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Analysis of ATPase Activity of Mitochondria Intima in Exercise-induced Fatigue

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With the development of society, competitive sports attracted a lot of attention. Coaches and athletes not only intend to improve the level of training, but also get the best performance by improving the auxiliary means of athletic ability, especially by applying some substances that contain no prohibited substances to enhance physical fitness, resist fatigue and give full play to the potential of physical exercise. In this paper, mitochondrial enzyme activity is investigated through 50 healthy SD male rats. After exercising, different groups of rats were killed one by one, decapitated, blood, then quickly skinned legs, remove the gastrocnemius, extracted mitochondrial were detected skeletal muscle mitochondrial Ca^{2+}-ATP enzyme and H^{+}-ATP activity changes, as well as indicators of muscle tissue superoxide dismutase (SOD) and malondialdehyde (MDA) to analyze the effect of fatigue which can guide the training of athletes.

1. Introduction

The decline of muscle movement ability is the basic sign and essential characteristic of exercise-induced fatigue (Yang et al., 2012; Lapray et al., 2009). For more than one century, researchers have done a lot of researches on exercise-induced fatigue from different aspects. It is suggested that the negative effect of exercise stress metabolism enhancement may be the root cause of exercise-induced fatigue, such as exhaustion of energy substances, accumulation of metabolic products, and generation of free radicals (Xu and Wu, 2014). Mitochondria is an important and unique organelle in eukaryote cells. Its function is to provide "power" for energy conversion, to supply energy needed for cells to exercise various life activities and to participate in fatty acid synthesis and some protein synthesis (Cui, 2015; Merry and Ristow, 2016). Mitochondria, as a place where cells can store and supply energy, convert energy contained in food into ATP, which can be directly used by the body, by means of oxidative phosphorylation. In a course of long-term exercise, the function of mitochondria decreases and cannot produce enough ATP for the utilization of contractile protein in muscle, thus leading to the decline of muscle working ability, which may be an important reason for fatigue caused by long-time exercise (Moore et al., 2012; Batson, 2013; Bullock and Giesbrecht, 2014.).

Mitochondria is an important organ of cellular respiration and a source of energy for muscle activity. At the same time, mitochondria is a Ca^{2+} reservoir. Ca^{2+}, as an important organelle regulating factor, plays an important role in maintaining normal physiological function of the body (Holtzer et al., 2011; Hu et al., 2005). It has been confirmed that mitochondria have two different calcium transport systems, one is responsible for Ca^{2+} internal flow, which needs energy and the other is Ca^{2+} external flow, which doesn't consume energy and completes via Na^{+}-Ca^{2+} permutoid. Ca^{2+}-ATPase of mitochondrial membrane is responsible for transporting Ca^{2+} in the cytoplasm into the mitochondrial matrix, and the required energy is provided by ATP hydrolysis. The function of mitochondrial H^{+} -ATPase is mainly to synthesize ATP and transport H^{+}. The ATP is synthesized from ADP and Pi by H^{+} ATPase using the energy released by H+ flowing back to mitochondrial endometrium matrix. It couples ATP synthesis at three locations on the mitochondrial respiratory chain. At each location, one ATP molecule can be synthesized by every two electrons. Mitochondria transport Ca^{2+} via Ca^{2+}-ATPase while the final synthesis of ATP must be involved in the H^{+}-ATP, which suggests that the exercise leads to the accumulation of calcium in mitochondria, and inhibition of ATP synthesis may be associated with changes in mitochondrial Ca^{2+}-ATPase and H^{+}-ATPase activities. This paper studies the

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effect of fatigue exercise on ATPase activity to understand the relationship between fatigue and exercise ability.

2. Experimental subjects and methods

2.1 Experimental subjects

30 healthy male Wistar rats with body weight of 150 ± 20 g and age of 8 weeks are selected as experimental subjects. The rats are fed with dry diet and drinking water of normal saline. During culture, they are fed and drink water freely in separate cages. The room temperature is controlled at 20±5 □ and the humidity is 50±10%. With natural lighting, the breeding room has weekly ultraviolet disinfection and sterilization.

2.2 Experimental methods

2.2.1 Basic process of experiment

In order to fully investigate mitochondrial ATPase activity under exercise-induced fatigue conditions, the experimental materials are compared in groups. The specific procedure is shown in Figure 1.

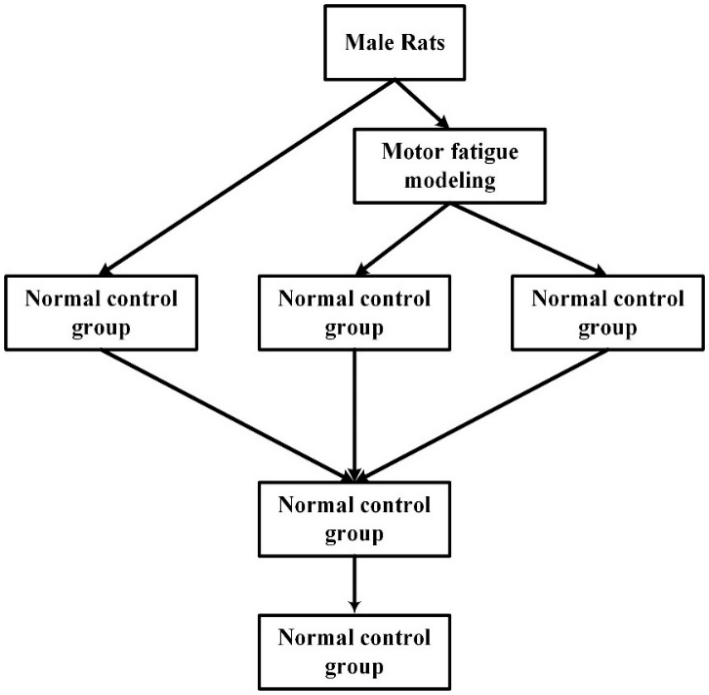

Figure 1: Flowchart of experiments

2.2.2 Rat grouping and training

The rats are subject to adaptive training within one week of purchase and are trained at a steady speed of 18 m/min on a horizontal treadmill every afternoon. After the training, several rats are randomly taken out to cut their tails for blood sampling and leave for 10min. Then, they are centrifuged by low temperature and high speed centrifuge under 4 □ at 3000 r/min for 20 minutes. The serum iss separated and then stored in -20 □ refrigerator. Six rats are then taken out as the normal control group and the remaining 24 rats as the exercise fatigue model group. The training scheme for the exercise fatigue model group is shown in Table 1.

Table 1: Training scheme for fatigue model group (m/min×min)

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	12 × 15	12 × 15	15 × 15	15 × 15	18 × 15	18 × 15
2	18 × 20	18 × 20	18 × 20	21 × 20	21 × 20	21 × 20
3	21 × 20	21 × 20	23 × 20	23 × 20	23 × 20	23 × 20
4	23 × 20	25 × 20	25 × 20	25 × 20	25 × 20	25 × 20

After 4 weeks of training, the rats are tested with indexes which indicate the fatigue state of the body such as blood hemoglobin (Hb), blood lactic acid (BLA), serum blood urea nitrogen (BUN), and creatine kinase (CK). If the results show: Hb is significantly lower than that of control group, and BLA is significantly higher than that of control group. Serum (BUN) increases significantly compared with the control group. Serum creatine kinase (CK) has a tendency to increase, and combining with test results of hemoglobin and blood lactate, it's suggested that the rats have shown obvious fatigue characteristics. Based on the above test results, it is judged that the modeling is successful. Then, 20 rats are selected and randomly divided into exercise fatigue quiet group and exercise fatigue exercise group, 8 rats in each group are fed in cages and the remaining rats are for reserve.

Normal control group live normally in cages without exercise. Exercise fatigue quiet group do not exercise and lead a normal life in the cage; exercise fatigue exercise group perform four weeks of active recovery exercise, and the exercise time increases from 10min to 20min gradually, the minimum intensity is controlled at 15m/min and the maximum intensity is controlled at 18m/min. Exercise every other day, and all trainings are at 4: 00 - 6: 00 pm, with a slope of zero. After training, the rats are free to drink and eat. The training programme is shown in Table 2.

The rats in the exercise group are killed immediately after exercise to exhaustion, and the intact iliac muscle is rapidly removed and stored at 0-4 □ for further study.

Table 2: Repair training plan for fatigue group. (m/min×min)

Number of days	Velocity (m/min)	Run duration (min/d)
1	15	10
3	15	11
5	15	12
7	15	13
9	15	14
11	16	15
13	16	16
15	16	17
17	16	18
19	16	19
21	16	20
23	17	20
25	17	20
27	17	20

2.2.3 Extraction of Mitochondria and Determination of ATPase Activity

The mitochondria extraction method is shown in Figure 3, the low-temperature preserved skeletal muscle is taken out and makes the homogenize in a buffer solution pre-cooled at 0 to 4 DEG C (buffer solution contains 70 mmol/L sucrose, 220 mmol/L mannitol, 0.5 mmol/L EDTA, and 5 mmol/L MOPS, pH = 7.4). Homogenize slurry (homogenate 30s, at the interval of 30s, and repeat 3 times) is added according to the volume ratio of 1: 5, the obtained homogenate slurry is centrifuged at 2000 rpm at 0□ for 15min, and the supernatant liquid is taken; the centrifugation is repeated once more; the supernatant obtained by two centrifugation is mixed and re-centrifuged (12000rpm, 15min). The supernatant is discarded. The obtained precipitate is fully suspended with appropriate buffer solution and centrifuged at 12000rpm for 15min. The obtained precipitate is mitochondria of rat skeletal muscle and suspended in suspension for test. Take bovine serum albumin as a standard, the content of mitochondrial protein is determined by Coomassie Brilliant Blue method, and the sample pretreatment is carried out strictly according to the instructions of ultra-micro ATP assay kit. The ATPase activity of the samples is analyzed by colorimetric phosphorus method.

In a certain protein concentration range (0-1000 ug/ml), the light absorption of the protein-pigment conjugate at a wavelength of 595nm is proportional to the protein content, so it can be used for the quantitative determination of protein. The protein content determined by Coomassie Brilliant Blue G250 is a kind of dye binding method, which is red in free-state and has a maximum light absorption at 465nm. When it binds to protein, it becomes cyan, and has a maximum light absorption at 595nm. Set concentration gradient of bovine serum albumin (BSA): in the test tube accurately suck 0.1 ml of solution from each tube and put it into a tube, add 5ml Coomassie Brilliant Blue G250 egg reagent, put the plug to the test tube, mix the solution in the test tube upside down, put quietly for 2 min, and make a standard curve with Origin 7.5 using a 10 mm cuvette at 595nm.

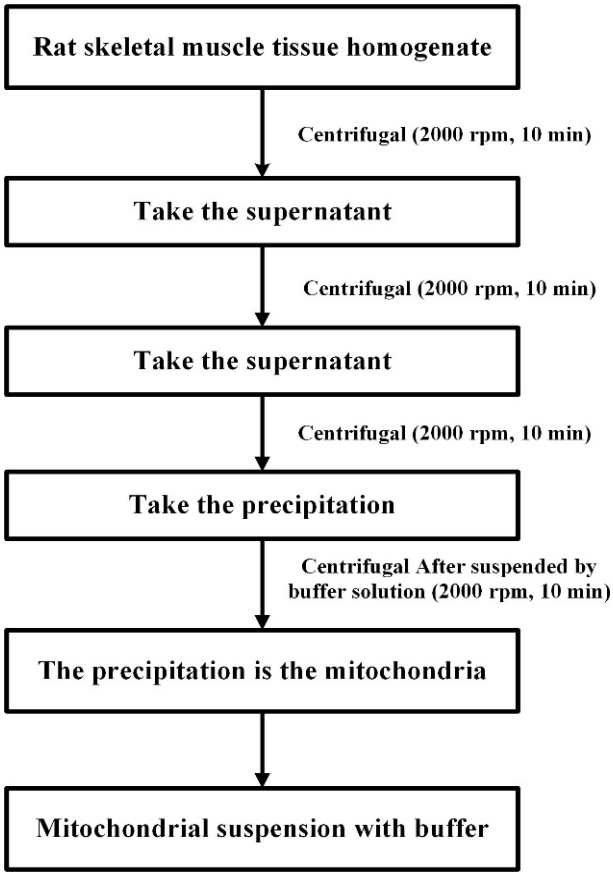

Figure 2: Mitochondrial extraction method

3. Experimental results and discussion

3.1 Changes of blood and serum content and activity in rats before and after experimental training

As shown in Table 3, after training, blood hemoglobin (Hb) is significantly decreased, and serum urea nitrogen (BUN) and creatine kinase (CK) are significantly increased. The test results indicate that the rats show obvious fatigue characteristics. According to the above behavior observation results, the modeling is successful. There are 18 exercise fatigue rats. The success rate of modeling is 90%, and 16 rats are selected to be divided into groups.

Table 3: Activity changes of rats' serum before and after training. (m/min×min)

	CK (IU/L)	BUN (nmol/L)	Hb (g/L)
Before training	1378.56±104.21	6.21±0.67	132.31±5.46
After training	2559.19±99.86	9.87±0.52	112.47±2.69

3.2 Changes of Ca2+-ATPase activity

As shown in Figure 3, after four-week exercise fatigue training, group training and fatigue elimination training, some changes of mitochondrial Ca^{2+}-ATPase occur in skeletal muscle of rats.

Compared with the normal group, there is a significant difference ($P < 0.01$) in the exercise fatigue quiet group, which decreases by 59.67%; there is significant difference in exercise fatigue group ($P < 0.05$), which decreases by 29.8%. There is a significant difference ($P < 0.05$) between the exercise fatigue quiet group taking medicine and exercise fatigue exercise group taking medicine ($P < 0.05$), which increases by 33.92%. The results show that after exercise fatigue, the hydrolytic activity of mitochondrial Ca^{2+}-ATPase hydrolysis of skeletal muscle decreases remarkably, which causes the fatigue of skeletal muscle. In detail, the decrease of mitochondrial Ca^{2+}-ATPase activity results in the decrease of mitochondrial calcium uptake ability, that's, the

decrease in the ability of transporting intracellular Ca^{2+} into mitochondria by Ca^{2+}-ATPase through the energy released from hydrolyzing ATP, causing the plasma Ca^{2+} concentration not to recover to the normal level quickly. The increase of cytosolic Ca^{2+} affects the function of skeletal muscle and thus causes fatigue of skeletal muscle;

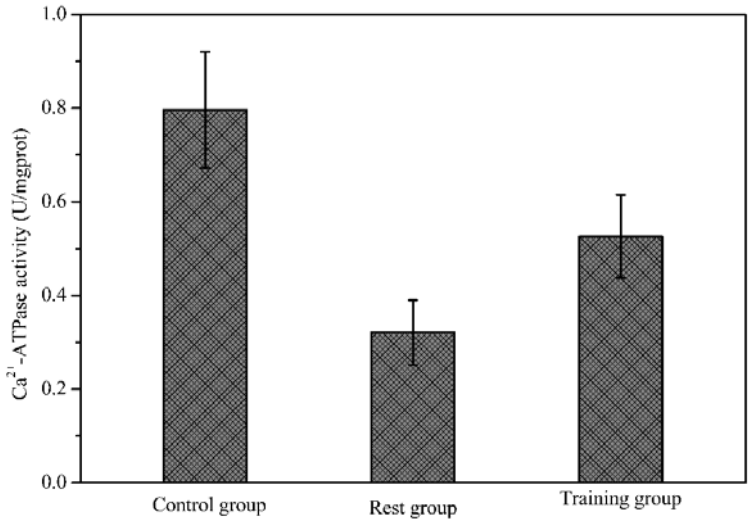

Figure 3: The Ca^{2+}-ATPase activity of different groups

3.3 Change of H^+-ATPase activity

As can be seen from Figure 4, after four weeks of exercise fatigue training, not only Ca^{2+}-ATPase but also H^+-ATPase of rat skeletal muscle mitochondria change.

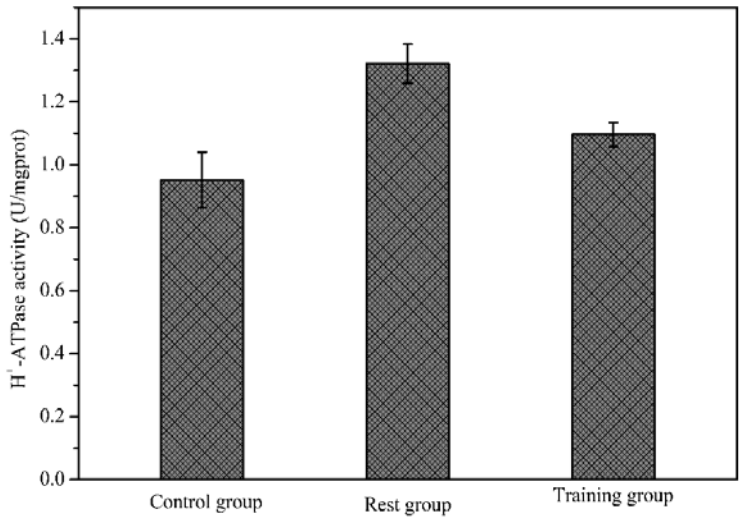

Figure 4: The H^+-ATPase activity of different groups

Compared with the normal group, there is a significant difference ($P < 0.01$) in the exercise fatigue quiet group (up by 38.76%). There is a significant difference in exercise fatigue exercise group ($P < 0.05$), which increases by 15.13%. The skeletal muscle has the characteristics of fatigue but no exhaustion, and the hydrolytic activity of H^+-ATPase increases very obviously. It has been found that exhaustive exercise can decrease the hydrolytic activity of H^+-ATPase of rat myocardial mitochondria and change the fluidity of mitochondria membrane, but exercise with a certain intensity is not enough to cause the change of H^+-ATPase structure. It is concluded that although exercise fatigue occurs in this study, the exercise intensity is not enough to destroy the integrity of the mitochondria membrane. The hydrolytic activity of H^+-ATPase increases very obviously,

which indicates that the ability of H^+-ATPase synthesizing ATP in skeletal muscle mitochondria after exercise is at a higher level, but it still needs to be further confirmed.

4. Conclusion

After introducing motor fatigue, the activity of Ca2+-ATP enzyme hydrolysis in skeletal muscle mitochondria decreased significantly. Detailed analysis showed that the activity of mitochondrial Ca^{2+}-ATP enzyme decreased, and the ability of mitochondrial calcium uptake decreased. That is, the ability of Ca^{2+}-ATP enzyme to hydrolyze ATP then release energy and transport Ca^{2+} into mitochondria in cytoplasm decreased, resulting in the difficulty of Ca^{2+} concentration in cytoplasm back to a normal level. Then cause exhaustion. Exhaustive exercise may not cause changes in the structure of H^+-ATP enzyme and inhibit its activity. Accordingly, in this study, although the sports fatigue, but the integrity of the exercise intensity is not enough to destroy the mitochondrial membrane, the hydrolysis activity of H^+-ATP enzyme increased significantly indicating that the synthesis of ATP in skeletal muscle mitochondrial H^+-ATP enzyme at a high level, but this still remains to be confirmed.

References

Batson G., 2013, Exercise-induced central fatigue: a review of the literature with implications for dance science research, Journal of Dance Medicine & Science Official Publication of the International Association for Dance Medicine & Science, 17(2), 53.

Bullock T., Giesbrecht B., 2014, Acute exercise and aerobic fitness influence selective attention during visual search, Frontiers in Psychology, 5, 1290.

Cui Z., 2015, Analysis of exogenous antioxidants supplementation on experimental exercise fatigue, Bulletin of Science & Technology, (6), 13-15.

Holtzer R., Shuman M., Mahoney J.R., Lipton R., Verghese J., 2011, Cognitive fatigue defined in the context of attention networks, Neuropsychology Development & Cognition, 18(1), 108-128.

Hu M., Cheng S.L., Zou L.C., Zhou W. L., 2005, Application of electrical nerve stimulation in assessing exercised-induced muscle fatigue, Journal of Guangzhou Physical Education Institute, 25(6), 43-46.

Lapray D., Bergeler J., Luhmann H.J., 2009, Stimulus-induced gamma activity in the electrocorticogram of freely moving rats: the neuronal signature of novelty detection, Behavioural Brain Research, 199(2), 350-354.

Merry T.L., Ristow M., 2016, Do antioxidant supplements interfere with skeletal muscle adaptation to exercise training, Journal of Physiology, 594(18).

Moore R.D., Romine M.W., O'Connor P.J., Tomporowski P.D., 2012, The influence of exercise-induced fatigue on cognitive function, Journal of Sports Sciences, 30(9), 841.

Xu G.Q., Wu M.Z., 2014, A review for microbial production of c_4 dicarboxylatic acids, China Biotechnology, 34(8), 97-104.

Yang D.S., Liu X.L, Qiao D.C., 2012, Dynamic Research of Electrocorticogram on the Development and Recovery of Exercise-induced Fatigue of Rats, China Sport Science, 32(4), 109-113.