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Research Article

Effect of increasing load exhaustive exercise on oxidative stress of skeletal muscle in different parts of rat

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Abstract: To explore oxidative stress condition of skeletal muscle in different parts of rat under the influence of the increasing load exhaustive exercise. Divide the 7-week-old SD male rats into two groups: quiet group(CG) and exhaustion group (EG). Conduct this experiment on the exhaustive group by gradually increasing load to skeletal muscle cells until exhaustive exercise and detect skeletal muscle cells' oxidative stress change.Quadriceps and gastrocnemius muscles' antioxidant activity increased, MDA content decreased, of which quadriceps CAT, GSH-PX activity increased more significantly($P<0.05$) after gradually increasing load exhaustive exercise; in contrast, flounder's antioxidant enzyme activity decreased, MDA content increased.Gradually increasing load exhaustive exercise can lead to oxidative damage of flounder muscle.
Keywords: increasing load, exhaustive exercise, skeletal muscle, oxidative stress.

INTRODUCTION

Research shows that after exhaustive exercise, body tissue, especially free radicals in skeletal muscle increased significantly, which result in oxidation and antioxidant system disorders, causing lipid peroxidation and affecting the structure and function of body tissue [1,2], this could be the reason of delayed onset muscle soreness(DOMS)[3]. Excessive exercise and skeletal muscle oxidative injury have been reported a lot, but most reports only studies one muscle's antioxidant system change or change of muscles antioxidant system at different time after movement[4-13]. However, how does different muscle tissue antioxidant system change after exercise and in a different sport mode, which muscle is more conducive to the future muscle experimental studies, these questions are still worth exploring.

Experimental Section

Animal grouping

Male SD rats (purchased from Hebei Medical University Animal Center; license number: SCXK2013-1-003; certificate number: 1409007), 7 weeks old, weigh 270±30g, were randomly divided into quiet group (CG) and exhaustive exercise group(EG), each group contain ten rats, two rats are bred in single cage, natural light, free water consumption, ambient temperature is 25±2℃, the national standard molar animal feeding, bedding replaced every 2-3 days.

Sports model creation

After one week acclimatization, rats of exhaustive exercise group were trained to run at nine o'clock every day, with the speed of 15m/min to run 15-20mins in 3 days of adaptation exercise. The incremental exhausting model were established after the end of the adaptive training, training model references to Bedford et al[14] treadmill training model slightly modified (Table 1), for a period of six weeks, and the last they were trained to exhausted before death. Exhausted standard is that rats can not adhere to the original speed, stuck in the end of treadmill; it is invalid to drive them by using electrical stimulation or sticks; eyes are dull and with the external stimulus there is no escape or revolt behavior after stop running.

Tissue samples drawn and specimen preparation

Exhaustive and control group rats were anesthetized with ether, then quickly removed its left leg quadriceps, gastrocnemius, soleus from the ice, transferred into liquid nitrogen for 30min, then shift them to -80℃ refrigerator for preservation. After muscles was removed, Clipping 0.3-0.5g tissue to dry surface tissue fluid and blood, adding 1ml ice-cold saline, fully homogenized, 3000r/min centrifugal 10min supernatant for testing.

Experimental Reagents and Instruments

TP, CAT, GSH-PX, T-AOC, SOD, MDA were determined in accordance with the kit instructions

operation, and the kit was provided by the Beijing Institute of Biotechnology Huaying.

ZH-PT animal treadmill(Huaibei Zhenghua, biological Equipment Co., Ltd.), CF15RXⅡ vertical high-speed refrigerated centrifuge(Beijing Aike Bang Technology Co., Ltd.), BCD-216TMZL Haier refrigerator(Qingdao Haier Co., Ltd.), Japan's Hitachi 7160 automatic biochemical analyzer.

Index detection

MDA content was measured by thiobarbituric acid(TBA) assay, SOD activity by xanthine oxidase method. CAT activity was determined by measuring ammonium molybdate method, GSH-PX activity by using thiosulfate nitrobenzoic acid(DTNB) determination, and T-AOC activity by using Fe^{3+} reduction method.

Statistical methods

The data were analyzed by using spss17.0, and results are represented by mean±SD.

Table-1: Movement program of laboratory animal

Week	Speed/(m·min^{-1})	Slope(°)	Time/(min)	VO_{2max}
1	15	0	20	58.40±1.70
2	15.2	5	20	58.40±1.70
3	15.2	5	20	58.40±1.70
4	26.8	5	20	74.30±2.90
5	26.8	5	30	74.30±2.90
6	26.8	10	20	81.00±3.50

RESULTS AND DISCUSSION

RESULTS

The effect of Incremental exercise to exhaustion on MDA content in rat skeletal muscle.

MDA content level can reflect the degree of lipid peroxidation, indirectly reflecting the degree of cell damage. A large number of studies shown that after acute or inappropriate exercise training, free radicals production increased in the body [15]. Figure 1 shows, quadriceps and gastrocnemius's MDA concentration in the EG is lower than that in the CG, and the soleus's EG is higher than that in the CG. Quadriceps CG's MDA concentration is significantly different from that in the EG's($P < 0.05$).

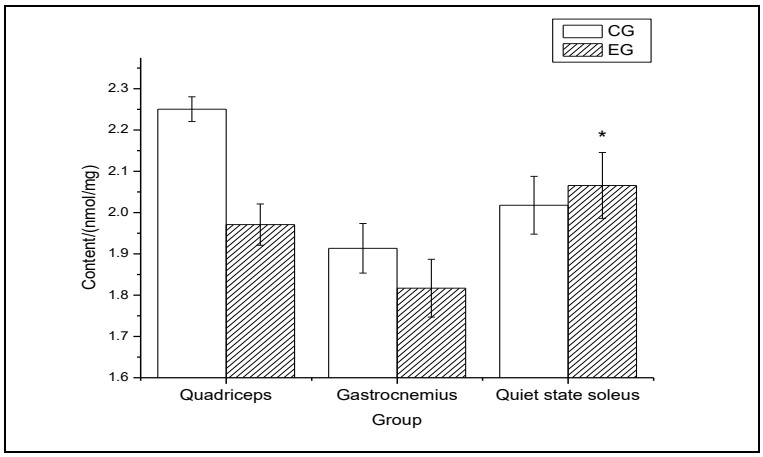

Fig-1: The effect of Incremental exercise to exhaustion on MDA content in rat skeletal muscle

The effect of incremental exercise to exhaustion on SOD activity in rat skeletal muscle

SOD is the first line of defense against free radicals [16]. Figure 2 shows, quadriceps and gastrocnemius's SOD activity in the EG is higher than that in the CG, and soleus's SOD activity in the EG is lower than that in the CG, but there is no significant difference ($P > 0.05$) between the two groups. At the same time, the soleus's SOD activity is higher than the other two groups, suggesting that SOD activity is higher in the soleus.

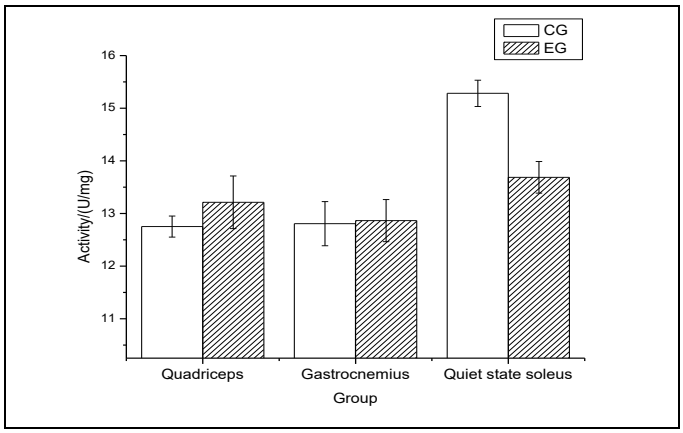

Fig-2: The effect of incremental exercise to exhaustion on SOD activity in rat skeletal muscle

The effect of incremental exercise to exhaustion on CAT activity in rat skeletal muscle

Jiang zhao-Feng, Yang Han-yi[17] studies shown that oxygen free radicals decrease the binding capacity of CAT and substrate, change the catalytic effect, reduce activity, but can not damage it completely without limitation. Figure 3 shows, quadriceps and gastrocnemius's CAT activity in the EG is higher than that in the CG, soleus's CAT activity in the EG is lower than that in the CG, quadriceps of CG and EG have a significant difference (P<0.05).

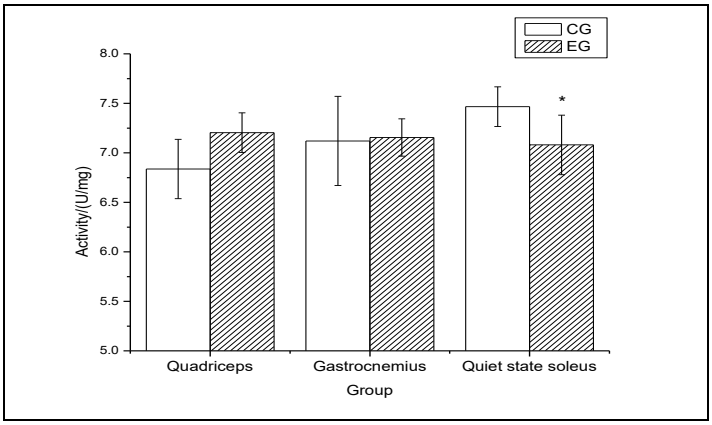

Fig-3: The effect of incremental exercise to exhaustion on CAT activity in rat skeletal muscle

The effect of Incremental exercise to exhaustion on GSH-PX activity in rat skeletal muscle

GSH-PX is a selenium-containing antioxidant enzym. GSH can be use as a hydrogen donor to scavenge free radicals, protect the normal function of the cell membrane [18]. As can be seen from Figure 4, quadriceps and gastrocnemius's GSH-PX activity in the EG is higher than that in the CG, soleus 's GSH-PX activity in the EG is lower than that in the CG. There was a significant difference between the quadriceps groups (P<0.05).

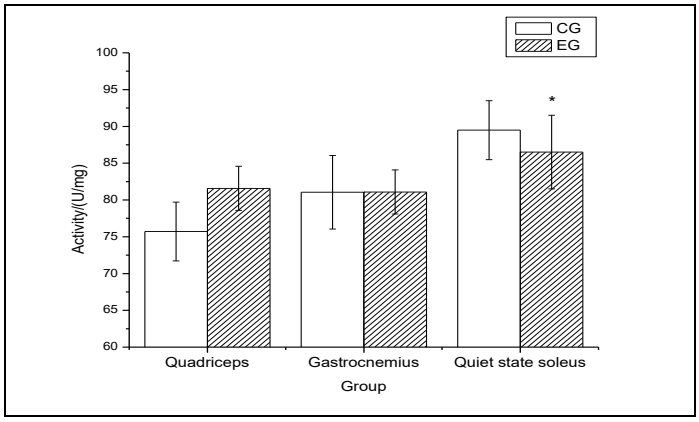

Fig-4: The effect of Incremental exercise to exhaustion on GSH-PX activity in rat skeletal muscle

The effect of incremental exercise to exhaustion on T-AOC activity in rat skeletal muscle.

T-AOC as the total antioxidant evaluation index, compared with a single antioxidant enzymes has unparalleled advantage [19]. As shown in Figure 5, stars quadriceps's T-AOC activity higher in the EG than that in the CG, soleus's T-AOC activity in the EG is lower than that in the CG. There was no significant difference between the groups (P>0.05).

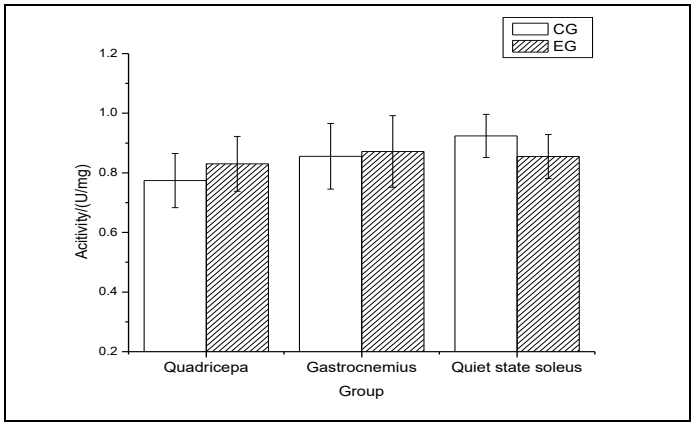

Fig-5: Incremental exercise to exhaustion of T-AOC activity in rat skeletal muscle

DISCUSSION

In quiet state, oxidation and antioxidant system is in dynamic equilibrium. Excessive exercise cause free radical accumulation and damage the cell membrane [20], leading to decreased exercise capacity. But a large number of active oxygen production and activation of antioxidant enzymes systems coexist [21], Schreek R [22] propose that radical action as a second messenger in cell's signal transduct system, activate the relevant gene expression. Gomez-Cabrera[23] et al reports, MnSOD expression increased in rat after exhaustive exercise; Franco [24] et al reported that when the myocardial cells were exposed to H_2O_2, the CAT, GSH-PX, CuZnSOD and MnSOD mRNA expression increased, suggesting that reactive oxygen species caused by movement are related with antioxidant enzyme expression increase; Ma Guodong, Gao Qi's [25] Study shown that endurance training can improve the expression of non-alcoholic fatty liver's MnSOD activity and mRNA expression. These studies suggest that free radicals generated during exercise as a signal molecule caused to cause antioxidant system gene expression. Regular exercise can improve oxidative capacity of SOD and GSH-PX's gene expression in skeletal muscle, thereby further adapt to the stronger oxidative stress [26].

The experiment results show that after the incremental load exhaustive exercise, rats' quadriceps and gastrocnemius MDA content is lower than that of the quiet group, and SOD、CAT、GSH-PX、T-AOC's activity increase, namely oxidative stress activate antioxidant enzymes system so as to make antioxidant enzymes expression happen to eliminate free radicals produced by the body. This result is consistent with Li Ai-chun [27], Shen Ning's [28] study, suggesting that its antioxidant gene is activated by external stimulation and expression of its antioxidant gene thereby eliminates free radicals, it belongs to a positive adjustment for the body to the external stimuli. The soleus's MDA content after exhausted movement immediately is higher than that of the quiet group, but the antioxidant enzymes activity decreased, .the result is consistent with Armstrong RB's result[29], but has discrepancy with Sen's [30] et al conclusion, which may be the result from differences sports models. Free radical damage antioxidant enzymes produced by the soleus muscle after exhaustive exercise reduce its activity. The mechanism of damage may be caused by changing its enzyme protein and prosthetic group 's structure and state[31], or it may also be caused by activating expression of antioxidant enzymes, but the generated free radicals are more than the antioxidant enzymes, which cannot offset the newly formed free radicals, so as to decrease activity of antioxidant enzymes. Movement caused large quantity of free radicals which can damage the body, and oxygen free radicals can cause damage to antioxidant enzyme.

CONCLUSION

After incremental load exhaustive exercise immediately, rats' quadriceps and gastrocnemius MDA content decreased、antioxidant enzyme activity increased, while the soleus muscle's MDA concentration increased, the activity of antioxidant enzymes decreased. There is no literature report about this conclusion. We recommend in-depth study of soleus muscle in rats after immediately increasing load exhaustive exercise in the future.

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