

ANTIOXIDANT CAPACITY AND PHYSICAL EXERCISE

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Abstract. The aim of this article is a presentation of current knowledge regarding the changes of plasma antioxidant capacity observed in response to physical exercise. Human body created the enzymatic and non-enzymatic systems, which play a protective role in the harmful impact of free radicals. Those two systems constitute what is known as the plasma total antioxidant capacity. The amount of reactive oxygen species (ROS) and reactive nitrogen species (NOS) in combination with oxidation processes increases in some tissues during physiological response to physical exercise. These changes are observed after single bout of exercise as well as after regular training. The response of human body to physical exercise can be analysed using various models of exercise test. Application of repeated type of exhaustion allows for characterizing the ability of human body to adjust to the increased energy loss and increased oxygen consumption. This article presents the characteristics of components of plasma antioxidant capacity, the mechanisms of free radicals production and their role in human body. It discusses also the currently used methods of detecting changes in total antioxidant capacity and its individual elements in response to single bout of exercise and regular training. It presents the review of literature about research performed in groups of both regularly training and low exercise activity individuals as well as in group of healthy subjects and patients with circulation diseases. *(Biol.Sport 26:197-213, 2009)*

Key words: Antioxidant capacity – Exercise - Free radicals - Oxidative stress

1.1 Characteristics of antioxidant capacity in cells and in plasma

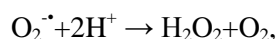
The use of oxygen for breathing by living organisms is connected with production of its reactive forms, the so-called free radicals. In order to protect the

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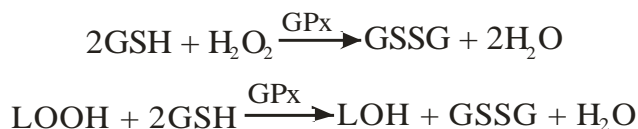


body against harmful impact of free radicals, different neutralizing molecules are created. Among many chemical substances, enzymatic and non-enzymatic systems are the most important in scavenging of free radicals. Those two systems constitute what is known as the plasma oxidant capacity.

Enzymatic system in the human body plays a key defensive role against their detrimental action. It consists of: superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase (SOD) exists inside the cells, e.g. in the cytosol and mitochondria, as well as in plasma. In cytosol and in plasma there are forms of SOD, which consist of copper and zinc: SOD 1 and SOD 3 (Cu, Zn SOD). In mitochondria there is a form of SOD, which consists of manganese ions (Mn SOD). All forms of SOD catalyze reaction of superoxide anion dismutation to hydrogen peroxide:



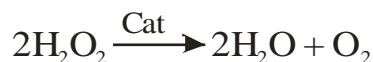
Glutathione peroxidase (GPx) occurs mainly in mitochondria and possesses selenocysteine in its catalytic site. This enzyme is responsible for scavenging of hydrogen peroxide and other organic peroxides:



Glutathione peroxidase requires glutathione as a substrate in the process of peroxides decomposition. Oxidized glutathione is reduced by NADPH in the presence of glutathione reductase:

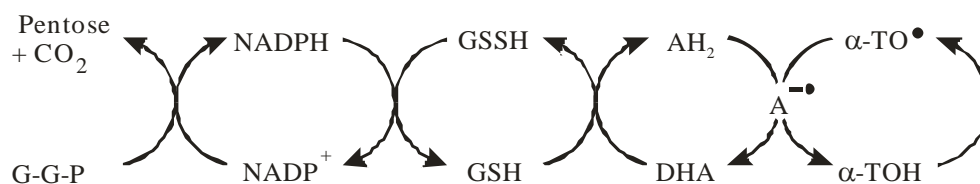


Another important enzyme is catalase, which decomposes hydrogen peroxide:

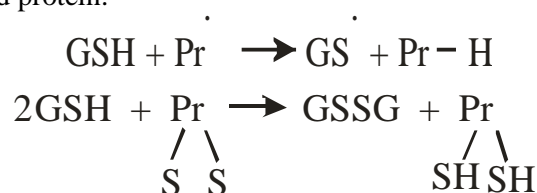


The most relevant components of non-enzymatic system are: ascorbic acid (vitamin C), ubiquinon, taurine, glutathione, β -carotene, tocopherols (vitamin E), albumin, uric acid, bilirubin and flavonoids. Glutathione is the most abundant antioxidant in cytosol. Its concentration is much higher than other antioxidants (1-2mM). GSH interacts with other low molecular weight antioxidants such as ascorbic acid and α -tokoferol.





Glutathione can protect cellular components (protein and lipids) against peroxidation initiated by hydroxyl radical or other radicals and reduces -S-S-bridges in oxidized protein:



Similar action in reduction of disulfides possesses thioredoxin. Ascorbic acid effectively inhibits membrane lipid oxidation and helps recycle oxidized vitamin E. Moreover, vitamin C can reduce lipid or protein radicals:



Ascorbyl radical produces ascorbic and dehydroascorbic acid in the reaction of disproportionation:



In the reduction of dehydroascorbic acid to ascorbic acid glutathione plays another important role:



Plasma is composed of low molecular weight antioxidants such as ascorbic acid, tocopherols (α, β, γ), β -caroten, and high molecular weight components such as albumin, ceruloplasmin, ferritin, lactoferrin, which play an important role in storing the transition metals, e.g. copper and iron ions. Labile ions in reduced state take part in Fenton or Haber-Weiss reactions, which lead to the generation of hydroxyl radicals:





Tocopherols and β -carotene, due to their lipophilic specificity, protect cell membrane and plasma lipoproteins against oxidation. Diet components additionally may have influence on antioxidant amount of non-enzymatic system [1,2,18,29,36].

Currently, plasma antioxidant capacity is one of the most intensively investigated scientific problems for many researchers and its diverse roles in cell protection are proved [28]. In the existing literature, the role of plasma antioxidant capacity in ageing processes as well as in different pathologies was presented in the context of disturbance of antioxidant capacity components efficiency [29].

The aim of this article is a presentation of a current knowledge regarding the changes of plasma antioxidant capacity observed in response to exercise as well as in people with different level of physical activity and different health status.

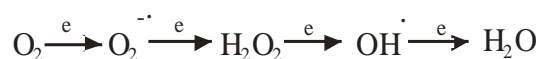
1.2 Free radicals production

During the past 20 years, the field of 'free radical research' has risen to become a mainstream element of biomedical science. 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^\cdot is $t_{1/2}=10^{-9}\text{s}$, whereas organic free radicals with delocalized electron, like a melanin, are characterized by low reactivity and their $t_{1/2}$ equals a few days [9]. 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 lipoxigenase, 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 O₂/kg/min, which makes 350l O₂/day (15 mol/day). It is assumed that 2-3% of O₂ 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. Chapter 1 describes enzymatic and nonenzymatic defensive systems in cells. 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 ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{\cdot}). 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 (NO_2) and peroxyntirite ($ONOO^-$). Peroxyntirite anion can be transformed to peroxyntiric 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 [19]. 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 [29]. In higher concentration, nitric oxide has antimicrobial, antitumor and cytotoxic effect. Many diseases are also related to the



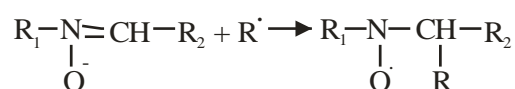
over- and underproduction of NO and to the direct participation of NO in some pathological mechanisms [7,8,45].

1.2 Methods used in the detection of free radicals during physical exhaustion

Concentration of free radicals in conjunction with the process of peroxidation increases in some of the tissues due to physiological response of the body to physical exhaustion. Biochemical measurements of the level of generated free radicals are mainly based on indirect assessments of oxidative stress. These methods use only co-products detection of free radicals reactions.

More sophisticated method, which can be utilized in exercise physiology and medicine, is electron paramagnetic resonance (EPR). EPR allows for direct detection of free radicals in physiological and biochemical systems. For many researchers EPR technique is known as the most specific, sensitive and direct method for measuring free radicals [4,13,20]. It was demonstrated that free radicals production detected by EPR in the circulation is connected with extracellular hydroxyl radical production [4,20]. Moreover, it is believed that detecting of the circulating EPR radical provides a non-invasive method of evaluation hydroxyl radical generation in skeletal muscle [4,20].

Spin trapping method is used to transform short life radicals to the stable paramagnetic adduct, which can be measured in room temperature. The reaction of free radical with spin trap is as follows:



where R can be hydroxyl radical, superoxide, alcoxyl, or peroxide radical.

EPR in conjunction with spin trapping method was applied in the investigation of maximal physical exhaustion (with the usage of cycle ergometer) according to progressive and incremental exercise protocol [4,5]. Studies showed a statistically significant increase of EPR signal intensity, which derived from tert-butyl nitron (PBN) adducts after exercise [4,5]. Spectrum analysis in both studies showed that alcoxyl radical was generated during oxidative damage of membrane lipids. It can be explained by the fact that lipid peroxidation is a chain reaction, which in consequence leads to the free radicals formation. Authors presented three-fold increase of the free radicals concentration after exercise [4]. The highest elevation was detected among the subjects with the highest maximal oxygen uptake ($\text{VO}_{2\text{max}}$). Furthermore, a statistically significant increase of lipid peroxidation as



well as total antioxidant capacity (TAC) was observed [4]. Another study was performed with healthy volunteers, who for 8 weeks before entering the investigation were supplemented with 1000 mg of ascorbic acid [5]. Results were compared with the subjects without vitamin C supplementation. Volunteers were subjected to a single bout of exhaustive exercise. EPR analysis presented a significant increase of signal intensity, which derived from PBN adduct immediately after exercise. This was connected with the process of free radicals production. EPR signal intensity was correlated with free radicals concentration in the sample. Spectra analysis as well as comparison of hyperfine coupling constants recorded from EPR suggested that trapped free radical was alcoxyl radical derived from the reaction between oxygen-centered radicals with membrane phospholipids. EPR signal intensity after exercise in supplemented subjects was similar to control samples before exercise. Ascorbic acid appeared to be a very efficient antioxidant. Results from this study suggest that the supplementation of high dosage of vitamin C significantly influences the decrease of EPR signal intensity as well as inhibits lipid peroxidation. A diet rich in vitamin C can effectively protect body against reactive species production induced by physical exhaustion. However, overdosing of the vitamin C is not neutral for the body. Therefore, high dosages of this vitamin are nowadays not recommended [13]. Results described by Asthon *et al.* [4,5] were confirmed by the experiment from another study [13], in which EPR technique was similarly used for direct free radicals assessment. Hyperfine coupling constants analysis revealed that alcoxyl radical was trapped and derived from the membrane lipids peroxidation. In this study also copper-derived radical was detected. It can suggest the albumin deterioration upon physical exhaustion. A significant increase of reactive oxygen species was observed following downhill running occurred 72 h post exercise. Furthermore, this increase in ROS production occurred at a time when the peak of muscle soreness decreased and muscles functions returned to the baseline values. This may suggest that ROS are not responsible for initiating the damage but may play role in mediating the recovery.

Based on the existing literature, EPR in conjunction with spin trapping method in physiological model of oxidative stress generated by physical exercise can be utilized in this research area and can be recognized as the direct and specific assessment of free radicals.

The biochemical method, which is commonly used in the investigation of oxidative stress, is the indirect markers analysis of lipid peroxidation like malondialdehyde (MDA), diens, expired pentane, lipid hydroperoxides, conjugated diens, isoprostanes. 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. The most common indirect detection of generated oxidative stress to assess changes in MDA with exercise is the thiobarbituric acid (TBARS) assay. TBA can react with several aldehydes to form yellow or orange complexes. Santos-Silva *et al.* [38] described increase of resting MDA level in trained adolescent swimmers compared with control subjects. Also Marzatico *et al.* [33] found higher MDA level in marathon runners compared with subjects. Several studies showed that single bout of exercise increase blood level of MDA [21,34]. However strenuous endurance training was shown to reduce indices of oxidative stress [34]. Moreover, in response to exercise in trained skiers and runners immediately after the exercise the decrease of MDA was presented [23,37]. 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. It is a matter of discussion whether the discrepancies between different studies are within acceptable limits, and it seems that some of the reported values may not reflect true levels of thioarbituric acid reactive substances (TBARS) in vivo. Criticism of this method is mainly connected with lack of specificity and repeatability [27]. Diversity of the results interpretation may be connected with different modifications of the method by many authors [20,30,32]. One reason why no significant amounts of MDA are detected using classical method is that MDA binds to proteins [11]. To detect free and bound MDA the TBARS-assay has been modified to realise protein-bound MDA, which can be achieved by hot alkali digestion [16]. However spectrophotometric spectra analyses show the lack of specificity of TBA in binding aldehydes.

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 [39,41]. 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.* [49] 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 [26]. During the performance of such a test it is possible to estimate physical capacity of the body as well as VO_{2max} [29].

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 radicals 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.

1.4 Antioxidant capacity in people with regular physical activity

Changes of enzymes activity under physical exhaustions in regularly trained subjects are ambiguous.

Analysis of superoxide dismutase activities showed contradictory results. Some studies showed that SOD activity increased during exercise tests performed on cycle ergometer or treadmill [10,40,46], but no significant changes among cyclists were detected [6]. However a decrease during exercise on cycle ergometer was described by Groussard [20].

Glutathione peroxidase level increased significantly after exercise tests with the usage of treadmill and cycle ergometer [29,40,46]. However tests where participants were subjected to maximal exhaustion within the next 5 days the decrease of enzyme concentration was observed. Return to the baseline level was observed 3h after performance of physical test. However glutathione concentration measured in the same time did not confirm results obtained for glutathione peroxidase concentration. Tests performed on cycle ergometer presented a decrease



of glutathione concentration [20,46], whereas other tests (sport exercises) showed an increase of glutathione concentration [24,29].

Results from catalase activity are more univocal. Analyses of results revealed an increase of its activity under physical exhaustion [6,29,46], or no statistically significant difference after exercise [40].

Creatine kinase activity mostly increased immediately after exercise test [12,29,31]. However no statistically significant difference among people with regular physical activity was observed. It can support the theory of muscle cells damage after exercise [29]. Some of investigations indicate a higher level of kinase concentration at rest in trained people [29].

Only few studies focused on alterations of non-enzymatic system of antioxidant capacity. Some reports presented an increase of tocopherol and ascorbic acid [6,10,29]. However results obtained from weight lifting women were contradictory [31]. Also in cyclists no differences in concentration of retinol and β -carotene were detected [6].

Regardless of the existing differences in reported results, various studies tend to indicate a significant impact of physical exercise on free radicals production. It may be necessary to pay more attention on individual total antioxidant capacity in physical training planning.

1.5 Antioxidant capacity in people with low physical activity

Interpretation of results concerning people with the so-called sedentary lifestyle is difficult because there exist very few studies, which are focused particularly on that group. Usually subjects characterized by low physical activity are used as control group.

In many studies the analysis of total antioxidant capacity level shows that its initial value is lower in untrained group than in the trained one [10,17,40]. Exercise tests or series of training allow for conclusions that level of total antioxidant capacity increases in group without regular physical activity [15,17,40].

It was demonstrated that SOD concentration increases after physical exhaustion [15,40]. Similar effect was observed in case of glutathione peroxidase [40]. However results from the same study showed that concentration of both enzymes was lower in subjects with low physical activity than in the trained group. Using cycle ergometer in the sedentary patients the level of reduced glutathione decreased immediately after physical exhaustion, but it significantly increased in recovery [25]. Similarly, there was no difference in catalase activity after exercise test [40].



Another study performed among volunteers with low physical activity (with the usage of cycle ergometer) showed the results from indirect detection of ROS production with the application of TBARS assay. The concentration of thiobarbituric acid reactive substances increased during exercise with the maximum level in recovery. Positive correlation between TBARS value in recovery and the subject's age was found. The older the subject, the higher the TBARS value was estimated [25].

Components of non-enzymatic system were investigated during exercise in studies with group of low physical activity. Few of them indicated the decrease of ascorbic acid concentration [25] and no changes in tocopherol level [14].

In other studies, the changes of lipid peroxidation in blood plasma were analysed (mainly HDL and LDL cholesterol fraction). No statistically significant influence of physical exhaustion on lipid peroxidation in group with sedentary patients was detected [14,40].

Due to the fact that people with sedentary lifestyle have lower total antioxidant capacity, it may be necessary to plan and increase the intensity of physical exercises more carefully, so that human body can adapt defensive mechanisms to the increased production of free radicals.

1.6 Antioxidant capacity during physical exhaustion in people with cardiovascular diseases

Oxidative stress can cause changes in function of endothelial lining of internal side of blood vessels. Endothelium is responsible for vessel function, it secretes substances regulating gases and chemical substances transport as well as hormones and molecules determining vessel contraction (e.g. nitric oxide). Aerobic exercise exacerbates oxidative stress, which is connected with an increase of lipid peroxidation, which significantly induces processes of atherosclerosis inside blood vessels [35,43]. On the other hand it is well known that exercise performed on regular basis supports angiogenesis and arteriogenesis, improves tissues blood flow and activates intracellular defence mechanisms against atherosclerosis evolution. Regular physical activity is related to antiapoptotic and fibroblasts antiproliferation activities. It also increases availability of nitric oxide, which improves diastolic reaction on elevated blood pressure [28]. Antioxidant system protects generated nitric oxide and enables its more efficient use [22].

Existing studies about efficiency of supplementation with ascorbic acid and tocopherol in cardiovascular diseases indicate favourable decrease of lipid peroxidation as well as positive increase of total antioxidant capacity [22,47].



In subjects with arterial hypertension a higher level of oxidative stress in rest and during physical activity is reported. In that group the lower activity of superoxide dismutase and catalase was detected comparing to healthy subjects. Furthermore, the increase of oxidative damage protein products was reported after physical exercise [44].

In subjects with coronary heart disease an increase of superoxide dismutase and glutathione peroxidase activity was detected. The level of oxidized LDL molecules in blood serum also was higher in rest [50]. It may indicate that this group of patients is characterized by increase of chronic oxidative stress despite medical treatment and lack of clinical symptoms. However other experiments showed a decrease of glutathione peroxidase, superoxide dismutase and glutathione with a significant increase of TBARS level in patients with coronary heart disease after exercise test [3]. Similar results were obtained in group of patients with myocardial infarction after cardiogenic shock complications. A decrease of antioxidant enzymes activity in blood serum and in concentration of ascorbic acid, tocopherol and β -carotene were observed [42].

Results published in existing literature indicate an important role of antioxidant capacity components in pathogenesis of cardiovascular diseases and their evolution. Results interpretation and comparison with healthy population has to be done with particular attention to differences connected with changes in antioxidant capacity related to the age of examined subjects and also to their pharmacological treatment. It should be additionally considered that physical exercise performed by people with cardiovascular diseases, in secondary prevention as well as in assisting treatment, can also change antioxidant capacity of blood plasma.

1.7 Summary

The described results from different studies indicate that the role of components of antioxidant capacity is not well established, especially in the context of exercise performed by patients in different clinical states or in subjects with different physical activities. Various methods, which are applied in antioxidant capacity investigations as well as various exercise protocols do not allow for making an univocal statement about a profile of changes, which occur in the human body upon physical exhaustion. We would like to suggest that planning the intensity of physical activity in healthy subjects with different exercise level and in patients with diseases, the individual scavenging and protecting ability of the body against detrimental influence of free radicals should be carefully analyzed. Therefore we



would like to emphasize that antioxidant capacity should be considered in the patients classification to physical effort.

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