THE EFFECTS OF SELENIUM AND TOCOTRIENOL SUPPLEMENTATION ON THE MUSCLE CONTRACTILE PROPERTIES, FATIGUE, EMG AND ANTIOXIDANT STATUS OF GASTROCNEMIUS MUSCLE OF TRAINED AND UNTRAINED RATS

MOHAND R. ALWAN

Degree of Master of Science

UNIVERSITI SAINS MALAYSIA

2009

THE EFFECT OF SELENIUM AND TOCOTRIENOL SUPPLEMENTATION ON THE MUSCLE CONTRACTILE PROPERTIES, FATIGUE, EMG AND ANTIOXIDANT STATUS OF GASTROCNEMIUS MUSCLE OF TRAINED AND UNTRAINED RATS

BY

MOHAND R. ALWAN

Thesis Submitted In Fulfillment of Requirement
For The Degree of Master of Science
(Sports Science)

UNIVERSITI SAINS MALAYSIA

2009

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my Supervisor, Dr. Oleksandr Krasilshchikov and co-supervisor, Dr. Tengku Muzaffar bin Tengku Mohamad Shihabudin for their continuous advice and guidance throughout this research project.

I would like to thank Dr. Ooi Foong Kiew for their assistance in designing the rats jumping training programme. I would also like to acknowledge all the staff of the Sports Science Unit, Universiti Sains Malaysia and the laboratory staff: Ms. Jamaayah bt. Meor Osman, Ms. Pn Nik Sakinah, Mr. Nawawi b. Yasin, Mr. Rozaid b. Musa and Ms. Ham Siew Ling for their helpful assistance throughout this research. Also thanks to all staff of Physiology Laboratory for helping me and also the staff of Animal House, Universiti Sains Malaysia.

To my family, my Father and Mother for your support and encouragements, brothers and sister and also my nieces, thank you for your moral support, patience and all of your love during my MSc study. Thank you to all my friends for helping and supporting me and to all my classmates for working hard and for co-operating in this MSc. Lastly, I would like to thank Universiti Sains Malaysia for supporting my research project.

Table of Contents

Page

Table of Contents

APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SC…….....Sedentary Control

EC……Exercise Control

T ……Tocotrienol Supplementation Group

S…… Selenium Supplementation Group

TE……..Tocotrienol Supplementation Exercise Group

SE…….....Selenium Supplementation Exercise Group

TS……....Combined Selenium and Tocotrienol Supplementation Group

TSE……. Combined Selenium and Tocotrienol Supplementation Exercise Group

MG…… Gastrocnemius Muscle

Pt……Peak Twitch Tension

Po…….. .Peak Tetanus Tension

CT…….....Contraction Time

HRT……Half Relaxation Time

FI……. Fatigue Index

MW…….. Muscle Weight

ML…… Muscle Length

GPX……Glutathione Peroxidase

SOD…… Superoxide Dismutase

CAT…….. …… Catalase

MDA…….....Malondialdehyde

ABSTRACT

Exercise induces radical-mediated oxidative damage of skeletal muscle membranes which has been implicated in the fatigue process. Selenium (S) is an important component of cellular selenocompounds and is an integral component of glutathione Peroxidase (GPx), which catalyze the reduction of harmful peroxides. Tocotrienol (T) is a major chain breaking antioxidant that has been shown to reduce contraction mediated oxidative damage. We hypothesized that S and T supplementation would positively affect muscle contractile function and increase antioxidant enzymes activities during exercise. To test this postulate, were used sixty-four male Wistar-Kyoto rats at weight between 300-350g randomized into eight groups with eight rats per group rats these sample size was calculated using PS software based on comparing two means of superoxide dismutase (SOD).

The rats were fed with either S (80 µg/kg body weight) or T (8 mg/kg body weight) and also with combination of S and T diet in sedentary and jumping exercise which consisted from 40 jumps for 40 cm in height for 5 days per week for 6 weeks. Upon completion of the feeding period, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg); the sciatic nerve was located and cut and tendon of the TA muscle was cut close to the bone and attached to an isometric force transducer (AD Instrument, model 400) with computer-based data acquisition system (AD Instrument, Power Lab® and Chart software™.

The muscle contractile properties were measured, fatigue protocol consisted of trains of pulses composed of fourteen pulses recurring at 40 Hz repeated every 1 s for at least 2 min in order to assess sensitivity to fatigue. There were significant ($P<0.05$) differences in the muscle contractile properties (Pt, Po, Pt/Po Ratio and CT), also decrease in EMG failure and increase of fatigue index between S, T and control animals and this difference was still greater in combined supplementation of S and T group of exercised rats. The stimulated MG muscle from the exercised S and T supplemented animals had significantly ($P<0.05$) higher antioxidant enzymes activity in both GPx, CAT and decreased lipid hydroperoxidation as compared to the same muscles in control animals as well as in comparison with sedentary supplementation. Most effective results were observed in combined S and T supplementation animals in exercise group as compared with sedentary supplementation groups. This data support the hypothesis that S and T supplementation in exercised animals increases muscular endurance and improves muscle contractile properties following a prolonged series of contractions.

ABSTRAK

Kerosakan oksidatif membrane otot skeletal akibat daripada radikal telah memberi kesan kepada proses ketahanan. Komponen penting dalam selular kompaunselono ialah Selenium. Selenium juga merupakan komponen penting GPx dimana ia memangkin pengurangan peroksida yang berbahaya. Tokotrienol(T) merupakan rantaian huraian antioksida utama yang telah membantu daJam pengurangan kerosakan pengecutan akibat daripada oksidatif. Kami membuat hipotesis bahawa gantian S dan T akan memberi kesan positif kepada fungsi pengecutan otot dan meningkatkan ketahanan otot semasa bersenarn. Untuk menguji prinsip ini, tikus-tikus diberi diet samada S, T dan kombinasi kedua-duanya semasa fasa sedentari dan senaman. Selepas 6 minggu, tikus-tikus itu dibius dan pengudaraan secara mekanikal dilakukan.

Daya ketahanan otot dan ciri-ciri pengecutan dinilai menggunakan persediaan otot "gastrocniemus" tengah. Ciri-ciri pengecutan otot MG ditentukan dan protokol daya ketahanan dilakukan. Protokol daya ketahanan merupakan deretan denyutan nadi dimaua 14 denyutan berlaku /berulang pada tahap 40 Hz setiap 1 saat untuk sekurang-kurangnya 2 minit bagi tujuan menaksir tahap kesensitifan kepada daya ketahanan. Sebelum memulakan protokol daya ketahanan, terdapat perbezaan signifikan ($P<0.05$) yang tinggi wujud dalam ciri-ciri pengecutan otot, EMG dan penentangan daya ketahanan antara kumpulan diberi S, kumpulan diberi T dan tikus yang dikawal.

Sementara itu bagi kumpulan tikus bersenam yang diberi kombinasi S dan T, perbezaannya menunjukkan satu peningkatan. Otot MG tikus -tikus yang dirangsang daripada kumpulan yang diberi pemakanan S dan T menunjukkan aktiviti antioksidan yang tinggi dan penurunan dalam penanda peroksidasi lipid berbanding otot yang sama tikus-tikus daJam kumpulan yang dikawal ($P<0.05$). Hasil ini juga akan lebih baik keputusaannya bagi tikus-tikus yang sentiasa aktifberbanding dengan tikus yang berada dalam keadaan sedentari. Data ini menyokong hipotesis bahawa penggantian S dan T meningkatkan ketahanan otot dan mengubah ciriciri pengecutan otot selepas siri pengecutan yang lama.

CHAPTER 1

INTRODUCTION

1.1 Reactive Oxygen Species

Oxygen-derived free radicals or reactive oxygen species (ROS) are atoms or groups of atoms with an odd (unpaired) number of electrons formed when oxygen interacts with certain molecules (Evans, 2000). ROS are naturally produced in the body through oxidative metabolic processes, some time being useful as in situations where the activation of the immunologic system is required. For example, macrophages use the hydrogen peroxide to destroy bacteria and other microorganism. According to Halliwell, (1999) the most part of oxygen (O_2) that we breathe is metabolized in body organism and 2 to 5% remaining is univalent reduced into metabolites called reactive species of oxygen. ROS may be essential for normal physiologic control processes in muscle under quiescent conditions of low levels ROS appear to induce endogenous antioxidant production (Mohan *et al*., 1995; Atalay *et al., 1999*; Fang *et al.,* 2002; Aguilo *et al.,* 2005). Once formed these highly reactive radicals can start a chain reaction, like dominoes and their chief danger comes from the damage as they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs (Asayama and Kato, 1990; Andrade *et al.,* 1998; Ascensao *et al*., 2003).

ROS has been implicated in the pathogenesis of a wide spectrum of diseases as well as in the aging process. In addition playing a role in direct tissue damage and promote further cell injury (Thomas *et al*., 1999; Thomas and Stocker 2000; Ascensao *et al*., 2003). Production of reactive oxygen species have known to occur because of a few different mechanisms: Firstly, mitochondrial origins in which free radicals either escape scavenging enzymes or develop due to an error in oxidative processes. Secondly, inside the endothelium capillary where a hypoxic and reoxygenation process is created during intense exercise which are commonly mobilized as a result of the muscle or tissue damage which is well-documented with extended or eccentric-based exercise (Evans, 2000; Chariot and Bignani,2003; Boveris, and Navarro, 2008).

These products of oxidation formed within mitochondria during electron transport and cross cell membranes via anion channels (Reid *et al.,* 1992; Diaz *et al.,* 1993; Bejma and Ji, 1999; Bejma *et al.,* 2000). The intracellular and extracellular ROS increase during muscle contraction (Supinski *et al*., 1997; Reid *et al.*, 1998; Supinski and Callahan, 2006; Da Silva *et al*., 2009). An interesting new hypothesis regarding the source of ROS in contracting skeletal muscle has originated from the laboratory of Supinski *et al*., (1997) who has shown that ROS may be formed because of phospholipase A2 activation. Relatively specific phospholipase A2 inhibitors nearly eliminate the intracellular ROS produced during muscle contraction (Supinski *et al*., 1997; Devries *et al.,* 2008). However, oxidant stresses has been demonstrated under numerous pathological conditions in skeletal muscle, during periods of intense contractile activity (Anzueto *et al*., 1993; Finaud *et al.,* 2006; Pimenta *et al.,* 2007).

The ROS peroxidize polyunsaturated fatty acids, which cause loss of cell membrane integrity and increase microvasculature permeability (Reid *et al*., 1992; Finaud *et al.,* 2006; Devries *et al.,* 2008). Strenuous exercise may also produce levels of ROS high enough to overcome normal antioxidant defenses and denature proteins important in the contractile and excitation-contraction coupling (ECC) processes (Kolbeck *et al*., 1997; Bejma *et al.,* 1999; Margaritis *et al.,* 2003; Powers *et al.,* 2004; Devries *et al.,* 2008).

1. 2 Antioxidants

To protect cells from oxidative effect, there are many antioxidant factors. Antioxidant means against oxidation. Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules have been damaged. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. Although there are several enzyme systems within the body that scavenge free radicals like glutathione peroxidase, superoxide dismutase (SD), and catalase, antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil. There are two lines of antioxidant defense within the cell: The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene, and coenzyme Q (Wissam *et al*., 2000; Fang *et al.,* 2002; Fiskin *et al.,* 2006).

The second line is found inside the cell, where water-soluble antioxidant scavengers are present. These include vitamin C, glutathione peroxidase, superoxide dismutase (SOD), and catalase (Chow, 1992; Yu, 1993; Desai *et al.,* 2001; Chow, 2004; De Moffarts *et al.,* 2005; Dundar *et al.,* 2005). Vitamins A, C, E and the mineral selenium are the only antioxidants that can be commonly supplemented and those are required for proper functioning of one of the body's antioxidant enzyme systems (Goldfarb *et al*., 2005; Ji *et al*., 2006; Gomez-Cabrera e*t al*., 2008).

Major antioxidant mechanisms include: (1) interaction with oxidants and oxidizing agents by ascorbic acid and reduced glutathione (GSH); (2) scavenging of free radicals and singlet oxygen by vitamin E, ascorbic acid, beta-carotene, and superoxide dismutase (SOD). (3) Reduction in hydroperoxides by GSH peroxidases and catalase. (4) Binding of transition metals by various creators; and 5) repair of resultant damage via metabolic activities (Chow, 1992; Kim *et al*., 1996; Fang *et al.,* 2002; Yu *et al.,* 2003). Oxidative damage, however, may occur when antioxidant potential is decreased and/or when oxidative stress is increased. Free radical induced oxidative damage has implicated in the pathogenesis of a number of injuries and diseases states (Wissam *et al*., 2000; Gaeini *et al.,* 2006; Galassett *et al.,* 2006). Evolution of organisms in an oxygen-rich atmosphere has led to the development of endogenous physiological defense systems that cooperate to scavenge and detoxify ROS. In addition, a second line of defense is provided by exogenous antioxidants primarily obtained as nutrients or nutritional supplements.

An antioxidant may be defined as any substance that when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell *et al*., 2005; El-Demerdash *et al.,* 2004; Alvarado *et al.,* 2006). In this definition, the term “oxidizable substrate” embraces almost everything found in living cells, including proteins, lipids, carbohydrates, and DNA. Broadly, the possible mechanisms by which antioxidants may protect against oxygen toxicity are as follows: First - prevention of ROS formation. Second - interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack. Third - facilitate the repair of damage caused by ROS and triggering the expression of genes that encode antioxidant proteins and fourth-providing a favorable environment for the effective functioning of other antioxidants. Physiological substances have been known to have “antioxidant-like” functions (Sen and Hanninen, 1995; Sen, 2001; Alvarado *et al.,* 2006). However, the primary contributors are the enzymes superoxide dismutase, catalase and the glutathione system. Antioxidant acts as chain reaction like vitamins E, C, dihydrolipoic acid, and glutathione known to act synergistically in the form of an antioxidant chain reaction (Beilstein and Whanger, 1992; Sen *et al*., 1994, Sen and Hanninen, 1995; El-Demerdash *et al*., 2004; Leo *et al*., 2008).

1.3 Antioxidants supplementation

Glutathione dependent Selenium functions as a cofactor of glutathione peroxidase, an enzyme family that requires GSH as its substrate for the scavenging of hydrogen and other peroxides (Brady *et al.,* 1979; Burk, 1983, Apostolski *et al*., 1998; El -Demerdash, 2004; Battin *et al*., 2006; Catal and Bolkent, 2008; Burk *et al*., 2008; Battin and Brumaghim, 2009). Selenium deficiency had little effect when vitamin E was present (Brady *et al.,* 1978; Di Leo *et al.,* 2003; De Moffarts *et al*., 2005). The effect of combination of several antioxidants as supplements has only been examined in relatively few studies, the rats supplemented with vitamin E and Selenium supplementation increased the activity of the hydroperoxide metabolizing enzyme glutathione peroxidase (Brady *et al.,* 1979; Dzhandzhgava and Shakarishvili, 1991; Avanzo *et al*., 2001; Nagyova *et al.,* 2004; Goldfarb *et al.,* 2005; Fiskin *et al.,* 2006; Mikhal'chik *et al.*, 2006).

Leeuwenburgh and Ji, (1996) found supplementation of individuals with a vitamin mixture containing, beta-carotene, vitamin C, and vitamin E for 5 wk resulted in decreased serum MDA and breath pentane, decreased lipid peroxidation at rest and that induced by exercise at both 60 and 90% Vo_2 max, both vit E and C decrease oxidative stress parameters during exercise and this agree with more recent studies (Paduraru *et al*., 1997, Carr, 1999; Chao *et al.,* 2002). Design of antioxidant supplementation protocols should be guided by the requirements of the antioxidant chain reaction; such an approach will maintain a favorable redox status of each of the constituent antioxidants and avoid the accumulation of reactive oxidized antioxidant by-products (e-g., chromanoxyl radicals)**.**

However, it is still unclear whether antioxidant supplementation with exercise can improve muscle contractile properties and fatigue resistance. In addition, there have been no studies undertaken to determine effects of combined supplementation of selenium and tocotrienol on muscle contractile properties, muscular fatigue and changes in oxidative stress and antioxidant enzymes in sedentary and exercise supplementation.

1.4 Objectives of the Study

The main objective of the study was to determine the effects of supplementation of selenium and tocotrienol on gastrocnemius muscle properties of trained and untrained rats.

To achieve this general objective, the following specific objectives:

1. To evaluate the muscle contractile properties, EMG changes and fatigue resistance of gastrocnemius muscle under different supplementation schemes in sedentary and exercise protocols.
2. To evaluate the changes in antioxidant enzymes, glutathione peroxidase (GPx), catalase (CAT) and Superoxide dismutase (SOD) in gastrocnemius muscle under different supplementation schemes and without supplementations.
3. To evaluate the changes in lipid peroxidation in gastrocnemius muscle as an oxidative biomarker with sedentary and exercise protocols.

1.5 Hypothesis

The hypothesized are:

1. Jumping exercise combined with tocotrienol and selenium supplementation might be significantly increase muscle contractile properties and fatigue resistance in comparison with other exercise groups.

2. Supplementation with tocotrienol and selenium might be lead to significant change of lipid peroxidation parameters and antioxidant enzymes activity in exercise groups as compared to sedentary groups.

1.6 Significance of the Study

Research on dietary supplementation with tocotrienol and selenium and with or without jumping training is important to investigate their effect on the skeletal muscle contractile properties, resistance to fatigue and peroxidation phenomena. We compared malondialdehyde content as oxidative biomarker (as product of lipid peroxidation), and antioxidant enzymes activity (GPX, SOD and CAT) to demonstrate effect of tocotrienol and selenium with/or without exercise on lipidperoxidation and fatigue. The efective protocols of supplementation and exercise are developed and may be recommended to improve muscle endurance and fatigue resistance for exercise and sports activities with further research in humans that may contribute towards the development of athletic performance.

1.7 Limitations of the study

There were some limitations with respect to the analysis and data that may affect the accuracy of the results.The first limitation was related with the age of animals because in this study we have been used growing up animals at age range between 12-18 weeks, that mean we could not strictly control on weight of animals, muscle weight and muscle length.

CHAPTER 2

LITERATURE REVIEW

2.1 Free Radicals and Oxidative Stress

Except for strict anaerobes, most organisms utilize oxygen as an electron acceptor to oxidize the various metabolic substrates, so that stored energy is released for biological activities. During this process, most oxygen molecules are reduced to water, but a fraction of oxygen (2-5%) is univalent and is reduced to various intermediates representing the electron reductants of oxygen such as: Superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) (Halliwell *et al.,* 1999). These reactive oxygen species (ROS) have strong tendency of extracting electrons to reach chemically more stable structure and therefore are capable of eliciting serious damage to the various cellular components (Ames *et al.,* 2000; Ji *et al*., 2009). Cells also utilize ROS to assist in the elimination of xenobiotic compounds and organisms through phagocytosis, which involves a respiratory burst and $O_2^{\cdot-}$ production (Cannon, 1993; Alvarado *et al*., 2006; Cannon *et al*., 2007). Aerobic organisms would not survive without protective mechanisms counteracting the detrimental effects of ROS.

Thus, higher organisms have developed effective antioxidant systems during the course of evolution (Halliwell *et al*., 1999). In general, the cell has adequate antioxidant reserves to cope with the production of ROS under physiological conditions such that these toxic compounds do not accumulate.

The system consists of antioxidant vitamins (water-soluble ascorbic acid and fat-soluble a-tocopherol and b-carotene), thiol-containing, low-molecular weight compounds, mainly glutathione (GSH), and antioxidant enzymes, such as Superoxide dismutase (SOD), GSH peroxidase (GPX), and catalase (CAT). Each of these antioxidants plays a unique role in the cell and complements one another geographically and functionally (Ji *et al*., 1998; Urso and Clarkson ,2003; Alvarado *et al.,* 2006; Ji *et al*.,2009).

These antioxidant defense systems preserve homeostasis for normal cell function at rest and perhaps during mild oxidative stress. However, the protective margin of most antioxidants is probably very small. Therefore, when ROS production is excessive, or when the antioxidant defense is severely compromised due to nutritional deficiency or biochemical inhibition, extensive cell and tissue damage may occur, leading to various pathogenic conditions and/or aging (Ames *et al*., 2000; Andrade *et al.,* 1998; Ascensao *et al*., 2003; Urso and Clarkson ,2003; Ji, 2008; Ji *et al*.,2009).

2.1.1 Chemistry of Free Radicals

Free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently. Molecular oxygen is a di-radical, containing two unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction (Yu, 1993; Sen, 2001), The complete reduction of oxygen to H_2O requires four steps and the generation of several free radicals and H_2O_2, which is not a free radical itself because it contains non-paired electrons. H_2O_2 is considered a reactive oxygen species (ROS) because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive transition metals (Aruoma, 1994; Sen, 2001).

The complete reduction of oxygen is summarized in the following equations:

$O_2 + e^- \rightarrow O_2^{-\bullet}$ superoxide radical (1)

$O_2^{-\bullet} + H_2O \rightarrow HO_2^{\bullet} + OH^-$ hydroperoxyl radical (2)

$HO_2^{\bullet} + e^- + H \rightarrow H_2O_2$ hydrogen peroxide (3)

$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^-$ hydroxyl radical (4)

All ROS considered highly reactive because their unstable electron configurations allow for the attraction of electrons from other molecules, resulting in other free radicals that is capable of reacting with more molecules. This chain reaction contributes to lipid peroxidation (Hochstein and Ernster, 1963; Cadenas *et al.,* 1992; Paschalis *et al*., 2007; Priscilla and Prince 2009).

2.1.2 Evidence of Association of Lipid Peroxidation and Diseases

Oxidative stress can cause oxidative modification of LDL, believed to be one of the key determinants of the development of atherosclerosis (Lynch *et al*., 1996; McCall and Frei, 1999; Steinberg and Witztum, 2002). Antioxidant intervention studies in animals and humans have found that antioxidants reduce LDL oxidation and susceptibility to oxidation, increase the lag period of in vitro LDL oxidation, and reduce lesion formation (Lynch *et al.,* 1996; Rifici and Khachadurian, 1996; Harats *et al.,* 1998; Carr and Frei, 1999; Carr and Frei, 2000). In addition to this well-established role of oxidative stress in the pathogenesis of atherosclerosis, increased lipid peroxidation (as measured by F_2-isoprostanes another biomarker of lipid peroxidation) in urine or plasma have been found in patients with a variety of disease conditions such as hypercholesterolemia (Cracowsk *et al.,* 2000), also established in diabetes (Mezzetti *et al.,* 2000), Alzheimer's disease (Pratico *et al.,* 2000), chronic obstructive pulmonary disease (Montuschi *et al*., 2000). Increased plasma MDA levels have also been found in many clinical conditions (Madazli *et al,* 1999; Meraji *et al*., 2000; Singh *et al*., 2001; Skrzydlewska *et al.*2001; Sharma *et al.,* 2001; Shimizu *et al*., 2001; Sahin and Gumuslu*.,* 2007). Mechanisms linking oxidative stress with disease include free radical inhibition of prostacyclin synthetase, prevention of nitric oxide inhibition of release of endothelium-derived relaxing factor (EDRF) (Vallance *et al.,* 1992; Solzbach *et al.,* 2003), promotion of endothelial prostacyclin production (Hashimoto, and Shimizu, 2006), also the effects on smooth muscle contractility of peripheral blood vessels and improvement of vasomotor dysfunction (Yoshioka *et al*., 1982; Levine *et al*., 1996).

Research on animal models has shown that lipid peroxidation was greater in myocardial tissue of obese rats when compared to lean rats (Vincent *et al.,*1999; Vincent *et al.,2002*), and that plasma concentrations of oxidation biomarkers increased significantly while total antioxidant status decreased in rats fed a high calorie diet (Beltowsk *et al*.,2000; Beltowsk *et al*.,2002).

2.1.3 Free Radicals Generation during Exercise

It is well established that regular aerobic exercise has some protective effects against atherosclerosis development (Berlin and Colditz, 1990) by reducing cholesterol levels and high blood pressure (Hollmann, 1994), by enhancing nitric oxide production (Gattullo *et al*., 1999) and antioxidant defense systems (Lawler and Powers, 1998; Goldfarb *et al.,* 2005). Although certain exercises can benefit health, too much may cause a deleterious effect on the body. The rate of oxygen uptake by the body during exercise may increase by 10- to 15-fold, which results in increased oxygen flux by 100-fold in the active peripheral skeletal muscle tissue with a 30-fold increase in blood flow. Although delivery of increased amounts of oxygen to active tissues during exercise fuels oxidative metabolism, maximizing energy yield per unit substrate and avoiding lactate accumulation, aerobic organisms have to pay a price for such a metabolic advantage (Halliwell, 1999; Goldfarb *et al.,* 2005). An elevated metabolic rate because of exercise can dramatically increase free radical/ROS production (Davies *et al.,* 1982; Kanter *et al.,* 1988; Alessio, 1993; Kanter *et al.,* 1994; Ji, 1995a; Davies *et al.,* 2009).

The direct detection of ROS in biological systems is difficult because of their high reactivity and low steady-state concentration. Numerous studies have been devoted to the impact of exercise on the oxidant–antioxidant balance in man and animals (Criswell *et al*., 1994; Sen and Packer, 2000; Chang *et al*., 2004; De Moffarts *et al.,* 2005). Exercise has shown to induce tissue damage by oxidation of cellular components, such as membrane lipids, proteins, carbohydrates and (desoxy) ribonucleic acids (Clarkson and Thompson, 2000; Elhaimeur *et al.,* 2003). Exercise-induced oxidative stress is believed to contribute to accelerated muscle fatigue and muscle fiber damage, leading to exercise intolerance and poor performance in different animal species (Sen and Packer,2000; Pimenta., *et al.,* 2007; DA Silva *et al.,* 2009). The intake of antioxidants in order to prevent exercise-induced oxidative lesions is widely practiced in athletes; despite a consistent lack of information about the real need of antioxidants and the advantage, these micronutrients might provide (Maughan, 1999; De Moffarts *et al*., 2005; Labonte *et al*., 2008).

2.1.4 Biomarkers of Oxidative Stress Induced by Exercise

The extent of lipid peroxidation in tissue and plasma by ROS can be monitored by Malondialdehyde (MDA) levels (Sumida *et al.,* 1989; Konishi *et al.,* 2006). Because the products of peroxidation affected by both the chemical composition of the tissue being studied and the presence or absence of metal ions, there is no single biomarker that is considered the “gold standard” of lipid peroxidation (Clarkson and Thompson, 2000; Speranza *et al.,* 2007).

Increases in oxidative damage biomarkers such as protein carbonyls and thiobarbituric acid reactive substances, defected mitochondrial function (Sen *et al.,* 1994; Willis and Jackman, 1994; Ravalec *et al*., 1996; Sen *et al.,* 1997;) also decreases in levels of antioxidants and antioxidant enzymes in the heart (Reznick *et al.,* 1982; Somani *et al.,* 1995a; Husain and Somani, 2005), blood (Ji, 1993; Lew *et al.,* 1985; Viguie *et al.,* 1993), lung (Salminen *et al.,* 1984; Yoon et al., 1991), liver (Brady *et al.,* 1979; Lew *et al.*, 1985; Ji, 1993; Husain and Somani, 2005), brain (Somani *et al.,* 1995b), and muscles (Criswell *et al.,* 1993; Jenkins and Goldfarb, 1993; Ji, 1993; Powers *et al*., 1994; Sen *et al.,* 1994)as well as increase of lipid peroxidation that lead to defect on mitochondrial function and tissue damage.

The results from human and animal studies have suggested that the formation of ROS and urinary excretion of peroxides increase during physical activity (Davies *et al.,* 1982; Packer, 1984; Zerba *et al.,* 1990; Clarkson, 1995; Olinescu, 1995; Messina *et al.,* 2006; Packer *et al.,* 2006).

Increase in blood MDA was found after an 80-km race (Kanter *et al.,* 1988), after a 30-min treadmill test at 60% and 90% VO^2 max (Kanter *et al.,* 1994), after downhill running (Maughan *et al.,* 1993) and after incremental cycling tests to exhaustion in sedentary and moderately trained men (Lovlin *et al.,* 1987; Sumida *et al.,* 1989; Konishi *et al.,* 2006). In contrast, no increases in MDA were found after a half-marathon (Duthie *et al.,* 1990), after 60-min of bench stepping exercise (Maxwell *et al.,* 1993; Selman *et al*., 2002), after maximal cycle ergometry exercise (Sen *et al.*, 1994), and after maximal cycle ergometry exercise in elite athletes (Viinikka *et al.,* 1984). Furthermore, MDA amounts found to decrease immediately after a marathon (Rokitzki *et al*., 1994) and immediately after a graded exercise test in long-distance skiers (Selman et al., 2002; 6Packer *et al.,* 2006; Fazio *et al.,* 2009). The discrepant results may be due to the high inter-subject variability in MDA formation and the non-specificity of the assay used in these studies. However, the above results still showed that strenuous exercise, depending on the mode of exercise, intensity and duration of the activity can lead to harmful effects on the body, especially low density lipoprotein (LDL) and plasma oxidation after ROS released into circulation (Maxwell *et al.,* 1993; Devries *et al.,* 2008).

Several studies indicate that lipid peroxidation increased in skeletal (Davies *et al.,* 1982; Alessio *et al*., 1988; Ji, 2008) and cardiac (Rajguru *et al.,* 1994) muscle, in liver (Davies *et al.,* 1982; Alessio *et al.,* 1988), and in erythrocytes (Rajguru *et al*., 1994) of untrained rats after acute exercise. In agreement with the above reports, Venditti *et al*., (1996) found that exhaustive exercise increases lipid peroxidation products, such as MDA and hydroperoxides, in all examined tissues and also found that chronic exercise does not modify the MDA and hydroperoxides levels in the tissues of both control and exhausted rats are only in part supported by results of other authors.

In fact, it has been reported that liver and white muscle MDA levels were not affected by training (Alessio *et al.*, 1988; Di Meo, and Venditti 2001), whereas those of heart (Kihlstrom, 1990; Venditti *et al*., 2001) and red muscle (Alessio *et al.,* 1988) decreased. Furthermore, increases in lipid peroxidation during acute exercise were found in white muscle, but not in red muscle or in liver of trained rats (Alessio *et al.,* 1988). Actually, the increase in lipid peroxidation depends on exercise duration (Davies *et al*., 1982; Lawler and Song, 2002; Venditti *et al*., 2005).

Venditti *et al*., (2005) show a protective training effect, and the effect consists of a lack of lipid peroxidation in rats running for 20 min (Alessio *et al.,* 1988) and of a slowing down in lipid peroxidation in rats swimming to exhaustion. Meo, and Venditti (2001) found that a low rate of lipid peroxidation is associated with a slower appearance of SR and ER damage, supports the view that the reduced sensitivity of tissues of trained animals to exercise-induced damage is due to their lower susceptibility to lipid peroxidation. There is a wealth of data, often conflicting, concerning the effects of training on cellular antioxidant systems. Oxidative stress suggested implicating in the generation of oxidative skeletal muscle fatigue (Barclay and Hansel, 1991; Kaikkonen *et al.,* 1998; Meo, and Venditti 2001; De Moffarts *et al.,* 2005) and muscular atrophy (Kondo *et al*., 1993; Speranza *et al.,* 2007). With the use of pump-perfused, mouse soleus muscle and canine gastrocnemius-plantaris muscle preparations it was shown that xanthine oxidase-generated superoxides may attenuate the function and enhance the fatigue rate of contracting muscles. Such effects of $-O_2$ were not observed in the presence of a hydroxyl radical scavenger, a xanthine oxidase activity blocker, or a Fe'+ chelator. It was concluded that free radicals might be one of the factors that contribute to oxidative skeletal muscle fatigue (Barclay and Hansel, 1991; De Moffarts *et al.,* 2005).

Sen and Hanninen, (1995) observed that skeletal muscle-derived cells are highly active in glutathione synthesis (Sen *et al.,* 1994; De Moffarts *et al*., 2005). Based on the estimated intracellular water content of the cells, skeletal muscle glutathione metabolism may be playing a crucial role in whole body glutathione homeostasis (Sen and Packer, 2000). Sen, (1999) observed that in skeletal muscle-derived cells certain membrane Kf transport proteins are highly sensitive to oxidant exposure (Sen and Packer, 2000). Reznick *et al*., (1992) had reported the first evidence that exhaustive exercise does increase skeletal muscle protein oxidation in rats. In another recent study carried out with rats, 10-15 min swim exercise resulted in oxidation of erythrocyte membrane protein. After exercise, skeletal muscle microsomes contained decreased sulfhydryls, and protein cross-linking was extensive (Pereira et al., 1994; Rajguru *et al.,* 1994; Messina *et al*., 2006; Speranza *et al*., 2007).

2.2 Antioxidants

2.2.1 Antioxidants Properties

An antioxidant can be defined as a substance that helps to reduce the severity of oxidative stress either by forming a less active radical or by quenching the damaging ROS chain reaction on substrates such as proteins, lipids, carbohydrates or DNA (Dekkers *et al*., 1996; Powers and Shanely, 2002; McClung *et al.,* 2008). Muscle cells contain complex defense mechanisms to protect against oxidative stress (Powers *et al*., 1999). The two classes of endogenous protective mechanisms are: 1) enzymatic and 2) non-enzymatic antioxidants.

A range of antioxidants is active in the body including enzymatic (endogenous) and non-enzymatic (mainly brought by food) antioxidants (Vincent *et al*., 2000; Armutcu *et al*., 2005; Deruisseau *et al.,* 2006). All of them can be intracellular or extracellular antioxidants. Antioxidant enzymes include SOD, catalase (CAT) and glutathione peroxidase (GPx).

Non-enzymatic antioxidants include a variety of ROS quenchers such as vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol), flavonoids, thiols (including glutathione [GSH], ubidecarenone (ubiquinone QIO), uric acid, bilirubin, ferritin) and micronutrients (iron, copper, zinc, selenium, manganese), which act as enzymatic co-factors. The antioxidant system efficiency depends on nutritional intakes (vitamins and micronutrients) and on endogenous antioxidant enzyme production, which can be modified by exercise, training, nutrition and aging (Dekkers *et al*., 1996; McClung *et al.,* 2007). Moreover, the antioxidant system efficiency is important in sport physiology because exercise increases the production of ROS.

2.2.2 Antioxidant Enzymes Activity during Exercise

Antioxidant enzymes, which provide the primary defense against ROS generated during exercise, may be activated selectively during an acute bout of strenuous exercise depending on the oxidative stress imposed on the specific tissues, as well as the intrinsic antioxidant defense capacity (Jenkins, 1988; Ji, 1995a; Sen, 1995; Cunnane *et al.,* 2001; Gaeini *et al*., 2006).

Superoxide dismutase reduces superoxide to hydrogen peroxide; and catalase and glutathione peroxidase reduce hydrogen peroxide from the SOD reaction to water. In addition, glutathione peroxidase can reduce lipid peroxides directly. There is still insufficient knowledge about the kinetics or molecular regulation of these enzymes in mammalian tissues (Ji, 1995b; Blache *et al.,* 2007; Galassetti et *al.,* 2006).

An acute bout of exercise has shown to increase SOD activity in number of tissues including the liver (Lang *et al.,* 1987; Alessio and Goldfarb *et al.,* 1989; Ji *et al*., 1992; Karanth and Jeevaratnam, 2005; Kakarla *et al.,* 2005.); skeletal muscle (Quintanilha *et al*., 1982; Ji *et al.,* 1991; Ji *et al.,* 1993; Lawler *et al.,* 1993; Packer *et al.,* 2006; Kaczor *et al*., 2007; Ji, 2008), heart (Quintanilha and Packer, 1983; Kim *et al.,* 1996; Ji *et al.,* 2006), and red blood cells (Ohno *et al*., 1988; Mena *et al.,* 1991; Karanth and Jeevaratnam, 2005). Many studies have reported a training-induced increase in total SOD activity (Jenkins, 1988, Leeuwenburgh *et al.,* 1994; Powers *et al.,* 1993; Powers *et al.,* 1994; Powers *et al*., 1994; Leeuwenburgh and Ji, 1995; Leeuwenburgh and Ji, 1996; Leeuwenburgh *et al.,* 1996; Leeuwenburgh *et al*., 1997; Oh-Ishi *et al.,* 1997; Powers and Shanely, 2002; Lambertucci *et al.,* 2007). This activation of SOD was thought to result from increased superoxide production during exercise (Ji, 1993; Miyazaki *et al.,* 2001). the studies that have investigated the effects of endurance exercise on GPX activity in skeletal muscle found that regular endurance exercise training results in increased GPX activity in active skeletal muscles (Criswell *et al*., 1993; Hellsten *et al.,* 1996; Ji *et al.,* 1988; Leeuwenburg *et al*., 1994; Leeuwenburg *et al.,* 1997; Powers *et al.,* 1993; Powers *et al*., 1994; Sen *et al*., 1994; Venditti and Meo, 1997; Powers and Shanely, 2002; Venditti *et al.,* 2005; Lambertucci *et al.,* 2007).

GPX activity has demonstrated variable responses to an acute bout of exercise. Some studies show no change in this enzyme in skeletal muscle after acute exercise (Brady *et al.,* 1979; Ji *et al.,* 1990; Leeuwenburgh and Ji, 1995). Others studies reported significant elevation of GPX activity (Ji *et al.,* 1992; Ji and Fu, 1992; Leeuwenburgh and Ji, 1996; Oh-Ishi *et al.,* 1996; Selamoglu et al., 2000; Selman et al., 2002; Leeuwenburgh and Prolla, 2006). Criswell *et al.* (1993) studied the effect of 12-wk interval training, observed favorable changes in the skeletal muscle of rats, and proposed that 5-min interval high intensity training was superior to moderate-intensity continuous exercise in regulating muscle antioxidant defenses. In another study, sprint training of rats observed significantly increased total glutathione pool of skeletal muscles, and glutathione peroxidase activity of the heart and skeletal muscle (Atalay *et al.,* 1996).

Skeletal muscle or heart superoxide dismutase activity was not influenced; the contention that moderate-intensity endurance exercise training may enhance the physiological antioxidant defenses has received substantial support. On the other hand, activity restriction has been significantly compromising such defenses, rendering the tissues more susceptible to oxidative damage (Sen *et al.,* 1992; Kondo *et al*., 1993; Selamoglu *et al.,* 2000; Sen, 2001; Selman *et al.,* 2002; Tauler *et al*., 2006). Thus, habitual physical exercise is crucial to maintain and promote natural capacity to defend against the ravages of reactive oxygen.

Exercise training is involved in a chronic and intermittent increase in the exposure of active tissues such as the skeletal muscle and heart to oxygen flux. In prokaryotes, some mechanisms of ROS-dependent induction of antioxidant defense proteins have been unraveled (Sen and Hanninen, 1995; Sen, 1995). There is little evidence to suggest that exercise training promotes an increase in catalase activity in skeletal muscle (Higuchi *et al.,* 1985; Leeuwenburg *et al*., 1994; Powers *et al.,* 1994; Powers *et al*., 1994). In fact, several studies have shown that exercise training results in reduced catalase activity in some locomotor muscles (Alessio and Goldfarb *et al,* 1988; Laughlin *et al.,* 1990; Leeuwenburg *et al*., 1997; Greathouse *et al*., 2005). The explanation for the training-induced reduction in catalase activity in selected muscles remains unclear. Most of the previous studies revealed no significant alteration in CAT activity with acute exercise (Meydani *et al.,* 1993; Ji, 1995a).

However, there are exceptions; CAT activity found to increase significantly in rat muscle after an acute bout of exercise to exhaustion or at high intensity (Ji *et al.,* 1992; Ji and Fu, 1992; Greathouse *et al.,* 2005; Goldfarb *et al.,* 2005). Activation by either enzyme molecules is possible because for some enzymes, such as SOD and catalase, partial occupancy of the enzyme molecules by their substrates over a wide range of concentrations known to increase their catalytic activity (Chance *et al.,* 1979; Pinho *et al.,*2006; Rinaldi *et al*., 2006). The investigations in erythrocyte antioxidant enzymes may show different results because erythrocytes are unable to repair damaged components by resynthesis, and their membranes made up of components that are vulnerable to peroxidative decomposition (Kaczmarski *et al.* 1999; Tauler *et al.,* 1999; Tauler *et al.,* 2002).

The factors that regulate acute changes of antioxidant enzyme activities in erythrocytes "*in vivo*" are attributable to covalent modification of proteins or other protein interactions, because erythrocytes do not have the machinery to synthesize proteins. Physical exercise can cause oxidative stress on erythrocytes because of increased generation of ROS (Senturk *et al.,* 2005; Ji *et al*., 2006; Vaanholt *et al*., 2008). Antioxidant enzymes may need to enhance their activities to reduce ROS to safe compounds during physical activity. However, the increase of activities of erythrocyte antioxidant enzymes by physical exercise is still controversial.

A session of acute exhaustive exercise has shown to increase SOD activity, indicating increased Superoxide production during exercise in erythrocytes (Somani *et al.* 1995a, Sureda *et al*., 2005; Itoh *et al.,* 2004; Tauler *et al., 2006*). The decrease in catalase activity was in accordance with an increase in oxidative stress, probably because the oxidative defenses were overwhelmed (Aguilo *et al.,* 2000; Sureda *et al*., 2005). The level of cardiac antioxidants in trained rats was found decreased or unchanged (Kihlstrom, 1990), but the GSH level remained higher after heart perfusion with cumene hydroperoxide. Vitamin E was not modified by training in several tissues, among which were muscles, heart, and liver (Gohil *et al*., 1987). With regard to antioxidant enzymes, catalase (CAT) was found unaffected in the liver and decreased in muscle by training; superoxide dismutase (SOD) was found unaffected in both tissues (Alessio and Goldfab*,* 1988).