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**REVIEW ARTICLE**

# SIGNS OF OXIDATIVE STRESS AFTER EXERCISE

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**Abstract.** Exercise is one of the factors that stimulate the aerobic metabolism, leading to an increased generation of reactive oxygen species (ROS). Mammals, including humans, have a complex antioxidant structure, which protects them against the toxic effects of ROS. This structure includes antioxidant enzymes and non-enzymatic scavengers of oxygen derived free radicals (ODFR). A disturbance in the pro- and antioxidant balance leads to oxidative stress, which often accompanies strenuous exercise. As a result of the excessive generation of ODFR, damage occurs to lipids, nucleic acids and the modification of proteins. Physical training alleviates the results of oxidative stress, mainly through an adaptable increase in the activity of antioxidant enzymes.

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*Key words:* Exercise - Lipid peroxidation - Antioxidant enzyme – Non-enzymatic antioxidant

## Introduction

The consequence of organisms living in aerobic conditions is the constant contact of cell structures with the toxic derivatives of the metabolism of oxygen [63]. These substances are called reactive oxygen species (ROS): these include $O_2^{\cdot-}$ - superoxide, $H_2O_2$ - hydrogen peroxide, $^1O_2$ - singlet oxygen, HOCl - hypochlorous acid, $^{\cdot}OH$- hydroxyl radical, $ROO^{\cdot}$ - peroxyl radical, $ONOO^-$ - peroxynitrate and $NO^{\cdot}$ - nitric oxide [19, 29]. Some of the reactive oxygen species are oxygen derived free radicals - ODFR [5]. ODFR are molecules capable of existing independently and possess one or more unpaired electrons [5]. They can receive and emit electrons, playing the role of oxidants or reducers in the organism.

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## Main sources of oxygen derived free radicals during exercise

*Respiratory chain:* The main source of oxygen derived free radicals in a cell is the mitochondria [19]. Almost all the oxygen absorbed by a cell is converted into $H_2O$ by way of reduction in the respiratory chain located in the inner membrane of the mitochondrion. A molecule of oxygen reduced by 4 electrons undergoes protonisation and two molecules of water are formed. Part of the oxygen which penetrates into the mitochondrion undergoes a reduction by not 4 but one electron with the production of ROS as the final products. During the reduction of oxygen by one electron, superoxide is formed:

$O_2 + e^- \rightarrow O_2^{\cdot-}$

by two electrons, hydrogen peroxide:

$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$

and by three electrons, hydroxyl radical and a molecule of water:

$O_2 + 3e^- + 3H^+ \rightarrow {}^{\cdot}OH + H_2O$

About 1-3 % of the oxygen absorbed in the process of respiration is converted into $O_2^{\cdot-}$ [8]. During aerobic exercise the amount of oxygen absorbed increases 10- or even 20-fold [17]. The increased use of oxygen accompanying exercise intensifies the processes of mitochondrial oxidation, which leads to an increased generation of ODFR [31]. The consequence of physical training is an increase in the maximum oxygen absorption and even an increased number of mitochondria in muscle cells [11].

Mitochondria also generate nitric oxide ($NO^{\cdot}$), which is a free radical [18]. This radical probably has a regulatory function in the process of mitochondrial respiration during exercise [18]. Nitric oxide can also react with superoxide, creating peroxynitrate ($ONOO^-$), which is a strong oxidant [27]:

$O_2^{\cdot-} + NO^{\cdot} \rightarrow ONOO^-$

In addition, nitric oxide impedes the activity of cytochrome oxidase and the flow of electrons in the region of ubiquinone-cytochrome $bc_1$ of the respiratory chain. It leads in turn to a rise in the level of ubisemiquinone and an increased generation of superoxide [56].

*Xanthine oxidase and others enzymes:* Oxygen derived free radicals also appear during enzymatic processes catalysed through the participation of xanthine oxidase, aldehyde oxidase, or cyclooxygenase [19]. Another source of ODFR is the auto-oxidation of some biologically active compounds, e.g. hydroquinones and haemoglobins [19]. Xanthine oxidase becomes a source of ODFR during anoxia and reoxygenation (Fig. 1) [5]. In properly oxygenated tissues this enzyme

appears as xanthine dehydrogenase, which catalyses the reaction of oxidation of substrates by $NAD^+$:

hypoxanthine + $H_2O$ + NAD → uric acid + NADH + $H^+$

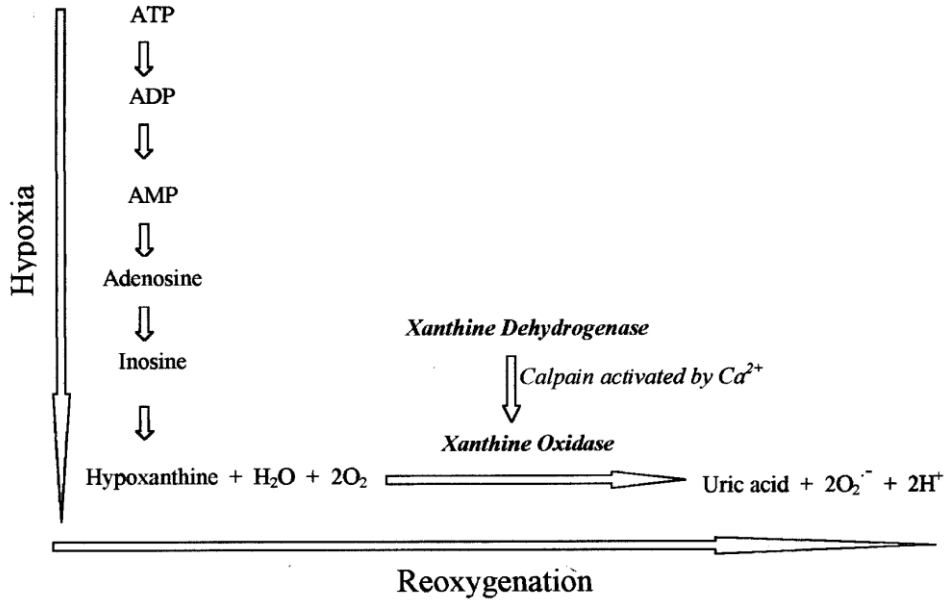


**Fig. 1**
Superoxide generation under hypoxia/reoxygenation conditions catalysed by xanthine oxidase

In states of oxygen deficiency, oxidative phosphorylation takes place at a low level of efficiency, and ATP resolves into AMP, which in turn is catabolised into hypoxanthine [12]. In states of anoxia and reperfusion, xanthine dehydrogenase is converted into xanthine oxidase. In a reaction catalysed by xanthine oxidase, superoxide is formed [34] according to the pattern below:

hypoxanthine + $H_2O$ + $2O_2$ → uric acid + $2O_2^{\cdot-}$ + $2H^+$

It has been shown that very intensive exercise can be accompanied by anoxia [12]. As a result of exercise, the flow of blood through the blood vessels of the muscles increases, which automatically reduces the flow of blood to other tissues and organs and causes hypoxia in, for example, the liver, kidneys and intestines [12]. During exercise in which the oxygen requirement exceeds $VO_2$max, the fibres of the working muscles are also subject to anoxia. After exercise the renewed flow of blood to the anoxic tissues leads in turn to reoxygenation [12].

*Respiratory burst:* The increased generation of ODFR during exercise may also be linked to the respiratory burst of phagocytes [44,64]. The release of $O_2^{\cdot-}$ takes place on the outer surface of the plasma membrane and progresses outside phagocytising cells or into their interior according to the equation [5]:

$$NADPH + O_2 \rightarrow NADP^{+} + H^{+} + 2O_2^{\cdot-}$$

The enzyme that catalyses the above reaction is NADPH oxidase. The oxygen derived free radicals released by the phagocytising cells facilitate the degeneration of atrophied tissues [3]. Many studies indicate the occurrence of inflammation and micro-damage in the skeletal muscles as a result of exercise [19,41].

In most reactions accompanied by the generation of ODFR, mainly superoxide is formed [5]. This radical penetrates the cell membrane and diffuses over a significant distance from where it was formed. As a result of dismutation of $O_2^{\cdot-}$ hydrogen peroxide is formed [5]. $H_2O_2$ is a signalling molecule involved in the regulation of diverse cellular functions e.g. gene expression activation [40], regulation of cell cycle progression [49] and apoptosis [48]. Hydrogen peroxide may also be involved in signaling of the macrophage itself or other nearby cells after release to the extracellular medium [67]. In the presence of $H_2O_2$ and ions of transitional metals, superoxide is, in turn, the source of hydroxyl radical (Fenton's and/or Haber-Weiss's reaction). $^{\cdot}OH$ radical is characterised by the highest reactivity of all the oxygen derived radicals [19]. It enters into a reaction with every particle that is nearby and is capable of forming so-called "secondary radicals".

## Oxidative stress and lipid peroxidation

Excessive generation of ODFR in muscle fibers can lead to oxidative stress, or in other words, to the disturbance of the pro- and antioxidant balance towards intensification of the oxidation processes.

*Oxidative stress in skeletal muscle:* Increased generation of ODFR in the muscles of the back legs of rats after they had run intensively on a treadmill until exhausted was demonstrated by Davies *et al.* [11]. The direct discovery of the presence of ROS in tested material became possible thanks to the utilisation of electron paramagnetic resonance (EPR). Davies *et al.* [11] found a higher absorption of the variable electromagnetic field, or in other words, an increased generation of ODFR which were identified as semiquinones. The intensified generation of semiquinone radicals was accompanied by increased membrane lipid peroxidation, mitochondrial uncoupling, and changes in sarcoplasmic reticulum permeability to $Ca^{2+}$.

A higher generation of ODFR in the muscles of rats subjected to exercise compared to animals from the control group was also shown by Jenkins *et al.* [29]. The authors used chemiluminescence as the technique for marker generation of ODFR in cells. In the back leg muscles of rats subjected to exercise (running to exhaustion) the intensity of chemiluminescence after stimulation with tert-butyl hydroperoxide was significantly statistically higher than in rats which did not exercise.

The intensified production of ROS in muscle fibers is also testified to by studies which show a rise in the level of products of lipid peroxidation after exercise. Lipid peroxidation is one of the consequences of the generation of oxygen derived free radicals. Free radical chain reactions lead to the breakdown of polyunsaturated fatty acids, which build cell membranes [19]. The increased concentration of thiobarbituric acid-reactive substances (TBARS) was shown in samples of the gastrocnemius muscle and of the soleus muscle of rats subjected to exercise [4]. The rise in the TBARS level in the soleus muscle was not, however, statistically significant. The authors of this paper believe that changes in the generation of oxygen derived free radicals under the influence of exercise depend on the muscle type studied, which explains the above discrepency in the statistical significance. The majority of muscles have a mixed character and are built of slow twitch (ST) red fibers and fast twitch (FT) white fibers. The soleus muscle of rats is composed exclusively of slow twitch fibers [37]. ST-type fibers differ from FT-type fibers in the greater density of the network of capillary vessels, the larger number of mitochondria and the larger number of enzymes of oxidative energy conversion. Histochemical research showed that during exercise of low and moderate intensity, glycogen reserves decreased only in ST type fibers, while during speed and speed-strength exercise of high intensity, glycogen is exhausted in FT fibers [37]. This research shows that the involvement of particular types of fibers depends on the type of exercise, and so changes in the generation of ODFR are also closely linked to the intensity and type of exercise.

Increased lipid peroxidation after exercise is observed not only in skeletal muscles. A statistically significant rise in the concentration of TBARS also occurs in the blood plasma of weightlifters after exercise on a cycloergometer [26] and in runners after a half-marathon run on a treadmill [10]. A statistically insignificant increase of TBARS was found, in turn, in weightlifters after exercise equal to 80-90% $VO_2$max [14]. Some studies show even a decrease in the level of TBARS [38], or of MDA (the main product of peroxide reacting with thiobarbituric acid) in the blood plasma of athletes after exercise [25]. The different results shown by the authors probably depend on the type of exercise, its intensity, duration and the

physical condition of the people being tested. It is to be assumed that long-lasting, intensive exercise generates oxygen derived free radicals and leads to micro-damage of the muscle fibers while exercise of an intensity below 70% $VO_2max$ impedes the generation of ODFR and consequently reduces lipid peroxidation. Evidence of the veracity of this reasoning is provided by the results of research carried out on mice and rats subjected to exercise of moderate intensity. Salminen and Vihko [59] and Suzuki *et al.* [66] found less production of and a lowered accumulation of lipid peroxides in the brain and skeletal muscles after exercise of moderate intensity.

The increase in the level of TBARS and MDA in plasma is the effect of intensified peroxidation of lipoproteins of the plasma, mainly low-density lipoprotein (LDL) and lipids of the biological cell membranes [19]. The differences observed in the concentration of malondialdehyde may result not only from the amount of MDA produced, but also from the varied rates of its metabolisation, depending on the general condition of the organism and the age of the person being tested. MDA is metabolised in the liver and probably in exercised human skeletal muscles [28]. It is therefore difficult to explain changes in the concentration of thiobarbituric acid-reactive substances by oxidative damage to tissues alone.

A raised level of other products of lipid peroxidation also testifies to an intensified generation of oxygen derived free radicals. A rise in the concentration of conjugated dienes (CD) occurred in muscle biopsies in male volunteers after 45 min. of eccentric running on a treadmill [46]. CD are formed in the process of lipid peroxidation after a hydrogen atom is broken away from the rest of the polyunsaturated fatty acid, when regrouping of double bonds takes place. In Jenkin's *et al*. [29] opinion the determination of the concentration of CD is a marker of oxidative stress.

*Damage of proteins and nucleic acids:* Increased generation of ODFR after exercise may also cause numerous modifications of proteins and damage of nucleic acids [31]. Purine bases of DNA undergo oxidation in many positions. However most studied are the modifications in carbon 8 of the purine ring. One of the products arising as a result of such damage is nucleoside 8-hydroxy-2'-deoguanosine [5]. A higher content of 8-hydroxy-2'-deoguanosine in urine is observed in competitors after a marathon run [2].

As a result of hyperthermia induced by exercise, greater synthesis of heat shock proteins occurs in the skeletal muscle, heart and liver of rats [60]. HSP prevents the denaturation of proteins which increases under the influence of the

effects of environmental stress [5]. Some authors believe that ROS can induce the synthesis of heat shock proteins [5].

*Oxidative stress in erythrocytes:* ODFR that appear during strenuous exercise cause phospholipid peroxidation and polymerisation of cell membrane proteins of erythrocytes [28]. Superoxide is generated within the erythrocyte during oxidation of oxyhaemoglobins to methemoglobins (Fig. 2) [39]:

$HbO_2(Fe^{2+}) + O_2 \rightarrow MetHb(Fe^{3+}) + O_2^{\cdot -}$

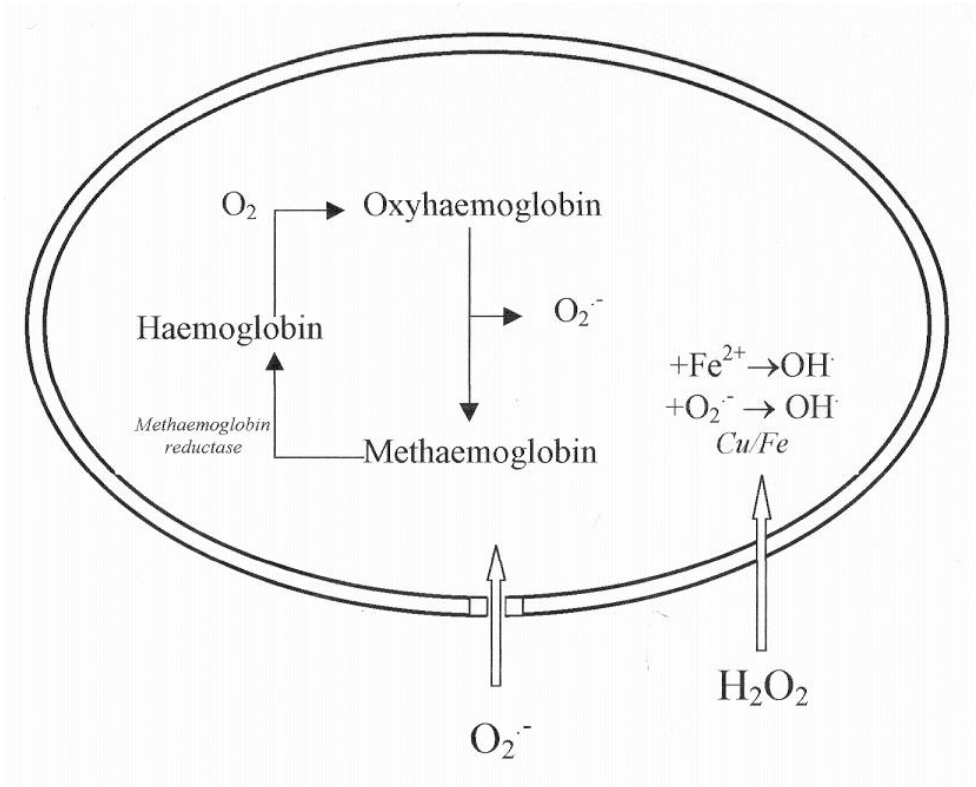


**Fig. 2**
Main sources of oxygen derived free radicals in erythrocytes

In physiological conditions about 3% of oxyhaemoglobin is converted into methemoglobin. Increased oxygen intake accompanying exercise can increase the amount of methemoglobins being produced and thus intensify the generation of superoxide. Red blood cells are exposed to the effect of ODFR generated both within the erythrocytes and free radicals originating from outside the cell [28].

Superoxide originating from outside blood cells can enter the erythrocytes through a special "channel" in the cell membrane [19]. Hydrogen peroxide, being electrically neutral, easily penetrates the membrane. $H_2O_2$ can be transformed into hydroxyl radical in Fenton's and/or Haber-Weiss's reaction. The final radical $^{\cdot}OH$ is directly responsible for the initiation of the lipid peroxidation [19].

Intensified lipid peroxidation in erythrocytes, expressed as an increase in the concentration of TBARS, was demonstrated by Drewa *et al*. [14] in weightlifters after exercise with an intensity of 80-90% $VO_2$max and by Dudek *et al*. [15] in untrained men after exercise on a cycloergometer with an intensity of 75% $VO_2$max.

Despite increased oxygen uptake during various forms of exercise, and thus intensified generation of reactive oxygen species, the risk of damage to muscle cells is relatively low [44]. Physical training lowers the susceptibility of muscles to micro-damage induced by exercise, which is associated with an increase in the expression of antioxidant enzymes [44].

It has not yet been explained exactly what this beneficial effect of training consists of. According to Salo *et al*. [60], increased generation of ODFR leads to oxidative stress in muscle cells and induces the synthesis of heat shock proteins, which protect against further stress. These authors suggest, moreover, that synthesis of HSP is a deciding factor in the biogenesis of mitochondria during exercise. During isometric exercise after which damage to muscle cells was not observed, the rise in the generation of superoxide was accompanied by only a short-lasting increase in the number of oxidised thiol groups (-SH), which quickly returned to normal [44]. Thiol groups are functional groups consolidating the spatial structure of proteins. As a result of the oxidation of -SH groups, a change in the spatial structure of proteins takes place, which leads to partial or total denaturation of cell proteins. Proteins in this state lose their biological activity and undergo aggregation. This process is prevented by heat shock proteins acting like molecular chaperones (protective proteins). They recognise fragments of denatured proteins and bind with them, preventing their aggregation and creating an environment for their renaturation to biologically active forms [44]. The production of ROS therefore seems to be a signal for the commencement of adaptation processes in muscle cells. ODFR oxidise -SH groups of cell proteins, which activates the response to stress and leads to synthesis of HSP. After increased transcription of mRNA of heat shock proteins, an increase in the activity of antioxidant enzymes was observed [44].

## Antioxidant enzymes

Human tissues have a complex antioxidant system which protects them against the toxic effect of ROS [5,19]. This system is composed of antioxidant enzymes and non-enzymatic scavengers of free radicals. The main antioxidant enzymes include e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and glutathione reductase (GR). Superoxide dismutase eliminates superoxide ($O_2^{\cdot-}$) catalysing a reaction of dismutation (Fig. 3):

$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$

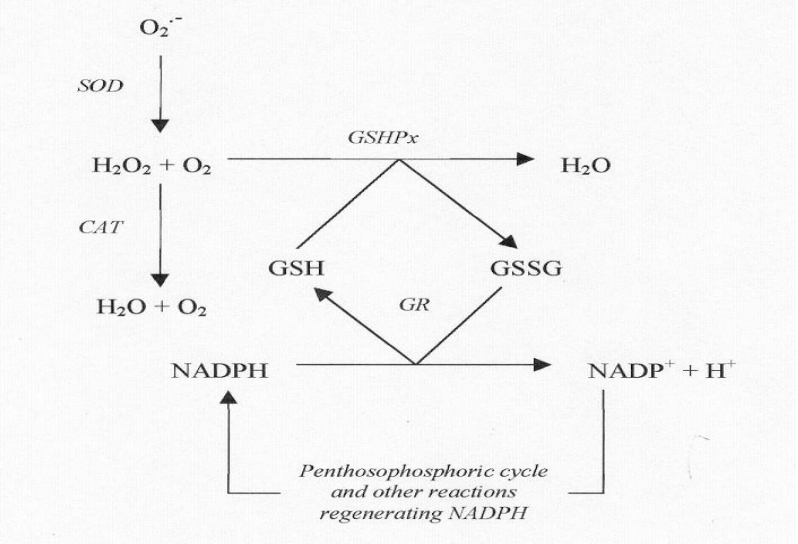


**Fig. 3**
Enzymatic antioxidant barrier: SOD – superoxide dismutase, CAT – catalase, GSHPx – glutathione peroxidase, GR – glutathione reductase, GSH – reduced glutathione, GSSG – oxidised glutathione

Catalase takes part in removing hydrogen peroxide, catalysing a reaction of disproportionation of this compound:

$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$

Glutathione peroxidase also takes part in removing hydrogen peroxide, reducing $H_2O_2$ with the participation of reduced glutathione (GSH):

$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$

GSSG - oxidised glutathione.

Four different glutathione peroxidases occurring in humans are known [6]. Glutathione peroxidase of phospholipid peroxides is also capable of breaking up phospholipid peroxides. Glutathione reductase cooperates with glutathione peroxidase, creating a reduced form of glutathione at the expense of oxidation of NADPH:

$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$

Under the influence of exercise or training, the activity of antioxidant enzymes is changed [29]. The mechanism's response for the change in the activity of these enzymes have not been fully investigated yet. Knowledge on the subject of kinetics or molecular regulation of the activity of these enzymes in mammalian tissues is still insufficient. As a result of covalent or allosteric modification, an increase in the activity of antioxidant enzymes probably occurs [31].

As a result of oxidative stress, a more rapid activation of the synthesis of enzymes at the transcription stage has been observed in Prokaryota. It has not been, however, ascertained whether these mechanisms also function in the tissues of mammals. The activation of superoxide dismutase was demonstrated in the skeletal muscles of rats after exercise [23]. Hollander *et al.* [23] showed that intensive exercise leads to a rise in the level of the nuclear factor-κB and the activator of proteins-1 (AP-1), which can stimulate the transcription of MnSOD. However, despite the increased amount of mRNA for MnSOD, the concentration of MnSOD protein in the muscles was unchanged. The rise in the level of CuZnSOD protein in the muscle of rats was neither accompanied by an increase in the amount of mRNA of this enzyme. The activity of CuZnSOD did not change either. It suggests that the activation of genes encoding antioxidant enzymes and a higher content of their transcripts is not directly connected to the activity of antioxidant enzymes.

There are data which indicate both the rise in the activity of catalase in the muscle cells of humans and/or animals after exercise [30], and the absence of changes to the activity [35], or the reduction of the activity of that enzyme [70]. Various diverse results of studies on the activity of superoxide dismutase [35,61] and glutathione peroxidase are also presented by other authors [9,24]. Changes in the activity of antioxidant enzymes after exercise and training are observed not only in muscles but also in the liver, heart [30], lungs [9], and in erythrocytes [53].

A great number of studies indicate an increased activity of antioxidant enzymes in erythrocytes after exercise [76,77,79]. Woźniak *et al*. [77]

demonstrated a statistically significant increase in the activity of superoxide dismutase an hour after the supramaximal exercise (Wingate test) in both untrained men and footballers. A significantly higher activity of glutathione reductase in erythrocytes of rowers was observed both directly after the end of the test on a rowing ergometer and after 24 hours' rest [79]. Woźniak *et al*. [76] demonstrated a statistically significant increase in the activity of SOD and CAT in erythrocytes of kayakers and rowers after physical training carried out in high mountain conditions.

The increase in activity of antioxidant enzymes in red blood cells testifies to an increased generation of ODFR, but cannot be the result of the synthesis of these enzymes once again, since erythrocytes do not possess a cell nucleus. It is assumed that the rise in activity of SOD in erythrocytes after exercise is caused not by its synthesis but by the lack of inhibitors or the presence of activators of that enzyme [53].

There are also data which do not show an increase in the activity of antioxidant enzymes in erythrocytes after exercise [25,52]. The variety of results probably depends, as in the case of the concentration of the products of lipid peroxidation, on the physical condition of the people being tested, the degree of training of athletes, and the different types of exercise carried out by those being studied.

In athletes of some sports disciplines, e.g. cyclists [45] and weightlifters [13], there occurs a higher resting activity of superoxide dismutase in erythrocytes than in untrained people from the control group. It can be assumed that the higher activity of SOD before exercise (resting activity) in trained people results from the adaptation of the organism to physical training. The rise in SOD activity testifies to the increased concentration of superoxide, a substrate of the dismutation reaction catalysed by SOD. The most frequently generated ODFR in the cells of the organism is $O_2^{\cdot-}$ [5], which explains the higher resting activity of this enzyme in athletes.

Very often a rise in the activity of one antioxidant enzyme is accompanied by an absence of change of activity or even a reduction in the activity of another antioxidant enzyme. Khassaf *et al.* [35] demonstrated a rise in the activity of SOD and an unchanged activity of CAT in material from the biopsy of the vastus lateralis muscle, after strenuous aerobic exercise in 7 volunteers. There is probably a connection between the activity of antioxidant enzymes and the concentration of reactive oxygen species. The activity of SOD, for example, is inhibited by $H_2O_2$ [22], and the activity of CAT and GSHPx is inhibited in the presence of superoxide [7,36].

## Non-enzymatic antioxidants

Non-enzymatic scavengers of free radicals have also an effect on oxidative stress induced by exercise. Non-enzymatic antioxidants include some proteins, e.g. albumin and so-called low-molecular-weight antioxidants. Low-molecular-weight antioxidants are divided into two groups: hydrophilic antioxidants protecting the aqueous environment of cells, e.g. glutathione, vitamin C (ascorbic acid), uric acid and cysteine, and hydrophobic antioxidants protecting the inside of cell membranes, e.g. α-tocopherol (vitamin E), β-carotene and reduced coenzyme Q (ubiquinone) [5,19]. The major natural antioxidants, most of them derived from natural sources by dietary intake, are vitamins C, E and A (retinol) and carotenoids. Also, numerous small molecules are synthesized or produced within the body that have antioxidant capacity (e.g., glutathione and uric acid).

*Glutathione:* Feeding mice with glutathione increases endurance to swimming [51]. The authors of this paper did not prove that the result obtained was connected with scavenging free radicals, but they showed that the presence of spin-trappers also increases the endurance of mice and enables them to perform exercise for a longer period [50]. Spin-trappers are most often the relevant nitrogen compounds which form paramagnetic adducts in reactions with free radicals [5]. The resulting adducts are more stable than the original free radicals. Moreover, the delocalisation of an unpaired electron in the resultant particle and its interaction with the atoms composing the original radical means that the EPR spectra of the products of the addition of various radicals are different. Thanks to this, it is possible to identify free radicals that react with spin-trappers [5].

Exercise of maximal intensity in junior female rowers caused a decrease in the concentration of reduced glutathione (GSH) in erythrocytes with an accompanying increase in the concentration of TBARS in blood plasma [33]. It is interesting that an identical exercise test on a group of seniors caused a lower post-exercise rise in the level of TBARS in blood plasma, and the drop in the concentration of GSH was not statistically significant. On this basis it can be assumed that a higher degree of training has a beneficial effect on preserving the pro- and antioxidant balance in erythrocytes and skeletal muscles of female rowers subjected to exercise.

*Uric acid:* Uric acid is the final product of purine metabolism in humans (Fig. 4) [74]. Uric acid is a weak acid distributed throughout the extracellular fluid compartment as sodium urate, and cleared from the plasma by glomerular filtration [73]. This acid has important antioxidant properties in vitro, by scavenging free radicals and chelating iron, the latter preventing iron-catalyzed oxidation [74].

Serum uric acid possesses antioxidant properties, and contributes about 60% of free radical scavenging activity in human serum [43,73]. Uric acid interacts with peroxynitrite to form a stable nitric oxide donor, thus promoting vasodilation and reducing the potential for peroxynitrite - induced oxidative damage [62,73].

Hellsten *et al*. [20] showed an increase in muscle allantoin level after two exhaustive cycling bouts at a work-rate of 90% of maximal oxygen uptake in seven men. It suggests that urate is utilized as antioxidant in human skeletal muscle during intense submaximal exercise. During one electron oxidation of urate by strong oxidants eg. $^{\cdot}OH$, $O_2^{\cdot-}$ a urate radical was formed. One electron oxidation of the urate radical leads to allontoin forming. A statistically significant increase in the serum allantoin/utate ratio after exercise at 100% $VO_2$max in humans was shown [47]. On the contrary, after 40% $VO_2$max of exercise, no significant changes in the levels of urate and allantoin in serum were observed. Mikami *et al*. [47] suggest that allantoin levels in serum may reflect the extent of oxidative stress in vivo.

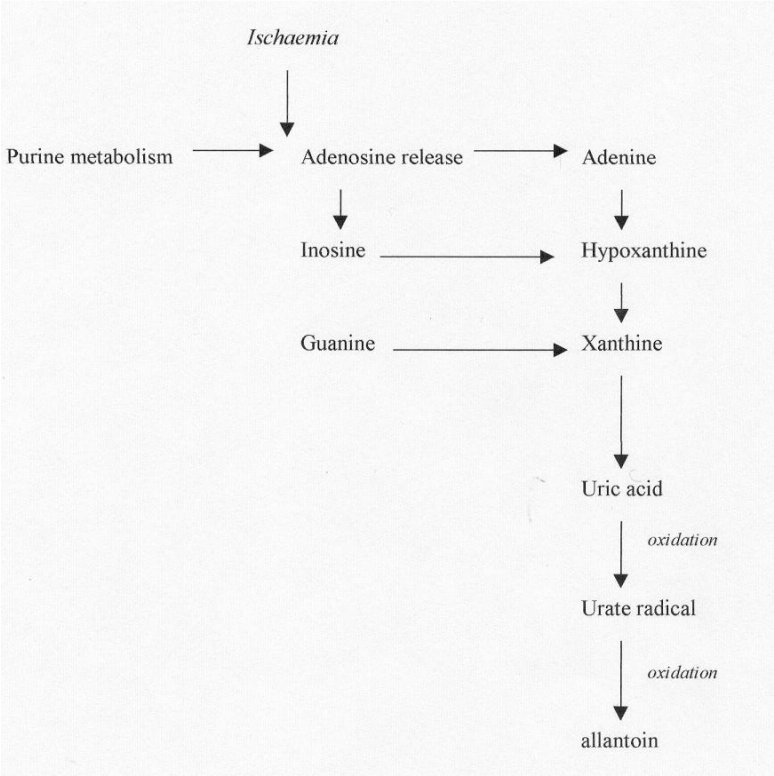

**Fig. 4**
Purine metabolism; uric acid formation during hypoxia

Uric acid administration substantially increases ex vivo antioxidant capacity in healthy volunteers with low baseline serum concentrations, consistent with normal antioxidant defenses and low oxidant stress [16]. Waring *et al.* [74] observed a significant increase in serum free radical scavenging capacity from baseline during uric acid and vitamin C infusion in healthy volunteers. The effect of uric acid was substantially greater than that of vitamin C.

*Ubiquinone (coenzyme Q):* The major form of ubiquinone in human subjects is ubiquinone-10 [57]. A reduced form of ubiquinone, ubiquinol is an antioxidant. It inhibits lipid peroxidation in biological membranes and protects mitochondrial inner-membrane protein and DNA against oxidative damage accompanying lipid peroxidation. Coenzyme Q supplementation decreases plasma lipid peroxidation in vivo, as assessed by the increased proportion of plasma ubiqinol of total coenzyme Q. Coenzyme Q supplementation with a high dose of vitamin E decreased this proportion. This may suggest regeneration of tocopheryl radicals by ubiquinol [32]. A lower concentration of coenzyme Q and vitamin A in plasma of rats after exercise compared to sedentary animals was shown by Quiles *et al.* [58]. The concentration of coenzyme Q and vitamin A in the liver and skeletal muscle mitochondria of rats was higher than in sedentary animals. This may suggest the existence of a balance between plasma and mitochondrial membrane for these antioxidants as a response to an oxidative attack. An increase in the ubiquinone level in slow muscle of rats after exercise was also shown by Liu *et al.* [42]. Coenzyme Q supplementation had no significant effect on oxygen uptake, anaerobic and respiratory compensation thresholds, blood lactate, and glucose and triglyceride kinetics after graded cycling exhaustion in endurance athletes [75].

*Vitamins:* Vitamin E is a particularly important antioxidant because of its capacity to convert superoxide, hydroxyl and lipid peroxyl radicals to less – reactive forms [57]. Vitamin E can also break lipid peroxidation chain reactions which occur during ROS – mediated damage to cell membranes [57]. However, the interaction of vitamin E with ROS results in a reduction of functional vitamin E and the creation of a vitamin E radical. Nonetheless, the vitamin E radical can be "regenerated" to its native state by several other antioxidants e. g. vitamin C [55].

Vitamin C (ascorbic acid) is the main water-soluble scavenger of ODFR and an important antioxidant occurring in the extracellular compartment of an organism [21]. Vitamin C can directly scavenge superoxide, hydroxyl and lipid

hydroperoxide radicals. However, it should be noted that recycling vitamin E, native vitamin C is converted to a vitamin C radical [54]. It was demonstrated in vitro that ascorbic acid in the presence of $Fe^{3+}$ ions can strengthen the generation of the hydroxyl radical and hydroxyethyl radical $–CH_3CHOH^{\cdot}$ [1]. It has not yet been confirmed unequivocally, however, whether its prooxidant properties can appear in vivo.

Carotenoids (e.g. β-carotene) are located in cellular membranes and protect lipids against peroxidation by quenching free radicals and other ROS, notably singlet oxygen [78].

Administration of glutathione, vitamin C or vitamin E protects against the damaging effects of free radicals during exercise [72]. Tiidus and Houston [69] found a decrease in the concentration of vitamin E in skeletal muscles, liver and in the heart of rats after endurance training. At the same time, increased vitamin E intake raised tissue resistance to lipid peroxidation induced by exercise [31]. The protective effect of vitamin E supplementation on exercise-induced oxidative muscle damage in male volunteers was observed by Meydani *et al.* [46]. Sumida *et al.* [65] showed a decrease in the plasma β-carotene concentration after acute exhaustive exercise in male volunteers. It suggests that β-carotene may contribute to the protection of the increasing oxidative stress during exercise. Diet supplementation with a vitamin E, vitamin C and β-carotene cocktail enhances the antioxidant enzyme activity of superoxide dismutase and catalase in neutrophils of athletes [68]. Vitamin E, vitamin C and ubiquinone supplementation raised the LDL antioxidant potential (TRAP), serum TRAP and serum α-tocopherol concentration in endurance athletes [71].

## Final conclusions

In spite of the many varied results on the pro- and antioxidant mechanisms during exercise it can be stated that physical exercise increases the generation of reactive oxygen species and leads to oxidative stress in muscle and other tissues. This is particularly the case in untrained people during intensive exercise. Physical training attenuates prooxidative processes, mainly thanks to an adaptable increase in the activity of antioxidant enzymes. A significant antioxidant role is also played by small-particle scavengers during exercise. Their skillful supplementation appears partially to protect the cells from damage caused by reactive oxygen derived species.

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