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Dedicated to Professor Florin Dan Irimie on the Occasion of His 65th Anniversary

CHANGES OF OXIDATIVE STRESS CAUSED BY PHYSICAL ACTIVITY

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ABSTRACT. Free radicals and reactive oxygen species are produced in the human body as a part of metabolic processes. Reactive species in low levels are important for cellular activities. Excessive amounts of reactive species can be harmful because they can produce lipid peroxidation, proteins and ADN oxidation. For reduce these harmful effects the organism requires an antioxidant defence. Oxidative stress is involve in atherosclerosis, coronary heart disease, metabolic syndrome, type 2 diabetes mellitus. The aerobic and anaerobic exercises have different effects on the muscles, but both influence positively the biomarkers of oxidative stress. Studies prove that aerobics increase endogenous antioxidant status. Regular moderate exercises produce an increase in antioxidant activity, due to the changes in redox homeostasis. The aim of this review is to discuss the importance of a constant physical activity for increase the body's antioxidant system and to protect against oxidative stress.

Keywords: *Free radicals, reactive oxygen species, oxidative stress, metabolic syndrome, aerobic exercises, anaerobic exercises, antioxidant system*

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INTRODUCTION

Oxidative stress (OS) is characterized by an imbalance between the production or inactivation of reactive oxygen species, and imbalance between oxidants and antioxidants in favour of oxidants with a destructive and pathogenic effect [1]. Free radicals are substances that are derived from incomplete oxidized compounds having in their structure oxygen groups capable of initiating aggressive oxidation reactions on the cells.

Reactive species such as reactive oxygen and nitrogen species (ROS and RNS) in low levels are important for gene expression, cellular growth, infection defense, regulating cell signalling pathways, regulating blood flow, and controlling superior nerve activity. Excessive amounts of ROS and RNS can be harmful because they can produce lipid peroxidation, proteins and ADN oxidation [2]. The most active free radicals are ions: superoxide, peroxide, hydroxide, nitric acid [2].

Most endogenous free radicals occur at the level of: mitochondria (during generation the ATP-adenosine triphosphoric acid), peroxisomes, cytochrome P450, and phagocytes. At the mitochondria level, following the molecular oxygen reduction reaction by cell respiratory cytochromes, about 2% of the total amount of ROS occurs. Superoxide is produce by NADH dehydrogenase, cytochrome C oxidoreductase or succinate dehydrogenase [3].

Peroxisomes are primarily responsible for oxygen consumption in the cell and maintain low ROS production by the balance of enzyme concentration or activity, such as catalase. Thus, destruction of peroxisomes allows the release of hydrogen peroxide in the cell and oxidative stress. Phagocytes play an important role in the inflammatory response of the body. Increased oxygen consumption and activation of NADPH-oxidase accompany the main bactericidal, oxygen-dependent mechanism [2]. Cytochrome P450 is a group of enzymes present in almost every cell of the body that plays a role in the metabolism of steroids, fat soluble vitamins, fatty acids, prostaglandins and alkaloids. Some enzymes of this group detoxify drugs and some environmental pollutants [2].

Under abnormal environmental conditions such as excessive heat, ultraviolet radiation, pollutants, the levels of ROS increase dramatically, resulting in serious cellular damage. Nitrogen reactive species are a family of antimicrobial molecules derived from nitric oxide and superoxide, produced by the activity of NOS2 enzymes and NADPH-oxidase, resulting peroxynitrite [2]. NOS are present under three isoforms: NOS-1 (in the nervous tissue), NOS-2 inducible enzyme (expressed primarily in macrophages) and NOS-3 (in endothelial cells).

For fight against the ROS are antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and other antioxidant molecules: ascorbic acid (vitamin C), tocopherol (vitamin E),

vitamin A, flavonoid, and ubiquinone [4]. Glutathione protects cells against free oxygen radicals by formation of glutathione disulphide (GSSG) which is reduced to free glutathione (GSH) under the action of glutathione reductase. The GSH / GSSG ratio can be used as a marker of increased intracellular oxidative stress.

The measurement of free radicals is very difficult because they are in low concentrations and have a short life span. For determination are used different methods such as: the total oxidative status, total antioxidant capacity, oxidative stress index (ISO- ratio of oxidants to antioxidants), evaluating lipid peroxides by determining Malonyldialdehyde (MDA), measure carboxylates proteins by determining carbonyl groups, measure of serum thiols by determination of total sulfhydryl groups (SH), determination of serum glutathione [5].

Determination of loss of unsaturated fatty acids is useful for evaluating lipid peroxidation stimulated by certain pro oxidants. The system studied has to be decomposed (lipids extracted from cells or lipoproteins) and the lipids have to be hydrolysed to release fatty acids that can be measured by high performance liquid chromatography (HPLC) or converted to volatile products and separated by gas-liquid chromatography (GLC) [5].

Direct methods such as determination of iodine concentration, oxidation of ferric ions detected with xylene-orange (FOX), determination of glutathione peroxidase, cyclooxygenase (COX) determination, degradation of peroxides by hem, gas chromatography (GC) and mass spectrometry (MS) are used for the determination of lipid peroxides (aldehydes, isoprostanes, cholesterol peroxides / cholesterol esters) [6].

Other methods used are methods which determine antioxidant activity such as total peroxyl radical trapping antioxidant parameter (TRAP method), determines the ability of plasma antioxidants (vitamin E, urate, ascorbate but not albumin) to reduce Fe (III) to Fe^{2+} at low pH, incubating ABTS® [2, 2-azabis (3-ethylbenzothiazoline-6-sulfonate)] with a peroxidase-meth globin and H_2O_2 to produce $ABTS^{+}$ cation [6]. In all these methods a radical is generated and purified by antioxidants. When all the antioxidant systems have been depleted, the radical reacts with a target molecule to produce colour, fluorescence, chemiluminescence, loss or gain of electron spin resonance signal [5, 6]. Methods for determining the total antioxidant capacity in biological fluids are useful in measuring relative antioxidant activity and changes occurring under clinical conditions.

Nowadays the involvement of oxidative stress in pathogenic processes with socio-economic impact such as atherosclerosis, coronary heart disease, metabolic syndrome, type 2 diabetes mellitus, hypertension, is accepted and partially demonstrated [7].

The metabolic syndrome is present when three or more of the following signs are occurring: increased blood pressure, abdominal circumference, serum triglycerides, and low levels of HDL cholesterol and insulin resistance. This syndrome is often characterized by increased generation of reactive oxygen species and decreased activity of antioxidant systems such as low serum vitamin C levels, α-tocopherol and decreased superoxide dismutase activity. In addition, these patients have an increase in malondialdehyde and carbonyl groups levels [8].

Oxidized LDL-cholesterol, a biomarker of oxidative stress, activates circulating monocytes and increases their ability to infiltrate the vascular wall, in the initial stage of plaque formation. Lipid peroxidation can produce changes in permeability of membranes and also enzymatic membrane equipment can be damage. Cytotoxic products resulting from lipid peroxidation contributes to endothelium damage, platelet aggregation, release growth factors stimulating proliferation of smooth muscle cells, and inflammatory response. ROS production stimulates the release of proinflammatory cytokine such as, interleukin-1 (IL-1), IL-6, leptin, and adiponectin, by monocytes and macrophages through activation of the transcription factor nuclear kB (NFkB) [9]. Cytotoxic products may also increase the release of chemotactic factor for polymorphonuclear cells and alter phospholipase A2 activity with the subsequent formation of prostaglandin and endoperoxides [9]. Through these mechanisms oxidative stress may trigger an inflammatory response directly involved in the pathogenesis of atherosclerosis. High plasma levels of oxidized LDL-cholesterol were associated with an increased risk of myocardial infarction, independent of LDL-cholesterol and other cardiovascular risk factors, indicating that LDL-oxidized would be a predictive value biomarker [9].

Oxidative stress can damage proteins and may produce changes in their aggregation, in enzymes activity, ions transport, and also can induce proteolysis. ROS contribute to the activation of protein, tyrosine kinases, protein kinase C, and the MAP kinase cascade which play an important role in cellular responses such as activation, proliferation, and differentiation. Also high levels of ROS can inactivate mitochondrial enzymes, causes DNA damage and hence higher frequency of mutation [10]. ROS can activate NFkB which regulates the expression of genes which are associated with atherosclerosis and diabetes (Figure 1).

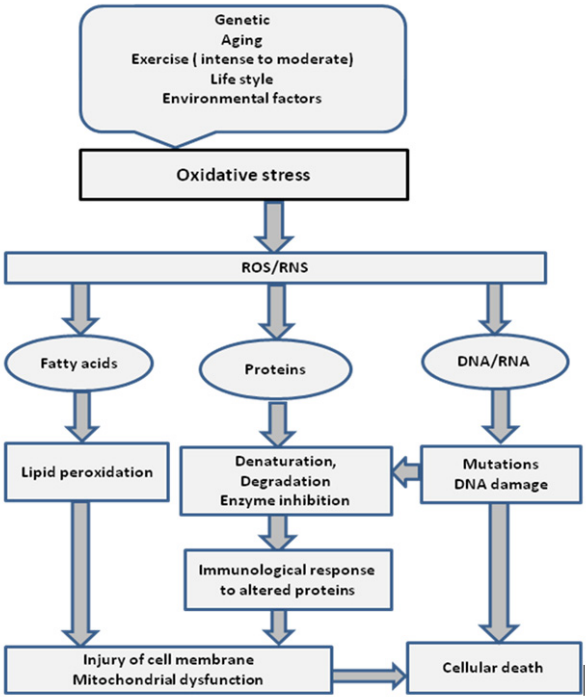

Figure 1. The pathophysiological mechanism by which oxidative stress contributes to the occurrence of various diseases

High levels of plasma glucose can increase oxidative stress through activation of protein kinase C (PKC), increased hexosamine pathway flux, increased advanced glycation end products (AGEs), and increased polyol pathway flux [11]. Increased AGEs will determine to bind to AGEs receptors (RAGEs) on endothelial cells and increase the production of growth factors and cytokines which will contribute to endothelial dysfunction [11]. High glucose level and free fatty acid stimulate reactive oxygen species production which can directly alter the production of NO or reduce the bioavailability of nitric oxide already produced reducing vasodilatation that contributes to hypertension. Nitric oxide is synthesized from L-arginine under the influence of the enzyme NO synthase. NO inhibits oxidation of LDL-cholesterol, proliferation and migration of smooth muscle cells, adhesion and platelet aggregation and produce vasodilatation. NO can reacting with superoxide anion forming peroxynitrite, which can oxidize NOS making it unstable. NOS inhibitors, such as the asymmetric dimethyl-arginine (ADMA), can contribute to hypertension through vasoconstriction [12]. Hyperglycaemia increases the production of endothelin-1, a powerful vasoconstrictor involved in endothelial dysfunction.

Muscles consume high quantities of oxygen and can generate ROS and RNS during muscle contraction. ROS and RNS have multiple effects on muscle excitability, contractility, and calcium homeostasis. On the other hand, they are involved in the muscular fatigue during strenuous physical activity.

Nowadays it is discussed that ROS and RNS plays a role in the development of sarcopenia leading to a reduction in muscle mass quantity and strength [13]. OS act on mitochondria leading to alteration of oxidative phosphorylation which contribute to decreased intracellular ATP. OS can reduce acetylcholine release at the synaptic cleft and impair neuromuscular transmission, also can lower the release of calcium from sarcoplasmic reticulum and impair excitation–contraction coupling and contractile properties of myofilaments [13, 14].

A good understanding of the link between oxidative stress and disease can be a real support in discovery of targeted treatment strategies for a better health.

Oxidative Stress and Physical Activity

There are several types of physical effort and each type has a different effect on oxidative stress. According to the type of contraction are isotonic (dynamic), isometric (static), and isokinetic effort. According to effort intensity are high, moderate, low intensity efforts. Based on the oxygen supply of the body there are aerobic, anaerobic, mixed effort.

Inactivity and high-intensity physical exercise increases OS, but moderate intensity exercise is linked to a reduction in OS levels [15]. Physical activities with intensities between 50% and 80% of VO_2max (the maximum rate of oxygen consumption) and with a frequency of three sessions per week are indicate for prevention OS [15].

Regular physical activity is recommended for prevention cardiovascular diseases. It is known that approximately 30 minutes per day of exercise training at moderate-intensity decrease the cardiovascular events [3]. For sedentary subjects the physical activity should be increased progressively in intensity and duration, to avoid excessive fatigue, muscle pain, or injuries.

The regular physical activity decreased blood pressure, cholesterol and body mass index which are important especially in prevention of the clinical sequel of atherosclerosis [16]. The 20 elderly women who were over 65 years of age had 30% body fat, underwent a 12-week healthy life exercise program. To evaluate the effects of the healthy life exercise program, measurements were performed before and after the healthy life exercise program in all the subjects. After the healthy life exercise program, MCP-1 and the arteriosclerosis adhesion molecules sE-selectin and sVCAM-1 were statistically significantly decreased. The 12-week healthy life exercise program

reduced the levels of arteriosclerosis adhesion molecules. Therefore, the results of the study suggest that a healthy life exercise program may be useful in preventing arteriosclerosis and improving quality of life in elderly obese women [16].

Further studies demonstrate the beneficial effects of the regular physical training on myocardial vasodilation improving O2 consumption and produces cardio protection. Two-hundred coronary artery disease (CAD) patients (LVEF > 40%, 90% men, mean age 58.4 ± 9.1 years) were randomized to a supervised 12-week cardiac rehabilitation program of three weekly sessions of either AIT – aerobic interval training (90–95% of peak heart rate (HR) or ACT – aerobic continuous training (70–75% of peak HR) on a bicycle. Primary outcome was peak VO2; secondary outcomes were peripheral endothelial function, cardiovascular risk factors, quality of life and safety [17]. Peak VO_2 (ml/kg/min) increased significantly in both groups (AIT 22.7 ± 17.6% versus ACT 20.3 ± 15.3%; $p < 0.001$). In addition, flow-mediated dilation (AIT +34.1% (range –69.8 to 646%) versus ACT +7.14% (range –66.7 to 503%); $p < 0.001$) quality of life and some other cardiovascular risk factors including resting diastolic blood pressure and HDL-C improved significantly after training. Improvements were equal for both training interventions. Contrary to earlier smaller trials, it was observed similar improvements in exercise capacity and peripheral endothelial function following AIT and ACT in a large population of CAD patients [17].

On the other hand, it is well known that aerobic exercise can increase endothelial function and can mediate the alternation between vasoconstriction and vasodilatation. In literature there is a lack of information as far as that goes the effects of exercise on vascular endothelial function particularly throughout the post exercise period and relation to oxidative stress, production of endothelin-1 (ET-1) and exercise intensity. Twenty one healthy, young men (24 ± 5 years) underwent assessment of brachial artery FMD (flow-mediated dilatation) using high-resolution ultrasound before and after 30-min of moderate-intensity cycle exercise (80% maximal heart rate). Subsequently, subjects performed five 30-min cycle exercise bouts at 80% maximal heart rate across a 2-week period, followed by repeat assessment of resting brachial FMD post-training [18]. Correcting for changes in diameter and shear, FMD did not change after the initial exercise bout ($P = 0.26$). However, a significant correlation was found between post-exercise changes in FMD and adaptation in resting FMD after training ($r = 0.634$, $P = 0.002$), where an acute decrease in post-exercise FMD resulted in a decrease in baseline FMD after 2 weeks and vice versa. We also found a positive correlation between shear rate during exercise and change in FMD% after acute exercise and after exercise training ($r = 0.529$ and 0.475, both $P < 0.05$) [18].

It has been observed a reduction of potent vasoconstrictor ET-1 after aerobic exercise training and an increase of endothelin-1 at the end of acute intense exercise [19]. Moreover, hard aerobic exercise is associated with transient reductions of flow-mediated dilation and moderate-intensity exercise confers good effects which improve production of reactive oxygen species at higher effort [20].

During exercise the production of ROS is increased by 50-100 times more than during rest [1]. It was found that MDA and lipid peroxidation were elevated after high-intensity physical activity [21]. But in other study the ROS have been shown to decrease among participants with regular exercise training or low-intensity physical activity [22]. Also higher physical activity was associated with a decreased SOD activity and an inverse relationship between physical activity and SOD levels was found [23].

Other activities such as intermittent team sport activities, short durations rock-climbing, cycling exercise were linked with high levels of markers of the oxidative stress [24, 25].

The type, intensity and duration of exercise being utilized are dependent in ROS production. The ROS were found elevated at greater exercise intensities 25 vs. 50 vs. 75 % of percentage of maximal exercise capacity VO2max after 30 min stationary cycling [26]. Also longer durations (120 vs. 60 vs. 30 min) of stationary cycling at 75 % VO_2 produced more oxidative stress marker [27].

During strenuous exercise IL-6 is produced in muscles proportionally with duration of the exercise and acts as a myokine [28]. Beside IL-6 other pro-inflammatory molecules such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β and macrophage inflammatory protein (MIP)-1 are found [29].

The aerobic and anaerobic exercises have different effects and responses on the muscles, but both influence positively the biomarkers of oxidative stress. It has not been elucidated the method that would explain benefits from daily aerobic and anaerobic exercises. Some studies prove that aerobics increase endogenous antioxidant status and protect against oxidative stress [30]. In a study with 113 untrained subjects undergoing treadmill exercise the authors evaluated total antioxidant capacity, peroxide, oxidative stress index and DNA damage. They found that during exercise ROS increased, antioxidant capacity and vitamin C decreased [31]. In another study were investigated if half-marathon or marathon run in healthy hobby runners can alter DNA, antioxidant capacity in lymphocytes and plasma. Increases in the levels of oxidative DNA damage in lymphocytes were found. Also the number of granulocytes and monocytes able to generate oxidative burst were significantly increased after both races, but the lytic activity of NK cells was significantly increased at the end of the half-marathon [32].

The anaerobic intense physical activity can induce OS and results in damage of proteins, glucose, lipids and nucleic acids in muscle cells. In aerobic exercise the ROS and RNS are produce during mitochondrial respiration, but in anaerobic exercise oxidative stress may result from the ischemia/reperfusion cycle of muscle contraction and/or immune system responses following muscle damage [33].

Recent studies have proved that free radicals produced during exercise are keys to adaptive processes [34]. Regular moderate exercises produce an increase in antioxidant activity and resistance to oxidative stress, due to the changes in redox homeostasis [35]. On the other hand, it has been show that subjects expose to large amount of physical activity have impaired cardiovascular health.

Exercise causes oxidative stress only in overload training (in large volume or long-term period) and can lead to an impaired antioxidant defense. But if exercise is practiced in moderation, it can increase the antioxidant enzymes. During exercise MAP kinases are activate and in turn activated the NF-kappa B pathway and the expression of superoxide dismutase, an antioxidant enzymes [36]. For that exercise it can be considered an antioxidant [36].

Overload training may lead to inflammation which is also associated with increased oxidative stress. In overload training in untrained subjects exercise leads to increases of ROS, which cannot be eliminated by endogenous antioxidants. During regular exercise, in trained persons, ROS increased production contribute to body adaptation by improving antioxidant capacity, mitochondrial biogenesis, insulin sensitivity and aerobic capacity of skeletal muscle [37].

Oxidative stress can be detected immediately after exercise but the long-term effects are better observed when we measure antioxidant activity. For example, decreased levels of enzymatic antioxidants have been reported 19 days after an ironman triathlon competition [38].

During physical overtraining increased levels of urinary isoprostanes, serum levels of TBARS, protein carbonyls, CAT, GPX, and GSSG and decreased levels of GSH, the GSH/GSSG ratio, and total antioxidant capacity in blood serum were found [39].

Chronic exercises, during a long period of time, were found to increase the antioxidant capacity (Figure 2).

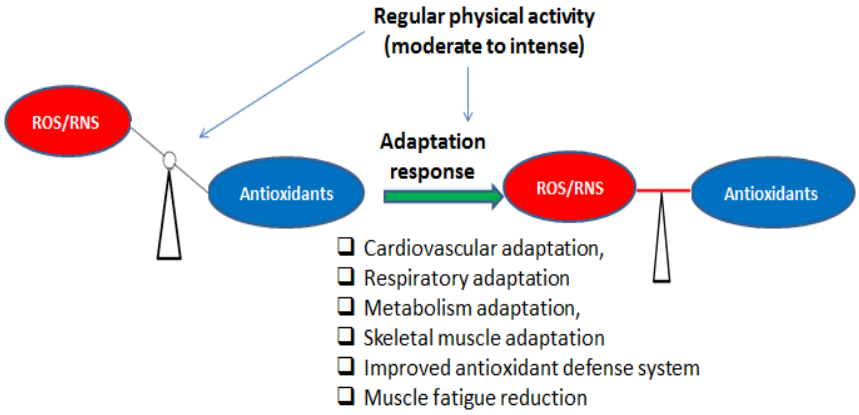

Figure 2. The physiologic process of body adaptation to regular physical activities

Beside physical training the increase of exogenous antioxidant defence, can be obtained by administrations a natural antioxidant nutrition or supplementation with non-nutritional antioxidants.

The exogenous antioxidants such as vitamin C, E, and carotenoids, have the positive effect and can protect against oxidative stress. Was demonstrating that a diet poor in antioxidants, during intensive short-term exercise can increases oxidative stress, and supplementation with multivitamins, before and during the marathon, was found to prevent the increase of lipid peroxidation [40].

To find out if a diet supplemented with vitamin C and E can be considered beneficial during exercise, Ristow et al. [41] investigated the effects on exercise induces insulin sensitivity as established by glucose infusion rates during a hyperinsulinemic euglycemic clamp before untrained and pre-trained healthy young men. Has been demonstrated that exercise increase parameters of insulin sensitivity only in default of antioxidants, both for trained and untrained individuals. At the same time, it was observed the increase expression of ROS-sensitive transcriptional regulators of insulin sensitivity and ROS defense capacity. The exercise induced molecular mediators of endogenous ROS defense (Mn-SOD, Cu, Zn-SOD, GPX) and this effect was again blocked by antioxidant supplementation. The authors concluded that exercise induced oxidative stress ameliorates insulin resistance and causes an adaptive response promoting endogenous antioxidant defense capacity and that supplementation with antioxidants may preclude these health-promoting effects of exercise in humans. The exercise causes an activation of mitogen-activated protein kinases which activates nuclear factor

in the muscles and consequently the expression of important enzymes associated with defense against ROS (SOD) and adaptation to exercise – endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) [36, 42].

The advantages of exercise are widely known, but there is a lack of information about correct mode, type, length, frequency of exercise necessary to gain such benefits. The quality of low volume exercise performed with high intensity has become more and more noteworthy with facts showing comparable efficiency of high intensity interval workout and traditional training based on resistance in metabolic control of skeletal muscle and cardiovascular system activity [43, 44]. Besides promotions of physical exercise, having an antioxidant-rich diet with healthy eating habits can prevent oxidative stress. A dietary with caloric restriction and surplus of vitamins are known in relieve of oxidative stress.

CONCLUSIONS

Cardiovascular pathology is linked to oxidative stress, inflammation and endothelial dysfunction. Physical activity is recognized as an important component of healthy lifestyle but when are practiced strenuously it causes OS and cell damage. Moderate exercise is recommended to improve the physiological and functional capabilities because it increases the expression of antioxidant enzymes. Mechanistic analysis of free radicals may be useful for physiotherapists and health professionals, in particular when comparing different exercise doses, trying to outline appropriate recommendation for physical exercise in guidelines for health.

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