THE EFFECTS OF EXERCISE INTENSITY ON BLOOD MARKERS OF OXIDATIVE STRESS IN RECREATIONAL MALE ATHLETES

WONG YEE YAN

SCHOOL OF HEALTH SCIENCES

UNIVERSITI SAINS MALAYSIA

2013

THE EFFECTS OF EXERCISE INTENSITY ON BLOOD MARKERS OF OXIDATIVE STRESS IN RECREATIONAL MALE ATHLETES

By

WONG YEE YAN

Dissertation submitted in partial fulfilment

of the requirements for the degree of Bachelor of Health Sciences

(Exercise and Sport Science)

JUNE 2013

CERTIFICATE

This is to certify that the dissertation entitled

THE EFFECTS OF EXERCISE INTENSITY

ON BLOOD MARKERS OF OXIDATIVE STRESS

IN RECREATIONAL MALE ATHLETES

Is the bona fide record of research work done by

WONG YEE YAN

During the period of October 2012

to June 2013-05-26

under my supervision

ACKNOWLEDGEMENTS

First of all, I would like to convey my greatest gratitude and appreciation to my supervisor, Dr. Ang Boon Suen for his continuous guidance, encouragement, support and patience throughout this research project. His expertise, teaching and direction are invaluable.

I would also like to express my sincere appreciation to the subjects for their participation in this research project. Without their valuable co-operation, this research would never have been completed.

I would also like to take this opportunity to thank the laboratory staff of Exercise and Sports Science Lab PPSK and PPSP, Mr. Hafizie Mat Zain, Madam Mazra Othman and Madam Jamaayah for their helpful assistance throughout this research.

To my father and mother, thank you for all of your support, understanding and encouragement. Your constant advice for me to believe in success and myself has helped me out to complete this project. Last but not least, I owe my gratitude and appreciation to my course-mates for their support and help throughout this entire project.

TABLE OF CONTENTS

CHAPTER 3: METHODOLOGY

CHAPTER 4: RESULTS

CHAPTER 5: DISCUSSION

CHAPTER 6: CONCLUSION

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

VO_{2max}	Maximum oxygen consumption
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RM	Reactive metabolite
FR	Free radical
MDA	Malondialdehyde
GSH	Reduced glutathione
GSSG	Oxidized glutathione
MHR	Maximum heart rate
RPE	Rate of perceived exertion
TBARS	Thiobarbituric acid-reactive substances

ABSTRAK

KESAN INTENSITI SEMANAN KE ATAS TAHAP PENANDA TEKANAN OKSIDATIF DI DALAM DARAH DI KALANGAN ATLET REKREASI LELAKI

Tujuan kajian ini ialah untuk menyiasat kesan intensiti senaman 30 minit pada intensiti 50-60% dan 80-90% kadar denyutan jantung maksima ke atas tahap penanda tekanan oksidatif di dalam darah di kalangan atlet rekreasi lelaki. Seramai tiga belas atlet rekreasi lelaki (umur: 21.8 ± 1.5 tahun; berat badan: 64 ± 8.5 kg; ketinggian: 170.6 ± 7.3 cm) dari Kampus Kesihatan, Universiti Sains Malaysia telah menyertai kajian ini. Bentuk kajian ini adalah jenis bersilang secara rawak. Subjek dikehendaki melakukan dua percubaan larian selama 30 minit pada intensiti rendah (50-60% kadar maksima denyutan jantung (MHR)) atau intensiti tinggi (80-90% MHR) di atas treadmill bermotor. Kadar denyutan jantung dan penilaian tahap kepenatan (RPE) direkodkan pada setiap 5 minit. Sampel darah diambil sebelum, selepas senaman dan 24 jam selepas senaman. Sampel darah telah dianalisakan untuk hematokrit, "lipid peroxidation", "total antioxidant power", "reduced glutathione (GSH), oxidized glutathione (GSSG)" dan nisbah GSH:GSSG. Keputusan telah menunjukkan tiada perbezaan yang signifikan ke atas perubahan tahap penanda-penanda tekanan oksidatif selepas 30 minit bersenam pada intensiti rendah dan tinggi. Walau bagaimanapun, bersenam pada intensiti 80-90% MHR menunjukkan trend peningkatan tahap penanda tekanan oksidatif yang lebih tinggi berbanding intensiti 50-60% MHR. Di samping itu, juga tidak ada perbezaan dalam perubahan isipadu plasma yang signifikan di antara ujian senaman berintensiti rendah dan tinggi selama 30 minit. Kesimpulannya, data ini menunjukkan bahawa 30 minit bersenam pada intensiti rendah dan tinggi tidak mempunyai perbezaan yang signifikan dalam tahap penanda-penanda tekanan oksidatif.

ABSTRACT

THE EFFECTS OF EXERCISE INTENSITY ON BLOOD MARKERS OF OXIDATIVE STRESS IN RECREATIONAL MALE ATHLETES

The purpose of this study is to investigate the acute effect of 30 minutes exercise at 50-60% and 80-90% of maximum heart rate on blood oxidative stress markers in recreational male athletes. Thirteen male recreational athletes (age: 21.8 ± 1.5 years old; body weight: 64 ± 8.5 kg; height: 170.6 ± 7.3cm) from Health Campus of Universiti Sains Malaysia participated in this study. This was a randomized cross-over study. Subjects were required to perform two trials with a break of week, low (50-60% of MHR) VS high intensity (80-90% of MHR) exercise for 30 minutes. Heart rate and the rate of perceived exertion (RPE Borg's Scale) were measured at an interval of 5 minutes. Blood samples were drawn at pre-exercise, post-exercise and 24 hours post-exercise. The blood samples were analysed for haematocrit level, lipid peroxidation, total antioxidant power, reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH:GSSG ratio. Two way ANOVA with repeated measures was used to determine the differences between trials and changes of the measured parameters over time. The results showed there was no significant difference in the changes of oxidative stress markers between low and high intensity exercise. However, exercise at high intensity (80-90% MHR) showed a trend of higher oxidative stress markers levels than low intensity exercise (50-60% MHR). In addition, there were also no significant differences between trials in plasma volume changes between low and high exercise intensity workout for 30 minutes. In conclusion, these data demonstrated that 30 minutes of exercise at low and high intensity did not have statistically significant difference in oxidative stress markers' level.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Exercise training is associated with numerous health benefits. Regular physical activity is an important factor in preventing and treating cardiovascular diseases. However, acute and intense physical activity is associated with an increased production of free radicals and reactive oxygen species (ROS) to a point that can exceed antioxidant defence causing oxidative stress and cellular damage such as oxidation of lipids, proteins, and DNA. (Jenkins and Goldfarb, 1993).

Lipid peroxidation of cell membranes changes membrane integrity, leads to increased swelling, and reduces the ability of the cell to maintain ion gradients (Merry *et al.*, 1991). This oxidative damage to cell membranes has been associated with tissue inflammation, muscle fatigue, and impaired recovery following high-intensity exercise (Abuja, 2001; Pyne, 1994).

Study showed that 30 minutes of aerobic and anaerobic exercise performed by young, cross-trained men can increase protein and glutathione oxidation in human blood while having little impact on lipid or DNA oxidation. Protein oxidation appears to be more greatly affected by anaerobic exercise and the magnitude of protein oxidation is greater following anaerobic compared with aerobic exercise. Glutathione oxidation appears to be more greatly affected by aerobic exercise (Bloomer *et al.*, 2005).

A finding showed that the generated reactive oxygen species increased as the exercise intensity increased. As intensity of exercise increased, the superoxide dismutase (SOD) level increase but glutathione peroxidase (GPx) and catalase (CAT) decrease. However, at low exercise intensity of 50% VO_{2max}, the hydrogen peroxide (H_2O_2) generated from superoxide anions (O_2^-) is sufficiently high enough to activate CAT scavenging mechanism, which implies low oxidative stress status. It showed that for 10 minutes duration of exercise at the low intensity of 50%$VO_{2\ max}$, oxidative stress level would not be increased in sedentary healthy adults (Maryama Daud *et al.*, 2006).

On the other hand, there are numerous studies on the animal and human's response to sub-maximal intensity exercise, without reaching a state of exhaustion, which do not reach conclusive results. Many studies find increases in oxidative stress markers even in aerobic activities which vary between 65% - 75% VO_{2max} with exercise duration varying from several minutes to several hours (Clarkson and Thompson, 2000; Kanter *et al.*, 1993).

Characteristics of exercise such as intensity or duration seem to be associated with formation of ROS and oxidative stress which lead to oxidative damage.

The relationship between intensity of exercise and duration associated with oxygen radical production has not been clearly defined. Therefore the aim for this study is to determine the acute effect of 30 minutes exercise at 50 - 60% and 80 - 90% of maximum heart rate (MHR) on blood oxidative stress markers in recreational male athletes.

1.2 OBJECTIVES OF THE STUDY

1) To assess acute effect of different exercise intensity (50 - 60% and 80 - 90% of MHR) related to the levels of blood oxidative stress markers in recreational male athletes.
2) To compare the acute effect of different exercise intensities (50 - 60% and 80 - 90% of MHR) on oxidative stress markers.

1.3 SIGNIFICANCE OF THE STUDY

This study is essential to investigate the effects of different intensities of exercise which may result in increased oxidative stress on an individual. Also, to demonstrate whether it is beneficial for beginner or untrained individual to exercises at low intensities aerobic exercise which will not induced oxidative stress.

1.4 HYPOTHESIS

Ho : There is no significant difference in blood oxidative stress markers between high and low exercise intensities

HA : There is a significant difference in blood oxidative stress markers between high and low exercise intensities

CHAPTER 2

LITERATURE REVIEW

2.1 OXIDATIVE STRESS

There are several ways to define oxidative stress. Oxidative stress is the status of deterioration of the balance between oxidant formation and antioxidant defence, which leads to an increase in the amount of antioxidants (Urso and Clarkson, 2003). Many factors can lead to an increase in oxidative stress and antioxidant defence mechanisms control level of free radical (FR) and reactive oxygen species (ROS). However the balance is not perfect. When some of the protective systems of the organism against FR toxicity fail, the action of FR becomes uncontrolled, resulting in damage to molecules, cells and organs, and potentially to the death of the organism (Dˇuracˇková, 1998; Dˇuracˇková *et al.*, 1999). The consequence of the negative effects of FR and reactive metabolites (RM) is called oxidative stress. Oxidative stress also can be defined as an imbalance between production and elimination of reactive metabolites of oxygen and nitrogen, in favor of their production, leading to potential damage (Figure 2.1) (Sies, 1991).

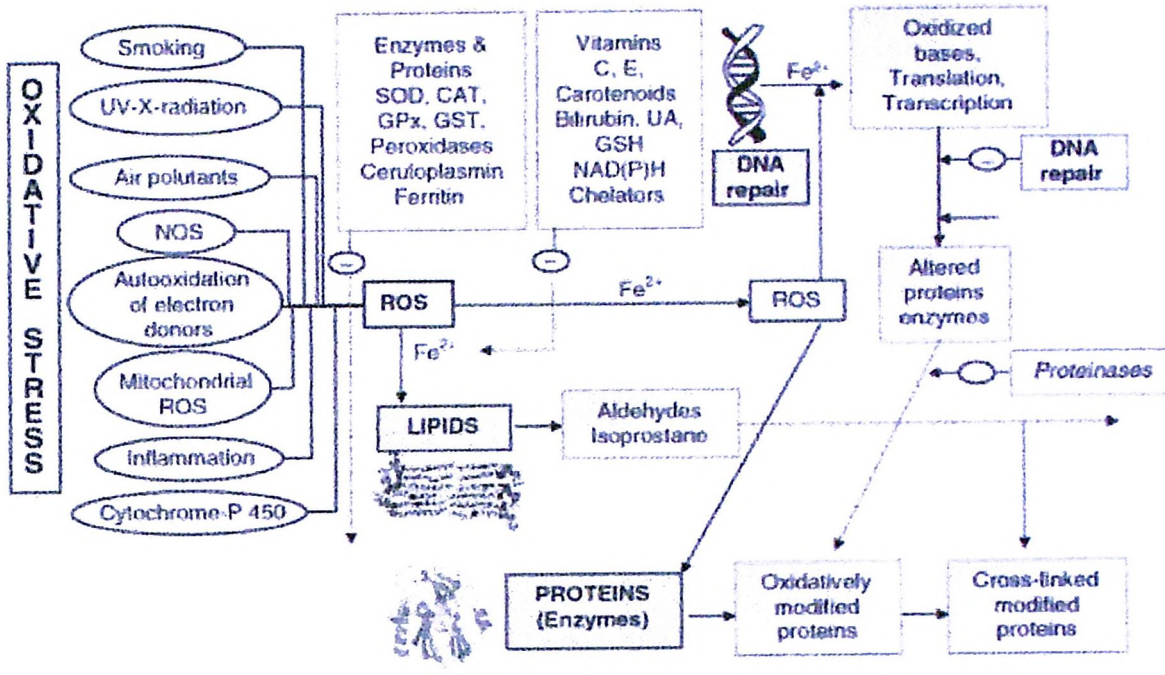

Figure 2.1 Oxidative stress and its impact on cell

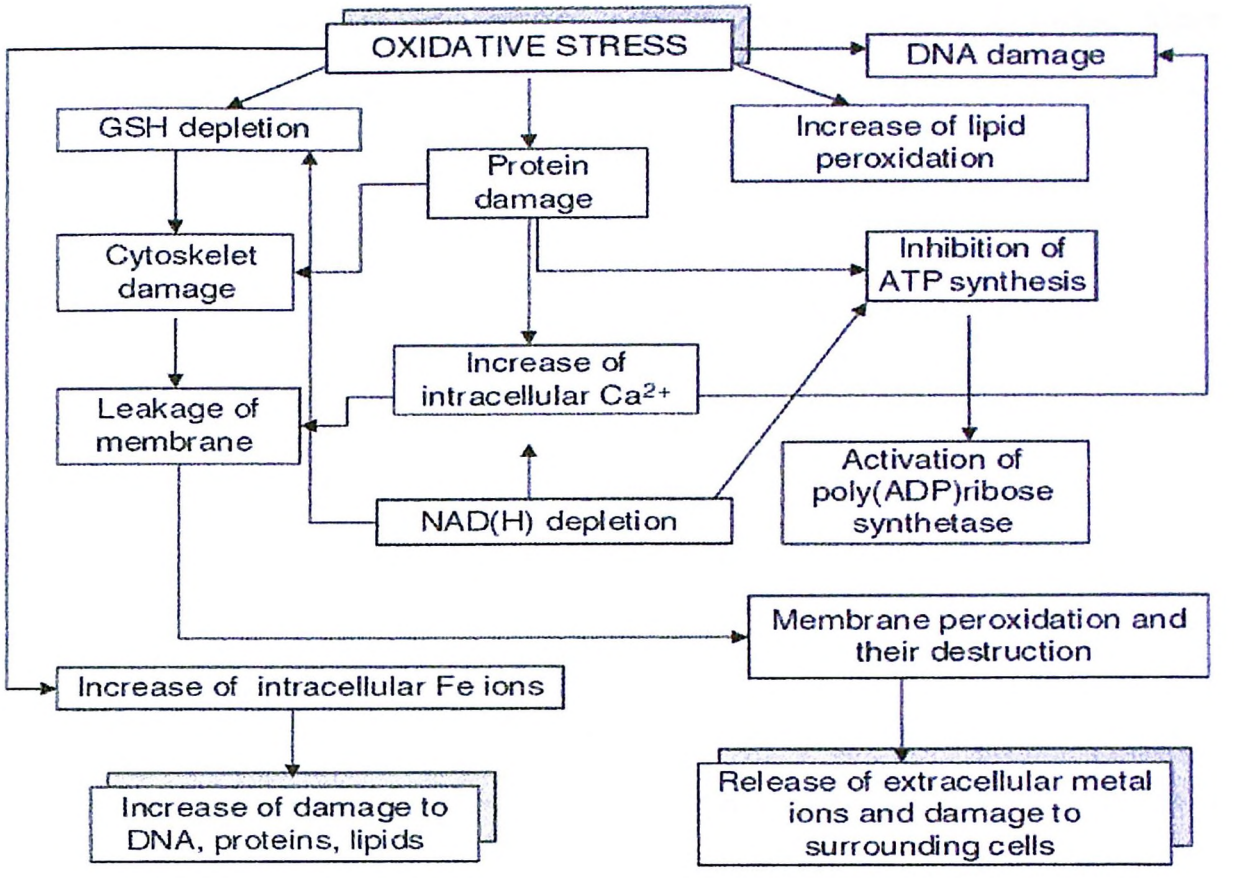

Figure 2.2 Associations between oxidative stress and damage to the organism

There was well documented serious imbalance between free radical spesies and antioxidant defense leading to oxidative stress and oxidative damage (Sies, 1991), these will cause biologically important molecules and cells damage and this can be significant in the pathogenesis of many diseases. Figure 2.2 shows associations between cell damage and oxidative stress (Aruoma, 1988). Imbalance between oxidant formation and antioxidant defense that lead to oxidative stress can result from several conditions (Halliwell and Whiteman, 2004):

I. Increased production of RM, as in hyperoxia, increased exposure to toxic RM, e.g. NO_2•, or promotors of RM formation, e.g. paraquat, or by increased activation of systems producing FR and RM, e.g. activation of neutrophils during inflammation.

II. Diminished levels of antioxidants, e.g. by increased exposure to toxins, which themselves need not be a source of FR but which are eliminated from the organism by glutathione transferase cooperating with GSH, thus leading to its depletion.

Further it may concern insufficient supply of some metal ions in food which are necessary for the function of some protective mechanisms (Zn^{2+}, Se^{4+}, Cu^{2+}, Mg^{2+}), by gene mutation encoding for antioxidative enzymes, e.g. superoxide dismutase, glutathione peroxidase.

FR and RM can damage biologically important molecules either primarily through the attack of FR or RM on the molecule leading to its damage, or secondarily, when products of FR or RM (e.g. aldehydes) further react with biomolecules (e.g. with proteins and nucleic acids) leading to the formation of conjugates and changing the structure and biological function of lipids, proteins and nucleic acids (Fig. 2.2).

Depending on the given conditions, the intensity and type of oxidative attack, cells and the organism can react differently:

I. Cells and the organism adapt to the oxidative stress either completely, e.g. by increased activity of protective antioxidative systems, or partially when they eliminate only one type of the oxidative attack, or the cell builds up its "oxidative immunity" by gradual exposure to a particular oxidant.

II. Cells can be injured by oxidative modification of lipids, saccharides, proteins and nucleic acids or by the following nonoxidative reactions as a consequence of the primary oxidative damage (cross-linkage of molecules), change in the level of intracellular ions by the change of integrity of the cell membrane, e.g. Ca^{2+}ions.

III. Damaged molecules in cells can be repaired or replaced by repair systems. In such a case, the cell can survive with a partial damage, yet when the repair ability of the organism fails and the cell, especially DNA, is constantly exposed to oxidants, then cell death can be triggered by apoptosis or necrosis.

The term "oxidative stress" is an incorrect term also from the chemical point of view. Oxidation never occurs alone but in association with reduction. Oxidation and reduction are shortly called the redox reaction (Valko *et al.*, 2006). Therefore "oxidative stress" should be correctly named "redox stress". For the understanding of the chemical nature of "oxidative stress", the use of terms such as oxidant/prooxidant and antioxidant have their significance. Concerning the redox balance, for the terms "oxidant and antioxidant" there are more precise expressions, such as a strong oxidant and a weak oxidant. A stronger oxidant (a higher positive redox potential) can extract hydrogen atom or electron from a weaker oxidant (antioxidant), transferring the radical character to the weaker oxidant (antioxidant).

According to redox potentials of individual redox pairs, it can be assessed whether a particular antioxidant can regenerate the other one. An antioxidant or a weak oxidant with a lower redox potential can regenerate an antioxidant with a higher potential. According to these indicators, dihydrolipoic acid (DHLA) is the most versatile antioxidant with the lowest oxidative properties. This redox potential indicator strictly follows chemical rules in in vitro systems and does not take into account the influence of other cell components and the effect of other antioxidants. The redox balance has thus to be evaluated in the total context of a living organism (Fig. 2.2).

The degree of oxidative stress damage depends on various factors. These include the type of the injured molecule (proteins, lipids, nucleic acids), the mechanism by which the damage is performed (fenton type chemistry, induction by a certain drug or a xenobiotic, activation of enzymes, e.g. NO-synthase, xanthine oxidase) and the type of the oxidative stress.

2.2 FREE RADICAL AND REACTIVE OXYGEN SPECIES

Chemically, a free radical is any molecule containing a single, unpaired electron. Usually, paramagnetic transition metal ions are not considered to be free radicals, although by technical definition they are. An example of free radical is oxygen itself. It is a diradical with two electrons, which are not spin paired, and each resides in an orbital of its own. Unpaired electron is responsible for paramagnetic properties of free radicals and their high reactivity toward molecules. Free radicals, which are metabolites of oxygen biological systems, have diverse reactivity. For example, life-span of hydroxyl radical OH• is $t_{1/2}=10^{-9}$s, whereas organic free radicals with delocalized electron, like a melanin, are characterized by low reactivity and their $t_{1/2}$ equals a few days (Boveris, 1998). From the chemical point of view, free radicals are active molecules participating in chain reactions, in which free radical substrate leads to the production of another free radical molecule, which in the another reaction gives the product, which is a free radical as well. This process is defined as propagation of free radicals reactions. Free radicals are therefore very unstable molecules with variable reactivity. By obtaining electrons from the molecules situated nearby and triggering a cascade of reactions, they can lead to the alteration of cellular structure and inhibit activity of different enzymes. Free radicals are generated in numerous processes in living organisms. For example in respiratory chain in mitochondria in the presence of coenzyme Q reductase and NADH nicotinamide adenine dinucleotide, in microsome in the

presence of cytochrome P-450 reductase and endoplasmatic reticulum (cytochrome P-450 reductase). Free radicals can be also generated in the membrane in presence of lipoxygenase, or prostaglandine synthase.

Another source of free radicals is hemoglobin (Hb). During its oxidation to methemoglobin (metHb) the superoxide anion radical is generated. During one day 3% of total mass of hemoglobin is converted to metHb. The most important source of superoxide anion radicals is an electron transport chain. 85-90% of oxygen is metabolized in mitochondria. One electron reduction of oxygen generates superoxide anion free radical. Next stages of reduction lead to generation of hydrogen peroxide and hydroxyl radicals. However, most of oxygen (approx. 95%) is reduced to water molecules during synthesis of ATP in mitochondria. In normal conditions, a man of weight 70 kg uses 3.5 ml of O_2/kg/min, which makes 350l O_2/day (15 mol/day). It is assumed that 2-3% of O_2 is converted to superoxide anion radical (0.30-0.45 mol). During extensive exercise the consumption of oxygen can increase by about 100%, so the leakage of superoxide anion in respiratory chain can also be elevated by about 100%. An increase of superoxide anion is also possible during oxidation of hemoglobin.

In healthy organisms free radical production and their inactivation by antioxidative systems stay in equilibrium. When production of free radicals exceeds cellular antioxidant capacity the oxidative injury occurs, which leads to the damage of biological materials. The main cellular components, which are susceptible to the activity of free radicals, are unsaturated lipid acids, proteins, nucleic acids and carbohydrates. It is believed that free radicals are generated in aging process and in all diseases. However, these molecules can have different functions. They take part in the regulation of many physiological processes, e.g. regulation of immunological and enzymatic processes, oxidizing and reducing processes, gene

transcription, as well as in cellular signaling. Not much is still known, which process and which factor establish boundaries between positive and detrimental functions of these molecules in the body. Free radicals are divided into two different groups: reactive oxygen species (ROS) and reactive nitrogen species (NOS). Reactive oxygen species include these free radicals, which are formed in the reaction of molecular oxygen reduction as well as these molecules, which are not free radicals but possess oxidative or reductive properties. Due to uncompleted molecular oxygen reduction, the generation of some of its reactive forms, named reactive oxygen species, takes place in the cells. The family of reactive intermediates resulting from the incomplete reduction of oxygen therefore includes: superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$). These molecules are produced under external factors like, for example, irradiation as well as under oxidative process in the presence of oxydase and oxygenase or under nonenzymatic autooxidations (e.g. catechole amine, thiol groups). Despite free radicals toxicity, they play an important antimicrobial role through the phagocytes.

Nitric oxide (NO) with unpaired electron is a small, unstable, hydrophobic molecule with a high diffusion coefficient and a short life-span. In spite of its chemical properties connected with limited reactivity, this molecule can easily react with hemoglobin, myoglobin, oxygen, superoxide anions, –SH groups of proteins, and various cellular components. Nitric oxide properties allow the effect of NO to occur close to its site of production. The reactions with molecular oxygen and superoxide anions lead to the formation of reactive nitrogen species (RNS) like nitrogen dioxide (NO2) and peroxynitrite ($ONOO^-$). Peroxynitrite anion can be transformed to peroxynitric acid, which decays by formation cytotoxic hydroxyl radical and then nitrates are the final products of the reactions. Cellular damage depends on the created

factors involved in the reaction. Some of the created RNS present higher reactivity with biological molecules than nitric oxide itself.

It was suggested that the major vascular pool of NO in vivo is nitrite, which is present at concentrations of 0.5-10 μM in plasma, erythrocytes and tissues (Gladwin *et al.*, 2004). NO is synthesized from L-arginine by a family of isoenzymes called nitric oxide synthases (NOSs). Depending on the concentration of NO in biological environment, the effect of this molecule changes dramatically. Nitric oxide is considered to be an endogenous modulator of numerous cellular functions in a variety of tissues with properties of cell signalling molecule. It is also proven to be a modulator of several aspects of skeletal muscle functions and, on the other hand, a mediator of injury and disease. Lower concentration stimulates guanylate cyclase and it influences blood pressure regulation, blood flow, neuronal transmission, neuroendocrine activity (Kozlowski *et al.*, 1999). In higher concentration, nitric oxide has antimicrobial, antitumor and cytotoxic effect. Many diseases are also related to the over- and underproduction of NO and to the direct participation of NO in some pathological mechanisms (Beckman, 1996; Bergendi *et al.*, 1999; Stamler and Meissner, 2001).

2.3 ANTIOXIDANTS

An antioxidant is 'any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate' (Halliwell and Gutteridge, 1989). This definition includes compounds of a non-enzymatic as well as an enzymatic nature. Clearly, the diversity of antioxidants matches that of pro-oxidants (Sies, 1993).

A first line of defence against reactive oxygen species is, of course, protection against their formation, i.e. prevention. There are numerous strategies in biology designed to evade oxidative stress, ranging from the plankton that descends from the surface of the seawater to lower levels of solar irradation, to the packaging of DNA in chromatin to shield the genetic material by providing alternative targets. Microbes have developed specialized strategies to prevent oxygen dependent killing by phagocytes.

Regarding radical formation, first it should be mentioned that some of the enzymes prone to generate free radical species are ingeniously designed. Cytochrome oxidase, which carries out most of the cellular oxygen reduction, does not release superoxide or other radicals, even though it contains iron and copper ions. Likewise, the three-dimensional structure of the enzyme ribonucleotide reductase keeps the radical character of the tyrosyl function in subunit B from spreading to the environment by forming an appropriate 'cage'.

Furthermore, the prevention of initiation of chain reactions includes the binding of metal ions, in particular iron and copper ions. Metal chelation is a major means of controlling lipid peroxidation and DNA fragmentation. Thus, the metal-binding proteins ferritin, transferrin, coeruloplasmin and others, e.g. metallothionein, are of central importance in the control of potential radical-generating reactions. Another strategy to increase the resistance to metal ion dependent oxidation is to modify the potential target site.

Protection of cells from incident radiation may occur through specialized pigments, e.g. the melanins for ultraviolet radiation or the carotenoids for electronically excited states such as singlet oxygen. However, these and other strategies are not completely preventative, because

they operate by decreasing the yield of a given challenging agent with less than 100% efficiency.

In this regard, there are many enzymatic systems in cells and body fluids to control the level of reactive species which otherwise might generate a cascade of products which, in turn, would lead to attacking oxidants. One important group of such enzymes is the glutathione S-transferases. This family of enzymes catalyses the reaction of the major low molecular mass thiol, glutathione, with reactive electrophiles to form thioethers, called S-conjugates. Biologically reactive electrophilic intermediates can be formed in a variety of metabolic pathways, notably those involving cytochrome P450, and are of interest in toxicology and pharmacology (Schulz *et al.*, 1996).

A strategy of preventative antioxidation could therefore be formulated as prevention by diversion, i.e. by channelling an attacking species into a less harmful product, hence lowering the risk of further damage. In the extreme, this could involve whole cells, one example being the intestinal mucosal cells. These cells are exposed to a variety of reactive intermediates and xenobiotics, and the rate of accumulation of products of oxidative damage in these cells is high. The turnover and elimination of whole cells prevents further spread of the challenging species. This type of prevention overlaps in part with the concept of interception.

Non-enzymatic antioxidant is the domain of the antioxidants as defined in a more narrow sense. The basic problem is to intercept a damaging species, once formed, to prevent it from further deleterious reactions. This is the process of deactivation. For radical compounds, the final deactivation consists of the formation of non-radical and non-reactive end-products.

Due to the nature of the free radicals, there is a tendency towards chain reaction, i.e. a compound carrying an unpaired electron will react with another compound to leave an unpaired electron in that compound ('radicals beget radicals').

A second objective of biological importance is to transfer the radical function away from more sensitive target sites to compartments of the cell in which an oxidative challenge would be less deleterious. In general, this means transferring the oxidizing equivalents from the hydrophobic phases into the aqueous phases, e.g. from the membrane to the cytosol or from lipoproteins to the aqueous phase of the plasma. Biologically, the most efficient intercepting antioxidants combine optimal properties for both these objectives: first, they react with initial free radicals, such as lipid peroxyl radicals, at suitable rates; and second, they are capable of interacting with water-soluble compounds for their own regeneration. This combined action then transfers the radical function away from further potential targets. In biological membranes, where a high-efficiency back-up system is present, there may be the need for only one to three antioxidant molecules per 1000 potential target molecules.

Such intercepting chain-breaking antioxidants are often phenolic compounds. (R,R,R)-α-Tocopherol is probably the most efficient compound in the lipid phase (Traber and Sies, 1996). This biological antioxidant contains shielding methyl groups in the vicinity of the phenolic hydroxyl group of the chromane moiety, and it is optimally positioned in the membrane by its phytyl side-chain.

The maintenance of a steady-state rate of peroxyl-radical reduction by tocopherol in the membrane is dependent on the reduction of the tocopheroxyl radical, once formed, by external reductants. These include ascorbate and thiols (Briviba and Sies, 1994). A

prerequisite for efficient interception by the phenolic antioxidants is that the lifetime of the radical to be intercepted must not be too short. The peroxyl radicals are therefore major reaction partners, since their lifetime extends into the range of seconds. In contrast, the hydroxyl radical, with its high reactivity and extremely short lifetime, cannot be intercepted with reasonable efficiency. It has been shown that up to 100 mm of an intercepting compound would be required for 90 % efficiency, eliminating interception as a useful strategy for defense against the hydroxyl radical, if only for osmotic reasons. Highly efficient biological polyene quenchers for singlet molecular oxygen, notably carotenoids and oxy-carotenoids, provide a suitable defense system against this oxygen species, in spite of its reactivity and short lifetime (Sies and Stahl, 1995; Stahl and Sies, 1996). The local concentrations of the carotenoids are decisive in determining the efficiency of the quenching of singlet oxygen and other electronically excited states.

As for enzymatic antioxidants, all cells in eukaryotic organisms contain powerful antioxidant enzymes. The three major classes of antioxidant enzymes are the superoxide dismutases, catalases and glutathione (GSH) peroxidases. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds. Indirect antioxidant functions carried out by enzymes are: (a) the back-up function, e.g. the replenishment of GSH from glutathione disulphide (GSSG) by the flavoprotein GSSG reductase; and (b) the transport and elimination of reactive compounds, e.g. the glutathione S-transferases and the transport systems for the glutathione S-conjugates. Different subcellular sites and different cell types may contain varying amounts of the antioxidant enzymes (Soboll *et al.*, 1995).

2.4 OXIDATIVE STRESS MARKERS

The most common method utilized to indicate exercise induced oxidative damage in regards to non-eccentric aerobic exercise has been the assessment of lipid peroxidation, with Malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) representing the most commonly used assays. Malondialdehyde is a three carbon chain aldehyde produced during decomposition of a lipid hydroperoxide. Lipid peroxidation is a chain reaction, which in consequence leads to the free radicals formation, studies showed that alcoxyl radical was generated during oxidative damage of membrane lipids (Ashton *et al.*, 1998). The highest elevation was detected among the subjects with the highest maximal oxygen uptake (VO_{2max}). Furthermore, a statistically significant increase of lipid peroxidation as well as total antioxidant capacity (TAC) was observed (Ashton *et al.*, 1998).

Most studies have used MDA as a measure of oxidative stress imposed by exercise. Generated free radicals can attack membrane polyunsaturated lipids acids and develop process of lipid peroxidation. Santos-Silva *et al.*, (2001) described increase of resting MDA level in trained adolescent swimmers compared with control subjects. Also Marzatico *et al.*, (1997) found higher MDA level in marathon runners compared with subjects. Several studies showed that single bout of exercise increase blood level of MDA (Hartman *et al.*, 1995; Miyazaki *et al.*, 2001). However strenuous endurance training was shown to reduce indices of oxidative stress (Miyazaki *et al.*, 2001). Moreover, in response to exercise in trained skiers and runners immediately after the exercise the decrease of MDA was presented (Hubner *et al.*, 1994; Rokitzki *et al.*, 1994). Despite the fact that this method is widely used in the sport science for lipid membrane peroxidation, the method itself and the obtained results are questioned in the existing literature. There are also studies showed there was no increase in MDA following maximal (Bloomer *et al.*, 2007; Leaf *et al.*, 1997; Niess *et al.*, 1996; Quindry

et al., 2005) or submaximal (Bloomer *et al.*, 2005; Buczynski *et al.*, 1991; Kanaley and Ji. 1991; Orhan *et al.*, 2004) exercise, with fewer investigations reporting a significant increase (Ashton *et al.*, 1998; Ashton *et al.*, 1999; Bailey *et al.*, 2001; Fatouros *et al.*, 2004; Kanter *et al.*, 1993). However, those studies reporting significant increases typically utilized maximal (GXT) (Ashton *et al.*, 1998; Ashton *et al.*, 1999; Bailey *et al.*, 2001;Braun *et al.*, 1991; Fatouros *et al.*, 2004) or near maximal (~75%VO2max) (Bryant *et al.*, 2003; Goldfarb *et al.*, 2007; Kanter *et al.*, 1993) exercise protocols, indicating a role of intensity in MDA formation.

Glutathione is another important marker of oxidative stress. It plays an important role in the elimination of organic peroxide and hydroxide peroxide. The GSH-GSSG ratio decreases under oxidative conditions. To detect both forms of glutathione HPLC and spectrophotometric techniques can be applied. Some studies presented that blood GSSG and GSH-GSSG ratio decreases in response to exercise (Sastre *et al.*,1992; Sen, 1999). Recently it has been suggested that oxidatively modified hemoglobin (OxHm) may be useful as an indicator of a specific form of oxidative stress, more than described above lipid peroxidation and glutathione redox status. Vollaard *et al.*, (2005) demonstrated that in vivo oxidative modification to haemoglobin is a normal occurrence in human blood, and is enhanced by exercise. Due to the fact that OxHm is produced in erythrocytes by peroxidation of haemoglobin, the changes in its concentration may provide direct mechanistic and diagnostic information.

In the response of the body to physical exercise, the changes in plasma antioxidant capacity can be analyzed based on the model of experimental exercise test. All applied model exercise tests are very simple and repeatable. During a single bout of exercise the basic hemodynamic

factors are changed, e.g. the increase of heart contractions frequency, systolic blood pressure, and minute volume of the heart (Jegier *et al.*, 2005). During the performance of such a test it is possible to estimate physical capacity of the body as well as VO_{2max} (Kozlowski *et al.*, 1999).

Physical exhaustion induces production of free radicals in different ways. The level of oxidative phosphorylation increases, which is responsible for ATP production. This reaction as a response of the body to physical exhaustion is connected with free radical production. Also exercises performed on the regular basis induce elevation of oxygen uptake, which is correlated with 10 up to 20-fold higher increase of cellular metabolism and with intensive oxygen radical production.

Problems, which appear in interpretation of the results of various studies, are mainly connected with different procedures according to which they were performed. Authors describe changes in enzymatic and non-enzymatic environments after single bout of exercise with the usage of cycle ergometer as well as after long term exercise like marathon or mountain cycling. Another reason for the discrepancies in results interpretation is a difference in used methodologies as well as a variety of substances, which are applied in the detection of the alterations in the body.

Typically, a decrease in reduced glutathione (GSH) (Laaksonen *et al.*, 1999; Michailidis *et al.*, 2007; Nikolaidis *et al.*, 2006; Steinberg *et al.*, 2006; Steinberg *et al.*, 2007), an increase in oxidized glutathione (GSSG) (Goldfarb *et al.*, 2005; Laaksonen *et al.*, 1999; Michailidis *et al.*, 2007; Nikolaidis *et al.*, 2007; Sen *et al.*, 1994),with no change to total glutathione concentration (TGSH) (Goldfarb *et al.*, 2007; Laaksonen *et al.*, 1996; Laaksonen *et al.*, 1999;

Sen *et al.*, 1994) has been reported following a variety of non-eccentric aerobic exercise protocols. Glutathione status typically returns to basal levels within 15–30 minutes of recovery (Gohil *et al.*, 1988; Steinberg *et al.*, 2006; Steinberg *et al.*, 2007; Viguie *et al.*, 2001). Studies reporting null findings for glutathione redox status (Inayama *et al.*, 2002; Laires *et al.*, 1993; Marin *et al.*, 1990; Nikolaidis *et al.*, 2006) may be partially related to the timing of sampling, as GSSG is rapidly reduced in vivo by way of glutathione reductase (Valko *et al.*, 2007), in addition to the trained status of the subjects (Kretzschmar *et al.*, 1991) or an insufficient intensity of exercise (Laires *et al.*, 1993; Marin *et al.*, 1990).

2.5 EXERCISE AND OXIDATIVE STRESS

Acute exercise-induced oxidative stress has been well documented over the last decade. A single bout of physical exercise has been shown to induce formation of ROS and nitrogen species and the related oxidative damage. On the other hand, regular training is known to increase the resistance against ROS induced lipid peroxidation, and to decrease the accumulation of oxidative protein and DNA damage (Radak *et al.*, 2001).

Previous studies have identified elevations in blood oxidative stress markers after acute exercise, indicating that oxidative stress is not limited to the cellular compartment. Furthermore, very high intensity exercise appears to exaggerate the blood oxidative stress response (Quindry *et al.*, 2003). A number of potential pathways exist for exercise-related oxidant production (Deaton and Marlin, 2003):

I. Oxygen consumption increases several-fold with exercise. Electron leak from the mitochondrial electron transfer chain results in the production of superoxide anions.

Free radical production measured by electron spin resonance spectroscopy correlates strongly with maximal oxygen consumption.

II. Xanthine dehydrogenase oxidizes hypoxanthine to xanthine and xanthine to uric acid using NAD^+ as the electron acceptor forming NADH. During ischemia, in active muscles xanthine is formed via anaerobic metabolism of ATP and xanthine dehydrogenase is converted to xanthine oxidase. During reperfusion, with the resulting increase in oxygen load, xanthine oxidase still converts hypoxanthine to uric acid, but utilizes oxygen as the electron acceptor forming superoxide.

III. Tissue damage resulting from exercise may induce the activation of inflammatory cells such as neutrophils, with the subsequent production of free radicals by NADPH oxidase.

IV. Catecholamine concentrations are increased during exercise, and ROS can result from their auto-oxidation.

V. Muscle mitochondria undergo increased uncoupling and superoxide generation with increasing temperatures. Therefore, exercise-induced hyperthermia may cause oxidative stress.

VI. Auto-oxidation of oxyhemoglobin to methemoglobin results in the production of superoxide and the rate of formation of methemoglobin can increase with exercise.

Aerobic–anaerobic exercises result in a higher degree of oxidative stress and females are able to tolerate oxidative stress more effectively than males (Ilhan *et al.*, 2004). One explanation for the observed gender difference is a higher metabolic rate in men leading to increased mitochondrial flux and increased production of ROS, the female hormone estrogen, another possible explanation, is known to exhibit antioxidant properties (Mastaloudis *et al.*, 2004).

Alessio *et al.*, (2000) found that after exhaustive aerobic (AE) and isometric exercise (IE) protein carbonyls increased 67% immediately and 1 h after AE, and 12% immediately after IE and returned to baseline 1 h after IE. Malondialdahyde (MDA) did not increase significantly with either treatment. Lipid hydroperoxides increased 36% above rest during IE compared with 24% during AE. Oxygen Radical Absorbance Capacity (ORAC) increased 25% after AE, compared with 9% after IE.

Alessio *et al.*, (2000) and Ashton *et al.*, (1999) have also found immediate postmaximal exercise rises in lipid hydroperoxides (42% and 20%, respectively) with no alteration in MDA levels. Alessio *et al.*, (1988) reported that 1 min high-intensity running (45 m/min) in rats resulted in 167% and 157% elevation of TBARS in red slow-twitch and white fast-twitch muscle, respectively. LH increased 34% and 31% in red slow-twitch and white fast-twitch muscle, respectively.

A study by Ortenblad *et al.*, (1997) had subjects perform six bouts of 30-s strenuous jumping with 2-min rest between bouts. Biomarkers of lipid peroxidation did not increase significantly, but several key antioxidants (e.g. superoxide dismutase, glutathione peroxidase, and glutathione reductase) significantly increased.

Groussard *et al.*, (2003) showed that short-term supramaximal anaerobic exercise (Wingate test of 30-s) induced an oxidative stress and that the plasma TBARS level was not a suitable marker during this type of exercise. Short-term supramaximal anaerobic exercise has been associated with a substantial lactic acidosis both in blood and muscle and also with a major increase in plasma catecholamine levels. Moreover, such exercise stimulates the catabolism of purines to xanthine and urate, as evidenced by plasma urate accumulation. In rats, short

intense exercise has been found to induce an increase in either lipid (Alessio *et. al.*, 1988) or protein oxidation (Radak *et al.*, 1998).

Thirty minutes of aerobic and anaerobic exercise performed by young, cross-trained males can increase protein and glutathione oxidation while having little impact on lipid or DNA oxidation; protein oxidation appears to be more greatly affected by anaerobic exercise and the magnitude of protein oxidation is greater following anaerobic compared with aerobic exercise; and glutathione oxidation appears to be more greatly affected by aerobic exercise (Bloomer *et al.*, 2005).

Mastaloudis *et al.*, (2001) reported that plasma F2-isoprostane levels increased significantly (57%) during the 50 km ultramarathon and returned to baseline at 24 h post-race. In untrained humans and animals, one bout of intensive exercise may cause muscle damage, followed by activation of neutrophils in response to inflammation. The activated neutrophils produce ROS, such as superoxide and hydrogen peroxide, which damage the neighbouring cells as well as the neutrophils themselves (Umegaki *et al.*, 2000).

Oh-ishi *et al.*, (1997) reported that superoxide production by neutrophils was increased after intensive exercise in untrained but not in trained rats. These findings may indicate that intensive exercise induces oxidative DNA damage in muscle and blood cells not only by increased uptake of oxygen, but also by muscle damage. High neutrophil counts and neutrophil-generated superoxide levels immediately after maximal treadmill exercise suggests that exercise-induced neutrophilia may have contributed to the observed oxidative stress (Quindry *et al.*, 2003).

Liu *et al.*, (2000) have found acute exercise induced increases in MDA content and decrease in glutamine synthetase activity in liver. Acute exercise did not induce any significant increase in protein carbonyl levels in any organs. For kidney contents, mean values of MDA, protein carbonyl, or glutamine synthetase activity did not change as a result of acute exercise. In the fast muscle, acute exercise induced some decrease in glutamine synthetase activity and vice versa for slow muscle. The differences among organs may be dependent on several factors, such as oxygen consumption, susceptibility to oxidants and to antioxidant enzyme activation, antioxidant levels, and other repair systems. Muscle and heart appear to respond to oxidative stress quite differently than other organs, such as brain and liver, possibly due to the difference in mitochondrial biogenesis and the occurrence of oxidant-induced degeneration.

Carbohydrate ingestion during exercise is associated with reduced levels of stress hormones, which may influence oxidative stress and plasma antioxidant potential. In contrast, McAnulty *et al.*, (2005) have reported that exhaustive resistance exercise did not result in increased oxidative stress as measured by F2-isoprostanes. Furthermore, carbohydrate administration did not affect blood antioxidant capacity or result in differences in F2-isoprostane levels.

From studies and articles reviewed in this chapter, it was speculated that acute aerobic exercise contributes to oxidative stress, especially when performed at high intensity levels. Many studies find increases in oxidative stress markers even in aerobic activities which vary between 65 - 75% of VO_{2max} with exercise duration varying from several minutes to several hours. The relationship between intensity of exercise and duration associated with oxygen radical production has not been clearly defined. Hence, the present study was carried out to