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Transmission Electron Microscopy Used to Observe the Transversal Tension Transfer of Skeletal Muscle Caused by Acute Centrifugation

Xudong Peng[1,*]

[1]*College of Teacher Education, Captial Normal University, Beijing 100048, China*
[*]*Corresponding Author*

Abstract: *The change of lateral tension of skeletal muscle can have a direct impact on the injury of skeletal muscle. Acute centrifugal exercise is a major factor to promote the transverse tension transfer of skeletal muscle. The purpose of this study is to determine a scientific and reasonable centrifugal movement mode by studying the changes of lateral tension transfer of skeletal muscle caused by acute centrifugal movement. In this paper, the relevant theories and concepts of transmission electron microscope, acute centrifugal exercise and skeletal muscle are explained at first, especially the acute responses of acute centrifugal exercise in three aspects of metabolic cardiopulmonary response, molecular level response and repetition effect are analyzed. The change of lateral tension transfer of skeletal muscle caused by acute centrifugal movement was analyzed concretely by rat model experiment. The results showed that: compared with the intensity of acute centrifugal exercise, the lateral tension of skeletal muscle increased significantly after acute exercise, from 0.36 to 0.65, with a change range of 0.29. The lateral tension before acute exercise increased from 0.24 to 0.29, with a change of only 0.05. Taken together, acute centrifugation can promote an increase in skeletal muscle transverse tension transfer.*

Keywords: *transmission electron microscope, cute centrifugation, skeletal muscle*

1. Introduction

Centrifugal exercise makes the ultrastructure of skeletal muscle change to a certain extent, resulting in decreased muscle strength and delayed muscle soreness. Generally, the above conditions are referred to as motor muscle microlesion, which hinderedthe improvement of human's motor ability to a certain extent. Changes in skeletal muscle tension of horizontal transfer is the type of the main causes of damage produced, based on the field of sports medicine for acute changes of skeletal muscle lateral tension transfer caused by centrifugal movement has been maintained a high popularity, in recent years, widely used for the study on transmission electron microscope observation provides important technical support.

Based on the direct effect of skeletal muscle transverse tension transfer changes on motor muscle injury, scholars at home and abroad have conducted a series of studies on it and obtained relevant research results [1]. In the literature [2], the author studied the characteristics and acute reactions of centrifugal movement, which were mainly manifested in mechanical characteristics, analytical characteristics and neurological characteristics. The acute reactions were mainly manifested in metabolism and cardiopulmonary reactions, molecular level reactions and repetitive effects. In the literature [3], the author studied the specific structure and function of the extracellular matrix of skeletal muscle, and found that the muscle bundle membrane is the main line of skeletal muscle, which provides certain theoretical support for the exploration of the tension transfer process of skeletal muscle. In the literature [4], the author studied the specific pathway of skeletal muscle tension transmission. Through a large number of transmission electron microscope observation experiments, it was proved that there were two transmission pathways: longitudinal and transverse. In the literature [5], the author explored the specific mechanism of massage in the recovery of motility muscle injury, and finally proved that the receptor of α7β1 integrin was activated based on the massage effect, thus significantly improving the sensitivity of muscle-membrane mechanical receptor to a certain extent.

In order to explore the changes in transverse tension transfer of skeletal muscle caused by acute centrifugal movement, this paper made relevant observation and research with the help of transmission

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electron microscope technology [6]. In this paper, the relevant theories and concepts of transmission electron microscope, acute centrifugal exercise and skeletal muscle are explained at first, especially the acute responses of acute centrifugal exercise in three aspects of metabolic cardiopulmonary response, molecular level response and repetition effect are analyzed. Moreover, by means of the rat model experiment of centrifugal movement, the change of transverse tension transfer of skeletal muscle caused by acute centrifugal movement was analyzed concretely [7]. The study in this paper not only promoted the in-depth understanding of the changes in the transverse tension transfer of skeletal muscle caused by acute centrifugal movement, but also laid a theoretical foundation for the subsequent studies in related aspects [8].

2. Theoretical basis

2.1 Transmission electron microscope

Transmission electron microscope, also known as "TEM", has a long history of development and is an important breakthrough in the traditional optical microscope technology. Compared with the traditional optical microscope, this microscope can clearly display the fine structure less than 0.2um, so the transmission electron heart microscope can also be called an ultrastructure microscope [9]. The light source of the microscope directly determines the minimum object structure that can be recognized. In general, the wavelength of the electron microscope is inversely proportional to its resolution, that is, the shorter the wavelength of the electron microscope, the higher the resolution of the electron microscope [10]. In 1932, based on the electron beam light source, foreign scholars first established the transmission electron microscope, whose wavelength of electron beam is shorter than that of ultraviolet ray and other visible light [11]. At present, 0.2nm is the highest resolution of transmission electron microscope, and the specific imaging principle of this type of microscope is consistent with that of conventional optical microscope. The difference is that electron beam is the light source of transmission electron microscope, and electromagnetic field is lens [12]. In addition, due to the extremely weak penetration of electron beam, the thickness of ultrathin section should be kept at about 50nm during the preparation of electron microscope specimens. The successful preparation of such ultrathin section requires the help of an ultrathin slicer. A transmission electron microscope can magnify an object up to a million times. Lighting, imaging, vacuum, recording and power supply are the five main components.

2.2 Acute centrifugal exercise

The centripetal contraction of the muscle has a priming effect, but the perspective of the centrifugal contraction of the muscle needs to bear extra load, thus producing a certain braking effect. In this case, a movement similar to moving or holding the body is produced, but the muscles undergo centrifugal contractions commensurate with resistance to gravity and braking, a process known as acute centrifugal motion. Acute centrifugal exercise often results in obvious acute reactions, which are mainly reflected in three aspects: metabolic cardiopulmonary reaction, molecular level response and repetitive effect. Metabolism and cardiopulmonary reaction, that is, on the basis of the same specific mechanical output, the metabolism and cardiopulmonary reaction after acute centrifugal exercise are different from that of centripetal exercise. In particular, the specific training load and training intensity of centrifugal exercise should be strictly controlled in the process of exercise. Based on the output of a particular mechanical function, the metabolism required for acute centrifugation is lower than that required for centripetal exercise, and when working at the same efficiency, fewer units are required to participate in acute centrifugation. On molecular level reaction, the existing research has proved that the sports effect on the activation of satellite cells, and verified the acute eccentric exercise can promote muscle growth in the number of nuclei and the concrete content of the satellite cells, this means that based on the stimulation of acute eccentric exercise, activate more satellite cells, the activation can promote maximum muscle satellite cells to rebuild and repair demand satisfaction. The repetitive effect and after repeated acute centrifugal exercises of the same degree will gradually reduce the degree of motor muscle injury. The function of this process is related to the reconstruction of neural adaptation and the mechanism of the external matrix. Therefore, compared with those who did not undergo acute centrifugation for a long time, the trainers were less likely to suffer from motor injuries. Both acute centrifugal exercise and repeated centrifugal exercise can improve creatine kinase activity and oxidative stress level to a certain extent, and the level of the above two substances under acute centrifugal exercise is higher than that under repeated centrifugal exercise, which indicates that the

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degree of muscle injury caused by acute centrifugal exercise is deeper.

2.3 Skeletal muscle

The ultrastructure of skeletal muscle will change under the action of inhabitual exercise, especially centrifugal exercise, and a series of uncomfortable symptoms will appear, such as decreased muscle strength and delayed muscle pain, which are collectively called motor muscle microinjury. This has caused a serious obstacle to the improvement of the overall competitive level of the athletes. Therefore, effective prevention and treatment of skeletal muscle injury has always been the focus of sports medicine research. Current studies show that the changes in skeletal muscle structure caused by acute centrifugation can be quickly restored to the original state by means of relevant interventions. Skeletal muscle tension in essence belongs to a complex, the thread of the skeletal muscle is perimysium, on the one hand, with the aid of perimysium trunk collateral organic cohesion endomysium and perimysium bridging plate membrane, on the other hand also can connect with the aid of perimysium perimysium backbone mainly at the ends of the tendon, so as to realize the skeletal muscle tissue can become a rich tension structure of organic whole. At the present stage, it has been proved that the tension formed by sarcomere contraction is transmitted to the tendon in part by means of longitudinal transmission between sarcomere and sarcomere, and in part by means of transverse transmission of costal body to the endomycoid, and then by means of endomycoid, tunica fascicularis and outer membrane to the tendon. Way of the first pass is called longitudinal tension transmission way, a way of passing is called a transverse tension after transmission mode, horizontal tension of transmission can make function of skeletal muscle as a whole to maintain in a relatively stable state, in the case of sarcomere produce damage, not damage the tension can be formed between adjacent sarcomere lateral transfer with the rib shape body in the form of transmission to the extracellular matrix, and then the transmission of tension to the tendon, so as to avoid have damage of sarcomere again pull over. In addition, in the muscle-membrane costal body of skeletal muscle, there are also unilateral substances that can resist myotrophic dystrophy and α7β1 integrin. The above two are essentially mechanoreceptors, which can realize the body's effective sense of mechanical tension and minimize the possibility of mechanical damage to the muscle-membrane. The above two kinds of material can not only combine with external muscle membrane matrix, also can carry on the combination with myofascial sarcomere, then with the help of mediated internal and external mechanical force signal, realize the whole function of bone and stable function, this also means that, the sensitivity of the rib shape body mechanical receptors on concrete and the horizontal tension of skeletal muscles have correlation between transmission capacity. Present some research has shown that the shear force between two adjacent muscle fibers directly determines the horizontal tension of skeletal muscle eventually transfer efficiency, namely the adaptability of endomysium itself exists, in general there is a proportional relation between the compliance and transverse tension, the greater the compliance of the endomysium, will have the bigger the horizontal tension transfer efficiency, the overall speed of skeletal muscle injury repair will be accelerated. Acute centrifugation can promote the change of skeletal muscle transverse tension to a large extent. Existing studies have shown that acupuncture effect can achieve specific regulation of skeletal muscle transverse tension transfer ability to a certain extent, so as to achieve effective stability of skeletal muscle. Therefore, in this paper, with the help of the animal model of skeletal muscle microinjury caused by acute centrifugal exercise, the author conducted a specific research on the changes of skeletal muscle transverse tension transfer caused by acute centrifugal exercise.

3. Acute centrifugation experiment of rats

3.1 Rxperimental subjects

In this paper, with the aid of the animal experiment in rats, selected the total of 120 healthy male rats, rats for SPF level, weight about 215 g, is provided by a particular animal laboratory rats, and then put the rats in the animal experiment room uniform cage, each cage raise 3-4 of rats, raising the temperature of the environment to keep moderate between 20-23 degrees Celsius, light and darkness once every 12 hours, the rat free diet. First, the rats were allowed to adapt to the feeding environment for a week, during which the rats did not need to undergo any kind of targeted training. This study was carried out under the approval and supervision of the experimental animal management and animal welfare ethics management committee of an undergraduate university.

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3.2 Experimental grouping and specific scheme

After a week of adaptive feeding, the 120 participants were randomly divided into four groups: the quiet group (C,n=12), the centrifugal exercise group (E,n= 36), the acute centrifugal exercise group (EA,n= 36) and the centrifugal exercise intervention group (EAI,n= 36). The above after the completion of the group to establish animal model of acute eccentric exercise its specific plan is as follows: the rats in the animal small slope on the battery run eccentric exercise, exercise time is 90 minutes, run a specific slope as - 16 degrees, centrifugal movement speed is 16 m/min, each group of eccentric exercise for 5 minutes, each group of sports rest 2 min, a total of 18 groups. Prior motor adaptation is not required before the rats begin formal exercise. The specific centrifugal forms and loads of the E, EA and EAI groups were consistent. Rats in group E did not need to take any measures to recover after exercise. The rats in the EA group were subjected to the intervention of toe long and extensor acupuncture after exercise, and the specific effect of the acupuncture was observed. After exercise, the rats in the EAI group were injected with the blocker at the venous position of their tail with the content of the blocker at 0.2% GdCl3. Then the rats were treated with acupuncture 30 minutes later. The specific method of acupuncture was consistent with that of the rats in the EA group, and the blocking effect was then focused.

3.3 Collection and treatment of experimental specimens

At the end of the rat acute eccentric exercise 1 h, 48 h, 120 h after the experiment specimens based, will be in group E, EA and EAI rats were divided into two groups, respectively, in random, that is, a team will be in the rats on the left side of the position of EDL use in measurement of skeletal muscle lateral tension transfer (n = 6), another group of rats on the right side of the EDL under transmission electron microscope to observe (n = 6). After to measure the weight of rats, the concentrations of 2% pentobarbital sodium (65mg/kg) intraperitoneal injection, and then with the help of transmission microscope, the team exposed the right hind leg muscle of rats were observed, and the faster will be the right parts of the EDL, eliminate excess fat and connective tissue, and then obtains the length of 5 mm long muscle belly, placed in an ice bath and concentration of 3% glutaraldehyde were fixed, in the specimens were observed using transmission electron microscopy (TEM). In vivo measurement of skeletal muscle transverse tension transfer group, the specific schemes of anesthesia and the same group, and then to the left hind leg muscle separation processing, remove to the tibialis anterior muscle, allowing exposure of EDL, with an eye will cut the maximum EDL near some tissue to remove excess (pay attention to in the process of experiment in order to avoid damage to muscle scratches, cannot use a scalpel); Then, from the position of the EDL proximal tendon to the position of the terminal tendon, the epidermis of the rat foot was slightly pulled with forceps, so that the ophthalmic scissors and the glass minute needle could fully expose the proximal tendon, and the damage to the blood vessels and connective tissue between the tendons should be minimized during the separation. Finally, the tendon at the proximal end of the EDL was clipped first, and the operative line was connected to the muscle tension sensor to measure the transversal tension. The experiment in this paper measured the specific transverse tension transfer of skeletal muscle in rats by EDL, which was based on the unique physiological structure of the tendon of EDL. Four tendons head together constitute the proximal end tendon of EDL and stop at the position of each phalangeal bone of the 2nd to 5th toe. The proximal tendon is closely connected to the aponeurosis, and the inlet tendon and aponeurosis are in a relatively independent state. The tendon after cut, based on their specific cut order toe Ⅴ ~ toe Ⅱ, arrangement of the specific mode of operation is as follows: first, after weighing the rat body weight, by intraperitoneal injection will use, in the form of pentobarbital sodium (65mg/kg) in the anesthesia in rats. After anesthetizing the rats, the rats were placed in a supine position on a temperature platform with a constant temperature of 37 degrees Celsius. During the experiment, pentobarbital sodium was continuously used to keep the rats in a state of deep anesthesia and prevent them from responding to subtactile stimuli. Second, the left hind limb of the rat was separated to expose the EDL, and the glass minute needle was gently separated from the tendon at the inlet side, so as to avoid damage to the connective tissue between the blood vessels and the tendon to the greatest extent. Thirdly, the surgical line was attached to the inlet side of the EDL, and then the tendon was cut at the proximal side of the EDL, so that the surgical line was connected to the muscle tension sensor, and the site connected by the first, muscle and muscle tension sensor was always on the same level. In order to maintain the excitability of the muscles, ringer's fluid was added. Fourth, stimulate with a stimulating electrode. After the start of electrical stimulation, the experimental data were collected with the help of the biological skills experimental system. Under the current stimulation with the intensity of 6v, the muscle could form the maximum operative tension through the change of the stimulation frequency, and the

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stimulation intensity, specific frequency and the maximum contraction tension of EDL were recorded at that time. Fifth, remove the operative wire connected to the muscle tension sensor at the proximal end of the EDL

3.4 Observation of skeletal muscle structure under transmission electron microscope

In this paper, the preparation and sectioning of transmission electron microscope were carried out by an ultrastructure testing center. Firstly, the fixed EDL was cut into 1mm size and fixed before the concentration of glutaraldehyde. Then, the sections were rinsed with 0.1 mol/L phosphate buffer. Then the relative fixation was carried out by 1% osmium tetroxide. With transmission electron microscope to observe changes of skeletal muscle ultrastructure of the specific situation, specific include between cells and stir outside adjacent matrix structure and changes of the muscle membrane connecting point, and with the help of Image software, observed by transmission electron microscope images for uniform quantization process, specific quantitative treatment scheme is as follows: first, the toe long extensor line structure change; Firstly, the specific area was determined based on the low-power visual field, and then the specific injury of muscle fibers was observed based on the ×1000 business. A total of 10 transmission electron microscope images were selected for each sample, with a total of 10 groups and a total of 60 images for each group. The total number, integrity, fracture and blur of the line were quantified in detail, and the proportion of the number of various categories in the line was calculated. The average number of lines in each sample was 611±30. Secondly, the structure of extracellular matrix between adjacent cells of the extensor longus. Third, the number of PJPs between the two muscle cells of extensor longus was measured.

3.5 Statistical analysis of experimental data

After the completion of the experiment, the experimental data of the above parts were statistically processed with SPSS statistical analysis software, and the statistical results were drawn and formed into a data chart with the help of computer graphics software. On the basis of detailed analysis of the data chart, relevant experimental conclusions were drawn.

4. Discussion on experimental results

4.1 Experimental results

Through the above acute centrifugal movement model experiment on rats, the author sorted out the following experimental data. The specific data are shown in the chart. The data in the chart are the results of the author's experiment.

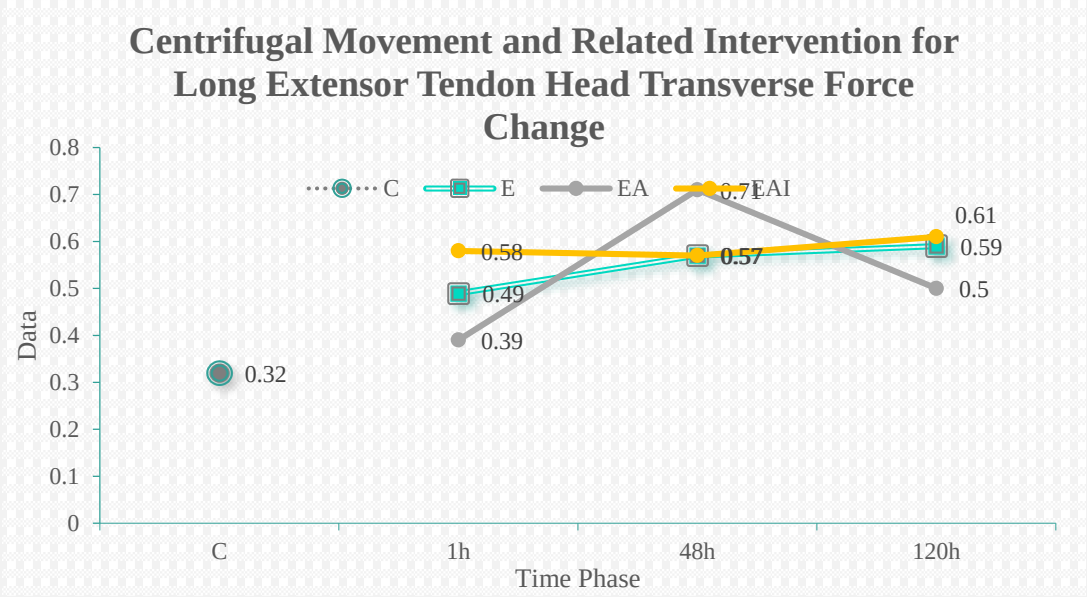

Figure 1. The centrifugal movement and related intervention for long extensor tendon head Ⅲ transverse force change

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By data we can see in Figure 1, tendons head Ⅲ transverse force change under the influence of processing factors of the most significant, time factors on the impact is not obvious, based on the interaction of both, its obvious influence the variation of tendon head Ⅲ transverse force, and its specific changes are as follows: first, compared with C group of rats, the values for the other three groups each time to show a rising trend, and the rats in group E, 48 h and 120 h period transverse force is obviously increased, the EA group rat tendon transverse force in 48 h period improved significantly, and reached the peak, peak of 0.71; Second, compared with the same group at different time periods, the transverse force of rat tendons in group E showed a stable increase, with the maximum value at 120h. The overall trend of transverse force in EA group was first up and then down. The transverse force reached the highest value in 48h period, and its horizontal force was significantly higher than that in 1h period. In 120h period, there was a rapid fall, from 0.71 to 0.5. Thirdly, the comparison of different time periods between different groups showed that the transverse tendon force of rats in the EA group was significantly lower than that in the EAI group at 1h, and significantly lower than that in the E and EAI groups at 120h, and higher than that in the E and EAI groups at 48h. In summary, it can be concluded that after 48h of intervention, the transverse force of the tendon was significantly increased and gradually returned to normal after 120h, which means that appropriate intervention can help the effective recovery of the overall function of skeletal muscle by increasing the transverse tension transfer.

4.2 Discussion of experimental results

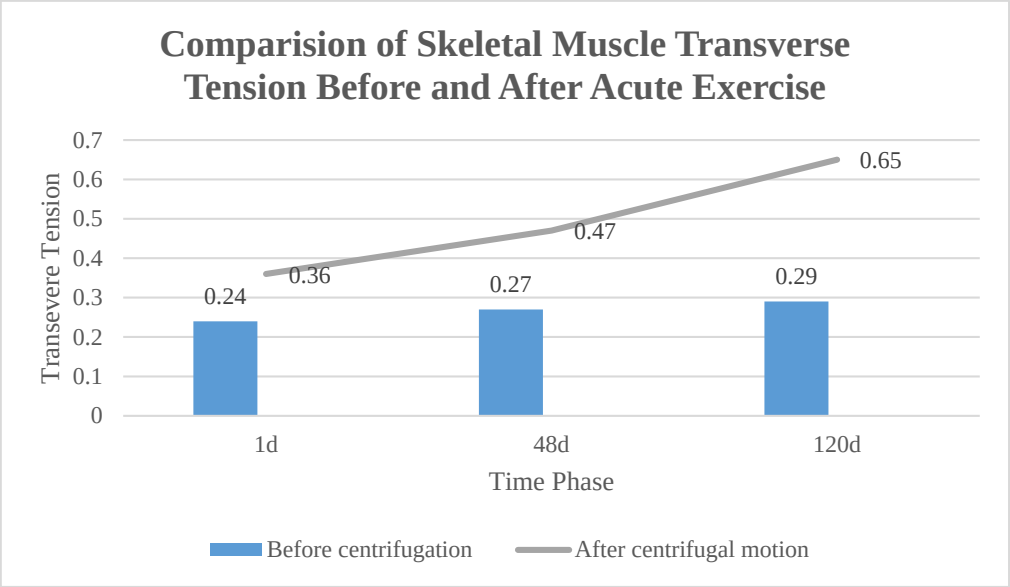

Figure 2.Comparison of skeletal muscle transverse tension before and after acute centrifugation

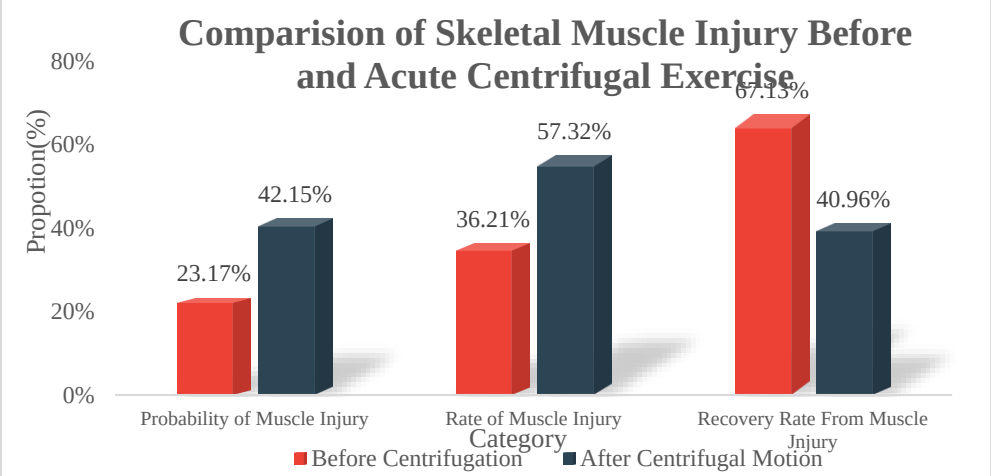

Figure 3. Comparison of skeletal muscle injury before and after acute centrifugal exercise

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The data in Figure 2 showed that, compared with the intensity of acute centrifugal exercise, the transverse tension of skeletal muscle increased significantly after acute exercise, from 0.36 to 0.65, with a change range of 0.29. The lateral tension before acute exercise increased from 0.24 to 0.29, with a change of only 0.05. The data in Figure 4 showed that the probability and speed of skeletal muscle injury before centrifugal exercise were significantly lower than those after acute centrifugal exercise, and the recovery rate of skeletal muscle injury was significantly higher than that after acute centrifugal exercise. Combined with the above data, it can be concluded that in the experiments conducted in this paper, acute centrifugal exercise significantly increased the transverse tension transfer of skeletal muscle. The results of the study showed that acute centrifugal exercise could increase the expression of it, because it is a feedback regulator capable of effective mechanical force transduction, and plays an important role in the transmission of motor muscle injury and lateral muscle force. This suggests, to some extent, that after the completion of acute centrifugation, the increase in the transfer of total lateral force and tension is largely due to the activity or increased expression of α7β1integrin on the musclemembrane. The existing research data indicate that calc-dependent proteolytic enzyme activation can increase the activity of related enzymes by further integration of skeletal muscle. Furthermore, acute centrifugation has been shown to activate Ca2+ and calpain in skeletal muscle cells. The experiments in this paper do not prove whether or not α7β1integrin increased expression. The results showed that the proportion of transverse tension transfer of skeletal muscle was more than 70% in the sarcomere contraction tension, and the increase of transverse tension transfer played an important role in the stability of sarcomere structure and the overall maintenance of skeletal function. Therefore, the gradual increase of the total lateral force of skeletal muscle after acute centrifugation is closely related to the repair of skeletal muscle injury. However, the value of skeletal muscle transverse tension transfer was the largest at 1h after the end of acute centrifugation, which gradually decreased after 48h and rose after 120h, which was largely related to the degree of skeletal muscle microinjury and the specific phase of Ca2+ concentration change. In this experiment, after the acute centrifugal movement in skeletal muscle related muscle line of a certain degree of fracture and fuzzy, the collagen area also increased, this shows that the eccentric exercise can make endomysium inside and outside the structure produce a certain degree of change, making the ultrastructure of skeletal muscle in a certain degree of damage. Acute after 48 h after eccentric exercise its skeletal muscle lateral tension is lower than at the end of 1 h, thanks in large part because of eccentric exercise 48 h, the skeletal muscle cytoplasm Ca2 + concentration within the rapid ascension, which makes its dependence of calpain greatly enhance, promote further degradation of skeletal muscle α7β1integrin, which reduces the horizontal tension. After the end of the centrifugation, the transverse tension at 120h was slightly higher than that at 48h, which should be related to the reduction of the damage degree of the zen position structure. Based on the changes in the external matrix of skeletal muscle cells, some scholars have found that acute centrifugal exercise can improve the concentration of the collagen band to a certain extent, and the muscle bundle membrane is the main attachment site of collagen fiber. This conclusion has supported the conclusion drawn in this paper to a certain extent. In addition, the increase of the thickness of the external matrix, especially the muscle bundle membrane, also leads to a decrease in the transverse tension transfer to some extent. To sum up, acute centrifugation can promote the continuous improvement of transverse tension transfer of skeletal muscle, which is caused by the increased activity or expression of α7β1integrin on the musclefilm of skeletal muscle.

5. Conclusion

To sum up, this paper studied the specific effect of acute centrifugal exercise on lateral tension of skeletal muscle through acute centrifugal exercise model experiment in rats. The research in this paper not only promotes the further understanding of the relationship between the two, but also lays a theoretical foundation for the subsequent research on the relevant aspects. This paper draws the following conclusions through research:

(1) Acute centrifugal exercise can promote the increase of skeletal muscle transverse tension;

(2) There is a close relationship between the transverse tension transfer and the degree of skeletal muscle injury. It can be said that as an indicator of the body's adaptation to exercise intensity, it has a relatively important practical significance.

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