Experimental Research on the Effect of L-carnitine and Physical Exercise on the Rats' Skeletal Muscle Mitochondrial Respiratory Chain Enzyme Complex Activity

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***Abstract* — Mitochondria are where the oxidative phosphorylation of eukaryotic cells provides energy and generates more ATP for the movements of organism. To discuss the effect of L-carnitine and physical exercise on the rat's skeletal muscle mitochondrial respiratory chain enzyme complex activity, 40 male Whister rats were classified into four groups at random, each consisting of 10 ones. The groups were control group (I), physical exercise group (II), L-carnitine group (III) and L-carnitine+ physical exercise group(IV). Results show that L-carnitine or physical exercise may improve the function of rats' skeletal muscle mitochondrion and respiratory chain immediately after their exhaustive exercise, however, combination of L-carnitine with physical exercises bring better results; and Physical exercise and L-carnitine have synergistic effect in improving the function of rats' skeletal muscle Mitochondrion and respiratory chain immediately after their exhaustive exercise. The experiment results demonstrate that experimentation basis and data can be provided for supplementing nutrients and exercise loads can be applied to improving the respiratory functions of the mitochondria and relieving the fatigue from sports.**

Keywords* - *L-carnitine; Physical exercises; Skeletal muscle; Mitochondrion; Respiratory chain enzyme complex activity; Effect

I. INTRODUCTION

Background for researching the issue: Mitochondrion is a place where the oxidative phosphorylation of eukaryotic cells provides energy. Oxidative phosphorylation is mainly carried out on the internal membrane of the mitochondrion. The components directly contributing to energy conversion on the internal membrane are classified into two classes:(1) Respiratory chain enzyme system (energy releasing device). The respiratory chain enzyme system is composed of many enzymes on the internal membrane and other components to act as electron transmitters, so it is also called "Electron Transmission Chain" [1]. The electron transmission chain of the mitochondrion consists of several multimolecular complexes, wherein, complex I (NADH-CoQ reductase), complex II (succinic acid-CoQ reductase), complex III (CoQ- cytochrome C reductase), complex IV (cytochrome c oxidase) are important constituents of the enzyme complexes, and the change in the activity may reflect the change in the respiratory function of the mitochondrion, whether directly or indirectly. Under normal conditions, the oxidative phosphorylation of the mitochondrion is closely coupled with the electron transmission, in this way, electrons are transmitted to molecular oxygen from NADPH in an ordered way gradually through the electron transmission chain, thus completing the respiratory metabolism;(2)ATP enzyme complex (energy converter), which is also called F1-F0 coupling factor, which is a complex consisting of several peptides, and plays an important role in synthesis of ATP. The two classes of energy conversions in mitochondrion are supplementary to each other and work together to produce ATP for the physical exercises of organs[2]. In this experiment, the combination of L-carnitine and physical exercises is adopted to observe the activity I-IV of rats' skeletal muscle mitochondrion and respiratory chain enzyme complex, research the effect of physical exercise and L-carnitine and discuss the mechanism of improving the physical exercise induced capability of skeletal muscle, and provide experimental basis and data support for properly using nutrition and physical exercise load to improve the respiratory functions of mitochondrion, and relieving exercise-induced fatigue[3].

II. MATERIALS AND METHOD

For simplification, we divided fractures into two kinds according to their sizes. Macroscopic big fractures should be built particularly as parts of the model, but small distributed fractures may be taken just as material remediation, such as Young's modulus drop or permeability growth.

(1)Animal in the experiment and grouping

40 male Whister healthy rats with their average weight of 130±20g are used and experiment is conducted on them after one-week balanced diet. The rats are classified into four groups at random: A quiet control group (I, n=10), a physical exercise group (II, n=10), an L-carnitine group (III, n=10) without doing any physical exercises, and an L-carnitine+physical exercise group (IV, n=10) which runs on treadmill with its exercise load ascending for six weeks in total. The L-carnitine group and the L-carnitine+physical exercise group take L-carnitine (300mg/kg of rat's weight) from 8:00-9:00 Am every morning. The quiet control group and the physical exercise group take equivalent amount of distilled water.

(2)How the animals do exercises

The experiment begins after balanced diets of the rats for one week. The physical exercise group (II) and the L-carnitine+physical exercise group (IV) do adaptive treadmill running exercises on horizontal treadmills at 5-10m/min for

5-10 minutes each time every day and for three days in total before the experiment. At the beginning of the experiment, the rats are quiet, and then run on the slop downwards continuously at increasing speed, and the speed increases to (20±1)m/min within 30 minutes and then the speed of (20±1)m/min is maintained, the total duration of exercise is 120 minutes[4,5].

(3)Sampling and testing

On Monday of the seventh week, all of the rats run until exhaustion in batches on the treadmill and then are guillotined. The skeletal muscles of their rear limbs (quadriceps femoris) are sampled quickly, and connective tissues such as fat and fascia are removed in the saline solution pre-cooled, and then frozen in liquid nitrogen for several hours, and then stored at the temperature of －40□ for use. After the frozen rats' skeletal muscle tissues are molten under normal conditions, they are cut into pieces in ice bath, and buffer homogenate is filled at the ratio of 1:5 by volume (homogenized for 30s at an interval of 30s, three times) at the temperature of 0-4□. The mixture is centrifugalized for 10 minutes (12000 rpm), and the deposit is discarded and the supernatant liquid is taken; the supernatant liquid from the two centrifugalizations is mixed and then the mixture is centrifugalized for 15 minutes at 12000rpm, the supernatant liquid is discarded, right amount of buffer liquid is mixed with the deposit to fully suspend the deposit, and then the mixture is centrifugalized for 15 minutes at 12000 rpm, the resulting deposit is the rats' skeletal muscle mitochondrion ball.

(4)Measurement of skeletal muscle mitochondrial respiratory chain enzyme complexes (I, II, III and IV)

The separated and purified mitochondrion is frozen and molten again and again three times at -20□ and 20□ to destroy the mitochondrion membranes and get the free mitochondrial respiratory chain enzyme. The activities of the enzymes are measured at 30□. The differential spectrophotometry is used to measure the activity of the respiratory chain enzyme complexes I-IV in reference to the Galina Method.

(5)Main drugs and reagents

L-carnitine(98%), BSA, Coomassie brilliant blue (CBB) G250, CytC, NADH, GSH, DB(CoQ0), DTNB, antitoxin A(an), Rotenone, Dodecyl-β-D-maltoside(β-cracking agent) are all Sigma agents.

(6) Calculation of the results

Enzyme complex activity (μmol/min.mgpro)=[(△A/min*reaction volume)/absorbence coefficient]/Protein content of mitochondrion[6].

III. RESULTS OF THE EXPERIMENT

Change in the duration of rats' running on treadmill until exhaustion. Seen from Table 1, compared with the quiet control group, right amount of physical exercises may significantly prolong the duration of rats' running on the treadmill until exhaustion; just L-carnitine has exerted little influence on the duration of rats' running on the treadmill until exhaustion. Only the combination of L-carnitine and physical exercises may effectively prolong the duration of the rats' running on the treadmill until exhaustion.

TABLE I DETAILS OF DURATION OF RATS' RUNNING ON THE TREADMILL UNTIL EXHAUSTION

Group	Duration of running until exhaustion（min）
Ⅰ（n=10）	161.20±10.35
Ⅱ（n=10）	202.08±14.99☆☆★★
Ⅲ（n=10）	186.02±21.01☆☆
Ⅳ（n=10）	229.01±29.89☆☆★★

Note:☆☆means that compared with I, the difference is very significant (P<0.01);★★means that compared with III, the difference is very significant (P<0.01).

Change in the rats' skeletal muscle mitochondrion and respiratory chain enzyme complex activity. Table 2 shows that immediately after exhaustive exercises, compared with group I, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group II rises by 11.32% but without significant difference (P>0.05); the CII activity rises by 8.47% without significant difference either(P>0.05); the CIII activity rises by 16.18% with significant difference (P<0.05); the CIV activity rises by 11.28% without significant difference (P>0.05).

TABLE II CHANGE IN THE RATS' SKELETAL MUSCLE MITOCHONDRION AND RESPIRATORY CHAIN ENZYME COMPLEX ACTIVITY（MMOL/MIN/MG PROT）

Group	Complex Ⅰ (n= 10)	Complex Ⅱ (n= 10)	Complex Ⅲ (n= 10)	Complex Ⅳ(n= 10)
Ⅰ	0.16±0.10	0.06±0.31	0.14±0.42	0.17±0.01
Ⅱ	0.18±0.01	0.06±0.01	0.16±0.01☆	0.20±0.22
Ⅲ	0.20±0.11☆☆	0.07±0.04	0.18±0.01☆☆	0.19±0.90
Ⅳ	0.23±0.11☆☆★¤	0.07±0.76☆	0.21±0.88★★¤¤	0.22±0.01¤

Note:☆means that compared with I, the difference is significant(P<0.05);☆☆ means that compared with I, the difference is very significant（P<0.01）;★ means that compared with II, the difference is significant(P<0.05); ★★means that compared with □, the difference is very significant(P<0.01);¤means that compared with III, the difference is significant(P<0.05); ¤¤means compared with III, the difference is very significant(P<0.01)

Table 2 shows that immediately after exhaustive exercises, compared with group I, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group II rises by 11.32% but without significant difference (P>0.05); the CII activity rises by 8.47% without significant difference either(P>0.05); the CIII activity rises by 16.18% with significant difference (P<0.05); the CIV activity rises by 11.28% without significant difference (P>0.05).

Immediately after exhaustive exercises, compared with group I, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group III rises by 20.12% but without significant difference (P<0.01); the CII activity rises by 13.55% without significant difference either(P>0.05); the CIII activity tends to rise by 28.68 % with significant difference (P<0.01); the CIV also tends to rise by 8.67% without significant difference (P>0.05).

Immediately after exhaustive exercises, compared with group I, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group IV rises by 31.44% with significant difference (P<0.01); the CII activity rises by 20.34% with significant difference(P<0.05); the CIII activity rises by 41.91 % with significant difference (P<0.01); the CIV also rises by 21.97% with significant difference (P<0.01).

Immediately after exhaustive exercises, compared with group II, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group IV rises by 18.08% with significant difference (P<0.05); the CII activity tends to rise by 10.93% without significant difference (P>0.05); the CIII activity tends to rise by 22.51 % with significant difference (P<0.01); the CIV also rises by 8.21% without significant difference (P>0.05).

Immediately after exhaustive exercises, compared with group III, the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CI of group IV rises by 19.9% with significant difference (P<0.05); the CII activity tends to rise by 5.97% without significant difference (P>0.05); the CIII activity tends to rise by 21.71 % with significant difference (P<0.01); the activity of CIV also rises by 17.55% with significant difference (P<0.05).

IV. ANALYSIS AND DISCUSSION

As an example, an underground tunnel under a fractured aquifer is calculated. Figure 1 is the model setting, in which the aquifer thickness is 50m and 45m far from the tunnel. The left, right and bottom boundaries have 0 displacements at their vertical directions, and the tunnel walls is overflow boundaries. The mesh includes $20\times20\times2=800$ linear triangle elements and 400 square elements of stochastic materials. Fig. 2~7 are the distributions of stress and flow rate, the stochastic pattern of waterheads, and the variance of them. The model parameters are shown in table 1.

(1) Effect of L-carnitine and physical exercises on the duration of rats' running until exhaustion.

The duration of rats' running on the treadmill until exhaustion is a commonly used index to reflect the athletic abilities. The athletic abilities may effectively reflect the macro anti-fatigue ability[7,8]. The experimental results show that compared with the quiet control group, right amount of long-term physical exercise, and combination of physical exercises and L-carnitine may significantly prolong the duration of rats' running on the treadmill until exhaustion, and it means that physical exercises, and combination of physical exercise and L-carnitine may help to resist fatigue, and play a role in improving the athletic abilities. However, simply L-carnitine may not effectively improve the rats' anti-fatigue abilities probably because after L-carnitine is fed while the synthetic capability of skeletal muscle mitochondrion ATP is improved, the content of free radicals of mitochondrion generated is also increased, consequentially, the duration of the rats' running until exhaustion is not increased.

(2) Effect of L-carnitine and physical exercises on the rats' skeletal muscle mitochondrial respiratory chain enzyme complex activity after the rats' exhaustive exercise

According to the experimental results: Physical exercise, L-carnitine and combination of physical exercises and L-carnitine all may exert positive effect on the rats' skeletal muscle mitochondrial enzyme complexes and improve the bodies' exercise stress abilities. Compared with simply L-carnitine, the combination of physical exercise and L-carnitine may significantly improve the activities I-IV of

rats' skeletal muscle mitochondrion enzyme complexes, and it means that the combination of physical exercise and L-carnitine may further improve the activity of rats' skeletal muscle mitochondrial enzyme complexes and provide energy for oxidative phosphorylation of the mitochondrion and then synthesis of the energy substance required by the bodies' physical exercise-ATP. Compared with the physical exercise group, combination of physical exercise and L-carnitine mainly affect CIII, and exerts little influence on the enzyme complex activities CI, CII and CIV[10-12].

Tips:The part of respiratory chain sensitive to L-carnitine should be CIII. The combination of L-carnitine and physical exercises has no great synergistic effect on improving the activities CI, CII and CIV, namely, combined with physical exercises, L-carnitine can not significantly improve the activities of the rats' skeletal muscle mitochondrion enzyme complexes CI, CII and CIV. For CI, CII and CIV, physical exercise, and the combination of physical exercise and L-carnitine may significantly improve their activities. However, by comparing the physical exercise+ L-carnitine group with the physical exercise group, CI, CII and CIV activities tend to rise, but the difference is not so significant as that of CIII. It means that based on physical exercises, even exogenous L-carnitine may help to improve the activities of CI, CII and CIV but without significant difference probably because L-carnitine is more effective to improve the activity of CIII. Exogenous L-carnitine may significantly improve the content of the combination of L-carnitine of the skeletal muscle mitochondrion and significantly improve the ATP synthetic abilities. In an exhaustive exercise, as the duration of exercise increases, the active oxygen of mitochondrion generated increases, and the peroxidation improves, and there are sufficient carriers on the respiratory chains of the mitochondrion. It means that the "speed limiting steps" in electron transmission are removed, in this way, the electrons from the NADH and electrons from succinic acid are successfully transmitted to CoQ through NADH dehydrogenase complexes (CI) and succinic acid dehydrogenase complexes (CII) respectively, accelerating the transmission of electrons from CIII to CIV, and then the next reaction follows to further accelerate the oxidative phosphorylation of the mitochondrion.

V. CONCLUSION

The experiments prove that the exercise training may help the respiratory chain enzymes of the skeletal muscle mitochondria to change significantly in an adaptive way and improve its activity. Seen from the experimental results, compared with the quiet control group, the L- carnitine may significantly improve the activity of the rats' skeletal muscle mitochondrial respiratory chain enzyme CIII immediately after their exhaustive exercise is significantly improved. Although the activities of CI, CII and CIV are not improved so significantly as that of CIII, they tend to rise. Increasing load exercise may significantly improve the activities of CI-CIV of rats' skeletal muscle mitochondrial respiratory chain enzyme immediately after their exhaustive exercises. The combination of physical exercises and L- carnitine may also improve the activity of the rats' skeletal muscle mitochondrion enzyme complexes. It means that the physical exercises and L- carnitine have synergistic effects in improving the functions of the skeletal muscle mitochondrion immediately after the exhaustive exercises.

Furthermore, it is found through research that by comparing the L-carnitine+physical exercise group with the exercise group, although the effect of the combination on the activities of the rats' skeletal muscle mitochondrion respiratory chain enzymes CII and CIV tend to rise, but not so evident as that of CI and CIII. The reason may be: exogenous L- carnitine may help to increase the content of the combination of endogenous L- carnitine of the skeletal muscle mitochondrion. While accelerating the electron transmission during biological oxidation, the electron leak and the proton leak are also accelerated during electron transmission, thus generating more superoxide anions.More superoxide anions may probably affect the functions of CIII and CIV directly to reduce their activities. In addition, the carrier for transmitting electrons between the complex CIII and CIV is Cyt-C. So, there may be other mechanism to regulate and control the electron transmission between CIII and CIV, thus affecting the oxidative phosphorylation of the mitochondrion. These issues are to be verified by others through experiments in the future.

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