RESEARCH ARTICLE

Effects of free radicals induced by football exercise on the structure of biomembrane

Xiaofan Fan*

Sports Department, Jilin Technology College of Electronic Information, Jilin 132021, Jilin, China

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In order to reduce the structural abnormalities and dysfunction of biological membranes (cell membranes and mitochondrial membranes) caused by excessive free radical production in animals and human under stress, and to avoid the occurrence of diseases, this study investigated the effects of free radicals on the structure of biofilms. The purpose of this study was to investigate the effects of free radicals induced by football exercise on the structure of biomembrane. However, due to the difficulty in controlling football exercise in experimental animals, swimming exercise that enhanced cardiovascular function and overall muscle strength with a focus mainly on the ankle, which was similar to football, was employed as a substitute for football exercise. A total of 30 rats were raised as research subjects. Superoxide dismutase (SOD), malondialdehyde (MDA), and other indicators were detected. The results showed that, compared with the control group, the MDA content in the muscle cell membrane of the high-load training group increased by 0.92 nmol/mg. The membrane fluorescence polarization, microviscosity, and fluorescence anisotropy were significantly higher than those of the control group ($P < 0.05$). The levels of mitochondrial adenosine triphosphate in the high load training group were higher than those in the control group and the low load training group ($P < 0.05$). The MDA content of red blood cell membrane was positively correlated with the fluorescence polarization (P), microviscosity (ŋ), and fluorescence anisotropy (r) with the correlation coefficients of 0.726, 0.737, and 0.719, respectively. The ATP content in the muscle cell membrane of the light load training group was 0.0575 mg, which was higher than that of the control group, while the ATP content of muscle cell membrane in the high-load training group was 0.0198 mg, which was lower than that in the control group. The experimental results showed that appropriate training could improve the antioxidant capacity of skeletal muscle cell membrane and the fluidity of membrane lipids. Proper training could maintain a reasonable level of free radicals and prevent damage to cell membranes and mitochondrial membrane structures.

Keywords: football exercise; free radicals; band structure; SOD activity; membranes; mitochondria; bioactive.

***Corresponding author:** Xiaofan Fan, Sports Department, Jilin Technology College of Electronic Information, Jilin 132021, Jilin, China. Email: 15688900345@163.com.

Introduction

The free radical theory was proposed by Harman in 1956 [1, 2]. People believe that free radicals can exist independently in organisms and react with various living macromolecules in the body, causing tissue and cell damage, which is also the fundamental cause of aging in organisms. With the deepening and breakthrough of free radical research, Dillard introduced the theory and technology of free radical biomedicine into the field of sports medicine for the first time in 1978, promoting the development of sports medicine, enabling people to understand and study sports

injury, fatigue, and sports nutrition issues at the submolecular level, especially in prevention, recovery, and maintenance of sports fatigue in sports training [3]. The importance of reasonable nutritional matching is increasingly being valued by people. Cell membrane, also known as plasma membrane, is a membrane structure located on the periphery of protoplasts and near the cell wall. Its function is to protect the interior. Cell membrane is a semipermeable membrane mainly composed of lipids (mainly phospholipids), proteins, and sugars with a thickness of 7-8 nanometers. For animal cells, the outer side of the membrane contacts the external environment [4, 5]. The outer membrane of mitochondria is smooth, and the inner membrane folds into ridges. In the outer membrane of mitochondria, it surrounds the entire organelle. It contains a large number of integrated membrane proteins called channel proteins, which form channels and allow 5,000 Daltons or fewer molecules to freely diffuse from one side of the membrane to the other. If most of the subunits bound at the *N*-terminus of its signal sequence are referred to as translocation outer membrane and then actively move them through the membrane, the larger proteins can enter mitochondria. The main function of cell membrane is to selectively exchange substances, absorb nutrients, discharge metabolic wastes, secrete and transport proteins. Cell membranes have important physiological functions, not only maintaining stable metabolism of the intracellular environment, but also regulating and selecting substances both inside and outside of the cell [6, 7]. The cell membrane absorbs, digests, and discharges substances inside and outside the cell membrane through pinocytosis, phagocytosis, or exocytosis. The plasma membrane also plays an important role in cell recognition, signal transduction, cellulose synthesis, and microfiber assembly. In mitochondria, promoter proteins are introduced through specific translocation complexes [8]. The outer membrane also contains enzymes related to the extension of various active fatty acids, oxidative adrenaline, and tryptophan degradation. These enzymes include monoamine oxidase, rotenone insensitive NADH cytochrome C conduction enzyme, canine urine ammonia hydroxylase, and fatty acid coenzyme A ligase.

Free radicals, also known as reactive oxygen species (ROS), are substances with high activity that have one or more unpaired electrons in their outer orbitals. Free radicals are intermediate products of aerobic metabolism and electron transfer chains in the body, catalyzed by enzyme systems. They are also intermediate products of certain chemical damage processes in biological tissues or cells [9-10]. It can directly or indirectly act as a strong oxidant in the body, damaging large molecules and many cellular components of the organism. It can also react with other substances such as nucleic acid, homemade eggs, lipids, *etc*. to produce oxides or peroxides of these substances, causing damage to the organism. Free radicals include superoxide anion radical (O^{2-}), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), singlet oxygen radical (O_2), hydrogen peroxide radical (HOO), peroxide radical (ROO), nitric oxide radical (NO), semiquinone radical (QH), peroxynitrite (ONOO-); alkoxy group (RO); lipoxy (LO), and lipoxy free radicals (LOO). Malondialdehyde (MDA) is an intermediate product of lipid peroxidation, a representative product of lipid peroxides, and a sensitive indicator for measuring free radical metabolism. When the ROS level in mitochondria is too high, it will oxidize the lipids, proteins, and other components on the mitochondrial membrane, leading to mitochondrial structural damage and affecting its function. Exhausted football activities often lead to sports injuries and fatigue, which is a common concern in the scientific and medical communities. However, the specific mechanism is still unclear. This study focuses on the cell membrane and mitochondrial membrane of biofilms to explore the effects of free radicals on the structure of biofilms induced by football exercises. In the study of the effect of free radicals on cell membranes, indicators including Na^+- K^+- ATPase, MDA, SOD, and lipid fluidity of erythrocyte membranes were measured.

Materials and Methods

Experimental animals

Thirty (30) male healthy 2-month-old Wistar rats were provided by the experimental animal center of Lanzhou University Medical College, Lanzhou, Gansu, China with average body weight of about 130 g. All animals were raised in separate cages with 5 rats in each cage. The animals were fed with national standard feed and free to eat and drink water. The animal room temperature was 23±2°C with the relative humidity of 50±10% and natural lighting. The room and utensils were sterilized once a week with ultraviolet lamp.

Establishment of animal sports models

Due to the difficulty in controlling football exercise in animal experiments, swimming exercise that enhanced cardiovascular function and overall muscle strength with a focus mainly on the ankle, which was similar to football exercise, was chosen as a substitute research method for football exercise. The experimental animals were randomly divided into three groups according to their weights as control group, small load swimming training group (L), and large load swimming training group (H) with 10 rats in each group. The animals in L group were trained 6 days a week, once a day for 4 weeks. In the first week, the animals swam 30 minutes a day, and then 1 hour on the first weekend. On the second week, animals swam 1 hour a day, and 1.5 hours at the second weekend. On the third week, animals swam 1.5 hours per day and 2 hours on the third weekend. And then, the animals swam 2 hours a day until the fourth weekend. For the animals in group H, they swam 1 h on the first day and then 90 min every day to the first weekend. In the second week, animals swam 2 h every day. In the third week, animals swam 2 h per day with 1% of the weight load on the tail. In the fourth week, animals swam 2 h daily with 3% of the weight load on the tail. The training load was determined by measuring the exhausted swimming time with a preset time of 3 hours and a fluctuation range within 15 minutes. 80% and 50% of the values were preset as the load of the first week in the large and small load training groups. The blood lactate values of each group were measured immediately before and after the first load.

Preparation of cell membrane of skeletal muscle

48 h after the training, the gastrocnemius muscles of both hind limbs of rats were separated rapidly. According to Klip's method, after washing in ice cold physiological saline, removing fat, blood vessel, nerve, and connective tissues, 2 g of muscle tissues were obtained and were immersed in 50 M ice cold Tris·HCl solution (pH 7.4) in a ratio of 1:10 for homogenization. The homogenate was then centrifuged at 2,500 rpm for 10 mins at 4°C. The supernatant was collected and centrifuged at 5,000 rpm for 10 more mins at 4°C. After that, the supernatant was centrifuged again at 14,000 rpm for 6 min at 4°C before discarded. The precipitate was collected and resuspended with 50 mmol Tris·HCl. Additional centrifugation was carried out at 14,000 rpm for 60 mins at 4°C. The precipitate was taken and then resuspended and stored at -20°C. The extracted membrane protein was determined by Coomassie brilliant blue staining method.

Cell membrane index determination

(1) Na^+-K^+-ATPase

The measurement of Na^+-K^+-ATPase in skeletal muscle cell membrane was done by using ATPase kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China) and colorimetry following the manufacturer's instruction.

(2) MDA

The membrane MDA was determined by using MDA measurement kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China) and colorimetry technology. Briefly, 1 ml of experimental animal blood was placed in an anticoagulation tube and centrifuged at 700 rpm for 10 minutes at 4°C. The upper layer yellow color liquid phase was collected. 10 µl of the supernatant was applied for MDA measurement following the manufacturers' instruction.

(3) SOD

The method of monohydroxytryptamine was used for the determination of membrane SOD enzyme. Briefly, 0.5 g of liver tissue was homogenized with 2.5 mL of PBS. After homogenization, an additional 2.5 mL of PBS was added and mixed well before centrifugation at 10,000 rpm for 15 min at 4°C. The supernatant was the crude enzyme solution. A portion of the supernatant was appropriately diluted and used for enzyme activity determination by using SOD kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China) following the manufacturer's instructions.

(4) Lipids fluidity of erythrocyte membranes

Orlov method was employed to prepare erythrocyte membrane. The blood samples were centrifuged at 1,500 rpm for 10 min at 4°C. The plasma was removed, and the erythrocytes were washed repeatedly with physiological saline and centrifuged again until the red blood cells were washed thoroughly. The membranes of erythrocytes were broken by adding equal amount of distilled water, centrifuged at 10,000 rpm at 4°C, and washed repeatedly until the milky white color erythrocyte membrane was obtained. 1, 6-dipheny l-1,3,5-hexat riene, 1,6-diphenyl-i-1,3,5-hexatreene (DPH) (Sigma, Saint Louis, Missouri, USA) was used to prepare fluorescence probe. 23 mg of DPH was dissolved with 5 mL of tetrahydrofuran, and then diluted with PBS to 1,000 times. 25 mL of dilution was added to 100 µl of red blood cell membrane sample and kept at 30°C for one hour. The fluidity of erythrocyte membrane lipids was determined by using the factors of fluorescence polarization (P), microviscosity (ŋ), and fluorescence anisotropy (r) measured by DPH fluorescence polarization method using Hitachi 850 Fluorescence Spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). The excitation wavelength (Ex) and emission wavelength (Em) were 360 nm and 430 nm, respectively. The excitation slit and emission slit width were 5 nm and 10 nm, respectively. The fluorescence polarization (P) and microviscosity (ŋ) were calculated according to the following formula:

$$P = \left(I_{VV} - GI_{VV}\right) / \left(I_{VH} - GI_{VH}\right) \quad (1)$$

$$\eta = 2P\left(0.46 - P\right) \quad (2)$$

where I_{VV} and I_{VH} were fluorescence intensity of the polarizing axis in the vertical direction (V) and the polarizer optical axis in the vertical direction (V) or the horizontal direction (H), respectively. *G* was the correction coefficient and equaled to I_{HV} / I_{HH}, where I_{HV} and I_{HH} were the fluorescence intensity of the polarizing axis in the horizontal direction (H) and the polarizer optical axis in the vertical direction (V) or the horizontal direction (H), respectively. The larger the fluorescence polarization (P), the greater the microviscosity (ŋ), and the smaller the membrane fluidity, on the contrary, the greater the membrane fluidity.

Isolation of mitochondria from animal liver

Mitochondria were isolated from rat liver by conventional differential centrifugation method. The rats in each group were sacrificed by cervical dislocation and the liver was quickly excised. Briefly, the liver tissues were cut into pieces by scissors on the ice and then suspended in mannitol buffer before ground by homogenizer. After centrifugation for 10 minutes, the supernatant was discarded. About 30 mL of PBS was applied to resuspend pellet and then precipitated. The step for homogenization was repeated one more time before centrifugation again for 15 minutes. The pink part on the surface of the dark precipitate was removed and about 10 mL of PBS was added to the tube and mixed well.

Detection of ATP and ROS in Liver

The ATP and ROS contents in liver were detected by using ATP and ROS test kits (Beijing Baiaoleibo Technology, Beijing, China) following manufacturer's instructions. The ATP content in the sample was calculated according to the standard curve, and the intracellular ROS concentration was determined by using NovoCell

2060R Flowcytometry (Essen BioScience, Inc., Ann Arbor, Michigan, USA). All experiments were repeated three times.

Determination of lipid peroxide in rat liver mitochondria

Malondialdehyde (MDA) was used to reflect the content of lipid peroxide, and tetraethoxypropane was used as the standard control. The samples of each rat liver mitochondria group were incubated with the detection reagent of MDA measurement kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, Jiangsu, China) at room temperature for 60 mins. The products after reaction were determined by using DR3900 spectrophotometer (Hach, Loveland, Colorado, USA), and the content of MDA in each sample was obtained by comparing with the standard curve.

Determination of mitochondrial respiratory chain enzyme complex activity

The activities of mitochondrial respiratory chain enzyme complex I to IV were determined by using spectrophotometry. About 10-20 μg of mitochondrial protein was added into 2 mL of buffer solution with the distilled water as blank tube. The absorbance was measured at 340, 550, and 600 nm, respectively.

Statistical analysis

SPSS 18.0 software (IBM, Armonk, New York, USA) was used for statistical analysis. The experimental results were all expressed as mean ± SD. Student t test was employed to detect the difference between the groups with $P < 0.05$ as significant difference and $P < 0.01$ as very significant difference. The overall comparison among independent groups was performed by using one-way ANOVA, while the multiple comparisons among groups employed LSD-t test.

Results

Blood lactate values in different load training

The blood values before exercise were 1.23 ± 027 mmol/L and 1.44 ± 0.35 mmol/L in groups L and H, respectively, while the blood values immediately after exercise were 3.32 ± 0.55 mmol/L and 5.62 ± 0.62 mmol/L in groups L and H, respectively. The level of blood lactate after exercise increased by 2.09 mmol/L and 4.18 mmol/L in groups L and H, respectively, which could be considered as the results of different load training.

Free radical metabolism of skeletal muscle cell membrane

The results for free radical metabolisms on skeletal muscle cell membrane were shown in Table 1. The Na^+-K^+-ATPase activity on the membrane in the small load training group (L) demonstrated a very significant increase comparing to that in the control group ($P < 0.01$). After four weeks of large load training (H), the Na^+-K^+-ATPase activity on the membrane decreased very significantly compared with that in the small load training group ($P < 0.01$). The MDA results showed that, compared with the control group, the MDA content of the muscle cell membrane in the L group was significantly reduced by 2.48 nmol/mg, while that in the H group increased by 0.92 nmol/mg compared to that in the control group. The SOD enzyme activity of the muscle cell membrane in the L group was significantly increased by 27.08 U/mg, while it in the H group was reduced by 3.62 U/mg. The ATP content in the muscle cell membrane of the L group was 0.0575 mg higher than that in the control group, while that in the H group was 0.0198 mg less than that in the control group.

Analysis of membrane fluidity and MDA content of erythrocytes

The lipid peroxidation product, MDA, on erythrocyte membrane of both exercise groups was significantly higher than that in control group ($P < 0.01$), which indicated that lipid peroxidation reaction on erythrocyte membrane was strengthened after exercise. Because of the different fluidity of membrane lipid, the three factors, P, ŋ, and r of erythrocyte membrane were different. The larger the three factors, the

Table 1. Effects of different load exercise on SOD, MDA, Na^+-K^+-ATPase of skeletal muscle cell membrane.

Free radical metabolism index	Control group	Small load training	Heavy training	*P*
SOD (U/mg)	75.26	102.34	71.64	< 0.01
MDA (nmol/mg)	8.74	6.26	9.66	< 0.01
Na^+-K^+-ATPase (mg)	0.1668	0.2243	0.1470	< 0.01

Table 2. Changes of membrane lipid fluidity and MDA content of erythrocytes after different load exercises.

Free radical metabolism index	Control group	Small load training	Heavy load training
Fluorescence polarization	0.237	0.246	0.285
Microviscosity	2.366	2.981	3.726
Fluorescence anisotropy	0.158	0.203	0.235

Table 3. Comparison of ATP, SOD, and lipid peroxide (LPO) levels of liver mitochondria in three groups.

Mitochondrial index	Control group	Small load training	Heavy load training
ATP (mg)	509	2,160	8,310
ROS	249	540	769
LPO (kda)	4.75	4.88	5.31

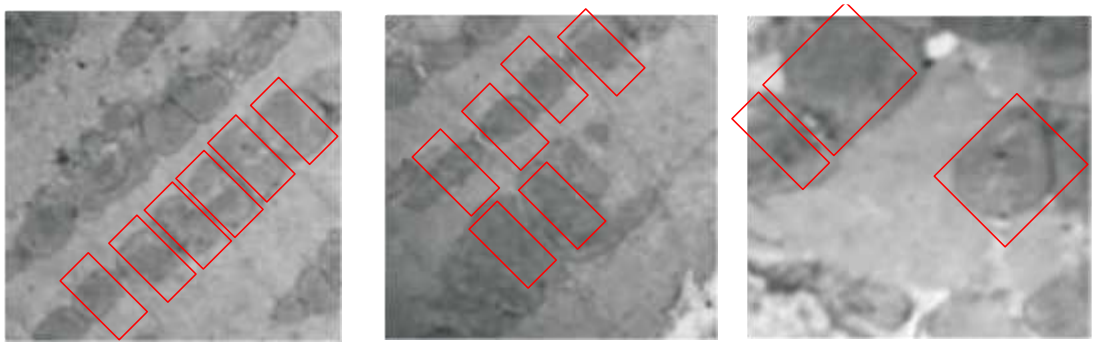
Table 4. Overall comparison and multiple comparison of ATP, SOD, and lipid peroxide (LPO) in rat liver mitochondria.

Category	Multiple groups comparisons (*P* value)		
	Small load group vs. control	Large load group vs. control	Large load group vs. small load
ATP (mg)	0.0021	0.002	0.004
SOD (U/mg)	0.0034	0.0042	0.0028
LPO (kda)	0.001	0.004	0.003

smaller the fluidity and the worse the deformability of erythrocyte membrane lipid. The results showed that the three factors of erythrocyte membrane in both exercise groups were significantly higher than that in the control group ($P < 0.05$), and the difference between ŋ and r in the H group was also very significant ($P < 0.01$), indicating that the fluidity of erythrocyte membrane lipid and the deformability of erythrocyte decreased in the H group (Table 2). The correlation analysis showed that the MDA content on the erythrocyte membrane was positively correlated with P, ŋ, and r, and the correlation coefficients were 0.726, 0.737, and 0.719, respectively, which indicated that the MDA content on the erythrocyte membrane was negatively correlated with the fluidity of the erythrocyte membrane.

Mitochondrial membrane index and ultra-structural analysis

The ATP level of liver mitochondria in the heavy load training group (H) was higher than that in the control group and the small load training group (L). The concentration of lipid peroxide (LPO) and ROS in rat liver mitochondria in H group were significantly higher than that in other groups, but within a reasonable range. The overall comparison results among the three groups and between every two groups were all statistically significant (Tables 3 and 4). The structure of mitochondrial membrane in different groups was shown in Figure 1. As shown in Table 3, in the comparison of the ATP, ROS, and LPO contents in the rat liver mitochondria of the three groups, the H group was the highest. The results of statistical analysis showed that the



(a) Heavy load training group (b) Small load training group (c) Control group

Figure 1. Ultrastructure of liver mitochondria in different groups of rats.

Table 5. Comparison of activities of mitochondrial respiratory chain complex I- IV in different groups of rats.

Complex I-IV activities	Control group	Small load training	Heavy load training
Complex I activity (IU)	0.686	0.663	0.700
Complex II activity (IU)	0.069	0.073	0.074
Complex III activity (IU)	0.276	0.304	0.234
Complex IV activity (IU)	0.231	0.223	0.307

comparison of the ATP contents in the liver mitochondria of both L group and the control group demonstrated the *P* value as 0.007, which indicated a significant difference between the two groups with the confidential probability of 99.3%. The *P* values of each group comparison in Table 4 were all less than 0.005, which could be considered that there were very significant differences in the results of the comparison between the compared two groups ($P < 0.01$). The morphology results demonstrated that the arrangement of mitochondria in the liver of H group was orderly with complete mitochondrial membrane, while the mitochondria in L group showed basically complete membrane with relatively scattered arrangement and low uniformity. The inner chamber of mitochondria in the liver in the control group was slightly swollen, and the membrane was not broken.

Effects of different hypoxia training on complex I-IV activity of mitochondrial respiratory chain

Compared with the control group, there was no significant change in complex I and II activities of liver mitochondrial respiratory chain in the heavy load training group. However, it did show certain increasing trends of about 2.04% and 7.24%, respectively. The activity of complex III was decreased by 15.21% ($P < 0.05$), while the activity of complex IV was increased by 27.27% ($P < 0.05$). There was no significant change being observed in complex I and II activities of liver mitochondrial respiratory chain in the small load training group (Table 5).

Effects of different hypoxia training on SOD activity and MDA content of liver mitochondria

Compared with the control group, there was no significant difference in SOD activity of liver mitochondria between the H group and the L group. However, the MDA contents were significantly reduced by 28.50% and 24.18%, respectively ($P < 0.05$) (Table 6).

Discussion

Effects of different training intensity on SOD, MDA, Na^+-K^+-ATPase free radical metabolism of skeletal muscle cell membrane *in vivo*

Under normal circumstances, the generation and elimination of free radicals in the body maintain

Table 6. Comparison of SOD activity and MDA content of liver mitochondria after exercise under different load training.

Grouping	Control group	Small load training group	Heavy load training group
SOD(U/mg)	22.200	19.6203	23.410
MDA content (nmol/mg)	20.140	15.270	14.400

a dynamic balance and will not cause damage to the body. This study showed that, in the process of high-intensity experimental animal swimming exercise, which was equivalent to football exercise, the oxidation metabolism was strengthened, the oxygen consumption of the body was increased, and the production of free radicals in the body was also increased. When the generation of free radicals is increased or the scavenging ability of free radicals is decreased, the accumulated oxygen free radicals will directly attack the polyunsaturated fatty acids in the biofilm system, and lead to the lipid peroxidation reaction, producing the metabolite malondialdehyde (MDA), which is a kind of active crosslinking agent that can enter into the membrane and cross-linked with membrane protein and amino groups on the membrane to form Shiff's base, therefore, reduces the fluidity and increases the rigidity of the membrane, and hinders the fluidity and permeability of the membrane and affects the normal function of the membrane [11]. Jenkins studied the rat and human skeletal muscles and found that the peak of intracellular free radical signal increased by 70% due to over contraction of muscle. Chen, *et al*. found that the free radical signal of white muscle increased significantly after high-intensity football exercise, and there was a characteristic free radical peak positively related to fatigue degree. In this study, it was also found that MDA content in heavy load exercise group increased significantly [12, 13]. As for the mechanisms of lipid peroxidation of skeletal muscle cell membrane caused by the increase of free radical production during the exercise, it might be attributed to: (1) during exercise, the oxygen consumption of the body was increased, and the respiratory function of muscle mitochondria was enhanced. Through the redox cycle of coenzyme Q in mitochondrial respiratory chain, the generation of O_2 was increased. (2) Ischemia-reperfusion. During the exercise, the blood in the body was redistributed. After the blood flow was temporarily supplied by hemostatic flow, reperfusion could induce the oxidation of xanthine to produce O_2. Xanthine oxidation is one of the important factors for the formation of free radicals in the body and the peroxidation damage of skeletal muscle. (3) Catechol could also self-oxidize to form oxygen free radicals. (4) The aerobic level was low, and the exercise was more intensive.

The production of free radicals is very important for improving the body's ability to football exercise. In this study, combined with the measured MDA contents, it was found that, after four weeks of swimming endurance training, the SOD activity of the skeletal muscle cell membrane of the L group was significantly increased by 27.08 U/mg, while the MDA content was significantly reduced by 2.48 nmol/mg, which was comparable to other related animal and human studies. On the other hand, the SOD activity on the skeletal muscle cell membrane of group H decreased by 3.62 U/mg, while the MDA content increased by 0.92 nmol/mg, which indicated that, after appropriate endurance training, the defense system of free radicals on the membrane of skeletal muscle cells could be strengthened, while the anti-oxygen system of the membrane of skeletal muscle cells could be improved if the training was over a certain load. Chemical defense system has certain damage effects [14]. Compared with other tissues, the change of antioxidant capacity caused by football exercise training was the most obvious in muscle, which may be due to the lower level of basic antioxidant enzyme activity and/or more free radicals produced in football exercise. However, after appropriate load training, the body will

produce adaptive changes, which is because a higher level of free radicals will stimulate the increase of free radical scavenging enzymes, and the body will maintain a higher ability of free radical scavenging, so that the increase of free radicals will not cause damage to the cell membrane of skeletal muscle [15]. However, this adaptive change of the body is only relative.

Na^+-K^+-ATPase, also known as sodium pump, is an intrinsic protein widely existing in eukaryotic cell membrane, an enzyme requiring phospholipid to maintain its activity, and an important ion carrier on cell membrane. Some studies showed that the cell membrane allowed a small amount of Na^+ to enter the membrane continuously when it was quiet, and the amount of Na^+ in the cell could be doubled in one hour. Therefore, there must be a mechanism on the membrane of muscle cells to pump Na^+ out of the leaking cells and absorb K^+ out of the cells at the same time. This process depends on the hydrolysis of ATP by Na^+-K^+-ATPase on muscle cell membrane. The results of this study showed that the effect of the exercise on Na^+-K^+-ATPase activity varied with the exercise loads. After four weeks of training, Na^+-K^+-ATPase activity in the small load group was significantly higher than that in the control group. However, in the large load group, Na^+-K^+-ATPase activity was significantly higher than that in the small load group (Table 1), indicating that the appropriate load training could improve the working ability of Na^+-K^+-ATPase on the muscle cell membrane, enhance the ability of the skeletal muscle cell membrane to maintain the distribution of ions, weak the trend of depolarization of resting potential and decline of action potential, and prolong the exercise time [16, 17].

Effects of different training intensity on membrane fluidity and MDA content of erythrocytes *in vivo*

The fluidity of erythrocyte membrane lipids is an important factor to determine erythrocyte deformability and a necessary condition to maintain effective microcirculation perfusion. The decrease of erythrocyte membrane lipids fluidity may participate in the occurrence of football exercise-induced fatigue. There are many possible reasons for the decrease of erythrocyte membrane fluidity caused by exhausted football exercise. Through this study, it was found that the increase of membrane lipid peroxidation during the exercise might be an important reason for the decrease of membrane lipid fluidity, which could be concluded from the significant negative correlation between MDA content and membrane fluidity. Hemoglobin interaction in normal people is also a source of free radicals. In addition, lipid peroxides from different tissues and organs in plasma also have toxic effects on erythrocytes [18]. However, there are a whole set of enzyme and non-enzyme antioxidants in the body that can scavenge free radicals. Under normal circumstances, the production and scavenging of free radicals are in a balanced state [19]. During long-term vigorous football exercise, energy metabolism and oxygen consumption of tissues and organs are increased about 20-40 times of that in resting time. The activity frequency and quantity of hemoglobin transporting oxygen molecules in erythrocytes are increased, and the production of free radicals is also significantly increased, so that a large number of free radicals cannot be removed in time, thus causing damage to the membrane of erythrocytes.

Effects of different training intensity on ATP, SOD, and lipid peroxide of mitochondrial membrane *in vivo*

The results of this study were consistent with previous studies that the exercise training could improve the ability of rat liver mitochondria to produce ATP and the level of total ROS in cells. As a special organelle, mitochondria can not only produce ATP through oxidative acidification to provide energy for the body, but also produce ROS under abnormal conditions. The results showed that ROS produced by proper training did not damage the structure and function of mitochondria, so the ability of ATP production was not affected. However, the exhaustive exercise caused ROS accumulation in rats, especially in the liver, which would cause lipid

oxidation of membrane components, affect the production of energy, and harm to the body [20]. Biomembrane, especially mitochondrial membrane, is an important part of cell function, and the lipids, especially unsaturated lipids, are necessary for cell function [21]. In this study, malondialdehyde level was used as the content of peroxidized lipids to speculate the concentration of oxidized lipids in rat liver mitochondria. The results demonstrated clinical significance for sports training.

The effect of different training intensity on the activity of mitochondrial respiratory chain enzyme complex *in vivo*

Mitochondria are the place where eukaryotic cells provide energy for oxidative phosphorylation. Oxidative phosphorylation is mainly carried out on the inner part of mitochondria. The components directly related to energy conversion on the inner part of mitochondria can be divided into two categories. The first is ATPase complex (energy exchange device), also known as f1-f0 coupling factor, which is a complex of various phthalides that plays a role in the synthesis of ATP. The second is the respiratory chain (energy releasing device) [22], which is composed of many enzymes and molecules bound to the inner membrane and taken on the role of electron transfer, so it is also called electron transfer chain. The mitochondrial electron transport chain is composed of several multi-molecular complexes, among which complex I (NADH-CoQreductase), complex II (succinic acid CoQreductase), complex III (CoQ cytochrome c reductase), and complex IV (cytochrome oxidation) are the important components of the complex, and their activity changes can directly or indirectly reflect the changes of mitochondrial respiratory function. The role of electron transfer chain is to make the sugar, fat, and amino acid in aerobic cells pass through their respective decomposition pathways, forming the reduced coenzyme including NADH and FADH2. Under the action of complex I or complex II, the electron is transferred to complex III through CoQ, then to complex IV through cytochrome C, and finally to reduce oxygen to generate water. At the same time of electron transfer, the oxidation potential energy (EH) of substrate (NADH or succinic acid) is converted to the proton electrochemical gradient potential energy (μH^+) by the proton pump action of complexes I, III, and IV, and the latter is converted to the higher phosphate bond energy (GP) of ATP by the complex V (H^+-ATPase). The free energy released by the oxidation-reduction reaction catalyzed by complex II is not enough to form ATP [23]. Its function is to remove the electrons of FADH2 and incorporate them into the electron transfer chain. The change of the enzyme activity of the transport chain complex will affect the synthesis of ATP. The results of this study showed that the blood flow and function of the whole right ventricle increased significantly during hypoxic acclimation. After 15 days of hypoxia, the capillary density of the right ventricle did not change significantly, but the ratio of the number of capillaries per unit volume to the number of muscle fiber cones (C/M) increased significantly. After 30 days of hypoxia, the capillary density and C/M ratio of the left ventricle increased significantly. The main effects of hypoxia treatment and exercise training on blood vessel volume density, surface area density, and length were different. Chronic intermittent hypoxia exposure could significantly increase the activities of Ca^{2+}, Mg^{2+}-ATPase and respiratory chain enzyme complexes I, II, and IV in rat liver mitochondria, so as to improve the function of mitochondrial respiratory chain, maintain the normal energy metabolism of liver, and finally improve the systolic and diastolic function of heart.

The results showed that immediately after exhaustive exercise, the activity of respiratory chain enzyme complex IV in liver mitochondria of heavy load training group was significantly increased, while the activities of complexes I-IV in the small load training group had no significant change. The results indicated that the high load training group could improve the activity of some enzymes in the respiratory chain of liver mitochondria and promote the activity of

complex IV, which was related to the training mode and might also be related to the lower MDA content in liver tissue and mitochondria and the less damage of membrane lipid bilayer in the heavy load training group [24, 25]. Under the same experimental conditions, the activity of complex II was easily damaged by exercise. The mechanism of ROS might be related to the increase of ROS production due to the mitochondrial complexes being the main sites of ROS production, which was very vulnerable to free radical damage. The results of this study showed that the activities of complexes I-IV in the respiratory chain of rat liver mitochondria needed 3 weeks in the process of acclimation in the small load training group with the activities of complexes I, II, and IV increased significantly in the fourth week, while the activity of complex III had no significant effect. The results were different to that in the small load training group that showed no significant changes in complexes I, III, and IV activities. The possible explanation was that it might be related to the degree of hypoxia, exercise intensity, exercise mode, and control group used in the experiment. In the process of hypoxic training, the body undergoes the double stimulation of hypoxia and exercise, which produces a strong stress response to mobilize the body's functional potential and produce a series of anti-hypoxia physiological reactions that are conducive to improving the exercise ability. In order to fully mobilize the body's functional potential, hypoxia training methods are constantly updated and improved, and a variety of hypoxia training methods are proposed [26, 27]. With the deepening of the research, it was found that different hypoxic training methods had their negative effects such as heavy load training was not conducive to protein synthesis, muscle working ability decline, and hard to increase the training load, while the small load training was not conducive to the elimination of exercise-induced fatigue. Under the condition of football exercise training, the ischemia and anoxia of body tissue and the state 3 and state 4 respiration of mitochondria in cells are continuously alternating. Therefore, the electron leakage of mitochondrial respiratory chain is the main constant source of superoxide anion in cells. Immediately after exercise, the MDA content of liver mitochondria in high load training group and low load training group decreased significantly. There was no significant change of SOD activity in the heavy load training group and the small load training group. The results showed that the high load training group could reduce the MDA content of liver mitochondria more than that in the low load training group.

Conclusion

This study conducted different load training intensities on experimental rats. The changes in free radicals in the body caused by exercise further affected the structural indicators of the biofilm. This study mainly analyzed the changes in cell membrane and mitochondrial membrane structural indicators under different load training intensities. The results showed that the SOD activity of skeletal muscle cell membrane in group L significantly increased by 27.08 U/mg, while the MDA content significantly decreased by 2.48 nmol/mg, which was comparable to other related animal and human experiments. On the other hand, the SOD activity on the membrane of skeletal muscle cells in group H decreased by 3.62 U/mg, but the MDA content increased by 0.92 nmol/mg. Compared with the control group, there was no significant change in complexes I and II activities of liver mitochondrial respiratory chain in the heavy load training group. However, the results did show certain increasing trends of complexes I and II by 2.04% and 7.24%, respectively. The activity of complexes III was decreased by 15.21%, while complex IV increased by 27.27% ($P < 0.05$). There was no significant change in complexes I and II activities in liver mitochondrial respiratory chain in the small load training group. The cell membrane and mitochondrial membrane indicators after exercise indicated that appropriate training could stabilize the production of free radicals in the body, maintain good growth status of biofilms, and avoid internal diseases caused by biofilm

rupture and dysfunction. Generally speaking, football can cause changes in free radicals in the body and alter the structure of biofilms. However, there are still some problems in the development level of football and free radicals. First, in the study of football and free radicals, the application scope of research results is very small. Second, research on the effects of exercise nutrition on free radical scavenging is mostly qualitative, but less quantitative. Third, there are many mechanisms by which football generates free radicals. Fourth, in the experiments on football and free radicals, there has been more detailed research on exhausted football and aerobic endurance football, while there has been less investigation on anaerobic football. Therefore, in future research, from the perspective of solving the above problems, further studies on the relationship between motion and free radicals were imperative to obtain more scientific and accurate research results.

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