Effects of Lycopene on Oxidative Damage and Mitochondrial Function in the Skeletal Muscle of Mice After Strenuous Exercise

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Abstract. The main purpose of this study is to investigate the effects of lycopene on oxidative damage and mitochondrial function in the skeletal muscle of mice after strenuous exercise. After the establishment of animal models of exercise-induced fatigue, the mice were randomly divided into four groups: quiet control group, exercise fatigue control group, Low-dose lycopene (20mg/kg) + exercise fatigue group, and high dose lycopene (60mg/kg) + exercise fatigue group. After four weeks, the mice were sacrificed and the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), free calcium-ion (Ca2+), citrate synthase (CS), values of state 3 respiration (S3) and state 4 respiration (S4) were measured. The results show that lycopene could increase the levels of SOD and GPx in muscle, as well as the levels of free calcium-ion, CS, and values of S4 and respiratory control ratio (RCR) in mitochondria, and decrease the MDA levels in muscle. Lycopene has protective effects against strenuous exercise-induced muscle damage and improves mitochondrial function.

1. Introduction

Many studies have shown that exercise is often associated with increased production of reactive oxygen species (ROS) in various tissues [1-3]. These ROS may exceed the resistance of the body's antioxidant defense system, and as a result they may increase oxidative stress. Excessive ROS would lead to chain reaction, resulting in damage to cell structure, lipid peroxidation, and muscle tissue, and finally occurrence of physical fatigue [4,5]. ROS is mainly derived from mitochondria, which consumes oxygen through oxidative phosphorylation, and 2%-3% of oxygen molecules are reduced to generate superoxide anions in this process [6]. Mitochondria are the regulatory center of life activities such as cell growth, proliferation, differentiation and apoptosis. The survival of cells depends largely on the functional state of mitochondria. Studies have shown that complex 1 in the electron transport chain releases superoxide to the mitochondrial matrix, while complex 3 releases superoxide to both sides of the inner membrane of mitochondria, which is considered to be the main site of ROS generation [7]. Mitochondria are not only the main source of ROS, but also its main target. ROS damage to mitochondria is mainly related to changes in respiratory chain enzymes, calcium ion overload, decreased mitochondrial membrane potential, overexpression of pro-apoptotic proteins, and opening of mitochondrialpermeability transition pore (mPTP). Several previous studies have shown that exogenous antioxidants supplementation could reduce exercise-induced oxidative stress by scavenging free radicals and destroying certain peroxides, thereby maintaining mitochondrial function and preventing skeletal muscle damage and the formation of lipid peroxides [8-10].

Lycopene belongs to the carotenoid family, widely found in various fruits and vegetables, especially in tomatoes. Lycopene is a highly unsaturated hydrocarbon compound that contains 11
conjugated and 2 unconjugated double bonds [11]. This structural feature gives lycopene a very strong antioxidant activity and it is by far the strongest antioxidant in nature of carotenoids, and its rate of scavenging superoxide anion can reach one hundred times that of vitamin E and about two times that of beta-carotene [12]. Numerous pharmacological studies have confirmed that lycopene has a variety of biological activities, such as anti-oxidation, anti-inflammatory, anti-cancer, anti-radiation, anti-coagulation, hypoglycemic, preventing macular degeneration and coronary heart disease, and eye protection [13-15]. To date, no toxic or side effects in humans and animal models have been found. However, there is little information on the effects of lycopene on strenuous exercise-induced muscle and mitochondrial damage. Therefore, this study was conducted to investigate whether supplementation with lycopene has any protective effects on muscle and mitochondrial damage induced by exercise using mice models.

2. Materials and methods

2.1 Materials and reagents
Lycopene (C40H56, purity>90%) was purchased from the Mansite Biotechnology Co., Ltd. (Chengdu, China) and stored in a dark place at 4°C. The commercial assay kits for blood lactic acid (BLA), blood urea nitrogen (BUN), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) were purchased from Jiancheng Biologic Project Co. (Nanjing, China). The commercial assay kit for citrate synthase (CS) was purchased from Meilian Biotechnology Co., Ltd. (Shanghai, China). The commercial assay kit for respiratory control ratio (RCR) was purchased from Jiemei Gene Medicine Technology Co., Ltd. (Shanghai, China). All other chemicals and solvents used in this study were of analytical grade and obtained from Hunan Reagent Company (Changsh, China). Laboratory water is deionized water.

2.2 Experimental animals
Healthy male Kun-Ming mice, weighing 18-22 g, were purchased from the Laboratory Animal Center of Hunan (Changsha, China). The mice were housed in separate cages in the animal room under controlled conditions with temperature maintained at 21°C to 25°C, and relative humidity maintained at 40% to 60% on a 12 h light: 12-h dark cycle (lights on from 6:00 to 18:00 h). The animals had free access to standard laboratory chow and tap water. The experimental procedures were in accordance with Guiding Opinions on Treating Experimental Animals issued by the Ministry of Science and Technology of China with the approval of the Institutional Animal Ethics Committee of Central South University.

2.3 Establishment of animal models of exercise-induced fatigue
The mice adapted to the experimental environment by feeding a standard chow for one week. After one week, the mice were divided into non-exercise control (n=10) and exercise (n=40) groups, and the mice in the exercise group were subjected to exercise on a rodent treadmill at a slope of 10%, speed of 15 m/min and duration of 15 min for two weeks. After two weeks of exercise, when the mice showed such behaviors as cold expression, sluggish response, decreased ability to escape and hindlimb kicking, they were stopped from training and blood samples were collected from the tail for the blood lactic acid and blood urea nitrogen analyses. If the results show a significant increase in blood lactic acid and blood urea nitrogen compared to the non-exercise control group, this indicates that the exercise-induced fatigue model was successfully established.

2.4 Exercise protocol
The mice were randomly divided into four groups of 8 mice each: quiet control group (QC), exercise fatigue control group (EC), low-dose lycopene + exercise fatigue group (LLE), and high dose lycopene + exercise fatigue group (HLE). The mice in QC group and EC group were intragastrically
administered with 0.5 ml corn oil, and the mice in LLE group and HLE group were administered with the same volume of lycopene and corn oil mixed solution at a dose of 20 mg/kg and 60 mg/kg. Lycopene or corn oil was daily given for the entire four-week period. The dose of lycopene used in this study was selected on the basis of previous studies.

2.5 Analysis of biochemical parameters
At the end of the experimental period, the mice were sacrificed by cervical dislocation, and the quadriceps muscles were immediately taken out, washed in physiological saline, blotted with filter paper and weighed. A part of the muscle tissue was made into 10% homogenate, centrifuged down, and the clear supernatant was used for the determination of the levels of SOD, GPx, and MDA. The other part of the muscle tissue and 10 times the volume of mitochondrial lysate are mixed and homogenized, and the undissolved cells and nucleus are removed by centrifugation, then the mitochondrial precipitate is obtained by centrifugation again. Finally, an appropriate amount of mitochondrial storage solution is added to dissolve the mitochondria, and stored at -80°C for determination of the levels of free calcium-ion, citrate synthase (CS), respiratory control ratio (RCR). The test method and calculation formula are strictly performed according to recommended procedures provided by the commercial diagnostic kits.

2.6 Statistical analysis
SPSS software is used for statistical analysis of experimental data. All data are expressed as mean ± SD, analyzed by using Analysis of Variance (ANOVA) and p values <0.05 are considered statistically significant.

3. Results and discussion
3.1 Effects of lycopene on the levels of SOD, GPx, and MDA in muscle of mice
Fig. 1 demonstrates the effect of lycopene on the levels of SOD, GPx, and MDA in muscle. The SOD levels of the EC and LLE groups, as well as the GPx levels of the EC, LLE and HLE groups were significantly lower than that of the QC group (p<0.05). Although the SOD levels of the HLE group also decreased, no significant difference was observed (p>0.05). The levels of SOD and GPx of the LLE and HLE groups were significantly higher than that of the EC group (p<0.05). The MDA levels of the EC, LLE and HLE groups were significantly higher than that of the QC group (p<0.05). The MDA levels of the LLE and HLE groups were significantly lower than that of the EC group (p<0.05).

SOD and GPx are the body's first defense system against excessive ROS produced by strenuous exercise. SOD can catalyze the disproportionation of superoxide radicals to oxygen and hydrogen peroxide, and GPX can reduce hydrogen peroxide or organic hydroperoxide to water and alcohol respectively. These endogenous antioxidant enzymes become weaker under fatigue conditions [16]. If they can increase the activities of SOD and GPx, it will help to eliminate excessive ROS and reduce oxidative stress. MDA is a lipid peroxidation product of free radical metabolism in the body, and MDA level indirectly reflects the severity of cells attacked by free radicals [17]. In this study, the data showed that lycopene can enhance antioxidant enzyme activities and reduce lipid peroxidation, which indicates that lycopene has protective effects on exercise-induced skeletal muscle damage.
Fig. 1. The effect of lycopene on the levels of SOD, GPx, and MDA in muscle of mice. Data are expressed as mean ± SD. *p<0.05 compared with QC group; #p<0.05 compared with EC group.

3.2 Effects of lycopene on the free calcium-ion levels in mitochondria of mice

Fig. 2 demonstrates the effect of lycopene on the free calcium-ion levels in mitochondria. The free Ca$^{2+}$ levels of the EC, LLE and HLE groups were significantly higher than that of the QC group (p<0.05). The free Ca$^{2+}$ levels of the LLE and HLE groups were significantly lower than that of the EC group (p<0.05).

Mitochondrion are important respiratory organs of cells, and also an important Ca$^{2+}$ reservoir for cells and plays an important role in maintaining cell function. Ca$^{2+}$ homeostasis disorder is one of the important causes of mitochondrial damage, and the irreversible changes in muscle cells are often closely related to the abnormal distribution of Ca$^{2+}$ in various cell structures [18]. It has been reported that after strenuous exercise, the generation of a large number of ROS leads to the opening of mitochondrial permeability transport channels, which releases a large amount of ca$^{2+}$, leading to an increase in the Ca$^{2+}$ content in the cell, resulting in calcium overload. A large amount of Ca$^{2+}$ accumulates in the mitochondria, inhibiting its own oxidative phosphorylation process, reducing ATP production and content, and then causing cellular Ca$^{2+}$ metabolism disorders. Metabolic disorders of Ca$^{2+}$ can reduce muscle contractility through muscle contracture, phosphatase A2, and inhibition of mitochondrial oxidative phosphorylation [19]. Therefore, metabolism disorder of Ca$^{2+}$ is an important mechanism leading to muscle and mitochondrial damage. In this study, the data showed that lycopene is an important antioxidant and can play a role in homeostasis regulation in the body. When the free Ca$^{2+}$ levels is higher than normal, lycopene can act as a Ca$^{2+}$ antagonist, reduce the intracellular
calcium ion level, and play a steady-state regulation role on it, thereby protecting muscle and mitochondrial damage.

3.3 Effects of lycopene on the CS levels in mitochondria of mice
Fig. 3 demonstrates the effect of lycopene on the CS levels in mitochondria. The CS levels of the EC, LLE and HLE groups were significantly lower than that of the QC group (p<0.05). The CS levels of the HLE group were significantly higher than that of the EC group (p<0.05). Although the CS levels of the LLE group also increased, no significant difference was observed (p>0.05).

Fig. 3. The effect of lycopene on the CS levels in mitochondria. Data are expressed as mean ± SD. *p<0.05 compared with QC group; #p<0.05 compared with EC group.

It has been reported that different types of exercise can change the quantity and quality of mitochondria. Among them, aerobic endurance exercise can increase the number and volume of skeletal muscle mitochondria, and one or repeated strenuous exercise can cause serious damage to the mitochondria and decrease the number [20]. CS catalyzes the first reaction of the tricarboxylic acid cycle (TCA cycle), which is considered to be the rate-limiting enzyme of the TCA cycle. It catalyzes the condensation of acetyl-CoA with oxaloacetate to produce citric acid and CoA, so its activity affects the entire oxidative metabolism of mitochondria process. CS has been used as an important indicator to reflect changes in the number of mitochondria [21]. In this study, the data showed that repeated strenuous exercise can significantly reduce the CS levels in mitochondria, reflecting that the mitochondrial functional state has decreased at this time, accompanied by a decrease in circulating levels. Lycopene can significantly increase the activity of citric acid synthase, indicating that lycopene can enhance the level of aerobic metabolism and promote the recovery of mitochondria.

3.4 Effects of lycopene on the values of S3, S4 and RCR in mitochondria of mice
Fig. 4 demonstrates the effect of lycopene on the values of S3, S4 and RCR in mitochondria. Compared with the QC group, the S4 values of the EC, LLE and HLE groups were significantly higher (p<0.05), and RCR values of the same groups were significantly lower (p<0.05). Compared with the EC group, the values of S4 and RCR of the LLE and HLE group were significantly higher (p<0.05). The S3 values were not significantly different of all groups (p>0.05).
**Fig. 4.** The effects of lycopene on the values of S3, S4 and RCR in mitochondria. Data are expressed as mean ± SD. *p<0.05 compared with QC group; #p<0.05 compared with EC group.

The mitochondrial respiratory chain is composed of a series of enzymes located on the inner membrane of the mitochondria, which has more than 15 kinds of components. It is mainly divided into two kinds of mobile electronic carriers and four kinds of enzyme complexes [22]. According to the mitochondrial respiration control mechanism, when the phosphate group and ADP are present in the matrix, the respiratory chain electrons can be transferred at the maximum rate in the complete mitochondria. In the absence of ADP, phosphorylation does not occur and is referred to as state 4 respiration (S4). If ADP is added to the system, the oxygen consumption increases sharply to the maximum, and ADP is phosphorylated to ATP, which is called state 3 respiration (S3) [23]. Respiratory control ratio (RCR) refers to the ratio of S3 to S4, which is a sensitive indicator for evaluating the integrity of mitochondria and the degree of oxidative phosphorylation [24]. In this study, the data showed that strenuous exercise leads to a significant increase in S4 values, but does not cause significant changes in S3 values, which results in a significant reduction in RCR values. Lycopene significantly inhibited the increase of S4 values in mice, and also relieved the decrease of RCR values, which indicate that lycopene protects strenuous exercise-induced muscle damage by affecting mitochondrial respiratory function.

**4. Conclusions**

The data demonstrates that lycopene could increase the levels of SOD and GPx in muscle, as well as the levels of free calcium-ion, CS, and the values of S4 and RCR in mitochondria, and decrease the MDA levels in muscle. These results suggest that lycopene has protective effects against strenuous exercise-induced muscle damage and improves mitochondrial function.

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**References**

[1] Y. F. Wei, H.H. Tao, J. Food Agric. Environ. 581, 11 (2013)
[2] D.D. Liu, X.W. Ji, R.W. Li, Iran. J. Pharm. Res. 115, 12 (2013)
[3] Y.F. Xu, Int. J. Med. Mushrooms 1083, 18 (2016)
[4] Z. Chen, S. Li, X. Wang, C.L. Zhang, Exp. Ther. Med. 5, 4 (2013)
[5] A.J. Niu, J.M. Wu, D.H. Yu, R. Wang, Int. J. Biol. Macromol. 447, 42 (2008)
[6] V. Adam-Vizi, C. Chinopoulos, Trends. Pharmacol. Sci. 639, 27 (2006)
[7] A.J. Lambert, M.D. Brand, Methods Mol. Biol. 165, 554 (2009)
[8] H.J. Yang, X.H. Zhang, Trop. J. Pharm. Res. 795, 16 (2017)
[9] B. Qi, L. Zhang, Z. Zhang, J. Ouyang, H. Huang, Pharmacogn. Mag. 458, 10 (2014)
[10] J.C. He, R.W. Li, H.Y. Zhu, Biomed. Res-India. 122, 28 (2017)
[11] K.K.D. Campos, G.R. Araújo, T.L. Martins, J. Nutr. Biochem. 9, 48 (2017)
[12] G. Báthegyi, Orv. Hetil. 1621, 146 (2005)
[13] C.M. Chan, J.Y. Fang, H.H. Lin, C.Y. Yang, C.F. Hung, Biochem. Biophys. Res. Commun. 172, 388 (2009)
[14] A. Bayramoglu, G. Bayramoglu, H. Senturk, Biochem. Biophys. J. Med. Food. 128, 16 (2013)
[15] M. Kelkel, M. Schumacher, M. Dicato, M. Diederich. Free Radic. Res. 925, 45 (2011)
[16] X.L. Zhang, F. Ren, W. Huang, R.T. Ding, Q.S. Zhou, X.W. Liu. Molecules. 28, 16 (2010)
[17] Q.P. Chen, P. Wei. Food Sci. Biotech. 1405, 22 (2013)
[18] U. Schlattner, M. Tokarska-Schlattner, T. Wallimann. Biochim. Biophys. Acta. 1762, 2 (2006)
[19] B. Glancy, W.T. Willis, D.J. Chess, R.S. Balaban. Biochemistry. 2793, 52 (2013)
[20] S. Lee, M. Kim, W. Lim, T. Kim, C. Kang, Biochem. Biophys. Res. Commun. 354, 461 (2015)
[21] A. Vigelsø, N.B. Andersen, F. Dela. Int. J. Physiol. Pathophysiol. Pharmacol. 84, 6 (2014)
[22] R.Z. Zhao, S. Jiang, L. Zhang, Z.B. Yu, Int. J. Mol. Med. 3, 44 (2019)
[23] B. Korzeniewski, PLoS One. e0117145, 10 (2015)
[24] M.D. Brand, D.G. Nicholls, Biochem. J. 297, 435 (2011)