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PHYSICAL EXERCISE AND OXIDATIVE STRESS

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Abstract

The health benefits of a moderate regular physical exercise are well documented in the subject literature. However, strenuous physical exercise or physical activity increases concentration of reactive oxygen species (ROS), generated within mammalian cells as a consequence of strongly increased consumption of molecular oxygen in respiration. The ROS include some molecules containing oxygen, e.g., superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), hypochloride ion (ClO^-), nitric oxide radical ($NO^{\bullet}$), peroxynitrite ($ONOO^-$). Increased aerobic metabolism during exhaustive exercise can cause an imbalance (so-called oxidative stress) between the ROS production and the antioxidant defense system capacity. Knowledge of the ROS formation mechanism during exercise is important because these species can effect both the tissue damage and adaptation to the oxidative stress, accompanying exercise. Because ROS can stimulate the development of cancer and other diseases, reducing of oxidative stress is important in strategies of prevention of the ROS-related diseases. This paper provides a brief summary of the present state of knowledge dealing with mechanisms of ROS formation during exercise or post-exercise, methods of their detection, role of ROS in cell signaling, and of the effect of regular exercise training on adaptation to oxidative stress.

Key words: *reactive oxygen species-sources, oxidative stress, exercise, training, adaptation*

Introduction

The benefits from regular moderate physical exercise (40-60% maximal oxygen uptake, $\dot{V}O_2max$) is recommended for primary and secondary prevention of a number of chronic diseases and psychological disorders (e.g., cancer, cardiovascular disease, diabetes, osteoporosis, depression, obesity) [1-4]. Nonexhaustive physical exercise reduces the formation of reactive oxygen species (i.e. oxygen molecules or some compounds of oxygen, hydrogen and nitrogen showing higher reactivity than molecular oxygen) (ROS) and other oxidants, improves antioxidant defense system and enhances the resistance of tissues against the toxic action of ROS [5-8]. The benefits of regular moderate intensity physical activity are especially important for older people. They can experience improved flexibility strength, balance, coordination, motor control, and mental health [9,10]. For more information on health gain, economic and social benefits through participation in regular moderate physical exercise the reader is referred to the recent reviews [3,11,12].

However, it is now clear that physical exercise, especially too intense or sporadic could cause damage to muscle cells or inflammatory reactions within them; some of this damage is due to the formation of ROS [13-17]. The intense exercise is related to higher consumption of molecular oxygen (O_2) and resulting increased generation of ROS in cells and tissues [18-20]. It is important to underline that Davies and co-workers were the first who reported that ROS are formed during exercise [21]. Long-term exercise can cause oxidative stress, i.e., an imbalance between increased production of ROS and the antioxidants scavenging capacity (Figure 1). The unbalanced ROS species could damage protein receptors, enzymes, and lipid membranes. In addition, ROS are powerful DNA damaging species causing strand breaks, alterations in guanine and thymine bases [22-25]. The ROS species are continuously formed within mammalian cells, thus the cells are under persistent oxidative stress [26-28]. Brown and Bicknell

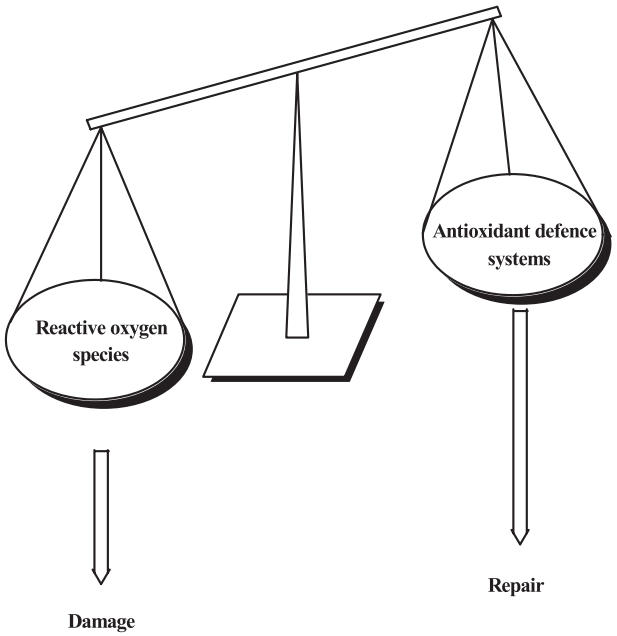

Fig.1. *An imbalance between the formation of reactive oxygen species (ROS) and the antioxidant defence capacity called as oxidative stress*

[28] in their review summarized the consequences of breast carcinoma cell oxidative stress. The authors reported the following ROS actions: increased mutation rate and accelerated tumor progression; activation of growth-promoting signaling pathways, resistance to therapy, increased blood supply to tumor cells by triggering vasodilatation and increased risk of metastasis. The epidemiological and laboratory studies indicate that both intensity and duration of physical exercise affect the initiation and the promotion/progression phases of carcinogenesis [22,23,29-31].

Although good evidence exists for formation of ROS during physical exercise, the complexity of the human body and of the prooxidant-antioxidant systems causes that the exact mechanism of their generation, similar as exercise-induced adaptation, remains only partly understood, and is a topic of extensive study [32]. Having that in mind, the present paper will address sources of ROS in physical exercise and the effects of regular exercise training on adaptation to oxidative stress.

Sources of ROS in physical exercise

Molecular oxygen (O_2) is vital for life; its major role in normal metabolism is oxidative phosphorylation. About 95% of the O_2 we breathe undergoes reductions to water. The remain percentage of O_2 is the substrate for the production of ROS, that include superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), nitric oxide ($NO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), singlet oxygen ($^1O_2$), ozone (O_3), hypochlorous acid (HOCl). Nitric oxide may react with $O_2^{\bullet -}$ to form peroxynitrate ($ONOO^-$) – powerful oxidant [24,25]. Among these species, $O_2^{\bullet -}$, $HO^{\bullet}$, $ROO^{\bullet}$ and $NO^{\bullet}$ are free radicals, i.e., molecules with at least one unpaired electron and are capable of independent existence. Due to the presence of an unpaired electron, free radicals are highly reactive towards various biological macromolecules. In turn, $^1O_2$ is an excited state of molecular oxygen. Singlet oxygen is one of the most active having at least an energy 22 kcal/mol above that of O_2 in a ground state. This energy determines its much higher reactivity than O_2 in the ground state. Among free radicals $HO^{\bullet}$ is the most reactive species, the reaction rate constant towards most biomolecules is in the order of 10^9-10^{11} L·mol^{-1}·s^{-1}. In biological systems is mainly formed by Fenton's reaction [25]:

$$Fe(II) + H_2O_2 \rightarrow HO^{\bullet} + OH^- + Fe(III) \qquad (I)$$

An important role in the $HO^{\bullet}$ production in the Fenton reaction is also played by the interaction of $O_2^{\bullet -}$ with Fe^{3+}:

$$O_2^{\bullet -} + Fe(III) \rightarrow Fe(II) + O_2 \qquad (II)$$

The generation of ROS is necessary for a number of important physiological processes in cells, for example, muscular contraction, the intracellular signaling, and the immune function [17,24,33]. The problem arises when total concentration of ROS formation is higher than the cellular defense systems capacity to neutralize and eliminate them. Defense mechanisms include those enzymatic such as cytochrome oxidase system, superoxidase dismutases (SODs), catalases, peroxidases, glutathione peroxidase, cerruloplasmin, transferin and low molecular weight compounds (glutathione, vitamin C, vitamin E, carotenoids, bioflavonoids etc.) [24,25].

Among stressors that can cause the oxidative stress is aging and exhaustive exercise [5,16,34-37]. It has been estimated that the metabolic rate resulting from the high intensity physical exercise may increase molecular oxygen consumption ($\dot{V}O_2max$) even 20 times in comparison with values at rest [33]. The oxygen consumption during moderate exercise increases 8-10 times [38].

Several mechanisms for the ROS formation during exercise have been proposed [8,39]. Exercise mode, intensity and duration can impact the extent of oxidative stress. A single bout of physical effort is a potential factor leading to a high level of ROS, followed by acute oxidative stress. Among several sources of ROS formation, the following are proposed as the most important: ***higher formation of ROS by mitochondria in respiratory electron transport chain***.

Even in a resting state the release of electrons occurs in the electron transport chain and O_2 (2-4% consumed by human beings) is reduced to give $O_2^{\bullet -}$. A small fraction of $O_2^{\bullet -}$ exists in the protonated form as $HO_2^{\bullet}$. The spontaneous or catalyzed dismutation of $O_2^{\bullet -}$ or $HO_2^{\bullet}$ generates H_2O_2. The later species then can react with the reduced metal ion, e. g., Fe^{2+} to form an extremely reactive species $HO^{\bullet}$, by the Fenton reaction (reaction I). The main reactions responsible for the $O_2^{\bullet -}$ production in mitochondria are as follows [25]:

$$NADH \rightarrow \text{dehydrogenaza NADH (e)} + O_2 \rightarrow O_2^{\bullet -} \text{(III)}$$

$$UH_2 + O_2 \rightarrow UH^{\bullet} + O_2^{\bullet -} + H^+ \qquad (IV)$$

$$UH^{\bullet} + O_2 \rightarrow U + O_2^{\bullet -} + H^+ \qquad (V)$$

where UH_2 denotes reduced form of ubiquinone; $UH^{\bullet}$ - ubisemiquinone, U is oxidized form of ubiquinol, and NADH-nicotinamide-adenine dinucleotide, reduced form.

The efficiency of the $O_2^{\bullet -}$ production by electron leaks in respiratory chain is the greatest during state IV respiration, then the adenosine triphosphate (ATP)/

adenosine diphosphate (ADP) ratio is the highest [8]. During heavy aerobic exercise the most of muscle mitochondria is in state III, when all substrates of the respiration chain are present. Their presence is followed by a lower level of ROS release than at rest [40,41]. However, the suggestion that the mitochondrial formation of $O_2^{\bullet-}$ is higher during heavy aerobic exercise given by Davies and coworkers [6] finds confirmation in earlier findings of Brooks and coworkers [42] and Salo et al. [7]. The former researchers reported that the muscle and core temperatures rise up to 41-45°C in athletes. In turn, the latter group of researchers observed that $O_2^{\bullet-}$ concentration increased proportionally to mitochondrial uncoupling with temperature. They stated that temperatures of 41-45°C cause extensive mitochondrial uncoupling with accompanying higher consumption of molecular oxygen, which undergoes direct one-electron reduction to $O_2^{\bullet-}$.

ROS formation by xanthine oxidase

The other source of ROS that can contribute to exercise induced damage is xanthine oxidase [18,43-45]. During intensive exercise a temporary ischemia (O_2 deficit) or hypoxia (O_2 excess) can occurs in certain tissues. During these states ATP is converted into hypoxanthine by 4-steps reduction via adenosine monophosphate (AMP) (Figure 2). During ischemia xanthine dehydrogenase can be converted to xanthine oxidase (XO). Under normal physiological conditions XO oxidizes both enzymes hypoxanthine and xanthine to uric acid followed by reduction of NAD^+ to NADH. With intense exercise, under ischemia-like conditions, intercellular levels of XO and hypoxanthine are rised, and during reperfusion XO converts hypoxanthine to xanthine and uric acid by using molecular oxygen. These reactions are accompanied by the formation of $O_2^{\bullet-}$ and H_2O_2 [8]. Under conditions of ischemia reperfusion the proteins containing iron may release the Fe-ions and convert H_2O_2 into $HO^{\bullet}$, by the Fenton's reaction. An important role for xanthine oxidase in the ROS production has been confirmed in human and laboratory animal studies that used allopurinol (4-hydroxy-pyrazole (3,4-pyrimidine)) – an excellent inhibitor of the enzyme [44,46]. Results from these studies showed significantly decreased concentration of the damaging reactions products, such as oxidized glutathione and malonyldialdehyde (MDA) in animals and humans that were administered allopurinol prior to exhaustive exercise. Also, recent paper by Kuwahara and co-workers reported that a single administration of antioxidant has increased the cellular ATP concentration and improved exercise efficiency [47].

ROS formation by neutrophils and other phagocytes

The next hypothesized source of ROS production especially during eccentric, exhaustive or prolonged

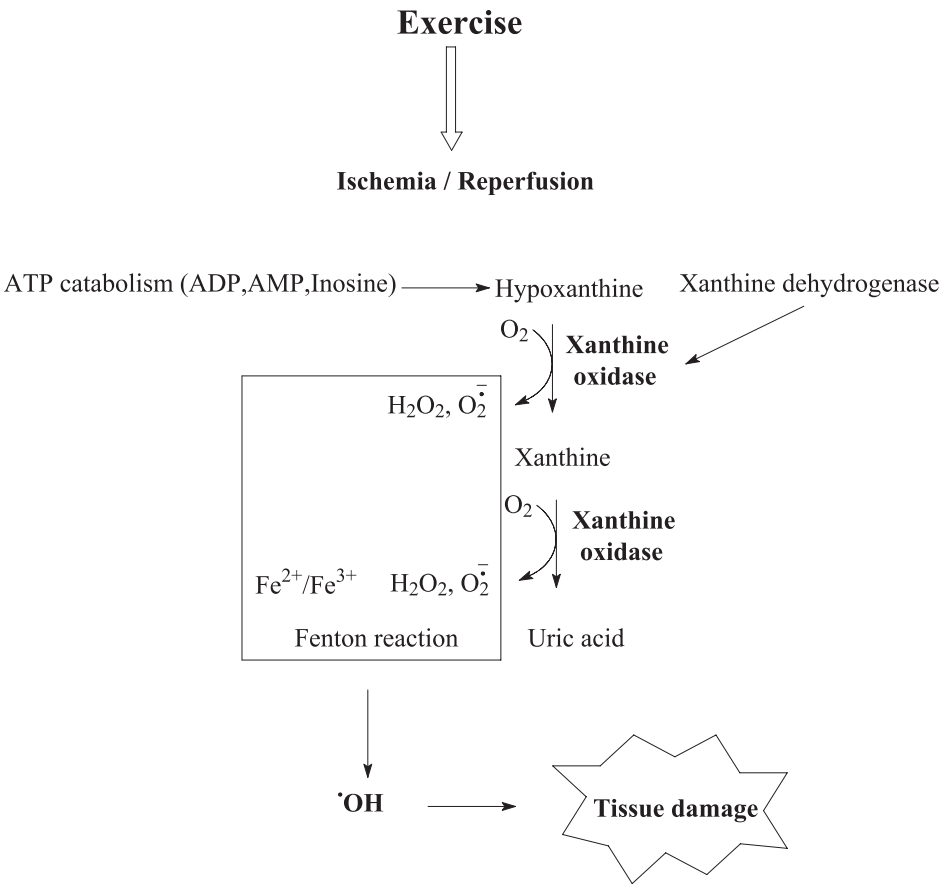

Fig.2. *Reactive oxygen species formation by xanthine oxidase during exhaustive physical exercise*

exercise is the respiratory burst, i.e., fast consumption of O_2 by phagocytic cells, mainly neutrophils and macrophages. The phagocytes play an important role as a primary defense cells in an acute inflammatory reaction. Respiratory burst is involved in the killing of invading bacteria, viruses, and other microorganisms by the formation of ROS and further destruction of a microbe by enzymes. During respiratory burst NADPH directly reduces O_2 to $O_2^{\bullet-}$ [25] using nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) oxidase, as a cofactor.

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2\,O_2^{\bullet-} \qquad (VI)$$

Reaction (VI) is catalyzed by NADPH oxidase. Superoxide anion radical can be dismutated by SOD to H_2O_2 [25].

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (VII)$$

In neutrophils, peroxidases may oxidize chloride ions using H_2O_2 to form hypochlorous acid (HOCl). The anion OCl^- reacts then with H_2O_2 to generate the extremely reactive $^1O_2$ [25]:

$$H_2O_2 + OCl^- \leftrightarrow {}^1O_2 + HCl + OH^-$$

Additionally, HO radicals may also be generated by the Fenton reaction.

Although exercise-induced neutrophils activation leads to the removal of damaged biomolecules and dead cells, if the process is not well controlled the increased generation of pro-inflammatory mediators, such as interleukin-1, interleukin-8, tumor necrosis factor-alpha (TNF-α) and prostaglandins is responsible for acute inflammation [35]. The intensive short-duration exercises induce stress hormones (cortisol and adrenocorticotropic hormones) [48], serum growth hormone and prolactin [49]. These substances affect the neutrophils functions. Yamada et al. [50], for example, examined the effect of exhaustive exercise on human neutrophils in eight male cross-country skiers. They found elevated level of cortisol one hour after exercise and also of serum growth hormone just after exercise. The elevated levels of salivary cortisol, TNF-α and $NO^\bullet$ (during acute exercise) was also reported by Rahman et al. [51] in males.

It is important to underline, that Pepe et al. [52] found similar responses to exercise of men and women at the same absolute workload at different running distances. The authors reported similar significant changes in lipid hydroperoxide (a marker of lipid peroxidation), SOD and catalase levels independent of gender, despite differences in physical activity characteristics.

Another source of $O_2^{\bullet-}$ is a leakage of electrons from NADPH cytochrome P450 reductase. Also, cycloxygenase, lipoxygenase and other enzyme systems (reductases, hydroxylases, dehydrogenases) are essential sources of $O_2^{\bullet-}$ and other reactive oxygen species [24,25]. Another site of ROS formation is autoxidation of catecholamines and their oxidation by ceruloplasmin or in the presence of the transition metal ions, and synthesis of prostaglandins and tromboxans.

Reactive nitrogen species

The NO radical is generated from arginine on a five-electron oxidation way catalyzed by nitric oxide synthase (NOS) [25]. Of the three isoform of NOS: the endothelium (eNOS), the neuron (nNOS), and the immunologic (iNOS), iNOS is the most effective in the $NO^\bullet$ production and considered as responsible for pathophysiological action of $NO^\bullet$[53]. Nitric oxide is an important biological signaling molecule involved in neutransmission, blood pressure regulation, immune regulation, defense mechanisms, muscle contraction and metabolism during physical effort. However, overgeneration of $NO^\bullet$ is called nitrosative stress. Recently, acute exercise has been confirmed as a factor elevating levels of salivary NO, using a standard treadmill test and enzyme immunoassay kits [54]. Nitric oxide is generated in mitochondria during inflammatory process; the species reacts rapidly with $O_2^{\bullet-}$ to produce peroxynitrite anion $ONOO^-$ [39,55]. The ONOO anion is a potent oxidizing agent able to oxidize lipids and DNA and inhibit their functioning. The NO radical can also exert its effects by a direct interaction with cellular components, e.g., with iron-sulfur clusters [56]. For exhaustive detail related to nitric oxide and other reactive oxygen species generation, physiological properties and reactivity, the reader is referred to the excellent book of Bartosz [25] and article by Valko et al [57].

Both moderate and extensive shifts in redox potential toward oxidation reactions resulting in chronic oxidative stress have been suggested to increase susceptibility to ROS-mediated tissue damage in older adults [36]. For more information on the exercise mode, intensity, duration, the training status, and oxidative stress consequences the reader is referred to the review of Fisher-Wellman and Bloomer [16] and Jethon [58].

At present, despite of the research done over the past 30 years, there are many inconsistencies within the literature dealing with the relationship between exercise and oxidative stress [16,59], Progress in this area requires well-equipped laboratories. Monitoring the amounts of free radicals *in vivo* and resulting oxidative stress is a major problem in the ROS research [60,61]. Investigation in this area has been dominated by animal studies. It is necessary to expand to human

populations. The development of modern, noninvasive direct techniques of free radicals detection is needed.

Methods of reactive oxygen and nitrogen species detection in exercise

The methods used to detect free radical formation during or after exercise are divided on indirect and direct ones. The first group of the methods is based on detection of by-product or end products of the ROS reactions, "examining the reaction path of the radicals" [8], because oxidative stress induces modifications of DNA, lipids and proteins that can be monitored through biomarkers assay development as well by glutathione oxidation. The indirect methods include detection of isoprostanes (isomers of prostaglandins) - products of the tissue phospholipids oxidation by oxygen radicals, using the enzyme-linked immunosorbent assay (ELISA) or by mass spectrometry [62-64]. Other method of lipid peroxidation includes the measurement of malonyldialdehyde (MDA) using thiobarburic acid reactivity in tissues or those end products (ethane and pentane) in exalted breath [25].

DNA oxidation by ROS involves more than 100 different products of DNA damage [65]. The most sensitive biomarker for cellular oxidative stress and genotoxicity of DNA is 8-hydroxy-2'-deoxyguanosine ELISA kit and its oxidation product 8-oxo-2'-deoxyguanosine (8-OHdG) [66].

Another noteworthy marker of inflammation and $NO^{\bullet}$ production is nitrotyrosine. Nitrotyrosine is a stable product of $ONOO^{-}$ oxidation. Both free and protein bound tyrosine undergo nitrosylation. The quantitative measurement of nitrosylated protein can be done using the nitrotyrosine ELISA kit. Reactive oxygen species can oxidize reduced glutathione to an oxidized form, i.e., glutathione disulfide dimer (GSSG). The ratios GSH/GSSG decreases after exhaustive exercise [16,34,67]. The GSSG turnover to GSH is catalyzed by glutathione peroxidase and glutathione reductase, measurements of these enzymes level indicates the amount of oxidative stress. In addition, the generation of ROS in excess can also be estimated by the measurement of other antioxidants deficiency, e.g., ascorbic acid (a scavenger of $HO^{\bullet}$, $O_2^{\bullet-}$, H_2O_2, HOCl, $^1O_2$, $ROO^{\bullet}$), vitamin E (inhibitor of lipid peroxidation) or selenium (a scavenger of hydroperoxides and a cofactor of glutathione peroxidase) supplementation [25,68,69]. The antioxidants level depends on physiological changes accompanying physical effort, like endurance, fatigue, muscle force [8,47,70]. Similarly, end products of free-radical damage of lipids, proteins and DNA may be determined using spectrophotometric and fluorometric methods. Many studies reported on determination of MDA directly by gas chromatography e.g. [71], or high-pressure liquid chromatography [72]. A basic problem with usage of the indirect methods is their lack of specificity. For example, the concentrations of oxidation products in biological material are low and reagents used for determination of the products react often with a wide variety of compounds, e.g., tiobarbituric acid reacts with sugar and DNA, and not only with malondialdehyde. Also, declined performance, blood flow and fatigue endurance capacity accompanying overtraining belong to indirect methods detection of oxidative stress [8].

Direct detection of free radicals in tissues can be done with electron paramagnetic resonance spectroscopy (EPR) [25,73,74]. Unfortunately, the sensitivity of this method to detect free radical species in biological systems present at low concentration is often too low [75]. The second complication results from a high dielectric absorption of microwave energy by aqueous solutions. Indeed, the signal quenching problem by water may be overcome by the use of compounds known as spin traps. The spin trapping compounds stabilize free radicals by formation with them a long-lived radical adducts showing an absorption signal of high intensity [76-78].

Reactive oxygen species can form electronically excited products (e.g., $^1O_2$) during an exoergic reaction. The reaction releases energy in sufficient amount to cause the electron jump from ground state to an excited state to form molecules in their excited electronic states. The electron transition to the ground state is often accompanied by light emission (this phenomena is called chemiluminescence) [25]. Despite of high sensitivity, simple and inexpensive instrumentation, safety, relative high frequency of usage in biochemical studies, the chemiluminescence techniques have rarely been used in the exercise – oxidative stress relationship. Lu et al. [79] and Vladimirov and Proskurnina [80] recently reviewed information on cell chemiluminescence detection systems useful for the $O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$, $^1O_2$ and $ONOO^{-}$ species. It should be mentioned that many inconsistencies in the exercise – induced oxidative stress literature are caused mainly by low sensitivity of the analytic techniques employed, the tissues sampled, and the exercise protocols [59].

The role of reactive oxygen species in signal transduction

It has been recognized that ROS influence the expression of a number of genes and signal transduction (cell signaling) [81,82]. Many redox pathways are recognized to be directly or indirectly redox-sensitive to ROS. Cell signaling enables information to be transmitted from the outside a cell to its internal functional elements. Lander [81] divided the cellular responses stimulated by ROS into the following categories: modulation of cytokine; growth factors, hormone

action and secretion, iron transport; transcription; neuromodulation, and apoptosis. Signals to the transcriptional apparatus in nucleus are regulated by a class of certain proteins called transcriptional factors. The role of ROS in cell signaling is reported as a rapid response of a cell to an external stimulus by production of ROS in a cell (acting as second messenger), changes within the messenger that lead to activation of transcription factor, and response of genetic expression to oxidative stress [53].

The transductional factors regulate the activity of RNA polymerase II by binding to specific DNA sequences [57]. The intracellular signaling can induce muscle contraction, gene expression, cell growth and differentiation.

Although the mechanisms of the transcription factors redox regulation and cell signaling are not well understood, several regulatory factors sensitive to ROS are reported [53,82]. A variety cytokines and growth factors induce changes in cell behavior and stimulate most types of cells following by ROS production. The most significant effect of ROS on signal transduction pathways is recognized in the mitogen-activated protein kinase (MAPK) pathway. ROS production results from stimulation of epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor, vascular endothelial growth factor (VEGF) [57]. The next growth factor-cytokines receptor such as tumor necrosis factor–α (TNF-α), interleukin-1 (IL-1) and interferon (IFN) did not have kinase activity although they also generate ROS in nonfagocytic cells [57]. In addition several nonreceptor protein tyrosine kinases (PTKs) and serine/threonine of the MAPK family can be regulated by ROS and are important in cell proliferation, differentiation, and apoptosis. One of the four MAPK families is c-jun-NH_2-terminal kinase JNKs in which serine/threonine kinase is important in the process of cancer formation. A number of studies reported that $O_2^{\bullet -}$, H_2O_2 can activate the MAPKs [57,82].

The MAPK pathways involve activation of nuclear transcription factors that control the expression of genes repairing damaged DNA, power of immune system, arrest the proliferation of damaged cells and induce apoptosis [57]. Among nuclear transcription factors sensitive to ROS are the following: nuclear transcription factors (NF-κB) – involved in regulation of apoptosis, survival, differentiation, inflammation and growth; activator protein-1 factor (AP-1) – can bind the tumor-promoting agent; p53 - protects a cell from tumor genesis (tumor suppressor); nuclear factor of activated T cells (NFAT) – regulates muscle growth and differentiation, angiogenesis, cytokine formation; heat shock proteins (HSP) factor-regulates the expression of the heat shock genes, and hypoxia-inducible factors HIF-1 and HIF-2 [83].

Effect of regular exercise training on adaptation to oxidative stress

The beneficial effects of regular, moderate physical exercise have been documented by numerous review reports [3,11,12]. The health benefits from regular exercise come from an improvement of antioxidant defense system, reduction of basal formation of oxidants and increased resistance of tissues against the ROS damage [8,39,83-86]. Sachdev and Davies [8] stated that "the adaptive responses include a dramatic increase in muscle mitochondria following endurance training, and altered expression of a large number of genes". According to the authors' statement the increase in muscle mitochondria is mainly responsible for increased endurance capacity and ROS stimulate the mitochondrial biogenesis. In the light of current knowledge on this subject, it is clear that the undesirable effect of exhaustive physical exercise may by prevented, at least partially, by training. For example, Salminen and Vihko [87] showed that endurance moderate training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation. Leeuwenburgh and Heinecke [39] reported that training increases antioxidant defense, reduces formation of oxidants and the oxygen free radicals during oxidative phosphorylation. Furthermore, Viña et al. [18] showed that training partially prevents against glutathione oxidation connected with exhaustive bouts of high-intensity physical exercise. In turn, Salo et al. [7] discovered a several-fold increase in the expression of various protein, in it of the 70-kDa heat-shock protein 70 in skeletal muscle, heart and liver during exhaustive physical exercise. In contrast, the authors observed very little heat-shock protein 70 induction in trained rats at the same level of exercise that caused the strong protein responses in untreated rats. According to the authors suggestion, a much greater level of oxidative stress is required in trained animals to cause the genes response. Moreover, Davies et al. [5] suggested that during non exhaustive daily training the production of ROS can stimulate to mitochondrial biogenesis and exercise adaptation. The hypothesis that exercise training increases both mitochondrial content and mitochondrial numbers finds also confirmation in previous study of Venditti et al. [84] who found slightly elevated amount of total H_2O_2 in tissues of wheel-running animals after exhaustive training. In skeletal muscle H_2O_2 at a low concentration enhances release of Ca^{2+} from sarcoplasmic reticulum and force formation. On contrary a large increase in H_2O_2 concentration results in a sharp decrease in force output [20,88]. Moreover, Holloszy et al. [89] much earlier reported the occurrence of both changes in mitochondria. As mentioned previously the next form of protection against oxidative stress and adaptation induced by training is the NF-

κB signaling pathway. The pathway is considered as one of the most important oxidative stress sensitive signaling pathways.

There is growing evidence that low concentration of ROS formed during muscle contraction plays an important role in the adaptation to physical exercise. These species are able to induce the expression of antioxidant enzymes and other protective systems [90]. In this respect the mitochondrial form of SOD containing of manganese (MnSOD), glutathione peroxidase may be cited as examples.

The antioxidant enzymes contain NF-κB signaling sites in their gene promoter region, thereby, they can be potential targets during exercise activated upregulation of the NF-κB transcription factor. This factor resides in an inactive form in cytosol and is bounded to the inhibitor-protein IκB (Figure 3). The NF-κB factor is the protein complex containing three subunits: IκB and dimer consisting of two DNA binding subunits p50 and p65 [25]. After activation (the direct binding reactive oxygen species) the dimer is translocated to the nucleus binding to target gene promoters (e. g., Mn-SOD) through its DNA binding subunits [8].

The following findings are the examples for exercise induced NF-κB activation. Hollander et al. [91,92]

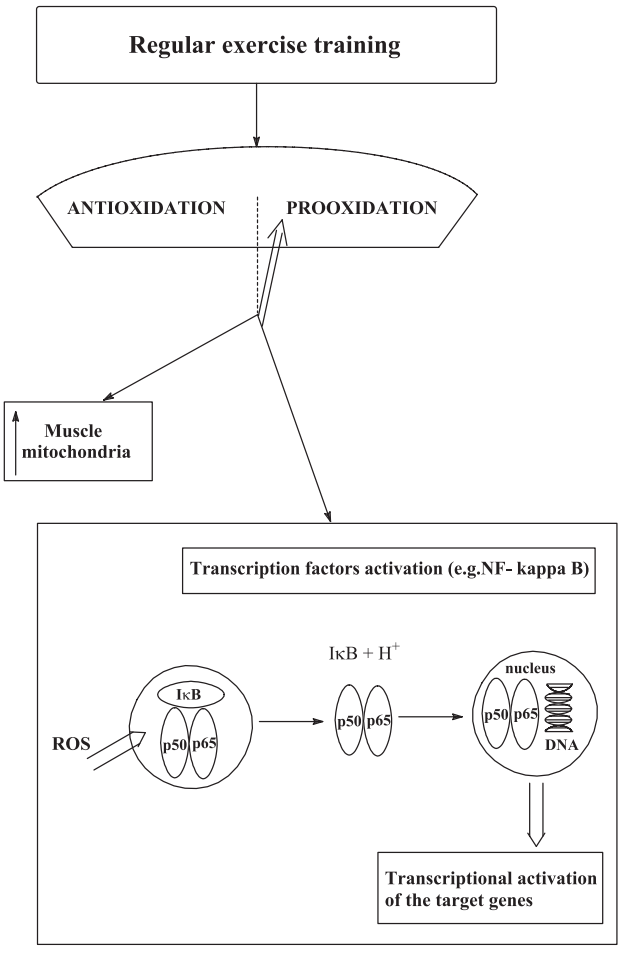

Fig.3. *Proposed mechanism of protection against oxidative stress induced by exercise training on the NF-kappa B signaling way*

found that expression of the Mn-SOD gene in rat skeletal muscle increased 2 hours after a single bout of treadmill running. The researchers found enhanced NF-κB binding in muscle nuclear extracts. The following study by Ji et al. [93] confirmed the effect of an acute bout of exercise on the NF-κB signaling pathway in rat skeletal muscle and in peripheral lymphocytes of marathon runners resulting from increased oxidant production. In addition, this group of investigators documented that marathon running induces activation of the p50 subunit of the NF-κB complex in lymphocytes [87] and that NF-κB can activate formation of not only Mn-SOD but other protective enzymes [92] as well. The NF-κB factor plays an important role in carcinogenesis. For review and extensive discussion dealing with NF-κB/IκB signaling, physiological significance of NF-κB activation and possible mediate of mammary carcinogenesis the paper of Wu and Kral is illustrative [94].

Skeletal muscle adaptation to training involves several changes that help the muscle to cope or minimize disruption of intracellular homeostasis. Skeletal muscle adaptation is connected with a several factors working simultaneously or in response to each other. It has been suggested [95] that local hypoxia is a metabolic stimulus for training response. An augmented increase in peripheral exercise adaptation during exercise is followed by reduced oxygen delivery as a result of capillarization and oxidative capacity. Despite massive research efforts detailed mechanism of mammalian oxygen sensing are still not clear. Oxygen homeostasis is preserved by regulation of a mass of red blood cells and respiratory ventilation [96]. Some studies suggested that changes in oxygen pressure include production hormones such as erythropoietin, VEGF, and IGF-II (insulin-like growth factor). These factors are controlled by HIF factors [97]. HIF-1 consist of two subunits, HIF-1α (the oxygen regulated subunit) and a constitutively expressed β-subunit HIF-1β [98]. This factor is activated in physiological conditions and also in various pathophysiological conditions. In nonhypoxic conditions HIF-1α is rapidly degraded, whereas the degradation of this subunit is suppressed in hypoxia conditions. Then HIF-1α rapidly accumulates, dimerizes with HIF-1β, translocates into the nucleus and activates the transcription of genes that control angiogenesis, glucose metabolism, cell proliferation and survival, metastasis and drug resistance [98]. HIF-2 is composed of the HIF-2α and HIF-1β subunits [99]. Both HIF-1α and HIF-2α subunits are regulated by intratumoral hypoxia, growth factors and genetic alterations. HIF-1α regulates pathways that increase oxygen delivery or allow a cell to adopt do decreased O_2 – availability and mediates processes in response to change in the O_2-level. The adaptation to hypoxia can promote carcinogenesis by induction of angiogenesis,

and treatment resistance [100]. It has been shown that various diseases are related to dysfunction of HIF, for example, cancer, stroke, heart ischemia, peripheral vascular disease or the aerobic glycolysis observed in cancer cells [101-103]. Hypoxia and oxidative stress may co-operatively promote cancer. Hypoxia can stabilize HIF-1α by ROS production and by glycolysis activation and cause the accumulation of HIF-1, which in turn promotes factor VEGF and IL-8 formation. VEGF similarly as aldolase, enolase, lactate dehydrogenase are examples of cancer related genes of which expression is regulated by HIF-1 [57,97]. Other regulatory mechanisms induced by exercise/physical activity may also modulate HIF-activity, for example, calcium levels, AMP-activated kinase (AMPK) (an intracellular sensor of energy status), and redox potential [102,104-106]. The sensor is sensitive to alteration the cellular AMP: ATP ratio.

The mitochondrial electron transport chain-generator of $O_2^{\bullet -}$, H_2O_2 as well as $ONOO^-$ is closely linked to HIF-1α activity and stability. One of the hypothetical mechanisms underlines a role of Fe(II)/Fe(III) ions in the HIF-1α activity as follows: a hypoxia state is followed by an increase in mitochondrial ROS generation what may lead to accumulation of the Fe(III) ion is the cell, for example, by reaction (I). The Fe(II) ion is known as a cofactor for hydroxylation of HIF-1α by prolyl hydroxylases (HPH) [102]. It seems that the Fenton chemistry plays important roles in the regulation of hydroxylases level [100,107].

Recent data [99] showed that tumor cell proliferation was inhibited by disruption of HIF-1α and HIF-2α genes. Similarly, other researchers [107,108] reported that receptor tyrosine kinases may regulate the HIF activity in hypoxia and normoxia. The modulation of HPH/HIF regulating pathways was reported as a potential therapeutic approaches in the diseases where hypoxia plays a key role in their development [99].

Skeletal muscle adaptation is due to several factors working commonly, such as, the intracellular level of calcium, free phosphate, ATP/ADP ratio, redox potential and hypoxia. All these factors regulate gene expression and protein activity. A key role in energy metabolism has been described to peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). PGC-1α increases mitochondrial biogenesis and respiration rates, uptake and utilization of substrates for energy generation [109,110]. PGC-1α regulates components of adaptive termogenesis and mitochondrial biogenesis, respiration, gene expression stimulates genes of fatty acid oxidation in adipocytes and in heart and is transcriptional regulator of hepatic gluconeogenesis. For a review and extensive discussion reference [109] is illustrative. PGC-1α is a coactivator that activates multiple transformation factors: nuclear hormone receptors, nuclear respiratory factors (e.g. peroxisome proliferator-activated receptors, estrogen receptors), and muscle-specific factors. Two key cellular sensors AMP-activated protein kinase (AMPK) and silent information regulator T1, SIRT1 (an enzyme that mediates NAD^+ – dependent deacetylation) strongly activate PGC-1α and control energy and nutrient homeostasis. An implication of PGC-1α in the pathogenesis of type 2 diabetes mellitus (T2DM) obesity and metabolic syndrome are the most frequent consequences during misbalance of energy homeostasis [111]. Therefore perturbation in the AMPK and SIRT1 controlling the PGC-1α activity may contribute to whole-body metabolism. However, the PGC-1α biology needs future studies. The actions of the AMPK and SIRT1 sensors affecting PGC-1α may explain the beneficial effects of exercise/physical activity and caloric restriction on energy homeostasis and prevention against obesity and T2DM.

Concluding Remarks

In the light of what has been presented we can conclude that regular, moderate endurance exercise training protects from oxidative stress induced by exhaustive high-intensity physical exercise. A mild tissue trauma followed by recovery has been reported [112]. Thus, an adequate recovery could improve athletic performance. The proposed mechanism includes an increase of the antioxidant enzymes activity, reduction of basal oxidants formation, and increased resistance of tissues against the ROS damage. Although evidence that ROS may stimulate training-induced adaptation is yet inconclusive because an optimal concentration of oxygen species produced during exercise needed to drive the desired adaptive response has not been recognized. On the contrary, physical exercise of extreme duration and/or extreme intensity, and with lack of training generates ROS in amounts that overwhelm cellular antioxidant defenses and cause muscle damage. In this case the mild trauma can develop into a more chronic and severe form of tissue trauma [113].

As several relationships exist between oxidative stress and severe training indirect oxidative stress biomarkers together with other biochemical indices measurement in human is important for diagnostic marker for overtraining. Margonis et al. [114] have studied this problem in men to measure performance and muscle damage indices, GSH and GSSG concentrations, indices of lipids and proteins peroxidation, level of antioxidant enzymes, and total antioxidant capacity. They found that overtraining is followed by a marked response of oxidative stress biomarkers "which, in some cases, was proportional to training load". However, further studies are needed to continue markers for overtraining and to assess the exact mechanisms operating in the relationship between exercise and oxidative stress to quantify the ROS

source significance. In addition the protective role of antioxidants supplementation in damage caused by the oxygen species needs their specification in view of individual age and level of physical exercise.

References

1. Atalay M, Laaksonen DE. Diabetes, oxidative stress and physical activity. *J Sports Sci Med* 2002; 1: 1-14.
2. Bucksch J, Schlicht W. Health-enhancing physical activity and the prevention of chronic diseases – An epidemiological review. *Soz Preventiv Med* 2006; 51: 281–301.
3. Warburton DER, Nicol C, Bredin SS. Health benefits of physical activity: the evidence. *Can Med Assoc J* 2006; 174: 801-9.
4. Hen K, Bogdanski P, Szulinska M, et al. Ocena wpływu regularnej aktywności fizycznej na stres oksydacyjny u kobiet z otyłością prostą. *Pol Merk Lek*. 2010; XXVIII,166: 284-8.
5. Davies KJA, Packer L, Brooks GA. Exercise bioenergetics following sprint training. *Arch Biochem Biophys* 1982; 215: 260-5.
6. Davies KJA, Maguire JJ, Brooks GA, et al. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am J Physiol* 1982; 242: E418-E427.
7. Salo DC, Donovan CM, Davies KJA. HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radic Biol Med* 1991; 11: 239-46.
8. Sachdev S, Davies KJA. Production, detection, and adaptive responses to free radicals in exercise. *Free Radic Biol Med* 2008; 44: 215-23.
9. *Noncommunicable Diseases and Mental Health. Health and Development Through Physical Activity and Sport*. Geneva, U.S. Department of Health and Human Services, Physical Activity Fundamental to Preventing Disease, June 20, 2002. World Health Organization 2003.
10. Van der Bij AK, Laurent MGH, Wensing M. Effectiveness of physical activity interventions for older adults: a review. *Am J Prev Med* 2002; 22: 120-33.
11. Warburton DER, Katzmarzyk PT, Rhodes RE, et al. Evidence--informed physical activity guidelines for Canadian adults. *Appl Physiol Nutr Metab* 2007; 32: S16-S68.
12. Kruk J. Physical activity and health. *Asian Pacific J Cancer Prev* 2009; 10: 721-8.
13. Armstrong RB, Ogilvie RW, Schane JA. Eccentric exercise--induced injury to rat skeletal muscle. *J Appl Physiol* 1983; 54: 80-93.
14. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med* 2001; 31: 911-22.
15. Kayatekin BM, Gönenç S, Açikgöz O, et al. Effects of sprint exercise on oxidative stress in skeletal muscle and liver. *Eur J Appl Physiol* 2002; 87: 141-4.
16. Fisher-Wellman KH, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dynamic Medicine* 2009, 8:1 http://www.dynamic-med.com/content/8/1/1
17. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* 2010; 48: 749-62.
18. Viña J, Gomez-Cabrera M-C, Lloret A, et al. Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. *IUBMB Life* 2000; 50: 271-7.
19. Coto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 2003; 108: 530-5.
20. Reid MB. Free radicals and muscle fatigue: of ROS canaries, and the IOC. *Free Radic Biol Med* 2008; 44: 169-79.
21. Davies KJA, Quintanilha AJ, Brooks GA, et al. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 1982; 107: 1198-205.
22. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. *Eur J Cancer* 1996; 32A: 30-8.
23. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem* 1996; J 313: 17-29.
24. Halliwell B, Gutteridge IMC. *Free Radicals in Biology and Medicine*. New York: Oxford University Press, 1999.
25. Bartosz G. Druga twarz tlenu. Warszawa: PWN, 2003.
26. Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res* 1991; 51: 794-8.
27. Toyokui S, Okamoto K, Yodol J, et al. Persistent oxidative stress in cancer. *FEBS Lett* 1995; 358: 1-3.
28. Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Res* 2001; 3: 323-7.
29. Thompson HJ. Effect of exercise intensity and duration on the induction of mammary carcinogenesis. *Cancer Res* 1994; (Suppl.) 54: 1960S-1963S.
30. Slatter TF. Free-radical mechanisms in tissue injury. *Biochem J* 1984; 222: 1-15.
31. Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 2000; 29: 222-30.
32. Packer L, Cadenas E, Davies KJA. Free radicals and exercise: an introduction. *Free Radic Biol Med* 2008; 44: 123-5.
33. de Pinho RA, de Araujo MC, de Melo Chisi GL, et al. Coronary heart disease, physical exercise and oxidase stress. *Arg Bras Cardiol* 2010; 94: 515-21.
34. Sastre J, Asensi M, Gasco E, et al. Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. *Am J Physiol* 1992; 26: R992-R995.
35. Bejma J, Ji LL. Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J Appl Phys* 1999; 85: 465-70.
36. Mecocci P, Fano G, Fulle S, et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. *Free Radic Biol Med* 1999; 26: 303-8.
37. Vollaard NB, Shearman JP, Cooper CE. Exercise-induced oxidation stress: myths, realities and physiological relevance. *Sports Med* 2005; 35: 1045-62.
38. Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr* 2000; 72: 6535-695.
39. Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. *Curr Med Chem* 2001; 8: 829-37.
40. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 1979; 59: 617-30.
41. Kehrer JP. Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol* 1993; 23: 21-48.
42. Brooks GA, Hittelman KJ, Faulkner JA, et al. Tissue temperatures and whole-animal oxygen consumption after exercise. *Am J Physiol* 1971; 221: 427-31.
43. Hellsten Y, Frandsen U, Ortehenblad N, et al. Xanthine oxidase in human skeletal muscle following eccentric exercise. A role in inflammation. *J Physiol* (London) 1997; 498: 239-48.
44. Heunks LM, Viña J, van Herwaarden CL, et al. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. *Am J Physiol* 1999; 277: R1697-704.
45. Romagnoll M, Gomez-Cabrera MC, Perrelli MG, et al. Xanthine oxidase induced oxidative stress causes activation of NF-κB and inflammation in the liver of type I diabetic rats. *Free Radic Biol Med* 2010; 49: 171-7.
46. Viña J, Gimeno A, Sastre J, et al. Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life* 2000; 49: 539-44.
47. Kuwahara H, Horie T, Ishikawa S, et al. Oxidative stress in skeletal muscle causes severe disturbance of exercise activity without muscle atrophy. *Free Radic Biol Med* 2010; 48: 1252-62.
48. Farrell PA, Garthwaite TL, Gustafson AB. Plasma adrenocorticotropin and cortisol responses to submaximal and exhaustive exercise. *J Appl Physiol* 1983; 55: 1441-4.

49. Freund BJ, Shizuru EM, Hashiro GM, et al. Hormonal electrolyte, and renal responses to exercise are intensity dependent. *J Appl Physiol* 1991; 70: 900-6.
50. Yamada M, Suzuki K, Kudo S, et al. Effect of exhaustive exercise on human neutrophils in athletes. *Luminescence* 2000; 15: 15-20.
51. Rahman ZA, Abdullah N, Singh R, et al. Effect of acute exercise on the levels of salivary cortisol, tumor necrosis factor-α and nitric oxide. *J Oral Science* 2010; 52: 133-6.
52. Pepe H, Balci SS, Revan S, et al. Comparison of oxidative stress and antioxidant capacity before and after running exercises in both sexes. *Gend Med* 2009; 6: 587-95.
53. Gabbita SP, Robinson KA, Steward CA, et al. Redox regulatory mechanisms of cellular signal transduction. *Arch Biochem Biophys* 2000; 376, 1-13.
54. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 2001; 81:209-37.
55. Bartosz G. Peroxynitrite: mediator of the toxic action of nitric oxide. *Acta Biochim Pol* 1996; 43: 645-60.
56. McMahon TJ, Stamler JS. Concerted nitric oxide/oxygen delivery by hemoglobin. *Methods Enzymol* 1999; 301: 99-114.
57. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cells Biol* 2007; 39: 44-84.
58. Jethon Z. *Możliwe niebezpieczeństwa spowodowane niewłaściwą lub nadmiernie intensywną aktywnością ruchową*. Studia i Monografie. Wrocław: Wyd AWF 2005; 75: 215-46.
59. Jenkins RR. Exercise and oxidative stress methodology: a critique. *Am J Clin Nutr* 2000; 72: 670S-4S.
60. Hageman JJ, Bast A, Vermeulen NP. Monitoring of oxidative free radical damage in vivo. Analytical aspects. *Chem Biol Interact* 1992; 82: 243-93.
61. Jackson MJ. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? *Free Radic Biol Med* 2008; 44: 132-41.
62. Chu X, Ageishi Y, Nishimura K, et al. Development of enzyme-linked immunosorbent assay for 8-isoprostaglandin $F_{2\alpha}$, a biomarker of oxidative stress in vivo, its application to the quantification in aged rats. *J Pharm Biomed Anal* 2009; 50: 911-6.
63. Ziech D, Franco R, Georgakilas AG, et al. The role of reactive oxygen species in oxidative stress in environmental carcinogenesis and biomarker development. *Chem – Biol Inter* 2010; 188: 334-9.
64. Yin H. New techniques to detect oxidative stress markers: mass spectrometry-based methods to detect isoprostanes as the gold standard for oxidative stress in vivo. *Biofactors* 2008; 34: 109-24.
65. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Safety* 2006; 64: 178-89.
66. Saito S, Yamauchi H, Hasui Y, et al. Quantitative determination of urinary 8-hydroxydeoxyguanosine (8-OH-dg) by using ELISA. *Res Commun Mol Pathol Pharmacol* 2000; 107: 39-44.
67. Pyke S, Lew H, Quintanilha A. Severe depletion in liver glutathione during physical exercise. *Biochem Biophys Res Commun* 1986; 139: 926-31.
68. Schroder H, Navarro E, Mora J, et al. Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress and markers in habitual training activity of professional basketball players. *Eur J Nutr* 2001; 40: 178-84.
69. Bloomer RJ, Fisher-Wellman KH. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gender and Medicine* 2007; 5: 218-28.
70. Zerba E, Komorowski TE, Faulkner JA. Free radical injury to skeletal muscles of young, adult and old mice. *Am J Physiol* 1990; 258: C429-35.
71. Bird RP, Hung SO, Hadley M, et al. Determination of malondialdehyde in biological materials by high-pressure liquid chromatography. *Anal Biochem* 1983; 128: 240-4.
72. Ichinose T, Miller MG, Shibamoto T. Gas chromatographic analysis of free and bound malondialdehyde in rat liver homogenates. *Lipids* 1989; 24: 895-8.
73. Jackson MJ, Edwards RH, Symons MC. Electron spin resonance studies of intact mammalian skeletal muscle. *Biochim Biophys Acta* 1985; 847: 185-90.
74. Asmus KD, Bonifacic M. Free radical chemistry. In: Sen CK, Packer L, Häninem O, eds. *Exercise and oxygen toxicity*. New York: Elsevier; 1994: 1-48.
75. Tarpey MM, Fridovich I. Methods of detection of vascular reactive species nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite. *Circ Res* 2001; 89: 224.
76. Ashton T, Young IS, Peters JR, et al. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. *J Appl Physiol* 1999; 87: 2032-6.
77. Khan N, Swartz H. Measurement *in vivo* of parameters pertinent to ROS/RNS using EPR spectroscopy. *Moll Cell Biochem* 2002; 234-5, 341-57.
78. Stolze K, Rohr-Udilova N, Rosenau T, et al. Free radical trapping properties of several ethyl-substituted derivatives of 5-ethoxycarbonyl-5-methyl-1-pyrroline N-oxide (EMPO). *Bioorg Med Chem* 2007; 15: 2827-36.
79. Lu C, Song G, Lin J-M. Reactive oxygen species and their chemiluminescence – detection methods. *Trends in Anal Chem* 2006; 25: 985-95.
80. Vladimirov YA, Proskurnina EV. Free radicals and cell chemiluminescence. *Biochemistry* (Moscow) 2009; 74: 1545-66.
81. Lander HM. An essential role for free radicals and derived species in signal transduction *FASEB J* 1997; 11: 118-24.
82. Allen RG, Tresini M . Oxidative stress and gene regulation. *Free Radic Biol Med* 2000; 28: 463-99.
83. Davies KJA, Packer L, Brooks GA. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch Biochem Biophys* 1981; 209: 539-54.
84. Venditti P, Masullo P, Di Meo S. Effect of training on H_2O_2 release by mitochondria from rat skeletal muscle. *Arch Biochem Biophys* 1999; 372: 315-20.
85. Gomez-Cabrera MC, Domenech E, Ji LL, Viña J. Exercise as an antioxidant: it up-regulates important enzymes for cell adaptations to exercise. *Science & Sports* 2006; 21: 85-9.
86. Gomez-Cabrera MC, Martinez A, Santangelo G, et al. Oxidative stress in marathon runners: interest of antioxidant supplementation. *Br J Nutr* 2006; 96 Suppl. 1: S31-3.
87. Salminen A, Vihko V. Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. *Acta Physiol Scand* 1983; 117: 109-13.
88. Andrade FH, Reid MB, Westerblad H. Contractible response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. *FASEB J* 2001; 15: 309-11.
89. Holloszy JO, Oscai LB, Don IJ, et al. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. *Biochem Biophys Res Commun* 1970; 30: 1368-73.
90. Gomez-Cabrera MC, Borras C, Pallardo FV, et al. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol* 2005; 567: 113-20.
91. Hollander J, Fiebig R, Gore M, et al. Superoxide dismutase gene expression in skeletal muscle: fiber-specific adaptation to endurance training. *Am J Physiol* 1999; 277: R856-62.
92. Hollander J, Fiebig R, Gore M, et al. Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pfügers Archiv Eur J Physiol* 2001; 442: 426-34.
93. Ji LL, Gomez-Cabrera MC, Steinhafel N, et al. Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. *FASEB J* 2004; 18: 1499-506.
94. Wu JT, Kral JG. The NF-κB/IκB signaling system: a molecular target in breast cancer therapy. *J Surg Res* 2005; 123: 158-69.
95. Hoppeler H, Vogt M. Muscle tissue adaptation to hypoxia. *J Exp Biol* 2001; 204: 3133-9.
96. Acker H, Xue DH. Mechanisms of O_2 sensing in the carotid body in comparison with other O_2 sensing cells. *News Physiol Sci* 1995; 10: 211-6.
97. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia – inducible factor 1 is a basis helix-lop-helix PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acod Sci USA* 1995; 92: 5510-4.

98. Greenberger LM, Horak ID, Filpua D, et al. A RNA antagonist of hypoxia-inducible factor-1α, EZN-2968, inhibits tumor cell growth. *Mol Cancer Ther* 2008; 7: 3598-608.
99. Burkitt K, Chun SY, Dang DT, Dang LH. Targeting both HIF-1 and HIF-2 in human colon cancer cells improves tumor response to sunitinib treatment. *Mol Cancer Ther* 2009; 8: 1148-56.
100. Lu H, Dalgard CL, Mohyeldin A, et al. Reversible inactivaction of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. *J Biol Chem* 2005; 280: 41928-39.
101. Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell* 2006; 125: 1241-52.
102. López-Lázaro M. HIF-1: hypoxia-inducible factor or dysoxia--inducible factor? *FASEB J* 2006; 20: 828-32.
103. Calzada MJ, del Peso L. Hypoxia - inducible factors and cancer. *Clin Transl Oncol* 2007; 9: 278-89.
104. Taylor CT. Mitochondria and cellular oxygen sensing in the HIF pathway. *Biochem J* 2008; 409: 19-26.
105. Mason SD, Rundqvist H, Papandreou I, et al. HIF-1α in endurance training: suppression of oxidative mechanism. *Am J Physiol Regul Integr Comp Physiol* 2007; 293: R2059-69.
106. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res* 2007; 100: 328-41.
107. Nilsson MB, Zage PE, Zeng L, et al. Multiple receptor tyrosine kinases regulate HIF-1α and HIF-2α in normoxia and in hypoxia in neuroblastoma: implications for antiangiogenic mechanisms of multikinase inhibitors. *Oncogene* 2010; 29: 2938-48.
108. Sandoel A, Kohler I, Fellmann C, et al. HIF-1 antagonizes p53--mediated apoptosis through a secreted neuronal tyrosinase. *Nature* 2010; 465: 577-83.
109. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator. *Endocrine Rev* 2003; 24: 78-90.
110. Cantó C, Auwerx J. PGC-1α, SITR1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol* 2009; 20: 98-105.
111. Soyal S, Krempler F, Oberkoffer H, Patscch W. PGC-1alpha: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia* 2006; 49: 1477-88.
112. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exercise* 2000; 32: 317-31.
113. Smith LL. Tissue trauma: the underlying cause of overtraining syndrome. *J Strength Cond Res* 2004; 18: 185-93.
114. Margonis K, Fatouros IG, Jamurtas AZ, et al. Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. *Free Radic Biol Med* 2007; 43: 901-10.

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