IMPACT OF HIGH INTENSITY INTERVAL TRAINING (HIIT) AND / OR SELENIUM (SE) SUPPLEMENTATION ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN ACTIVE FEMALES.

KAREN KEANE

MRes **2014**

Impact of HIIT (High Intensity Interval Training) and/or Selenium Supplementation (Se) on Oxidative Stress and Anti-Oxidant Status in Active Females

Karen Keane

A thesis submitted in partial fulfilment of the

Requirements of the

Robert Gordon University

For the degree of Doctor of Philosophy

February 2014

Abstract

Impact of HIIT (High Intensity Interval Training) and/or Selenium supplementation (Se) on oxidative stress and anti-oxidant status in active females

Keane, K

Background: Cells continuously produce free radicals (FR) and reactive oxygen species (ROS) as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defence system consisting of enzymes. An imbalance between ROS / FR and antioxidants is often referred to as oxidative stress and is involved in the pathogenesis of various disorders including cardiovascular disease, neurodegenerative diseases, diabetes and several types of cancer (Campbell *et al.* 2010). Selenium (Se), as part of the glutathione peroxidase (GPx) family of enzymes, has the ability to detoxify these ROS / FR, increasing protection against this stress. Regular training has been shown to increase antioxidant capacity; it is through adaptation that increased antioxidant capacity may alleviate oxidative stress. **Objectives:** The present investigation is designed to test the hypothesis that Se supplementation and / or regular High Intensity Interval Training (HIIT) can alleviate exercise - induced oxidative stress following a single bout of HIIT. **Design:** Randomised Open Label Trial**. Method:** Twenty-two healthy female participants, who participated in intermittent sports, were recruited. In a randomised manner, participants were equally allocated to either a Se only group (250 ug sodium selenite/day) or a Se + HIIT group (250 ug sodium selenite / day + 2 sessions HIIT / week) for 3 weeks. Measures of fitness, malondialdehyde (MDA), glutathione peroxidase (GPx) in plasma and red blood cells (RBC) and total antioxidant capacity (TAC) were assessed before and after an initial (baseline) and last bout of HIIT. **Results:** No statistically significant changes were identified for any of the measured markers of oxidative stress or antioxidant capacity, although a number of trends toward altered activity were noted. Following the intervention, Se + HIIT group demonstrated a 9% decrease in MDA response to a single bout of HIIT, whilst the Se only group decreased by 12%. GPx3Activity in plasma increased by 15% in the Se group and decreased in the Se + HIIT group by 2% pre to post intervention. Additionally, GPx1 Activity in RBC increased in both groups by 6% and 2% respectively. TAC elicited similar responses increasing by 40% in the Se + HIIT group and 66% in the Se only group. Furthermore, there was an improvement in a

number of fitness components in the HIIT trained group post intervention, namely speed and maximal aerobic capacity. **Conclusion:** This is the first study to examine the impact of HIIT and/or Se supplementation on oxidative stress and antioxidant capacity in active females. Whilst there were no significant differences observed, there were some promising trends highlighting a potential benefit of Se (and possibly HIIT) in reducing oxidative stress and increasing antioxidant status post high intensity interval exercise in females engaged in intermittent sports.

References:

CAMPBELL, P. T., GROSS, M. D., POTTER, J. D., SCHMITZ, K. H., DUGGAN, C., MCTIERNAN, A. & ULRICH, C. M. 2010. Effect of exercise on oxidative stress: a 12-month randomized, controlled trial. Med Sci Sports Exerc, 42, 1448-53.

Key Words: ***Free Radicals, Reactive Oxygen Species, Oxidative Stress, High Intensity Interval Training, Selenium.***

Authors Declaration

I hereby declare that this thesis has been composed by myself and has not been presented or accepted in any previous application for a degree, and is a record of work carried out by myself unless otherwise stated; all quotations have been distinguished by quotation marks and all sources of information acknowledged.

Date 12.12.13

Karen Keane

Acknowledgements

I would like to take this opportunity to thank everyone that has helped and supported me during the past year. Without doubt, this last year has been host to some of my most memorable times to date.

Firstly, I would like to thank my Principal Supervisor, Giovanna Bermano, for all her input, guidance and suggestions throughout the past year.

A massive thank you to Katherine Burgess and Eimear Dolan for their endless support, friendship and help over the last year.

I would like to thank the other staff members, particularly in the Sport and Exercise Science Department for their help and support over the past year. Whether it was pesky stats or awkward timing gates, there was always someone there to offer guidance.

Ba mhaith lion an taighde a thiomnú dom chlann, ach go háirithe do mo tuismitheoirí, mar siombail dom bhuíochas as uct a chuid tacaíocht mhorálta agus fhoghne trí mo bhlianta scolaíochta ar fad. Gan ian ní bheinn in ann é seo a dhéanamh.

Thanks to the RGU Gaelic Football Team, in particular the AMAZING ladies team for keeping me sane the past year. From getting involved in the study and listening to me go on about it – it truly wouldn't have been possible with you all. #ilovemyteam #britishchamps

To all the amazing friends I met in Aberdeen and all the forgettable nights! It has been short but very sweet!

I would like to thank my fellow MRESearchers for all their help, advice and friendship throughout the year. I wish them the very best and have no doubt they will all go on to do great things.

“Knowing is not enough; we must apply. Willing is not enough; we must do.”

—Goethe

Table of Contents

List of Abbreviations

ACSM	American College of Sports Medicine
AREDS	Age Related Eye Disease Study
ATP	Adenosine-5'-Triphospate
AV	Antioxidant Vitamin
CAT	Catalase
CHAOS	Cambridge Heart Antioxidant Study
CK	Creatine Kinase
CMJ	Countermovement Jump
CS	Citrate Synthase Activity
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DOMS	Delayed Onset Muscle Soreness
EIMD	Exercise Induced Muscle Damage
ET	Endurance Training
FITT	Frequency, Intensity, Type, Time
FR	Free Radicals
FRAP	Ferric Reducing Antioxidant Potential
GPPAQ	General Practice Physical Activity Questionnaire
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Glutathione
GSH-E	Glutathione Ethyl Ester
GSSG	Glutathione Disulphide
H^2O	Water
H^2O^2	Hydrogen Peroxide
HDL	High Density Lipoprotein

HIIT	High Intensity Interval Training
HK	Hexokinase
ICC	Interclass correlation coefficient
IL – 6	Interleukin – 6
LDL	Low Density Lipoprotein
MDA	Malondialdehydes
MI	Myocardial Infarction
MUFA	Monounsaturated Fatty Acids
NADPH	Nicotinamide Adenine Dinucleotide Phosphohydrolase
$O^{\cdot}2^{-}$	Superoxide Anion Radical
ORAC	Oxygen Radical Absorbance Capacity
PAI	Physical Activity Index
PARQ	Physical Activity Readiness Questionnaire
PBS	Phosphate Buffered Saline
PC	Protein Oxidation
PFK	Phosphofructokinase
PUFA	Polyunsaturated Fatty Acids
RBC	Red Blood Cells
RDA	Recommended Daily Allowance
RGU	Robert Gordon University
ROS	Reactive Oxygen Species
RT	Room Temperature
SD	Standard Deviation
Se	Selenium
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Sciences
TAC	Total Antioxidant Capacity
TAG	Triacylglycerol
TBARS	Thiobarituric Acid

USOTC	United States Olympic Training Centre
VO_2	Oxygen Uptake
VO_2max	Maximal Oxygen Uptake
WANT	Wingate Anaerobic Cycle Test
WAVE	Women's Angiographic Vitamin and Estrogen Study
WBC	White Blood Cells

List of Figures

List of Tables

Chapter One: Introduction

Introduction.

During the resting state the human body produces both reactive oxygen species (ROS) and free radicals (FR). Low concentrations of both ROS/FR are necessary for several physiological processes including maintenance of normal redox status, apoptosis and other intracellular signalling processes (Halliwell & Gutteridge 1989). However, it is the increased generation and accumulation of these species that can potentially lead to a state of oxidative stress. There is an abundance of evidence suggesting that this increase in radical production and the subsequent increase in oxidative stress is involved in the pathogenesis of various disorders including cardiovascular, neurodegenerative diseases, diabetes and several types of cancer (Bjelakovic *et al.* 2010; Campbell *et al.* 2010). In order to protect the body against this potential stress, a highly complex antioxidant protection system has evolved (Kallora *et al.* 2006). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. They have the ability to prevent or reduce the extent of oxidative damage to other molecules (Young & Woodside 2001). Selenium (Se) is described as an antioxidant due to its incorporation into selenoproteins as discussed below (Navarro Alarcón & López – Martinéz 2000)

Se is a non- metal that is found in the oxygen series and exists in multiple oxidation states. There have been approximately 25 selenoproteins identified within the human body, all of which are Se dependent, generally with selenocysteine at the active site. The most studied selenoprotein is Glutathione Peroxidase (GPx), which is thought to have anti-oxidant properties. GPx is the generic name of the GPx family of enzymes, which have been described as six different multiple isozymes in mammals (Rotruck *et al.,* 1973). Table 1.1 shows a description of each of the mammalian GPx isozymes (De Zoysa et al, 2008) :

Table 1.1: Description of mammalian GPx isozymes

GPx	Name
GPx1	Cellular/cytosolic glutathione peroxidase
GPx2	Gastrointestinal glutathione peroxidase
GPx3	Plasma/extra cellular glutathione peroxidase
GPx4	Phospholipids hydroperoxide glutathione peroxidase
GPx5	Epididymal glutathione peroxidase
GPx6	Olfactory epithelium glutathione peroxidase

With GPx, Se functions as a redox centre. The best known example of this GPxs redox function is the decomposition of H_2O_2 and damaging lipid and phospholipids to harmless productions (water and alcohols), thereby dampening the propagation of FR and ROS (Spallholtz *et al*. 1990). A review carried out by Navarro – Alarcón and López – Martínez (2000) examined the essentiality of Se in the human body and its relationship with various diseases. Yang *et al* (1984) identified a role for Se in the prevention of endemic cardiomyopathy in humans. Additionally, Ortenblad *et al.* (1997) demonstrated how GPx3 activity increases with chronic training. They used a short – term maximal exercise protocol. Free radical muscle damage was determined after a continuous 30-second maximum jump test in both a trained (volleyball) and untrained population. The resting levels of glutathione reductase (GR) were significantly higher in the trained groups, furthermore, creatine kinase (CK), an indicator of muscle damage was significantly higher in the untrained group post exercise ($p<.05$). This research supported the idea that the GR defence system is up-regulated and has a greater ability to protect against oxidative stress, in this case lipid peroxidation in trained individuals. This study prompted Tiidus (1998) to suggest that Se may somewhat play an indirect, but significant role in exercise recovery. As a result of these studies focusing on Se and other antioxidants and the general conclusion, highlighted by Diplock (1994), that the subsequent damage to structures such as lipid membranes and genetic material caused by an increase in oxidative stress increases the risk of conditions such as cancer and atherosclerosis, extensive research has been carried out to investigate the role of Se within the human body.

Exercise, in particular strenuous exercise, has been associated with an increased generation of ROS/FR due to a dramatic increase in oxygen uptake. Exercise is associated with an increase in oxygen uptake by the whole body and particularly skeletal muscle (Ji 1999). It has also been suggested that adaptation to regular exercise may result in an enhanced antioxidant defence system (Oztasan *et al.* 2004). Regular exercise causes adaptation of the antioxidant and repair systems, which could result in a decreased base level of oxidative damage and increased resistance to oxidative stress (Radak *et al.* 2001). A number of mechanisms have been proposed to explain this response including the up regulation of endogenously produced antioxidant enzymes (Ji & Fu 1992), for example Fisher *et al.* (2010) demonstrated that regular training increased the up-regulation of catalase activity following exercise. A secondary mechanism often used to explain this adaptation response is the increase in de novo production of endogenous antioxidant molecules including glutathione (Ji 1999). However, the majority of this research has focused on endurance training (ET) only and further research may be required to examine the influence of other exercise and training modalities.

HIIT consists of repeated sessions of brief intermittent bursts of vigorous exercise performed at intensities >90% maximal oxygen uptake (VO_{2max}) interspersed by periods of rest or low intensity activity (Smith *et al.* 2009). VO_{2max} is the maximal oxygen uptake or the maximum volume of oxygen that can be utilized in one minute during maximal or exhaustive exercise (Evans & White, 2009, pp.9). HIIT has gained recent popularity due to research that suggests that this method of training may lead to physiological and performance gains that are similar to endurance training in both trained and untrained individuals (Laursen and Jenkins 2002). They found that HIIT has the ability to improve a range of physiological parameters including VO_2 peak anaerobic power output and time to fatigue to a greater extent than continuous ET in the majority of the 180 studies examined. One such study included was carried out by Billat and associates in 13 untrained individuals. They found that HIIT (5×4 minutes at 100% VO_{2max}, with 2minutes rest) enhanced oxidative capacity (succinate dehydrogenase and cytochrome oxidase) of type 2 fibres when compared with continuous exercise who worked for a similar duration at a mean intensity of 79% VO_{2max}. They concluded that this is largely due to an up-regulated contribution of anaerobic and aerobic metabolism. Furthermore, the maximal enzyme activities of citrate

synthase activity (CS), hexokinase (HK), phosphofructokinase (PFK), succinate dehydrogenase and malate dehydrogenase also significantly increased following HIIT training. These findings were in contrast to submaximal endurance training, which had little or no effect on glycolytic enzyme activity.

In summary, cells continuously produce FR and ROS as part of everyday metabolic processes. This amount produced is usually well within the capabilities of our antioxidant defence system; however when there is a shift in balance in favour of these radicals, oxidative stress can occur. This potential stress is involved in the pathogenesis of various disorders including cardiovascular, neurodegenerative diseases, diabetes and several types of cancer (Bjelakovic *et al*. 2010; Campbell *et al.* 2010). A number of strategies have been suggested to attenuate the oxidant stress response in individuals. These include both antioxidant supplementation (Sutherland *et al.* 2007) and exercise training (Fenster *et al.* 2002).

Chapter Two: Literature Review

Review of the Literature.

2.1. Oxidative Stress

During the resting state the human body produces both ROS and FR. A FR can simply be defined as any species capable of independent existence that contains one or more unpaired electrons (Halliwell & Gutteridge 1989), whereas the term ROS is used to describe both FR such as nitric oxide (NO) as well as some non- radical derivatives of O_2, e.g. hydrogen peroxide (H_2O_2) (Spurway & MacLaren 2007). The two terms are often used interchangeably in the literature. Hormesis is a term used frequently by toxicologists referring to a biphasic dose-response curve with beneficial or stimulatory effects at low doses but adverse or inhibitory effects at high doses (Mattson 2008). ROS/FR are believed to possess these properties. The presence of low concentrations of ROS/FR are involved in several important functions including normal cellular redox status, tissue function, apoptosis, stimulation of antioxidant defence molecules, and intracellular signalling processes (Spurway & MacLaren 2007). The importance of small concentrations of FR/ ROS production is exemplified when dealing with patients who suffer with granulomatous disease. These subjects have defective a membrane-bound NADPH oxidase system which makes them unable to produce the superoxide anion radical (O^{2-}.), thereby resulting in multiple and persistent infection (Valko *et al*. 2007). Other beneficial effects of FR and ROS involves there role in the function of several cellular signalling systems (Wilcox *et al.* 2004). Nitric Oxide (NO), for example is an intercellular messenger for modulating blood flow, thrombosis, and neural activity However, it is the generation and increased accumulation of both ROS/FR that can inevitably lead to a state of oxidative stress. This elevated state can occur as a result of deleterious health states (Kennett and Kuchel 2006). The term oxidative stress is an expression used to describe a range of deleterious states that result from an imbalance between the excessive formation of ROS/FR and limitations in the antioxidant defence system (Fisher 2009). There is an abundance of evidence suggesting that this rise in radical production and the subsequent increase in oxidative stress causes damage to internal structures such as DNA, lipids and proteins. Therefore, it has been implicated in the pathogenesis of various disorders including cardiovascular disease, neurodegenerative disease, diabetes and cancer (Bjelakovic *et al.* 2010; Campbell *et al*. 2010). Oxidative stress

markers in humans are most commonly measured in blood (Dekkers *et al.* 1996) and urine (Hellsten-Westing *et al.* 1991). The mode of detection is critical in determining what level of oxidation is present.

2.2. Antioxidant Defence System

The precise nature of the health benefits obtained from consuming antioxidant rich foods is yet to be fully understood (Wootton-Beard and Ryan 2011). As previously mentioned in section 1.1, excess production of ROS/FR can lead to a state of oxidative stress, with numerous associated consequences to health. In order to protect the body against this potential stress, a highly complex antioxidant protection system has evolved (Kaliora *et al.* 2006). Antioxidants are defined as molecules that can be present in smaller concentrations when compared to other oxidizable biologically relevant molecules. They have the ability to prevent or reduce the extent of oxidative damage to other molecules (Halliwell & Gutteridge 1989). Antioxidants are effective because they can donate their own electrons to ROS, thereby neutralizing the adverse effects of the latter (Kunwar and Priyadarsini 2011). The balance between oxidation and antioxidation is believed to be essential in maintaining a healthy biological system (Bouayed & Bohn 2010).

The total antioxidant capacity (TAC) of an individual consists of both endogenous molecules e.g. superoxide dismutase (SOD), catalase (CAT) and GPx, and exogenous sources mainly derived from diet e.g. vitamin A, E,C and Se. The endogenous anti-oxidant defence system is not capable of independently preventing development of oxidative stress and the intake of exogenous dietary anti-oxidants may be required to diminish the cumulative effects of oxidative damage throughout the lifespan. There are several classes of antioxidants useful for human beings; these include vitamin A, C, E, Se Manganese, Chromium and Zinc. They are abundant compounds, found primarily in fresh fruits and vegetables (Bjelakovic *et al.* 2003) and it must be noted that the human endogenous antioxidant defence system is incomplete without these exogenously originating reducing compounds, such as Vitamin C or Vitamin E. These play an essential role in many antioxidant mechanisms in living organisms (Bouayed & Bohn 2010). The FR and ROS levels produced by the body during rest

are usually within the capacity of the body's antioxidant defence system. However, due to the consistent oxidative stress being placed on the body, there is a continuous requirement for exogenous antioxidants to prevent disequilibrium in favour of oxidation.

In terms of mechanistic functions, antioxidants can work in several ways. They can be classified as preventing antioxidants, scavenging antioxidants and repair and de novo antioxidants (Niki 2010). The preventing antioxidants work as the first line of defence against ROS/FR, while the scavenging antioxidants essentially work as the second defence line (Niki 2010). The preventing antioxidants suppress the formation of ROS by, for example, reducing H_2O_2 to water. Meanwhile, the scavenging antioxidants remove the active species before they attack biologically essential molecules (Niki 2010). An illustration of the antioxidant defence network is provided in figure 2.1. Therefore, the extent of oxidative stress experienced will be determined by the level of production of both ROS / FR and the capacity of the antioxidant system to neutralise them.

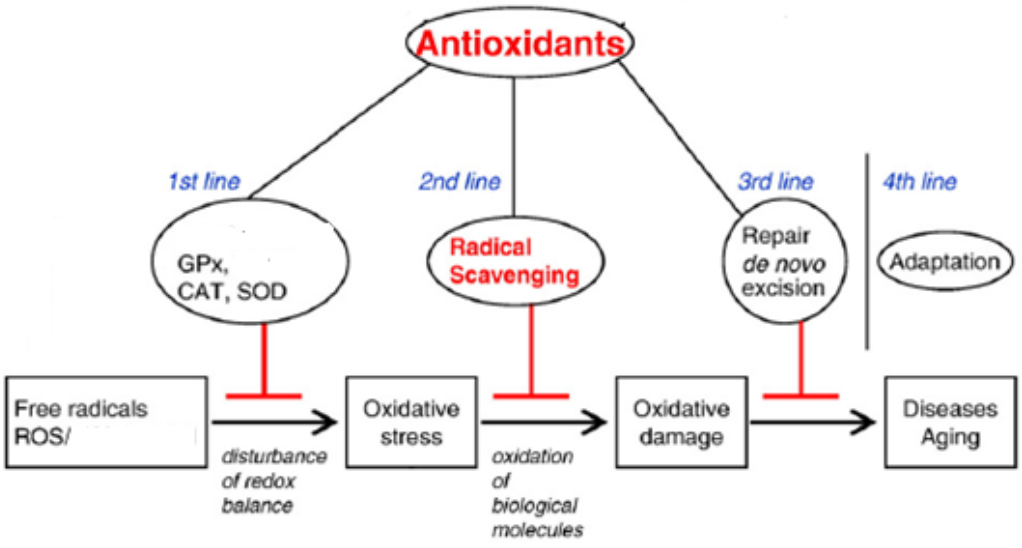


Figure 2.1: Defence network in vivo against oxidative stress. 1st line = prevention of build – up of free radicals, 2nd line = scavenging of radicals prior to damage, 3rd line = DNA repair after damaged occurred.

2.3. Antioxidant Status and Exercise

A number of animal studies have shown antioxidant supplementation to have positive effects on exercise performance and oxidative stress and have become more widespread in recent years (Larcombe 2008: 2010; Huang 2009). Previous work carried out by Leeuwenburgh & Ji (1998) examined the effect of glutathione (GSH) and glutathione ethyl ester (GSH-E) supplementation on exercise induced oxidative stress. They found that both GSH and GSH-E improved endurance performance and prevented muscle lipid peroxidation in mice during prolonged exercise. Animal studies examining the impact of induced anti-oxidant deficiency on exercise capacity are difficult to replicate in a controlled human environment due to the ethical implications of this type of research. As a result, human studies have primarily focused on increasing antioxidant intakes above usual daily reference values through antioxidant supplementation as a means of reducing oxidative stress and enhancing exercise capacity (Schnass & Pabst 1998; Goldfarb *et al.* 2005). A study by Schnass & Pabst (1988) examined the effect of 400mg of vitamin E daily (and a multivitamin / multi-mineral supplementation) on 12 mountain climbers who were on a 10 week expedition at high altitude. They found that not only did anaerobic threshold increase in the treatment group and decrease in the placebo (control) group but that pentane, a determinant of lipid peroxidation, showed no significant increase in the treatment group and yet, increased by more than 100% in the controls.

2.4. Antioxidant Supplementation

Nutritional supplements are products that are used to increase the nutritional content of the diet. Examples of popular supplements include vitamins, minerals and herbs amongst others. Supplements are used for many purposes some of which include boosting overall health and energy, providing immune system support, improving athletic performance and reducing the risk of illness (Ransley *et al.* 2001). Due to the possible large health benefits of a diet high in antioxidants highlighted in section 2.3, supplement use is becoming more widespread.

Collectively, for the most part, clinical trials have failed to demonstrate a beneficial effect of antioxidant supplementation. Many reviews have found that antioxidant supplements did

not reduce risk of disease, but in fact increased it (Bouayed & Bohn 2010). Most of the trials presented investigated the effects of supplements administered at higher doses than those commonly found in a balanced diet, and some of the trials used doses well above the recommended daily allowances and even above the tolerable upper intake levels, with deleterious results.

Excessive antioxidant intake can adversely affect key physiological processes. The primary concern regarding supplements is their potentially deleterious impact on ROS production, especially when, as previously mentioned, exact modulation of ROS levels are required to allow normal cell function (Bouayed & Bohn 2010; Kunwar and Prijadarsini 2011). Studies have shown that antioxidants may exert pro-oxidant activity, depending on the specific set of conditions. This is subject to the dosage, redox conditions and the presence of free transition metals (Galati *et al.* 2002). This suggests that supplemental antioxidants must be taken with caution and not taken in place of natural occurring dietary antioxidants.

Many primary and secondary prevention trials of antioxidant supplements have been conducted to prevent several diseases, many yielding conflicting results. Negative findings were reported in the Women's Angiographic Vitamin and Estrogen Study (WAVE), in which 423 postmenopausal women with coronary disease on hormone replacement therapy were given 400IU vitamin E twice daily plus 500mg of vitamin C twice daily or a placebo. Results suggested that those in the treatment group had an unexpected significantly higher all-cause mortality rate and a trend for an increased cardiovascular mortality rate compared with the vitamin placebo women. Likewise, in the HDL-Atherosclerosis Treatment Study, 160 subjects with angiographically demonstrated coronary artery disease were allocated to either a simvastatin/niacin group or an antioxidant vitamin (AV) group (consisting of vitamin E 800 IU/d, vitamin C 1000 mg/d, beta carotene 25mg/d, and Se 100ug/d). Results showed a 0.7% progression in stenosis after 3 years in the AV group, compared with 0.4% regression in the group on only simvastatin/niacin.

Null findings have also been reported in a systematic review and Meta – analysis recently carried out by Bjelakovic *et al. (*2007). This study aimed to analyse the effects of antioxidant supplements on all-cause mortality of adults. They included all randomized trials involving adults comparing beta carotene, vitamin A, vitamin C (ascorbic acid), vitamin E and Se either singly or combined vs. placebo or vs. no intervention. 68 randomized trials with 232,606

participants (385 publications) in total were included. Antioxidant supplements at any dose, duration and route of administration were included also. They found that antioxidants supplements, with the potential exception of Se, were without significant effects. The potential reasoning for their results reiterates the findings of Bouayed & Bohn (2010), that by eliminating free radicals from our organism, we interfere with some essential defensive mechanisms like apoptosis, phagocytosis, and detoxification. However, interestingly Bjelakovic and his colleagues concluded that there was not enough substantial evidence to state that antioxidant supplements could be used for primary or secondary prevention of disease in healthy participants or patients with various diseases. In spite of this, they added that Se given singly or in combination with other supplements seemed to significantly decrease mortality, but after exclusion of high-bias risk trials, the effect disappeared. Further research and randomized trials involving Se will likely increase our understanding of the effects of this trace nutrient.

Lastly, positive findings have also been reported. Although the preponderance of clinical trial evidence has not shown beneficial effects of antioxidant supplementation, evidence from some smaller studies documents a benefit of antioxidant supplements. The Age-Related Eye Disease Study (AREDS), found a beneficial effect of antioxidant supplements. 3600 individuals who took part in the study for an average of 6.3years concluded that high levels of antioxidants (vitamin C, vitamin E, and beta carotene) reduced the risk of developing advanced stage of age-related macular degeneration by 17% in people who had the intermediate stage of this disease or who had the advanced stage in only one eye. “High levels” were defined as vitamin C, 500mg; vitamin E, 400IU; and beta carotene, 15mg. Similarly, 2002 patients with angiographically proven coronary atherosclerosis were recruited by the Cambridge Heart Antioxidant Study (CHAOS): 1035 of whom subjects were assigned provided with α-tocopherol (capsules containing 800 IU daily for first 546 patients; 400 IU daily for remainder) whereas; 967 participants received identical placebo capsules. Patients were followed up for a median of 510 days. Results suggested that plasma α-tocopherol concentrations (measured in subsets of patients) rose in the actively treated group (from baseline mean 34.2 µmol/L to 51.1 µmol/L with 400 IU daily and 64.5 µmol/L with 800 IU daily) but did not change in the placebo group. A-tocopherol treatment significantly reduced the risk of the primary trial endpoint of cardiovascular death and non-

fatal myocardial infarction (MI) (41 vs. 64 events; relative risk 0.53 [95% Cl 0.34-0.83; p=0.005). In general, the studies showing either positive or adverse effects (especially for vitamins C and E) are much smaller studies than the larger clinical trials that consistently have not shown any beneficial effects of antioxidant supplements. Furthermore, studies that examine the relationship between exercise, oxidative stress and supplementation have demonstrated more positive results A study by Goldfarb *et al.* (2005) looked at the effect of a combined antioxidant treatments (400IU Vitamin E, 1g vitamin C and 90ug Se per day for 14days) on blood oxidative stress following eccentric endurance exercise in 18 women aged 19 -31y. They found an apparent antioxidant response. The data suggest that this type of endurance exercise can increase blood biomarkers of oxidative stress in trained females and that this combination of vitamin E, C and Se can assuage the rise in blood protein oxidation and MDA.

To summarise, inconsistencies are present in the literature with regards the relationship between supplements and exercise performance. Furthermore, there is little theoretical basis to believe they would have a positive effect. It is very difficult to solely detect the effects of antioxidant supplementation on the basis that several factors govern human performance (Sen 2011). Based on positive findings, the only statement that can be made until further research is carried out is that the type, dosage and timing of the supplement as well as the outcome measures are crucial when implementing a supplement intervention.

2.5. Antioxidant Status and Disease

Cardiovascular disease and cancer are ranked as the first and second leading causes of death in the Western World (Liu 2003). Studies investigating the use of antioxidant therapy to combat the onset of these diseases are becoming widespread. Vitamin E, and its antioxidant properties, has been found to inhibit low density lipoprotein (LDL) oxidation, which in turn reduces the risk of atherosclerosis and associated cardiovascular problems (Kaliora et al. 2006; Wang et al. 2011). Seifried (2007) estimated that one in three of all cancer deaths can be credited to dietary factors. They attributed this finding to the lack of protective factors gained from antioxidative substances in the diet. The role that antioxidants have to play in the prevention of cancer has long been debated; a review by

Block et al. (1992) examined a range of cancer types including colon, breast and lung and determined that 128 out of 156 epidemiological studies found a statistically significant protective effect from fruit and vegetable consumption. They concluded that this protective effect was due to the high polyphenol content of both fruit and vegetables. Polyphenols are naturally occurring compounds found in fruit, vegetables, cereals and beverages. Studies have found that these compounds can provide antioxidant benefits in human health and provide protection against many chronic diseases (Pandey et al. 2009). Trend data demonstrating an inverse relationship between declining fruit and vegetable consumption and increasing rates of coronary heart disease (CHD) across Europe can be tracked back to the 1970s, when Armstrong and Doll (1975) reported that CHD mortality rates were higher in areas of the UK where consumption of fruit and vegetables was lowest. An analysis by Denny & Buttriss (2007) produced a synthesis report on behalf of the European Food Information Resource (EuroFIR) consortium examining the relationship between plant foods and health. The relationship between fruit and vegetable intake and CVD incidence in the American Nurses' Health Study and the Health Professionals' Follow-up Study prospective cohorts has been investigated by Hung *et al.* (2004). They found that risk of cardiovascular disease was reduced by 28% in men and women who had at least five servings a day compared to those who had less than one and a half. As fruit and vegetables have a variety of nutrients and non-nutrients, it is nearly impossible to detect the cause of these protective effects. A recent theory is that food components with antioxidant properties may prevent the processes involved in the development of disease by protecting against oxidative damage (Pandey *et al.,* 2009*).*

2.6. Selenium (Se)

Selenium (Se) is a non-metal that is found in the oxygen series and exists in multiple oxidation states. Within biological systems the element is a constituent of the amino acid, selenocysteine that is incorporated into specific proteins called selenoproteins. There have been about 25 identified selenoproteins in total, all of which are Se dependent, generally with selenocysteine at the active site. Over the past 180 years, the perception of the biochemical effects and function of Se has changed dramatically. In fact, when Swedish chemist Berzelius first discovered Se in 1817, it was considered to be toxic to both humans

and animals. However, Shwartz and Foltz (1957) later demonstrated that Se was in fact an essential nutrient for animal health. They found that, Se as an antioxidant singularly and through its interaction with vitamin E prevented liver necrosis in rats.

2.6.1. Physiological Role of Se

Following this initial discovery by Shwartz and Foltz (1957), Se essentiality for humans was later shown in a number of clinical studies as reviewed by Navarro Alarcón & López – Martinéz (2000). Yang *et al.* (2006) for example, showed that Se was able to prevent endemic fatal cardiomyopathy. They concluded that because GPx3is found in all mammalian cells (Lawrence and Burke 1976), Se may provide a defence against the accumulation of peroxides and FR that damage membranes and macromolecules, such as DNA, thus preventing the onset of cardiomyopathy. As a consequence of the expansive research carried out to investigate unknown roles of Se within the human body, dietary recommendations were established. An adequate Se intake was set by the US National Academy of Science for adults as 50-200ug/daily, though the absolute requirement of Se for man is still unknown (Institute of Medicine, Food and Nutrition Board 2000).

The results from studies focusing on antioxidant supplementation in humans have been somewhat inconsistent as previously noted. However, these studies focused primarily on antioxidant vitamins, in particular the water soluble vitamin C and the lipid soluble vitamin E as a means of protecting against ROS production in response to exercise (Schnass & Pabst 1998; Goldfarb *et al.* 2005: Sacheck *et al.* 2003). Certain studies have shown some potential benefits associated with Se supplementation (Bjelakovic *et al.* 2007). Inflammation is believed to play a role in the pathogenesis of cardiovascular events. Walston *et al.* (2005) carried out a combined cross-sectional and longitudinal analysis on 619 older females. They found an inverse relationship between specific carotenoids and the trace element Se, which both have antioxidant compounds, and IL-6, suggesting that these specific antioxidant nutrients aid in reducing inflammation. These antioxidant compounds mechanistically decrease levels of H_20_2 and lipid peroxides and therefore play a vital role in suppressing inflammation (Walston *et al.* 2005). Furthermore, those with the lowest Se levels had a significantly higher risk of total mortality over a period of 5 years (hazard ratio = 1.54, 95%

confidence interval: 1.03, 2.32). Altogether, these findings suggest that specific antioxidant nutrients play an important role in suppressing IL-6 in disabled older women.

However, the biochemical functions of some of the other selenoproteins have yet to be fully determined (Reeves & Hoffman, 2009). The major class of selenoproteins that has important physiological characteristic and provides anti-oxidant properties is GPx3which protect cells against potentially damaging effects of peroxides. It does this by helping to prevent FR – mediated attacks on biological molecules (Arthur *et al.* 1997). Within the cell total is distributed in a ~2:1 ratio between the cytosol and the mitochondrial matrix (Ji 1995). The distribution of the enzyme allows for increased efficiency in scavenging free radicals by the GPx system. These enzyme systems are well established as being the major antioxidant defence systems within the body. With GPx, Se functions as a redox centre. The best-known example of this GPx redox function is the decomposition of H_2O_2 to harmless products (water and alcohols), thereby dampening the propagation of FR and ROS (Spallholtz *et al*. 1990). Table 2.1 details additional selenoproteins of physiological importance

Table 2.1.: Examples of Selenoproteins and their functions

(Sperm) mitochondrial capsule selenoproteins	Form of GPx4: shields developing sperm cells from oxidative damage and later polymerises into structural protein required

	for stability / motility of mature sperm (Ursini *et al.* 1999).
Iodothyronine delodinases	Production and regulation of level of active thyroid hormone, T3, from thyroxin, T4 (Sunde 1997).
Thioredoxin reductases	Reduction of nucleotides in DNA synthesis; critical for cell viability and proliferation (Allan *et al.* 1999).
Selenoprotein P	Found in plasma and protects endothelial cells again damage prom peroxynitrite (Allan *et al.* 1999).
Selenoprotein W	Needed for muscle function (Sunde 1997).

Optimal Se status is therefore important to maintain optimal levels of activity of the different selenoproteins and in order to reduce the further oxidative damage to biomolecules such as lipids, lipoproteins, and DNA. As highlighted by Diplock (1994), damage to these structures increases the risk of conditions such as cancer and atherosclerosis amongst others.

2.6.2. Sources of Se

Sea-foods and organ meats are the richest food sources of Se (Sunde *et al.* 2012, pp. 225-37). Other sources include muscle meats, cereals and other grains, and dairy products. However, Se content of food is largely dependent on the soil where the plants are grown and the animals are reared. As a result, Se content of food varies widely between regions throughout the world. Unsurprisingly, estimates of adult daily dietary Se intakes vary in different parts of the world, with the most extreme values of deficiency and toxicity been reported in the People's Republic of China (7 and 38000ug respectively). As a result of this high variability, severe deficiencies are not uncommon (Patrick 1999).

2.6.3. Effects of low Se intake on health

As alluded to previously, adequate intakes of Se are necessary to maintain health in both humans and animals. The most common clinical symptoms of poor selenium intake include nail abnormalities, skin and hair depigmentation, skeletal muscle myopathies and a cardiomyopathy which has been shown to be both fatal in humans and animals (Cohen *et al.* 1988). The negative effects of a diet low in Se was first established back in the late 70's

where it was shown that children living in areas that were regarded as Se – deficient were more likely to suffer from a Keshans disease than those who did not, indicating that Keshan disease develops partly as a result of Se deficiency (Fairweather – Tait et al 2011) Se supplementation was successful as a preventative measure against the disease (Yang 2006). Furthermore, Kashin-Beck disease, an endemic disease of cartilage that occurs in preadolescence or adolescence, has been reported in some of the low-Se areas of Asia (Yang 2006). However, unlike the Keshan disease there has been no demonstration that increased Se intake can prevent Kashin – Beck disease from manifesting, so involvement of Se deficiency in its pathogenesis remains uncertain. Se status has also been associated with some neurological disorders and ageing. Se has been shown to preserve the progressive failure of the immune system and the accumulation of peroxides and free radicals features associated with human ageing (Neve 1989; Pryor 1987). Tolenen et *al.* (1989) found that a combination of both Se and other antioxidants had beneficial effects on general health status in an elderly population.

2.6.4. Selenium in Exercise Recovery

Another role of Se that has come under recent debate is its involvement in exercise recovery. During the initial repair of muscle damage, an acute inflammatory process takes place. The immune system, especially the neutrophils are believed to use FR to establish bad or damaged tissue for removal from the body (Butterfield *et al.* 2006). In cases where severe muscle damage occurs, this leads to infiltration of these neutrophils into the tissue, the same occurs with macrophages. Both of these varieties of white blood cells have the ability to overkill in order to get all of the damaged tissue. However, due to the duplicitous roles of neutrophils and macrophages (Butterfield *et al.* 2006), it is most likely, during this process, that healthy tissue is in fact damaged as a result. There is a lack of evidence examining the relationship between the delayed onset of muscle damage and response to antioxidant defence system. Therefore, it has been hypothesized that control of post – exercise FR production could end up being a counterproductive measure. By removing the FR that neutrophils have established to mark tissue, the body may in fact be delaying the exercise recovery process (Tiidus 1998). Removal of the oxidative markers has the potential to decrease the amount of damaged tissue target by the immune system. Endogenous

regulation explored in further detail in section 2.8, of the reduced glutathione system could play an important role in maintaining the balance of oxidative by-products to antioxidants,. Several studies have demonstrated that glutathione reductase levels are elevated in skeletal muscle and erythrocytes following 10weeks of chronic exercise (Leeuwenburgh *et al.* 1994). NADPH, using GR as a catalyst converts oxidised glutathione back to its reduced form. The endogenous increase in the reduced glutathione antioxidant defence system associated with chronic training is likely to protect against oxidative stress within the body. Ortenblad *et al. (*1997) demonstrated how GPx3 activity increases with this type of exercise. They used a short – term maximal exercise protocol. Free radical muscle damage was determined after a continuous 30-second maximum jump test in both a trained (volleyball) and untrained population. The resting levels of GR were significantly higher in the trained groups, furthermore, CK, an indicator of muscle damage was significantly higher in the untrained group post exercise ($p<.05$). The research supported the idea that the GR defence system is up-regulated and has a greater ability to protect against oxidative stress, in this case lipid peroxidation in trained individuals. Therefore, it has been suggested that Se may play an indirect, but significant role in exercise recovery. However, strong research addressing this possibility does not currently exist. This current study has the potential to address some of these issues relating to exercise recovery.

2.7. Exercise and Oxidative Stress

The benefits of exercise have been well established, these include physiological (Pollock *et al.* 2000); psychological (MacMahon 1990) and social benefits (Elward & Larson 1992). Physical inactivity is widely accepted as both a primary and secondary risk factor for the development of chronic diseases including cardiovascular disease, obesity and diabetes amongst others (Nimmo *et al.* 2013)

There is a strong body of evidence to suggest that physical exercise, in particular strenuous activity, may be associated with an increased generation of ROS/FR due to the dramatic increase in oxygen uptake (Reid 2001). Exercise is associated with an increase in oxygen uptake by the whole body and particularly skeletal muscle, due to the increase in metabolic activity within the working muscles. This increase in metabolic processes is also associated with an increased production of ROS and FR (Ji 1999). Most of the oxygen consumed by the

body is utilized in the mitochondria for substrate metabolism and Adenosine-5'-triphosphate (ATP) production and 2-5% of this forms FR (Sohal and Weindruch 1996). Therefore, as oxidative phosphorylation increases in response to exercise, there is a simultaneous increase in FR/ROS. Unless oxygen homeostasis is maintained, through oxidative-reduction reactions, the cellular environment shifts from a reduced state to a more oxidised state. The initial rise in ROS during exercise, can lead to an additional secondary generation of proxidants via phagocytic respiratory burst, a loss of calcium homeostasis and/or the destruction of iron containing proteins (Fisher -Wellman & Bloomer 2009).

The first suggestion that physical exercise results in ROS/FR mediated damage to tissues appeared in 1982 and the past three decades have resulted in a large growth of both knowledge and further research regarding exercise and oxidative stress. Davies *et al.* (1982) used electron paramagnetic resonance spectroscopy to measure carbon-based FR in rabbit muscle both before and after exhaustive exercise. They found that there was a 2 to 3 fold increase in FR concentrations in the muscle following exercise to exhaustion. Some three years later, Jackson *et al*. (1985) subsequently extended this observation in humans by adopting the same technique to highlight the increased production of ROS/FR in limbs following exercise. These findings demonstrated that exercise, paying particular attention to exhaustive exercise, increased the biochemical indices of oxidative stress when measured in the working muscle. Following these initial observations, there have been an abundance of studies investigating the relationship between exercise and oxidative stress as measured by lipids, proteins and even genetic material (Hartmann *et al.* 1994). Hartmann and his colleagues studied peripheral blood cells from 3 volunteers following an exhaustive test on a treadmill. The results showed an increase in DNA Damage as illustrated by the Comet Assay in all subjects.

However, there are still conflicting results in the literature regarding oxidative stress response post exercise. Sacheck *et al.* (2003) reported no difference in oxidative stress levels when examining 11 female collegiate rowers. They concluded that there was no difference in performance capacity or oxidative stress markers following a 45minute sub - maximal run. Therefore, results from these studies have been conflicting and inconsistent. Sacheck *et al. (*2003) utilized collegiate rowers who trained regularly. It has been

hypothesized that athletes often consume low fat diets and may be more susceptible to oxidative stress produced by exercise due to the low availability of vitamin E. However, the results showed otherwise; their vitamin E intake appeared to protect against oxidative stress produced by moderate intensity exercise. Baseline antioxidant nutritional status, and indeed training level has the potential to produce conflicting results (Schulz et al. 2007) which may have been the case in the above study. Trained individuals likely experience attenuated muscular damage in response to exercise compared to untrained subjects, in turn blunting the oxidative stress response.

Specific ROS production most likely depends on the FITT principle (Frequency, Intensity, Type, Time) as all exercise varies in their respective energy requirements, level of oxygen consumption as well as the presence or amount of mechanical stresses imposed on the tissues (Fisher – Wellman & Bloomer 2009). Therefore, it is likely that exercise mode, intensity, duration as well as the subject population selected all impact on the extent of oxidation (Fisher – Wellman& Bloomer 2009) and each of these parameters will be explored within the forthcoming sections.

2.7.1. Exercise Mode

The majority of the research that assesses the relationship between oxidative stress and exercise has focused on the influence of aerobic exercise, including jogging, cycling and swimming (Ashton *et al.* 1998). Typical protocols have included submaximal or maximal exercise to induce oxidative stress. Performing aerobic exercise requires a level of VO_2 which is sufficient to increase ROS/FR production. However, Laaksonen *et al*. (1996) found that physical fitness as measured by VO_{2max}, was strongly inversely correlated with plasma thiobarbituric acid reactive substances (TBARS) in men with type 1 diabetes, suggesting a protective effect of fitness against the damaging effect of oxidative stress. Fewer studies have investigated the production of ROS as a result of anaerobic exercise protocols such as intermittent running, resistance exercise, and Wingate cycle tests (Bloomer *et al.* 2004). They reported that unlike aerobic exercise, where increased mitochondrial respiration is thought to be the main reason for increased ROS production, that this increase during anaerobic exercise may be meditated a large degree by the activities of certain radical generating enzymes (xanthine and NADPH oxidase), prostanoid metabolism, phagocytic

respiratory burst, disruption of iron containing proteins, as well as altered calcium homeostasis. Despite the lack of information in this respective field it has been shown that acute anaerobic exercise serves as a sufficient stimulus to elicit an increase in ROS/FR production and accumulation (Tauler *et al.* 1999). The increase in ROS/FR during anaerobic exercise may be mediated similarly to aerobic exercise which is via electron leakage along the electron transport chain, or through other mechanisms, such as ischemia reperfusion, or phagocytic respiratory burst (McBride *et al*. 1998).

2.7.2. Intensity

Evidence suggests that exercise of varying intensities increases the production of free radicals and oxidative stress in both animals (Jackson *et al.* 1985; Liu *et al.* 2000) and humans (Marzatico *et al.* 1997; Sacheck *et al.* 2003). During low-intensity protocols, antioxidant defences appear sufficient to meet the ROS production. Lovelin *et al.* (1987) concluded that intensities less than 50% of VO_{2max} did not elicit a damaging oxidative stress response, therefore, implying that during exercise of this low intensity nature, the antioxidant defence system has the ability to match the increased ROS/FR production. However, as the intensity of the exercise increases (>50% VO_{2max}), these defences are no longer adequate, potentially resulting in oxidative damage to surrounding tissues (Knez *et al*. 2007). Ashton *et al.* (1998) demonstrated a strong correlation between aerobic exercise intensity and the extent of oxidative stress. He and his colleagues found that the more intense the bout of exercise is, the greater the production of ROS / FR and subsequent oxidative stress. However, contradicting studies have shown no increase in oxidative stress following high intensity exercise (Nieman *et al.* 2003). The reason for this null finding could potentially be due to the lack of sampling during the actual running protocol itself. This could have impeded investigators ability to detect oxidative stress, as elevations in oxidative stress have been reported during such protocols (Child *et al.* 1998)

2.7.3. Duration

Similar to exercise intensity, exercise duration in relation to oxidative stress response has yielded some contradictory results. During short duration protocols (<2hours), the antioxidant system is capable of coping with the increased accumulation of ROS and as a

result, no oxidative stress response was shown to be present (Chevion *et al.* 2003). However, during longer duration events (>2hours), an exacerbated proxidant production with the potential to overwhelm these defences has been shown (Bloomer *et al.* 2007), which may in turn lead to oxidative damage to surrounding tissues. The majority of long duration exercise induced oxidative stress has been assessed following full, half or ultra - marathons. This increased duration exercise appeared to promote an acute state of oxidative stress detected by increases in several oxidative stress biomarkers including DNA damage (Tsai *et al.* 2001), lipid peroxidation (Palazzetti *et al.* 2004) and protein oxidation (PC) (Tauler *et al.* 2006). However, other studies with similar exercise duration have contradicted these findings. Ozawa (1999) found a decrease in both ROS/FR following long duration events but postulated that the response may be directly dependant on exercise intensity. The participants in this study were working at a relatively moderate intensity. It was suggested that while the duration of some exercise protocols may have been sufficient for the induction of ROS production, the intensity was likely so low that highly trained individuals may have possessed sufficient antioxidant defences to combat such radical production, thus masking any potential accumulation of oxidative stress biomarkers (Margaritis *et al*. 1997).

2.7.4. Subject Population

Furthermore, the subject population selected may have a bearing on oxidative response. Gender is thought to effect oxidative stress levels. Ruiz-Larrea *et al.* (1993) highlighted that the female sex hormone, oestrogen, was shown to exhibit antioxidant properties in vitro. Therefore, as females possess a larger concentration of this hormone compared to the opposite sex, they are believed to be less susceptible to oxidative stress. Oestrogen has not been the only factor involved in gender differences of oxidative stress. Vitamin C, vitamin E and glutathione levels were also reported to be higher in female rats when compared to their male counterparts following an acute exercise bout. Tiidus *et al.* (1999) investigated if, apart from oestrogen, there were any other gender based differences in tissue antioxidants. It was reported that female rats had significantly higher levels of vitamin E in liver and heart tissues when compared with their male counterparts post exercise even when baseline

levels were not significantly different between groups. These results appear to indicate that females may have lower resting levels of oxidative stress relative to males.

However, these results have not however been shown to be 100% transferable to humans. Goldfarb *et al.* (2007) examined the gender differences of exercise induced oxidative stress and the influence of antioxidant supplementation. They reported no difference in the exercise-induced oxidative stress response between men and women following submaximal aerobic exercise which consisted of running for 30mins at 80% VO_{2max}. However, they did report that females had higher resting reduced and total glutathione levels before supplementation. These results do appear to indicate that females may have enhanced anti-oxidant capacities to males. The reason for this could be in part due to their higher expression and activity of antioxidant enzymes (Sastre *et al.* 2002).

2.8. Benefits of Regular Training and Oxidative Stress

Despite the potential of both oxidative damage and stress that occurs during exercise as highlighted in section 2.7, it has been suggested that adaptation to regular exercise may result in an enhanced anti-oxidant defence system. This conflicting situation is often termed the exercise – oxidative stress (EXOS) paradox (Ashton *et al.* 1998). Regular exercise causes adaptation of the antioxidant and repair systems, which could result in a decreased base level of oxidative damage and increased resistance to oxidative stress (Radak *et al.* 2001). Radak and his colleagues examined the effect of regular exercise on cognitive functioning and oxidative stress in the brain of rats. They utilized four-week-old (young) and 14-month-old (middle aged) Wistar rats, both of which were randomly allocated to either an exercise or control group. Exercise groups were exposed to a swimming regime of 1h a day, 5 days a week for 9 weeks. The data showed that swim training improves some cognitive function in rats, with parallel attenuation of the accumulation of oxidatively damaged proteins. This derives from the significant decrease in protein carbonyls following training ($p<.05$). Following this initial study, Radak carried out a larger, follow up study that examined the consequences of regular exercise (swimming and treadmill based) on oxidative stress in the brain and liver of rats (Goto *et al*. 2007). They found that the same biomarker of oxidative stress, protein carbonyl, reduced significantly after 9 weeks of training. This reduction was accompanied by an up regulation of glutathione level and reduced ROS generation. These

findings suggest that moderate regular exercise causes an initial increase in oxidative stress, however up regulation of antioxidant mechanisms, including antioxidative and repair degradation enzymes for damaged molecules may indicate an overall beneficial effect on the oxidative profile of the individual. Regular participation in physical activity has been therefore suggested to trigger a later enhanced oxidative capacity in both animals and humans (Burke & Deakin 2006). There is an abundance of literature that states that antioxidant defences increase in response to regular athletic training, thereby improving the body's ability to defend against increased production of ROS induced during exercise (Levine & Levine, 2013,pp. 321). A number of mechanisms have been proposed to explain this response, and these will be explored within the forthcoming sections.

The up-regulation of endogenously produced antioxidant enzymes is one popular proposed mechanism for this adaptation (Ji & Fu 1992). Regular training has been found to increase the up regulation of antioxidant enzymes in human participants. Some authors have demonstrated an increase in CAT activity following a 20-min HIIT protocol (Fisher et al. 2010); however, others have not (Castro *et al.* 2009). Although both studies used participants with similar characteristics and aged 25±10y, they utilized very different methodological approaches to elicit an oxidative response. Castro *et al.* (2009) opted for a continuous incremental exercise approach of cycling for 20minutes at 60, 70, 80% VO_{2max}. Whereas, Fisher et al., 2010 who elicited not only an increase in CAT activity, but also SOD and GPx3 opted for a higher intensity training protocol which comprised of 4minutes cycling at 15% VO_{2max}, followed by 30s pedalling at 90%. $VO_{2max.}$ Evidence suggests that exercise of varying intensities increases the production of free radicals and oxidative stress in humans (Marzatico *et al*. 1997; Sacheck *et al.* 2003). During low-intensity and sometimes even average intensity protocols, antioxidant defences appear sufficient to meet the ROS production like in cases similar to Castro and colleagues. However, as the intensity of the exercise increases these defences are no longer adequate, potentially resulting in oxidative damage to surrounding tissues (Knez *et al*. 2007). Furthermore, a HIIT exercise design to examine the oxidative response during exercise and recovery from exercise allows the researcher to incorporate both an aerobic and anaerobic exercise component to maximize the oxidative stress response similar to that carried out by Fisher *et al.* (2010).

In contrast, a more consistent training adaption has been demonstrated on GPx1 activity. Powers *et al.* (1994) showed an increase of 45% in GPx1 activity in red gastrocnemius muscles after endurance training in rats. A slightly higher increase of 62% was reported by Leeuwenburgh *et al.* (1997) in response to treadmill training for 2h/d at moderate intensity for 10 weeks. Furthermore, Renacle *et al.* (1992) proposed that is the most important antioxidant enzyme for cell survival because of its higher sensitivity to ROS levels and its greater adaptability to oxidative stress. It is conceivable that an up-regulation of antioxidant enzymes offer greater protection to tissues during exercise. A higher antioxidant enzyme activity was reported to correlate positively with VO_2 max, and trained athletes were shown to have greater SOD and CAT activities in skeletal muscle (Jenkins *et al*. 1993).

Secondly, the increased de novo production of endogenous antioxidant molecules have been suggested as an additional mechanism that assists in the positive antioxidant response associated with regular training (Ji 1999). Kilstrom *et al.* (1990) demonstrated that endurance swim training provides enhanced protection to the heart against oxidative stress. They concluded that this extra capacity to detoxify ROS/FR was mainly due to elevated GSH levels and a more efficient nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) supplying system in the trained heart. GSH is an antioxidant molecule synthesised in almost all living cells from prokaryote organisms to the eukaryote kingdoms (Griffith and Mulcahy 1999). GSH has the ability to protect cells and tissues from oxidation by reducing power of the thiol group on the cysteine portion of the molecule. When GSH is oxidised to glutathione disulphide (GSSG), the enzyme GSH reductase rapidly reduces it back to GSH using NADPH as an electron donor, thus maintaining redox balance (Griffith 1999; Griffith and Mulcahy 1999). It is only when the oxidative load on the cell becomes too high to alleviate, typically associated with a variety of disease states, that a shift in this redox buffer can occur, and can result in oxidative damage to cellular constituents and the especially vulnerable plasma membrane (Kennett and Kuchel 2006). Furthermore, red blood cells (RBC) have been shown to be susceptible to oxidation due to its role as the main oxygen transporting cell. GSH is the main source of RBC protection against oxidative damage (Kennett and Kuchel 2006). In addition, RBC GSH has been shown to be the largest antioxidant pool, detoxifier and chemokine scavenger in the body (Keenoy & Vertommen 2001)

2.9. Regular Training and Oxidative Stress

The majority of the research examining oxidative stress response has focused on the well – established traditional means of exercise known as Endurance Training (ET). Oztasan *et al*. (2004) investigated whether ET had the potential to reduce exercise induced oxidative stress in rats. The ET program consisted of treadmill running for 1.5h 5days/week. Results suggested that MDA, a biomarker of oxidative stress, increased in the sedentary group, but not the trained group. Overall, they concluded that ET may be useful to prevent exercise induced oxidative stress following exhaustive exercise by up-regulating antioxidant enzymes and that this may have implications in exercising humans. Further studies carried out by Fatouros *et al*. (2004) supported this when they evaluated the oxidative stress marker response and antioxidant status in 19 older men aged 65-79y. Their ET program consisted of 3 sessions a week for 16 weeks of either walking or jogging at 50-80% $Vo_{2}max$. Results mimicked those presented in Oztasan *et al*. (2004); ET caused MDA to decrease by 16% post exercise in the training group. Furthermore, total antioxidant capacity (TAC) and GPx3 activity increased by 14% and 12% respectively. Additionally, the trained group increased their running time by 40% and VO_{2max} by 20% following the 16week intervention.

2.10. High Intensity Interval Training (HIIT)

High Intensity Interval Training (HIIT) has been increasingly adopted as a form of training amongst varying populations due to research that suggests that this method of training may lead to physiological and performance gains in both untrained and trained individuals with less training time when compared with ET (Laursen and Jenkins 2002). With current literature suggesting that —"lack of time" remains the most commonly cited barrier to regular exercise participation, a growing body of evidence suggests that HIIT induces numerous physiological adaptations that are similar to traditional ET, with a noticeable decreased time commitment (Gibala & McGee, 2008). HIIT consists of repeated sessions of brief intermittent bursts of vigorous exercise performed at intensities >90% of VO_{2max}, interspersed by periods of rest or low intensity activity (Smith *et al.* 2009). HIIT has been shown to be an effective alternative to conventional ET as it has the ability to produce both similar and in some cases enhanced changes in physiological, performance and health-related markers in both healthy individuals and individuals with a variety of pathologies.

(Burgomaster *et al.* 2008; Gibala *et al.* 2006). Gibala *et al.* (2006) examined both molecular and cellular adaptations in resting human skeletal muscle following six sessions of either HIIT or traditional ET over 2 weeks. As predicted both training time commitment and exercise volume was lower in the HIIT group. Additionally, skeletal muscle oxidative capacity and exercise performance was similar between groups. Practically, this meant that the two very diverse forms of training induced remarkably similar changes in exercise capacity and selected muscle adaptations related to exercise tolerance. Therefore, given the lower training volume in the HIIT group, the results suggested that intense interval training is a good time – efficient strategy to induce rapid muscle and performance adaptations comparable to traditional endurance training. Laursen and Jenkins (2002) compiled a literature review discussing a range of studies which compared the effects of ET and/or HIIT training in both sedentary and trained subjects. They found that HIIT has the ability to improve a range of physiological parameters including VO_2 peak, peak anaerobic power output and time to fatigue to a greater extent than continuous ET in the majority of the 180 studies. One such study included was carried out by Billat and associates in 13 untrained individuals. The found that HIIT (5×4 minutes at 100% VO_{2max} with 2minutes rest) enhanced oxidative capacity (succinate dehydrogenase and cytochrome oxidase) of type 2 fibres when compared with continuous exercise who worked for a similar duration at a mean intensity of 79%. $VO_{2max.}$ They concluded that this is largely due to an up-regulated contribution of anaerobic and aerobic metabolism. Furthermore, the maximal enzyme activities of citrate synthase activity (CS), hexokinase (HK), phosphofructokinase (PFK), succinate dehydrogenase and malate dehydrogenase also significantly increased following HIIT training. Thus, in contrast to submaximal endurance training, that had little or no effect on glycolyticenzyme activity.

HIIT results in diminished stores of ATP, which has been implicated as a cause of muscle fatigue (Allen *et al.* 2008). However, regular HIIT training allows the body to adapt to overcome this depletion through enhancing the ability of the muscle to re – synthesize ATP. This improves the working muscles energy status during the anaerobic, highly-intense intervals. In addition, during the recovery phase of HIIT, aerobic metabolism is important in phosphocreatine re - synthesis and lactic acid removal. Consequently, HIIT functions to improve the capacity for aerobic metabolism (Talanian *et al.* 2006).

2.10.1. HIIT and Oxidative Stress

Few studies have examined the oxidative response to a single bout of HIIT. Fisher (2009) examined the immune and oxidative stress responses following a HIIT program. Eight recreationally active males completed identical HIIT protocols. The HIIT protocol consisted of 3sessions/week for 1 week on an electrically braked cycle ergometer. It involved 4mins of cycling at 15% of $VO_{2max,}$ followed by 30s of pedalling at 90% VO_{2max}. This was repeated four times. Blood samples were obtained pre exercise, immediately post, 3h post and 24h post exercise. Significant increases in SOD, CAT or GPx1 activity were observed in lymphocytes following HIIT. However, a significant increase in MDA was found following the strenuous exercise bout immediately post exercise on days 1 and 2. More recently, Gabriel *et al.* (2012) compared the effects of HIIT and 30min of brisk walking on postprandial triaclyglerol (TAG), soluble adhesion molecules and markers of oxidative stress. Gabriel and his colleagues recruited 9 healthy male volunteers, all participants were regularly physically active but none were specifically trained, similar to the subjects examined by Fisher. They reported that protein carbonyl levels increased ($p<0.05$) in both walking and control groups, however, HIIT prevented this effect with no change in protein carbonyl levels reported. TBARS significantly increased in all three trials when compared with baseline; again, HIIT reduced the magnitude of this effect.

Even less researched is the effect of a HIIT training program on oxidative stress. Very recently a study by Ugras *et al. (*2012) examined the effect of HIIT on elite athletes' antioxidant status and on certain biomarkers of oxidative stress. The elite athletes used in this study were 21 Muay Thai boxers with regular exercise and training habits. Muay Thai boxing is said to be intermittent in nature. The results of this study suggested that HIIT and competition could affect the oxidative status of these athletes. The study reported a significant increase in MDA and a significant decrease in CAT post HIIT training ($p<.05$). However, it was found that the differences in GPx3 and SOD activities were not statistically significant. Ugras concluded that further studies were needed to identify if similar adaptations manifest after a couple weeks of a different type of interval training.

As previously discussed, there is strong evidence to support an increase in oxidative stress following both aerobic and anaerobic exercise (Bloomer *et al.* 2005; Magalhaes *et al.* 2007).

It has also been suggested that it may be that the magnitude of oxidative stress is dependent on the energy balance within the cell. Therefore, it is difficult to determine the specific sites of ROS/FR production during different duration and intensities of exercise (Fisher 2010). As a result, utilizing a HIIT exercise design to examine the oxidative response during exercise and recovery from exercise allows the researcher to incorporate both an aerobic and anaerobic exercise component to maximize the oxidative stress response. HIIT provides a stimulus that changes whole body and cellular function quite rapidly, providing a model that allows relationship between antioxidant response, oxidative stress and exercise to be examined.

2.11. Research Aim

The aim of this research is to evaluate if HIIT and / or Se supplementation will impact on exercise – induced oxidative stress and alter antioxidant status in physically active females aged 18-30y.

2.12. Research Objectives

- To examine the extent of oxidative stress caused by, and the antioxidant acute response to, a single bout of HIIT in physically active females.

- To provide a 3 week HIIT and / or Se supplementation intervention to each participant.

- To repeat exercise tests following the intervention period to detect any changes in indices of oxidative damage and antioxidant capacity.

- To examine the change in fitness levels following both interventions.

Chapter Three: Method

Methods.

3.1. Study Design

A randomised open label trial with a convenience sample and an intervention period of three weeks was carried out at Robert Gordon University (RGU) between January and April 2013. Physically active females aged 18 – 30 years, who participate in intermittent sports within RGU, were recruited and screened. They were then allocated to one of two groups, i.e. Se Only or Se + HIIT. Their baseline fitness levels were then assessed. Blood samples were obtained and used to measure markers of oxidative stress both before and after a

single bout of HIIT (see section 3.5.3.). The intervention period was then imposed and lasted 3 weeks in duration following which, both fitness levels and exercise induced oxidant response pre and post HIIT bout were reassessed. A coding system e.g. OS11 – Subject 1 pre bout, OS12 – Subject 1 post bout, OS13 pre bout post intervention and OS14 post bout post intervention etc. was utilized throughout.

3.2. Ethical Approval

Ethical Approval for the completion of this study was granted by the Chair of the School Research Ethics Committee, School of Pharmacy and Life Sciences at Robert Gordon University. Participants were asked to sign an institutionally approved informed consent document (Appendix A1) and provided with a participant information sheet (Appendix A2) prior to any testing processes.

3.3. Subject Recruitment

Team captains and managers of the various intermittent sports teams within RGU were contacted initially via face to face meetings and the research study was briefly outlined to them. After obtaining permission, players were contacted via email (Appendix A3), which contained a brief introduction to the research study. The participant information sheet was also attached with further details on purpose, time commitments and potential implications of the study. Players were asked to reply to the email if they were interested in taking part. An advertisement (Appendix A4) was also put in RGU's bulletin to further recruit for the study with contact details given, for those who were willing to participate.

3.4. Sample Population

A total of 21 physically active females aged 18 – 30y (Age: 21.7±5.7y, Height: 168.6±35.9cm, Body Mass: 65.7±16.5kg) who participate in intermittent sports within RGU were recruited. Intermittent sports can be defined as sports in which periods of exercise are characterized by fluctuations in exercise intensity over a given time frame (MacLaron & Morton 2012). Sports involving intermittent exercise are primarily composed of team sports including volleyball, football and basketball amongst others. A sample size of 22 subjects represents a

similar amount of participants as were utilized in previous research on both HIIT (Ingham *et al.* 2008) and nutritional intervention studies (Savory *et al.* 2012). Each student athlete volunteering in the study was required to complete a Physical Activity Readiness Questionnaire (PARQ) along with a General Practice Physical Activity Questionnaire (GPPAQ) (See Appendix A5 and A6) to determine their eligibility to actively participate in the study. The PARQ has been designed as a screening tool to identify people for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them. Furthermore, the GPPAQ was commissioned by the Department of Health and developed by the London School of Hygiene and Tropical Medicine as a validated short measure of physical activity screening tool for human's aged 16 – 74 y. The GPPAQ generates a simple, 4-level Physical Activity Index (PAI) which categorizes subjects as: Active, Moderately Active, Moderately Inactive, and Inactive. Furthermore, those who were already taking nutritional supplements and / or had signed up to a new training program in the last month were omitted from the study.

3.4.1. Inclusion Criteria

- Healthy, injury free females determined by the PARQ. Subjects were assumed healthy and injury free with a low risk of having any medical complications from exercise if they answered no to all PAR-Q questions.
- Age 18 – 30 years. These subjects were more likely to be fit and healthy and capable of completing the HIIT intervention which involved exercising at very high intensities. Recent studies examining the effect of HIIT have used a similar age range (Gabriel *et al.* 2012).
- Participants must be physically active. Those that attained either an active or moderately active index determined by a GPPAQ during the screening phase were included in the study.
- Engagement in intermittent sports at RGU.

3.4.2. Exclusion Criteria

- Those who have answered yes to any of the questions outlined by the PAR-Q and had not received medical clearance from a doctor to participate in exercise.
- Aged <18 or >30 years.
- Contraindication of indices specified by the GPPAQ, namely those who reside in either the moderately inactive or inactive index.
- Those who are already taking nutritional supplements and / or have signed up to a new training program in the last month. Both of these factors have the potential to mask true results. This was assessed during initial subject screening.

3.5. Procedures and Assessments

See figure 3.1 for visual outline of protocol. Participants were randomly assigned to the Selenium Only Group or the Selenium + HIIT Group. Subjects were then scheduled for an initial visit to RGU: Sport where their baseline fitness levels were assessed (see section 3.5.1.). Participants were asked to keep a food diary during the week of preliminary screening in order to obtain a nutrient intake profile for each individual (see section 3.5.2.). On a subsequent visit to the School of Health Sciences, subjects took part in a single bout of HIIT on a cycle ergometer (see section 3.5.3.). Blood samples were taken before and after this exercise bout (see section 3.5.4.). Following these preliminary tests, the interventions commenced, Selenium Only Group (see section 3.5.5.) or the Selenium + HIIT Group (see section 3.5.6.). Each intervention lasted 3 weeks in duration during which a second nutrient profile was submitted. After the subjects had successfully completed the assigned intervention of three weeks, both series of tests were repeated. Following re tests, the subjects were asked to answer a questionnaire (Appendix A7) relating to their experience, both positive and negative, to the selenium supplementation.

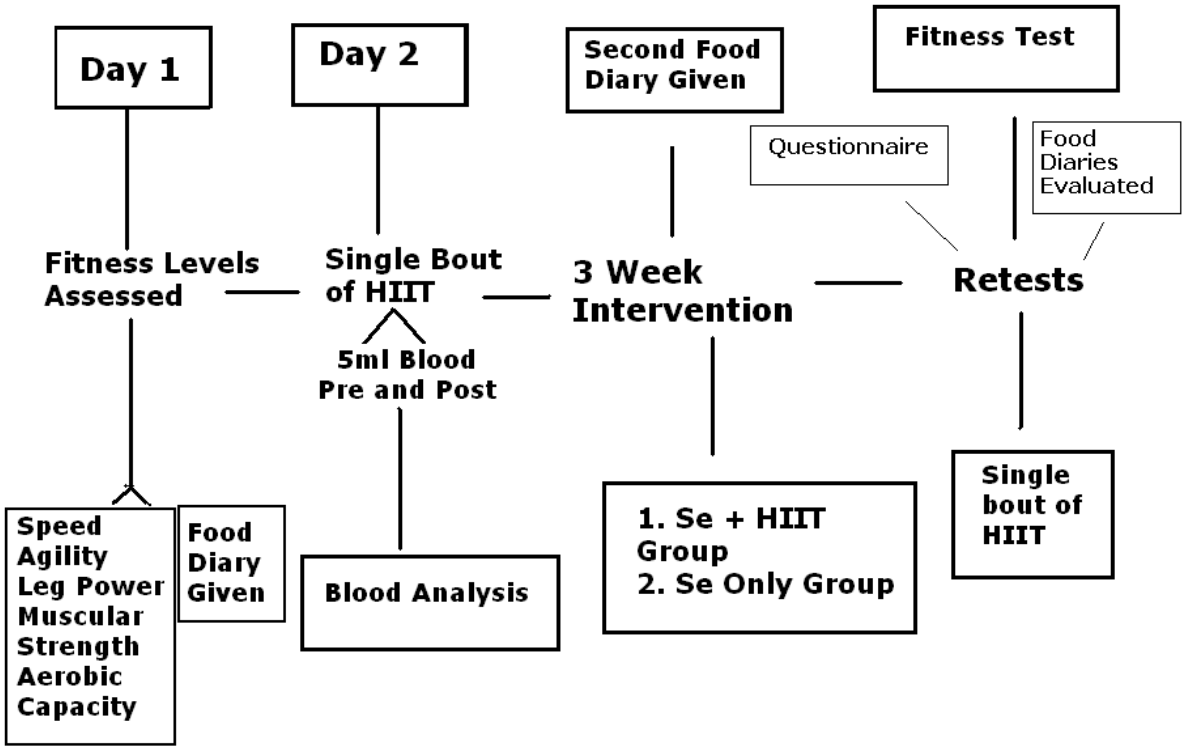


Figure 3.1.: Visual Outline of Protocol

3.5.1. Batteries of Fitness

Prior to all fitness tests, stretched stature was measured to the nearest 0.5cm using a portable stadiometer (Seca, Leicester Height Measure). Body Mass was measured to the nearest 0.1kg in minimal clothing and using a portable digital scales (Seca, Germany), minimal clothing was required when weighing. The first part of the fitness testing session involved a standardized warm up consisting of basic jogging to increase body temperature and a series of dynamic stretches including high knees, heel flicks, walking lunges, groin rotations and leg swings to reduce muscle stiffness. All fitness tests were then executed in the same orders both pre and post intervention. The following tests and rest intervals were included in the batteries of fitness (Appendix A8 for individual test protocols).

3.5.1.1. Speed – 10 and 20m Sprints. Subject performed 20m sprints and both 20m time and 10m split time was recorded. Times were recorded using timing gates located at the starting line and both distances. Each subject was allocated to a group of three. The subject was given a rest between reps while the other two members of their group were performing the test. Each subject had 3 trials and the lowest time was recorded.

3.5.1.2. Explosive Leg Power – Countermovement Jump (CMJ). To determine maximal jump height, the countermovement jump was used on a Smartspeed ™ jump mat. The peak height of the jump will be recorded in cm. Each subject was allocated to a group of 3 and was giving a resting period whilst other members of the group were performing the test. Each subject had 3 trials and the highest jump was recorded.

3.5.1.3. Muscular Strength and Endurance –Sit ups. Subjects were asked to complete as many sit ups as possible in one minute or until exhaustion, whichever occurred first.

3.5.1.4. Agility – T-Test. This test which consists of running laterally and backwards was performed at maximal effort 3 times. Each subject was allocated to a group of 3. The subject was given a rest between reps while the other 2 members of their group were performing the test. Data were recorded in seconds to the nearest hundredth of a second. Each subject had 3 trials and the lowest time was recorded.

3.5.1.5. Aerobic Capacity (VO_{2max}) – Level 1 Yo - Yo Intermittent Recovery Test. The Yo – Yo intermittent test is a variation of the regular beep test. There is an active recovery period (5 seconds respectively) interjected between every 40m. The running speed increases by 0.5km/hr every minute, representing a completed stage. Participants were asked to give maximal effort and go until exhaustion. The test terminates when they either voluntarily withdrew, or if they were unable to maintain the required pace. Not being able to maintain the required pace is defined as being unable to reach the correct line on two successive occasions when the beep sounded. The level at which the subjects dropped out or were deemed to have missed two consecutive lines was recorded and used to estimate of VO_{2max} using a conversion table. The Yo – Yo test was designed specifically for assessing maximal fitness in intermittent athletes. The Yo-Yo level 1 test focuses on the capacity to carry out intermittent exercise leading to a maximal activation of the aerobic system (Bangsbo *et al.* 2008)

3.5.2. Nutritional Analysis

Participants were asked to maintain a detailed food diary during the week of preliminary baseline testing in order to obtain a nutrient intake profile for each individual (Appendix A9). They were asked to record food intake over two week days and 1 weekend day. Food

diaries have been established as a good tool to estimate the intake of individual antioxidant nutrients. Pears *et al.* (2012) concluded that food diaries were not only an accurate estimate of dietary intake but also a reliable way to assess dietary behaviour change. Precise instructions were provided to each participant regarding the details to be recorded in this diary. Subjects were asked to record their food intake for a second time in the final week of their assigned intervention. The completed food diaries were analysed using a validated dietary analysis package (WinDiets Software). Food portion sizes were estimated using standard UK references. The purpose of the food diaries was to identify any major alterations in diet during the course of the study that could potentially impact on the results. However, this same paper (Pears et al., 2012) also noted the limitations for Se estimation from food diaries, as the Se content of food varies depending on the selenium content of the soil where plants are grown or where animals consume forage crops. The problem is similar food items can have 10-fold differences in selenium content, making dietary assessment of selenium intake from foods unreliable, therefore limiting the interpretation of baseline and post intervention Se levels.

3.5.3. Single bout of HIIT

All participants performed a single bout of HIIT both pre and post intervention. This consisted of a Wingate Anaerobic Cycle Test (WANT) (Inbar *et al*. 1974). A Wingate test is a supra - maximal exercise test that involves pedalling a cycle ergometer for 30 seconds at maximal speed against a high resistive force. This high force is determined by a subject's body weight (Bar – Or 1994). The issue of optimal force in the Wingate anaerobic test is not fully resolved. Inbar and his colleagues (1974) recommended a load of .075 × kg body mass when the Monark ergometer was used when testing sedentary adolescent males. However, this load was set for a 1 × 30 second Wingate test. A 4 × 30 second Wingate test with a minute of rest given between each trial was used in this study in order to measure anaerobic power and capacity, as well as recovery capabilities. This is a protocol commonly used by the United States Olympic Training Centre (USOTC) especially when working with elite off road endurance cyclers (Garrett & Kirkendall 2000). Therefore, a lower resistive force of .055 × kg body mass was set as it could be maintained over the four repetitions. The Windows based Monarch software that was connected to the cycle ergometer was used

when administering this test. This allowed the weight basket to be preloaded and applied immediately via a quick release lever controlled by the subject. Subjects were instructed to release the lever when they felt they had reached maximum rpm. This computer software also tracks and reports power, peak power, average power, minimum power, power drop, time and rpm's. Table 3.2 outlines the protocol and exercise conditions that were administered on the day of testing.

Table 3.2: Single Bout of HIIT Protocol

5ml of blood taken (2.5.6)

Period	Time Length	Activity
1. Warm Up	1 minute	Cycling at minimal resistance (1kg) whilst maintaining 60rpm.

2.	**Acceleration Phase**	Based on the individual	Subject built up speed, aiming for maximum rpm before starting the test themselves. The test was started when the subject presses the button on the handle of the ergometer dropping the preloaded weight.
3.	**Modified Wingate Test**	30 seconds	Continuous cycling at maximum rpm against a resistance equal to 5.5% of body mass.
4.	**Recovery**	1 minute	Rest– consisted of continuous pedalling against zero resistance

Steps 2 – 4 were repeated for an additional 3 repetitions

5.	**Cool Down**	2minutes	Cycling at minimal resistance (1kg)

5ml of blood taken after an additional 5minutes (2.5.6)

These 5 minutes were spent static stretching the leg muscles including the hamstrings, quadriceps and calves. The subject was also advised to drink water in order to re-hydrate. Preparation for the second blood draw also took place during this time.

3.5.4. Blood Sampling

Participants were asked have a light breakfast at least 2hours before bloods were taken. They were also asked not to consume any alcohol or caffeine and not to undertake any strenuous exercise 12 hours before the procedure. Blood samples were taken by a trained phlebotomist. A venous butterfly needle (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, 23G x ¾) was inserted into a superficial vein in the antecubital region of the left arm before exercise and the right arm following exercise. Blood samples were taken both pre and 5minutes post exercise, 10ml (5 + 5) of blood was drawn in total. Samples were processed to separate plasma and red blood cells within 4 hours of blood draw. Samples were aliquoted and stored anonymously in freezers for further analysis. The blood samples were processed in St. Andrew Street A17 and were waste disposed by following clinical waste disposal procedures.

3.5.5. Selenium Intervention

Participants were provided with and asked to take a tablet of Sodium selenite (250 ug) every day at the same time preferably. Tablets were sourced from Twinlab®, a trusted leader for innovative, high performance health and wellness products. Tablets are commercially available from shops such as Boots or Holland and Barrett. In order to monitor adherence to this particular intervention, tablets were counted by the principal investigator prior to dispatch. A total of 21 tablets were given to each subject. At the end of the intervention, subjects were asked how many tablets were left over. Those who answered zero were presumed to have adhered to the intervention.

3.5.6. Selenium + HIIT Intervention

The combination intervention involved the subjects ingesting a tablet of Se daily and attending the allocated exercise sessions for three weeks. The 3 week HIIT intervention took place indoors at RGU: Sport. There were three pre booked sessions per week; subjects were required to attend at least two sessions each week. The first part of the session involved a standardized warm up consisting of basic jogging to increase body temperature and a series of dynamic stretches including high knees, heel flicks, walking lunges, groin rotations and leg swings to reduce muscle stiffness. This was followed by the Sprint8 protocol (Appendix A10 for further details). The Sprint8 protocol is a workout originally designed by the ACSM (American College of Sports Medicine). It is a commonly used approach to interval training due to the ease at which it can be administered. This uses the 30s:90s series of sprint to rest ratio. The Sprint8 protocol involves 8 interval peaks in a time frame less than twenty minutes. The second part of the HIIT training involved replicating the Sprint8 protocol on a spin bike against a pre- determined standardised resistance that was set for every participant by the principal investigator. This resistance was determined via pilot testing by both the principal investigator and members of the supervisory team. A high resistance which would markedly stress the individual but which could be maintained for 8 reps of 30 seconds was set, following pilot testing to determine a suitable resistance. The time on: time off ratio was set to thirty seconds of all out pedalling, followed by a minute and a half rest, identical to the running procedure. Following completion, a 10 minute cool down was performed consisting of 5 minutes pedalling at a low resistance to decrease body temperature followed by a series of static stretches including hamstring, quadriceps, calves, adductor muscles to relax and realign muscle fibres (Mechelen et al., 1993).

During week two of the intervention, recovery time was decreased from 90seconds to 60seconds. During week three, there was an additional ten seconds added to the sprint time. As a result, participants were sprinting for 40 seconds instead of the original 30 whilst maintaining the decreased recovery time set the previous week. The reason for doing this is due to the principle of progressive overload. Progressive overload is the systematic increase in training frequency, volume, and intensity in various combinations (Baechle & Earle, 2008). It involves gradually increasing the stress / load placed on the musculoskeletal system by increasing the amount of work being performed in response to the continual adaptation of the body.

3.5.7. Questionnaire

Subjects were provided with a subjective questionnaire (Appendix A7) following supplementation. The aim of the questionnaire was to examine the subjects own experiences / thoughts and opinions on selenium supplementation.

3.6. Processing of Blood Samples

3.6.1. Plasma and Red Blood Cell (RBC) Preparation

Blood was centrifuged at 2500g for 12minutes at 4 °C (acc 9 and break 3). Plasma level was marked and then plasma was removed. Plasma was aliquoted in 2 × 0.5ml for assay, and where feasible 1 × 1 for spare. White blood cells were removed and cold phosphate buffered saline (PBS) was added up to marked level. Samples were then mixed and spun again. Above steps were repeated until the supernatant was clear. RBC was reconstituted to the original concentration with cold PBS and aliquoted in 2 × 0.5ml for assay. All samples were stored at -80°c.

3.6.2. Peripheral Blood Mononuclear Cell (PBMC) Isolation

3ml of room temperature (RT) Histopaque was pipetted into 2× 50ml falcon tubes. 2.5ml of blood was layered on top of the histopaque using a plastic Pasteur pipette. Tubes were then centrifuged at 2000rpm (Jouan Centrifuge) for 30 minutes at 20°c. The top layer of plasma was then collected using a plastic pipette and stored in eppendorfs at -80°c. The

buffy coat was collected and transferred into 15ml falcon tubes with a plastic pipette. RT PBS was added to final volume of 15ml. Cells were then mixed. Tubes were centrifuged again at 1200rpm for 10minutes at 20°c. The supernatant was removed and RT PBS was added to final volume of 15ml, and centrifuged one last time at 1200rpm for 10minutes at 20°c. The supernatant was removed a second time and excess liquid was removed using a pipette. The pellet was then mixed with 475 µL of serum and 25 µL of Dimethyl Sulfoxide (DMSO). These samples were then stored at -80°c.

3.6.3. Plasma Glutathione Peroxidase Activity

GPx3 activity in plasma was measured following the method described by Paglia and Valentine (1967). An aliquot of 50 µL of sample was added to 915ul of reaction mix (0.3mM NADPH, 5mM reduced glutathione, 4mM sodium azide, 0.7U/ml glutathione reductase, 0.05 M phosphate buffer pH 7.6 containing 5mM EDTA). The reaction was initiated by the addition of 35 µL of 2.2 mM H_2O_2 and carried out at 25°c. The rate of change of absorbance was followed at 340nm using an ATI Unicam UV/VIS UV4 spectrophotometer and a blank rate measured by substituting distilled water for the samples. For measuring GPx1 activity in red blood cells, it was necessary to produce red blood cell lysates. To produce lysates, 0.1ml of RBC was added to 0.9ml of distilled water, the solution kept cool on ice. The reaction was linear up to a total absorbance / minute change of 0.1. The results were calculated assuming that a unit of glutathione peroxidase is defined as that which oxidizes 1µmole of NADPH/minute and that the molar extinction coefficient of NADPH is 6220.

3.6.4. Thiobarbituric Acid Reactive Substances (TBARS) assay – Malondialdehyde (MDA)

All reagents were prepared according to the OxiSelect™ TBARS assay kit (Item No. STA-330). 2×TBA Diluent TBA was diluted 1:2 with distilled water (6.25ml 2×TBA Diluent TBA + 6.25ml H20). The SDS Lysis Solution was heated at 37°c to redissolve the SDS crystals. The TBA reagent was prepared just before use. 65mg of TBA reagent was added to 12.5ml of the TBA diluent. The pH was adjusted to 3.5 with sodium hydroxide solution. 100µl of unknown samples and MDA standards were added to microcentrifuge tubes. 1µl of antioxidant BHT was added to each sample to prevent further oxidation of lipid during sample processing and the TBA reaction. 100µl of SDS Lysis Solution was added to both the samples and

standards and mixed thoroughly. Both samples and standards were incubated for 5 minutes at RT. 250µl of TBA reagent was then added to each sample and standard to be tested and incubated at 95°c for 45 – 60minutes. Tubes were then removed and cooled to RT in an ice bath for 5minutes. All tubes were then centrifuged at 3000rpm for 15minutes. 200µl of the MDA standards and the samples were transferred to a 96 well plate. Absorbance was read at 532nm using a spectrophotometer.

3.6.5. Total Antioxidant Capacity (TAC)

All reagents were prepared in accordance with the Cayman Chemical Company "Antioxidant Assay Kit" (Item No. 709001). 3ml of Assay buffer was diluted with 27 ml of HPLC-grade water. 600µl of Assay Buffer was then used to reconstitute the vials containing Metmoyglobin. The Chromogen was diluted with 6ml of HPLC-grade water. Trolox was also diluted by adding 1ml of HPLC-grade water. All reagents were vortexed well before use. 10 µl of H_2 O_2 was diluted with 990 µl of water and further diluted by removing 20 µl and reconstitute with 3.98ml of water. All plasma samples were diluted 1:20 with Assay Buffer before assaying. Trolox standard wells were prepared accordingly. 10µl of Trolox standards, 10 µl of Metmyoglobin, and 150 µl of Chromogen were added to designated plate wells. Additionally, 10 µl of the plasma samples, 10 µl of the Metmyoglobin, and 150 µl of Chromogen were assayed in duplicate. Reactions were initiated by adding 40 µl of H_2 O_2 working solution to all the wells as quickly as possible. The plate was then covered and incubated on a shaker for 5minutes at RT. Absorbance was read at 750nm using a spectrometer.

3.7. Reliability

Five subjects of comparable descriptive statistics were recruited from the RGU Ladies Gaelic Football team (Age: 23.2 ± 0.83y, Height: 170.7 ± 3.63cm, Body Mass: 66.36 ± 6.40kg). All participants were contacted via email and text messages to take part in an intra - trial and inter - trial reliability study. This reliability study involved all subjects performing 3 consecutive countermovement jumps on the Smartspeed ™ jump mat, and 3

consecutive 20m sprints. Repeat measures were made within the same data collection session to assess intra trial reliability and within three days of each other to assess inter trial reliability.

3.8. Statistical Analysis

All data was analysed using the Statistical Package for Social Sciences (SPSS, version 17.0, SPSS, Inc., Chicago, IL.). Distribution of data was determined using the Shapiro Wilks test ($n<50$). Any variable which did not meet parametric assumptions was log transformed to normalise data distribution. All group characteristics were reported as means ± standard deviations, unless otherwise stated. Differences between groups were identified using an independent samples t-test. Pre-post within group difference were assessed using a paired sample t-test. The independent variables for this experiment were group (Se and Se + HIIT) and time (pre intervention and post intervention). The dependant variables for this study were speed, agility, muscular strength, explosive leg power, aerobic capacity, max power, min power, power drop, mean power, fatigue index, TBARS, GPx1 and 3 activity and TAC. The probability level for statistical significance was set at $p<.05$.

Both intra trial reliability (repeat measures made within the same data collection session) and inter trial reliability (repeat measures made within three days of each other) measures were taken for the timing gates (10, 20m sprints and Agility T-test) and the Smartspeed ™ jump mat (Explosive leg power). The purpose of this was to comprehensively test the reliability of both sets of equipment. SPSS was also used to analyse reliability using Interclass Correlation Coefficients (ICC). Using variance estimates obtained through ANOVA, a reliability coefficient was calculated. The value for an ICC can range between 0.00 – 1.0.

Chapter Four: Results

Results.

4.1. Descriptive Data

A total of 21 subjects were recruited to participate in the study, with 20 completing all test requirements, representing an attrition rate of 5%. The descriptive and anthropometric data of the subjects are presented in table 4.1. All variables were normally distributed with age being the only exception (p=.032). There were no baseline differences reported

between groups (P>0.05). Mean weight (kg) was reduced by 1.1±0.3kg in the Selenium and HIIT Group after intervention (P<.001). The selenium group subjects maintained mean body mass between trials.

Table 4.1: Subject Demographics

	Selenium + HIIT (n=9)		**Selenium (n=12)**	
Variable	***Pre***	***Post***	***Pre***	***Post***
Age (years)	22.7±4.05	-	22.7±2.9	-
Height (cm)	167.1±6.57	167.5±6.64	168.4±9.29	168.4±9.17
Body Mass (kg)	62.6±7.15	61.5±6.85**	66.1±8.62	66.0±8.83
BMI (kg/m²)	22.2±1.68	22.1±1.68	23.2±2.22	23.2±2.36

Data presented as mean ± SD, **p<.01 between trials.

4.2. Physical Activity Analysis

Physical activity (PA) was assessed using a categorical GPPAQ – 1=Inactive, 2=Moderately Inactive 3=Moderately Active and 4=Active. There were no differences observed in PA in the selenium supplementation only group. There was a slight increase in PA in the SE + HIIT group due to the training attended as part of the study as outlined in Table 4.2. However, this was not significant (p=.081)

Table 4.2: Physical Activity Analysis

	Selenium + HIIT (n=9)		**Selenium (n=12)**	
Variable	***Pre***	***Post***	***Pre***	***Post***
Physical Activity Index (PAI)	3.7±0	4±0	3.58±1	3.58±1

Data presented as mean ± SD, ♦p<.05 between groups **p<.01 between trials.

4.3. Nutritional Intake Analysis

All nutritional variables which were considered most relevant for this study were included in Table 4.3. These were total calories, fats, saturated fat, polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), carbohydrates, proteins and selenium. All nutritional variables were normally distributed. There was a decrease in protein intake in the Se + HIIT group over the course of the intervention (p=.007) as illustrated in Figure 4.1. There were no other differences observed either within or between the groups for any of the other variables shown (p>.05). Values of Se are below the recommended daily allowance (RDA) in both groups both pre and post intervention. The typical RDA for females aged 19-50y equates to ~55ug (Institute of Medicine, Food and Nutrition Board, 2000)

Table 4.3: Nutritional Intake Analysis

	Selenium + HIIT (n=9)		**Selenium (n=12)**	
Variable	***Pre***	***Post***	***Pre***	***Post***
Calories (kcal)	1629±165.5	1655±131.6	1687±201.0	1697±145.9
Fats (g)	58.9±13.6	58.6±14.8	51.6±11.2	50.3±10.1

Saturated Fat (g)	21.2±3.8	21.9±3.6	21.7±3.8	22.3±3.7
PUFA (g)	9.5±1.9	9.4±1.8	10.6±2.0	10.6±1.9
MUFA (g)	17.6±4.1	17.3±3.0	20.0±4.2	20.7±4.1
Carbohydrates (g)	158.8±22.1	155.8±21.6	146.6±16.7	147.7±12.7
Protein (g)	56.0±13.5	53.2±11.9**	54.2±11.0	56.3±12.5
Selenium (ug)	25.4±11.9	24.6±10.9	31.8±7.8	35.6±6.1

Data presented as mean ± SD**p<.01 between trials.

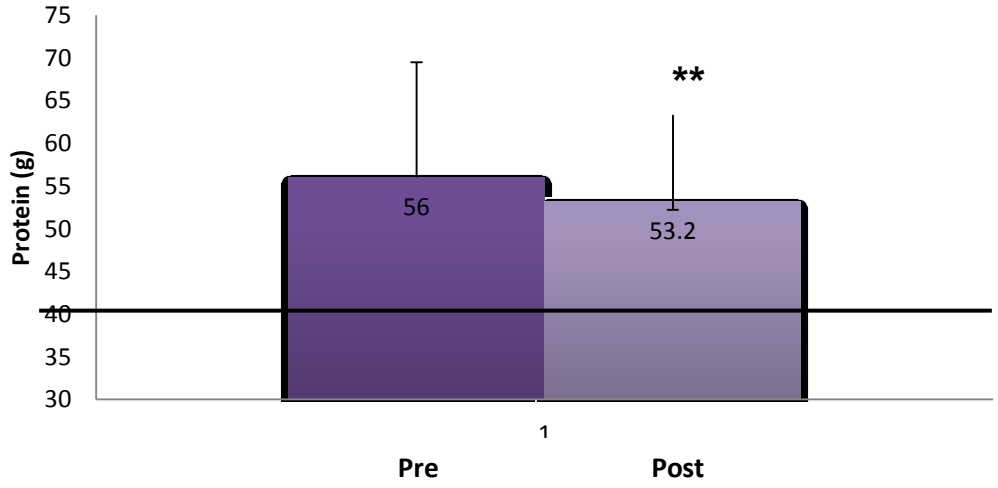


Figure 4.1 Changes in Protein Intake in Se + HIIT Group

Data presented as mean±SD, **p<.01 between trials, ▬ denotes protein RDA for women aged 19 -30y (Food and Nutrition Board, Institute of Medicine, 2005)

4.4. Intra - Rater Reliability

Reliability for the timing gates and Smartspeed ™ Jump Mat were found to have excellent reliability with the lowest ICC reported as .720 (>0.75 (Fleiss, 1986); when the ICC value is high, there is little variation found. Furthermore, the low mean differences between measurements for both intra trial and inter trial reliability demonstrates good agreement between measurements (.018, .084, 1.494 and 2.658 respectively).

4.5 Measures of Oxidative Stress following a Single Bout of HIIT

A summary of oxidative stress response to a single bout of HIIT pre intervention are summarised in Table 4.4. There was a change in mean value pre to post exercise but this was not statistically significant (p>.05).

Table 4.4: Oxidative Response to a Single Bout of HIIT

TBARS ASSAY (µM)		
Group	**Pre Bout**	**Post Bout**
Se + HIIT	1.01±.48	1.23±.42
Se Only	1.29±.42	1.30±51

Data presented as mean±SD

4.6. Blood Analysis

4.6.1. Oxidative Stress Response to Single Bout of HIIT – Post Intervention

A summary of the oxidative stress response is presented in table 4.7 below. MDA as indicated by the TBARS assay was similar at rest and after exercise for both groups. MDA levels were increased in both groups after exercise independent of treatment, although differences were not statistically significant (p=.395 and .904 respectively). After the intervention, the Se + HIIT group demonstrated similar MDA responses; however the magnitude of this effect was reduced, 10% reduction at rest and 2% following exercise. However, after intervention the Se Only group demonstrated a far greater decrease in MDA

at rest (12%) and attenuation of MDA response to exhaustive exercise (16%) as illustrated below However, none of these differences were significant. There was no difference between groups at baseline in any of the variables examined. Figure 4.5 illustrates the percentage changes that occurred in oxidative stress response in both groups pre to post intervention.

Table 4.5: Measures of Oxidative Stress

TBARS ASSAY (µM)				
Pre intervention		**Intervention**	**Post intervention**	
Pre Exercise	**Post Exercise**		**Pre Exercise**	**Post Exercise**
Selenium + HIIT				
1.01±.48	1.23±.42	⟶	1.11±.39	1.21±.47
Selenium Only				
1.29±.42	1.30±.51	⟶	1.13±.67	1.09±.56

Data presented as mean±SD

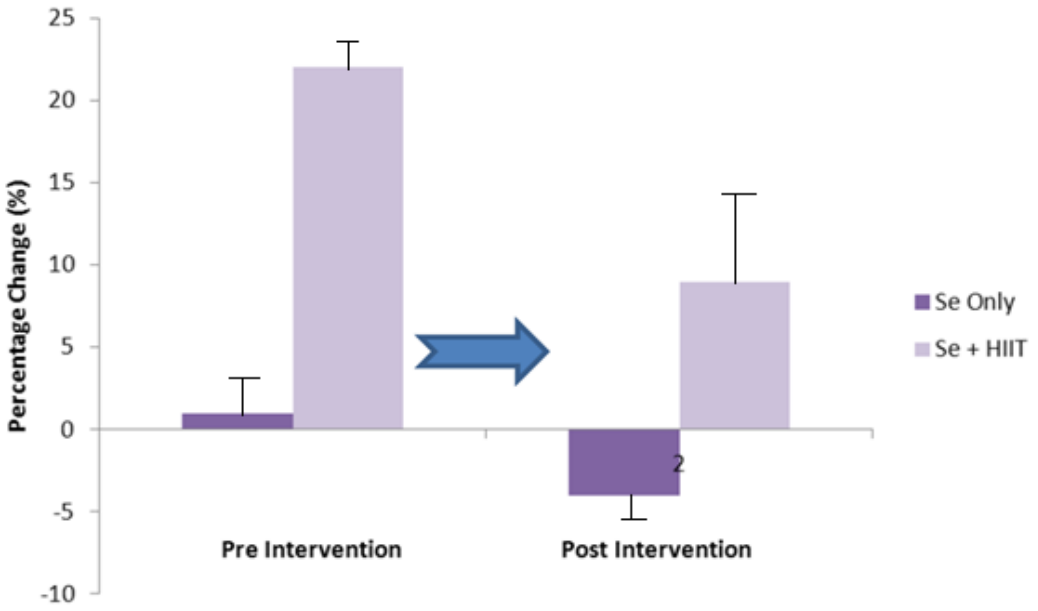


Figure 4.2: The extent of Oxidative Stress caused by a single bout of HIIT both pre and post intervention in both groups. ⟹ denotes intervention period. Data presented as % Change± SD

4.6.2. Antioxidant Status

A summary of the antioxidant status analysis is presented in table 4.6 below. One subject within the Se+ HIIT group did not complete the single bout of HIIT retest, therefore n=8. All variables were found to be normally distributed with the exception of TAC in the Selenium Group Post intervention. Mean GPx3 activity was shown to be higher following supplementation. GPx3Activity in Plasma increased by 15% in the Se only group and decreased in the Se + HIIT group by 2% pre to post intervention. Additionally, Mean GPx1Activity in RBC increased in both groups by 6% and 2% respectively, however none of these increases reached statistical significance. TAC did not differ between groups either at baseline or post intervention. Both supplementation and HIIT training elicited an increase in TAC (40% and 116%), although none of these differences proved significant. Figure 4.4 illustrates the percentage changes that occurred in the antioxidant status in both groups pre to post intervention.

Table 4.6: Measures of Antioxidant Status

	Selenium + HIIT (n=8)		**Selenium (n=12)**	
ANTIOXIDANT STATUS				
Variable	***Pre***	***Post***	***Pre***	***Post***
Total Antioxidant Capacity (mM)	40.02±33.3	56.31±32.0	25.68±17.0	42.74±35.5
Total GPx3Activity in Plasma (U/ml)	0.42±0.23	0.41±0.18	0.39±0.13	0.45±0.16
Total GPx1Activity in RBC (U/ml)	5.14±0.69	5.44±0.75	5.06±0.91	5.14±0.69

Data presented as mean±SD

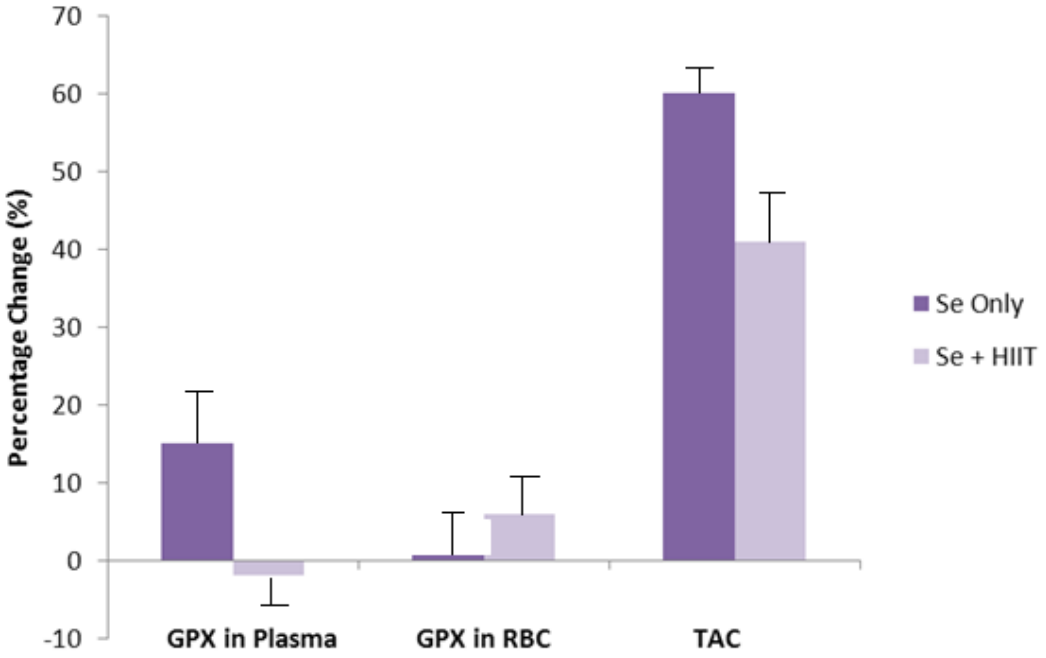


Figure 4.3: Percentage Changes between group pre and post intervention in measures of Antioxidant Status. Data presented as % Change± SD.

4.7. Wingate Analysis

A summary of the Wingate results are presented in Table 4.4. All variables were found to be normally distributed. There was no indication of baseline differences between the two groups. However, differences between trials were reported in the Selenium + HIIT group. In this group, the average power over the first 5 seconds increased by 6% pre to post intervention (p=.025). Furthermore, in this same group, the average over the last 5 seconds increased by 10% pre to post intervention (p=.012). Additionally, there was a 4% significant increase in mean power in the Se Only group after intervention (p=.047). Figure 4.2 illustrates the percentage changes that occurred in the Wingate variables in both groups pre to post intervention.

Table 4.7: Wingate Data Results

	Selenium + HIIT (n=8)		Selenium (n=12)	
Variable	***Pre***	***Post***	***Pre***	***Post***
Maximum Power (W)	369.40±47.75	391.73±49.71*	374.94±57.86	379.40±77.32
Minimum Power (W)	239.38±27.21	262.90±38.91*	274.93±69.38	258.12±62.30
Power Drop (W)	130.01±51.56	128.83±44.11	99.92±37.92	122.11±56.13
Mean Power (W)	319.39±26.93	325.57±36.18	319.53±63.85	332.47±62.31*
Fatigue Index (%)	34.29±10.97	30.58±10.33	31.85±11.61	27.24±11.40

Data presented as mean±SD, *p<.05between trials

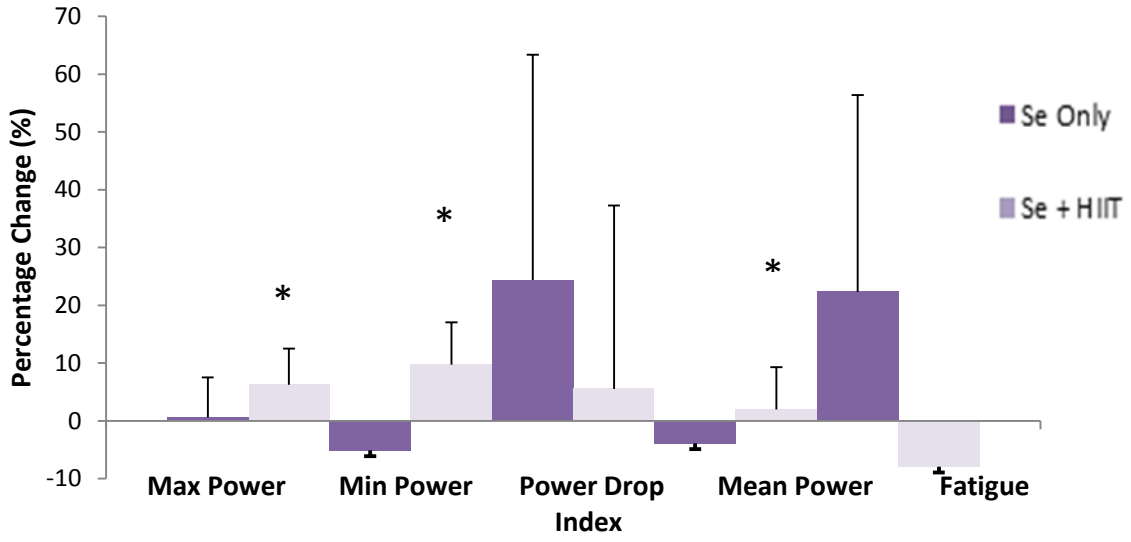


Figure 4.4: Percentage Changes between pre and post intervention for all Wingate Variables in both groups. Data presented as % Change ±SD*p<.05 between trials

4.8. Fitness Test Analysis

A summary of the fitness test values are presented in Table 4.5. All variables were normally distributed with the exception of the 10m sprint both pre and post intervention (p=.017 and .030) in the Selenium only group. These variables were log transformed so to normalise data distribution for further analysis. A number of differences were shown between the groups both at baseline and post assigned intervention. In particular, the Se + HIIT group

had a significantly lower sprint time over 10m (p<.001), and a significantly higher jump height at both pre (p=.046) and post (p=.036) stages. In contrast, 20m sprint times were not significantly different between groups. The Se + HIIT group significantly improved their 10m sprint (p=.027) and VO_{2max} scores (p < .001) following the intervention protocol. No other significant differences within group were identified between trials. Figure 4.3 illustrates the percentage changes that occurred in the Fitness variables in both groups pre to post intervention.

4.8: Fitness Test Results

	Selenium + HIIT (n=9)		**Selenium (n=12)**	
Variable	***Pre***	***Post***	***Pre***	***Post***
10m Sprint(s)	1.61±0.17	1.56±0.20*	1.94±0.15♦♦	1.98±0.14♦♦
20m Sprint(s)	3.43±0.10	3.42±0.12	3.53±0.14	3.50±0.21
T Test (s)	11.49±0.95	11.37±0.78	12.26±0.74	11.83±1.00
Countermovement Jump (cm)	27.66±2.22	28.34±1.83	24.85±3.44♦	25.99±4.05♦
Sit Ups (whole no.)	29 ±3	30±3	27±10	28±10
Vo_{2max}(ml.kg.min-1)	46.16±4.70	50.49±5.44**	46.04±3.53	47.80±4.08

Data presented as mean±SD, ♦p<.05 between groups ♦ ♦ p<.01 between groups *p<.05between trials **p<.01 between trials.

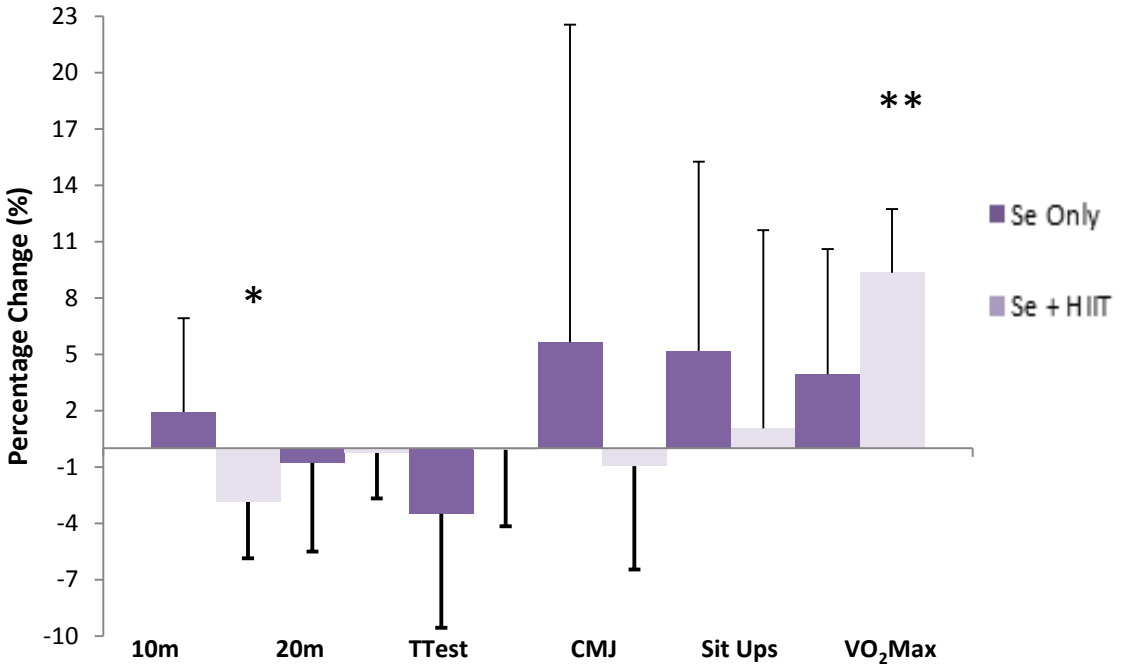


Figure 4.5: Percentage Change for all fitness variables.

Data presented as % Change *p<.05between trials **p<.01 between trials

4.9. Questionnaire

Subjects were provided with a subjective questionnaire (Appendix A7) following supplementation. The aim of the questionnaire was to examine the subjects own experiences / thoughts and opinions on selenium supplementation. Results of the questionnaire are briefly illustrated in figure 4.6. Thirty six percent of subjects (n=8), in their opinion, experienced positive responses to selenium and would consider supplementation again. This thirty six percent of subjects reported less muscle fatigue, decreased recovery time and muscle soreness following exercise with supplementation. Furthermore, 9 percent of this population (n=2) stated that selenium allowed them to "*exercise for longer periods of time*" and that they "*felt less tired during the day following exercise*" whilst supplementing. Fifty percent of subjects (n=10) reported no differences with supplementation. Additionally, 14 percent of subjects (n=3) reported negative responses following selenium supplementation with bowel problems and skin irritations cited as the main negative reactions experienced.

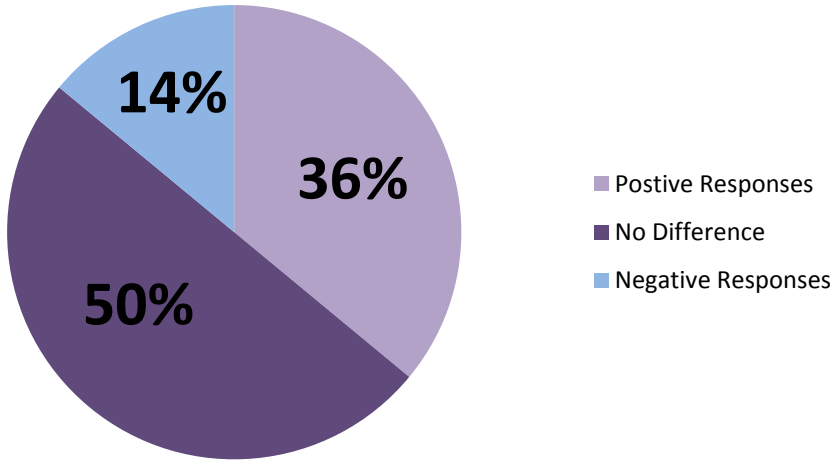


Figure 4.6: Results of Questionnaire. Data presented as percentages of total responses (%)

Chapter Five: Discussion

5.1. General Discussion

The purpose of this study was 1) To examine the extent of oxidative stress caused by, and the antioxidant acute response to, a single bout of HIIT 2) To provide a 3 week HIIT and / or Se supplementation intervention to each participant. 3) To repeat exercise tests following the intervention period to detect any changes in indices of oxidative damage, antioxidant capacity and 4) to examine the change in fitness levels following both interventions. The main findings of this investigation were that no statistically significant changes were

identified for any of the measured markers of oxidative stress or antioxidant capacity, although a number of trends toward altered activity were noted. More specifically, 1) There were no significant differences reported in MDA levels in either group post exercise pre intervention. 2) Following Se Only intervention, mean MDA levels attenuated in response to exercise, although not significantly 3) There were no changes reported in GPx1 and 3 activity and TAC in either groups following supplementation. 4) Several differences were observed in the fitness variables in both groups independent of treatment, however, a higher number of significant differences were observed in the HIIT trained group. Aerobic capacity, in particular significantly increased in the HIIT trained group.

Several strategies have been undertaken in an attempt to attenuate the enhanced oxidative stress response that occurs as a result of exercise. These include increased antioxidant intake (diet composition) (Rimm *et al.* 1996), exercise training (Fenster *et al.* 2002) and antioxidant supplementation (Sutherland *et al.* 2007). In this present study, the role of antioxidant supplementation and / or exercise training in minimizing oxidative stress was under examination.

Low response and drop out are problems commonly encountered in intervention trials (Groeneveld *et al.* 2009). An attrition rate of 5% in the current study was deemed successful and unlikely to have impacted results. An intervention of 3 weeks in duration was chosen as it was compliable and fitted with the time available to conduct the study within an MRes research project. Additionally, the choice was supported by a recent study carried out by Savory *et al*. (2012) who had also utilized a 3 week Se supplementation period.

All participants were instructed to retain usual physical activity habits during the course of the intervention as a change in these variables could influence results and mask true findings. Physical activity levels were assessed using a GPPAQ; this screening tool is widely used to assess physical activity levels for human's aged 16 – 74y. Results reported that there was no difference observed in the Se supplementation group pre to post intervention indicating that any results found were not caused by the confounding influence of changes in physical activity levels. There was however, a slight difference in PAI in the Se + HIIT group, which is likely to be due to the HIIT training attended, although this was not statistically significant (p=.081). The reasoning for this non - significant result may be part or largely due to the strict inclusion criteria outlined in the current investigation. All

participants in the study had to have obtained a 3 or 4 in the GPPAQ before recruitment. This suggests that all participants were already moderately or highly active prior to the investigation. Therefore it is possible that, increased and significant differences were not feasible. Furthermore, subjects were instructed to maintain regular dietary intake habits for the duration of the study. Savory *et al.* (2012) examined whether Se supplementation could be a potential effective therapy to reduce both obesity and exercise associated oxidant stress. 10 normal weight and 10 overweight subjects were assessed during a randomized double blind Se supplementation study with a similar dosage and duration to the current study (200ug/day for 3 weeks). However, they cited lack of dietary monitoring as one of the main limitations of their study. Additionally, their lack of GPx3 activity measurements was also alluded to. The present study utilized a 3 day food diary during both the preliminary stages and final week of testing. Pears et al (2012) concluded that food diaries were not only an accurate estimate of dietary intake but also a reliable way to assess dietary behaviour change. Furthermore, Food diaries have been established as a good tool to estimate the intake of individual antioxidant nutrients (Mayne et al., 2004). However, this same paper has also highlighted the uncertainty of food diaries when measuring Se intake. The main reason why food diaries are an inaccurate measure of Se intake is based on the fact that the Se content of food varies massively. This variation depends on the Se contect of the soil where plants grow or where animals consume crops. Therefore, similar foods may have varying levels of Se depending on where they originate. This limits the interpretation of baseline Se intake. Therefore, biomarkers are preferred for assessing selenium exposure. Some approaches used include plasma and/or toenail selenium concentrations neither of which was analysed in the current study.

5.2. The Extent of Oxidative Stress Caused by a Single Bout of HIIT

Exercise, in particular strenuous exercise, has been associated with an increased generation of ROS/FR due to the dramatic increase in oxygen uptake (Ji 1999). Strenuous exercise is known to increase perturbations to the immune system due to the accumulation of ROS/FR exceeding the capacity of the antioxidant defence system leading to oxidative stress. Fisher-Wellman and Bloomer (2009) reviewed a vast number of prior studies assessing oxidative stress following both aerobic and anaerobic exercise. They critically showed the conflicting

results of both the occurrence and non - occurrence of oxidative stress following exercise throughout the literature. In the present study, there was an increase in mean value pre to post intervention but this was not statistically significant. Both groups were shown to have low Se levels before supplementation; on the basis of low GPx3 results which were .42 and .39 respectively and low intake values identified in the food diaries. Several studies have reported that GPx3 activity is an appropriate indicator of human Se status. Additionally, several studies measuring GPx3 activity have demonstrated a positive correlation with Se levels in plasma. In the current study, subjects had generally low pre intervention GPx3 levels (>.43), this most likely led to a compromised antioxidant system and increased oxidative response following exhaustive exercise. Furthermore, these results could also potentially mean that the exercise itself was so exhaustive that the antioxidant status of the participants was not sufficient to fight the stress.

The current study is in agreement with other studies who did not report increases in MDA in response to exercise (Viinikka *et al.* 1984). Niess *et al.* (1996) measured MDA levels in plasma before and after an exhaustive bout of exercise on a treadmill in both trained and untrained subjects. They reported no significant in MDA in either group, either at 15 min or 24 h post exercise. These non-significant results contradict findings by Tauler *et al.* (2006) who measured both plasma MDA and lymphocyte protein carbonyl levels following a mountain stage cycling protocol. They reported a significant increase in both indices of oxidative stress. The reason the present results were not significant, may be due to the more stringent nature of exercise in the mountain stage utilized in Tauler's research. The exercise protocol was a cycling mountain stage (171.8 km). This mountain stage was the third stage of the "Challenge Volta Ciclista a Mallorca 2002", a 5-day competition for professional cyclists. Therefore, the duration of the exercise was far greater in this study than in the current investigation. Additionally, Kanter *et al.* (1988) reported increases in plasma MDA (~70%) following a 50 m run in elite athletes. Such discrepancy in findings is not unusual. These inconsistencies may be due the nature of the exercise in the certain studies (Kanter *et al.* 1988). This study opted for a longer duration exercise protocols compared to the formers. During short duration protocols (<2hours), the antioxidant system appears to be capable of coping with the increased accumulation of ROS and as a result, no oxidative stress response was shown to be present (Chevion *et al.* 2003).

However, during longer duration events (>2hours), an exacerbated proxidant production with the potential to overwhelm these defences has been shown (Bloomer *et al.* 2007), which may in turn lead to oxidative damage to surrounding tissues.

Additionally, these contradictory results are likely due to the measurement of oxidant stress. This present study and that undertaken by Niess *et al.* (1996), both of whom reported no significant differences detected lipid peroxidation end-products using the TBARS assay. Although the TBARS assay has been reported to correlate closely with MDA levels in samples (Hermes – Lima *et al.* 1995), it has however, been criticised for its lack of specificity over the years. The present study measured the end product MDA, a three carbon chain aldehyde that is produced as a result of lipid hydroperoxide decomposition. However, TBA has been found to react with several other biomolecules including prostaglandins and carbohydrates (Oh-ishi *et al.* 2000), thus interfering with the assay. The current study produced non -significant results across the board when using the TBARS assay to detect changes in MDA levels in response to exercise. McAnulty *et al.* (2007) suggested that perhaps F2-iso-protanes is a more specific index of lipid peroxidation. Mastaloudis *et al.* (2001) reported a 43% increase in F2-isoprostanes after a 50 km ultra-marathon. Additionally, expired pentane has been reported as an effective measure of lipid peroxidation. Leaf *et al.* (1997; 1999) have confirmed that aerobic exercise increased levels of expired pentane during and immediately post exercise.

Another contributor to the non-significant results may be in part due to the notion that the majority of studies that have shown a change in oxidative stress following interventions have used a direct ROS measurement. Electron spin resonance is the technique designed to directly measure ROS from their paramagnetic properties (Barnett *et al*. 1995). Furthermore, the majority of studies showing no change or insignificant changes in oxidant stress, measured the effect of ROS on molecules i.e. lipid and protein oxidation (Oh-ishi *et al.* 2000), similar to the current study.

Another potential reason for the non-significant results may be because only one sample was taken post exercise. Therefore, changes may have been missed. Howatson *et al.* (2009) reported that TBARS was not elevated immediately post marathon, or at 24h in recreational marathon runners. However, at 48h a marked increase in TBARS was apparent in the placebo group which demonstrates a similar response in the elevation of TBARS following

extended endurance activity reported by Machefer *et al. (*2007). Furthermore, the subject population selected may have a bearing on oxidative response and more importantly the non-significant results. Gender is also thought to effect oxidative stress levels. Ruiz-Larrea et al (1993) highlighted that the female sex hormone, oestrogen, was shown to exhibit antioxidant properties in vitro. Therefore, as females possess a larger concentration of this hormone compared to the opposite sex, they are believed to be less susceptible to oxidative stress. All the participants in the current study were female, therefore potentially influencing results.

5.3. Se Supplementation and its Effect on Oxidative Stress and Antioxidant Status

Studies investigating the effect of antioxidant therapy on exercise induced oxidative stress levels have been undertaken but only one was identified with Se (Savory *et al.* 2012). As understood antioxidants have the ability to neutralize the adverse effects of ROS. Se supplementation was found to be effective at producing a decreasing trend in MDA in all cases in the current study. The present investigation found that after Se supplementation, the Se + HIIT group demonstrated similar MDA responses to baseline following a single bout of HIIT, although the magnitude of this effect was reduced (17% - 8% post exhaustive bout). However, the Se Only group demonstrated an overall decrease in mean MDA value following intervention both at rest (10%) and post exercise (12%). However, none of these differences reached statistical significance. As mentioned, all subjects had low Se levels before supplementation as indicated by GPx3 and Se supplementation might have contributed to increase selenium status closer to optimal levels, contributing therefore to a diminished MDA response post exercise.

Similar studies have demonstrated that antioxidant therapy can alleviate indices of oxidant stress. Savory *et al.* (2012) reported a lipid hydroperoxide reduction of 2.7% in the normal weight group and 26% in the overweight group ($p<.05$) following 3 weeks of Se supplementation with similar dosages and duration to that of the current investigation (200ug/daily for 3 weeks). However, what did differ was the exercise protocol, unlike the current study that utilized a modified Wingate test, Savory and his associates opted for a more continuous exercise protocol consisting of a 5-min warm up (40% VO_{2max}) followed by

a constant workload at 70% VO_{2max} for a further 30mins. Likewise, Vincent et al (2004) found that 8 weeks of antioxidant treatment (800IU of vitamin E, 500mg of vitamin C and 10mg of beta carotene) significantly attenuated oxidative stress in overweight adults. Anderson et al (2006) also indicated protection against formation of oxidative stress with similar antioxidant cocktail which consisted of 9 weeks supplementation (24 mg beta carotene, 1000mg vitamin C and 800IU of vitamin E). They found a 30% reduction in MDA following treatment period. The current investigation supplemented with 250ug of Se demonstrated a 12% reduction in MDA following intervention period post exercise as highlighted above.

Changes in antioxidant enzyme activity in erythrocytes have been used to document oxidative stress (Urso & Clarkson 2003). This was achieved in the current investigation by measuring changes in antioxidant enzyme GPx3 activity. In the current investigation, GPx3 activity was measured in response to 3weeks of Se supplementation. Mean values of GPx 3 activity were shown to increase following supplementation in both the Se only group (15%) and the Se + HIIT group (2%). Similarly, mean values of GPx1 in RBC was shown to rise following supplementation by 6% and 2% respectively, however none of these increases reached statistical significance. As a result of Se supplementation, one would expect GPx3 activity to increase and it did as a percentage of baselines in the Se only group (See Table 4.6). Previous studies have reported an increase in both SOD and GPx3 activity following antioxidant supplementation. Ghaffari *et al.* (2011) examined the effect of vitamin E and Se supplementation on antioxidant defense in diabetic rats. The found a significant increase in SOD and GPx 3 activities in vitamin E and selenium supplemented diabetic group after 5 weeks of supplementation. Therefore, there is reason to believe we did not see an increase in plasma as the intervention was not long enough, as it takes time for the selenium to be accumulated in the body and then to be incorporated in the selenoproteins.

Also used as an indicator of oxidative stress is total antioxidant capacity (Cao *et al.* 1993). The body's antioxidant capacity may be temporarily decreased in response to strenuous physical activity as its components are used to quench harmful FR/ROS. This current study, did not measure the acute antioxidant response to a single bout of exercise unlike previous research (Child *et al*. 1999; Ginsburg *et al.* 2001; Santos-Silva *et al*. 2001). However, what this study did investigate was the effect that a 3 week Se supplementation had on TAC.

Similar to GPx1 and 3 activity, TAC increased by 40% in the Se + HIIT group and increased by 66% in the Se only group, however none of these differences reached statistical significance. These findings however, do not stand alone. Recently, publications have reported a failure of this approach with reports showing no effect of single antioxidant supplements on TAC of plasma. Loitio and Frei (2004) demonstrated that even a controlled consumption of apples which are known to possess antioxidant compounds, had no effect on TAC in plasma. Loitio and Frei (2004) examined the antioxidant capacity of representative apple-contained polyphenols and whole apple extracts by quantifying both their oxygen radical absorbance capacity (ORAC) and their ferric reducing antioxidant potential (FRAP). They concluded that despite the high TAC of individual apple polyphenols and apple extracts, ingestion of large amounts of apples by humans does not appear to result in equivalent in vivo antioxidant effects of apple polyphenols.

Following this, Serafini and Del Rio (2004), reviewed the literature around dietary antioxidants and TAC. They concluded that a person's overall antioxidant capacity is directly related to the antioxidant levels, ROS production and is under genetic control. They also showed that the environment, sex, age, hormones, life style and dietary factors are all involved in TAC and that it is maintained under tight regulation. The TAC measured in the current study quantified the total antioxidant activity of various constituents (endogenous in this case) such as vitamins, proteins lipids uric acid in plasma. The supplementation of Se itself does not necessarily add to the antioxidant capacity as such, but the selenium is used to produce more GPx3enzymes which then are part of the TAC as GPx3 activity. If the time to allow the incorporation of Se into selenoprotiens was not sufficient to reach statistical differences then there would not be significant improvements in TAC. These studies as well as this proposed mechanism provide rationale for the insignificant findings presented in the current study.

However, studies such as Fatouros *et al*. (2004) have demonstrated that following 16 weeks of ET, a population of older men experiences enhanced antioxidant defences by increasing enzyme activities and TAC. They reported an increase in both TAC (6%) and GPx3 activity (12%) following ET. These findings are in agreement with earlier work with younger subjects that suggested that systematic training induces positive adaptations in antioxidant enzyme activities. These enhancements have been found to be associated with increased protein

content and mRNA level in muscle. GPx3 activity has been shown several times to increase in response to training (Leewenburgh *et al.* 1994; Oh-ishi *et al*. 1997). GPx3facilitates H_2O_2 and lipid peroxides removal produced in the mitochondrial inner membrane as previously mentioned. Therefore, an increase in training increases the rate of removal thus decreasing the subsequent oxidative stress produced. The findings from the current study in relation to the Se only group are however, in agreement to results reported by Fatouros *et al.* (2004). The current study found after a single bout of HIIT a decrease of 12% at rest and 16% post exhaustive exercise in the Se Only group although these results were non - significant. Similarly, Fatouros *et al.* (2004) demonstrated that 16 weeks of ET may attenuate exercise induced oxidative stress. They reported a decrease in MDA post ET both at rest (10%) and following exhaustive exercise (16%). Furthermore Metin *et al.* (2003) examined the effect of regular training on plasma thiols, MDA and Carnitine Concentrations in Young Soccer Players, similar characteristics to those in the current study. They found that long term trained sportsmen seem to be under less oxidative stress, as their MDA levels were lower than those in control group. Kilstrom *et al.* (1990) demonstrated that endurance swim training provides enhanced protection to the heart against oxidative stress. They concluded that this extra capacity to detoxify ROS/FR was mainly due to elevated GSH levels and a more efficient NADPH supplying system in the trained heart. ET has been shown to up regulate mitochondrial respiratory chain proteins, leading to a decreased electron flux through each electron chain, electron leakage, and the resulting radical formation.

During aerobic exercise, mitochondria are in state 3. State 3 of the 1-5 respiration stages of mitochondria involves the respiratory substrate been added, respiration increases markedly to a high steady rate, and pyridine nucleotides become more reduced. Lower amounts of ROS/FR produced during this state 3 may employ a protective mechanism against oxidative stress by enhancing electron coupling (Leewenburgh *et al.* 2001). As mentioned previously, exercise is associated with an increase in oxygen uptake, particularly skeletal muscle. Enhanced coupling of electron transport and oxygen reduction to water in state 3 may constitute a significant mechanism for controlling free radical production. HIIT is thought to act in the same manner as ET, therefore potentially explaining why HIIT is thought to enhance protection against oxidative stress. The results of the current investigation,

however, did not demonstrate this protective effect. Therefore, further research is needed to support the notion that the up regulation of electron transport chain complexes reduces the oxidant formation.

5.4. HIIT and its Effect on Oxidative Stress and Antioxidant Status

In spite of the potential damage and stress that occurs as a result of exercise, it was shown that adaption to regular exercise results in an enhanced antioxidant defence system (Radak *et al.* 2001). As previously mentioned, few studies have examined the effect of a HIIT training program on oxidative stress. In the current investigation, MDA as indicated by the TBARS assay was similar at rest and after exercise for both groups. Mean MDA levels were increased in both groups after exercise independent of treatment prior to intervention, although these were not statistically significant (p=.395 and .904 respectively). After the intervention, the Se + HIIT group demonstrated similar MDA responses; however the magnitude of this effect was reduced, 9% increase at rest and 2% following exercise. The Se Only group, however, post intervention demonstrated a far greater decrease in MDA at rest (12%) and attenuation of MDA response to exhaustive exercise (16%). However, none of these differences were significant. The Se + HIIT group had unclear results indicating an increase in 9% at rest and decrease of 2% following intervention. This suggests that these contradictory results may be as a result of the additional training. Training had an opposite effect to Se alone in that changes were more pronounced in the Se only group suggesting that the exercise increased lipid peroxidation much like the results reported by Ugras *et al.* (2012) who found a significant increase in MDA and significant decrease in CAT post HIIT training for 3hours per day for 5 days. Contrary to the hypothesis in the current study, Urgas *et al*. (2012) reported significantly higher MDA levels following HIIT program. This was largely due to the intensive nature of the program. Metin *et al.* (2003) reported that excessive training can produce oxidative stress and organism's antioxidant elements were stimulated with this challenge. This was most likely the cause in the case of Ugras *et al.* (2012) and potentially contributed to the increase in MDA in the current investigation. Overall, the present study does not present evidence that HIIT has the ability to attenuate exercise induced oxidative stress.

5.5. The Effect of HIIT on Fitness Levels

Regular training has been widely accepted as a means of increasing fitness levels. Metin *et al.* (2003) demonstrated that in a group of 26 soccer players, a sport of intermittent nature, similar to the current sample, VO_{2max} values were significantly increased in the trained group ($p<001$) when compared to the control group who did nothing. The training protocol consisted of their regular training sessions. Although, HIIT is fast becoming a topic of recent debate, the amount of research available on the effect HIIT has on individuals engaged in intermittent sports is limited. Previous results from studies have expressed a positive correlation between oxygen uptake and antioxidant defence enzyme activity (Jenkins *et al.* 1984). This finding along with Child *et al.* (1999) who found that the ability to quench FR in serum is increased in relation to the maximum ability to consume oxygen. Both of these studies provide good rationale for the results of the current study. Subjects who were involved in the HIIT program in the present investigation demonstrated a significant improvement in VO_{2max} (9%) indicating a substantial increase in their aerobic capacity. Additionally, HIIT trained subjects significantly decreased their 10m sprint time by 3%. Subjects also demonstrated significant improvements in max and min power produced during a Wingate (6% and 10% respectively). These findings are in agreement with previous research suggesting that VO_{2max} increased in skiers, runners and swimmers post HIIT intervention (Breil *et al.* 2010; MacPherson *et al.* 2010). Therefore, as a result of the HIIT group demonstrating an increased exercise capacity, but not showing any further increases in the extend of oxidative stress experienced, this could potentially indicate that their antioxidant defence systems increased in accordance with their increased fitness level. This could perhaps indicate an increased tolerance to a more intense workout without any further increase in amount of oxidative stress experienced. Furthermore, there was a parallel significant decrease in body mass in the HIIT trained group ($p<.01$). Similar findings were reported in relation to weight loss in longer duration studies. A 2001 study from East Tennessee State University reported a decrease of 2% in body mass following an eight-week HIIT program (King 2001). The subjects in the HIIT group in the current study had a 1.7% decrease in body mass. Previous research has alluded to the fact that HIIT enhances the metabolic machinery in muscle cells that promotes fat-burning and blunts fat production. A study carried out at Laval University reported that the HIIT subjects' muscle fibres had

significantly higher markers for fat oxidation that those in a continuous exercise group. Talanlan (2007) reported that young females in particular, who performed seven HIIT workouts over a two – week period, the subjects experienced a 30% increase in fat oxidation. In the current study, subjects of similar characteristics performed 6 sessions in a three – week period. The results from these studies provide rationale for the loss of body mass experienced in the HIIT trained group in the current study.

5.6. The Effect of Se Supplementation on Fitness Levels

The involvement of Se enhancing fitness levels was not observed in the current study. Se, as previously discussed, has been suggested to play an indirect, yet significant role in exercise recovery. Recovery can be defined as the point at which the athlete is able to train without constraints of sore muscles or an increased risk of injury (Darr *et al.* 1987). It has been hypothesized (Tiidus 1998) that control of post – exercise FR production could end up being a counter – productive measure. By removing the FR that neutrophils have established to mark tissue, the body may in fact be delaying the exercise recovery process. Removal of the oxidative markers has the potential to decrease the amount of damaged tissue target by the immune system. Endogenous regulation discussed previously in section 1.4.1, of the reduced glutathione system could play an important role in maintaining the balance of oxidative by-products to antioxidants. Several studies have demonstrated that glutathione reductase levels are elevated in skeletal muscle and erythrocytes following 10weeks of chronic exercise (Leeuwenburgh *et al.* 1994). NADPH, using GR as a catalyst converts oxidised glutathione back to its reduced form. The endogenous increase in the reduced glutathione antioxidant defence system associated with chronic training is likely to protect against oxidative stress within the body. Ortenblad *et al.* (1997) demonstrated how GPx3 activity increases with this type of exercise. They used a short – term maximal exercise protocol similar to the one utilized in the current investigation. Free radical muscle damage was determined after a continuous 30-second maximum jump test in both a trained (volleyball) and untrained population. The resting levels of GR were significantly higher in the trained groups, furthermore, CK in plasma, an indicator of muscle damage was significantly higher in the untrained group post exercise ($p<.05$). The research supported the idea that the GR defence system is up-regulated and has a greater ability to protect

against oxidative stress, in this case lipid peroxidation in trained individuals. However, the current study did not support that hypothesis. There were no significant differences reported in the Se Only group in relation to any of the fitness variables assessed, with the exception of mean power (p=.047). Mean power increased by 4% in this group. A potential explanation for this finding could be related to a learning effect. As outlined by Schmidt (1991) practice can have two distinct kinds of influences on performance, one that is relatively permanent (due to learning) and one that is only temporary. This was the second time the subjects performed the Wingate trial and therefore, knew what to expect and what was expected of them.

Indirect measures of recovery including fatigue index proved non – significant. However, interestingly when asked, subjects felt as if they had performed better following supplementation and that they recovered quicker following exhaustive exercise and experienced less muscle soreness. This has the potential to be viewed as a placebo effect, however, no markers of muscle damage were examined in the present investigation and therefore the role of selenium in recovery from exercise may warrant further research.

6. Limitations

There was several identified limitation in this study. The participants in this study were female university level students involved in intermittent sports in RGU, so therefore the results may not be generalizable to other populations including males, children etc. However, the population chosen for this study was those aged 18-30y engaged in intermittent sports within RGU. The reason for choosing this particular sample population was formed on the basis that this study exposes individuals to a very high intensity form of exercise. HIIT training involves exercising at a value at or close to VO_{2max}. Furthermore, although a great deal of research outlines the benefits associated with utilizing HIIT in untrained and recreationally trained individuals, research on the effect HIIT has or could potentially have on the well-trained intermittent athletes is limited. According to Laursen and Jenkins (2002) much of this issue may be due to the fact that it is often difficult to persuade coaches and athletes to alter training programs, specifically in the name of research. Furthermore, the participants involved in the study were already fit, so therefore might have been less likely to show metabolic modifications.

Additionally, the study was comprised of 21 subjects, which is a relatively small number. The small sample size limits the statistical power and the possibility of observing more significant differences. Although small in number, this sample size is comparable to similar studies investigating both Se supplementation and HIIT. The aforementioned study on p.61 (Savory *et al*2012) recruited 20 subjects (10 = Overweight and 10 = Normal Weight) whereasa Ingham *et al*. (2008) analysed the physiological and performance effects associated with low versus mixed – intensity row training in 18 experienced male rowers.

All subjects were instructed to perform a 30 second "all out" effort for the Wingate protocol. The interpretation of the term "all out" effort is very subjective, and it could be a strong possibility that these university trained athletes thought they were giving 100%, when in fact they could have exerted themselves more.

The duration of the intervention is another potential limitation of this study. The bulk of both physical activity and nutritional intervention studies have included long duration interventions. Exercise interventions have typically lasted anywhere between 3 – 20 weeks depending on which physiological variables are being analysed, whereas antioxidant therapy interventions typically last even longer (Avellini *et al.* 1999). Unfortunately due to time constraints, this was not feasible for the current study. 3 weeks selenium supplementation was apparently too short to increase Se status as assessed by plasma GPx3. Therefore, the short duration of the study could have affected outcome of the research and potentially contributed to the common occurrence of non- significant results. Ideally, a longer intervention or indeed a washout period would have been included. Unfortunately this was not practical or feasible.

Lack of dietary monitoring on a continuous basis throughout the trials could be seen as a potential limitation. This lack of monitoring is a common limitation cited in intervention studies. Savory *et al.* (2012), cited lack of dietary monitoring as one of the main limitations of their study when investigating Se as a potential effective therapy for alleviating oxidative stress. Although all precautions that were possible were taking in the current study to ensure that physical activity and nutritional habits remained constant, there is still a possibility that either of these variables could still have influenced results found. The present study utilized a 3 day food diary during both the preliminary stages and final week of testing. Food diaries have been established as a good tool to estimate the intake of

individual antioxidant nutrients (Mayne *et al.* 2004). However, this same paper highlighted the uncertainty of food diaries when measuring Se intake. As identified earlier, the Se content of foods varies, depending on the selenium content of the soil where plants are grown or where animals consume forage crops. The problem is similar food items can have 10-fold differences in selenium content, making dietary assessment of selenium intake from foods unreliable. Therefore, biomarkers are preferred for assessing selenium exposure. Some approaches used include plasma and/or toenail selenium concentrations neither of which were analysed in the current study. This could be viewed as a potential limitation.

Another limitation of the current study is that only one blood sample was taking post exhaustive exercise. Initially, what was discussed was that an immediate sample be taken followed by subsequent samples at hourly intervals. However, given the already large commitment associated with the investigation, it was not feasible to have subjects do this. Because of this, the study may have missed changes that occurred following the initial blood draw. Previous studies, such as Howatson *et al*. (2009) reported that TBARS was not elevated immediately post marathon, or at 24h in recreational marathon runners. However, there was a significant increase in the same biomarker 48h after exercise. Furthermore, in the current study, there were only 2 groups as opposed to 3 desired groups. Due to lack of time and participants, a HIIT only group was no longer plausible. Therefore, the current research was unable to determine the effect a HIIT only intervention would have on antioxidant status, oxidative stress and fitness levels without the addition of antioxidant supplementation. There was also a desire to carry out additional measurements on the blood samples to gain a better understanding of the changes in oxidative stress and antioxidant capacities, however due to time constraints had to be omitted from the study. Other indicators of oxidative stress i.e. DNA damage and protein oxidation would have giving a clearer and more precise indication of oxidative stress response.

Indirect measures of recovery including fatigue index were taking to assess the effect of Se on exercise recovery. All these results were reported as non-significant. However, the current study did not examine any direct measures of muscle damage. There are a number of direct and indirect methods used to determine the extent of, and rate of recovery from exercise induced muscle damage (EIMD) and delayed onset muscle soreness (DOMS). Direct measurement is difficult and is only possible by muscle biopsies or magnetic resonance

imaging. Therefore, indirect measures are used widely in the literature, but differ from the current study in that they are coupled with the measurement of certain biochemical markers such as myoglobin and CK, these measures can provide a reasonably accurate measure of muscle damage and subsequent recovery time. As a result, this study did not fully explore the relationship between Se and exercise recovery.

Furthermore, the subject population selected may have a bearing on oxidative response and more importantly the non-significant results. Gender is thought to effect oxidative stress levels as oestrogen, the female sex hormone, is thought to have a protective effect against this potential stress.. Given that all the participants in the current study were female, therefore potentially influencing results. Future studies need to be carried out examining and more interestingly, comparing, both the male and female response to exercise induced oxidant stress and Se supplementation.

7. Conclusion

This study has highlighted a potential benefit of Se (and possibly HIIT) in reducing oxidant stress and increasing antioxidant status post exhaustive exercise in females engaged in intermittent sports. This study has also reiterated previous findings that HIIT has the potential to enhance some components of fitness including speed and aerobic capacity.

As demonstrated, several studies have found that antioxidant supplementation is effective at reducing exercise induced oxidative stress. This study has highlighted a potential benefit of Se (and possibly HIIT) in reducing oxidant stress and increasing antioxidant status post exhaustive exercise in females engaged in intermittent sports. Although many of the results reported were non - significant, this may have been largely or in part due to the several limitations identified. What the results of this study do highlight is a decreased trend when examining the effect of Se supplementation on biomarkers of oxidative stress. With that said, there still remains an on-going debate over whether this potential stress is indicative of a harmful stimulus. Although, it is thought that pro oxidant production has the ability to result in significant damage to lipids, proteins and even genetic material, there is limited evidence to suggest that it is the increase in ROS that actually causes ill health and disease (Lobo *et al.* 2010). In fact, the presence of low concentrations of ROS/FR are involved in

several important functions including normal cellular redox status, tissue function, apoptosis, stimulation of antioxidant defence molecules, and intracellular signalling processes (Spurway & MacLaren 2007).

Furthermore, the involvement of HIIT in elevating antioxidant enzyme activities and decreasing the extent of oxidative stress caused following exhausting exercise remains unknown. However, what can be said for HIIT training is that it has the potential to increase fitness levels. The participants in the current study who engaged in HIIT significantly increased several components of fitness over the course of the intervention. However, Se Only did not produce significant improvements in the fitness variables and therefore, the role of Se in recovery from exercise may warrant further research. This study also has the potential application in a different population such as overweight or obese where the effect of both training and supplementation might be more evident.

8. References

ALLAN, C.B., LACOUCIERE, G.M., STADTMAS, T.C. 1999. Responsivness of selenoproteins to dietary selenium. Annual Review of Nutrition, 19, 1-16.

ALLEN, D.G., LAMB, G.D. & WESTERBLAD, H. 2008. Skeletal Muscle Fatigue: Cellular Mechanisms. Physiol Rev, 88(1), 287-332.

ASHTON, T., ROWLANDS, C.C., JONES, E., YOUNG, I.S., JACKSON, S.K. DAVIES, B. & PETERS , J.R. 1998. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. Eur J Appl Physiol Occup Physiol, 77, 498-502.

AVELLINI, L., CHIARADIA, E., GAITI, A.1999. Effect of exercise training, selenium and vitamin E on some free radical scavengers in horses (Equus caballus). Comparative Biochemistry and Physiology Biochemistry and Molecular Biology, 123, 147–154

BAE, S. C., KIM, S. J. & SUNG, M. K. 2003. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. J Am Coll Nutr, 22, 311-5.

BAECHLE, T.R. & EARLE, R.W. 2008. Essentials of strength training and conditioning. (3rd ed.). Champaign: Human Kinetics.

BAKER, J.S., BAILEY, D.M. HULLIN, D., YOUNG, I. & DAVIES, B. 2004. Metabolic implications of resistance force selection for oxidative stress and markers of muscle damage during 30s of high intensity exercise. Eur J Appl Physiol, 92(3), 321-327.

BARNETT, Y.A. BRENNAN, L.A., O'FARRELL, F., HANNIGAN, B.M.1995. Oxidant – induced stress response in lymphoid cells. Biochem Molec Bio Int,37, 273 – 281.

BAR-OR, O. 1987. The Wingate anaerobic test: An update on methodology, reliability and validity. Sports Medicine, 4, 381–394.

BILLAT, V. L., FLECHET, B., PETIT, B., MURIAUX, G. & KORALSZTEIN, J. P. 1999. Interval training at $VO2_{max}$: effects on aerobic performance and overtraining markers. Med Sci Sports Exerc, 31, 156-63.

BJELAKOVIC, G., NIKOLOVA, D., GLUUD, L. L., SIMONETTI, R. G. & GLUUD, C. 2012. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev,* 3, CD007176.

BLOOMER, R.J., GOLDFARB, A.H., WIDEMAN, L. McKENZIE, M.J., CONSITT, L.A. 2005. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *Journal of Strength and Conditioning Research,* 19(2), 276–285.

BLOOMER, R. J. & SMITH, W. A. 2009. Oxidative stress in response to aerobic and anaerobic power testing: influence of exercise training and carnitine supplementation. *Res Sports Med,* 17**,** 1-16.

BLOOMER, R. J., DAVIS, P. G., CONSITT, L. A. & WIDEMAN, L. 2007. Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *Int J Sports Med,* 28**,** 21-5.

BOUAYED, J. & BOHN, T. 2010. Exogenous antioxidants - Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses:. *Oxid Med Cell Longev,* 3**,** 228-237.

BREIL, F.A., WEBER, S.N. KOLLER,S.K., HOPPELER,H. VOGT, M. 2010. Block training periodization in alpine skiing: Effects of 11 – day HIIT on $Vo_{2max}$and performance. European Journal of Applied Physiology, 109,1077 – 1086.

BURGOMASTER, K. A., HOWARTH, K. R., PHILLIPS, S. M., RAKOBOWCHUK, M., MACDONALD, M. J., MCGEE, S. L. & GIBALA, M. J. 2008. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol,* 586**,** 151-60.

BURKE, L. & DEAKIN, V. 2006 Clinical Sports Nutrition. Sydney, Australia: McGraw – Hill.

BUTTERFIELD, T. A., BEST, T. M. & MERRICK, M. A. 2006. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. *J Athl Train,* 41**,** 457-65.

CALABRESE, E. J. 2008. Hormesis: why it is important to toxicology and toxicologists. *Environ Toxicol Chem,* 27**,** 1451-74.

CAMPBELL, P. T., GROSS, M. D., POTTER, J. D., SCHMITZ, K. H., DUGGAN, C., MCTIERNAN, A. & ULRICH, C. M. 2010. Effect of exercise on oxidative stress: a 12-month randomized, controlled trial. *Med Sci Sports Exerc,* 42**,** 1448-53.

CHEVION, S., MORAN, D. S., HELED, Y., SHANI, Y., REGEV, G., ABBOU, B., BERENSHTEIN, E., STADTMAN, E. R. & EPSTEIN, Y. 2003. Plasma antioxidant status and cell injury after severe physical exercise. *Proc Natl Acad Sci U S A,* 100**,** 5119-23.

CHILD, R. B., WILKINSON, D. M., FALLOWFIELD, J. L. & DONNELLY, A. E. 1998. Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. *Med Sci Sports Exerc,* 30**,** 1603-7.

COHEN, H.J., BROWN, M., LYONS, J., AVISSAR, N., HAMILTON, D. & LIEGEY, P.1998. Clinical, physiological and biochemical consequences of human selenium deficiency, in: Essential and Toxic Trace Elements in Human Health and Disease, pp. 201–210. Alan R. Liss, New York.

COOMBS, G.F. & LU. J. 2006. Selenium as a cancer preventative agent. Selenium: its molecular biology and role in human health, 2nd Ed, Springer, New York, USA, pp. 205-218.

COOPERS, S.M., BAKER, J.S., EATON, Z.E. & MATTHEWS, N. 2004. Cooper, A simple multistage field test for the prediction of anaerobic capacity in female games players. *Br J Sports Med,* 38(6),784-9.

CORNELISSEN, V. A. & FAGARD, R. H. 2005. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension,* 46**,** 667-75.

DAVIES, K. J., QUINTANILHA, A. T., BROOKS, G. A. & PACKER, L. 1982. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun,* 107**,** 1198-205.

DE CASTRO, M.A.C., CAVALCANTI NETO, F.F., LIMA, L.M.C., DE SLIVA, F.M., OLIVERIA, R.J., ZANESCO, A. 2009. Production of free radicals and catalase activity during acute exercise training in young men. *Biology of Sport*, 26,2.

DEKKERS, J. C., VAN DOORNEN, L. J. & KEMPER, H. C. 1996. The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. *Sports Med,* 21**,** 213-38.

DENNY, A., & BUTTRISS, J. 2007. Synthesis Report No 4: Plant Foods and Health – Focus on Plant Bioactives Norfolk, British Nutrition Foundation.

DIPLOCK, A.T. 1994. Antioxidants and disease prevention. *Mol Aspects Med*, 15, 293–376.

DUTHIE, G. G., ROBERTSON, J. D., MAUGHAN, R. J. & MORRICE, P. C. 1990. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch Biochem Biophys,* 282**,** 78-83.

FAIRWEATHER-TAIT, S.J. BAO, Y., BROADLEY, M.R. COLLINGS, R., FORD, D., HESKETH, J.E., HURST, R. 2011. Selenium in human health and disease. *Antioxid Redox Signal*, 14(7), 1337-1383.

FATOUROS, I. G., JAMURTAS, A. Z., VILLIOTOU, V., POULIOPOULOU, S., FOTINAKIS, P., TAXILDARIS, K. & DELICONSTANTINOS, G. 2004. Oxidative stress responses in older men during endurance training and detraining. *Med Sci Sports Exerc,* 36**,** 2065-72.

FENSTER, C. P., WEINSIER, R. L., DARLEY-USMAR, V. M. & PATEL, R. P. 2002. Obesity, aerobic exercise, and vascular disease: the role of oxidant stress. *Obes Res,* 10**,** 964-8.

FISHER, G., SCHWARTZ, D. D., QUINDRY, J., BARBERIO, M. D., FOSTER, E. B., JONES, K. W. & PASCOE, D. D. 2011. Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise. *J Appl Physiol (1985),* 110**,** 730-7.

FISHER-WELLMAN, K. & BLOOMER, R. J. 2009. Acute exercise and oxidative stress: a 30 year history. *Dyn Med,* 8**,** 1.

GABRIEL, B., RATKEVICIUS, A., GRAY, P., FRENNEAUX, M. P. & GRAY, S. R. 2012. High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. *Clin Sci (Lond),* 123**,** 313-21.

GALATI, G., SABZEVARI, O., WILSON, J.X. & O'BRIEN, P.J. 2002. Proxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics, *Toxicology,* 177, 91-104.

GARRETT, W.E. & KIRKENDALL, D.T. 2000. Exercise and sport science. Philadelphia: Lippincott Williams &Wilkins.

GIBALA, M. J., LITTLE, J. P., VAN ESSEN, M., WILKIN, G. P., BURGOMASTER, K. A., SAFDAR, A., RAHA, S. & TARNOPOLSKY, M. A. 2006. Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol,* 575**,** 901-11.

GOLDFARB, A.H., BLOOMER, R.J. & MCKENZIE, M.J. 2005. Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Med Sci Sports Exerc,* 37, 234-239.

GOMEZ-CABRERA, M. C., BORRÁS, C., PALLARDÓ, F. V., SASTRE, J., JI, L. L. & VIÑA, J. 2005. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol,* 567**,** 113-20.

GOTO, C., NISHIOKA, K., UMEMURA, T., JITSUIKI, D., SAKAGUTCHI, A., KAWAMURA, M., CHAYAMA, K., YOSHIZUMI, M. & HIGASHI, Y. 2007. Acute moderate-intensity exercise induces vasodilation through an increase in nitric oxide bioavailiability in humans. *Am J Hypertens,* 20**,** 825-30.

GRIFFITH, O.W. & MULCAHY, R.T. 1999. The enzymes of glutathione synthesis: gammaglutamylcysteinesynthetase. Advances in Enzymology. New York, John Wiley & sons Inc. 73, 60-80.

GRIFFITH, O.W. 1999. Biologic and pharmacologic regulation of mammalian glutathionesynthesis. *Free Radical Biology and Medicine,* 27,(9-10), 922-935.

GROENEVELD, I.F., PROPER, K.I, VAN DER BEEK, A.J., HILDEBRANDT, V.H. & VAN MECHELEN, W. 2009. Factors associated with non - participation and drop – out in a lifestyle intervention for workers with an elevated risk of cardiovascular disease. *International Journal of Behavioural Nutrition and Physical Activity*, 6,80.

GÜZEL, N. A., HAZAR, S. & ERBAS, D. 2007. Effects of different resistance exercise protocols on nitric oxide, lipid peroxidation and creatine kinase activity in sedentary males. *J Sports Sci Med,* 6**,** 417-22.

HALLIWELL, B., GUTTERIDGE. 1989. Free Radicals in biology and medicine. Oxford: Clarendom Press.

HARTMANN, A., PLAPPERT, U., RADDATZ, K., GRUNERT-FUCHS, M., SPEIT, G. 1994. Does physical activity induce DNA damage? *Mutagenesis,* 9(3),269-272.

HELLSTEN – WESTING, Y., SOLLEVI, A. & SJODIN, B. 1991. Plasma accumulation of hypoxanthine uric acid and creatine kinase following exhaustive runs of differing durations in man. *Eur J Appl Physiol Occup Physio,l* 63,380-384.

HERMES-LIMA, M., WILLMORE, W. G. & STOREY, K. B. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. *Free Radic Biol Med,* 19**,** 271-80.

HOWATSON, G., MCHUGH, M. P., HILL, J. A., BROUNER, J., JEWELL, A. P., VAN SOMEREN, K. A., SHAVE, R. E. & HOWATSON, S. A. 2010. Influence of tart cherry juice on indices of recovery following marathon running. *Scand J Med Sci Sports,* 20**,** 843-52.

HUANG, S. C., LEE, F. T., KUO, T. Y., YANG, J. H. & CHIEN, C. T. 2009b. Attenuation of long-term Rhodiola rosea supplementation on exhaustive swimming-evoked oxidative stress in the rat. *Chin J Physiol,* 52**,** 316-24.

HUNG, H.C., JOSHIPURA, K.J., JIANG, R., HU., F.B., HUNTER, D., SMITH- WARNER, S.A., COLDITZ, G.A., ROSNER, B., SPIGALMAN, D. & WILETT, W.C. 2004. Fruit and Vegetable Intake and Risk of Major Chronic Disease. *Journal of the National Cancer Institute*, 96, 1577-1584.

INAL, M., AKYÜZ, F., TURGUT, A. & GETSFRID, W. M. 2001. Effect of aerobic and anaerobic metabolism on free radical generation swimmers. *Med Sci Sports Exerc,* 33**,** 564-7.

INBAR, O., BAR-OR, O, SKINNER, J. 1996.The Wingate Anaerobic Test. Human Kinetic; Champaign, IL: pp. 41–71.

INSTITIUTE OF MEDICINE, FOOD AND NUTRITION BOARD. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC, 2000.

JACKON, M.J. 2000. Exercise and oxygen radical production by muscle. In Handbook of oxidants and antioxidants in exercise. Edited by Sen CK, Packer L, Hanninen O. Amsterdam: Elsevier Science 1: 57-68.

JACKSON, M. J., EDWARDS, R. H. & SYMONS, M. C. 1985. Electron spin resonance studies of intact mammalian skeletal muscle. *Biochim Biophys Acta,* 847**,** 185-90.

JACOB, R.A. 1995. The Integrated Antioxidant System. *Nutr Res*, 15(5),755-766.

JENKINS, R. R., KRAUSE, K. & SCHOFIELD, L. S. 1993. Influence of exercise on clearance of oxidant stress products and loosely bound iron. *Med Sci Sports Exerc,* 25**,** 213-7.

JI, L. L. & FU, R. 1992. Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. *J Appl Physiol (1985),* 72**,** 549-54.

JI, L. L. 1993a. Antioxidant enzyme response to exercise and aging. *Med Sci Sports Exerc,* 25**,** 225-31.

JI, L. L. 1995a. Exercise and oxidative stress: role of the cellular antioxidant systems. *Exerc Sport Sci Rev,* 23**,** 135-66.

JI, L. L., LEEUWENBURGH, C., LEICHTWEIS, S., GORE, M., FIEBIG, R., HOLLANDER, J. & BEJMA, J. 1998. Oxidative stress and aging. Role of exercise and its influences on antioxidant systems. *Ann N Y Acad Sci,* 854**,** 102-17.

KALIORA, A.C., DEDOUSSIS, G.V.Z. SCHMIDT, H. 2006. Dietary antioxidants in preventing atherogenisis. *Atheroschlerosis,* 187, 1-17.

KEENOY, B.M., VERTOMMEN, J. 2001. Divergent effects of different oxidants onglutathione homeostasis and protein damage in erythrocytes from diabetic patients: effects of high glucose. *Molecular and Cellular Biochemistry* 225: 59-73.

KENNETT, E. C. & KUCHEL, P. W. 2006. Plasma membrane oxidoreductases: effects on erythrocyte metabolism and redox homeostasis. *Antioxid Redox Signal,* 8**,** 1241-7.

KIHLSTRÖM, M. 1990. Protection effect of endurance training against reoxygenation-induced injuries in rat heart. *J Appl Physiol (1985),* 68**,** 1672-8.

KING, J.W. 2001A comparison of the effects of interval training vs. continuous training on weight loss and body composition in obese premenopausal women (thesis). East Tennessee State University.

KNEZ, W. L., JENKINS, D. G. & COOMBES, J. S. 2007. Oxidative stress in half and full Ironman triathletes. *Med Sci Sports Exerc,* 39**,** 283-8.

KOZ, M., ERBAŞ, D., BILGIHAN, A. & ARICIOĞLU, A. 1992. Effects of acute swimming exercise on muscle and erythrocyte malondialdehyde, serum myoglobin, and plasma ascorbic acid concentrations. *Can J Physiol Pharmacol,* 70**,** 1392-5.

KUNWAR, A., PRIYADDARSINI, K.I. 2011). Free radicals, oxidative stress and importance of antioxidants in health. *Journal of Medicine and Applied Sciences,* 1(2), 53-60.

LAAKSONEN, D. E., ATALAY, M., NISKANEN, L., UUSITUPA, M., HÄNNINEN, O. & SEN, C. K. 1996. Increased resting and exercise-induced oxidative stress in young IDDM men. *Diabetes Care,* 19**,** 569-74.

LARCOMBE, S. D., COFFEY, J. S., BANN, D., ALEXANDER, L. & ARNOLD, K. E. 2010a. Impacts of dietary antioxidants and flight training on post-exercise oxidative damage in adult parrots. *Comp Biochem Physiol B Biochem Mol Biol,* 155**,** 49-53.

LARCOMBE, S. D., TREGASKES, C. A., COFFEY, J. S., STEVENSON, A. E., ALEXANDER, L. & ARNOLD, K. E. 2008a. The effects of short-term antioxidant supplementation on oxidative stress and flight performance in adult budgerigars Melopsittacus undulatus. *J Exp Biol,* 211**,** 2859-64.

LAURSEN, P. B. & JENKINS, D. G. 2002. The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. *Sports Med,* 32**,** 53-73.

LAWRENCE, R.S., BURKE, R.F. 1976. Gluthatione peroxidase activity in selenium deficient rat liver. *Biochem. Biophys. Res. Commun*. 71, 952 – 958.

LEEUWENBURGH, C., FIEBIG, R., CHANDWANEY, R. & JI, L. L. 1994. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. *Am J Physiol,* 267**,** R439-45.

LIU, R. H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr,* 78**,** 517S-520S.

LOVLIN, R., COTTLE, W., PYKE, I., KAVANAGH, M. & BELCASTRO, A. N. 1987. Are indices of free radical damage related to exercise intensity. *Eur J Appl Physiol Occup Physiol,* 56**,** 313-6.

LOBO, V., PATIL, A., PHATAK, A., CHANDRA, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*, 4(8), 118–126.

MAC PHERSON, R.E.K., HAZELL, T.J. OLVER, T.D. PATTERSON, D.H., LEMON, W.R. 2010. Run sprint interval training improves aerobic performance but not max cardiac output. *Medicine and Science in Sport and Exercise,* published ahead of print.

MACHEFER, G., GROUSSARD, C., VINCENT, S., ZOUHAL, H., FAURE, H., CILLARD, J., RADÁK, Z. & GRATAS-DELAMARCHE, A. 2007. Multivitamin-mineral supplementation prevents lipid peroxidation during "the Marathon des Sables". *J Am Coll Nutr,* 26**,** 111-20.

MACLARON, D. & MORTON, J. 2011. Biochemistry for sport and exercise metabolism. 1stEd: John Wiley & Sons LTD: Chichester: West Sussex UK.

MACMAHON, J. R. 1990. The psychological benefits of exercise and the treatment of delinquent adolescents. *Sports Med,* 9**,** 344-51.

MAFFETON, P. The Big Book of Endurance Training and Racing. Skyhorse Publishing, New York, NY/US.

MAGALHAES, J., FERREIRA, R., MARQUES, F., OLIVERA, E., SOARES, J., ASCENSAO, A. 2007. Indoor climbing elicits plasma oxidative stress. M*edicine and Science in Sports and Exercise,* 39(6), 955–963.

MANUEL Y KEENOY, B., VERTOMMEN, J. & DE LEEUW, I. 2001. Divergent effects of different oxidants on glutathione homeostasis and protein damage in erythrocytes from diabetic patients: effects of high glucose. *Mol Cell Biochem,* 225**,** 59-73.

MARGARITIS, I., TESSIER, F., RICHARD, M. J. & MARCONNET, P. 1997. No evidence of oxidative stress after a triathlon race in highly trained competitors. *Int J Sports Med,* 18**,** 186-90.

MARZATICO, F., PANSARASA, O., BERTORELLI, L., SOMENZINI, L. & DELLA VALLE, G. 1997. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J Sports Med Phys Fitness,* 37**,** 235-9.

MASTALOUDIS, A., LEONARD, S. W. & TRABER, M. G. 2001. Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med,* 31**,** 911-22.

MATTSON, M. P. 2008. Dietary factors, hormesis and health. *Ageing Res Rev,* 7**,** 43-8.

MAYNE, S. T., WRIGHT, M. E. & CARTMEL, B. 2004. Assessment of antioxidant nutrient intake and status for epidemiologic research. *J Nutr,* 134**,** 3199S-3200S.

MCANULTY, S.R., OWENS, J.T., MCANULTY, L.S., NIEMAN, D.C. MORROW, J.D., DUMKE, C.L., MILNE, G.L. 2007. Ibuprofen use during extreme exercise: effects on oxidative stress and PGE2. *Med Sci Sports Exerc,* 39(7),1075-1079.

MCBRIDE, J.M., KRAEMER, W.J., TRIPLETT – MCBRIDE, T. SEBASTIANELLI, W. 1998. Effect of resistance exercise on free radical production. *Med Sci Sports Exerc*, 30, 67-72.

MCCORD, J.M. FRIDOVICH, I. 1969. An enzymatic function for erythrocuprein (hemocuprein). *The J of Bio Chem,* 224, 6049 – 6055.

MEURET, J.R. 2007. A comparison of the effects of continuous aerobic, intermittent aerobic, and resistance exercise on resting metabolic rate at 12 and 21 hours post-exercise. *Medicine & Science in Sports & Exercise,* 39(5), 247.

NÉVE, J. 1989. Sélénium et vieillissement. *J. Med. esthet. Chirurg. Dermat,* 26, 185–190.

NIEMAN, D. C., HENSON, D. A., MCANULTY, S. R., MCANULTY, L., SWICK, N. S., UTTER, A. C., VINCI, D. M., OPIELA, S. J. & MORROW, J. D. 2002. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *J Appl Physiol (1985),* 92**,** 1970-7.

NIKI, E. 2010. Assessment of antioxidant capacity in vitro and in vivo. *Free Radic Biol Med,* 49**,** 503-15.

NIMMO, M. A., LEGGATE, M., VIANA, J. L. & KING, J. A. 2013. The effect of physical activity on mediators of inflammation. *Diabetes Obes Metab,* 15 Suppl 3**,** 51-60.

OH-ISHI, S., KIZAKI, T., OOKAWARA, T., SAKURAI, T., IZAWA, N., OHINO, H. 1997. Endurance training improves the resistance of rat diaphragm to exercise induced oxidative stress. *Am. J. Resp. Crit. Care Med,* 156,1579 – 1585.

ORTEGA, F., TORAL, J., CEJUDO, P., VILLAGOMEZ, R., SÁNCHEZ, H., CASTILLO, J. & MONTEMAYOR, T. 2002. Comparison of effects of strength and endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med,* 166**,** 669-74.

ORTENBLAD, N., MADSEN, K. & DJURHUUS, M. S. 1997. Antioxidant status and lipid peroxidation after short-term maximal exercise in trained and untrained humans. *Am J Physiol,* 272**,** R1258-63.

OZAWA, T. 1999. Mitochondrial genome mutation in cell death and aging. *J Bioenerg Biomemb*, 31,377-390.

OZTASAN, N., TAYSI, S., GUMUSTEKIN, K., ALTINKAYNAK, K., AKTAS, O., TIMUR, H., SIKTAR, E., KELES, S., AKAR, S., AKCAY, F., DANE, S. & GUL, M. 2004. Endurance training attenuates exercise-induced oxidative stress in erythrocytes in rat. *Eur J Appl Physiol,* 91**,** 622-7.

PALAZZETTI, S., ROUSSEAU, A. S., RICHARD, M. J., FAVIER, A. & MARGARITIS, I. 2004. Antioxidant supplementation preserves antioxidant response in physical training and low antioxidant intake. *Br J Nutr,* 91**,** 91-100.

PANDEY, K.B., RIZVI, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2(5), 270 – 280.

PATRICK, L. 1999. Nutrients and HIV: part one – beta carotene and selenium. *A Journal of Clinical Therapeutic*, 4(6), 403-413.

POLLOCK, M.L. 2000. Resistance exercise in individuals with and without cardiovascular disease: Benefits, rationale, safety, and prescription. *Circulation*, 10, 828–33

POWERS, S.K., LENNON, S.L. 1999. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *The proceedings of Nutrition Society*, 58, 1025-1033.

PRYOR, W.A. 1987. The free radical theory of aging revisited: A critique and a suggested disease specific theory, in: Modern Biological Theories of Aging, Eds H. R. Warner et al. Raven Press, New York pp. 89–112.

RADÁK, Z., NAITO, H., KANEKO, T., TAHARA, S., NAKAMOTO, H., TAKAHASHI, R., CARDOZO-PELAEZ, F. & GOTO, S. 2002. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Pflugers Arch,* 445**,** 273-8.

RADAK, Z., SASVARI, M., NYAKAS, C., KANEKO, T., TAHARA, S., OHNO, H. & GOTO, S. 2001. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. *Neurochem Int,* 39**,** 33-8.

REEVES, M.A. & HOFFMAN, P.R. 2009. The human selenoproteome: recent insights into functions and regulation. *Cell Mol Life Sci,* 66(15), 2457 – 2478.

REID, M.B. 2001. Invited Review: Redox modulation of skeletal muscle contraction: what we know and what we don't. *Journal of Applied Physiology,* 90(2), 724 – 731.

REMACLE, J., MICHIELS, C., RAES, M. 1992. In: Emeritt, I., Chance, B. (Eds.). Free Radicals and Aging. Birkhauser,Basel

RIMM, E.B., ASCHERIO, A., GIOVANNUCCI, E. 1996. Vegetable, fruit and cereal fibre intake and risk of coronary heart disease among men. *JAMA* 275, 447 – 451.

RISTOW, M., ZARSE, K., OBERBACH, A., KLÖTING, N., BIRRINGER, M., KIEHNTOPF, M., STUMVOLL, M., KAHN, C. R. & BLÜHER, M. 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A,* 106**,** 8665-70.

RUIZ-LARREA, M. B., GARRIDO, M. J. & LACORT, M. 1993. Estradiol-induced effects on glutathione metabolism in rat hepatocytes. *J Biochem,* 113**,** 563-7.

SACHECK, J. M., DECKER, E. A. & CLARKSON, P. M. 2000. The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes. *Eur J Appl Physiol,* 83**,** 40-6.

SANG-CHEOL, B., SOO, J.K., MI, K.S. 2003. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. J. *Am. College Nutr,* 22, 311-315.

SASTRE, J., BORRAS, C., GARCIA-SALA, D., LLORET, A., PALLARDO, F.V., VINA, J. (2002) Mitochondrial damage in aging and apoptosis. *Ann N Y Acad Sci* 959:448-451.

SAVORY, L.A., KERR, C.J., WHITING, P., FINER, N., MCENENY, J., ASHTON, T. (2012) Selenium supplementation and Exericse: Effect of Oxidant Stress in Overweight Adults. *Obesity (Silver Spring*), 20(4),794-801

SCHULZ, T.J., ZARSE, K., VOIGT, A., URBAN, N., BIRRINGER, M., RISTOW, M. 2007. Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab*, 6(4),280-293

SEIFRIED, H.E., ANDERSON, D.E., FISHER, E.I., MILNER, J.A. 2007. A review of the interaction among dietary antioxidants and reactive oxygen species. *Journal of Nutritional Biochemistry,* 18,567-579

SHWARTZ, K., FOLTZ, C.M. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J Am Chem Soc,* 79, 3292-3293.

SIMON-SCHNASS, I., PABST, H. 1988. Influence of vitamin E on physical performance. *Int J Vitam Nutr Res*, 58, 49-54.

SMITH, A.E., WALTER, A.A., GRAEL, J.L., KENDALL, K.L., MOON, J.R. 2009. Effects of b-alanine supplementation and high intensity interval training on endurance performance and body composition in men: a double blind trial. *Journal of international society of sports nutrition*, 6:5.

SPALLHOLZ, J.E., BOYLAN, L.M., LARSEN, H.S. 1990. Advances in understanding selenium's role in the immune system. *Ann NY Acad Sci*, 587, 123–39.

SPURWAY, N., MACLAREN, D. 2007) Nutrition and Sport, 1st Ed, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto,153 – 173.

SUNDE, R.A. 2012Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. Modern Nutrition in Health and Disease. 11th ed. Philadelphia, PA, Lippincott Williams & Wilkins,225-37

SUTHERLAND, W.H., MANNING, P.J., WALKER, R.J. 2007. Vitamin E supplementation and plasma 8-isoprostane and adionectin in overweight subjects. *Obesity (Silver Spring*), 15,386 – 391.

TALANIAN, J. L., GALLOWAY, S. D., HEIGENHAUSER, G. J., BONEN, A. & SPRIET, L. L. 2007. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol (1985),* 102**,** 1439-47.

TAULER, P., AGUILÓ, A., GIMENO, I., FUENTESPINA, E., TUR, J. A. & PONS, A. 2006. Response of blood cell antioxidant enzyme defences to antioxidant diet supplementation and to intense exercise. *Eur J Nutr,* 45**,** 187-95.

TAULER, P., AGUILÓ, A., GIMENO, I., NOGUERA, A., AGUSTÍ, A., TUR, J. A. & PONS, A. 2003. Differential response of lymphocytes and neutrophils to high intensity physical activity and to vitamin C diet supplementation. *Free Radic Res,* 37**,** 931-8.

TIIDUS, P. M. 2000. Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol,* 25**,** 274-87.

TIIDUS, P. M., BOMBARDIER, E., HIDIROGLOU, N. & MADERE, R. 1999. Gender and exercise influence on tissue antioxidant vitamin status in rats. *J Nutr Sci Vitaminol (Tokyo),* 45**,** 701-10.

TIIDUS, P.M. 2000. Estrogen and gender effects on muscle damage, inflammation, and oxidative stress.*Can J Appl Physiol*, 25(4),274-287.

TOLONEN, M., SARNA, S., KEINONEN, M., HALME, M., NORDBERG, U.R. 1989. Antioxidant supplementation reduces lipid peroxides in the elderly. A randomized double blind one year study, in: Selenium in Medicine and Biology, Eds J. Nève and A. Favier. W. de Gruyter, Berlin/New York pp. 329–332.

TSAI, K., HSU, T. G., HSU, K. M., CHENG, H., LIU, T. Y., HSU, C. F. & KONG, C. W. 2001. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic Biol Med,* 31**,** 1465-72.

URGAS, A.F. 2012. Effect of high intensity interval training on elite athletes' antioxidant status. *Sci Sports*, 28 (5), 253-259.

URSINI, F., HEIM, S., KIESS, M. 1999. Dual function of the selenoprotein PHGPx3during sperm maturation. *Science,* 285, 1393 – 1396.

URSO, M.L., CLARKSON, P.M. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189(1–2),41-54

VAN MECHELEN, W., HLOBIL, H., KEMPER, H. C., VOORN, W. J. & DE JONGH, H. R. 1993. Prevention of running injuries by warm-up, cool-down, and stretching exercises. *Am J Sports Med,* 21**,** 711-9.

VINCENT, H. K., MORGAN, J. W. & VINCENT, K. R. 2008. Effects of Antioxidant Supplementation on Insulin Sensitivity, Endothelial Adhesion Molecules and Oxidative Stress in Normal Weight and Overweight Young Adults. *Metabolism,* 58(2), 254-262.

WANG, Y., CHUN, O. K. & SONG, W. O. 2013. Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. *Nutrients,* 5**,** 2969-3004.

WATSON, T. A., CALLISTER, R., TAYLOR, R. D., SIBBRITT, D. W., MACDONALD-WICKS, L. K. & GARG, M. L. 2005. Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. *Med Sci Sports Exerc,* 37**,** 63-71.

WOOTEN-BEARD, P.C. RYAN, L. 2011. Improving Public Health? The role of antioxidant rich fruit and vegetables. *Food Research International*, 44,3135-3148.

YANG, G. Q. 2006. Keshan disease and mitochondrial cardiomyopathy. *Science in China Series C: Life Sciences*, 49(6), 513-518

YANG, G.Q. CHEN, J.S., WEN, Z.M. GE, K.Y. ZHU, L.Z., CHEN, X.C. 1984. The role of selenium in Keshan disease. *Adv Nutr Res,* 12, 203-231.

YOUNG, I.S., WOODSIDE, J.V. (2001) Antioxidants in health and disease. *J Clin Pathol*, 54, 176-186.

Appendix A1

Patient Identification Number for this trial_____

CONSENT FORM

Title of Project: Impact of HIIT (High Intensity Interval Training) and/or selenium supplementation (Se) on oxidative stress and anti-oxidant status in active females.

Principal Investigator: Karen Keane

Supervisors: Dr. Giovanna Bermano, Dr. Katherine Burgess and Dr. Eimear Dolan**.**

	Please initial box
1. I have read the participant information sheet on the above study and have had the opportunity to discuss the details with and ask questions. The principal investigator has explained to me the nature and purpose of the tests to be undertaken. I understand fully what is proposed to be done.	☐
2. The individual results of all testing will not be available to anyone outside the researchers undertaking the study, including yourself the participant.	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.	☐
4. Anonymised data collected during the study may only be accessed by members of the research team from Robert Gordon University. I give permission for these individuals to have access to my records.	☐
5. I agree to take part in the above study.	☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent (If different from Researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix A2

Participant Information Sheet

Title: Impact of HIIT (High Intensity Interval Training) and/or selenium supplementation (Se) on oxidative stress and anti-oxidant status in active females.

We would like to invite you to help us with some research investigating the effect of High Interval Intensity Training (HIIT) and Selenium Supplementation on alleviating oxidative stress and improving antioxidant defences. This research is being carried out at Robert Gordon University. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The over production of modified oxygen molecules, known as oxidative stress, has been shown to damage the cells that form our body. Oxidative stress is thought to be involved in the development of several chronic diseases including diabetes, rheumatoid arthritis, and muscular dystrophy among others. When we exercise, the body produces some of these molecules and if our body is not able to deal with them promptly, oxidative damage may occur. Several studies have looked at different methods of reducing exercise induced oxidative stress. Both antioxidant supplementation and regular training have been shown to increase our ability to counteract these molecules and in turn reduce the extent of oxidative stress. There is very little literature on HIIT training and even less on supplementation with the mineral selenium as regards alleviating this potential stress. Both are methods that could easily be incorporated into a person's daily life, through diet or training if shown to be successful.

Why have I been chosen?

We are approaching all females aged between 18 – 30y who are involved in intermittent sports within RGU. We are aiming to recruit 40 volunteers in total.

Do I have to take part?

No. It is up to you to decide whether to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw from the study at any time and without giving a reason. If you do participate, you can be assured that the information that you provide us will be treated with confidentiality and that the study has been submitted to the Robert Gordon University Ethics Committee to ensure that the correct procedures are being followed.

What will happen if I decide to take part?

If you decide to take part in this study, you would be allocated to one of three groups. Sometimes because we do not know which intervention is best, we need to make

comparisons. Subjects will be put into groups and compared. The groups are selected randomly, with no information about the individual. Participants in each group have a different treatment and these are compared. This is known as a randomized trial. The groups are as follows;

1. A Selenium Group 3. A HIIT Group 4. A Selenium + HIIT Group.

We would then ask you to take part in a series of fitness tests consisting of 10 and 20m sprints, T – test, Countermovement Jump, Sit ups, and a Yo-Yo intermittent shuttle run to test the different components of fitness.. After an initial visit we would ask that you come back for a second visit to perform a single bout of HIIT exercise. This would comprise of a modified Wingate test which involves 30 seconds of cycling at 60 revolutions per minute (rpm) against a high resistive force on a specialized ergometer. Each rep will be performed four times, separated by four minutes of recovery. You will be asked to give a sample of blood before and after the bout of exercise (15ml in total equivalent to a table spoon) from which we can identify certain markers of oxidative stress and anti-oxidant status.

Participants will then be asked to take part in a 3 week intervention depending on their group assignment. The supplementation group will involve ingesting a tablet daily, one containing a selenium salt, the other a dummy treatment, which looks like the real thing but contains no active ingredient for 21days. The exercise groups will be asked to attend a specialized HIIT training session at least twice a week for 3 weeks. The HIIT sessions will consist of the "Sprint8" protocol and a similar protocol replicated on a cycle ergometer. This "Sprint8" protocol is a series of sprints, which will vary in both duration and recovery time depending on intervention stage. Initially both protocols will involve 30seconds of all out maximal effort sprints followed by a minute and a half rest. This will be repeated 8 times on both modes of exercise, 16 sprints in entirety. After the period of intervention you will be asked to come back for two more visits where both the fitness test(visit 3) and the single bout of HIIT (visit 4) will be re-tested with blood samples.

What do I have to do?

If you agree to take part, we will ask you to sign a consent form. We will ask that you try and maintain your normal diet and physical activity levels over the course of the study. However, we will also ask you to record a food diary to monitor any changes in habit. This will involve recording what you eat and drink over a 3 day period including on day of the weekend. The purpose of this is to monitor any significant changes in diet that may directly impact results.

What are the possible risks of taken part?

You could experience some minor discomfort when the blood is taken but this tends to pass quickly. This procedure is very safe. HIIT training involves exercising at a value at or close to your maximum aerobic capacity ($Vo_{2\ max}$) and is said to be rather challenging and exhausting. However, research has shown that HIIT training is suitable for de - conditioned individuals of all ages (Hwang et al., 2011), and therefore should be well within the capabilities of physically active individuals. Selenium supplementation has been used in several studies and the tablets used are available in health shops or chemists to be bought and used by the general public without any guidance.

What are the possible benefits of taken part?

The information we get from the study will help us to determine whether HIIT and / or Selenium supplementation has the ability to alleviate exercise induced oxidative stress and improve antioxidant defences. For these particular participants who are involved in intermittent sports teams, this information may be of use and results may have long lasting effects on dietary intake and training.

Will my taken part in this study be kept confidential?

All information, which is collected, about you during the course of the research will be kept strictly confidential. The blood samples will be immediately coded so that your identity will be erased and anonymity ensured. The personal data will not be held in any format that would allow anyone to trace information back to you. Only the people involved in the study will have access to this information.

What will happen to the samples in the future?

The blood samples you provide are very valuable and we would like to keep them for use in further studies. If you do not wish us to keep your sample after the end of the study, they will be destroyed immediately upon completion of the study.

Thank you for taking the time to consider participating in our study. If you need any more information about the study please contact: Karen Keane at 1211745@rgu.ac.uk or 01224 262871.

If a problem should arise please feel free to discuss it with myself or my principal supervisor, Dr Giovanna Bermano. Our contact details can be found at the bottom of this letter. If you have a complaint please send details of this to Dr Giovanna Bermano in the first instance.

Appendix A3

Dear Student,

You have been invited to take part in a study investigating the effect of High Interval Intensity Training (HIIT) and Selenium Supplementation on alleviating oxidative stress and improving antioxidant defences. You have been chosen based primarily on your involvement in intermittent sports within RGU.

Contact has already been made with your manager and / or captain(s) and they have given this study their full support. There are many potential benefits associated with this study. The study may have direct impact on aspects such as your diet, health, training and performance. Have important competitions coming up in the next few months? Those who take part may find it a good way to get back into shape and get fit following the festive season. If nothing else, this study offers two free fitness testing sessions.

For more in depth detail and information on the current study, please find the patient information sheet and advertisement attached. Before you decide if you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the attached information carefully and discuss it with others if you wish. Contacts are provided so ask us if there is anything that is not clear, or if you have any further enquiries.

Please respond to this email if you are interested in taking part.

I look forward to hearing from you.

Karen Keane

Appendix A4

VOLUNTEERS WANTED

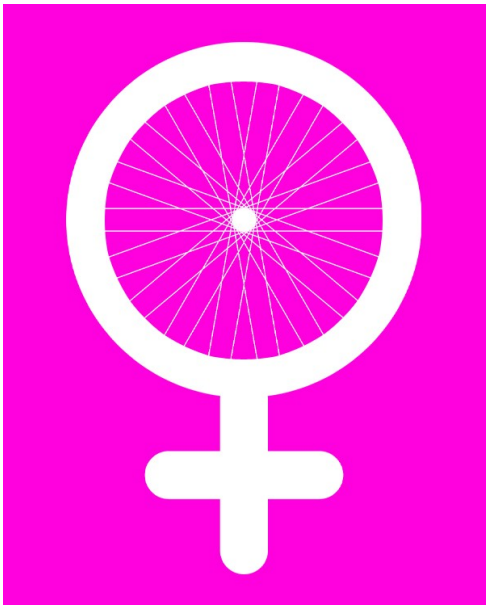
Who are we looking for?

If you are a physically active female involved in intermittent sports at RGU, injury free, aged between 18 – 30y and would like to get involved in a project aiming to examine the impact of Selenium Supplementation and High Intensity Interval Training on oxidative stress and fitness levels, volunteer for this project.

Where?

Robert Gordon University, Garthdee Campus

For more information or enquiries, please contact Karen Keane (1211745@rgu.ac.uk)



Appendix A5

Physical Activity Readiness Questionnaire (PAR-Q)

Many health benefits are associated with regular exercise, and the completion of PAR-Q is a sensible first step to take if you are planning to increase the amount of physical activity in your life.

For most people, physical activity should not pose any problem or hazard. PAR-Q is designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them. Common sense is the best guide in answering these few questions.

1. Has your doctor ever said that you have a bone or joint problem, such as arthritis, that has been aggravated by exercise or might be made worse with exercise? **Yes/No**
2. Do you have high blood pressure? **Yes/No**
3. Do you have low blood pressure? **Yes/No**
4. Do you have Diabetes Mellitus or any other metabolic disease? **Yes/No**
5. Has your doctor ever said that you have raised cholesterol (serum level above 6.2mmol/L)? **Yes/No**
6. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by your doctor? **Yes/No**
7. Have you ever felt pain in your chest when you do physical exercise? **Yes/No**
8. Is your doctor currently prescribing you drugs or medication? **Yes/No**
9. Have you ever suffered from unusual shortness of breath at rest or with mild exertion? **Yes/No**
10. Is there any history of Coronary Heart Disease in your family? **Yes/No**
11. Do you often feel faint, have spells of severe dizziness or have lost consciousness? **Yes/No**
12. Do you currently drink more than the average amount of alcohol per week (21 units for men and 14 units for women)? **Yes/No**
13. Do you currently smoke? **Yes/No**
14. Do you currently exercise on a regular basis (at least 3 times a week) and work in a job that is physically demanding? **Yes/No**
15. Are you, or is there any possibility that you might be pregnant? **Yes/No**
16. Do you know of any other reason why you should not participate in a programme of physical activity? **Yes/No**

If YES please give details _____

_____

If you answered: **YES to one or more questions:**

If you have not recently done so, consult with your doctor by telephone or in person before increasing your physical activity and/or taking a fitness appraisal. Tell your doctor what questions you answered “yes” to on PAR-Q or present your PAR-Q copy. After medical evaluation, seek advice from your doctor as to your suitability for:

1) Unrestricted physical activity starting off easily and progressing gradually, and

2) Restricted or supervised activity to meet your specific needs, at least on an initial basis.

NO to all questions:

If you answered PAR-Q accurately, you have reasonable assurance of your present suitability for:

1) A graduated exercise programme

2) A fitness appraisal

Assumption of Risk

I hereby state that I have read, understood and answered honestly the questions above. I also state that I wish to participate in activities, which may include aerobic exercise, resistance exercise and stretching. I realise that my participation in these activities involves the risk of injury and even the possibility of death. Furthermore, I hereby confirm that I am voluntarily engaging in an acceptable level of exercise, which has been recommended to me.

Client's Name:	Trainer's Name:
Client's Signature:	Trainer's Signature:
Date:	Date:

Additional note: I have taken medical advice and my doctor has agreed that I should exercise.

Signature:_____ Date: _____

Appendix A6

General Practice Physical Activity Questionnaire

1. Please tell us the type and amount of physical activity involved in your work. Please tick one box that is closest to your present work from the following five possibilities:

		Please mark one box only
a	I am not in employment (e.g. retired, retired for health reasons, unemployed, full-time carer etc.)	
b	I spend most of my time at work sitting (such as in an office)	
c	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, childminder, etc.)	
d	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
e	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. During the *last week*, how many hours did you spend on each of the following activities?

Please answer whether you are in employment or not

Please mark one box only on each row

		None	**Some but less than 1 hour**	**1 hour but less than 3 hours**	**3 hours or more**
a	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
b	Cycling, including cycling to work and during leisure time				
c	Walking, including walking to work, shopping, for pleasure etc.				
d	Housework/Childcare				
e	Gardening/DIY				

3. How would you describe your usual walking pace? Please mark one box only.

Slow pace (i.e. less than 3 mph)		Steady average pace	
Brisk pace		Fast pace (i.e. over 4mph)	

Hit 'Return' to calculate PAI

Appendix A7

Selenium Questionnaire

Private and Confidential

Congratulations on completing your assigned intervention successfully. This questionnaire is a follow up procedure focusing on Selenium.

Whilst supplementing, did you experience any of the following:

□ Allergies □ Changes in Appetite □ Asthma or breathing problems □ Bowel problems □ Ear, Eye, Nose or Throat problems □ Eczema or skin conditions □ Menstrual / Menopausal problems □ Sleep problems □ Urinary tract conditions

Please provide as much details as possible with regards the above condition(s) e.g. details including onset, and if applicable cessation dates, symptoms etc.

_____
_____
_____
_____
_____

In your opinion, did you experience any <u>positive</u> responses to selenium? Would you consider selenium supplementation again in the future? Explain.

_____
_____
_____
_____
_____

Appendix A8

Individual Test Protocols

Speed – 10 and 20m Sprints

Subjects will perform both 10 and 20m sprints. Both markers and timing gates will be placed at 0, 10 and 20metres. Timing gates use infra – red signal and detectors to record when the beam is broken. Subjects will use a standing start at 5m behind the starting line (0m) and when instructed they will perform a maximal sprint over 20m, passing through the 10m en route. Times at both the 10m and 20m will be recorded. Each subject will be allocated to a group of three. The subject will be given a rest between reps while the other two members of their group are performing the test. Each subject will perform the test three times.

Agility – T-Test

The set - up of the T-Test is shown in the Figure 2.1. Each participant will begin at cone A, where there will be electronic timing gates set up. Timing gates use infra – red signal and detectors to record when the beam is broken. Subjects will use a standing start. Subjects will be required to sprint to cone B. Continuing facing forward, the subject will shuffle sideways to touch cone C, then shuffle sideways again till they reach cone D. The subject will then shuffle laterally back to cone B, before running backwards through the gates and past cone A. The test is to be performed at maximal effort 3 times.. Each subject will be allocated to a group of 3. The subject will be given a rest between reps while the other 2 members of their group are performing the test. Data will be recorded in seconds to the nearest hundredth of a second.

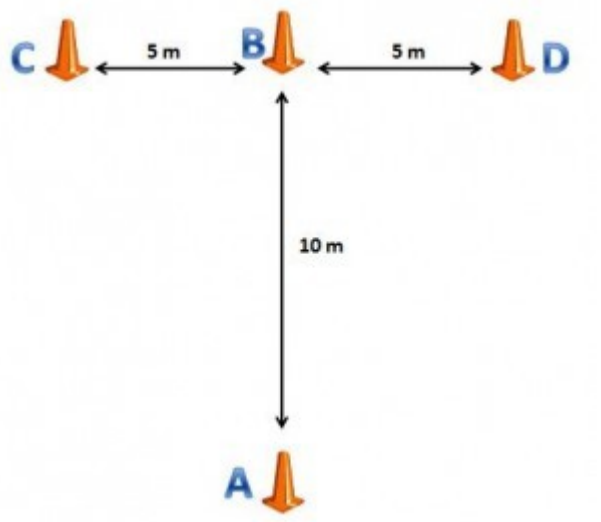


Fig 2.1 Agility T-Test Set up

Explosive Leg Power – Countermovement Jump (CMJ)

To determine maximal jump height, the countermovement jump will be used on a Smartspeed ™ jump mat. For the CMJ technique, the subject starts from an upright standing position, making a preliminary downward movement by flexing at the knees, hips and ankles, then immediately extending the knees, hips and ankles to jump vertically up off the ground. The subject will be instructed to keep their arms on their hips during the trial to

ensure power is coming from the lower extremity only. The peak height of the jump will be recorded in cm. Each subject will have 3 trials and the best height will be used.

Muscular Strength and Endurance –Sit ups

Subjects will be instructed to lie in a supine position, with knees bent at 90°. The arms are held flat across the chest, with the hands placed on opposite shoulders. The subject raises the trunk, keeping the arms in position, and curling up to touch their elbows to thighs and then lowers back to the floor so that the shoulder blades (upper back) touch the floor. They will be asked to complete as many sit ups as possible in one minute or until exhaustion, whichever occurs first.

Aerobic Capacity (Vo_{2Max}) – Yo Yo Intermittent Beep Test

The Yo - Yo intermittent test is a variation of the regular beep test. Cones will be used to mark out three lines as illustrated in Figure 2.2. Participants will be required to start on the middle line, and begin running the 20m on the first signal, which comes in the form of a loud beep. The subjects will run forward at a speed of ~8.5km/hr. The running pace will be regulated by a CD through a series of beeps. The subject turns and returns to the starting point when signalled by the next recorded beep. There is an active recovery period (5 seconds respectively) interjected between every 40m. During this recovery phase, subjects must either jog or walk around the other cone marked 5m away and return to the starting line ready to go again. Participants will be instructed prior to the assessment that it is beneficial to adjust their pace so that they turn at the line at the same time as the emitted tones. The running speed increases by 0.5km/hr every minute, representing a completed stage. Participants will be asked to give maximal effort and go until exhaustion. The test terminates when they either voluntarily withdraw, or if they are unable to maintain the required pace. Not being able to maintain the required pace is defined as being unable to reach the correct line on two successive occasions when the beep sounded. The level at which the subjects drop out or were deemed to have missed two consecutive lines will be recorded and later used to calculate an estimate of VO2max using a conversion table.

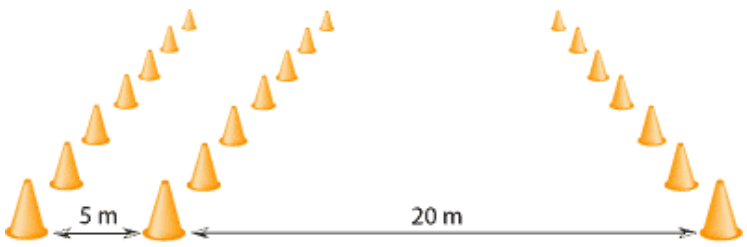


Fig 2.2 Yo – Yo Intermittent Beep Test Set u

Appendix A9

3 – Day Food Journal

[Must include: 2 weekdays and 1 day at the weekend]

Name: _____

Dates: _____

Time	Food Item	Amount
Record time of meal or snack *	Include brand names, restaurant names and method of preparation (such as baked, boiled or fried). Include all condiments i.e., ketchup, dressings. Include all beverages.	Use common kitchen terms: cup, teaspoon, ounces, cans etc.
Breakfast – day1		
AM Snack – day 1		
Lunch – day1		
MID Snack – day1		
Dinner – day1		
PM Snack – day1		

**if meal or snack was skipped, simply leave blank space*

Appendix A10

Sprint 8 Protocol – Running and Cycling

Week 1

Time	Intervals
0 – 30	**Sprint #1**
30 – 2	Rest
2 – 2.30	**Sprint #2**
2.30 – 4	Rest
4 – 4.30	**Sprint #3**
4.30 – 6	Rest
6 – 6.30	**Sprint #4**
6.30 – 8	Rest
8 – 8.30	**Sprint #5**
8.30 – 10	Rest
10 – 10.30	**Sprint #6**
10.30 – 12	Rest
12 – 12.30	**Sprint #7**
12.30 – 14	Rest
14 – 14.30	**Sprint #8**
Finish	

Week 2

Time	Intervals
0 – 30	**Sprint #1**
30 – 1.30	Rest
1.30 – 2	**Sprint #2**
2 – 3	Rest
3.30 – 4	**Sprint #3**

4 – 5	Rest
5 – 5.30	**Sprint #4**
5.30 – 6.30	Rest
6.30 – 7	**Sprint #5**
7 -8	Rest
8.30 – 9	**Sprint #6**
9 – 10	Rest
10 – 10.30	**Sprint #7**
10.30 – 11.30	Rest
11.30 – 12	**Sprint #8**
Finish	

Week 3

Time	Intervals
0 – 40	**Sprint #1**
40 – 1.40	Rest
1.40 – 2.20	**Sprint #2**
2.20 – 3.20	Rest
3.20 – 4	**Sprint #3**
4 – 5	Rest
5 – 5.40	**Sprint #4**
5.40 – 6.40	Rest
6.40 – 7.20	**Sprint #5**
7.20- 8.20	Rest
8.20 – 9	**Sprint #6**
9 – 10	Rest
10 – 10.40	**Sprint #7**
10.40 – 11.40	Rest
11.40 – 12.20	**Sprint #8**
Finish	