# *Rats with Myocardial Ultrastructure Changes Caused by Heavy Exercise*

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***Keywords:*** Heavy exercise, Myocardial ultrastructure, Keep moving, Mouse experiment

***Abstract:*** Objective: To observe and study the ultrastructural changes of myocardium in the normal training mice and the overactive mice by establishing the exercise models of the mice with different amount of exercise. Methods: 90 rats were randomly divided into control group, general exercise group and high exercise group. All the mice began to carry out the experiment after a one-week adaptation cycle. The normal control group lived normally and did not exercise. The ordinary exercise group trained for 5 days a week. The limit speed was set at 20m/min, and the slope of the platform was 5%. The high-exercise group trained 7 days a week with a limit speed of 35m/min and a platform slope of 10%. During the whole exercise experiment, the electric stimulation method was used to force the rats to carry out exercise training, which lasted for 10 weeks. After the training, the mice lived normally for 20 weeks. Light microscopy, electron microscopy and immunohistochemistry were used respectively. The experimental data were processed by Sigmaplot scientific plotting system, and the experimental results were expressed by means of mean±standard deviation. Results: The common movement group rats myocardial ultrastructure changes in myocardium will be physiologic hypertrophy, myocardial mitochondria of rats changed much, myocardial volume larger, myocardial swelling volume, myocardial structural integrity, cardiac muscle wire overall and orderly, mitochondrial membrane are clear and complete the nucleus has increased obviously and the number of nuclei increased significantly. The ultrastructure of the myocardium of rats in the high-intensity exercise group was distorted, the myofilament was cracked, the mitochondrial membrane was damaged and obviously incomplete, the granulosity was obvious, and the myocardial structure was damaged. Conclusion: The suitable aerobic exercise makes the cardiomyocytes adapt to the combination of cardiomyocytes and cardiomyocytes, which is helpful to the health of the heart and improves the function of the heart. Excessive exercise will make the heart to the unhealthy direction of development, damage the performance of the heart; the damage degree of myocardial structure was significantly correlated with the amount of exercise. Excessive exercise over a long period of time can lead to pathological changes in the heart muscle and an increased risk of sudden death.

## 1. Introduction

The effect of high exercise on the ultrastructural changes of myocardium has been a common concern. The periodic endurance sports mostly adopt the training method of large amount of exercise. However, in recent years, the sudden cardiac death of athletes in the process of sports competition is common. There have also been cases of cardiac arrest in recreational activities outside of sports because of overload. The phenomenon of cardiac arrest and sudden cardiac death has attracted wide attention from various groups. However, there are few studies on the effects of overloading exercise on the ultrastructure of myocardium. It must be recognized that the health of the heart not only affects the health of people's lives, but also directly relates to the safety of life. Overload exercise causes damage to the cardiac structure, ischemia and hypoxia, as well as chromatin abnormalities and mitochondrial thickening. Therefore, through animal experiments to explore the characteristics of ultrastructural changes in cardiac muscle caused by high-exercise training and the possible damage to cardiac muscle, it can provide useful guidance for scientific training and provide morphological basis for cardiac health care.

Recent studies have shown that the heart is not only a circulatory organ used to complete the function of blood ejection, but also produces a series of cardiac hormones by means of paracrine, autocrine, perisecretion and cellular endocrine [1]. Literature [2] reported that cardiovascular regulation plays an important role in regulating the adaptive remodeling of cardiac function and structure. The study also mentioned the influence of different amounts of exercise on the ultrastructure of myocardium, providing a training basis for the study of overload amount of exercise. In the literature [3], the authors note that exercise can cause changes in cardiac hypertrophy, myofibril thickening, the number of mitochondria in cardiac myocytes, and sarcoplasmic reticulum expansion, which are considered compensatory changes in the fitness of the heart. According to literature [4], pathological changes such as mitochondrial swelling of cardiomyocytes, loss of stepped structure of leap disk, abnormal nuclear chromatin and serpentine curling were observed after heavy exercise. Literature [5] pointed out that the correlation between exercise intensity and changes in myocardial ultrastructure was still rare, so the pathogenesis of sudden cardiac death and syncope could not be clearly determined. Literature [6] report found that the mice swimming running training in the same conditions of left atrial perfusion pressure, the reaction of myocardial performance index ejection phase of myocardial fibers maximum shortening rate, left ventricular pressure rise rate, cardiac output, and ejection fraction in a large increase, and the training group rats cardiac pump function and myocardial contractility and not exercise training is significantly higher than the control group rats. Studies in literature [7] indicate that the maximum rate and the maximum contractile tension of the ventricular papillary muscle unit in swimming trained rats are significantly higher than those in control rats without exercise training, suggesting that exercise training can enhance the contractile performance of the rats' hearts, but excessive load exercise will produce the opposite effect. According to the report in literature [8], the mechanical indexes of all aspects of the myocardium of rats after heavy exercise showed a significant decline, indicating that the diastolic and systolic properties of the myocardium were seriously affected.

There have been sufficient studies on cases of cardiac arrest or sudden cardiac death caused by overloading exercise in the sports and entertainment industries, but there are few studies on the effects of heavy exercise on changes in myocardial ultrastructure [9]. The mechanism of action of different exercise loads on the body is quite different, and the heart function is directly related to the life safety of the body [10]. In order to fully understand the influence of high exercise on myocardial ultrastructure, this study established the movement model of rats with different exercise amount to find out the changes of cardiac ultrastructure under different loads, and provided a supervisory basis for medical intervention under overtraining [11].

## 2. Literature Review on Exercise and Muscle Cell Research

### 2.1 Myocardial

In the mature myocardial tissue, the intermediate fibrin is mainly the knot protein, which accounts for more than 90% of the whole myocardial intermediate fiber composition, and is uniformly distributed in the myocardial cells. In addition to the knot protein, vimentin is next, accounting for about 10% of the cardiac muscle cells, which is mainly distributed in the cardiac muscle connective tissue. In the muscle fiber gap, the fibrin longitudinal fiber and muscle fiber show a parallel trend, connecting the mitochondria and terminating the desmosomes of the myocardial leap disk. The crosslinked network of protein nodes forms a ring band, which forms a lateral connection between the z-lines, encircles the muscle fibers, and connects the nuclear membrane with the muscle membrane.

In cardiomyocytes, the knot protein is most closely related to the nuclear skeleton, forming a unique intermediate fibride-nuclear skeleton - nuclear lining system, which means that the knot protein plays an important role in the nuclear signal transmission and material transport. In addition, defibrin plays a very important role in the localization of organelles and contractile devices of cardiomyocytes. It keeps the normal structure of the muscle cells in the process of relaxation and contraction, and integrates the cardiomyocytes into a contractile whole.

How to observe the function of intermediate fiber in the body has been a hot topic in recent years. It has been pointed out that knot protein may be related to leap disc membrane biosynthesis, and has certain influence on the physiological activities of cardiomyocytes. The most common method to observe the function of the intermediate fiber in the body is to knock out the intermediate fiber gene by means of gene recombination technology, observe the changes brought by the dominant genetic deficiency of the intermediate fiber in the body, and analyze the function of the intermediate fiber in the body. Gene recombination technology can also be used to remove the knot protein in the tissue and observe the effect of the knot protein free state on the body. Studies have shown that experimental mice with a lack of glucoprotein dominant inheritance can survive, but multiple organs of the mice were damaged, and the myocardial tissue was the most severely damaged. These findings were most pronounced in 2- to 3-week-old mice, where large areas of myocardial tissue necrosis and calcification were observed. Myocardial fibers are dispersed and then surrounded by cytoplasmic fibrous tissue, and the leap disk of adjacent tissue is also affected. There are also corresponding changes in organelles, especially in mitochondria, and the abnormal accumulation of mitochondria in myofibril makes it more obvious after myocardial overload.

When measuring the mechanical function of the rat myocardium, it was found that the ventricular diastolic blood pressure increased as the ventricular weight increased, but the myocardial systolic protein muscle and actin did not change. These results indicate that the ventricular wall thickens, cardiac function deteriorates, cardiomyocytes are disordered, and cardiac structure is remodeled after the loss of fibrin. It can be seen that knot protein has an important influence on the generation and transmission of cardiac muscle cells.

### 2.2 Effects of Different Exercise Loads on Myocardial Contractility

Myocardial contractility is closely related to the biochemical reaction and energy conversion in the process of myocardial contractility, and it is also an internal factor that determines the pumping function of the heart. The pumping of blood by the heart depends on the combined effects of three variables of the myocardium itself: preload, afterload, and contractility. The current research results suggest that myocardial contractility is a property closely related to the internal energy conversion rate and biochemical reaction in the process of myocardial contractility, and it is an internal

function that can change the rate and force of myocardial contractility without depending on the length and load state of the myocardium.

In order to directly measure the myocardial systolic performance, exclude the influence of exercise load, and determine that the myocardial systolic performance is the core factor determining the cardiac function, many scholars, based on the theory of myocardial mechanics, proposed to measure the myocardial systolic performance by myocardial shortening speed, isovolemic systolic left ventricular pressure change speed and other indicators. Some scholars also believe that these indicators have a weak recognition of changes in myocardial systolic performance without excellent interference and exclusion factors, so they propose to measure the myocardial systolic performance with the instantaneous acceleration index of left ventricular pressure. Some other scholars took endurance swimming training as a model and observed and studied the histological changes and left ventricular systolic performance indexes of experimental mice under different loads. The results showed that with the increase of exercise load, the left ventricular hypertrophy of the mice gradually increased, and the physiological compensatory phenomenon of the myocardium was obvious. After swimming training rats heart in the same conditions of left atrial perfusion pressure, the ejection phase of myocardial fibers maximum shortening rate, ejection fraction and left ventricular pressure rise rate and cardiac output were largest than swimming training rats increased, the unit of papillary muscle contraction area of isometric contraction tension was also significantly higher than not swimming training with a control group. Under hypoxia condition, the maximum rate and cross-sectional area of ventricular papillary muscle units in mice after swimming training were also significantly higher than those in the control group, suggesting that moderate exercise training enhanced the contractility of the experimental mice. However, excessive amount of exercise may also cause damage to the myocardial diastolic performance and systolic performance. After the rats underwent strenuous exercise, the myocardial mechanical indexes of the rats showed a significant decline, indicating that the myocardial diastolic performance and systolic performance were seriously damaged.

### 2.3 Pathological Mechanism of Sports Myocardial Injury

Existing studies have confirmed that mRNA expression of vascular endothelial growth factor (VEGF) increases in hypoxic pathological models, regardless of whether multiple tissues are in a state of ischemia or hypoxia. In the ischemia model, the mRNA of vascular endothelial growth factor (VEGF) was expressed rapidly after 30min and lasted for several hours after perfusion for about 5 minutes, and this phenomenon also existed in the heart. After the left coronary artery was ligated in mice, the mRNA of vascular endothelial growth factor could be measured at 30min and expressed rapidly for 3 h, and then began to decline after 6 h. Further analysis revealed that the mRNA content in the ischemic left ventricle was significantly higher than that in the right ventricle, suggesting that vascular endothelial growth factor was associated with myocardial ischemia and hypoxia related diseases. Abnormal expression of vascular endothelial growth factor can be found in animal models of myocardial injury caused by various animal types and forms, suggesting that VEGF is related to the repair of myocardial injury. To observe the effect of vascular endothelial growth factor on hemodynamics, intravenous injection of recombinant vascular endothelial growth factor into rats that were awake caused an increase in heart rate and a decrease in mean arterial pressure, but did not affect the output. In the isolated rat heart, vascular endothelial growth factor had no effect on heart rate and contractility, suggesting that vascular endothelial growth factor resulted in a decrease in the amount of cardiac resuscitation, and the decrease in the amount of exudation was caused by its vasodilation.

In the cardiovascular system, a large number of cells are withered, accompanied by the process

of cardiovascular differentiation, proliferation and maturation. These cells are induced by a variety of thorn source factors to participate in the pathological changes of cardiovascular diseases, and jointly constitute the cytological basis for the occurrence and development of cardiovascular diseases. Of rats caused by excessive training rats myocardial apoptosis study found that compared with appropriate training and the excessive training is not trained rats significant differences fall cells, was the hypoxia training in the training process, the phenomenon such as ischemia, heart overload capacity, with a large number of free radical and cytokine production, a combination of these factors make the damage morphology changes have taken place in heart structure and function. Flow cytometry showed that the number of myocardial cell apoptosis in rats was closely related to the intensity of exercise, and both high-intensity exercise and exhaustion exercise could significantly increase the number of myocardial cell apoptosis. It can be concluded that it is related to the pathogenesis of sports myocardial injury, and appropriate exercise can stimulate cardiac hypertrophy and remodeling, but the specific mechanism still needs to be further studied.

## 3. Experiment on Mice with Myocardial Ultrastructure Changes Caused by Exercise Training

### 3.1 Selection of Experimental Subjects

Ninety 3-week-old mice were selected, all of which were provided by the laboratory animal management center of a medical university. The weight of the mice was all 70±10g, and there was no restriction on male or female magnetism. Mice were partially caged for the experiment, drinking water freely and eating freely within a week of adaptation, and were fed with solid mixed feed according to national standards. The temperature inside the cage is set at 20-25 degrees Celsius, and the environmental relative humidity is maintained at 50-60%. These mice were randomly divided into three groups, 30 mice in each group, and named as the normal control group, the normal exercise group and the high-exercise group.

### 3.2 Sports Training Program

At the end of the one-week adaptive cycle, the mice began to exercise. The control group continued to live in the cage without any exercise intervention. The normal exercise group and the high-exercise group adopted the method of increasing intensity for platform exercise. The initial platform speed of the training was 10m/min, and the time was set at 10 minutes. The normal exercise group trained 5 days a week, with an increasing speed of 3m/min and an increasing speed every 5 minutes. The limit speed was set at 20m/min, the exercise time was set at 60 minutes a day, and the platform slope was 5%. The high-exercise group trained 7 days a week with an increasing speed of 3m/min and an increasing speed every 5 minutes. The limit speed was set at 35m/min, and the exercise time was set at more than 60 minutes but not more than 90 minutes a day. The slope of the platform was 10%. During the whole exercise experiment, the electric stimulation method was used to force the rats to carry out exercise training, which lasted for 10 weeks. After the training, the mice lived normally for 20 weeks. Light microscopy, electron microscopy and immunohistochemistry were used respectively.Experimental Instruments and Reagents is show in Table 1.

*Table 1: List of main experimental reagents and instruments*

| Reagent/instrument | Manufacturers |
|---|---|
| Urethane | Shanghai chemical reagent co. LTD |
| Anhydrous ethanol | Xi 'an mipu fine chemical plant |
| Xylene | Beijing Oriental chemical factory |
| Heparin sodium | Shanghai greenbird technology co. LTD |
| Formaldehyde | Chongqing beipei fine chemical plant |
| Glutaraldehyde | Shanghai xingzhi chemical plant |
| Sodium dihydrogen phosphate | Henan jiaozuo no. 3 chemical plant |
| Disodium hydrogen phosphate | Xi 'an chemical reagent factory |
| Hematoxylin | Shanghai chemical reagent procurement and supply station |
| Type 201-3 wet thermometer | Shanxi medical instrument factory |
| Thermostatic water bath | Oriental electrical instrument factory |
| Siemens refrigerator | Siemens electric appliance co. LTD |
| Electronic balance | Yuyao jinnuo tianping co. LTD |
| 6240 physiological multiple recorder | Chengdu instrument and equipment factory |
| Olympus optical microscope | OLYMPUS, Japan |
| H-1 mini vortex mixer | Shanghai jingke industrial co., LTD |
| Ultrapure water meter | MILLIPORE products, USA |
| Hh-60 constant temperature display tank | Shenzhen guohua instrument factory |
| BX51 digital image capture system | Japanese OLYMOUS product |

### 3.3 Index Testing and Methods

The body temperature of mice was measured with the type 201-3 wet thermometer produced by shanxi medical instrument factory, and the weight of mice was measured with the electronic balance produced by jinnuo tianping co., LTD. The ecg adopts standardized type ii limb lead, and the ecg signal input sensitivity is set at 10mm/1mv. The lead is connected with 75% alcohol-sterilized head acupuncture into the limbs, and the alligator clip is connected with the lead. The observed indicators included t-wave voltage, QRS duration, heart rate, QT interval and QRS voltage, and these indicators were compared with the normal ecg, so as to obtain the ecg characteristics caused by different training intensity. The whole test process includes the following steps.

Rats were anesthetized with pentobarbital injection at 40mg/kg through the abdominal cavity. The rats were fixed on the laboratory table on their back. Intravenous heparin was injected at 0.2ml /100g, intubated into the left ventricle with polyethylene catheter, and the blood stasis was irrigated with normal saline. The pulmonary artery was opened for bleeding and fixed by the infusion of 2.5% glutaraldehyde phosphate buffer, with the perfusion pressure of 1.8x105~ 2x105kPa, pH=7.3. After 0.5h of perfusion, the heart was removed, the atria were removed, and 1/3 ventricular muscle crosscutting mass was cut along the spindle.

Dehydration and immersion: dehydration was carried out with acetone dehydrating agent, EPON812 mixture and propylene oxide were selected as the immersion solution.

Fixation: 1% osmium tetroxide was used for anterior fixation and 2.5% glutaraldehyde for posterior fixation.

Polymerization and embedding: polymerization was completed by adjusting the temperature, embedding agent EPON812 was selected, and the sample was put into a dry oven with automatic

temperature control to perform embedding operation.

Section and staining: LKB - NOVA hot expansion ultrathin slicer made in Sweden was used for slicing and double staining with uranium dioxy acetate and lead citrate.

Observation: JEM-2000EX transmission electron microscope made in China was used for observation and photographing.

### 3.4 Statistical Methods

All the measured data were processed and input into the statistical analysis software SPSS 18.0. One-way ANOVA program was used for one-way ANOVA analysis. The experimental data were processed by Sigmaplot scientific plotting system.

## 4. Experimental Results

### 4.1 Changes of Myocardial Ultrastructure in Rats at 20 Weeks after the End of Training

It was found that the myocardial structure of the control group and the normal exercise group was normal. The euchromatin is uniformly distributed in the nucleus, which is elliptical or irregular, and distributed in the center of the cell. The heterochromatin is distributed along the subnuclear membrane, and the nucleoli are clearly visible. The z-line has clear structure and regular arrangement, and the myofibrils have regular sarcomere. Mitochondria are located between myocardial fibers with dense cristae and large volumes. In the vicinity of the z-line, it can be seen that sarcoplasm is scattered among muscle fibers, and a small amount of scattered collagen fibers are distributed among cardiomyocytes. The leap disc structure of cardiomyocytes is clear.

The intermuscular mitochondria of rats in the high-intensity exercise group were obviously proliferated and hypertrophy, the myofibril structure was loose, and some cardiomyocytes and mitochondria were mildly swollen. The collagen fibers between cardiac muscle cells increased significantly, and the leap disk structure was basically clear. Part of the collagen fibers were inserted into cardiac muscle cells, resulting in fibrosis in the heart.

### 4.2 Changes in Cardiac Physiological Function of Rats after 20 Weeks of Training

The changes in cardiac physiological function of rats in each group after stopping training for 20 weeks were shown in Table 2 and Figure 1.

*Table 2: Experimental results of changes in cardiac physiological function of mice*

| Group | Mean arterial pressure(Kpa) | Left ventricular pressure (Kpa) | +dp/dtmax(Kpa.s$^{-1}$) | -dp/dtmax(Kpa.s$^{-1}$) |
|---|---|---|---|---|
| Control group | 11.82±0.53 | 5.37±0.47 | 504.67±11.21 | 352.68±21.37 |
| General exercise group | 11.42±0.45 | 5.82±0.42 | 500.73±18.26 | 365.28±12.42 |
| Heavy exercise group | 12.36±0.57* | 5.66±0.64* | 483.19±12.68** | 337.26±20.27** |
| *P<0.05 and **P<0.01 were compared with the control group | | | | |

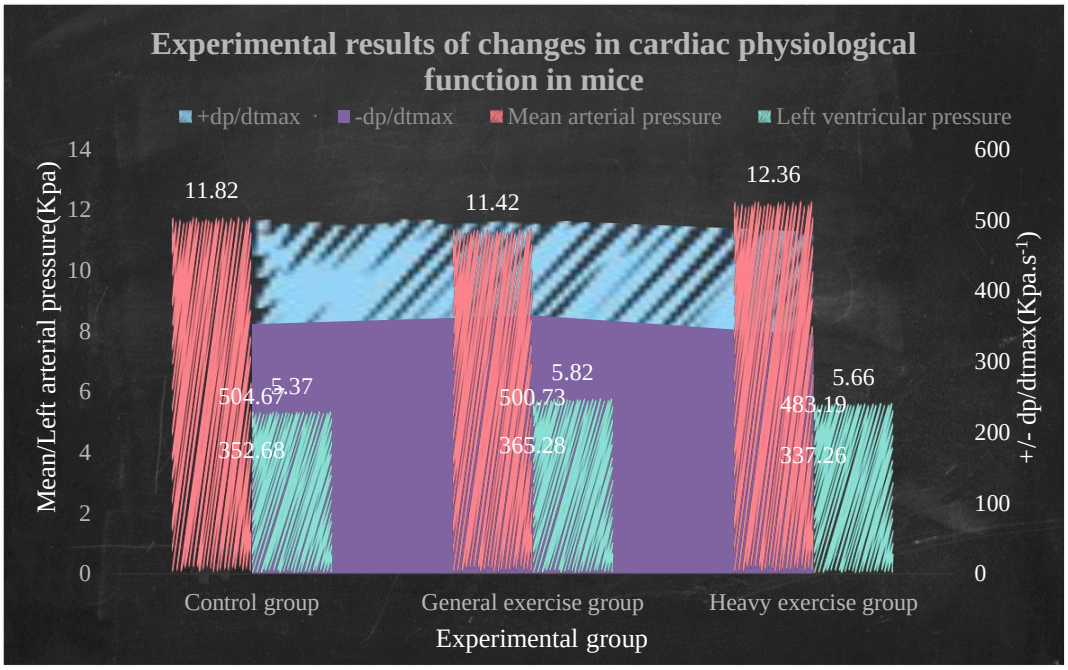


*Figure 1: Experimental results of changes in cardiac physiological function in mice*

It can be seen from the test results that, compared with the control group, there is no significant difference in the indicators of the ordinary exercise group, but the differences between the high-exercise group and the control group and the ordinary exercise group are statistically significant. The mean arterial pressure of rats in the high-exercise group increased, suggesting that the cardiac pressure reached the overload state. There were significant changes in -dp/dtmax reflecting the myocardial diastolic function and +dp/dtmax reflecting the myocardial systolic function. Full stop training after 20 weeks, under normal exercise load, the changes of the above indicators are not obvious, the cardiac function of mice also failed to return to normal levels, tip overload exercise training can lead to the diastolic function and myocardial contractility of mice serious damage, and even stop high intensity training hard to return to normal levels.

## 4.3 Changes of Electrocardiogram in Rats after 20 Weeks of Training

The results of electrocardiogram changes of rats in each group after stopping training for 20 weeks are shown in Table 3 and Figure 2.

*Table 3: Experimental results of electrocardiogram changes in mice*

| Group | Heart rate (seconds/min) | QRS duration (MS) | QT interphase (MS) | QRS voltage ($MV \times 10^{-2}$) | T-wave voltage ($MV \times 10^{-2}$) |
|---|---|---|---|---|---|
| Control group | 433.64±26.77 | 20.67±2.06 | 38.2±5.27 | 19.66±1.52 | 6.27±0.83 |
| General exercise group | 421.48±26.95 | 20.65±2.41 | 38.28±2.82 | 19.79±2.58 | 6.92±1.18 |
| Heavy exercise group | 476.36±25.54*# | 22.62±2.18 | 41.53±6.16 | 17.63±1.73*# | 2.74±0.35*# |
| *P<0.05 was compared with the control group, and #P<0.05 was compared with the high-exercise group | | | | | |

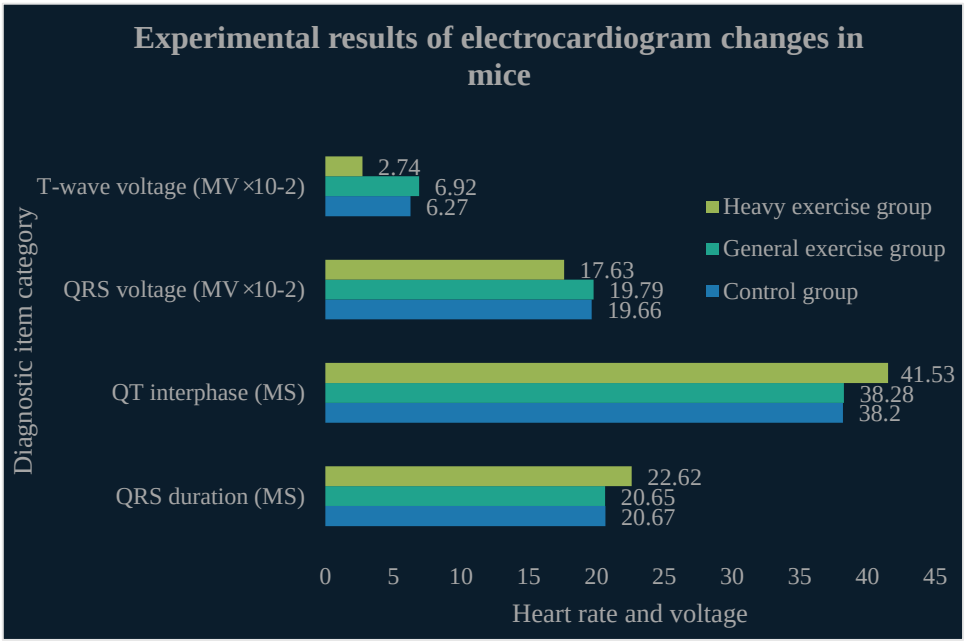


*Figure 2: Experimental results of electrocardiogram changes in mice*

As can be seen from Table 3 and Figure 2, there were significant differences in heart rate, T-wave voltage and QRS voltage between the control group and the high-exercise group, while there were no significant differences in QT interval and QRS duration between the experimental mice. After T test, there was no significant difference between the control group and the normal exercise group (P>, 0.05), while there was a significant difference between the high-exercise group and the normal exercise group and the control group (P<0.05). Therefore, the increased QRS wave group voltage was often clinically diagnosed as left ventricular hypertrophy.

## 4.4 Changes in Body Weight and Heart Weight of Rats after 20 Weeks of Training

The changes of body weight and heart weight in each group were shown in Table 4 and Figure 3 after stopping training for 20 weeks.

*Table 4: Weight and heart weight of mice*

| Group | Body weight (g) | Heart weight (mg) | Heart weight/body weight (mg/g) |
|---|---|---|---|
| Control group | 563.48±21.57 | 1542.56±89.25 | 2.74 |
| General exercise group | 567.36±71.45 | 1568.26±52.58 | 2.76 |
| Heavy exercise group | 565.27±36.81 | 1668.92±37.31*# | 2.95 |

*P<0.05 was compared with the control group, and #P<0.05 was compared with the high-exercise group

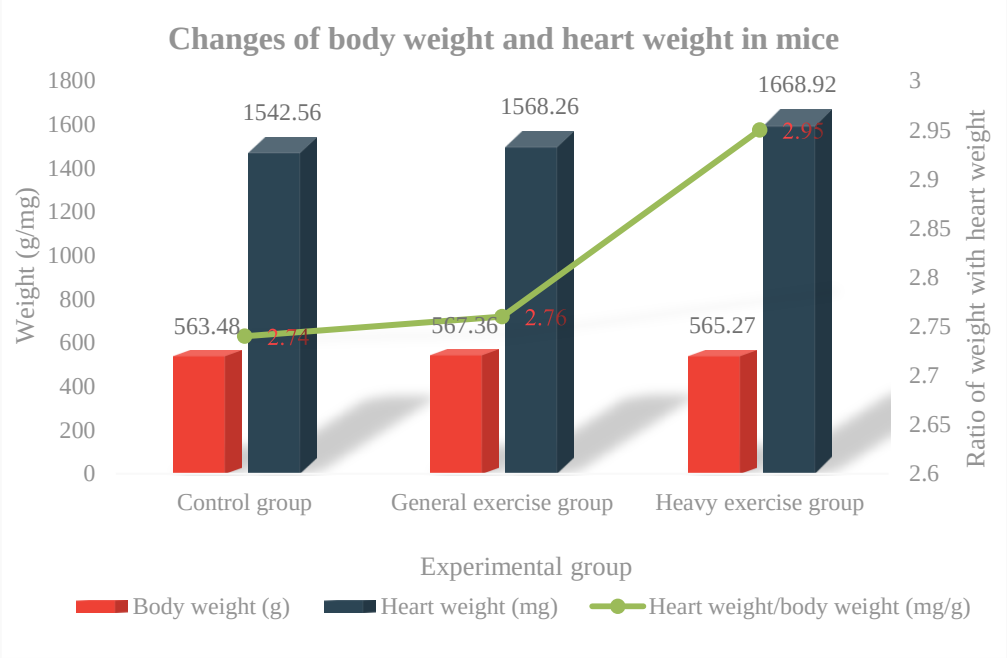


*Figure 3: Changes of body weight and heart weight in mice*

From the results it can be seen on the chart, the changes of mice heart heavy weight/body weight coefficient is the great physiological load of exercise group > normal group > the control group, between groups increase trend, the change of the T test shows, there is no significant difference between control group and normal movement group ($P > 0.05$), while the great physiological load of exercise group with significant difference between normal exercise group and control group ($P < 0.05$). It was suggested that after 20 weeks of recovery, the hypertrophy of the mice in the normal exercise group could return to the normal level, while the hypertrophy of the mice in the overload exercise group was non-recoverable.

## 4.5 Changes in Immunohistochemical Index of Mouse Ventricular Myocytes after 20 Weeks of Training

The gray values of the expressions of nNOS, iNOS and eNOS in the ventricular myocytes of rats in each group were compared after 20 weeks of stop training, as shown in Table 5 and Figure 4.

*Table 5: Comparison of the gray values of the expressions of nNOS, iNOS and eNOS in mouse ventricular myocytes*

| Group | Number of cases | Left ventricular nNOS grey value | Right ventricular nNOS grey value | Left ventricle iNOS gray value | Right ventricle iNOS grey value | Left ventricle eNOS grey value | Right ventricular eNOS grey value |
|---|---|---|---|---|---|---|---|
| Control group | 30 | 73.26±2.33 | 79.32±1.09 | 80.56±1.97 | 80.84±2.75 | 74.49±2.17 | 86.42±1.72 |
| General exercise group | 30 | 67.71±3.54* | 78.15±4.61 | 79.72±1.73 | 80.65±2.19 | 72.42±1.42** | 84.73±1.58 |
| Heavy exercise group | 30 | 78.27±2.09**$^{\#\#}$ | 82.31±2.17**$^{\#\#}$ | 71.36±2.05**$^{\#\#}$ | 78.73±1.14*$^{\#}$ | 79.58±1.26**$^{\#\#}$ | 89.51±2.09*$^{\#\#}$ |
| *P<0.05, ** P<0.01 compared with the control group, and $^{\#\#}$P<0.01 compared with the normal exercise group | | | | | | | |

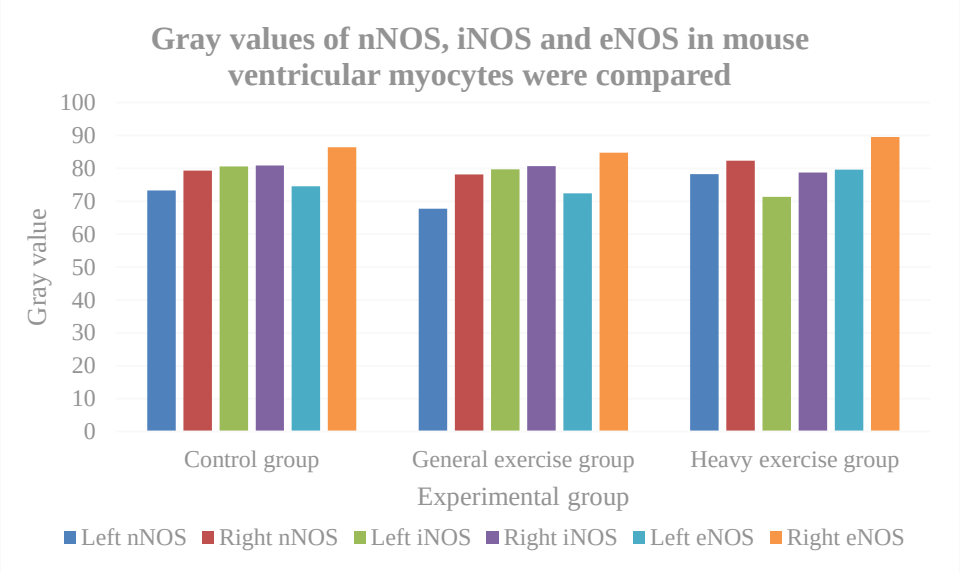


*Figure 4: Comparison of the gray values of the expressions of nNOS, iNOS and eNOS in mouse ventricular myocytes*

The results showed that in terms of the nNOS table of mouse left ventricular muscle cells, there was an extremely significant difference between the high-exercise group and the normal exercise group and the control group, and there was a significant difference between the control group and the normal exercise group. In the right ventricle, there was an extremely significant difference between the high-exercise group and the control group and the ordinary exercise group, while there was no significant difference between the control group and the ordinary exercise group.

In the expression of left ventricular muscle cells in mice, there was a significant difference in iNOS between the high-activity group, the control group and the normal exercise group. In the right ventricle, there was a significant difference in iNOS expression between the high-activity group, the control group and the normal exercise group, while there was no significant difference between the control group and the normal exercise group. In other words, the gray value of iNos in the left ventricle of rats in the high-activity group was significantly different from that in the control group and the normal exercise group, and there was a significant difference in the right ventricle. There was no significant difference in the expression of iNos in the left ventricle and the right ventricle between the normal exercise group and the control group.

In terms of the expression of eNOS in mouse left ventricular muscle cells, there was a significant correlation between the large exercise group, the normal exercise group and the control group, and there was a significant difference between the control group and the normal exercise group. In the right ventricle, there was a significant difference between the high-exercise group and the normal exercise group, and a very significant difference between the control group and the normal exercise group, while there was no significant difference between the control group and the normal exercise group.

## 5. Analysis and Discussion on Changes of Myocardial Ultrastructure in Mice

Prior to this study, a large number of literatures have reported the changes in myocardial ultrastructure that may be caused by high-intensity exercise, but they mainly focus on the degenerative changes in the structure of mitochondria and sarcoplasmic reticulum, such as the disorder or fracture of myofibril, unequal or asymmetric sarcomere length and so on. In this study,

the ultrastructural changes of rats with different training intensities were observed and compared after the complete suspension of training for 20 weeks. The results showed that the structure of cardiac muscle cells in the normal exercise group and the control group was basically normal. The nucleus was located in the center of the cell, with an oval or irregular shape. The euchromatin was evenly distributed in the nucleus. The z-line has clear structure and regular arrangement, and the myofibrils have regular sarcomere. Mitochondria are located neatly between myocardial fibers with dense cristae and large size. In the vicinity of the z-line, it can be seen that sarcoplasm is scattered among muscle fibers, and a small amount of scattered collagen fibers are distributed among cardiomyocytes. The leap disc structure of cardiomyocytes is clear.

The results indicated that after 20 weeks of exercise training, the effect of exercise training on the ultrastructure of rat heart was completely stopped and returned to the normal level. The results are consistent with the hypothesis of previous studies and also confirm the refolding theory of the heart after exercise. However, in the high-exercise group, there were obvious proliferation and hypertrophy of mitochondria between muscle fibers, loose structure of myofibril, and slight swelling of some cardiomyocytes and mitochondria. The collagen fibers between cardiac muscle cells increased significantly, and the leap disk structure was basically clear. Part of the collagen fibers were inserted into cardiac muscle cells, resulting in fibrosis in the heart. These results suggest that prolonged overloading exercise can lead to heart damage and failure to recover spontaneously over a long period of time.

## 6. Summary

In order to explore the influence of high exercise on myocardial ultrastructure, this study established the exercise model of rats with different exercise amount to find out the changes of cardiac ultrastructure under different loads. In this study, 90 experimental mice were randomly divided into the normal control group, the normal exercise group and the high-intensity exercise group for a period of 10 weeks. The subjects were selected by light microscopy, electron microscopy and immunohistochemistry, respectively. The study finally realized the purpose of providing supervision basis for medical intervention under overtraining.

Mice research shows that regular exercise can appear the changes of myocardial ultrastructure in cardiac muscle physiologic hypertrophy, myocardial mitochondria of rats increased, myocardial volume larger, myocardial swelling volume, myocardial structural integrity, cardiac muscle wire overall and orderly, mitochondrial membrane are clear and complete the nucleus has increased obviously and a significant increase in the number of nuclei. The ultrastructure of the myocardium of rats in the high-intensity exercise group was distorted, the myofilament was cracked, the mitochondrial membrane was damaged and obviously incomplete, the granulosity was obvious, and the myocardial structure was damaged.

The research of this paper mainly draws the following important conclusions :(1) The suitable aerobic exercise can cause the adaptive change of the combination of cardiomyocytes and cardiomyocytes, which is conducive to the health of the heart and the improvement of the function of the heart; (2) Over the great physiological load of exercise will make the heart to the unhealthy direction, destroy the heart performance, long continuous excessive exercise can cause a pathological myocardial phenomenon and lead to an increased risk of sudden death (3) The structure of the myocardial damage significantly related with the size of the amount of exercise, exercise, the greater the damage to the structure of the heart, the more obvious.

## References

*[1] Yousif A A, Al-Naqeeb M M N.(2019). "Ultrastructural Changes in the Ileum of White BALB/C*

*Mice Experimentally Infected with Salmonella Hadar", American Journal of Animal and Veterinary Ences, 5(3),pp.196-201. DOI: 10.3844/ajavsp.2010.196.201*

*[2] Antonova I N, Goncharov V D, Bobrova E A.(2017). "Ultrastructural Changes of Human Dental Hard Tissues During Orthodontic Treatment with Fixed Appliances", Stomatologiia, 96(3),pp.5. DOI: 10.17116/stomat20179635-10*

*[3] Ma F, Zhai Z, Zhu S.(2018). "Ultrastructural Changes in Human Masseter Muscles after Botulinum Neurotoxin a Injection", Muscle & Nerve, 57(1),pp.96-99.*

*[4] Gowda B C, Kokila G.(2017). "Ultrastructural Changes in Cell Death", Journal of Oral & Maxillofacial Pathology, 21(2),pp.299.*

*[5] Mahmoud Y I, Abo-Zeid F S.(2018). "Epididymal Ultrastructural Changes Associated with Chronic Cholestasis after Bile Duct Ligation in Adult Rats", Ultrastructural Pathology, 42(4),pp.344-349.*

*[6] Fedchenko S, Zadorozhnyy O S, Molchaniuk N I.(2017). "Ultrastructural Changes in the Rabbit Chorioretinal Complex Following 577-nm Laser Photocoagulation", Journal of Ophthalmology, 4(477),pp.55-59.*

*[7] Das T, Goswami M.(2018). "Histopathological and Ultrastructural Changes in the Gill and Liver of Fresh Water Fish Channa Punctatus Exposed to Sodium Arsenite", Bioence Biotechnology Research Communications, 11(3),pp.434-441. DOI: 10.21786/bbrc/11.3/12*

*[8] López-Alonso, B, Hernáez, A, Sarnago H.(2019). "Histopathological and Ultrastructural Changes after Electroporation in Pig Liver Using Parallel-Plate Electrodes and High-Performance Generator", Scientific Reports, 9(1),pp.2647.*

*[9] Ferreira F F, Nazari E M, Yara M. R.(2018). "Müller. MeHg Causes Ultrastructural Changes in Mitochondria and Autophagy in the Spinal Cord Cells of Chicken Embryo", Journal of Toxicology, 2018(5),pp.1-12.*

*[10] Samosudova N V, Reutov V P.(2018). "Ultrastructural Changes in the Frog Brain in the Presence of High Concentrations of Glutamate and an NO-Generating Compound", Biophysics, 63(3),pp.402-415.*

*[11] Zhou Y, Hong F, Wang L.(2017). "Titanium Dioxide Nanoparticle-induced Cytotoxicity and the Underlying Mechanism in Mouse Myocardial Cells", Journal of Nanoparticle Research, 19(11),pp.356.*