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Original Article

Oxidative stress in athletes after occasional smoking

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Abstract:
Normally, in the tissues of living organisms, freeradical processes run continuously, and their intensity is insignificant. With increased physical activity, bad habits intensify freeradical processes. We studied the intensity of freeradical processes in the blood of sportsmen (track and field athletes) who smoked tobacco once a week. The results were compared with the values of men who do not practice sports and smoke daily, with track and field athletes who do not use tobacco at all, and with a control group in which men do not smoke and do not practice sports. The intensity of free radical processes was determined in venous blood samples using the induced chemiluminescence method; the activities of malonicdialdehyde, superoxide dismutase, and catalase were also determined. The activity of malonicdialdehyde in the serum of the track and field athletes who smoked a cigarette once a week was elevated compared with that of the control group. In the group of track and field athletes who did not smoke, the level of malonicdialdehyde was also slightly higher than in the control group, which is associated with active exercise and accelerated metabolism. There were also differences between superoxide dismutase activity levels. In the group of athletes who smoked once a week, the level was reduced. In the group of athletes who do not smoke at all, the level of superoxide dismutase was, in contrast, higher. The activity of catalase was decreased in the group of athletes who smoked once a week compared to that of the control group. A similar result was found for the group of smokers. In athletes who do not smoke at all, the level of catalase was higher than that in the control group. Correspondingly, the index of maximum intensity of induced biochemiluminescence was increased in the group of athletes who smoked and in the group of smokers who did not participate in sports. In athletes who did not smoke, the level was elevated insignificantly. Thus, the oxidative stress activity in athletes who smoke once a week was higher than in the regular smokers. Thus, sporadic tobacco use also leads to the development of oxidative stress in athletes.
Key Words: **antioxidant, catalase, malondialdehyde, physical overexertion, superoxide dismutase**

Introduction

Prolonged, high-intensity exercise is known to induce oxidative stress in highly trained athletes. Oxidative stress during exercise can lead to endothelial dysfunction and is one of the causes of cardiovascular dysfunction. Oxidative stress plays an important role in the development and maintenance of vascular damage. In this case, the initiation of oxidative stress begins after an increased formation of free radical compounds in the body and/or a disturbance in the antioxidant systems, which leads to a decrease in their effectiveness (Veliz et al. 2017; Antunes et al. 2020; Vladimirov et al. 2021).When the efficiency of antioxidant systems at the level of erythrocyte membranes decreases (Pingitore et al. 2015; Beschasnyi et al. 2020), cellular metabolic processes are inhibited and the structural integrity and functional activity of cytoplasmic membranes are impaired.Thus, a "vicious circle" phenomenon is observed - the above-mentioned changes lead to a dysfunction of mitochondrial complexes (Frediani et al. 2020; Taherkhani et al. 2020), which leads to the blockage of aerobic adenosine triphosphate resynthesis pathway.

Prolonged training can negatively affect the antioxidant status of the athlete (Boccatonda et al. 2016; Nocella et al. 2019). The development of endothelial dysfunction in athletes under the influence of high training loads is increasingly being reported (Martins et al. 2020; Alonso et al. 2020). It has been shown that tissue hypoxia eventually causes a significant change in the pro-oxidant-antioxidant balance, increasing the oxidation processes of metabolites and suppressing the antioxidant system (Poortinga, 2007). The activation of lipid peroxidation processes is a physiological response of the body to stressful situations. After the activation of peroxidation processes, the reaction products of lipoperoxides can significantly damage the endothelium and provoke the development of subsequent radical reactions on cell membranes (Abrahams et al. 2019;Biagini et al. 2020). Deposition of free radicals reduces the bioavailability of nitric oxide and causes vascular dysfunction. It is equally recognized that free radicals inhibit cellular function and regulation of intracellular signal transduction and gene expression (Khan et al. 2020).

High levels of oxidatively modified low-density lipoproteins have been shown to adversely affect endotheliocytes, as these lipoproteins can induce necrosis and apoptosis of endothelial cells, while also causing

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immune cell activation, which leads to auto-sensitisation and autoantibody deposition.Chronic overexertion of the cardiovascular system in athletes may be accompanied by abnormalities in the lipid spectrum. It should be taken into account that the lipid profile of athletes differs significantly from that of healthy but untrained individuals. For example, athletes have lower total cholesterol, low- and very-low-density lipoproteins and triglycerides, but high-density lipoproteins are higher (Halliwell, 2007;Bojkowski et al. 2020).

Tobacco smoking is a bad habit that causes cardiovascular diseases and cancer and affects the respiratory system (Sugdenet al. 2019). Tobacco smoking is known to lead to the development of oxidative stress (Alzoubi et al. 2019; Khan et al. 2020; Viktor et al. 2020). Oxidative stress is a state of the physiological system characterized by increased levels of reactive oxygen species, which can cause molecularlevel disruption of vital structures and functions. Oxidative damage, especially lipid peroxidation and protein and DNA damage, develops when the accumulated amount of reactive oxygen species exceeds the antioxidant capacity of the body (Tkachenkoet al. 2014).

During the action of stress factors (especially physical exercise) on the body, the level of reactive oxygen species briefly increases(Ortenburgeret al. 2020; Sybil et al. 2020). It is known that two types of reactive oxygen species are formed in cells. The first type is very active low molecular weight radicals, which quickly decay (superoxide anionradicals, nitric oxide, andperoxynitrite). The second type is formed after the interaction of the first type of radicals with biomolecules in the cell (lipid radicals appear and hydroxyl radicals). Both types of reactive oxygen species exist in the cell for a long time (Liguoriet al. 2018).

One component of cigarette smoke is carbon monoxide (CO). CO binds highly to haemoglobin and myoglobin. This substance in the body leads to decreased oxygen transport from the lungs to the cells (Ernst et al. 1998). Of note, a small amount of CO is produced in the human body because of the breakdown of haemoglobin and other haem-containing proteins (Tenhunen et al. 1968). It is known that in the process of combustion in a cigarette, three zones are formed, smouldering, pyrolysis, and distillation, which is the source of CO production and its further inhalation. After quitting smoking for 12 h, the oxygen content in the blood and oxygen supply to tissues increases, and within 5–7 days, blood viscosity normalizes. Thus, it is indisputable that cigarette smoke affects blood parameters. In addition, blood contains antioxidant enzymes and lipid peroxidation products, the activity and level of which indicate the activity of the oxidant–antioxidant system in the body (Tkachenkoet al. 2014).Athletes who smoke tobacco infrequently can also be negatively affected by cigarette smoke. It is not known how infrequent tobacco use affects the oxidative status an athlete's body.

Materials& methods

To study the intensity of free radical processes in blood, four groups of men (ages 23–27 years) were formed. The first group was men who smoked at least 20 cigarettes a day and had a smoking history of more than 3 years. The second group included track and field athletes who had never smoked. The third group were track and field athletes who had smoked one cigarette a week for at least 3 years. The fourth group was men who neither smoked tobacco nor exercised (control). After the subjects gave written consent for the study, venous blood samples were taken from them.

The work was carried out in accordance with the ethical standards of the Human rights Committee of the Helsinki Declaration of 2008 (World Medical Association Declaration of Helsinki, 2013). The study did not infringe on the rights or endanger the well-being of the study participants.

Venous blood samples were taken from the ulnar vein on an empty stomach, at rest, in the morning. The intensity of freeradical processes was determined using an induced chemiluminescence spectrophotometer, ULAB-102UV. For the induced chemiluminescence reaction, serum, divalent iron solution and hydrogen peroxide were added to phosphate buffer solution (pH = 7.4). The light sum (S under the curve) and fast flash amplitude (max) were recorded. The value of “max” and “luminosum” were calculated in conventional units.Lightsum is the area under the chemiluminescence curve from the beginning of slow flash amplitude rise until it reaches the maximum.It describes the number of branching chains or the number of peroxide radicals formed. The luminosum reflects the ability of the components of the system to undergo oxidation chain processes when initiated by divalent iron ions.The fast flash amplitude reflects the concentration of hydroperoxides and is proportional to the initial hydroperoxide content of the sample

We also studied the concentration of malonicdialdehyde (MDA) (de Zwartet al. 1999) in blood plasma and superoxide dismutase activity (SOD) and catalase of erythrocytes (Hadwan&Kadhum, 2018). The state of antioxidant defense was assessed by the levels of SOD, catalase, and light sum S (Tkachenkoet al. 2014). To determine the MDA activity, 2 ml of 0.5% thiobarbituric acid solution was added to 1 ml of supernatant and incubated for 45 min in a water bath at 60 °C. The absorption spectrum was recorded on a spectrophotometer. When calculating the amount of MDA at 535 nm the molar absorption coefficient of the product was taken as 156000.

Activity of antioxidant system enzymes was determined in erythrocyte hemolysates. A 0.01 % solution of saponin in 0.01 M Na-K-phosphate buffer (pH 7.4) was used as a lysis agent. The activity of catalase (EC 1.11.1.6) was determined by the rate of hydrogen peroxide utilization. The amount of undecomposed H_2O_2 was determined using FOX reagent. The erythrocyte catalase activity was the amount of μmol of substrate (H_2O_2) converted by the enzyme per unit time (min) calculated per mg of haemoglobin (Hb) in the sample, i.e.

μmol/(min·mgHb). Tissue catalase activity was expressed in μmol/(min·mg protein). Superoxide dismutase activity (EC 1.15.1.1.) was determined by a method based on the ability of SOD to inhibit the autoxidation reaction of quercetin at pH 10 in the presence of tetramethylenediamine. SOD activity was measured spectrophotometrically at 406 nm by recording a kinetic curve reflecting the oxidation inhibition reaction of quercetin. SOD activity was expressed in μmol/min mg Hb. In this case the activities reflect the true nature of the relationship between the activities of antioxidant system enzymes.Statistical processing was performed using the Mann–Whitney and Wilcoxon tests. Statistical analysis was performed using SPSS (SPSS-17, Chicago, IL, USA).

Results

After the study, it was found that the MDA activity in the serum of athletes who smoked a cigarette once a week (group II) was increased by 1.7 nmol/L compared with that of the control group (group IV) (Figure 1.A).

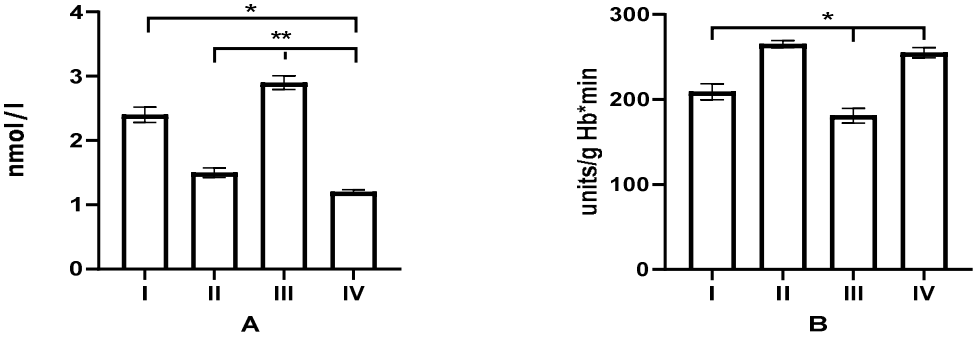

Fig 1. Results of malonicdialdehyde (A) and superoxide dismutase (B) in the blood of the experimental groups

Notes: Group I - men who smoke at least 20 cigarettes a day and have been smoking for more than 3 years; Group II - track and field athletes who have never smoked; Group III - track and field athletes who smoke once a week for at least 3 years; Group IV - men who do not smoke and do not participate in sports (control).
Credibility of Differences: * $p \leq 0.05$** $p \leq 0.01$.

In the regular smokers (group I), MDA levels were elevated by 1.2 nmol/L. Interestingly, in group II (athletes who did not smoke at all), the MDA level was also slightly higher than that in the control group by 0.3 nmol/L.

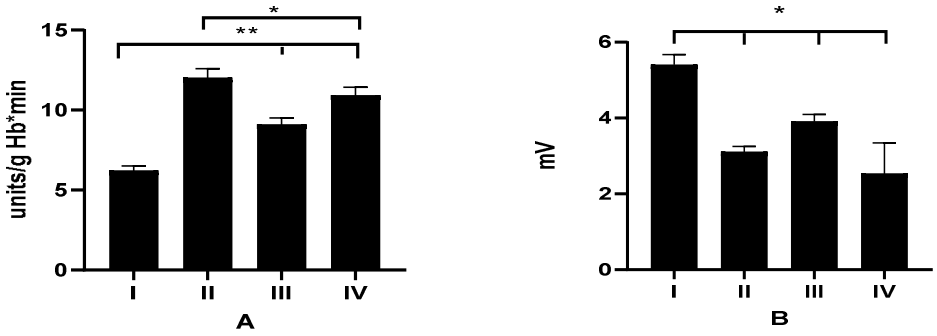

Fig 2. Activity of catalase (A) and indicators of maximum intensity of biochemiluminescence (B) of the experimental groups

Notes: Group I - men who smoke at least 20 cigarettes a day and have been smoking for more than 3 years; Group II - track and field athletes who have never smoked; Group III - track and field athletes who have smoked once a week for at least 3 years; Group IV - men who do not smoke and do not participate in sports (control).
Credibility of Differences: * $p \leq 0.05$** $p \leq 0.01$.

Superoxide dismutase activity also showed differences in the different groups. Compared with the control, in athletes who smoked (group II), the level was reduced by 74 units/g Hb*min, and in the group of smokers (group I), it was reduced by 46 units/g Hb*min. In the group of track and field athletes who did not smoke (group II), the level of SOD was higher by 10 units/g Hb*min (Figure 1.C).

Catalase activity in group III was decreased (by 1.8 units/g Hb*min compared to that of the control group). A similar result was obtained for the group of smokers (group I), which showed a decrease of 4.7 units/g Hb*min. (Figure 2.A).Moreover, the index in group II (athletes), unlike that in other groups, was higher than that in the control group by 1.1 units/g Hb*min.

The index of maximum intensity of induced biohemiluminescence (I_{max}) was increased in comparison with the control in all studied groups (Figure 2.C). In group I (men who smoke and did not exercise), the increase was 2.87 mV, in group III, (athletes who smoke) the increase was 1.37 mV, and in group II (athletes who exercise), the level of I_{max} was slightly increased by 0.57 mV.

Discussion

In all tissues of living organisms, freeradical processes occur continuously under normal conditions. Under normal conditions, their intensity is insignificant (Beschasnyi, 2013). Increased intensity of freeradical processes in tissues begins due to hyperproduction of free radicals and (or) insufficient functioning of the antioxidant system. This physiological state of cells, which is associated with a disruption in the normal regulation of freeradical reactions is termed “oxidative stress”, which is a universal mechanism of cell damage (Halliwell, 2007). This leads to the development of a variety of pathological conditions (Bojkowski et al. 2020). Thus, studying the development of oxidative stress is important.

The main processof oxidative stress involves freeradical oxidation of fatty acids or so-called lipid peroxidation (LP). It has been confirmed that the lipid peroxidation process begins with a chain initiation reaction, due to which superoxide and hydroxyl radicals are formed. If such a radical is formed near the cell membrane, it tends to react with polyunsaturated fatty acids (PFAs) of lipid side chains with the formation of a free carbon radical in the membrane. Afterwards, it reacts with molecular oxygen to form a peroxy radical (LOO*) (Tkachenkoet al. 2014).

In the absence of an appropriate antioxidant, the lipid peroxide "extracts" hydrogen from another nearby PFA to form hydroperoxide (LOOH) and a new carbon radical. This reaction begins a new step in the freeradical chain process, when hydroperoxides decompose, initiating new chains. Not all radicals continue the chain, and some of them interact with each other; thus, inactive products are formed, which leads to chain termination (Tkachenkoet al. 2014).

The self-accelerating freeradical oxidation reaction produces many LP products, which include lipid hydroperoxides (primary products), unstable substances that are easily subjected to further transformations with the formation of a number of more stable secondary oxidation products, such as aldehydes, ketones, and several low molecular weight acids (formic, acetic, and butyric acid). These substances are toxic to the cell and lead to disruption of membrane functions and metabolism in general. One of them, malonicdialdehyde, is formed during oxidative degradation of lipids and is part of the secondary products of LP (de Zwartet al. 1999).

The quantitative determination of malonicdialdehyde (MDA) is most commonly used to assess LP intensity. Its increase is one method of early detection of metabolic disorders in the body, even at the preclinical stage of a disease (de Zwartet al. 1999).

MDA activity in the serum of athletes who smoked a cigarette once a week was elevated compared to that of the control group. MDA levels were also higher compared to those of regular smokers. Interestingly, in the group of athletes who did not smoke, the MDA level was also slightly higher than that in the control group. This may be because high physical activity is accompanied with an increase in the formation of free radicals and the development of oxidative stress because of active exercise and increased metabolism.

As opposed to freeradical processes in the body, there is an antioxidant system, which is a set of protective mechanisms of cells, tissues, organs, and systemsaimed at preserving and maintaining homeostasis in the body. The equilibrium between these two opposing components in the physiological optimum state keeps peroxidation at a certain low level, preventing the development of a chain oxidative process and characterizing the antioxidant status of the body.

Enzymes that protect cells from the action of reactive oxygen species include superoxide dismutase, catalase, and glutathione peroxidase. Superoxide dismutase (SOD) converts superoxide anions into hydrogen peroxide. SOD isoenzymes are found in both the cytosol and mitochondria and are the first line of defense because superoxide anionsare usually the first reactive oxygen species to be formed when electrons leak from the respiratory chain (Hadwan&Kadhum, 2018).

In this study, differences were found between SOD activity depending on the length of smoking and the degree of physical activity. In the group of athletes who smoked once a week, the level was decreased. In contrast, the SOD level was higher in the group of athletes who did not smoke at all. We hypothesize that smoking is a factor that reduces the body's ability to counteract the effects of reactive oxygen species on cells during oxidative stress.

Hydrogen peroxide, which can initiate the formation of the most active form of OH-, is broken down by the catalase enzyme. Catalase is found mainly in peroxisomes, where most hydrogen peroxide is formed, and in white blood cells, where it protects cells from the effects of “respiratory explosion” (Rago et al. 2016).

Catalase activity was decreased in the group of athletes who smoked once a week. A similar result was obtained for the group of smokers. Athletes who do not smoke at all had higher catalase levels compared to that

of the control group. Accordingly, the index of the maximum intensity of induced biohemiluminescence in the group of athletes who smoked was higher compared with that of the control. Similarly, this index level was elevated in the group of smokers who do not participate in sports. In athletes who do not smoke, the level of I_{max} was elevated insignificantly. Thus, the oxidative stress activity in athletes who smoked once a week was higher than that in regular smokers. This suggests that infrequent smoking in combination with high physical activity increases the intensity of oxidative stress in athletes.

Training and exposure to cigarette smoke during prolonged physical activity lead to the development of adaptive shifts in an athlete's body. However, the positive training effect of hypoxia is contrasted by a number of negative aspects of its effects, each of which can lead to a deterioration in sports performance. Excessive hypoxia is a universal pathological process and the cause of impaired cellular metabolism, which is based on an insufficiency of the main energy-producing system, mitochondrial oxidative phosphorylation. The dysfunction of mitochondria as the main source of free radical formation is believed to play the leading role in the inhibition of the antioxidant protection system of an organism as well as activation of freeradical oxidation processes. A vicious cycle occurs: lack of oxygen disrupts energy metabolism and stimulates freeradical oxidation, and activation of freeradical processes, which damages mitochondrial and lysosomal membranes, increases energy deficiency in the cell, leading to reduced physical performance, as indicated by different researchers (Liguoriet al. 2018). Thus, oxidative stress was observed in the group of subjects who are daily exposed to CO from cigarette smoke. This was evidenced by the increased synthesis of endogenous antioxidants, which neutralize oxidative reactions. Increased levels of SOD and catalase were also indicative of oxidative stress.

Conclusions

The biochemical mechanisms of the body's antioxidant defense are a complex system characterised by both synergism and antagonism of its effects. A decrease in the activity of one antioxidant leads to a corresponding change in the other antioxidants, so that the overall activity of free-radical processes is maintained. This study has showed that athletes in track and field have a significantly increased risk of oxidative stress due to accelerated metabolism and hyperproduction of free radicals. This risk is higher in athletes who use tobacco once a week. This was confirmed by the fact that the group of athletes who smoked once a week had increased levels of malondialdehyde, an index of maximum chemiluminescence intensity. In these athletes, the levels of catalase and superoxide dismutase were reduced, which confirmed reduced activity of the antioxidant system.

The study of oxidative stress indicators in athletes can detect the presence of bad habits, detect the onset of overtraining and distress phenomena at an early stage. It is advisable to use antioxidants to eliminate oxidative stress to prevent disruption of the body's adaptation to physical overexertion.

Conflicts of interest

The authors have no conflicts of interest to declare.

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