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Influence of football activities on the amount of lipid peroxidation products and enzyme activity in adolescent saliva

I.S. Aliyev, S.A. Aliyev

Azerbaijan State Academy of Physical Education and Sport, 98 Fatali Khan Khoyski Str., AZ1072 Baku, Azerbaijan;
**For correspondence: ilgar.aliyev@sport.edu.az*
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The article concerns the study of the role of regular physical activity in adaptive changes in the lipid peroxidation process and the antioxidant reaction of the organism in 10–15-year-old adolescents involved in football. Measurements and analysis of lipid peroxidation products (heptan- and isopropanol-soluble products) were performed in the saliva of athletes in various age groups|: 10-11, 12-13 and 14-15 years old. The obtained data indicate that the biochemical analysis of saliva can characterize not only the level of training loads received by the athlete, but also assess the reserve possibilities of adaptation to future loads, as evidenced by the indicators of the enzymatic activity of saliva measured for the antioxidant enzyme catalase and the metabolic enzyme α-amylase. Determining the nature of adaptive changes in indicators of lipid peroxidation in saliva in response to physical exercise will be of significant practical importance, especially, in light of the demand for modern functional and laboratory diagnostics methods that allow fast and effective non-invasive, painless testing.

Keywords: *Adolescent football players, physical training loads, saliva, lipid peroxidation, enzyme activity*

INTRODUCTION

As with some sports, the constant increase in training loads in football leads to a number of functional and biochemical changes of an adaptive nature, which cause improving both of working capacity and effectiveness of recovery processes at different stages of training. It should be noted that the most important task in the preparation of athletes is the gradual intensification of the training process, i.e. a consistent increase in the intensity of training loads. In the practice of sports, an effective increase in the intensity of training loads is achieved on the basis of an individual approach to each athlete. However, physical loads used in football training can be highly effective only when they are scientifically justified, and are implemented in a certain sequence, taking into account the functional capabilities of the organism, recovery potential and diet (Михайлов**,** 2016; Əliyev və b., 2018a). Nutrition and oxygen requirements of the organism must meet its energetic and plastic needs. The main negative effect of physical activity is considered to be a discrepancy between the amount of oxygen entering the body from the external environment and its use in mitochondria for energy production, which leads to the formation of oxidative stress.

Under the influence of oxidative stress, free radical oxidation of lipids (lipid peroxidation - LPO) develops in cells. In normal physiological conditions, this process is controlled by regulatory antioxidant systems (Baraboy, 1989; Alessio, 1993; Əliyev və b., 2018b). Excessive development of free-radical reactions in tissues and cells is opposed by a special antioxidant system consisting of various enzymes and low-molecular compounds. Normally, this system is able to prevent excessive growth of the LPO and maintains its rate at a certain level characteristic of a particular tissue. However, with significant activation of peroxide processes in the body, antioxidant protection is ineffective and free radical oxidation has a pronounced damaging effect on the membranes, thereby causing a certain pathology. However, within the "capacity" of antioxidant protection, the level of

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LPO processes can vary in a certain interval and characterize urgent metabolic features of a functioning organism (Владимиров, 2000).

For muscular activity, an increase in the level of LPO above the endogenous level of rest occurs during physical exertion, which seems to be a functional feature of the motor system. Thus, it is shown that the level of LPO products in blood plasma and erythrocytes membranes is an important indicator of the adaptive processes of metabolic adjustment in the organism of athletes under the influence of intense physical activity in basketball players (Луцик и др., 2001). Moreover, there is evidence that the specifics of the training and competition process in skiing leads to an optimal intensification of the LPO, in which the content of lipoperoxides increases to values that optimize aerobic metabolism (Дятлов и др., 1997).

The relationship between the two types of action of LPO processes, namely the action that leads to the pathology of the functions of cellular structures and the action that optimizes cellular processes to perform an increased function, is a special subject of free radical biology. At the same time, for the biochemistry of sports, these issues are of crucial importance; almost any sports activity is accompanied by the activation of peroxide reactions. Therefore, research in this area should be aimed at identifying patterns of LPO development, depending on both the nature of the physical work performed, and the orientation of training processes.

Taking into account the fact that at various physical exertions we will face either the pathological effect of LPO, or its “improving” effect, it is necessary to clarify both the possibility of preventing free-radical reactions by using exogenous antioxidants, and the use of LPO indicators (in minimally invasive or non-invasive objects, for example, in blood or saliva) as a criterion for an objective assessment of the athlete’s special training level.

An equally important reason for the activation of LPO processes is the stressful nature of physical activity in modern sports. According to many researchers, excessive activation of the sympathoadrenal system is one of the main causes of structural and functional changes in organs and systems during extreme physical exertion (Meerson, 1985; Дятлов и др., 1997; Григорьева, 2003). The adaptive lipotropic effect of the stress reaction can also turn into a damaging effect due to increased free radical production and subsequent activation of free radical oxidation (Львовская, 1998; Baraboy, 2006; Стаценко и Алькевич, 2009). It should be taken into account that the organism's systems, including the antioxidant defense system, adapt not to physical activity in general, but to a specific type of muscle activity. The orientation of the training process significantly affects the state of the oxidant-antioxidant system, since adaptive changes in metabolism that are characteristic of different sports are specific. Specific metabolic changes are formed in the body of athletes, which are manifested both at rest and in response to physical activity (Львовская, 1998**;** Baraboy, 2006; Базагин, 2013).

The effect of stress on the human organism leads to the activation of the sympathetic nervous system (SNS), which innervates all organs. Activation of SNS is correlated with changes in the activity of α-amylase in saliva (van Stegeren et al., 2006). So monitoring the activity of this enzyme in the saliva of athletes is of considerable interest in terms of reaction to the effects of physical activity.

Based on the above, we have devoted this study to studying the role of regular physical activity in adaptive changes in the processes of lipid peroxidation, antioxidant and metabolic reactions in adolescent footballers, studying the corresponding indicators in saliva.

MATERIALS AND METHODS

The work with athletes was carried out in accordance with the standards of training of young athletes (Nabatnikova, 1984**).**

Saliva was used for biochemical analyzes. Before taking a saliva sample, the oral cavity was rinsed with saline solution, and then about 2 ml of saliva was collected in test tubes for 35 minutes. Saliva samples were centrifuged at 3000 rpm for 15 minutes. Supernatant was used for further research. The LPO level was assessed by the content of primary (diene conjugates of hydroperoxides) and secondary (ketodienes and conjugated trienes) products of LPO in heptan-isopropanol extracts of saliva. Determination of LPO products in hepta-

ne-isopropanol extracts of saliva was performed by the spectrophotometric method according to I.A.Volchegorsky et al. (Волчегорский и др., 1989). The results were calculated in the form of oxidation indices-E232/E220 and E278/E220, which reflect the relative level of primary (diene conjugates) and secondary (ketodienes and conjugated trienes) LPO products, respectively.

The lipid fraction of 0.5 ml samples was extracted in 5 ml of a mixture of equal volumes of heptane and isopropanol by shaking for 15 minutes. The lipid extract was separated by centrifugation and diluted with 5 ml of the heptane-isopropanol mixture (3:7 by volume) and divided into phases by adding 2 ml of an aqueous HCl solution (pH=2). After 30 minutes, the heptane (upper) phase was transferred to a separate test tube, and 1 g of dry sodium chloride was added to the water-alcohol (lower) phase to separate the water phase. After that, they were shaken again for 5 minutes. After 20 minutes, the obtained extracts were selected and measured at the appropriate wavelengths of the ultraviolet range.

Determination of catalase activity was performed by a method based on the reaction of hydrogen peroxide with catalase and determination of light absorption of a complex of hydrogen peroxide with ammonium molybdate at a wavelength of λ=410 nm. For this purpose, 25 ml of drinking water was mixed with 2 ml of 0.03% hydrogen peroxide. The reaction was stopped by adding 2 ml of 2% ammonium molybdate after 10 minutes. In parallel, control experiments were conducted without the participation of enzyme. The difference in the optical densities of the control and experimental samples was used to calculate the enzyme activity (molar absorption coefficient - 22.2×10^3 $cm^{-1}M^{-1}$).

The activity of α-amylase was determined by the test using 2-chloro-4-nitrophenyl-maltotrioside (CNPG3) as a substrate. The reaction is catalyzed directly by α-amylase. The formation of 2-chloro-4-nitrophenol (CNP) leads to an increase in optical density over time. The activity was determined by the rate of accumulation of CNP, which is proportional to enzyme concentration in sample. Saliva samples were diluted 200 times.

SPSS for Windows version 22.0 package program was used for statistical analyses of data. Shapiro–Wilk test was used to check whether the variables for studied groups fit normal distribution. Differences between control and experimental measurements were tested using paired samples t-test. Mean ± standard error values were given as the descriptive statistics and $p<0.05$ was accepted as the statistically significant value.

RESULTS AND DISCUSSION

The table illustrates data on the age dynamics of saliva biochemical parameters in adolescent football players in comparison with adolescents who do not engage in active sports. Biochemical indicators are products of lipid peroxidation, namely, the concentration of neutral lipids heptan_1, heptan_2, as well as phospholipids isopropanol_1, isopropanol_2. In addition, the activities of the antioxidant enzyme catalase and the amylase enzyme in saliva of adolescent football players of the same age groups of 10-11, 12-13 and 14-15 years are shown in the table. The analysis of these indicators characterizes the functioning of antioxidant factors of non-specific protection and the state of LPO in organism of young athletes.

The analysis of LPO indicators in young football players shows that the content of heptan-soluble products in saliva does not significantly change in different age groups, which implies independence from the experience of playing football. A similar pattern was observed in the amount of LPO products solubilized in isopropanol. This means that the antioxidant system of non-specific adaptation functions optimally, that is, there is no reliable increase in LPO products under the influence of various physical loads to the organism of adolescents, with no damaging effect on the membrane of cells and tissues.

A comparative analysis of the results of non-invasive biochemical study of the saliva in adolescent athletes and non-athletes of the same first age group showed that heptane-soluble primary and secondary peroxide products of lipids (Heptan_1 and Heptan_2) have significant differences ($p<0.05$) in subjects aged 10-11 years: the excess in adolescent football players is 10.9% for Heptan_1, and 17.9% - for Heptan_2.

Table. Pecularities of biochemical analysis indicators of saliva in adolescent football players and non-sporting adolescents (M±m)

Indicators of biochemical analysis	I CG (n=12)	I EG (n=16)	II CG (n=12)	II EG (n=15)	III CG (n=12)	III EG (n=12)
LPO product Heptane_1	0.172±0.030 100%	0.190±0.016^{+} 100%	0.183 ±0.030 107.2%	0.184±0.020 97.0%	179.0±0.011 105.9%	0.181±±0.011 107.1%
LPO product Heptane_2	0.120±0.095 100%	0.144±0.060^{+} 100%	0.124±0.027 102.4%	0.138±0.035 97.9%	0.128±0.035 105.8%	0.134±0.032 95.1%
LPO Product Isopropanol_1	0.450±0.032 100%	0.560±0.025^{++} 100%	0.514±0.060 115%	0.547±0.016^{+} 99.8%	0.516±0.077 114.7%	0.542±0.022 98.9%
LPO product Isopropanol_2	0.310±0.045 100%	0.311±0.072 100%	0.313±0.024 100%	0.313±0.014 100%	0.325±0.019 104%	0.305±0.35 98%
Catalase. nmol/mg/min	140.0±3.20 100%	138.97± 2.20 100%	144.94± 3.35 103%	147.39±2.50^{++***} 106%	140.45±1.46 102%	153.68± 2.30^{++***} 108%
α-Amylase. nmol/mq/min	455.0±2.60 100%	456.95± 1.65 100%	451.36±2.10 98.5%	462.45±2.27^{++***} 101%	459.99±2.23 100.5%	466.97±2.40^{++***} 102.2%

I CG, I EG - control and experimental groups of 10-11 year old, initial training;
II CG, II EG - control and experimental groups of 12-13 year old, training for 1-2 years;
III CG, III EG - control and experimental groups of 14-15 year old, training for 3-4 years.
+ - $p < 0.05$, ++ - $p < 0.01$ - reliable changes in adolescent athletes compared to control group.
* - $p< 0.05$, *** - $p < 0.001$ - reliable changes to group I.

In other age groups, heptane-soluble lipoperoxide products in saliva do not have significant differences between athletes and non-athletes. This is due to the fact that the products of lipid peroxidation in the organism of young athletes accumulate in response to physical activity at the initial stage of sports training.

The content of isopropanol-soluble primary LPO products (Isopropanol_1) in the saliva of 10-11 year old football players was significantly higher by 24.4% than in the corresponding control group ($p<0.01$). In other age groups of football players, the content of Isopropanol_1 is also more or less significantly higher than the level of the corresponding control groups; in the group of 12-13 years by 6.4% ($p<0.05$), and in the group of 14-15 years – by 5.1% ($p<0.05$). Secondary products of isopropanol-soluble LPO products (Isopropanol_2) did not show any significant changes in all groups of adolescent football players when compared with adolescents who do not engage in active training.

Catalase, an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide formed during biological oxidation into water and molecular oxygen. This enzyme can give rise to more dangerous active forms of oxygen, including a highly reactive hydroxyl radicals that can easily induce oxidative stress in the cell, thereby damaging biological membranes.

The study of catalase activity in saliva of adolescent athletes, who play football and are at different levels of training, showed that 10-11 year olds do not show a significant difference in the activity of the enzyme in relation to the control group of adolescents who are not athletes. Contrary to this, athletes with a long experience of football training have increased catalase activity when compared with control groups. A group of 12-13 year old football players (1-2 years of football training) showed an increased level of catalase activity by 2.2% ($p<0.01$), and a group of 14-15 year old football players (3-4 years of football training) - by 9.3% ($p<0.01$) compared with control persons.

The activity of the α-amylase enzyme as well as catalase does not show significant changes in 10-11 year-olds engaged in football, in relation to control adolescents. At the same time, 12-13 year old football players with 1-2 years of experience have amylase activity in saliva by 2.4% ($p<0.01$) higher than the activity level for non – athletes, and 14-15 year old football players with 3-4 years of experience-by 1.7% ($p<0.01$) of the control level.

An analysis of changes in the activity of catalase, an antioxidant enzyme in adolescent football players revealed that the adaptation reactions to physical loads have a phased nature in functioning of the antioxidant defense system in 10-15 year old players. Reliable increase in activity of the ca-

talase and α-amilase enzymes ($p<0.05$) was observed in 12-13 year-old compared to non-athletes and an increase of about 3.7%. Such a positive growth trend was also observed in adolescents aged 14-15, with an increase of 6.9% (Figure 1 and Figure 2).

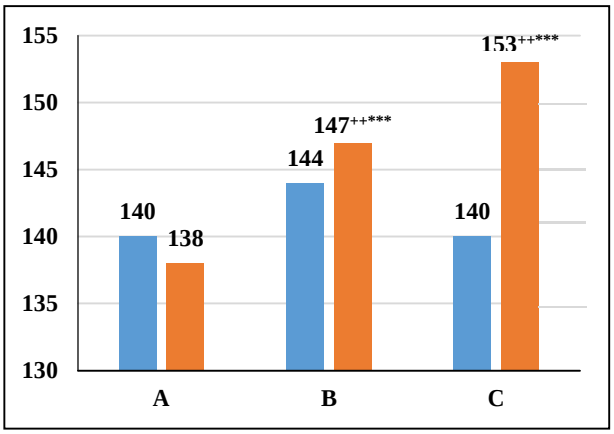

Fig. 1. Catalase activity (nmol/mg/min) in saliva of adolescent football players and non-sporting adolescents. A - I group, 10 - 11 year old; B - II group, 12 - 13 year old; C - III group, 14 - 15 year old. Blue - control, red - athletes. *** - $p<0.001$ - compared to I group athletes; ++ - $p<0.01$ - compared to control group.

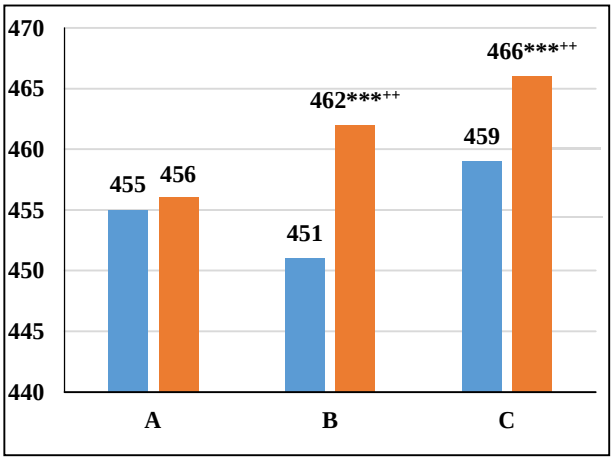

Fig. 2. α-Amylase enzyme activity (nmol/mg/min) in saliva of adolescent football players and non-sporting adolescents. All designations as in Figure 1.

The biochemical adaptation of organism to the muscle activity during the course of the training process extends to all functional systems related to the physical activity. Of course, this effect does not overlook the antioxidant system. The basic chemical mechanisms of adaptation changes in these systems and organs are identical to the biochemistry of adaptation changes that occur in the muscles (Meerson, 1985; Михайлов, 2016).

Strengthened energy processes in the body under the influence of intensified physical loads increase the amount of oxygen consumption by the organism, and its transport from the cell membrane is intensified. At this point, part of the oxygen transported is oxidized by contacting the membrane's structural compounds. As a result, particles or radicals, called free radicals, form on the cell membrane. As these particles have unused energy and unconnected electrons, they increase their ability to react highly, removing electrons from adjacent particles, complementing their outer electron shell, and converting the substance into free radical carrier. As a result, free radicals stimulates the chain development. It should also be noted that under normal physiological conditions, free-radical oxidation of lipids in the cells occurs partly and is under the direct control of these regulatory systems (Alessio, 1993; Григорьева, 2003). Moderate doses of free radicals are involved in the regulation of biological membrane functions and the renewal of their chemical composition by LPO products (peroxide oxidation of lipids) of biological membranes. It is well known that activation of sympatoadrenal and hypothalamic - hypophysis - renal systems under the influence of external factors (including physical loads) stimulates increased LPO levels (Meerson, 1985; Григорьева, 2003; Михайлов, 2016)

The stress reactions that occur in response to physical stress are accompanied by the activation of various stress-limiting reactions. Stress-limiting metabolites include classical hormones, neuromediators, and various enzymes (superoxidismutase, catalase, glutathione peroxidase, α-amylase ets.) (Stoney, 1997; Еликов и др., 2017**)**.

Our results indicate that the biochemical analysis of saliva can characterize not only the level of training loads received by the athlete, but also assess the reserve possibilities of adaptation to future loads, as evidenced by the indicators of the enzymatic activity of both antioxidant and metabolic nature.

CONCLUSION

Thus, the analysis of the biochemical parameters of saliva in adolescent soccer players showed that the amount of first and second products of peroxide oxidation of lipids were not significantly reduced. This also indicates that the response to anxious reactions under the influence of physical exercise loads has been more economical. More precisely, the performance of LPO products is more important than the effect of different physical loads. In addition, increases in the activity of the antioxidant enzyme catalase and the α-amylase enzyme confirms the increased antioxidant component of non-specific protection in the organism of adolescents. Positive adaptive changes result from the stress-damaging effects of physical loads. It should be noted that the positive changes in the training process with the players are aimed to adapting the antioxidant system to their physical activity.

Determining the nature of adaptive changes in indicators of lipid peroxidation in saliva in response to physical exercise will be of significant practical importance, especially, in light of the demand for modern functional and laboratory diagnostics methods that allow fast and effective non-invasive, painless testing.

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Futbol məşğələlərinin yeniyetmələrin ağız suyunda lipidlərin peroksidləşmə məhsullarının miqdarına və fermentativ aktivliyə təsiri

S.A. Əliyev, İ.S. Əliyev

Azərbaycan Dövlət Bədən Tərbiyəsi və İdman Akademiyası

Məqalə futbol idmanı ilə məşğul olan 10-15 yaşlı yeniyetmələrdə lipidlərin peroksidləşməsi proseslərinin adaptasiya dəyişikliklərində müntəzəm fiziki yüklənmələrin rolunun və orqanizmin antioksidant reaksiyasının öyrənilməsinə həsr olunmuşdur. Lipidlərin peroksidləşmə məhsullarının (heptanda və izopropanolda həll olunan məhsulların) ölçülməsi və təhlili 10-11, 12-13 və 14-15 yaşlı yeniyetmələrdən ibarət qrupları üzrə idmançıların ağız suyunda aparılmışdır. Alınan məlumatlar göstərir ki, ağız suyunun biokimyəvi analizi yalnız idmançının əldə etdiyi məşq yüklərinin səviyyəsini xarakterizə etməyə deyil, həm də gələcək yüklənmələrə adaptasiyanın ehtiyat imkanlarını qiymətləndirməyə imkan verir. Fiziki yüklənməyə cavab olaraq ağız suyunda lipidlərin peroksidləşmə göstəricilərində adaptiv dəyişikliklərin xarakterinin müəyyən edilməsinin, bugün qeyri-invaziv, ağrısız testlər aparılmasını operativ və effektiv həyata keçirməyə imkan verən funksional və laborator diaqnostikanın müasir metodlarına yüksək tələbatın olması baxımından müəyyən praktik (diaqnostik) əhəmiyyətə malik olması şübhə doğurmur.

Açar sözlər: *Yeniyetmə futbolçular, fiziki məşq yükləri, ağız suyu, lipid peroksidləşməsi, fermentativ aktivlik*

Влияние футбольных тренировок на уровень продуктов перекисного окисления липидов и ферментативную активность в слюне подростков

И.С. Алиев, С.А. Алиев

Азербайджанская государственная академия физической культуры и спорта

Работа посвящена исследованию роли регулярных физических нагрузок в адаптивных изменениях процесса перекисного окисления липидов и антиоксидантной реакции организма у 10-15 летних подростков, занимающихся футболом. Были проведены измерения и анализ продуктов перекисного окисления липидов в слюне спортсменов в различных возрастных группах 10-11, 12-13 и 14-

15 лет. Полученные данные указывают на то, что биохимический анализ слюны может характеризовать не только уровень тренировочных нагрузок, получаемых спортсменом, но и оценить резервные возможности адаптации к будущим нагрузкам, о чем свидетельствуют показатели ферментативной активности слюны, измеренной для антиоксидантного фермента каталазы и метаболического фермента α-амилазы. Установление характера адаптивных изменений показателей ПОЛ в слюне в ответ на физические нагрузки имеет практическое (диагностическое) значение, особенно, в свете востребованности в современной функциональной и лабораторной диагностике методов, позволяющих быстро и эффективно проводить неинвазивные, безболезненные тестирования.

Ключевые слова: *Футболисты подросткового возраста, физические тренировочные нагрузки, слюна, перекисное окисление липидов, ферментативная активность*