

The role of physical exercise in free radical processes

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The effect of physical exercise on free radical processes involved in oxygen metabolism is still not clear. However recent evidence suggests that elevated oxygen consumption may increase generation of reactive oxygen species.

During exercise there is an increase in the requirement for oxygen. The mechanism of delivering the oxygen to the working muscles may actually result in damage to polyunsaturated fatty acids in membrane structures (lipid peroxidation).

It was documented by numerous investigations presenting increases in the content of products of lipid peroxidation following exercise.

There are enzymes involved in protecting the cells from oxidative damage. Although these enzymes reduce reactive oxygen species and oxidative stress reactions like lipid peroxidation during exercise, it is evident that the activities of these enzymes are not always adequate in preventing exercise-induced lipid peroxidation.

Antioxidants are substances, which help to reduce the severity of the oxygen stress forming a less reactive radical species or by quenching the free radical reaction. Antioxidants are present in the body to reduce the activity of radical-induced reactions.

When oxygen free radical generation exceeds the antioxidant capacity of cells or extracellular fluids, oxidative stress develops. This phenomenon has been related to many pathological conditions, including ischaemia, reperfusion, inflammation, degenerative diseases, exposure to prooxidant chemicals (like iron mobilization, paraquat, acetaminophen), air pollutants, congenital disorders, or changes in the trace elements in the diet (like selenium - component of glutathione peroxidase, or the pathology of hepatic iron overload, etc.).

Oxidative stress can also occur as a result of normal physiological activities.

Oxygen radicals are generated in mitochondria in almost every tissue studied.

It can be expected that oxidative stress can potentially occur whenever the aerobic metabolic rate of a tissue increases.

The kind of risk should be much higher in tissues with a very large variation of total oxygen consumption between rest and maximal activity. For instance, an already classical example can be the skeletal of cardiac muscle during strenuous exercise.



It is known that sudden and strenuous exercise can damage muscle. The damage occurs at the level of sarcoplasmic membranes, leading to accumulation of intracellular calcium, impairment of muscle force generation, myofibrillar disruption and increases of enzymes activities such as: creatine kinase, beta-glucuronidase in plasma immediately after exercise.

According to Clarkson and Dedrick (J.Gerontol.43,1988), accumulation of cellular calcium could activate sarcoplasmic proteases, which may have serious consequences including focal damage and necrosis, leading to failure of sarcolemmal integrity and massive release of enzymes into the plasma compartment.

It should be emphasized that training reduces the sensitivity of muscle tissue to this exercise-induced damage.

Strenuous physical exercise is associated with increases in the size and number of mitochondria in the exercised muscle. Mitochondrial enzyme activities associated with respiratory chain occurring in the inner membrane are also enhanced. Over 98% of oxygen utilized by cells is efficiently reduced by the mitochondrial electron transport chain, resulting in the formation of ATP and water.

The remaining oxygen is available for one or two electron reduction, causing the formation of the superoxide anions and hydrogen peroxide. These two products can react to form the highly reactive hydroxyl radical. Activity of cytochrome oxidase, the final enzyme involved in electron transport, is increased over 30% in exercise-trained versus sedentary rats (Starnes J.W. et al.J.Appl.Physiol.1989,67-69).

The mitochondrial matrix is the site of fatty acid oxidation, oxidative decarboxylation of pyruvate and the reactions involved in the citric acid cycle (Krebs cycle).

Free radical damage to the inner membranes can increase the activities of cytochrome reductase, enzymes associates with oxidative pathways and lower production of ATP (Gohil K. et al.J.Appl.Physiol.1987,63,1638).

Muscle fibre sites of free radical generation, in addition to mitochondria, include lysosomes, peroxisomes, nuclear and sarcoplasmic reticulae and the sarcolemma.

Potential sites of oxidative damage to skeletal muscle including increased influx of calcium ions, can activate phospholipase A₂ and proteases.

Free radicals and their products are also present in the sarcoplasm. Molecular species involved include hydroxyl, peroxy, superoxide and alkoxy radicals and reactive molecules such as hydrogen peroxide and singlet oxygen, which are not free radicals but also cause cell damage. It is known that tissue is usually accompanied by lipid peroxidation.

Muscle fibres contain high concentration of arachidonic acid and their phospholipids as well as specific cytochrome-linked fatty acid desaturase systems (Salviati G., et al.Experientia 1980, 36,1140);

It is known that an important regulator of muscle protein turnover is prostaglandine E₂ (PGE₂) as the product of arachidonic acid oxidation. Products of lipoxygenase activation stimulate cells associated with inflammatory responses to move into the damaged muscle.

Endurance training may increase the capacity of skeletal muscle to oxidize long chain fatty acids, such as arachidonic acid.

Lipid peroxidation can result in a loss of membrane fluidity, ion channel permeability and cellular lysis.



Membranes become leaky, which is reflected in an increased plasma concentration of muscle-specific enzymes (such as pyruvate kinase etc).

Leakage of lysosomal enzymes, such as proteases can significantly disrupt skeletal muscle contractibility.

Calcium channels can be altered, resulting in calcium flux, activation of phospholipase A₂ and initiation of the arachidonic cascade.

Free radicals can attack nucleic acids and consequently DNA is damaged.

Intracellular reactive oxygen species are also generated from autooxidation and consequent inactivation of small molecules such as thiols and catecholamines, and as by-products of the activity of certain oxidases such as xanthine oxidase, which is present in skeletal muscle, cyclo-oxygenases, dehydrogenases, peroxidases and other intermediates (Korthius R. et al. *Circ. Res.* 1985, 57, 599).

It should also be emphasised that higher concentrations of free radicals can be utilized as part of the first line of defence against pathogens by leukocytes. Neutrophils the most abundant white blood cells, have the capacity to take up molecular oxygen and generate reactive oxygen-containing molecules when stimulated. This is often called respiratory burst.

Exogenous sources of oxygen free radicals include radiation (X-rays, ultraviolet light), tobacco smoke, air pollutants such as ozone, nitrogen dioxide and the products of automobile exhaust system, organic solvents, hyperoxic environments and pesticides.

I would like to emphasize that outdoor exercise programs, which result in exposure of the skin to greater than normal levels of ultraviolet light (UV), may also contribute to an increased burden of reactive oxygen species in the athletes as compared to the sedentary person.

Indirect indices of muscle membrane leakage include the increased concentration of the muscle enzyme, pyruvate kinase in the serum. Recently the increase in pyruvate kinase has been directly correlated with increased red blood cell haemolysis which is another index of membrane susceptibility to lipid peroxidation and liver malondialdehyde levels as well as with decreased concentrations of glutathione peroxidase (Chow CK, *Nutr Res.* 1990, 10, 183-194).

The thiobarbituric acid reactive substance (TBAR) and conjugated dienes have widely been used to measure lipid peroxidation in cell membrane and fatty acids. TBAR technique has been shown to be sensitive to malondialdehyde and be a good general index of oxidative stress in biological systems. More recently malondialdehyde has been measured by HPLC, although many investigators continue to use the TBAR assay.

Oxidative stress appears to be related to exercise intensity. It is generally accepted that high-intensity exercise induces muscle damage and acute neurotic myopathy (Vihko A. et al. *Acta Physiol. Scand.* 1978, 104, 74-81) in the contrary to submaximal physical exercise which stimulates the antioxidative mechanism against oxidative stress (Buczynski and Kedziora *J. Int. J. Sports Med.* 1991, 12, 52).

Alesio HM et al. (*A.M.J. Physiol.* 1988, 255, C878) reported an average 120% increase in TBAR following high-intensity and 68% increase in skeletal muscle following moderate intensity running.

The next problem is physical exercise and hydrocarbon production. When lipid peroxides split, the hydrocarbons ethane and pentane are formed.



Most of authors supported the notion that hydrocarbons can be used as biomarkers of oxidative stress; for instance ethane production can be a useful index of lipid peroxidation of tissue homogenates.

Research on the use of hydrocarbons as *in vivo* indicators of lipid peroxidation continues to progress and enhanced sensitivity of new methods may resolve the question of how exercises type may influence lipid peroxidation.

Damage to DNA may occur as a result of oxidative stress. The production of superoxide radicals is related to metabolic rate, the intensity of various metabolic pathways creating free oxygen radicals, and the cell defence system. Hruszkewycz A M (Biochem.Biophys.Res.Commun.1988,153,191-197) reported evidence for mitochondrial DNA damage by lipid peroxidation. Byproducts of lipid peroxidation include 4-hydroxy-alkenals and aldehydes, products known to react with thiol groups and inhibit DNA synthesis and cell division.

A study on oxidative stress associated with marathon running reported that the ratio of oxidized nucleosides per creatine increased 1.3 fold following a marathon race. The increase of 8-hydroxy-2'deoxyguanosine represented the steady-state unrepaired DNA damage level.

An exercise intensity threshold may exist above which oxidative stress increases dramatically. The percent of maximum effort at which the threshold may occur, might also depend on training or health status. For instance, the bed rest can affect antioxidative defence like physical effort (Kedziora J.et al.Acta Physiol.Pol.1990,34,161). It is clear that reports of oxidative stress during exercise depend on the intensity and type of exercise and the health and training status of the models being used.

Antioxidant defences include free radical scavengers and enzymes, which facilitate the decomposition of reactive oxygen species-molecules, which are precursors of free radicals.

These antioxidants can break the chain reaction or lower the free radical burden before a chain reaction begins.

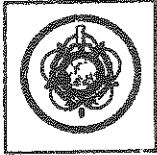
Two important antioxidants are metalloenzymes which can interfere with the production of free radicals during the initiation phase by inactivating precursor molecules. There are two types of superoxides dismutases: a Mn-containing enzyme located in mitochondria, and Cu, Zn-containing enzyme place in the cytoplasm, both of which catalyse the reduction of superoxide free radicals to hydrogen peroxide.

Catalase, a Fe-containing enzyme found in peroxisomes, catalyses the decomposition of the hydrogen peroxide produced as a result of superoxide dismutation or by other reactions. The final products of hydrogen peroxide breakdown are oxygen and water. Selenium is a trace element component of glutathione peroxidase, which causes the decomposition of hydrogen peroxide and lipid peroxides.

Recently, the effect of endurance training on the activities of these antioxidants enzymes in rat skeletal muscle has been examined. All four enzyme activities were significantly enhanced following training from 33 to 74% as compared to that seen in muscle from untrained animals, whereas liver enzyme activities remained unchanged (Krotkiewski M. et al.Scand.J.Med.Sci.Sports 1994,4,191).

Some of vitamins have antioxidant properties (Goldfarb A.H.,Med.Sci.Sports Exercise 1992,25,232).

Vitamin E (alpha-tocopherol), the lipid soluble antioxidant present in all cellular membranes, protects against lipid peroxidation (Pryor W.A.,J.Nutr.1991,53,702).



Vitamin C (ascorbic acid), is water soluble and along with vitamin E can quench free radicals as well as singlet oxygen. Ascorbate can also regenerate the reduced, antioxidant form of vitamin E (Frei B. et al. Proc. Natl. Acad. Sci. USA., 1989, 86, 6377), and (Bendich A., et al. Free Radic. Biol. Med. 1986, 2, 419).

Beta-carotene is an efficient quencher of singlet oxygen and can function as an antioxidant (Kaplan L.A. et al. Clin. Physiol. Biochem. 1990, 8, 1).

Also other compounds can play a role as antioxidants.

In addition to the vitamins and mineral-containing enzymes, there is a list of compounds with antioxidant potentials. Two of these compounds, glutathione (a tripeptide composed of glutamine, cysteine and glycine) and carnosine (a dipeptide composed of alanine and histidine) are especially important for protection of skeletal muscle from damage (Kohan R. et al. Proc. Natl. Acad. Sci. USA 1988, 85, 3175).

Glutathione is a source reducing equivalents and cosubstrate for glutathione peroxidase. Glutathione is also involved in inactivation of oxygen radicals as well as the regeneration of certain antioxidants, such as vitamin E (Gibson D.D. et al. Lipids. 1985, 20, 704).

Carnosine has recently been shown to scavenge peroxy radical, quench singlet oxygen, act as a reducing agent and is a critical chelator of copper.

The antioxidant properties as well as the chelation of copper by carnosine can help to protect muscle tissue from oxidative damage (Martenson J., and Meister A.: Proc. Natl. Acad. Sci. USA. 1989, 86, 471).

Calcium influx can initiate membrane enzymes, such as phospholipase A₂ resulting in the release of arachidonic acid from the membrane as it was mentioned above. The arachidonic acid cascade of oxidative reactions leads to the possible formation of prostaglandins (PGE₂) and leukotriens. Vitamin E has been shown to interfere with phospholipase A₂ activity and inhibit lipoxygenase activity and PGE₂ production.

Vitamin E also protects muscle membranes from oxidative damage. A very important question is the vitamin E supplementation. Young untrained males were exercised to exhaustion before and following supplementation for 4 weeks with 300 mg Vitamin E.

According to Sumida et al. (Int. J. Biochem. 1989, 21, 835) the vitamin E supplementation did not affect exercise time or other indices of endurance. Vitamin E supplementation did lower the pre-exercise, baseline level of serum thiobarbituric acid reactive substances and beta-glucuronidase (an index of cellular damage). It should be emphasized that following exercise, lipid peroxides were significantly increased in the control group, but were decreased significantly in the vitamin E-supplemented group.

Serum uric acid levels were increased in both groups, as were mitochondrial -GOT and beta-glucuronidase, although the relative increases were lower in the vitamin E supplemented group. In summary I would like to conclude:

1. Physical exercise (especially maximal and supramaximal effort) has been associated with dramatical increases in free radical production in skeletal muscle and in the consequences the overproduced reactive oxygen species cause damage to mitochondria.
2. Submaximal physical exercise protects skeletal muscle against free radicals by increasing antioxidative barriers i.e. it is superoxide dismutases (Cu/Zn-SOD, Mn-SOD) glutathione peroxidase (GP_x-SH) catalase (CAT) and carnosine in cells as well as elevation of ferritin (linked with iron) and ceruloplasmin (coupled with copper).



3. Vitamins supplementation (i.e. vitamin E, ascorbic acid, beta carotene) before endurance training protect muscle against free radical damage and allow to maintain the balance between reactive oxygen species generation and scavenging processes.
4. In order to protect against augmentation of lipid peroxidation proper diet (containing such ingredients like polyunsaturated fatty acids of the fish oil, low cholesterol and high content of cellulose together with trace elements) is recommended.
5. During sedentary life hypokinesia (bed rest) should be avoided.