cell membrane. All Phospholipids are amphipathic molecules and therefore, despite their structural differences, all contain hydrophobic and hydrophilic domains (McKee and McKee, 1999). The hydrophobic domain is composed largely of the hydrocarbon chains of fatty acids while the hydrophilic domain (polar head group) contains the phosphate and other charged or polar groups. There are two types of phospholipids known as phosphoglycerides and sphingomelins. Phosphoglycerides contain glycerol, fatty acids, phosphate and an alcohol (for example serine, choline and glycerol). The simplest form of a phosphoglyceride is phosphatidic acid, which is the precursor for all the other phosphoglyceride molecules (McKee and McKee, 1999). It is composed of glycerol-3-phosphate that becomes esterfied with two-fatty acids. The molecule is then classified according to which alcohol group becomes esterfied to the phosphate group. For example, if it is the alcohol serine that becomes esterfied then the molecule

is called phosphatidylserine (PS), other phospholipids include phosphatidylethanolamine (PE), phosphatidylcholine (PC), diphosphatidyglycerol (dPG) and phosphatidylinositol (PI).

2.1.3 Structure of Phosphatidylserine

Phosphatidylserine is a naturally occurring phospholipid that was first isolated and characterised as a constituent of the cephalin fraction of the brain by Folch (1942). Structurally, PS consists of 3 different parts: a glycerol backbone (which forms the centre of the molecule) a polar head group (which contains phosphorus and another chemical subgroup serine) and 2 hydrophobic tails each of which are fatty acids (Figure 2.1). The unique atomic and electronic topography of the hydrophilic head piece of the PS molecule destines it for a preferential association with membrane 'integral proteins' which include enzymes, receptors and ion channels that insert deep into the membrane.

PS is present in the biological membranes of all animals, higher plants and micro-organisms. Table 2.1 shows the phospholipid composition of various mammalian tissues. The % concentration of PS in the phospholipids pool varies in human tissue; skeletal muscle (3%), heart (3%) and in the brain where it is most concentrated (10%) (Diagne *et al.*, 1984).

Table 2.1 The lipid composition in a variety of human tissues (adapted from Cevc, 1993)

Tissue	Phospholipid Composition (% of lipid Phosphorus)				
	PC	PE	PI	PS	SM
Brain	34	13	3	11	14
Heart	22	14	4	3	6
Liver	45	28	7	4	7
Testis	39	16	5	6	11
Skeletal muscle	48	26	9	3	4
Erythrocytes	31	19	1	13	24
Blood Plasma	77	2	1	0	15

PC: phosphatidycholine; PE: phosphatidyethanolamine; PI: phosphatidylinositol; PS: phopsphatidylserine; SM: sphinomyelin.

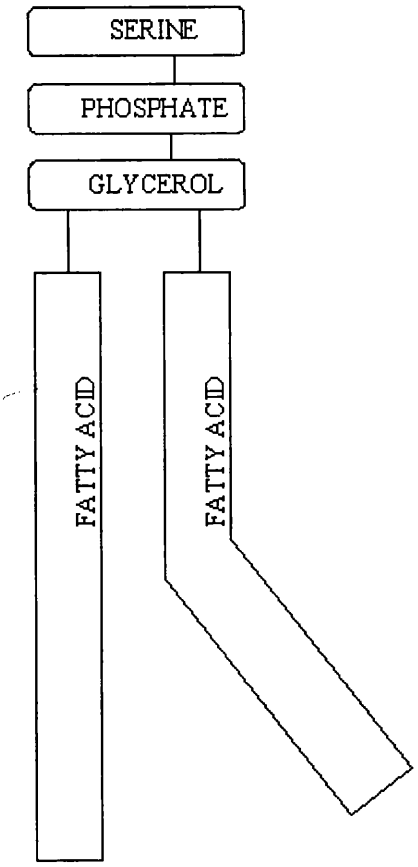


Figure 2.1 Schematic diagram depicting the structure of Phosphatidylserine, showing the glycerol backbone, the polar head group (consisting of phosphate and serine) and the hydrophobic tails. Adapted from McKee and McKee (1999)

Phosphatidylserine is located mainly in the inner leaflet of the cell membrane which is typical for phospholipids containing an acidic head group. It is this acidic head group that is responsible for PS having the tendency to associate with membrane proteins.

2.1.4 Functions of Phosphatidylserine

Phospholipids are not merely structural components of membranes, they are directly and actively involved in many cell membrane functions. Even though it is proteins that activate most membrane functions such as ion pumps, transport molecules, enzymes and receptors, they all depend on the phospholipid matrix to function correctly (McKee and McKee, 1999). All phospholipids, including PS, play an important role in the membrane of cells. Their unique structural properties influence individual membrane proteins that in turn regulate functions at the cellular level. Phosphatidylserine carries a negatively charged amino headgroup which tends to associate preferentially with adenosine triphosphatase (ATPase), kinases, receptors and other key membrane proteins (McKee and McKee, 1999). These specific PS-protein associations may be the ultimate key to the proposed global effects of PS on the brain as a whole (Pepeu *et al.*, 1996). Phosphatidylserine has many important functions in the cell membrane which have been demonstrated to include: (1) release of Acetylcholine (Vannucchi and Pepeu, 1987; Vannucchi *et al.*, 1990; Casamenti *et al.*, 1991); (2) activation of Protein Kinase C (PKC) (Takai *et al.*, 1979; Wrenn *et al.*, 1980; Kaibuchi *et al.*, 1981); (3) activation of Tyrosine Hydroxylase (TH) (Raese *et al.*, 1976); (4) activation of the sodium-potassium (Na^+-K^+) pump (Specht and

Robinson, 1973; Tsakiris and Deliconstantinos, 1984); and (5) increase total calcium uptake (Floreani *et al.*, 1991).

Acetylcholine (ACh) is a neurotransmitter that is released by neurons of the motor nerve cells to change the permeability or other properties of another cell membrane. Release of ACh from the synaptic terminal alters the permeability of the sarcolemma and triggers the contraction of the muscle fibre. Phosphatidylserine has been demonstrated to restore ACh levels in ageing rats (Vannucchi and Pepeu, 1987). The authors pre-treated aged cortical slices from rats with 15 mg kg BW^{-1} and found that PS restored ACh levels to that of younger rats. Also Vannucchi *et al.* (1990) showed that PS stimulated ACh release in both young and aged rat cortical slices when dosed 15 mg kg BW^{-1} intraperitoneally (i.p.) daily for one week. Similarly, Casamenti *et al.* (1991) demonstrated that ACh release was increased though supplementing rats with 15 mg kg BW^{-1} of PS i.p daily for 8 days. They suggested that this chronic PS treatment improved ACh release by increasing availability of choline for ACh synthesis. There are a number of studies that demonstrate that PS increase ACh levels (For example Vannucchi *et al.*, 1990), especially in aged rat cortical slices; however, these studies have been completed using animal models and care should be taken when extrapolating these data to humans.

Protein kinase C are a group of second messenger enzymes, associated with the cytoplasmic face of the cell membrane, that are stimulated by calcium and/or phospholipids (Silverthorn, 2001). Protein Kinase C complexes play a pivotal role in

mediating cellular responses to extracellular stimuli. They also catalyse the phosphorylation of a number of target neuronal proteins which control a number of functions in the cell membrane such as growth and cellular differentiation. Neuronal proteins, such as PKC, are also thought to regulate neurotransmitter release by way of controlling the hormone receptor-transduction mechanisms (Canonico and Scapagnini, 1989). Wrenn *et al.* (1980) demonstrated that the calcium dependent phosphorylation of a number of proteins, including PKC, in the cytosol of the rat or guinea pig cerebral cortex was profoundly stimulated by PS. Takai *et al.* (1979) demonstrated that PS was the sole phospholipid to be effective in activation of calcium activated PKC. Furthermore, Kaibuchi *et al.* (1981) demonstrated that PS plays a positive role in activating this unique protein kinase.

Tyrosine hydroxylase is the rate limiting enzyme involved in the synthesis of dopamine and noradrenaline. Raese *et al.* (1976) showed that the addition of PS to a incubation mixture purified from rat brain striatal synaptosomal fraction strongly activates TH. It is possible that an inactivation of TH is involved in the pathophysiology of psychiatric disorders and associations have been reported for TH gene markers in mood disorders. Serretti *et al.* (1998) showed TH variants may be related with depressive symptomatology in subjects affected by mood disorders. Therefore, an increase in TH activation would lead to an increase in dopamine. Dopamine has long been associated with euphoria, desire and motivation. Moreover,

dopamine disorder or imbalance can cause memory and cognitive loss as well as difficulty with problem solving (Diehl and Gershon, 1992; Ashby *et al.*, 1999).

The Na^+-K^+ stimulated enzyme ATPase is an integral membrane enzyme that is found in cells of all higher organisms. It is the enzyme responsible for the ATP-dependent transport of sodium (Na^+) and potassium (K^+) across the cell membrane (known as the Na^+-K^+ Pump). It is a multi unit enzyme that moves three Na^+ out of the cell along with two K^+ in to the cell at the expense of hydrolysed ATP (Westerblad *et al.*, 1991). This gradient needs to be maintained between the interior and the exterior of the cell for a number of reasons that include facilitated diffusion across the cell and maintenance of the action potential. The net result of intracellular ionic concentration changes during exercise will be a reduction in the intracellular strong ion difference and consequently a marked rise in intracellular hydrogen ion concentration (McKenna, 1992). This intracellular acidosis in humans has been linked with impairment of regulatory and contractile protein function, calcium (Ca^{2+}) regulation and metabolism. PS has been demonstrated to increase Na^+-K^+ stimulated ATPase. Tsakiris and Deliconstantinos (1984) demonstrated that synaptosomal plasma membrane from dogs treated with incubated PS showed an increase in Na^+-K^+ stimulated ATPase. Phosphatidylserine has not only been shown to increase Na^+-K^+ stimulated ATPase in the plasma membrane from dog, but it has also been demonstrated to increase the amount of ATPase in rabbit kidney preparations stimulated with small concentrations of PS (Specht and Robinson, 1973).

Phosphatidylserine has been shown to increase total Ca^{2+} uptake in rat brain synaptosomes (Floreani *et al.*, 1991). These authors incorporated PS vesicles into rat brain synaptosomes in varying amounts. They demonstrated that potassium depolarisation-induced-calcium uptake was stimulated at low amounts of incorporated PS (lower than 0.1 μmol PS mg $protein^{-1}$). At concentrations of PS above 0.1 μmol PS mg $protein^{-1}$, an increase in either Na^{+}/Ca^{2+} exchange activity or passive Ca^{2+} influx was evident and these accounted for the increase in total Ca^{2+} uptake by 40 and 60%, respectively. In short, this demonstrates that PS modifies Ca^{2+} uptake upon incorporation into the membranes of rat brain synaptosomes.

2.2 Phosphatidylserine as a Nutritional Supplement

2.2.1 Sources of PS

Phosphatidylserine can be derived for nutritional supplementation from several sources. Until recent times bovine-cortex PS (BC-PS) has been the most widely used source of PS (Blokland *et al.*, 1999). However, BC-PS is now considered unsuitable for human use due to the possibility of transferring infectious diseases, such as Bovine Spongiform Encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD).

Other sources of PS include soybean derived PS (S-PS) and egg derived PS (E-PS). These sources of PS are free from the health risks posed by BC-PS. Phosphatidylserine derived from these sources have a similar biochemical structure

with respect to the glycerol moiety and serine head group; however, the fatty acid components are different in S-PS, being virtually devoid of arachidonic and decosa hexenoic acid (Blokland *et al.*, 1999).

Approximately 100 clinical studies investigating the effects of both BC-PS and S-PS supplementation in humans have been published and to date no serious side effects have been reported. Cenacchi *et al.* (1993) retrospectively reviewed the results of common haematological variables taken from 130 subjects who underwent oral treatment with PS (300mg day^{-1} of BC-PS daily for 60 days) in an effort to explore the biochemical tolerability of PS. Even though significant reductions in uric acid and liver alanine aminotransferase levels were noted, these were considered clinically negligible.

Blokland *et al.* (1999) examined the behaviour of rats after treatment with a daily dose of 15 mg kg BW^{-1} PS derived from S-PS, E-PS, BC-PS or a placebo. It appeared that the cognition enhancing effects of S-PS were not different from those of BC-PS; however, those treated with E-PS did not differ from the placebo group. Therefore, it was concluded that S-PS, but not E-PS, may have comparable effects on cognition as BC-PS. The authors stated that the differences between S-PS and E-PS were unexpected, due to the similar molecule composition of S-PS and E-PS. They speculated that E-PS may have lost bioactivity due to breakdown of the bioactive compound during processing or storage.

2.2.2 Digestion and Absorption of Phosphatidylserine

Even though PS supplementation has been shown to have good bioavailability, it is currently unclear whether exogenous PS is able to reach the inner membrane of cells and exert the same biochemical effects as endogenous PS; these effects include the activation of PKC, increasing ATPase activity, activating TH and facilitating calcium uptake. It has been demonstrated that exogenous PS can be efficiently incorporated into cultured Chinese hamster ovary cells and then utilized for membrane biogenesis (Nishizuka, 1986). PS has also been shown to be incorporated into olfactory epithelium by incubating these epithelium with a PS-suspension containing radiolabeled PS (Taniguchi *et al.*, 1994). Also, when ^{14}C- labelled PS was directly administered into the cerebral ventricles of rats, the hypothalamus strongly attracted the isotope label (Toffano *et al.*, 1987). It has been demonstrated that intravenously (i.v) injected PS vesicles were rapidly removed from plasma since they are taken up by macrophages and internalised by lymphocyte and endothelial cells (Ponzin *et al.*, 1989). These actions show that exogenous PS directly interacts with cell membranes.

Administration of radiolabelled PS (20 mg kg BW^{-1}) by three different routes (i.v) (i.p) and oral was investigated in rats by Toffano *et al.* (1987). These authors found that, following oral administration, the appearance of the isotope label in the blood increased slowly and consistently over a 24-hour period. When the same dose was administered intravenously, the concentrations of PS in the blood were halved very rapidly (2-8 mins). This rapid clearance from the blood is probably reflective of tissue

uptake across a range of organs which appeared to be saturated within 10 min. Furthermore, it has been demonstrated that when mice were intravenously injected with 20 mg kg^{-1} BW of PS, the rapid clearance from the blood coincided with uptake by both the liver and the brain (Toffano *et al.*, 1987).

Twenty min after i.v. administration with 20mg kg^{-1} BW of ^{14}C-Labelled PS in mouse brain tissue 90-95% of the isotope labelled material was recovered from the brain and was identifiable on thin-layer chromatography as PS. This equated to about 0.25% of the injected dose, a significant proportion when comparing this to the fact that a mouse brain represents 1.5% of total body weight (Toffano *et al.*, 1987). Examination of separate brain areas showed that most radioactivity was present in the cortex, hypothalamus and hippocampus.

From the limited data available regarding the pathway of PS into the body, it seems that PS has good bioavailability via i.p, i.v and oral routes. Currently there is limited information on the uptake and bioavailability of PS following long-term supplementation in humans; however, the pharmacological effects of exogenous PS suggest that PS is able to reach the brain and other tissue areas. Even though the amount of PS incorporated may be small, a number of studies have demonstrated that exogenous PS, from oral route or injection, can have psychological and physiological effects that include enhanced cognitive function (Cenacchi *et al.*, 1993) and a reduction in cortisol concentrations following acute stress (Monteleone *et al.*, 1992).

In fact, most of the published clinical data following PS supplementation has utilised the oral route. These studies have shown that PS has varying pharmacological effects mainly on cognitive function. Doses of PS that have been shown to have positive effects have ranged from long term moderate dosing (For example, 300 mg day^{-1} for up to 3 months; Cenacchi *et al.*, 1993) to higher short term dosing (For example, 800 mg day^{-1} for 10 days; Monteleone *et al.*, 1992). It may be that only a small amount of exogenous PS actually reaches its target. However, it is probable that PS accumulates in the brain and other membrane sites through long term moderate and higher short term supplementations (Pepeu *et al.*, 1996). The plausible accumulation effect could explain the persistence of some of the effects of PS supplementation even after treatment has ended (Engel *et al.*, 1992). In this study, 33 patients with mild degenerative dementia were dosed for 8 weeks with 300 mg day^{-1} of PS in a cross-over study. The subjects showed improvements that continued over the 8-week washout period. This finding and the lack of available data on the appropriate washout period of PS suggests that care must be taken when interpreting published research that utilised cross-over designs.

2.2.3 Phosphatidylserine Supplementation and Cognitive Function in Humans

The effects of PS supplementation on the ageing brain have been extensively researched. Most of the studies that have been well controlled suggest that dietary

supplementation with PS can play an important role in the support of mental functions in the aging brain. Among the clinical trials conducted with PS, most were undertaken with patients who had experienced measurable losses in memory, judgment, abstract thought, and other higher mental functions; therefore, these findings may have limited relevance when considering the 'normal population'. Following searching in Pubmed, Web of Knowledge, Science Direct and Google Scholar using the following key words: PS, cognitive function, mood, and brain some activity on PS and various words including cognitive function, mood, double-blind and placebo, 13 double-blind and placebo controlled clinical trials were identified (Table 2.2). In addition approximately 100 studies were identified that investigated these areas but used inferior study designs and therefore were discarded. Of the studies that were more tightly controlled the findings strongly suggest that PS supplementation has positive effects and psychological and cognitive function. A double-blind study that was carried out by Cenacchi *et al.* (1993) investigated cognitive function in geriatric patients. They found that supplementation with 300 $mg \cdot day^{-1}$ BC-PS for six months statistically improved cognitive and behavioural patterns when compared with the placebo group. Maggioni *et al.* (1990) provided 300 $mg \cdot day^{-1}$ of BC-PS for 30 days to 10 elderly women with depressive disorders and reported that consistent improvements in depressive symptoms, memory and behaviour were observed. The only cross-over study that has indicated a wash out period following supplementation with PS was carried out by Engel *et al.* (1992). They supplemented thirty-three patients with mild degenerative dementia with 300 $mg \cdot day^{-1}$ of BC-PS. Supplementation lasted 8 weeks and was followed by an 8-week washout period.

Table 2.2 Main characteristics of human studies examining the effects of PS supplementation on various makers of cognitive function

Name	No of Subjects / Type of Study	Type of PS Used	Dosage	Results
Amaducci *et al.* (1988)	120 subjects with gradual progressive mental decline	BC-PS administered orally	200mg/day for 3 moths vs. placebo	Benefited memory, verbal ability and daily living
Benton *et al.* (2001)	48 male subjects / double-blind	S-PS administered orally	300mg/day for 1 month vs. placebo	Those defined as more neurotic showed feelings of being less stressed
Cenacchi *et al.* (1993)	494 patients with moderate – severe cognitive decline / double-blind	BC-PS administered orally	300mg/day for 6 moths vs. placebo	Cognitive and behavioural improvements
Crook *et al.* (1991)	149 patients meeting criteria for age-associated memory impairment	BC-PS administered orally	100mg/day for 12 weeks vs. placebo	Clinical global improvements in 'sub groups'
Crook *et al.* (1992)	51 patients meeting criteria for probable Alzheimer's disease / double-blind	BC-PS administered orally	100mg/day for 12 weeks vs. placebo	Benefited memory and recall
Delwaide *et al.* (1986)	42 hospitalised demented patients / double-blind	BC-PS administered orally	300mg/day for 6 weeks vs. placebo	Benefited daily living
Engel *et al.* (1992)	33 patients with mild primary degenerative dementia / double-blind	BC-PS administered orally	300mg/day for 8 weeks vs. placebo	Clinical global improvements

Hellhammer *et al.* (2004)	80 healthy / double-blind	Soy lecithin phosphatidic acid and PS complex (PAS)	400/600/800mg/day for 3 weeks vs. placebo	Decreased serum ACTH and cortisol and salivary cortisol with 400mg PAS
Jorissen *et al.* (2001)	120 elderly (>57 years) both sexes, age-associated memory impairment / double-blind	S-PS administered orally	300/600mg/day for 12 weeks vs. placebo	No significant interactions
Maggioni *et al.* (1990)	10 elderly women with depressive disorders / double-blind cross over	BC-PS administered orally	300mg/day for 30 days vs. placebo	Improvements in memory and concentration
Palmieri *et al.* (1987)	87 subjects with moderate cognitive deterioration / double-blind	BC-PS administered orally	100mg/day for 60 days vs. placebo	Benefited attention, concentration and short-term memory.
Rosadini *et al.* (1991)	8 healthy males / double-blind cross over	BC-PS administered intravenously	25/50/75mg vs. placebo	Increase in the alpha EEG rhythm
Villardite *et al.* (1987)	120 subjects with moderate cognitive deterioration	BC-PS administered orally	300mg/day for 90 days vs. placebo	Benefited memory, learning, concentration

Clinical global improvement ratings were significantly improved under the PS condition when compared to the placebo condition and this improvement carried on into the continuing washout period. If the positive affects of PS are carried over into a washout period and the duration of this period is unknown for PS, then the conclusions drawn from cross-over studies are difficult to interpret. Therefore, BC-PS, when supplemented for a range of time periods, has been demonstrated to improve cognitive and behavioural patterns in subjects who have shown depressive symptoms in these areas.

Not only has PS been shown to improve cognitive function; interestingly, supplementation has also been shown to boost electroencephalogram (EEG) rhythms (Rosadini *et al.*, 1990). These authors administered 25, 50 or 75 mg of BC-PS i.v. to eight healthy volunteers. It was noted that the highest dose of PS boosted the average alpha rhythm by 15-20% without any detectable side effects. Moreover, alpha rhythms are often found to be attenuated in the ageing and people with cognitive decline (Rosadini *et al.*, 1990). This study was carried out on what would be considered a 'normal' population and the results suggest that supplementation of BC-PS administered intravenously, increased the EEG alpha rhythm. The alpha rhythm as detected by EEG, is considered a direct measure of brain function and increases in alpha rhythm changes have been associated with improvements in general mood state (Kumano *et al.*, 1996).

To date there have been very few human studies that have looked at the effects of S-PS on various psychological and cognitive functions. However one such study that uses S-PS and is considered to employ a strong study designs has produced results that are in conflict with the majority of the previous research on PS. Jorissen *et al.* (2001) supplemented 120 elderly subjects who fulfilled the criteria for age-associated memory impairment (AAMI). These subjects were allocated in a random order to receive either 300, 600 mg S-PS or placebo for 12 weeks. Assessments of learning and memory, choice reaction time, planning and attention functions were carried out at the beginning of supplementation, after 6 and 12 weeks and 3 weeks post-supplementation. No significant differences were found in any of the outcome variables between the treatment groups. The reasons for the ineffectiveness of S-PS to show positive findings are unclear; however, it may be that the word list, which was the main outcome tested, was not capable of showing any true cognitive differences between the groups and only 'some' of the 120 elderly fulfilled the criteria for cognitive decline. Therefore, this study demonstrated that S-PS did not affect memory recall in elderly patients and may not of evaluated cognitive function.

There have been 2 studies to date which have investigated the effects of S-PS on subjects who are not experiencing some sort of cognitive decline, one of which investigated feeling states and the other mood and heart rate in response to stress. Benton *et al.* (2001) studied the effects of S-PS on feeling states in a 'normal' young student population. These authors supplemented 22 subjects with 300 $mg \cdot day^{-1}$ of S-PS for 30 days while a further 26 subjects received a suitably matched placebo.

Following supplementation with S-PS, those who were defined as having a more neurotic personality showed statistical significant improvements in feeling clear-headed, composed and confident, feeling energetic and elated when faced with the acute stressor of performing a demanding mental arithmetic task. During this study none of subjects supplemented with PS guessed that they had taken the supplement; therefore, suggesting that the double blind procedure was not compromised. Hellhammer *et al.* (2004) investigated the influence of soy lecithin phosphatidic acid and PS complex (PAS) supplementation on mood and heart rate when faced with an acute stressor. Four groups of 20 subjects were treated with 400, 600 or 800 mg PAS or placebo for 3 weeks prior to completing the Trier Social Stress Test (TSST). They demonstrated that supplementation with 400 mg PAS blunted serum ACTH and cortisol concentrations, and salivary cortisol responses to the TSST, while the placebo group showed the expected increase in distress post TSST. Interestingly, neither the 600 and 800 mg supplementation groups showed the positive effects demonstrated in the 400 mg group.

In summary, the majority of the available evidence available suggests that PS supplementation (provided in varying forms of administration and different dosing regimes) can positively affect cognitive and higher brain functions in subjects with cognitive decline. In addition, it seems that not only does it improve these abnormalities, it also has positive effects on mood, EEG alpha rhythm and endocrine response in subjects that were considered to have normal cognitive function. However, there are a limited number of studies that have been carried out using S-PS

and further research needs to be carried out with S-PS to confirm the effects supplementation has on cognitive function.

2.2.4 Phosphatidylserine Supplementation and its Effects during Exercise

To date there have been three peer reviewed published studies that have examined the effects of PS supplementation on humans during exercise (Table 2.3). Monteleone *et al.* (1990) studied the effects of PS on neuroendocrine responses to physical stress in humans. These authors supplemented eight healthy men who did not participate regularly in any kind of sport on three separate occasions with PS or placebo. Twenty min following i.v. administration (50, 75 mg of BC-PS and volume-matched placebo) the subjects completed three six-min bouts of exercise on a cycle ergometer at different workloads (1.5, 2.0, and 2.5 W kg BW^{-1}, respectively) interspaced with 1-min rest periods. Venous blood samples were collected prior to i.v. injection, 10 min following i.v. injection, at the end of each workload and then 20 and 60 min after the completion of exercise. They showed that the physical stress caused significant increases in plasma ACTH and cortisol concentrations and that cortisol peaked 20 min prior to the end of exercise. These responses were attenuated in both BC-PS treatment groups. Similarly, Monteleone *et al.* (1992) supplemented nine healthy inactive subjects, orally with 800 $mg \cdot day^{-1}$ of BC-PS, 400 $mg \cdot day^{-1}$ of BC-PS and a suitably matched placebo for 10 days.

Table 2.3 Main characteristics of human studies examining the effects of PS supplementation on various makers during physical exercise

Authors	No. of subjects /Type of study	Type of PS used	Dosage	Effect of PS supplementation
Monteleone, Beinat, Tanzillo, Maj and Kemali (1990)	8 healthy inactive males / double-blind cross over	BC-PS intravenously administered before exercise	50/75mg vs. placebo	Decreased cortisol and ACTH after exercise with both 50 and 75mg of PS
Monteleone, Maj, Beinat, Natale and Kemali (1992)	9 healthy inactive males / double-blind cross over	BC-PS administered orally	400/800mg/day for 10 days vs. placebo	Decreased cortisol and ACTH after exercise when supplemented with 800 mg/day
Fahey and Pearl (1998)	Twelve fit males with at least 4 years weight training experience / double-blind cross over	S-PS administered orally	800mg/day for 14 days vs. placebo	Increased perception of well being, decreased perception of muscle soreness, and decrease in cortisol after weight training regime

During this study the same exercise and venous sampling protocols were used as employed during the groups previous study (Monteleone *et al.*, 1990). The exercise induced increase in ACTH and cortisol concentrations were significantly blunted by 800 $mg \cdot day^{-1}$ BC-PS but not by 400 $mg \cdot day^{-1}$ BC-PS. Therefore, it was concluded that the blunting of the HPA- axis was dose specific and under these conditions 800 $mg \cdot day^{-1}$ was required to attenuate the ACTH and cortisol responses to exercise. Unfortunately, these authors offered no information about the order of supplementation; therefore, it may be possible that the subjects were supplemented, with a placebo, then 400 $mg \cdot day^{-1}$, and finally 800 $mg \cdot day^{-1}$. If this was the case, then accumulation of PS in the tissues may well have occurred and consequently this accumulation may have lead to the dose response reported for ACTH and cortisol response. Both Monteleone *et al.* (1990) and Monteleone *et al.* (1992) employed intermittent progressive exercise with absolute exercise intensities; therefore, the subjects were working at varying relative exercise intensities which could lead to different endocrine responses between subjects depending upon subject fitness. In support of this statement, Luger *et al.* (1987) reported that active subjects have been shown to have a lower cortisol response when compared with sedentary individuals exercising at equivalent absolute and relative exercise intensities. Therefore the large ACTH and cortisol responses to exercise reported by Monteleone *et al.* (1990) and Monteleone *et al.* (1992) probably reflected the training status of the subjects. By having larger responses of ACTH and cortisol to exercise any effect that PS may have on the endocrine system may be easier to indentify.

Fahey and Pearl (1998) orally administered 800 $mg \cdot day^{-1}$ of S-PS for 14 days to twelve resistance trained subjects during a resistance exercise induced over training regime. Subjects taking the S-PS reported a significantly greater sense of well-being during the 2-week training regime and a decreased perception of muscle soreness when compared with the placebo group. Cortisol concentrations were also reduced after training; furthermore, the authors suggested that this reduction in cortisol was not sufficient to change vital cortisol functions such as gluconeogenesis. A cross-over design was used during this study and as there is no information available on the washout period of PS an accumulation effect of PS could occur if subjects were supplemented with PS followed by the placebo. However, these results support the conclusions of Monteleone *et al.* (1990) and Monteleone *et al.* (1992) who suggested that PS supplementation suppressed the HPA-axis response to exercise.

In summary, these findings must be considered somewhat preliminary due to the small quantity of published data; however, findings are consistent with an influence of PS on brain function. From these studies it seems that PS supplementation, both in the form of BC-PS and S-PS, reduces blood cortisol concentrations during exercise. In addition, 800 $mg \cdot day^{-1}$ of S-PS for 14 days increased subject well-being and reduced perception of muscle soreness. Even though both endocrine responses and mood have been shown to be affected by PS, to date there are no peer-reviewed studies that have examined the effects of PS supplementation on exercise performance or exercise capacity. Furthermore, controlled trials are needed to investigate the effects of PS supplementation on the HPA – axis and mood during exercise on a trained population.

2.3 Physiological Responses to Exercise

2.3.1 Hormonal Responses to Exercise

Stress generated by a significant change from the 'normal' psychological and physiological state causes the hypothalamus to release corticotrophin–releasing hormone (CRH). This generic response has been demonstrated to be initiated during changes in self-rated emotional states (Gerra *et al.*, 1998), other psychological stressful situations (Aguilera, 1998) and physical activity (Davies and Few, 1973). Thus, cortisol has been used as a marker of overall stress and is sometimes called a 'stress hormone'. It is the stimulation of the hypothalamus and the release of CRH which leads to a change in the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis stimulates the anterior lobe of the pituitary gland to release Adrenocorticotrophin hormone (ACTH), which upon reaching the adrenal cortex causes cortisol to be released.

The glucocorticoids, including cortisol, have been known to increase during demanding physical activity for approximately 50 years (Staehelin *et al.*, 1955). More recently it has been demonstrated that cortisol secretion is dependent on the intensity and duration of exercise (Davies and Few, 1973). These authors demonstrated that plasma cortisol concentrations were maintained or decreased at exercise intensities below ~50% $\dot{V}O_{2max}$ on a treadmill; however, at exercise intensities of 65-90% $\dot{V}O_{2max}$ increases in plasma cortisol concentrations were shown after 10 min of

exercise. As the exercise intensity approaches anaerobic threshold, the relative cortisol increases from baseline (Port, 1991). In addition, during short-term high-intensity exercise the blood plasma cortisol response has been shown to increase with the intensity of leg power up to 100% of maximal leg power on a cycle ergometer (Kraemer *et al.*, 1989). Luger *et al.* (1987) found that the exercise induced increase in serum cortisol concentrations were attenuated in response to a given workload in trained subjects when compared to untrained subjects.

Hormones such as cortisol tend to be secreted into the blood in an episodic manner, where secretions may be separated by rest periods anywhere between 5 and 30 min (Brandenberger *et al.*, 1984); additionally the body's level of cortisol in the bloodstream displays what is known as a diurnal variation; that is, normal concentrations of cortisol vary throughout a 24-hour period (Edwards *et al.*, 2001). Following sub-maximal treadmill running in moderately trained young men, cortisol has been shown to remain elevated for as long as 2 hours after exercise (Kanaley *et al.*, 2001); however, peak cortisol concentrations have been reported to occur at approximately 10-40 min post-exercise (Davies and Few, 1973; Monteleone *et al.*, 1990; Monteleone *et al.*, 1992; Jacks *et al.*, 2002).

2.3.2 Fatigue during Exercise

Fatigue during exercise has been defined as an inability of muscle or a group of muscles to maintain a required or expected force (Edwards, 1981). Fatigue during voluntary muscular contraction is a complex phenomenon and may be caused by central nervous factors as well as changes in the peripheral site of the neuromuscular system (Allen, 2004).

Fatigue has been extensively researched; however, the research in this area has tended to concentrate on the extremes of exercise duration and intensity, such as supra-maximal and endurance exercise. For the purpose of this review supra-maximal exercise has been considered to be any exercise at an intensity above $\dot{V}O_{2max}$, to include exercises such as short-sprints or repeated lifting of heavy weights; whereas endurance exercise is considered exercise that exceeds an hour in length of time. There has been relatively little research on the mechanisms of fatigue during high-intensity exercise; which, for the purpose of this review was considered to be exercise at intensities above the anaerobic threshold but below $\dot{V}O_{2max}$. In addition, investigators who have studied fatigue have tended to use various stimulation schemes which makes it very hard to make direct comparisons between studies (Westerblad *et al.*, 1991). Both supra-maximal and high intensity exercise intensities cause a large increase in both blood and muscle lactate and both break down glycogen at a fast rate, and use large amounts of energy. When muscles are worked at high

work loads, stores of energy within the muscle are used up at a fast rate and the by-products of these reactions accumulate within the working muscle and blood.

Measurements of muscle lactate concentrations after exercise have shown that the maximum accumulation of lactate within the muscle occurs at the end of exercise that causes exhaustion in approximately 3-7 min (Maughan and Gleeson, 2004). High levels of intracellular lactic acid can lead to intracellular acidosis which causes intracellular pH to fall, have long been discussed as being a cause of fatigue (Westerblad *et al.*, 2002). Therefore, both supra-maximal efforts and high-intensity exercise will cause large amounts of lactic acid. There have been a number of studies that have shown a link between this decline of intracellular muscle pH and the reduction of force or power production (Fabiato and Fabiato, 1978). However, this link is coming under question as many studies are concluding the lowering of pH only leads to fatigue in lower temperatures and not the physiological temperatures that are found in the human body. Pate *et al.* (1995) found the reduction in fatigue to be minimal at 30°C on skinned rabbit fibres when compared to 10°C.

During supra-maximal intensities the phosphacreatine (PCr) content in the working muscle is all or nearly all depleted in a matter of seconds (Bogdanis *et al.*, 1996). As a result of PCr levels depleted, inorganic phosphate (P_i) is produced from the breakdown of PCr. Therefore, if PCr levels are being depleted in the working muscle

excess inorganic phosphate will be produced, which has been shown to a potential cause of fatigue (Westerblad *et al.*, 2002). As exercise intensity drops so does the amount of depletion of PCr in the working muscle. At intensities of approximately 80-90% $\dot{V}O_{2max}$ PCr levels have been shown to be depleted by up to as much of half there original resting values (Maughan and Gleeson, 2004); consequently producing high-levels of P_i in the muscle, which would potentially lead to fatigue.

Depending on different variables such as exercise duration/intensity and the muscular groups tested and the type of contractions involved, strength loss with fatigue can originate from several sites from the motor cortex through to contractile elements (Bigland-Ritchie, 1981; Edwards, 1981; McKay *et al.*, 1995). This phenomenon has been termed 'task dependency of muscle fatigue' (Millet and Lepers, 2004). Fatigue caused by exercise that occurs at intensities that require intense contraction, particularly with large muscle groups have been commonly attributed to what is known as peripheral fatigue (Green, 1997). In principle, the site of peripheral fatigue could occur along a number of sites including the neuromuscular junction, the process of excitation-contraction (E-C) coupling (which involves the activation of the surface membrane), the propagation of that activation down the T-tubules (which brings the activation into the depths of the cell), the release of calcium and, finally, the activation of the contractile elements involved in the generation of force and power. Therefore; fatigue can be attributed to any number of mechanisms during this process; however, the most probable causes could include: (1) a reduction in neurotransmitter release at the neuromuscular junction (Davis and Bailey, 1997); (2) disturbances in

the ionic changes that may lead to an inability to maintain the membrane potential and excitability (Green, 1997); and (3) a failure of normal calcium release from the sarcoplasmic reticulum (Allen and Westerblad, 2001).

2.3.2.1 Acetlycholine and Fatigue

Each motor neurone activates a group of muscle fibres and is collectively referred to as a motor unit. The chemical messengers, or neurotransmitters, that carry the nerve's excitation message to the muscle at the neuromuscular junction can become impaired with intense exercise. One of the main neurotransmitters in this case is ACh. At the neuromuscular junction an action potential causes release of ACh from the nerve ending, and this will cause a local depolarisation in the end-plate area of the surface membrane of the cell (Westerblad *et al.*, 1991). The local depolarisation will initiate the action potential that propagates along the surface membrane. Acetylcholine has been shown to be impaired during exercise and any inhibition would result in the inability of local depolarisation and as a consequence of this the action potential would not propagate along the surface membrane. This would lead to the muscle fibres being unable to contract, and therefore lead to cessation of exercise (Davis and Bailey, 1997). PS has been shown to increase ACh in rat cortical slices when supplemented with PS (Vannucchi and Pepeu, 1987; Vannucchi *et al.*, 1990; Casamenti *et al.*, 1991). If this increase in ACh is transferable to humans when supplemented with PS, then this increase in ACh could potentially delay the onset of fatigue during intense exercise. Increasing ACh availability during exercise could

potentially cause local depolarisation to carry on occurring and therefore the action potential would still propagate along the surface membrane for an extended period of time.

2.3.2.2 Ionic Balance across the Cell Membrane and Fatigue

Fatigue during maximal efforts has also been linked to Na^+ and K^+ disturbances in ionic balance across the cell membrane (Green, 1997). Ionic changes in the cell are central to the activity of a muscle due to the action potential being caused by rapid movements of Na^+ and K^+ in and out of the cell (Allen, 2004). Disturbances in these ionic balances play an important role in the development of muscular fatigue (Clausen and Nielsen, 1994). As previously mentioned intense muscular contraction is accompanied by the anaerobic breakdown of glycogen and PCr whose by products are lactate and inorganic phosphate (P_i). This combined with the increased muscle water content, which is distributed in both intracellular and extracellular spaces, modifies ionic balance across the cell membrane. Changes in muscle intracellular electrolyte concentrations with intense contraction may be summarised as including decreases in K^+ (6 to 20%), decreases in PCr (up to 70 to 100%), increases in lactate (more than 10-fold), increases in Na^+ (2-fold) and small, variable increases in chloride (McKenna, 1992). The net result of these intracellular ionic concentration changes with exercise will be a reduction in the intracellular strong ion difference, with a consequent marked rise in intracellular hydrogen ion concentration. This intracellular acidosis has been linked with fatigue via impairment of regulatory and contractile

protein function, Ca^{2+} regulation and metabolism. Potassium efflux from the contracting muscle cell dramatically decreases the intracellular to extracellular potassium ratio, leading to depolarisation of sarcolemmal and t-tubular membranes. During non-steady state incremental exercise up to about 60% $\dot{V}O_{2max}$, plasma K^+ increases proportionally to increases in oxygen consumption; thereafter, it increases disproportionately (Paterson *et al.*, 1990) until the point of exhaustion where it can reach 8-9 $mmol^{-1}$ in the arterial blood (Medbo and Sejersted, 1990). These transmembrane fluxes, under normal circumstances are counteracted by the Na^+ - K^+ pump, however, during intense muscular stimulation the efficacy of the Na^+ - K^+ pump may be reduced; consequently these Na^+ and K^+ ionic shifts transpire (Clausen and Nielsen, 1994). Fowles *et al.* (2002) investigated the hypothesis that reductions in (Na^+-K^+)- ATPase activity are associated with neuromuscular fatigue following isometric exercise. These results demonstrated that (Na^+-K^+)- ATPase activity is reduced by sustained isometric exercise in humans from and that this reduction in (Na^+-K^+)- ATPase activity is associated with loss of cell excitability. If these reductions in (Na^+-K^+)- ATPase activity could be reduce or if (Na^+-K^+)- ATPase activity could be increased in the cell membrane then potentially fatigue could be delayed. PS has been shown to increase (Na^+-K^+)- ATPase activity in both dog synaptosomal plasma membrane (Tsakiris and Deliconstantinos, 1984) and rabbit kidney preparations stimulated with small concentrations of PS (Specht and Robinson, 1973). Therefore, by acting as a buffer, PS could work to minimise these disturbances in ions across the cell membrane which are caused by intense exercise.

2.3.2.3 Calcium and Fatigue

As previously stated anaerobic metabolism in skeletal muscle involves hydrolysis of PCr to P_i. Inorganic phosphate has been shown to be an important factor in the causes of fatigue during high-intensity exercise (Westerblad *et al.*, 2002) and it has become evident in recent years that P_i may affect fatigue by effecting sarcoplasmic reticulum Ca^{2+} handling. Calcium plays an important and complex role in generation of force and power in the muscle and has been linked as a possible cause of tension decline during fatiguing situations such as high intensity short-term exercise (Allen, 2004). As a result of Ca^{2+} release from the sarcoplasmic reticulum (SR), Ca^{2+} binds to troponin C, causing an interaction between myosin and actin resulting in cross-bridge cycling and therefore cell contraction. Fryer *et al.* (1995) demonstrated that increased P_i might depress SR Ca^{2+} release in initial experiments on skinned fibers with intact transverse-tubular SR systems. Eberstein and Sandow (1963) reported that a fatigued muscle showed substantial recovery when K^+ or caffeine were applied, both of which are known to increase the SR Ca^{2+} release. They fatigued intact muscles with repeated tetani until force was greatly reduced. They showed that by increasing extracellular K^+ or caffeine muscular force was substantially increased in the fatigued muscle, suggesting that a reversible failure of activation was an important contributor to fatigue. Caffeine acts to increase the opening of the SR Ca^{2+} release channels to overcome fatigue, thus the partial failure of SR Ca^{2+} release is accepted to be one of the causes of muscle fatigue (Williams and Klug, 1995; Allen *et al.*, 1995; Favero, 1999). It is possible that PS can act in such a way as to increase Ca^{2+} release or

availability in a similar way to caffeine. PS has been shown to increase total Ca^{2+} uptake in rat brain synaptosomes (Floreani *et al.*, 1991) though incorporation of PS vesicles into rat brain. If this is the case in human cell and more Ca^{2+} is made available or more is released during exercise then PS may act in such a way to allow more Ca^{2+} to be made available when the myofibril and therefore prolonged cross-bridge cycling and delay the onset of fatigue.

3 Methods

3.1 Subjects

Fourteen active healthy male volunteers completed all the requirements of the study and were included in the subsequent analysis (Table 3.1). All subjects provided written consent for this study (Appendix 1), which was approved by a University of Wales Swansea ethics committee (Appendix 2). Subjects were initially assessed for eligibility for the study by interview and questionnaire (Appendix 3). The subjects were considered eligible for the study if they: (1) agreed to attend the laboratory when required; (2) had not taken nutritional supplements or medication for at least 4 weeks prior to testing; (3) agreed to abstain from ingesting caffeine 24 hours prior to testing; and (4) agreed to refrain from partaking in any additional exercise 24 hours prior to testing. Subjects were also made aware that they could withdraw from the study at any time without providing a specific explanation.

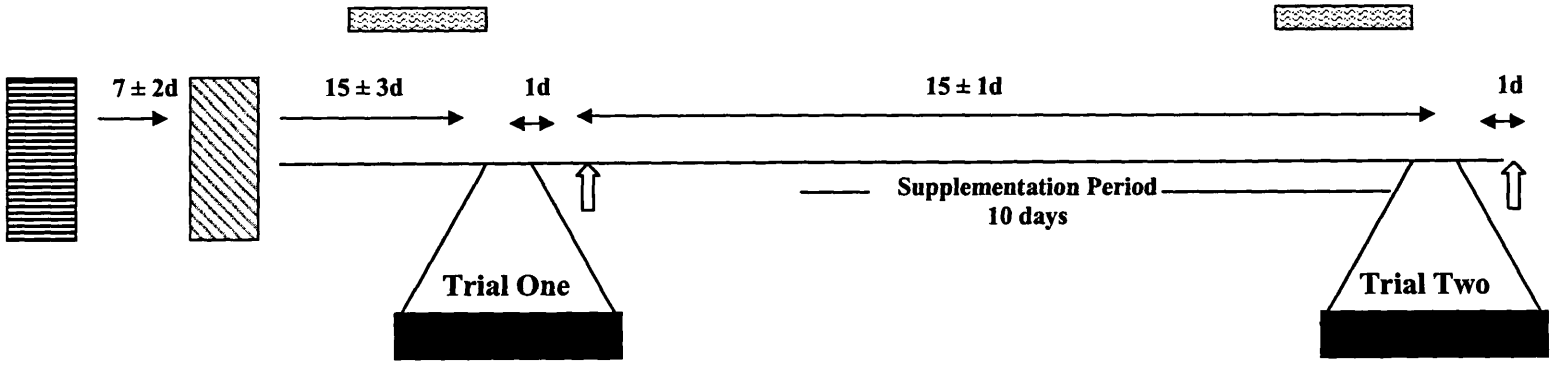
3.2 Experimental Design

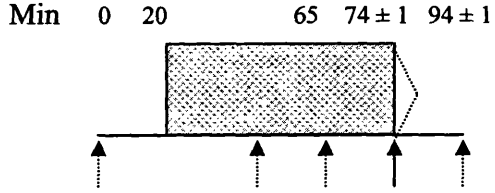
A schematic diagram of the main experimental design is presented in Figure 3.1. During the experimental period each subject attended the laboratory on 6 separate occasions over a period of 42 ± 4 days.

Table 3.1 Physical characteristics of subjects that completed all study requirements

Physical Characteristics	PS	Placebo
Age (dec.years)	23.4 ± 1.9	22.2 ± 1.1
Mass (kg)	84.9 ± 3.9	87.7 ± 3.2
Height (m)	1.79 ± 0.01	1.81 ± 0.02

Values are mean ± SEM (N=7). PS: phosphatidylserine group; P: placebo group.





3-day dietary control

$\dot{V}O_{2max}$ Test

Familiarisation

Blood sample and EFI

End of exercise capillary blood sample

24 post blood sample and EFI

Figure 3.1 Schematic diagram of the experimental procedure

During the initial session each subject completed preliminary assessments that consisted of anthropometric measurements and an incremental exercise test. Following a familiarisation session that consisted of the main trial exercise protocol (completed 7 ± 2 days after the preliminary assessments), each subject completed two main exercise trials separated by 15 ± 1 days. The main trial exercise protocol required the subjects to complete three 10-min stages of cycling followed by a final exercise bout that was continued until volitional fatigue. Breath-by-breath respiratory data and heart rate data were continually recorded during both main trials. Blood samples and the Exercise Induced Feeling Inventory (EFI) (Gauvin and Rejeski, 1993) were collected at pre-exercise (pre-ex), after each exercise bout and on the day following the main trial.

For the ten days prior to undertaking the second main trial the subjects received either 750 $mg \cdot day^{-1}$ S-PS (Appendix 5) or placebo capsule, which was similar in size and shape containing less than 1g of glucose powder. Dietary records were completed for the three days prior to the first main exercise trial and the subjects were requested to match their dietary intake as closely as possible prior to the second main trial.

3.3 Preliminary Procedures

3.3.1 Anthropometric Measurements

Body mass was determined using balanced beam scales (Seca 710; Vogel and Halke GmbH and Co., Germany) when subjects were dressed in minimal clothing (recorded to the nearest 0.1 kg) and height was measured (recorded to the nearest 1 cm) using a portable stadiometer (Harpenden stadiometer; Holtain Ltd, Wales) whilst the heals were flat against the back board and the head was supported in the Frankfurt plane.

3.3.2 Measurement of Maximal Oxygen Uptake ($\dot{V}O_{2max}$)

Subjects underwent an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode, Netherlands) which was carried out to measure maximal oxygen uptake ($\dot{V}O_{2max}$), from this the relative intensities for the main trial's exercise protocol were calculated.

Expired gases were analysed using an automated online gas analyser (Jaeger Oxycon pro; Erich Jaeger GmbH, Germany). The integrated gas analysers were calibrated before each test in accordance with manufactures instructions using a gas cylinder with known gasses (5.18% carbon dioxide in a nitrogen balance: β certified gas; BOC, UK) and a reference gas (room air). Flow rates were measured using a Triple V bi-directional turbine which was calibrated before each test using 2 reference flow rates

and weekly using a 3-litre syringe (Hans Rudolph, Canada), with a range of different flow rate profiles.

After the subjects were seated for at least 10 min on the cycle ergometer, resting respiratory data were recorded for 3 min whilst the subjects remained seated and in an upright position. This was followed by a 3-min warm-up at a work rate of 60 W and a subsequent 2-min pre-test rest period. The incremental test began at 60 W and increased in work rate by 30 W every 2 min until volitional fatigue or until the subject could no longer maintain a pedal cadence of at least 50 rev·min^{-1}. Breath-by-breath respiratory data and heart rates were recorded in 5-s intervals using short-range telemetry (Polar S810; Polar electro, Finland) throughout the protocol. Ratings of perceived exertion (RPE) were recorded immediately following each stage.

Oxygen uptake ($\dot{V}O_2$) data were averaged to 10-s intervals and maximum oxygen uptake ($\dot{V}O_{2max}$) was determined as the peak $\dot{V}O_{2max}$ recorded during exercise. All subjects were required to reach at least 3 out of the 4 following criteria: (1) RPE_{max} 18; (2) maximum recorded heart rate was greater than 90% of age predicted maximum (220-age) (Vanderburgh and Katch, 1996); (3) respiratory exchange ratio (RER) 1.10 (Londeree *et al.*, 1995); (4) change in $\dot{V}O_{2max}$ 2.0 ml kg^{-1} min^{-1} during the last min of exercise (Vanderburgh and Katch, 1996).

The average $\dot{V}O_2$ for the final 30-s period at each completed exercise stage was plotted against the respective work rate and the best fit linear relationship was determined for each subject. Subsequently, these individualised linear relationships and $\dot{V}O_{2max}$ values were used to calculate the exercise intensities for each stage of the main trial exercise protocol.

3.4 Main Trial Procedures

The subjects completed both main trials at approximately the same time (± 1 hour) of the day. The subjects were required to confirm that they had reported to the laboratory having: (1) not ingested caffeine within 24 hours; and (2) refrained from partaking in any additional exercise within 24 hours.

Figure 3.2 presents a schematic of the events completed during the main trials. On arrival to the laboratory the subjects were required to complete a pre-ex EFI, which required the subjects to rate 12 different feeling states, using a 5-point likert scale (Gauvin and Rejeski, 1993) (Appendix 4). Subsequently, a pre-ex venous blood sample was taken 20 min prior to the start of exercise. Sitting on a medical table, with the backboard in an upright position for approximately 10 min, a tourniquet was placed on the upper arm and a 21 gauge precision needle (Becton Dickinson Vacutainer Systems, UK) was inserted into an antecubital vein. Subsequently, two 3.5 ml serum separation tubes (SST) (Becton Dickinson Vacutainer systems, UK) and one

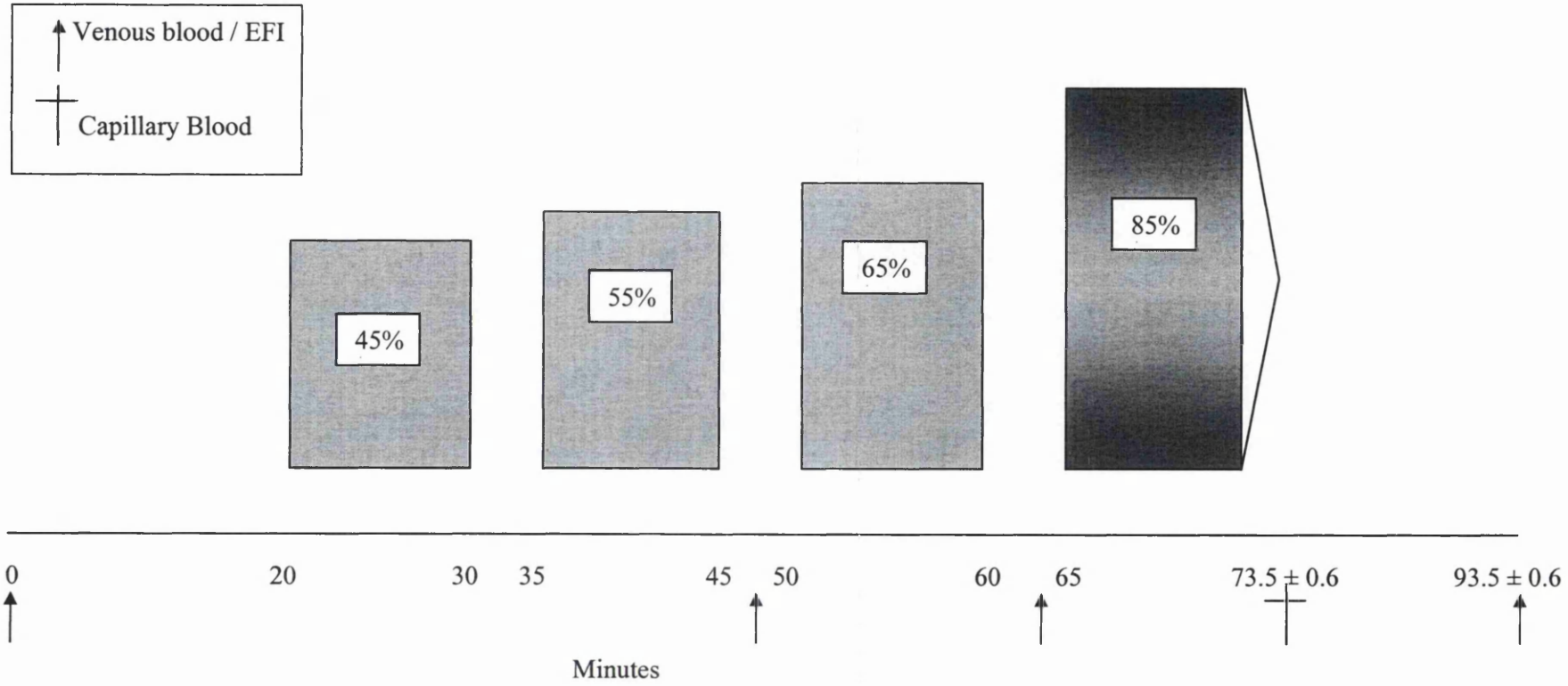


Figure 3.2 Schematic diagram of the main trials exercise protocol.

7 ml ethylenediaminetetraacetic acid (EDTA) tube (Becton Dickinson Vacutainer systems, UK) were filled with venous blood.

After the subjects were seated for at least 10 min on the cycle ergometer (Lode Excalibur Sport; Lode, Netherlands) resting respiratory data were recorded for 3 min whilst the subjects remained seated and in an upright position on the cycle ergometer. This was followed by a 3-min warm-up undertaken at the work rate of 60 W and a subsequent 2-min rest period. The subjects then completed three 10-min stages of cycling at approximately 45, 55 and 65% $\dot{V}O_{2max}$ (bouts 1-3, respectively). The exercise bouts were interspaced with 5-min passive rest periods. Directly after the completion of the 2nd and 3rd bouts of exercise EFI were completed and venous blood samples were taken whilst the subjects were seated upright on the cycle ergometer using the same procedures as previously described. This was followed by a final exercise bout at 85% $\dot{V}O_{2max}$ (bout 4) that was continued until volitional fatigue or until the subject could no longer maintain a pedal cadence of at least 50 rev·min^{-1}, the time of which was defined as time to exhaustion (TTE) and used as a measure of exercise capacity. Breath-by-breath respiratory data were continuously recorded throughout the protocol (Jaeger Oxycon pro; Erich Jaeger GmbH, Germany). Immediately after bout 4 an end of exercise capillary blood sample (end-ex) was collected whilst the subject remained upright on the cycle ergometer. Accu-Check Softclix Pro and lancets (Roche diagnostics GmbH, Germany) were used to collect approximately 50 µl of blood in a heparinised capillary tube from the index finger. Subjects remained seated in an upright position on the cycle ergometer for a rest

period of 20 min. Directly after this a post-exercise (post-ex) resting venous blood samples and EFI were completed with the subjects sitting on the medical table, as previously described.

Approximately 24 hours after both the main trials each subject reported to the laboratory having fasted for at least 4 hours and resting EFI and venous blood samples were obtained (24-post).

3.5 Supplementation

The subjects were matched in terms of body mass and $\dot{V}O_{2max}$ values and subsequently, they were randomly assigned, in a double-blind manner, to receive either S-PS (PS) or a matched glucose placebo which was similar in size and shape containing less than 1g of glucose powder (placebo).

The supplement used in the present study is one marketed by Lucas Mayer. The method used by Lucas Meyer to produce this supplement is one of transerterification of soybean lecithin and gives a total PS concentration of 46 to 54% in each capsule.

The PS group received 750 $mg \cdot day^{-1}$ S-PS administered in ten 75 mg S-PS capsules placed in generic packages to maintain double blind procedure. The placebo group received ten capsules filled with glucose powder. Subjects were required to take all the capsules with a pint of water first thing in the morning with food. Subjects were

supplemented for 10 days prior to undertaking the second main trial which included the day before attending the laboratory. Subjects were asked if they had taken all supplements prior to trial 2.

3.6 Blood Analysis

Venous blood was immediately drawn from the EDTA vacutainer to measure blood haemoglobin concentrations in duplicate and blood lactate, glucose concentrations and haematocrit in triplicate. The capillary blood samples were analysed in duplicate for glucose and lactate concentrations. The SST tubes were allowed to coagulate for 30 min and then centrifuged for 15 min at 3000 rpm (Labofuge 200; Kendro, Germany). Approximately 500 µl of serum was then placed in an eppendorf and frozen at -70°C for subsequent cortisol analysis.

3.6.1 Plasma Volume

Changes in plasma volume were calculated for each individual from the pre-ex values in trial one using haemoglobin and haematocrit values as previously described by Dill and Costill (1974).

(a) Haemoglobin

Haemoglobin was determined for each sample using an automated 2-wavelength photometer (570nm and 880nm) (Hemocue; Hemocue AB, Sweden) as described by the manufacturers instructions. The photometer was calibrated prior to each test using a micocuvette of known absorbance.

(b) Haematocrit

Heparinised capillary tubes were filled with whole blood (50-70 µl) and centrifuged for 5 min at 12000 rpm (Micro Haematocrit mk IV, Hawksley & Sons LTD, England). A microhaematocrit reader was then used to determine the haematocrit (Hawksley and Sons, England).

3.6.2 Serum Cortisol Concentration

Serum cortisol concentrations were determined using an automated time-resolved fluoroimmunoassay (AutoDELFIA™ Cortisol kit, Perkin Elmer, Life Sciences, UK). The dissociation enhanced lanthanide fluoroimmunoassay is based on time-resolved fluorescence lanthanide chemistry, where the Europium label is detected by excitation at 340 nm and emission at 615 nm.

3.6.3 Glucose and Lactate

Approximately 25 µl of whole blood was used to determine glucose and lactate concentrations using an automated immobilised enzyme analyser (YSI 2300D stat plus; YSI Incorporated, USA) as described by the manufactures instructions. The assays are based on the following principle:

Reaction 1 (glucose): β-D-glucose + O_2 —GOx⟶ Glucono-δ-lactone + H_2O_2

Reaction 2: H_2O_2 —Pt anode⟶ $2H^+ + O_2 + 2e^-$

3.7 Gas and Heart Rate Analysis

Average last min oxygen uptake ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) were calculated for bouts 1 to 3 from breath-by-breath data. Last min heart rate data were calculated for all exercise bouts from 5-s interval data. Rates of fuel utilisation were calculated using indirect calorimetry as described in (McArdle *et al.*, 2001).

3.8 Statistical Analysis

Statistical analysis was carried out using SPSS software (version 11.0; SPSS Inc., IL). Group values were expressed as mean ± standard error of the mean (SEM). Subject

characteristics, environmental conditions, and $\dot{V}O_{2max}$ values were compared using an independent samples t-test. Mixed model analysis of variance (ANOVA) with repeated measures were used to determine if differences existed between treatment (PS and placebo), trial (trial 1 and trial 2) or timing (stage during each trial). Statistical significance was accepted at the ($p<0.05$) level. Where significant timing effects or interactions including timing were identified, repeated measure ANOVA followed by Bonferroni pairwise comparisons were used to identify the location of these differences. Where significant trial effects or interactions including trial effects were identified paired sample *t*-tests were completed to locate these differences. Where significant treatment effects or interactions including treatments were identified independent *t*-tests were carried out as post-hoc analyses.

4 Results

4.1 Subject Characteristics

The physical characteristics of the subjects were similar for both treatment groups (Table 3.1). No significant differences in mass (*P=0.629*), height *(P=0.525*) and age (*P=0.644*) were identified between placebo and PS.

4.1.1 Measured Maximal Oxygen Uptake ($\dot{V}O_{2max}$)

Maximum oxygen uptake for placebo and PS were 42.4 ± 1.7 and 43.9 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. There were no differences in maximal oxygen uptake between PS and placebo (*P*=0.645).

4.1.2 Dietary Analysis

The total energy intake for the three days prior to each main exercise trial were similar between trials (*P*=0.493) and treatment groups (*P*=0.384). The total energy intake prior to trial 1 and trial 2 were 10285 ± 1198 and 10548 ± 1164 KJ, respectively for PS and 12287 ± 1387 and 11557 ± 983 KJ, respectively for placebo. There were no significant differences in the percentage energy intake from protein, fat

and carbohydrate between trials (P=0.296, P=0.377 and P=0.557, respectively) or treatments (P=0.214, P=0.919 and P=0.614, respectively).

4.2 Environmental Conditions During the Main Exercise Trials

4.2.1 Temperature

The ambient laboratory temperatures during the main exercise trials were similar between trials (P=0.765) and treatment groups (P=0.930). The ambient temperatures during trial 1 and trial 2 were 21.7 ± 0.6 and 21.4 ± 0.5 C, respectively for PS and 21.3 ± 0.6 and 21.7 ± 0.6 C, respectively for placebo.

4.2.2 Barometric Pressure

The laboratory barometric pressures during the main trials were similar between trials (P=0.346) and treatment groups (P=0.396). The barometric pressure during trial 1 and trial 2 were 763.1 ± 0.2 and 763.7 ± 0.4 mmHg, respectively for PS and 763.2 ± 0.2 and 763.1 ± 0.2 mmHg, respectively for placebo.

4.3 Relative Intensities, Work Rates and Heart Rate during the Main Exercise Trials

The mean relative oxygen consumption during the main trials were 47.0 ± 0.5, 57.7 ± 0.5 and 69.3 ± 0.6% $\dot{V}O_{2max}$ for the first 3 bouts of exercise, respectively (Table 4.1). The trial * timing * treatment interaction was non-significant (*P*=0.388). There were no significant differences between trials *(P*=0.25) or treatments (*P*=0.22). The corresponding work rates for the first three bouts of exercise were 91.3 ± 8.5, 127.4 ± 9.1 and 163.5 ± 9.9 W for PS and 90.5 ± 4.7, 124.8 ± 4.6 and 159.1 ± 4.6 W for placebo. During bout 4 the work rates were 235.7 ± 11.7 W for PS and 227.7 ± 5.1 W for placebo. The timing * treatment interaction was not significant *(P=0.456)*. There were no significant differences between treatment groups for work rate *(P=0.52)*. Average last min heart rate data for the 4 bouts of exercise were 117.9 ±3.2, 133.8 ± 3.1, 149.9 ± 3.5 and 180.9 ± 2.2 beats min^{-1} for PS and 120.3 ± 3.4, 139.9 ± 4.1, 159.0 ± 3.8 and 185.2 ± 2.5 beats min^{-1} for placebo. There were no significant differences between trials (*P*=0.59) or treatments (*P*=0.37).

Table 4.1 Relative intensity of exercise (% $\dot{V}O_{2max}$) achieved during the first three bouts of exercise during the main trials exercise protocol for phosphatidylserine supplementation group (PS) and placebo group.

Relative intensities of exercise (% $\dot{V}O_{2max}$)			
Trial	Bout 1	Bout 2	Bout 3
PS Trial 1	46.9 ± 0.1	58.4 ± 0.8*	68.9 ± 1.6*$
Placebo Trial 1	47.4 ± 0.1	58.5 ± 0.8*	70.9 ± 1.0*$
PS Trial 2	46.3 ± 1.4	58.4 ± 1.0*	68.9 ± 1.6*$
Placebo Trial 2	47.7 ± 1.0	58.3 ± 0.8*	69.7 ± 1.1*$

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from bout 1 ($P<0.05$)

$ Significant differences from bout 2 ($P<0.05$)

4.4 Exercise Capacity – Times to Exhaustion (TTE)

The TTE were not significantly different between the supplementation groups prior to supplementation (*P=0.87*). TTE for PS and placebo were 7.9 ± 1.6 and 8.2 ± 0.9 dec. min, respectively. TTE were similar between trials *(P=0.61)* for placebo. However, the times to exhaustion for PS during trial 2 (9.9 ± 1.7 dec. min) were 25 ± 6% longer than trial 1 (Figure 4.1) with the trial * treatment interaction being *P=0.002.*

4.5 Exercise Induced Feeling Inventory

There were no significant differences in the physical exhaustion, positive engagement, tranquillity and revitalisation subscales between trials (P=0.249, P=0.863, P=0.814 and P=0.246 respectively), treatments (P=0.661, P=0.465, P=0.922 and P=0.867, respectively) or the trial * timing * treatment interaction (P=0.462, P=0.825, P=0.236 and P=0.533, respectively) (Table 4.2, 4.3, 4.4, 4.5 respectively).

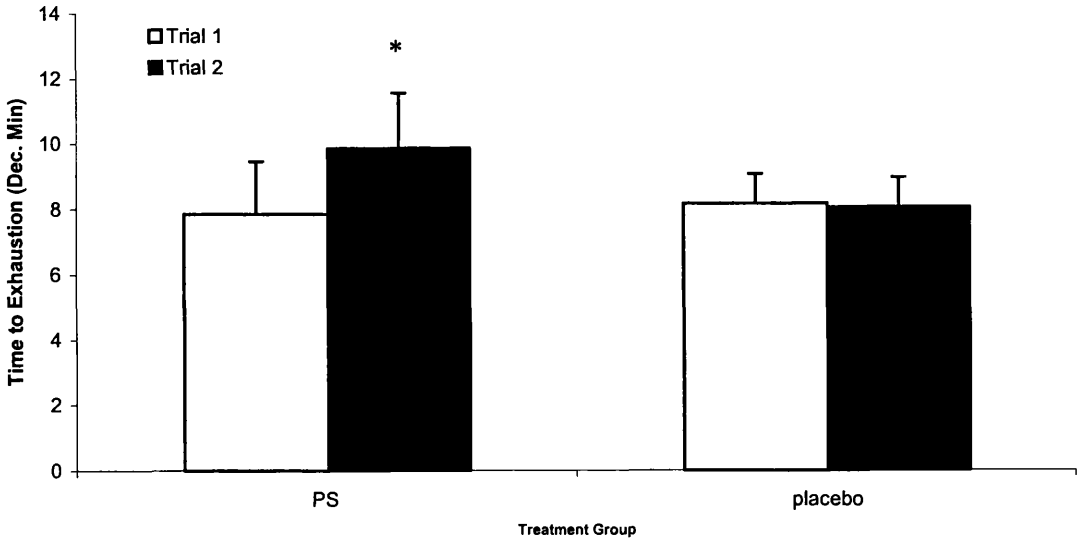


Figure 4.1 Exercise capacity during bout 4 of the main trials exercise protocol. Values represent mean ± SEM (N=7). PS: phosphatidylserine group. * Trial 2 significantly different from trial 1 for PS group ($P<0.01$).

Table 4.2 Physical exhaustion scores on a 5-point likert scale during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

	Physical Exhaustion				
Trial	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS Trial 1	1.28 ± 0.34	1.83 ± 0.11	2.33 ± 0.17	1.72 ± 0.39	1.00 ± 0.23$^{\$\&}$
placebo Trial 1	1.44 ± 0.31	1.78 ± 0.16	1.94 ± 0.18	2.22 ± 0.32	1.17 ± 0.24$^{\$}$
PS Trial 2	0.94 ± 0.16	1.56 ± 0.19	2.11 ± 0.20*$^{\$}$	1.78 ± 0.28*	1.06 ± 0.25
placebo Trial 2	1.44 ± 0.16	1.89 ± 0.25	1.56 ± 0.25	1.89 ± 0.51	1.50 ± 0.35

Values are mean ± SEM (N=6). PS: phosphatidylserine group.

* Significant differences from initial value (pre-ex) ($p<0.05$)

$ Significant differences from bout 2 ($p<0.05$)

& Significant differences from bout 3 ($p<0.05$)

Table 4.3 Positive engagement scores on a 5-point likert scale during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

	Positive Engagement				
Trial	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS Trial 1	3.11 ± 0.22	2.50 ± 0.17	2.39 ± 0.22	2.56 ± 0.36	2.94 ± 0.23
placebo Trial 1	2.78 ± 0.27	2.72 ± 0.22	2.50 ± 0.22	2.61 ± 0.26	2.72 ± 0.28
PS Trial 2	3.11 ± 0.20	2.67 ± .017	2.72 ± 0.18	2.83 ± 0.17	3.00 ± 0.24
placebo Trial 2	2.44 ± 0.31	2.28 ± 0.37	2.39 ± 0.46	2.56 ± 0.36	2.61 ± 0.34

Values are mean ± SEM (N=6). PS: phosphatidylserine group.

Table 4.4 Tranquillity scores on a 5-point likert scale during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

	Tranquillity				
Trial	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS Trial 1	2.67 ± 0.23	2.33 ± 0.24	2.11 ± 0.28	1.94 ± 0.45	2.78 ± 0.34
placebo Trial 1	2.78 ± 0.31	2.44 ± 0.14	2.06 ± 0.22	2.61 ± 0.28	2.78 ± 0.27
PS Trial 2	2.67 ± 0.32	2.28 ± 0.29	2.00 ± 0.38	2.56 ± 0.38	2.83 ± 0.35
placebo Trial 2	2.22 ± 0.27	2.28 ± 0.34	2.22 ± 0.43	2.56 ± 0.37	2.61 ± 0.32

Values are mean ± SEM (N=6). PS: phosphatidylserine group.

Table 4.5 Revitalisation scores on a 5-point likert scale during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

	Revitalisation				
Trial	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS Trial 1	2.22 ± 0.24	1.94 ± 0.13	1.61 ± 0.13	1.72 ± 0.41	2.67 ± 0.27*$&
placebo Trial 1	2.44 ± 0.31	2.22 ± 0.19	1.78 ± 0.25	2.28 ± 0.29	2.78 ± 0.28
PS Trial 2	2.67 ± 0.15	2.11 ± 0.28	1.83 ± 0.28	2.28 ± 0.23	2.78 ± 0.25
placebo Trial 2	2.39 ± 0.20	2.11 ± 0.34	1.83 ± 0.39	2.00 ± 0.42	2.56 ± 0.35

Values are mean ± SEM (N=6). PS: phosphatidylserine group.

* Significant differences from initial value (pre-ex) ($p<0.05$)

$ Significant differences from bout 2 ($p<0.05$)

& Significant differences from bout 3 ($p<0.05$)

4.6 Blood Analysis

4.6.1 Changes in Plasma Volume

Throughout the investigation there were no significant differences in the calculated plasma volume changes between treatment groups (*P=0.825*) or by trial *(P=0.427)* (Table 4.6). During trial 1 the plasma volume was increased by 4.9 ± 2.5% for the PS group and 4.5 ± 1.4% for the placebo group 24 hours post-ex. During trial 2 a 3.8 ± 2.3% increase for PS and 3.3 ± 2.2% for placebo was calculated at 24 hours post-ex. The trial * timing * treatment interaction was non-significant (*P*=0.430). There was no significant difference in the pre-ex plasma volume for trial 2 when compared with the trial 1 pre-ex values for both PS and placebo, which increased by 3.5 ± 2.7 and 0.5 ± 2.1%, respectively.

4.6.2 Blood Lactate Concentration

There were no significant differences in blood lactate concentrations between trials (*P=0.62*) or between treatments (*P=0.10*) (Table 4.7). The trial * timing * treatment interaction was non-significant (*P*=0.122). Blood lactate concentrations remained below 4 mmol L^{-1} for all trials during bout 2 and bout 3. The blood lactate

Table 4.6 Calculated percentage changes in plasma volume during the main exercise trials from the T1 pre value for phosphatidylserine treatment group (PS) and placebo group.

	T1 Pre-ex	T1 Bout 2	T1 Bout 3	T1 Post-ex	T1 24-post	T2 Pre-ex	T2 Bout 2	T2 Bout 3	T2 Post-ex	T2 24-post
PS	-	-9.3±1.3*	-11.0±1.3*	-4.5±0.7	4.9±2.5	3.5±2.7	-8.6±1.4	-8.2±1.2*	-6.1±1.8	3.8±2.3
placebo	-	-7.3±1.4	-8.9±1.4*	-3.1±1.7	4.5±1.4$	.05±2.1	-7.0±1.6	-9.8±1.5	-5.0±2.0	3.3±2.2

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from initial value (pre-ex) ($p<0.05$)

$ Significant differences from bout 3 value ($p<0.05$)

Table 4.7 Blood Lactate concentration during both exercise trials for phosphatidylserine supplementation group (PS) and placebo group.

	Blood Lactate Concentration (mmol L^{-1})					
Trial	Pre-ex	Bout 2	Bout 3	End-Ex	Post-ex	24-post
PS Trial 1	1.50 ± 0.21	1.57 ± .013	2.71 ± 0.32$^{\$}$	7.22 ± 0.81*$^{\$\&}$	2.49 ± 0.30$^{£}$	1.39 ± 0.15$^{£}$
placebo Trial 1	1.49 ± 0.32	1.95 ± 0.21	3.24 ± 0.14*	8.01 ± 0.45*$^{\$\&}$	3.13 ± 0.22$^{£}$	1.69 ± 0.33$^{£}$
PS Trial 2	1.25 ± 0.17	1.69 ± 0.19	2.42 ± 0.23*$^{\$}$	8.47 ± 0.86*$^{\$\&}$	3.05 ± 0.36*$^{£}$	1.12 ± 0.14$^{\&£\%}$
placebo Trial 2	1.38 ± 0.19	1.79 ± 0.20	2.69 ± 0.19*	7.02 ± 0.50*$^{\$\&}$	3.06 ± 0.24*$^{£}$	1.79 ± 0.31$^{£}$

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from initial value (pre-ex) ($p<0.05$)

$^{\$}$ Significant differences from bout 2 ($p<0.05$)

$^{\&}$ Significant differences from bout 3 ($p<0.05$)

$^{£}$ Significant differences from end-ex ($p<0.05$)

$^{\%}$ Significant differences from post-ex ($p<0.05$)

concentrations at bout 4 during trial 1 and trial 2 were 7.22 ± 0.81 and 8.47 ± 0.86 mmol L^{-1}, respectively for PS and 8.01 ± 0.45 and 7.02 ± 0.50 mmol L^{-1}, respectively for placebo (Figure 4.2). All bout 4 blood lactate values were significantly elevated from the pre-ex values ($P<0.05$).

4.6.3 Blood Glucose Concentration

Blood glucose concentrations during the main exercise trials are shown in Table 4.8. There were no significant differences by trial (*P=0.291*) or by treatment *(P=0.211)* during the main exercise trials. The trial * timing * treatment interaction was non-significant ($P=0.372$). Pre-ex values of blood glucose during the main exercise trials were 4.20 ± 0.11 for PS and 4.33 ± 0.14 mmol L^{-1} for placebo. Post-ex values were 4.12 ± 0.09 for PS and 4.38 ± 0.17 mmol L^{-1} for placebo. Blood glucose concentrations did not significantly change over the timing of the trials (*P=0.310*).

4.6.4 Serum Cortisol Concentration

Serum cortisol concentrations during the main exercise trials are presented in Table 4.9. The trial * timing * treatment interaction was non-significant ($P=0.189$). There were no significant differences in treatment groups for the pre-ex serum cortisol concentrations during trial 1 ($P=0.554$). The peak serum cortisol concentrations at 20 min post-ex for trial 1 and trial 2 were 626.6 ± 79.5 and 599.3 ± 50.5 nmol L^{-1}, respectively for PS and 492.4 ± 61.4 and 498.9 ± 38.2 nmol L^{-1}, respectively for

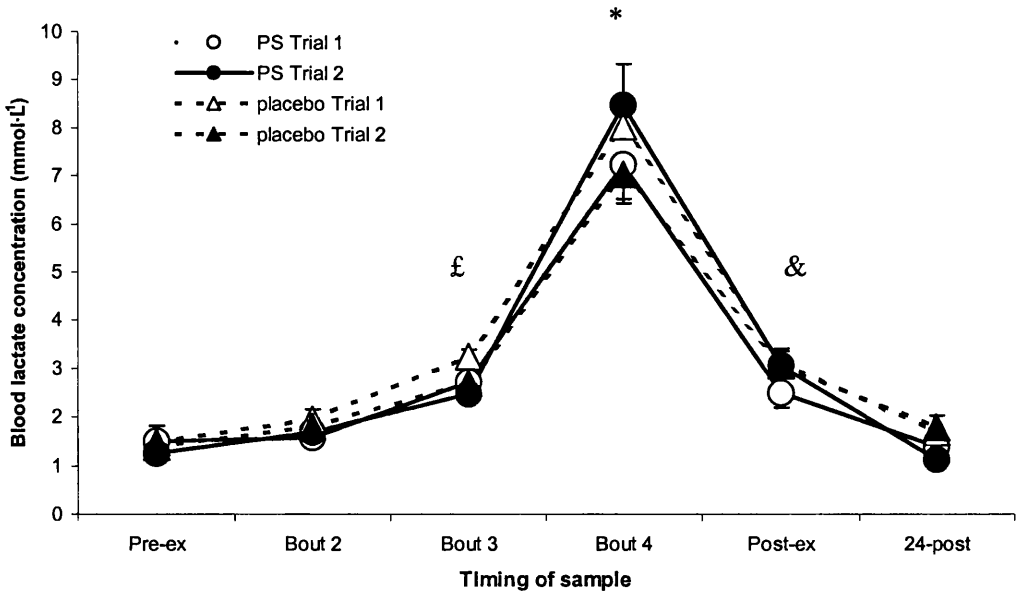


Figure 4.2 Blood lactate concentration. Values represent mean ± SEM (N=7). PS: phosphatidylserine group.

£ Significant differences from initial value (pre-ex) (P<0.05), placebo T1, T2; PS T2

* Significant differences from initial value (pre-ex) (P <0.05), placebo T1, T2; PS T1, T2

& Significant differences from initial value (pre-ex) (P <0.05), Placebo T2; PS T2

Table 4.8 Blood Glucose concentration during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

	Blood glucose concentration (mmol L^{-1})					
Trial	Pre-ex	Bout 2	Bout 3	Bout 4	Post-ex	24-post
PS Trial 1	4.22 ± 0.21	4.04 ± 0.09	3.95 ± 0.16	4.36 ± 0.25	4.13 ± 0.11	4.36 ± 0.29
placebo Trial 1	4.24 ± 0.10	4.24 ± 0.07	4.18 ± 0.11	4.49 ± 0.37	4.34 ± 0.20	4.35 ± 0.13
PS Trial 2	4.19 ± 0.10	4.06 ± 0.13	3.88 ± 0.06	4.40 ± 0.19	4.12 ± 0.14	3.93 ± 0.18
placebo Trial 2	4.42 ± 0.27	4.19 ± 0.12	4.08 ± 0.15	4.41 ± 0.31	4.41 ± 0.30	4.32 ± 0.11

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

Table 4.9 Serum cortisol concentration during the main exercise trials for phosphatidylserine treatment group (PS) and placebo group.

Serum Cortisol Concentration (nmol L^{-1})					
Trial	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS Trial 1	429.0 ± 41.2	336.0 ± 33.0	428.9 ± 62.2	626.6 ± 79.5[&]	267.6 ± 27.6
placebo Trial 1	390.9 ± 47.3	317.9 ± 43.9	350.9 ± 62.6	492.4 ± 61.4[$]	285.3 ± 44.4
PS Trial 2	331.9 ± 27.9	325.0 ± 22.1	339.9 ± 30.1	599.3 ± 50.5[$&]	263.7 ± 32.85
placebo Trial 2	469.1 ± 37.4	361.4 ± 49.4	346.6 ± 40.7*	498.9 ± 38.2	326.0 ± 36.7*

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from initial value (pre-ex) ($p<0.05$)

[$] Significant differences from bout 2 ($p<0.05$)

[&] Significant differences from bout 3 ($p<0.05$)

placebo. Cortisol concentrations then decreased 24 hours post-ex in both treatment groups and trials. Cortisol concentrations decreased to 267.6 ± 27.6 and 263.7 ± 32.9 nmol L^{-1} in trial 1 and trial 2 respectively for PS and 285.3 ± 44.4 and 326.0 ± 36.7 nmol L^{-1} in placebo.

4.7 Oxygen Uptake

Average last min $\dot{V}O_2$ data during both main exercise trials are presented in Table 4.10. There were no significant differences between trials (*P=0.57*) and treatment groups (*P=0.48*). The trial * timing * treatment interaction was non-significant (*P*=0.273). The mean oxygen uptake for the first 3 bouts of exercise during the main exercise trials were 1.72 ± 0.07, 2.11 ± 0.09 and 2.52 ± 0.09 l min^{-1} for PS and 1.75 ± 0.04, 2.22 ± 0.06 and 2.48 ± 0.08 l min^{-1} for placebo.

Table 4.10 Oxygen uptake ($\dot{V}O_2$) data during both exercise trials for phosphatidylserine supplementation group (PS) and placebo group.

Oxygen Uptake ($L \cdot min^{-1}$)			
Trial	Bout 1	Bout 2	Bout 3
PS Trial 1	1.73 ± 0.11	2.16 ± 0.13*	2.54 ± 0.15*$
placebo Trial 1	1.74 ± 0.05	2.22 ± 0.09	2.52 ± 0.12*$
PS Trial 2	1.71 ± 0.11	2.05 ± 0.13*	2.50 ± 0.12*$
placebo Trial 2	1.76 ± 0.06	2.22 ± 0.10	2.45 ± 0.13*$

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from bout 1 ($P<0.05$)

$ Significant differences from bout 2 ($P<0.05$)

4.8 Calculated Rate of Substrate Utilisation

4.8.1 Rate of Fat Utilisation

The rate of fat utilisation did not significantly differ during the timing of the trials (*P=0.84*) (Table 4.11). There were no significant differences by treatment (*P=0.91*) or by trial (*P=0.52*). The Trial * timing * treatment interaction was not significant (*P*=0.187). The rate of fat utilisation for the first three bouts of exercise during the main exercise trials were 0.28 ± 0.03, 0.28 ± 0.04 and 0.28 ± 0.04 g min^{-1} for PS and 0.28 ± 0.03, 0.26 ± 0.03 and 0.26 ± 0.03 g min^{-1} for placebo.

4.8.2 Rate of Carbohydrate Utilisation

The calculated rates of carbohydrate utilisation during the main exercise trials are shown in Table 4.12. Carbohydrate utilisation during bout 2 were increased 45% for PS and 40% for placebo from bout 1 during trial 1 and increased 23% and 27% for PS and placebo respectively from bout 2 to bout 3. During trial 2 carbohydrate utilisation increased 34% and 30% from bout 1 to bout 2 for PS and placebo respectively and increased 32% and 27% from bout 2 to bout 3 for PS and placebo respectively. The trial * timing * treatment interaction was not significant (*P*=0.596). There were no significant differences between trials (*P=0.41*) and treatment groups (*P=0.59*).

Table 4.11 Calculated rates of fat utilisation during both exercise trials for phosphatidylserine supplementation group (PS) and placebo group.

Rate of Fat Utilisation (g min^{-1})			
Trial	Bout 1	Bout 2	Bout 3
PS Trial 1	0.28 ± 0.04	0.31 ± 0.05	0.32 ± 0.06
placebo Trial 1	0.30 ± 0.02	0.27 ± 0.04	0.28 ± 0.04
PS Trial 2	0.28 ± 0.04	0.26 ± 0.07	0.24 ± 0.04
placebo Trial 2	0.26 ± 0.05	0.26 ± 0.05	0.24 ± 0.04

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

Table 4.12 Calculated rates of carbohydrate utilisation during both exercise trials for phosphatidylserine supplementation group (PS) and placebo group.

Rate of Carbohydrate Utilisation (g min^{-1})			
Trial	Bout 1	Bout 2	Bout 3
PS Trial 1	1.29 ± 0.11	1.88 ± 0.15*	2.31 ± 0.15*$
placebo Trial 1	1.39 ± 0.10	1.95 ± 0.11 *	2.49 ± 0.14*$
PS Trial 2	1.39 ± 0.08	1.87 ± 0.10 *	2.54 ± 0.12*$
placebo Trial 2	1.53 ± 0.16	1.98 ± 0.15*	2.61 ± 0.18*$

Values are mean ± SEM (N=7). PS: phosphatidylserine group.

* Significant differences from bout 1 ($P<0.05$).

$ Significant differences from bout 2 ($P<0.05$).

5 Discussion

The main findings of the present study revealed that chronic supplementation with PS (750 $mg \cdot day^{-1}$ S-PS for 10 days) administered to previously familiarised subjects significantly increased time to exhaustion during trial 2 when compared with trial 1, while the placebo group showed no significant changes; although, supplementation had no effect on cortisol concentrations or feeling states throughout the protocol.

There were no differences in time to exhaustion (TTE) between treatment groups prior to supplementation. This finding confirmed that the exercise capacity of both groups were similar prior to supplementation. During the present study TTE were 7.9 ± 1.6 and 8.2 ± 0.9 dec. min for PS and placebo, respectively during trial 1 and 9.9 ± 1.7 and 8.0 ± 0.9 dec. min for PS and placebo, respectively during trial 2. These exercise times are in general agreement with Bell *et al.* (1998), who reported TTE values of 12.6 ± 3.1 (S.D) min when subjects were exercising on a cycle ergometer at intensities of approximately 85% $\dot{V}O_{2max}$. Numerous mechanisms have been suggested to cause fatigue during exercise. Generally, fatigue during endurance exercise has been proposed to be caused by both central and peripheral components (Fitts, 1996), whereas short-term intense exercise is most likely to be associated with peripheral mechanisms (Westerblad *et al.*, 1991; Fitts, 1996). Peripheral fatigue might occur at a number of sites during the excitation contraction coupling process, these include: (1) a reduction in neurotransmitter release at the neuromuscular junction

(Davis and Bailey, 1997); (2) disturbances in the ionic changes that may lead to an inability to maintain the membrane potential and excitability (Green, 1997); and (3) a failure of normal calcium release from the sarcoplasmic reticulum (Allen and Westerblad, 2001). Therefore, during the final bout of exercise in the present exercise protocol, fatigue may have occurred at a number of plausible sites.

Interestingly, TTE for the PS group during trial 2 were 25 ± 6% longer than trial 1 (*P<0.01*). No previously published data has evaluated the effects of PS supplementation on exercise capacity or performance; therefore, this is a novel finding. Although the cause of fatigue and the mechanism(s) responsible for prolonging exercise after PS supplementation were not clearly identifiable, exogenous PS may have enhanced biochemical function at a number of the previously implicated sites of fatigue.

Acetylcholine (ACh) release has been shown to be impaired during exercise (Davis and Bailey, 1997). The reduction in ACh has the potential to lead to a failure in the activation of the action potential and thus fatigue at an organism level. Administration of PS liposomes (75-150 $mg \cdot kg^{-1}$ intraperitoneally (i.p.)) has been shown to stimulate cortical ACh release in both young and aged anaesthetised rat cortical slices using the cortical cup technique. Also, Vannucchi *et al.* (1990) reported that 15 $mg \cdot kg^{-1}$ i.p. PS injected daily for one week stimulated ACh release from both young and old rat cortical slices. Transferring these findings to the human neuromuscular junction, PS

may have delayed the onset of fatigue by maintaining sufficient ACh at the neuromuscular junction during the present study.

Repetitive activation of excitable cells, such as skeletal muscle cells, leads to ionic shifts across the cell membrane (Allen, 2004). Changes in ionic balance across the cell membrane may prevent the propagation of action potentials and ultimately result in fatigue (Fitts, 1996). These ionic shifts result in increases in extracellular potassium (K^+) and intracellular sodium (Na^+) and comparable decreases in intracellular potassium and extracellular sodium. Although these transmembrane fluxes are normally counteracted by the Na^+-K^+ pump, during intense muscular stimulation the efficacy of the Na^+-K^+ pump may be reduced; consequently, sodium and potassium ionic shifts are thought to transpire (Clausen and Nielsen, 1994). PS has been demonstrated to increase membrane bound (Na^+-K^+)- ATPase activity after incubation of brain synaptosomes plasma membranes with PS liposomes (Tsakiris and Deliconstantinos, 1984). Therefore, if a PS mediated increase in ATPase activity in human muscle membrane was evident, it is possible that supplementation with PS may have acted to sustain normal ionic balance for a longer duration and thus prolong TTE.

Alternatively, as a result of calcium (Ca^{2+}) release from the sarcoplasmic reticulum (SR), calcium binds to troponin C, causes an interaction between myosin and actin, cross-bridge cycling, and subsequently leads to muscle cell contraction. Consequently, it has been proposed that a failure in Ca^{2+} release from the SR may

contribute to fatigue, especially during high intensity exercise (Favero, 1999). PS may have a role in regulating calcium uptake through passive influx, depolarisation and $Na^+$and K^+ exchange (Floreani *et al.*, 1991). These authors demonstrated that when 0.1-0.3 μmol PS mg protein^{-1} were incorporated into rat brain synaptosomes, both Na^+- K^+ exchange activity and passive Ca^{2+} entry were increased. Assuming that this finding is transferable to human muscle cells, PS may have acted *in vivo* to increase the calcium availability in the myofibril, prolonged cross-bridge cycling and increased exercise capacity accordingly.

No differences were observed in serum cortisol concentrations between treatment groups throughout trial 1. Therefore, the subject groups had similar cortisol responses to intermittent exercise before supplementation. Following bout 4 the serum cortisol concentrations peaked in all trials. These findings are in agreement with Davies and Few (1973) who demonstrated that plasma cortisol concentrations increased when exercise intensities of approximately 60% $\dot{V}O_{2max}$ were exceeded. The results from the present study showed an attenuated cortisol response to intermittent exercise when compared with data from Monteleone *et al.* (1990) and Monteleone *et al.* (1992). These authors showed significantly increased cortisol concentrations during relatively low intensity exercise (~45% HR_{max}). These equivocal findings probably reflect the training status of the subjects in the respective studies. Active subjects, like those used in the current study, have been previously shown to have a lower cortisol response when compared with sedentary individuals exercising at equivalent absolute and relative exercise intensities (Luger *et al.*, 1987).

There present study does no show any differences in cortisol concentrations during trial 2 when compared to trial 1 or no differences between treatment groups in both trials. Previous studies have shown reductions in cortisol concentrations when supplemented with PS during intermittent exercise (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992) and following intense resistance exercise (Fahey and Pearl, 1998). Monteleone *et al.* (1992) demonstrated that supplementation with 800 $mg \cdot day^{-1}$ of BC-PS for 10 days significantly blunted the cortisol response during intermittent exercise. The present study used 750 $mg \cdot day^{-1}$ of S-PS for the same time period but found that PS did not blunt the cortisol response during intermittent exercise. Since no significant increases were observed in cortisol concentrations during exercise bouts 1-3 in the present study, any effects that PS may have had on cortisol during these points would have been difficult to detect. During the present study cortisol concentrations significantly increased during bout 4 of exercise and supplementation did not have any effect on cortisol concentrations at this point. Direct comparisons between the current study and Monteleone *et al.* (1990) or Monteleone *et al.* (1992) may be difficult due to the timing of the final blood sample. Cortisol was measured 20 mins following exercise in order to obtain peak cortisol values, whereas the previous studies used blood samples taken at the end of exercise. Furthermore, during the current study the final bout of exercise was continued to volitional exhaustion; therefore, differences in the total work done may have concealed any differences in the peak cortisol concentrations recorded. Fahey and Pearl (1998) showed that the cortisol concentration increases that follow intense resistance training were

significantly attenuated when these subjects were supplemented with 800 $mg \cdot day^{-1}$ of S-PS over a 2-week period. Subjects during the present study were supplemented with similar doses of S-PS over a 10-day period; however, the type, duration and intensity of exercise were not comparable between this study and previous studies carried out with PS.

There were no differences in blood lactate concentrations between trials, which indicates that subjects were working at similar intensities during all the trials. This was in agreement with previous work carried out by Monteleone *et al.* (1992) who found that administration with PS did not affect blood lactate concentrations during physical exercise. Blood lactate concentrations remained under 4 $mmol\ L^{-1}$ during bouts 2 and 3; therefore, the exercise intensity during these bouts were below the onset of blood lactate accumulation (OBLA) (Kindermann *et al.*, 1979). Therefore, the majority of energy production was sourced from aerobic mechanisms during the first 3 bouts of exercise. This enabled the calculation of non-protein substrate utilisation using indirect calorimetry from the last min oxygen uptake (McArdle *et al.*, 2001). There were no differences in substrate utilisation during all trials in the present study (Table 4.11, 4.12). Furthermore, there were no differences in blood glucose concentrations during all trials in the present study. These data were in agreement with previous research (Monteleone *et al.*, 1990; Monteleone *et al.*, 1992) and suggested that the changes in pre-exercise cortisol concentrations did not affect substrate metabolism or blood glucose concentrations.

As with any study of this nature the present study is not without limitations. Even though the sample size was sufficient to identify changes in exercise capacity, it is possible that a larger sample size might have been required to identify the neuroendocrine effects of this supplement in active individuals during maximal exercise. Therefore, it is a recommendation that future studies that employ a similar study design should have at least 10 subjects in each supplementation group. Phosphatidylserine supplements contain a range of other compounds (Appendix 5). Although PS is believed to be the main active ingredient of the supplement, the present study has investigated the combined effects of this supplement; consequently, care needs to be taken when interpreting these findings. Plausible mechanisms have been identified to explain why fatigue was delayed during this study; however, these mechanisms are speculative and future research is required to determine the biochemical actions of PS that led to the current findings.

5.1 Summary and Conclusions

Chronic supplementation with PS significantly increased times to exhaustion by 25 ± 6%. PS supplementation did not affect serum cortisol concentrations during intermittent exercise. Subjects reported no differences in feeling states during exercise as indicated on the Exercise Induced Feeling Inventory. These data demonstrated that PS has the potential to delay the onset of fatigue during intermittent exercise although the precise mechanism/s are unclear. Further research is required to investigate the mechanisms responsible for these findings.

5.2 Future Recommendations

(1) Fatigue was delayed during a predominately anaerobic bout of exercise that was preceded by aerobic exercise. Future research could evaluate the effects of PS supplementation on short-term high-intensity exercise.

(2) Evidence is presented that suggested PS delayed the onset of fatigue; however, it was not possible to identify the primary mechanism(s) responsible for this finding. Therefore, direct and/or indirect biochemical markers could be monitored in order to quantify mechanisms such as ATPase activity and trans-membrane ionic balances during exercise.

(3) Contrary to earlier studies, PS was not effective in reducing cortisol concentrations during exercise in this study. It was possible that the current dosing regime was not able to supply sufficient PS for these active individuals. Consequently, future studies could provide supplementation for a longer duration.

(4) PS supplementation has the potential to act on a number of neurotransmitters that have been associated with psychological mood status. Central mechanisms have been implicated in fatigue during prolonged exercise; therefore, it would be of interest to assess the effects of PS supplementation on exercise capacity during endurance activities.

Bibliography

Abood, L. G., Salem, N., Jr, MacNeil, M., Bloom, L. and Abood, M. E. 1977. Enhancement of opiate binding by various molecular forms of phosphatidylserine and inhibition by other unsaturated lipids. *Biochimica Et Biophysica Acta*, 468(1), 51-62.

Aguilera, G. 1998. Corticotropin releasing hormone, receptor regulation and the stress response. *Trends in Endocrinology and Metabolism*, 9(8), 329-336.

Allen, D. G. 2004. Skeletal muscle function: role of ionic changes in fatigue, damage and disease. *Clinical And Experimental Pharmacology and Physiology*, 31(8), 485-493.

Allen, D. G., Lannergren, J. and Westerblad, H. 1995. Muscle-cell function during prolonged activity - cellular mechanisms of fatigue. *Experimental Physiology*, 80(4), 497-527.

Allen, D. G. and Westerblad, H. 2001. Role of phosphate and calcium stores in muscle fatigue. *Journal of Physiology-London*, 536(3), 657-665.

Amaducci, L. 1988. Phosphatidylserine in the treatment of alzheimers-disease - results of a multicenter Study. *Psychopharmacology Bulletin*, 24(1), 130-134.

Ashby, F. G., Isen, A. M. and Turken, U. 1999. A neuropsychological theory of positive affect and its influence on cognition. *Psychological Review*, 106(3), 529-550.

Bell, D. G., Jacobs, I. and Zamecnik, J. 1998. Effects of caffeine, ephedrine and their combination on time to exhaustion during high-intensity exercise. *European Journal of Applied Physiology*, 77(5), 427-433.

Benton, D., Donohoe, R. T., Sillance, B. and Nabb, S. 2001. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. *Nutritional Neuroscience*, 4(3), 169-178.

Bigland-Ritchie, B. 1981. EMG and fatigue of human voluntary and stimulated contractions. *Ciba Foundation Symposium*, 82, 130-156.

Black, K. J., Hershey, T., Koller, J. M., Videen, T. O., Mintun, M. A., Price, J. L. and Perlmutter, J. S. 2002. A possible substrate for dopamine-related changes in mood and behavior: prefrontal and limbic effects of a D3-preferring dopamine agonist. *Proceedings of The National Academy of Sciences of The United States of America*, 99(26), 17113-17118.

Blokland, A., Honig, W., Brouns, F. and Jolles, J. 1999. Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: Comparison of bovine cortex PS with egg PS and soybean PS. *Nutrition*, 15(10), 778-783.

Bloom, M., Evans, E. and Mouritsen, O. G. 1991. Physical-properties of the fluid lipid-bilayer component of cell-membranes - a Perspective. *Quarterly Reviews of Biophysics*, 24(3), 293-397.

Bogdanis, G. C., Nevill, M. E., Boobis, L. H. and Lakomy, H. K. 1996. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J Appl Physiol*, 80(3), 876-884.

Brandenberger, G., Follenius, M. and Muzet, A. 1984. Interactions between spontaneous and provoked cortisol secretory episodes in man. *J Clin Endocrinol Metab*, 59(3), 406-411.

Canonico, P. L. and Scapagnini, U. 1989. Phosphatidylserine as a modulator of receptor-coupled signal transduction. In: Phospholipds in the nervous system: biochemical and molecular pathology. Fidia Reseach Series (L. A. Horrcoks and G. Toffano), pp. 11-20. Berlin: Springer.

Casamenti, F., Scali, C. and Pepeu, G. 1991. Phosphatidylserine reverses the age-dependent decrease in cortical acetylcholine-release - a Microdialysis Study. *European Journal of Pharmacology*, 194(1), 11-16.

Cenacchi, T., Bertoldin, T., Farina, C., Fiori, M. G., Crepaldi, G., Azzini, C. F., Girardello, R., Bagozzi, B., Garuti, R., Vivaldi, P., Belloni, G., Bordin, A., Durando, M., Lostorto, M., Bertoni, L., Battistoni, A., Cacace, C., Arduini, P., Bonini, A., Caramia, M. P., Vaglieri, G., Brusomini, A., Dona, G., March, A., Campi, N., Cannas, P., Casson, F., Cavallarin, G., Dellaloggia, M., Cristianini, G., Louvier, O., Mello, F., Fameli, R., Degheltoff, N. U., Decandia, O., Nante, G., Cattoni, C., Forte, P. L., Loreggian, M., Targa, A., Mansoldo, G., Noro, G., Meggio, A., Pedrazzi, F., Bonmartini, F., Ruggiano, C., Peruzza, M., Olivari, G., Recaldin, E., Bellunato, C., Rigo, G., Marin, M., Marinangeli, L., Saracino, A., Miceli, O., Lovo, G., Scarpa, R., Battistello, L., Tomat, E., Bernava, B., Olivo, P., Verga, G., Merli, G., Zerman, A. M., Crivellaro, R., Vozza, A., Ziliotto, G. R., Favaretto, V. and Allegro, L. 1993. Cognitive decline in the elderly - a double-blind, placebo- controlled multicenter study on efficacy of phosphatidylserine administration. *Aging-Clinical and Experimental Research*, 5(2), 123-133.

Clausen, T. and Nielsen, O. B. 1994. The Na^+,K^+-pump and muscle contractility. *Acta Physiologica Scandinavica*, 152(4), 365-373.

Crook, T., Petrie, W., Wells, C. and Massari, D. C. 1992. Effects of phosphatidylserine in alzheimers-disease. *Psychopharmacology Bulletin*, 28(1), 61-66.

Crook, T. H., Tinklenberg, J., Yesavage, J., Petrie, W., Nunzi, M. G. and Massari, D. C. 1991. Effects of Phosphatidylserine in Age-Associated Memory Impairment. *Neurology*, 41(5), 644-649.

Davies, C. T. and Few, J. D. 1973. Effects of exercise on adrenocortical function. *Journal Of Applied Physiology*, 35(6), 887-891.

Davis, J. M. and Bailey, S. P. 1997. Possible mechanisms of central nervous system fatigue during exercise. *Medicine and Science in Sports and Exercise*, 29(1), 45-57

Delwaide, P. J., Gyselynckmambourg, A. M., Hurlet, A. and Ylieff, M. 1986. Double-Blind randomized controlled-study of phosphatidylserine in senile demented patients. *Acta Neurologica Scandinavica*, 73(2), 136-140.

Diagne, A., Fauvel, J., Record, M., Chap, H. and Douste-Blazy, L. 1984. Studies on ether phospholipids. II. Comparative composition of various tissues from human, rat and guinea pig. *Biochimica Et Biophysica Acta*, 793(2), 221-231.

Diehl, D. J. and Gershon, S. 1992. The role of dopamine in mood disorders. *Comprehensive Psychiatry*, 33(2), 115-120.

Dill, D. B. and Costill, D. L. 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37(2), 247-248.

Eberstein, A. and Sandow, A. 1976. Fatigue mechanisms in muscle fibers. In: The effects and use and disuse in neuromuscular function (E. Guttman and P. Hnik), pp. 515-526. Amsterdam: Elsevier.

Edwards, R. H. 1981. Human muscle function and fatigue. *Ciba Foundation Symposium*, 82, (1-18).

Edwards, S., Evans, P., Hucklebridge, F. and Clow, A. 2001. Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology*, 26(6), 613-622.

Engel, R. R., Satzger, W., Gunther, W., Kathmann, N., Bove, D., Gerke, S., Munch, U. and Hippius, H. 1992. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. *European Neuropsychopharmacology: The Journal of The European College of Neuropsychopharmacology*, 2(2), 149-155.

Fabiato, A. and Fabiato, F. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *The Journal of Physiology*, 276, 233-255.

Fahey, T. D. and Pearl, M. S. 1998. The hormonal and perceptive effects of phosphatidylserine administration during two weeks of resistive exercise-induced overtraining. *Biology of Sport*, 15(3), 135-144.

Favero, T. G. 1999. Sarcoplasmic reticulum Ca^{2+} release and muscle fatigue. *Journal of Applied Physiology*, 87(2), 471-483.

Fitts, R. H. 1996. Muscle fatigue: The cellular aspects. *American Journal of Sports Medicine*, 24, 9-13.

Floreani, M., Debetto, P. and Carpenedo, F. 1991. Phosphatidylserine Vesicles Increase Ca-2+ Uptake by rat-brain synaptosomes. *Archives of Biochemistry and Biophysics*, 285(1), 116-119.

Folch, J. 1942. Brain Cephalin, a mixture of phosphatides. Separation from it of phosphatidylserine, phosphatidyethanolamine, and a fraction containing an inositolphosphatide. *J. Biol. Chem.*, 146(1), 35-44.

Fowles, J. R., Green, H. J., Tupling, R., O'Brien, S. and Roy, B. D. 2002. Human neuromuscular fatigue is associated with altered (Na^+-K^+)- ATPase activity following isometric exercise. *Journal of Applied Physiology*, 92(4), 1585-1593.

Fryer, M., Owen, V., Lamb, G. and Stephenson, D. 1995. Effects of creatine phosphate and P(i) on Ca2+ movements and tension development in rat skinned skeletal muscle fibres. *J Physiol (Lond)*, 482(1), 123-140.

Funfgeld, E. W. and Nedwidek, P. 1987. Neurohomologous phosphatidylserine in parkinsonian-patients with associated disorders of cerebral metabolism - results of a pilot-study. *Clinical Trials Journal*, 24(1), 42-61.

Gauvin, L. and Rejeski, W. J. 1993. The exercise-induced feeling inventory - development and initial validation. *Journal of Sport and Exercise Psychology*, 15(4), 403-423.

Gerra, G., Zaimovic, A., Franchini, D., Palladino, M., Giucastro, G., Reali, N., Maestri, D., Caccavari, R., Delsignore, R. and Brambilla, F. 1998. Neuroendocrine responses of healthy volunteers to 'techno- music'; relationships with personality traits and emotional state. *International Journal of Psychophysiology*, 28(1), 99-111.

Green, H. J. 1997. Mechanisms of muscle fatigue in intense exercise. *Journal of Sports Sciences*, 15(3), 247-256.

Gurr, M. I. and Harwood, J. L. 1991. Lipid biochemistry: An introduction. Bury St Edmunds: Chapman and Hall.

Hellhammer, J., Fries, E., Buss, C., Engert, V., Tuch, A., Rutenberg, D. and Hellhammer, D. 2004. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the Endocrine and Psychological Responses to Mental Stress. *Stress (Amsterdam, Netherlands)*, 7(2), 119-126.

Hirata, F. and Axelrod, J. 1980. Phospholipid methylation and biological signal transmission. *Science*, 209, 1082-1090.

Jacks, D. E., Sowash, J., Anning, J., McGloughlin, T. and Andres, F. 2002. Effect of exercise at three exercise intensities on salivary cortisol. *Journal of Strength And Conditioning Research / National Strength and Conditioning Association*, 16(2), 286-289.

Jorissen, B. L., Brouns, F., Van Boxtel, M. P., Ponds, R. W., Verhey, F. R., Jolles, J. and Riedel, W. J. 2001. The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. *Nutritional Neuroscience*, 4(2), 121-134.

Kaibuchi, K., Takai, Y. and Nishizuka, Y. 1981. Cooperative roles of various membrane phospholipids in the activation of calcium-activated, phospholipid-dependent protein kinase. *The Journal of Biological Chemistry*, 256(14), 7146-7149.

Kanaley, J. A., Weltman, J. Y., Pieper, K. S., Weltman, A. and Hartman, M. L. 2001. Cortisol and growth hormone responses to exercise at different times of day. *The Journal of Clinical Endocrinology And Metabolism*, 86(6), 2881-2889.

Kindermann, W., Simon, G. and Keul, J. 1979. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *European Journal of Applied Physiology and Occupational Physiology*, 42(1), 25-34.

Kraemer, W. J., Patton, J. F., Knuttgen, H. G., Marchitelli, L. J., Cruthirds, C., Damokosh, A., Harman, E., Frykman, P. and Dziados, J. E. 1989. Hypothalamic-pituitary-adrenal responses to short-duration high-intensity cycle exercise. *Journal Of Applied Physiology*, 66(1), 161-166.

Kumano, H., Horie, H., Shidara, T., Kuboki, T., Suematsu, H. and Yasushi, M. 1996. Treatment of a depressive disorder patient with EEG-driven photic stimulation. *Biofeedback and Self-Regulation*, 21(4), 323-334.

Londeree, B. R., Thomas, T. R., Ziogas, G., Smith, T. D. and Zhang, Q. 1995. Percent-Vo2max versus percent-Hr(Max) regressions for 6 modes of exercise. *Medicine and Science in Sports and Exercise*, 27(3), 458-461.

Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W. T., Montgomery, L. C., Gold, P. W., Loriaux, D. L. and Chrousos, G. P. 1987. Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *The New England Journal of Medicine*, 316(21), 1309-1315.

Maggioni, M., Picotti, G. B., Bondiolotti, G. P., Panerai, A., Cenacchi, T., Nobile, P. and Brambilla, F. 1990. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. *Acta Psychiatrica Scandinavica*, 81(3), 265-270.

Maughan, R. and Gleeson, M. 2004. The biochemical basis of sports performance. Oxford: Oxford University Press.

McArdle, W. D., Katch, F. I. and Katch, V. L. 2001. Exercise Physiology: energy, nutrition, and human performance. Baltimore, ML: Lippincott Williams & Wilkins.

McKay, W. B., Tuel, S. M., Sherwood, A. M., Stokicacute, D. S., Dimitrijevicacute and, M. R. 1995. Focal depression of cortical excitability induced by fatiguing muscle contraction: a transcranial magnetic stimulation study. *Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale*, 105(2), 276-282.

McKee, T. and McKee, J. R. 1999. Biochemistry: An introduction. WCB/McGraw-Hill

McKenna, M. J. 1992. The roles of ionic processes in muscular fatigue during intense exercise. *Sports Medicine*, 13(2), 134-145.

Medbo, J. I. and Sejersted, O. M. 1990. Plasma potassium changes with high intensity exercise. *The Journal of Physiology*, 421, 105-122.

Millet, G. Y. and Lepers, R. 2004. Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Medicine*, 34(2), 105-116.

Monteleone, P., Beinat, L., Tanzillo, C., Maj, M. and Kemali, D. 1990. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. *Neuroendocrinology*, 52(3), 243-248.

Monteleone, P., Maj, M., Beinat, L., Natale, M. and Kemali, D. 1992. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. *European Journal of Clinical Pharmacology*, 42(4), 385-388.

Nishijima, M., Kuge, O. and Akamatsu, Y. 1986. Phosphatidylserine biosynthesis in cultured chinese-hamster ovary cells .1. Inhibition of denovo phosphatidylserine biosynthesis by exogenous phosphatidylserine and its efficient incorporation. *Journal of Biological Chemistry*, 261(13), 5784-5789.

Nishizuka, Y. 1986. Studies and perspectives of protein-kinase-C. *Science*, 233(4761), 305-312.

Nunzi, M. G., Milan, F., Guidolin, D. and Toffano, G. Dendritic spine loss in hippocampus of aged rats. Effect of brain phosphatidylserine administration. *Neurobiology of Aging*, 8(6), 501-510.

Palmieri, G., Palmieri, R., Inzoli, M. R., Lombardi, G., Sottini, C., Tavolato, B. and Giometto, B. 1987. Double-blind controlled trial of phosphatidylserine in patients with senile mental deterioration. *Clinical Trials Journal*, 24(1), 73-83.

Pate, E., Bhimani, M., Franks-Skiba, K. and Cooke, R. 1995. Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol (Lond)*, 486(3), 689-694.

Paterson, D. J., Friedland, J. S., Bascom, D. A., Clement, I. D., Cunningham, D. A., Painter, R. and Robbins, P. A. 1990. Changes in arterial K^+ and ventilation during exercise in normal subjects and subjects with McArdle's syndrome. *The Journal Of Physiology*, 429, 339-348.

Pepeu, G., Pepeu, I. M. and Amaducci, L. 1996. A review of phosphatidylserine pharmacological and clinical effects. Is phosphatidylserine a drug for the ageing brain? *Pharmacological Research*, 33(2), 73-80.

Ponzin, D., Mancini, C., Toffano, G., Bruni, A. and Doria, G. 1989. Phosphatidylserine-induced modulation of the immune response in mice: effect of intravenous administration. *Immunopharmacology*, 18(3), 167-176.

Port, K. 1991. Serum and saliva cortisol responses and blood lactate accumulation during incremental exercise testing. *International Journal of Sports Medicine*, 12(5), 490-494.

Raese, J., Patrick, R. L. and Barchas, J. D. 1976. Phospholipid-induced activation of tyrosine hydroxylase from rat brain striatal synaptosomes. *Biochemical Pharmacology*, 25(20), 2245-2250.

Rosadini, G., Sannita, W. G., Nobili, F. and Cenacchi, T. 1990. Phosphatidylserine: quantitative EEG effects in healthy volunteers. *Neuropsychobiology*, 24(1), 42-48.

Serretti, A., Macciardi, F., Verga, M., Cusin, C., Pedrini, S. and Smeraldi, E. 1998. Tyrosine hydroxylase gene associated with depressive symptomatology in mood disorder. *American Journal of Medical Genetics*, 81(2), 127-130.

Silverthorn, D. U. 2001. Human physiology: An intergrated approach. New Jersey: Prentice Hill.

Sobrinho, L. G., Simoes, M., Barbosa, L., Raposo, J. F., Pratas, S., Fernandes, P. L. and Santos, M. A. 2003. Cortisol, prolactin, growth hormone and neurovegetative responses to emotions elicited during an hypnoidal state. *Psychoneuroendocrinology*, 28(1), 1-17.

Specht, S. C. and Robinson, J. D. 1973. Stimulation of the (Na^+-K^+)- dependent adenosine triphosphatase by amino acids and phosphatidylserine: chelation of trace metal inhibitors. *Archives of Biochemistry and Biophysics*, 154(1), 314-323.

Staehelin, D., Labhart, A., Froesch, R. and Kagi, H. R. 1955. The effect of muscular exercise and hypoglycemia on the plasma level of 17-hydroxysteroids in normal adults and in patients with the adrenogenital syndrome. *Acta Endocrinologica*, 18(4), 521-529.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. and Nishizuka, Y. 1979. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *The Journal of Biological Chemistry*, 254(10), 3692-3695.

Taniguchi, M., Kashiwayanagi, M. and Kurihara, K. 1994. Enhancement of the turtle olfactory responses to fatty-acids by treatment of olfactory epithelium with phosphatidylserine. *Brain Research*, 647(1), 10-14.

Toffano, G., Battistella, A. and Orlando, P. 1987. Pharmacokinetics of radiolabeled brain phosphatidylserine. *Clinical Trials Journal*, 24(1), 18-24.

Tsakiris, S. and Deliconstantinos, G. 1984. Influence of phosphatidylserine on (Na^+ + K^+)- stimulated ATPase and acetylcholinesterase activities of dog brain synaptosomal plasma membranes. *The Biochemical Journal*, 220(1), 301-307.

Vanderburgh, P. M. and Katch, F. I. 1996. Ratio scaling of $\dot{V}O_{2max}$ penalizes women with larger percent body fat, not lean body mass. *Medicine and Science in Sports and Exercise*, 28(9), 1204-1208.

Vannucchi, M. G., Casamenti, F. and Pepeu, G. 1990. Decrease of acetylcholine-release from cortical slices in aged rats - investigations into its reversal by phosphatidylserine. *Journal of Neurochemistry*, 55(3), 819-825.

Vannucchi, M. G. and Pepeu, G. 1987. Effect of phosphatidylserine on acetylcholine-release and content in cortical slices from aging rats. *Neurobiology of Aging*, 8(5), 403-407.

Villardita, C., Grioli, S., Salmeri, G., Nicoletti, F. and Pennisi, G. 1987. Multicenter clinical-trial of brain phosphatidylserine in elderly patients with intellectual deterioration. *Clinical Trials Journal*, 24(1), 84-93.

Westerblad, H., Allen, D. G. and Lannergren, J. 2002. Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci*, 17(1), 17-21.

Westerblad, H., Lee, J. A., Lannergren, J. and Allen, D. G. 1991. Cellular mechanisms of fatigue in skeletal-muscle. *American Journal of Physiology*, 261(2), 195-209.

Williams, J. H. and Klug, G. A. 1995. Calcium exchange hypothesis of skeletal-muscle fatigue - a brief review. *Muscle and Nerve*, 18(4), 421-434.

Wrenn, R. W., Katoh, N., Wise, B. C. and Kuo, J. F. 1980. Stimulation by phosphatidylserine and calmodulin of calcium-dependent phosphorylation of

endogenous proteins from cerebral cortex. *The Journal of Biological Chemistry*, 255(24), 12042-12046.

Yeagle, P. 1987. The membranes of cells. London: Academic Press, INC.

Appendix

Appendix 1 Subject information sheet and written consent

Contact Details:
Mark Miller
Department of Sports Science
Vivian Tower
University of Wales Swansea, SA28PP
Tel: 01792 295086
Mob:[redacted]

1. Study title
Chronic phosphatidylserine (PS) supplementation and its effect on recovery following exercise stress.

2. Invitation paragraph
You are being asked to volunteer for this research study. It is important that before you decide to take part you know what it will involve. Take time to read this information sheet and discuss, if you feel you need to with friends, relatives or even you're G.P. If you do not understand anything please feel free to ask.
Consumers for ethics in research (CERES) publish a leaflet entitled "medical research and you". This leaflet gives more information about medical research and looks at some questions you may wish to ask. A copy may be obtained from CERES, PO box 1365 London N16 OBW. Thank you for reading this.

3. What is the purpose of this study?

The study aims to investigate the effect of chronic supplementation of PS on recovery following exercise. It has been suggested that supplementation may increase the rate of recovery following exercise.

4. Why have I been chosen?

All subjects are volunteers from the University of Wales Swansea (UWS). They will consist of undergraduates and graduates. Approximately 24 subjects will take part.

5. Do I have to take part?

Taking part is entirely voluntary. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. Even if you decide to take part you are free to withdraw at any time without a reason. This will not affect the treatment you receive.

6. What will happen to me if I take part?

If you decide to take part in this study you will be required to visit the exercise physiology laboratory four times, each visit will last approximately 1 hour. Your first three visits will take place over the space of one to two weeks. During these visits you will complete preliminary tests, a familiarisation trial and the pre-supplement main trial. You will then be required to undertake a thirty-day oral supplementation period, during which time you will consume either 750 $mg \cdot day^{-1}$ of phosphatidylserine (PS) or a placebo. A placebo is a dummy treatment, which looks like the real thing but contains no active ingredients. You will be randomly assigned to either the PS or placebo group in such a way that neither yourself of the researcher will know which group you are in until the end of the study. Following the supplementation period you will complete the post-supplement main trial.

You will be required to complete the following:

1) Preliminary testing: Following the completion of a health screening questionnaire your weight, height and body composition will be measured. Following this you will complete an exercise test at progressively increasing intensity until you are unable to continue exercising. Heart rate, perceived exertion and respiratory data will be monitored throughout. This assessment will be used to identify your maximal exercise capacity and calculate the intensities you will complete each main trial.
2) Familiarisation: You will undergo an intermittent exercise test on an exercise ergometer. The test will require you to complete five 6-min bouts of exercise separated by approximately 10-min of recovery. The work rate for the first exercise bout will start at a relatively low intensity and progress to a moderately hard intensity for the last exercise bout.
3) Main trials: During both main trials you will complete the same intermittent exercise test as completed during the familiarisation. Your heart rate and expired respiratory gas will be monitored throughout the trials. In addition, 10 ml venous blood samples will be obtained by the means of an indwelling cannula (a thin, flexible plastic tube) or venepuncture (a small hollow needle) inserted in a forearm vein. Blood samples will be taken prior to exercise and after each recovery period.
4) Additional requirements: You will be required to consume oral supplementation (PS; 750 $mg \cdot day^{-1}$ or placebo) over a 10-day period and complete 3-day exercise and dietary records during the week prior to each main trial. You will be asked fast overnight before reporting to the laboratory.

8. What is the supplement that is being tested?

Phosphatidylserines are naturally occurring phosphalipids predominantly found in cell membranes and other structures. It is considered safe to use and is currently on the market as a memory aid.

9. What are the effects of taking part?

There are no known side effects of the supplement, however, if you experience any adverse effects please contact us straight away.

10. What are the possible disadvantages of taking part?
The acute risks associated with exercise are very small. However, this will be further minimised by the health screening you will complete prior to undertaking exercise. There is an extremely small risk that cannulation could result in an air or plastic embolism, but good practice minimises this risk. The samples will be taken by trained staff members.

11. What are the possible benefits of taking part?
If you receive the PS supplement it could benefit your recovery from exercise. Preliminary studies have shown this might be the case.

12. Will new information become available?
New information can always come to light about research areas. If this happens while the study is taking place you will be informed and a course of action will be decided.

13. What happens when the research study stops?
The supplement is available on the open market if you wish to carry on taking it.

14. Will my taking part in the study be kept confidential?
All information collected about you will be kept strictly confidential. Any information that is distributed by the Department of Sports Science will only be identifiable by number and not name.

15. What will happen to the results of the research study?
The results of the study will be disseminated to the wider Sports Science and Psychology community. It is intended that this will be available late 2002 early 2003.

16. Who is organising the research?
The Department of Sports Science UWS is carrying out the research in collaboration with the Department of Psychology UWS.

Contact Details:
Mark Miller
Department of Sports Science
Vivian Tower
University of Wales Swansea, SA28PP
Tel: 01792 295086
Mob: [redacted]

Chronic phosphatidylserine (PS) supplementation and its effect on recovery following exercise stress.

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 2 Ethical Committee Proposal

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 exercise stress.

Students Name: Mark Miller; 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:

Aims & purpose: To investigate chronic (PS) supplementation and its ability to enhance recovery following exercise stress.

No. of participants: Approximately twenty-four.

How and where are the participants to be recruited: Undergraduate and postgraduate student volunteers will be recruited.

Experimental procedure (brief details only): Chronic supplementation of (PS) will take place. Subjects will be allocated by a placebo controlled double blind procedure. Preliminary testing of subjects will include anthropometric testing and a progressive maximal exercise test on a cycle ergometer. Subjects will then undergo a familiarisation trail followed by 2 trials (pre and post supplementation). The exercise administration will be in the form of a repeated progressive exercise and recovery on a cycle ergometer. Measurement procedures will consist of heart rate monitoring, venous blood samples, respiratory gas analysis and other non-invasive procedures. A full description of the study design is attached (Appendix A).

Details of any payment given: None

Consent and Debriefing

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

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

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.

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 D

Students 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)

Appendix 3 Subject information checklist

This is a very important study and it is very important I have subjects that are willing to follow the instructions presented to them. Subjects should read the information sheet and decide very carefully whether they wish to take part in this study or not. If they choose not to this decision will be respected however if you do decide to help me out it is very important you follow the instructions given any deviation from these could result in my whole study being rendered useless. Could you please answer the question below honestly:

What other supplements are you currently taking if any?

What sport or physical activity do you take part in?

On what days do you take part in these activities?

Do you follow a strict diet?

Are you willing to forgo any alcohol 24 hours before any testing?

Are you willing to take at least 10 tablets a day for 10 days?

Are you willing to give blood for samples each on 5 separate occasions?

Are you willing to come into the lab on 5 separate occasions?

Are you willing to forgo caffeine on the day of testing?

Are you willing to write down what you have eaten 3 days prior to any testing and repeat this before any other testing?

Appendix 4: Exercise-Induced Feeling Scale

Instructions: Please use the following scale to indicate the extent to which each word below describes how you feel at this moment in time. Record your responses by placing an **X** in the appropriate box.

0 = Do Not Feel **[DNF]**
1 = Feel Slightly
2 = Feel Moderately
3 = Feel Strongly
4 = Feel Very Strongly **[FVS]**

	DNF				FVS
	0	1	2	3	4
1. Refreshed					
2. Calm					
3. Fatigued					
4. Enthusiastic					
5. Relaxed					
6. Energetic					
7. Happy					
8. Tired					
9. Revived					
10. Peaceful					
11. Worn-out					
12. Upbeat					

Appendix 5: Composition of the supplement

Table 1 shows the typical composition of the concentrated phosphatidylserine product as analysed by an independent consulting company. The remaining content will reflect the composition of the source soybean lecithin. Lecithin is usually used as a synonym for phosphatidylcholine, although the composition of lecithin is variable depending upon the source soybean. However, soybean lecithin also contains, to a lesser extent, a mixture of phospholipids, tocopherols, triglycerides, and free fatty acids. The approximate content of the final product (from commercial analysis) is listed in Table 2.

Table 1: Typical composition of concentrated phosphatidylserine product.

Phospholipids	**Composition**
Phosphatidylserine	45-54%
Phosphatidylcholine	5-7%
Phosphatidylethanolamine	6-10%
Phosphatidylinositol	1-3%
Phosphatidic acid	4-7%

Table 2: Approximate composition of final phosphatidylserine product.

Phospholipids	**Composition**
Phosphatidylserine	20%
Phosphatidylcholine	9%
Phosphatidylethanolamine	2%
Phosphatidylinositol	2%
Linoleic Acid	23%
Linolenic Acid	2%
Oleic Acid	2%
Stearic Acid	<1%
Palmitic Acid	5%
Capric Acid	10%
Caprylic Acid	26%
Phosphorus	2%
Potassium	<1%
Others (including tocopherol)	Not listed

Appendix 6 Raw data for subject characteristics and measured maximal oxygen uptake

Supp/Sub	Age (dec.years)	$\dot{V}O_{2max}$ (l/min)	$\dot{V}O_{2max}$ (ml/min/kg)	Mass (kg)	Height (m)
PS/1	23.38	3.64	40.9	89	1.83
PS/2	20.33	3.78	44.5	85	1.77
PS/3	21.28	3.73	35.2	106	1.76
PS/4	20.25	4.64	53.3	87	1.74
PS/5	20.89	3.73	49.1	76	1.76
PS/6	36.08	2.97	43.7	68	1.84
PS/7	21.8	3.34	40.2	83	1.8
Mean	23.43	3.69	43.8	84.9	1.79
SEM	2.15	0.19	2.3	4.5	0.01

Supp/Sub	Age (dec.years)	$\dot{V}O_{2max}$ (l/min)	$\dot{V}O_{2max}$ (ml/min/kg)	Mass (kg)	Height (m)
P/8	21.34	3.55	34.5	103	1.83
P/9	22.48	3.17	41.7	76	1.78
P/10	18.97	3.96	49.5	80	1.76
P/11	21.04	3.86	43.4	89	1.82
P/12	19.48	3.69	38.0	97	1.93
P/13	23.47	3.89	48.0	81	1.82
P/14	28.73	3.68	41.8	88	1.7
Mean	22.21	3.69	42.4	87.7	1.81
SEM	1.24	0.10	2.0	3.7	0.03

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 7 Raw data total energy intake and percentage protein, fat and CHO intake prior to main exercise trials

	Trial 1				Trial 2			
Supp/Sub	Energy intake (KJ)	Protein (% energy)	Fat (% energy)	CHO (% energy)	Energy intake (KJ)	Protein (% energy)	Fat (% energy)	CHO (% energy)
PS/1	10576	21	41	42	12143	18	42	42
PS/2	15446	15	45	37	14540	16	44	37
PS/3	6847	18	41	43	5994	18	36	49
PS/4	8762	16	40	46	9358	15	37	50
PS/5	9019	17	33	48	10505	18	39	42
PS/6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PS/7	11061	16	43	44	10748	16	41	41
Mean	10285	17.2	40.5	43.3	10548	16.8	39.8	43.5
SEM	1198	0.9	1.7	1.5	1164	0.5	1.3	2.0

	Trial 1				Trial 2			
Supp/Sub	Energy intake (KJ)	Protein (% energy)	Fat (% energy)	CHO (% energy)	Energy intake (KJ)	Protein (% energy)	Fat (% energy)	CHO (% energy)
PS/1	9909	17	40	36	10301	17	42	39
PS/2	17968	18	45	40	15256	18	44	39
PS/3	10667	16	47	39	9595	20	43	40
PS/4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/41A
PS/5	9870	16	45	42	9792	18	43	41
PS/6	15010	22	27	55	13922	22	25	57
PS/7	10301	17	42	35	10478	19	43	37
Mean	12287	17.7	41.0	41.2	11557	19	40	42.2
SEM	1387	0.9	3.0	3.0	983	0.7	3.0	3.0

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 8 Raw date for temperature (C) and barometric pressure (mmHg) during both main trials

	Trial 1		Trial 2	
Supp/Sub	Temp	Pressure	Temp	Pressure
PS/1	23	762.8	23	762.8
PS/2	23	762.8	23	762.8
PS/3	20	763.6	20	763.6
PS/4	20	763.6	20	763.6
PS/5	20	763.6	20	763.6
PS/6	23	762.8	22	763.6
PS/7	23	762.8	22	765.8
Mean	21.7	763.1	21.4	763.7
SEM	0.6	0.2	0.5	0.4

	Trial 1		Trial 2	
Supp/Sub	Temp	Pressure	Temp	Pressure
P/8	20	763.6	20	763.6
P/9	20	763.6	20	763.6
P/10	23	762.8	23	762.8
P/11	20	763.6	23	762.8
P/12	20	763.6	20	763.6
P/13	23	762.8	23	762.8
P/14	23	762.8	23	762.8
Mean	21.3	763.2	21.7	763.1
SEM	0.6	0.2	0.6	0.2

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 9 Raw data for the mean relative oxygen consumption during the main trials (%$\dot{V}O_{2max}$)

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
PS/1	49.3	61.3	71.6	40.9	52.6	64.8
PS/2	50.3	61.5	64.9	45.9	54.8	72.2
PS/3	47.5	58.4	71.2	48.3	58.3	69.1
PS/4	47.5	58.2	66.9	48.7	58.5	66.9
PS/5	44.1	59.2	76.2	46.5	55.5	67.0
PS/6	46.4	55.6	66.6	51.3	57.5	70.4
PS/7	42.9	54.6	64.8	42.4	51.7	64.9
Mean	46.9	58.4	68.9	46.3	55.6	67.9
SEM	1.0	1.0	1.6	1.4	1.0	1.1

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
P/8	48.4	58.0	68.0	49.0	56.5	66.1
P/9	48.4	59.3	75.1	48.1	59.5	71.2
P/10	45.2	54.3	66.9	42.5	54.3	65.3
P/11	49.1	60.2	71.6	50.8	62.2	75.2
P/12	46.1	58.6	73.3	46.4	57.9	69.5
P/13	49.7	61.6	71.6	51.4	60.2	68.3
P/14	44.6	57.4	69.5	45.7	57.8	72.1
Mean	47.4	58.5	70.9	47.7	58.3	69.7
SEM	0.8	0.9	1.1	1.2	1.0	1.3

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 10 Raw data for work rate during both main trials (W)

Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 4
PS/1	92	128	164	235
PS/2	93	134	175	257
PS/3	79	116	154	228
PS/4	144	182	219	295
PS/5	95	133	171	246
PS/6	69	99	128	187
PS/7	67	101	134	202
Mean	91.3	127.4	163.5	235.7
SEM	9.8	10.6	11.4	13.5

Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 4
P/8	85	119	153	221
P/9	81	112	143	204
P/10	100	136	172	244
P/11	109	143	176	244
P/12	81	119	157	232
P/13	105	138	171	237
P/14	71	107	142	213
Mean	90.5	124.8	159.1	228.0
SEM	5.4	5.3	5.4	5.9

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 11 Raw data for last minute heart rate (b.p.m) during both main exercise trials

	Trial 1				Trial 2			
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 4	Bout 1	Bout 2	Bout 3	Bout 4
PS/1	118.2	135.1	165.2	183.8	119.5	132.6	153.4	181.8
PS/2	114.7	129.5	151.5	181.2	115.2	127.5	146.1	179.8
PS/3	131.6	144.9	147.3	178.2	127.2	144	126.7	188.7
PS/4	131.2	144.6	152.8	174.9	133.8	152.7	169.4	192.4
PS/5	115.7	131.2	146.5	167.7	92.5	109.2	125.6	164.3
PS/6	104.3	119	141.2	176.2	103.5	124.6	147.2	188.3
PS/7	125.3	144.1	165	185.4	117.4	133.6	160.8	189.8
Mean	120.1	135.5	152.8	178.2	115.6	132	147	183.6
SEM	3.7	3.7	3.5	2.3	5.3	5.3	6.2	3.6

	Trial 1				Trial 2			
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 4	Bout 1	Bout 2	Bout 3	Bout 4
PS/1	118.2	135.1	165.2	183.8	119.5	132.6	153.4	181.8
PS/2	114.7	129.5	151.5	181.2	115.2	127.5	146.1	179.8
PS/3	131.6	144.9	147.3	178.2	127.2	144	126.7	188.7
PS/4	131.2	144.6	152.8	174.9	133.8	152.7	169.4	192.4
PS/5	115.7	131.2	146.5	167.7	92.5	109.2	125.6	164.3
PS/6	104.3	119	141.2	176.2	103.5	124.6	147.2	188.3
PS/7	125.3	144.1	165	185.4	117.4	133.6	160.8	189.8
Mean	120.1	135.5	152.8	178.2	115.6	132	147	183.6
SEM	3.7	3.7	3.5	2.3	5.3	5.3	6.2	3.6

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 12 Raw data for times to exhaustion (dec.min) during bout 4 of exercise during both main trials

	Trial 1	Trial 2
Supp/Sub	TTE	TTE
PS/1	8.12	11.9
PS/2	16.25	18
PS/3	4.47	5.6
PS/4	7.32	10.4
PS/5	4.78	5.03
PS/6	4.38	7.15
PS/7	9.63	10.85
Mean	7.85	9.85
SEM	1.6	1.69

	Trial 1	Trial 2
Supp/Sub	TTE	TTE
P/8	12.00	12.27
P/9	9.20	8.60
P/10	5.95	6.45
P/11	6.97	5.95
P/12	10.35	10.03
P/13	6.02	5.97
P/14	6.53	6.95
Mean	8.15	8.03
SEM	0.90	0.91

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 13 Raw data for enthusiastic feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	2	2	2	3	2	2	2	3
PS/2	3	2	3	3	2	3	2	2	2	3
PS/3	4	2	2	1	3	4	3	3	3	2
PS/4	3	3	2	1	3	3	3	3	3	2
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	4	2	3	4	4	4	3	3	4	4
PS/7	3	3	3	3	3	3	2	3	3	3
Mean	3.17	2.33	2.50	2.33	2.83	3.33	2.50	2.67	2.83	2.83
SEM	0.31	0.21	0.22	0.49	0.31	0.21	0.22	0.21	0.31	0.31

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	1	2	2	2	2	2	2	3
P/9	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
P/10	3	3	3	3	2	3	2	3	3	3
P/11	3	3	2	2	3	2	2	2	1	2
P/12	3	3	3	3	2	2	3	3	2	3
P/13	2	3	2	2	3	2	0	0	2	1
P/14	4	3	3	3	4	3	4	4	4	4
Mean	2.83	2.83	2.33	2.50	2.67	2.33	2.17	2.33	2.33	2.67
SEM	0.31	0.17	0.33	0.22	0.33	0.21	0.54	0.56	0.42	0.42

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 14 Raw data for upbeat feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	2	2	3	2	2	2	2	3
PS/2	3	2	2	2	2	3	2	2	2	2
PS/3	3	2	2	2	3	3	3	3	2	2
PS/4	3	3	2	2	3	2	3	3	3	3
PS/5										
PS/6	4	3	3	4	4	4	3	3	3	4
PS/7	3	3	3	3	3	3	3	3	3	3
Mean	3.0	2.5	2.3	2.5	3.0	2.8	2.7	2.7	2.5	2.8
SEM	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	2	2	2	2	2	2	1	3
P/9										
P/10	3	3	3	3	3	4	3	3	3	3
P/11	3	3	2	2	3	2	2	2	2	2
P/12	2	3	2	2	2	2	2	2	2	2
P/13	3	3	3	2	3	2	2	1	3	1
P/14	3	3	3	4	4	3	3	4	4	4
Mean	2.7	2.8	2.5	2.5	2.8	2.5	2.3	2.3	2.5	2.5
SEM	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.4	0.4	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 15 Raw data for happy feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	3	3	3	3	3	3	3	3	3	4
PS/2	3	3	2	3	3	3	2	2	3	3
PS/3	3	3	1	2	2	3	3	3	4	3
PS/4	3	3	2	2	3	3	3	3	3	3
PS/5										
PS/6	4	1	3	4	4	4	3	3	3	4
PS/7	3	3	3	3	3	3	3	3	3	3
Mean	3.17	2.67	2.33	2.83	3.00	3.17	2.83	2.83	3.17	3.33
SEM	0.17	0.33	0.33	0.31	0.26	0.17	0.17	0.17	0.17	0.21

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	1	2	2	2	2	3	2	2	3
P/9										
P/10	4	3	3	3	3	4	3	3	3	2
P/11	3	3	2	2	2	2	2	2	2	2
P/12	2	2	3	3	3	3	3	3	3	2
P/13	2	3	3	3	2	1	0	1	3	3
P/14	4	3	3	4	4	3	3	4	4	4
Mean	2.83	2.50	2.67	2.83	2.67	2.50	2.33	2.50	2.83	2.67
SEM	0.40	0.34	0.21	0.31	0.33	0.43	0.49	0.43	0.31	0.33

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 16 Raw data for Fatigued feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	2	1	1	1	2	2	1	1
PS/2	2	2	3	2	1	2	3	3	3	1
PS/3	2	2	2	3	1	1	1	2	2	2
PS/4	1	2	1	4	2	2	2	3	3	1
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	0	1	2	0	0	1	2	2	1	0
PS/7	1	1	2	2	1	1	1	2	2	1
Mean	1.3	1.7	2.0	2.0	1.0	1.3	1.8	2.3	2.0	1.0
SEM	0.3	0.2	0.3	0.6	0.3	0.2	0.3	0.2	0.4	0.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	2	3	2	1	1	3	2	2
P/9	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
P/10	2	2	1	3	2	1	2	2	3	1
P/11	2	2	1	1	2	2	2	1	1	2
P/12	2	2	2	2	2	2	2	2	2	1
P/13	2	2	3	1	1	1	3	1	3	2
P/14	0	1	2	1	0	1	2	1	0	0
Mean	1.7	1.8	1.8	1.8	1.5	1.3	2.0	1.7	1.8	1.3
SEM	0.3	0.2	0.3	0.4	0.3	0.2	0.3	0.3	0.5	0.3

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 17 Raw data for worn-out feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	3	3	3	1	1	1	1	2	2	1
PS/2	2	2	3	1	1	1	1	1	3	1
PS/3	1	2	3	3	1	1	1	1	1	1
PS/4	1	2	3	0	2	0	2	3	3	1
PS/5										
PS/6	0	2	2	0	0	0	1	2	0	0
PS/7	1	2	2	2	1	0	1	2	1	1
Mean	1.3	2.2	2.7	1.2	1.0	0.5	1.2	1.8	1.7	0.8
SEM	0.4	0.2	0.2	0.5	0.3	0.2	0.2	0.3	0.5	0.2

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	1	3	1	1	1	1	2	2
P/9										
P/10	1	2	3	3	1	2	2	2	4	2
P/11	3	1	2	2	1	2	1	1	1	2
P/12	1	2	3	3	1	2	2	3	2	2
P/13	2	2	3	3	1	2	3	1	3	3
P/14	0	1	1	1	0	1	1	1	0	0
Mean	1.5	1.7	2.2	2.5	0.8	1.7	1.7	1.5	2.0	1.8
SEM	0.4	0.2	0.4	0.3	0.2	0.2	0.3	0.3	0.6	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 18 Raw data for tired feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	1	1	1	2	1	2	2	2	1
PS/2	2	2	3	2	1	1	2	3	3	1
PS/3	1	2	3	3	1	1	1	2	2	2
PS/4	1	2	3	3	1	2	2	3	0	1
PS/5										
PS/6	0	1	2	1	0	0	2	2	2	0
PS/7	1	2	2	2	1	1	1	1	1	3
Mean	1.2	1.7	2.3	2.0	1.0	1.0	1.7	2.2	1.7	1.3
SEM	0.3	0.2	0.3	0.4	0.3	0.3	0.2	0.3	0.4	0.4

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	1	2	2	3	1	1	2	2	2	1
P/9										
P/10	2	2	3	3	1	1	2	2	3	2
P/11	1	3	2	2	2	1	2	1	1	1
P/12	1	1	2	2	1	2	2	2	2	1
P/13	2	2	1	3	2	2	3	1	3	3
P/14	0	1	1	1	0	1	1	1	0	0
Mean	1.2	1.8	1.8	2.3	1.2	1.3	2.0	1.5	1.8	1.3
SEM	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.5	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 19 Raw data for calm feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	4	2	2	2	2	3	2	1	2	3
PS/2	3	2	2	3	3	3	2	2	3	3
PS/3	3	2	1	2	3	3	2	2	3	2
PS/4	3	2	2	0	3	2	2	1	1	2
PS/5										
PS/6	4	2	2	3	4	3	2	2	3	4
PS/7	4	3	3	3	3	3	3	3	3	3
Mean	3.5	2.2	2.0	2.2	3.0	2.8	2.2	1.8	2.5	2.8
SEM	0.2	0.2	0.3	0.5	0.3	0.2	0.2	0.3	0.3	0.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	2	3	3	2	2	1	1	3
P/9										
P/10	4	3	2	3	3	3	3	3	3	4
P/11	2	2	1	1	2	2	2	1	2	2
P/12	3	2	3	3	2	1	2	2	3	2
P/13	2	1	1	3	3	2	1	1	2	2
P/14	4	3	3	4	4	3	3	4	4	4
Mean	2.8	2.2	2.0	2.8	2.8	2.2	2.2	2.0	2.5	2.8
SEM	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.5	0.4	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 20 Raw data for peaceful feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	2	2	1	2	3	2	3	3
PS/2	3	3	3	3	3	2	3	3	3	3
PS/3	2	1	2	2	2	3	2	1	3	2
PS/4	1	2	1	0	1	0	0	0	1	1
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	4	3	3	3	4	4	3	3	3	4
PS/7	3	3	3	3	3	3	3	3	3	3
Mean	2.5	2.3	2.3	2.2	2.3	2.3	2.3	2.0	2.7	2.7
SEM	0.4	0.3	0.3	0.5	0.5	0.6	0.5	0.5	0.3	0.4

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	1	1	2	2	2	2	2	2	2	1
P/9										
P/10	3	2	2	3	2	3	3	3	3	3
P/11	2	3	2	2	3	2	2	2	2	2
P/12	2	3	2	2	3	1	2	1	1	2
P/13	2	3	2	3	3	2	1	2	3	2
P/14	4	3	3	4	4	3	3	4	4	4
Mean	2.3	2.5	2.2	2.7	2.8	2.2	2.2	2.3	2.5	2.3
SEM	0.4	0.3	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 21 Raw data for relaxed feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	2	2	2	3	3	3	3	4
PS/2	2	3	2	3	3	2	2	2	3	3
PS/3	2	1	1	1	4	3	2	2	3	2
PS/4	2	3	1	0	2	2	1	0	0	2
PS/5										
PS/6	0	3	3	0	4	4	3	3	3	4
PS/7	4	3	3	3	3	3	3	3	3	3
Mean	2.0	2.5	2.0	1.5	3.0	2.8	2.3	2.2	2.5	3.0
SEM	0.5	0.3	0.4	0.6	0.4	0.3	0.3	0.5	0.5	0.4

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	3	3	1	2	2	2	2	2	1	2
P/9										
P/10	3	3	2	2	2	3	3	3	3	2
P/11	3	2	2	2	2	2	2	1	2	2
P/12	3	2	2	2	3	2	3	2	3	3
P/13	3	3	2	3	3	2	1	2	3	3
P/14	4	3	3	3	4	3	4	4	4	4
Mean	3.2	2.7	2.0	2.3	2.7	2.3	2.5	2.3	2.7	2.7
SEM	0.2	0.2	0.3	0.2	0.3	0.2	0.4	0.4	0.4	0.3

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 22 Raw data for energetic feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	1	1	1	3	2	2	2	2	3
PS/2	2	2	1	1	2	3	1	1	1	2
PS/3	3	2	1	2	3	2	3	2	3	3
PS/4	2	3	2	1	2	2	2	2	2	2
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	3	3	2	4	4	4	3	2	3	4
PS/7	2	2	2	1	3	3	3	3	3	2
Mean	2.3	2.2	1.5	1.7	2.8	2.7	2.3	2.0	2.3	2.7
SEM	0.2	0.3	0.2	0.5	0.3	0.3	0.3	0.3	0.3	0.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	3	2	1	3	3	3	2	1	3
P/9	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
P/10	3	2	2	2	2	3	2	2	1	2
P/11	3	2	1	1	2	2	2	1	1	2
P/12	3	2	3	2	2	2	3	3	3	2
P/13	2	2	1	2	3	2	0	1	2	1
P/14	4	3	2	3	4	3	3	3	4	4
Mean	2.8	2.3	1.8	1.8	2.7	2.5	2.2	2.0	2.0	2.3
SEM	0.3	0.2	0.3	0.3	0.3	0.2	0.5	0.4	0.5	0.4

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 23 Raw data for refreshed feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	2	2	1	1	2	3	2	1	2	3
PS/2	2	1	1	2	3	3	1	1	2	3
PS/3	2	3	2	2	3	3	2	2	3	3
PS/4	2	2	1	1	3	3	1	1	2	3
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	4	1	2	4	4	3	2	2	3	4
PS/7	2	2	2	2	2	2	3	3	3	3
Mean	2.3	1.8	1.5	2.0	2.8	2.8	1.8	1.7	2.5	3.2
SEM	0.3	0.3	0.2	0.4	0.3	0.2	0.3	0.3	0.2	0.2

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	2	3	3	2	2	1	1	3
P/9	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
P/10	3	3	2	2	3	3	3	2	2	3
P/11	2	2	1	2	2	2	1	1	2	2
P/12	2	2	2	3	3	2	3	2	2	2
P/13	2	1	1	3	3	2	1	1	2	2
P/14	3	3	2	4	4	3	3	3	4	4
Mean	2.3	2.2	1.7	2.8	3.0	2.3	2.2	1.7	2.2	2.7
SEM	0.2	0.3	0.2	0.3	0.3	0.2	0.4	0.3	0.4	0.3

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 24 Raw data for revived feelings on the EFI

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	1	2	2	1	2	2	2	1	1	2
PS/2	2	2	2	2	2	2	1	1	2	3
PS/3	2	2	2	1	2	3	3	1	2	2
PS/4	2	2	1	1	2	2	2	3	2	2
PS/5	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
PS/6	3	2	2	3	4	3	3	2	3	4
PS/7	2	1	2	1	2	3	2	3	2	2
Mean	2.0	1.8	1.8	1.5	2.3	2.5	2.2	1.8	2.0	2.5
SEM	0.3	0.2	0.2	0.3	0.3	0.2	0.3	0.4	0.3	0.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	2	2	2	2	2	2	2	2	2	3
P/9	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a	N/a
P/10	3	2	2	2	2	3	2	2	1	3
P/11	1	2	1	2	2	2	2	1	1	2
P/12	2	2	2	2	3	2	2	2	1	2
P/13	1	2	1	1	3	2	1	0	2	2
P/14	4	3	3	4	4	3	3	4	4	4
Mean	2.2	2.2	1.8	2.2	2.7	2.3	2.0	1.8	1.8	2.7
SEM	0.5	0.2	0.3	0.4	0.3	0.2	0.3	0.5	0.5	0.3

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 25 Raw data for changes in plasma volume (%) during both main exercise trials

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	-	-10.5	-8.2	-6.8	-2.0	0.4	-11.5	-6.7	-7.3	-1.3
PS/2	-	-5.7	-6.2	-1.5	3.8	-9.0	-11.5	-10.1	-12.9	-0.9
PS/3	-	n/a	-8.6	n/a	2.4	1.7	-10.7	-12.8	-9.8	1.7
PS/4	-	-11.2	-15.4	-3.4	14.7	14.5	-5.0	-3.3	-3.4	12.3
PS/5	-	-6.0	-10.4	-4.9	13.1	5.8	-3.5	-9.5	0.9	4.5
PS/6	-	-13.6	-14.6	-5.0	4.8	6.3	-12.0	-9.4	-2.1	12.4
PS/7	-	-8.6	-13.4	-5.2	-2.1	4.8	-6.1	-5.7	-8.2	-1.7
Mean	-	-9.3	-11.0	-4.5	4.9	3.5	-8.6	-8.2	-6.1	3.8
SEM	-	1.3	1.3	0.7	2.5	2.7	1.4	1.2	1.8	2.3

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	-	-2.9	-10.9	-5.6	0.0	2.6	-2.4	-13.0	-6.5	-3.8
P/9	-	N/a	-0.6	0.6	2.3	-4.6	-8.9	-10.5	-13.9	-5.4
P/10	-	-11.2	-11.7	-2.4	11.3	8.4	-10.3	-9.2	1.3	9.2
P/11	-	-10.8	-14.0	-9.3	5.3	-6.5	-8.6	-13.5	-8.6	7.7
P/12	-	-6.8	-11.9	-7.7	2.3	5.7	-1.3	-4.5	-1.3	4.1
P/13	-	-8.5	-8.4	2.2	6.3	1.9	-12.8	-13.7	-0.8	8.5
P/14	-	-3.8	-4.9	0.2	4.0	-4.2	-4.4	-4.4	-5.2	3.0
Mean	-	-7.3	-8.9	-3.1	4.5	0.5	-7.0	-9.8	-5.0	3.3
SEM	-	1.4	1.8	1.7	1.4	2.1	1.6	1.5	2.0	2.2

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 26 Raw data for Blood Lactate Concentrations (mmol L^{-1})

	Trial 1						Trial 2					
Supp/Sub	Rest	55	65	End of ex	End	Post	Rest	55	65	End of ex	End	Post
PS/1	1.01	1.19	1.65	4.9	1.51	1.14	0.64	1.16	1.77	5.99	1.77	0.86
PS/2	1.06	1.88	2.99	6.59	2.9	1.03	1.11	1.67	2.4	7.21	3.11	0.71
PS/3	2.25	n/a	4.18	9.35	n/a	2.11	1.7	2.5	3.4	12.9	3.06	1.89
PS/4	1.35	1.7	2.57	9.91	3.66	1.22	1.95	1.63	2.28	8.63	3.87	1.27
PS/5	1.1	1.93	3.23	n/a	2.52	1.28	0.96	1.72	2.81	7.56	2.16	1.03
PS/6	2.27	1.41	2	5.98	2.11	1.19	1.3	1.05	1.73	7.26	2.84	1.12
PS/7	1.44	1.29	2.36	6.6	2.26	1.75	1.13	2.06	2.87	9.73	4.52	0.99
Mean	1.50	1.57	2.71	7.22	2.49	1.39	1.25	1.69	2.47	8.47	3.05	1.12
SEM	0.21	0.13	0.32	0.81	0.3	0.15	0.17	0.19	0.23	0.86	0.36	0.14

	Trial 1						Trial 2					
Supp/Sub	Rest	55	65	End of ex	End	Post	Rest	55	65	End of ex	End	Post
P/8	2.72	1.31	3.85	5.84	2.51	2.89	1.44	2.30	1.85	7.00	2.38	2.20
P/9	0.47	n/a	2.70	7.20	3.46	1.88	1.23	1.45	2.28	6.12	2.77	2.66
P/10	2.30	2.12	2.85	8.03	2.70	1.68	0.98	1.23	2.60	5.90	2.67	1.34
P/11	0.94	2.01	3.26	8.89	3.63	1.06	1.25	2.50	3.20	7.66	4.22	1.85
P/12	1.94	2.80	3.48	8.29	2.60	2.71	2.32	2.02	3.02	5.31	2.77	2.83
P/13	0.68	1.92	3.25	8.35	3.03	1.00	1.63	1.86	3.25	8.20	2.97	0.88
P/14	1.38	1.53	3.31	9.46	4.00	0.63	0.79	1.17	2.66	8.95	3.65	0.77
Mean	1.49	1.95	3.24	8.01	3.13	1.69	1.38	1.79	2.69	7.02	3.06	1.79
SEM	0.32	0.21	0.14	0.45	0.22	0.33	0.19	0.20	0.19	0.50	0.24	0.31

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 27 Raw data for Blood Glucose Concentrations (mmol L^{-1})

	Trial 1						Trial 2					
Supp/Sub	Rest	55	65	End of ex	End	Post	Rest	55	65	End of ex	End	Post
PS/1	3.57	4.04	3.67	3.60	3.81	5.83	3.78	3.82	3.77	4.25	3.68	2.88
PS/2	4.69	4.34	4.50	5.11	4.53	4.08	4.03	4.13	3.98	4.49	3.88	4.22
PS/3	4.49	n/a	3.26	4.45	n/a	4.06	4.10	3.83	3.70	4.63	4.11	4.17
PS/4	4.50	4.27	4.24	4.80	4.33	4.24	4.23	3.81	3.73	4.00	3.86	3.82
PS/5	3.28	3.87	4.16	n/a	3.98	3.32	4.29	4.76	3.86	4.14	4.82	3.99
PS/6	4.47	3.89	3.66	3.69	3.95	4.29	4.59	4.04	3.94	3.89	4.21	4.30
PS/7	4.53	3.81	4.13	4.51	4.16	4.67	4.29	4.06	4.15	5.40	4.30	4.13
Mean	4.22	4.04	3.95	4.36	4.13	4.36	4.19	4.06	3.88	4.40	4.12	3.93
SEM	0.21	0.09	0.16	0.25	0.11	0.29	0.10	0.13	0.06	0.19	0.14	0.18

	Trial 1						Trial 2					
Supp/Sub	Rest	55	65	End of ex	End	Post	Rest	55	65	End of ex	End	Post
P/8	4.45	4.15	4.30	4.08	4.25	3.87	3.79	4.06	3.81	4.11	3.95	4.57
P/9	3.97	n/a	3.72	4.15	3.68	4.57	3.82	3.71	3.55	4.20	3.33	4.13
P/10	4.30	4.34	4.15	3.30	4.21	4.08	4.61	4.29	4.22	3.25	4.85	4.16
P/11	4.42	3.94	4.12	4.32	4.42	4.74	4.61	4.31	4.35	4.67	4.86	4.61
P/12	4.54	4.46	4.29	4.25	4.16	4.77	5.83	3.92	3.79	3.93	3.74	3.96
P/13	4.12	4.27	4.70	6.42	5.43	4.36	4.46	4.61	4.72	5.88	5.62	4.64
P/14	3.88	4.26	4.00	4.89	4.28	4.08	3.83	4.41	4.15	4.80	4.53	4.14
Mean	4.24	4.24	4.18	4.49	4.34	4.35	4.42	4.19	4.08	4.41	4.41	4.32
SEM	0.10	0.07	0.11	0.37	0.20	0.13	0.27	0.12	0.15	0.31	0.30	0.11

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 28 Raw data for Serum Cortisol Concentrations (nmol L^{-1})

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
PS/1	628	526	480	747	205	406	426	474	718	219
PS/2	478	274	640	862	195	247	242	265	812	187
PS/3	272	307	658	865	177	296	367	414	647	155
PS/4	382	272	280	653	310	330	321	348	589	225
PS/5	426	310	294	436	359	452	324	325	483	349
PS/6	381	339	302	344	321	266	289	259	467	356
PS/7	436	324	348	479	306	326	306	294	479	355
Mean	429	336	428.9	626.6	267.6	331.9	325	339.9	599.3	263.7
SEM	41.2	33	62.2	79.5	27.5	27.9	22.1	30.1	50.5	32.9

	Trial 1					Trial 2				
Supp/Sub	Pre-ex	Bout 2	Bout 3	Post-ex	24-post	Pre-ex	Bout 2	Bout 3	Post-ex	24-post
P/8	257	190	176	263	250	377	297	290	340	299
P/9	503	434	619	615	506	477	369	357	463	346
P/10	303	331	391	537	236	530	401	330	613	308
P/11	552	378	355	571	326	581	498	434	507	411
P/12	426	279	293	294	229	484	227	346	427	308
P/13	459	457	475	699	321	537	542	505	626	458
P/14	236	156	147	468	129	298	196	164	516	152
Mean	390.9	317.9	350.9	492.4	285.3	469.1	361.4	346.6	498.9	326.0
SEM	47.3	43.8	62.6	61.4	44.4	37.4	49.4	40.7	38.2	36.7

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 29 **Raw data for average last min oxygen uptake ($\dot{V}O_2$) data during both main exercise trials**

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
PS/1	1.8	2.23	2.61	1.49	1.92	2.36
PS/2	1.9	2.33	2.45	1.74	2.07	2.73
PS/3	1.77	2.18	2.66	1.8	2.18	2.58
PS/4	2.21	2.7	3.1	2.26	2.71	3.11
PS/5	1.65	2.21	2.84	1.74	2.07	2.5
PS/6	1.38	1.65	1.98	1.52	1.71	2.09
PS/7	1.43	1.83	2.16	1.42	1.73	2.17
Mean	1.73	2.16	2.54	1.71	2.05	2.5
SEM	0.11	0.13	0.15	0.11	0.13	0.13

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
P/8	1.72	2.06	2.41	1.74	2.01	2.35
P/9	1.54	1.88	2.38	1.52	1.89	2.26
P/10	1.79	2.15	2.65	1.68	2.15	2.59
P/11	1.90	2.32	2.77	1.96	2.40	2.90
P/12	1.70	2.16	2.71	1.71	2.14	2.57
P/13	1.93	2.40	2.79	2.00	2.34	2.66
P/14	1.64	2.56	1.92	1.68	2.66	1.85
Mean	1.74	2.22	2.52	1.76	2.22	2.45
SEM	0.05	0.09	0.12	0.06	0.10	0.13

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 30 Raw data for average last min carbon dioxide uptake ($\dot{V}$ CO_2) used in calculations for substrate utilisation data during both main exercise trials

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
PS/1	1.57	1.96	2.35	1.42	1.90	2.38
PS/2	1.75	2.23	2.36	1.53	1.93	2.63
PS/3	1.58	2.06	2.39	1.67	2.07	2.43
PS/4	1.96	2.44	2.81	1.98	2.39	2.86
PS/5	1.55	2.06	2.75	1.60	1.86	2.36
PS/6	1.27	1.50	1.87	1.38	1.60	2.00
PS/7	1.33	1.66	2.02	1.27	1.63	2.07
Mean	1.57	1.99	2.36	1.55	1.91	2.39
SEM	0.09	0.12	0.13	0.09	0.10	0.11

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
P/8	1.55	1.87	2.18	1.54	1.86	2.15
P/9	1.36	1.76	2.20	1.44	1.80	2.14
P/10	1.66	2.04	2.55	1.50	2.03	2.60
P/11	1.78	2.25	2.65	1.83	2.31	2.83
P/12	1.54	2.01	2.49	1.61	1.94	2.41
P/13	1.73	2.17	2.59	1.94	2.24	2.58
P/14	2.11	1.42	2.50	2.13	1.42	2.45
Mean	1.67	1.93	2.45	1.71	1.94	2.45
SEM	0.09	0.11	0.07	0.10	0.11	0.09

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 31 Fat utilisation during bouts 1-3 during both main exercise trials

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
PS/1	0.40	0.47	0.45	0.11	0.03	n/a
PS/2	0.27	0.17	0.17	0.37	0.25	0.18
PS/3	0.34	0.21	0.46	0.24	0.19	0.26
PS/4	0.43	0.47	0.52	0.49	0.58	0.43
PS/5	0.16	0.27	0.16	0.24	0.38	0.25
PS/6	0.19	0.26	0.20	0.25	0.20	0.17
PS/7	0.18	0.29	0.26	0.26	0.17	0.17
Mean	0.28	0.31	0.32	0.28	0.26	0.24
SEM	0.04	0.05	0.06	0.04	0.07	0.04

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
P/8	0.30	0.34	0.42	0.35	0.26	0.34
P/9	0.30	0.21	0.32	0.15	0.16	0.21
P/10	0.23	0.20	0.17	0.32	0.22	n/a
P/11	0.21	0.13	0.20	0.23	0.15	0.14
P/12	0.29	0.27	0.37	0.19	0.34	0.27
P/13	0.35	0.40	0.35	0.11	0.19	0.14
P/14	0.39	0.34	0.11	0.47	0.49	0.36
Mean	0.30	0.27	0.28	0.26	0.26	0.24
SEM	0.02	0.04	0.04	0.05	0.05	0.04

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo

Appendix 32 Raw data for carbohydrate utilisation (g min^{-1}) during bouts 1-3 during the main exercise trials

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
PS/1	1.20	1.56	2.07	1.52	2.24	2.93
PS/2	1.66	2.41	2.55	1.21	1.90	2.88
PS/3	1.33	2.13	2.10	1.60	2.16	2.48
PS/4	0.78	2.14	2.50	1.55	1.89	2.72
PS/5	1.60	2.02	3.05	1.52	1.59	2.42
PS/6	1.21	1.36	1.92	1.25	1.59	2.13
PS/7	1.29	1.51	1.99	1.09	1.69	2.21
Mean	1.29	1.88	2.31	1.39	1.87	2.54
SEM	0.11	0.15	0.15	0.08	0.10	0.12

	Trial 1			Trial 2		
Supp/Sub	Bout 1	Bout 2	Bout 3	Bout 1	Bout 2	Bout 3
P/8	1.35	1.67	1.91	1.27	1.79	2.01
P/9	1.12	1.76	2.10	1.47	1.90	2.23
P/10	1.60	2.12	2.80	1.26	2.08	3.19
P/11	1.78	2.50	2.86	1.81	2.53	3.18
P/12	1.35	1.95	2.37	1.81	1.77	2.45
P/13	1.50	1.93	2.54	2.15	2.38	2.88
P/14	1.05	1.73	2.83	0.91	1.40	2.34
Mean	1.39	1.95	2.49	1.53	1.98	2.61
SEM	0.10	0.11	0.14	0.16	0.15	0.18

Supp/Sub: Supplementation / Subject Number. PS: phosphatidylserine group, P: Placebo