

Original Article

Effects of different intensity of exercise on tissue injury and gene expression in obese rats

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Abstract: Weight loss is an important aspect of chronic disease prevention and management, with increasing rates of overweight and obesity worldwide. Drugs and surgery are popular approaches to lose weight. Given their potential harm to the body, exercise is an alternative widely accepted approach. However, participation in high intensity exercise entails an inherent risk of sports-related injuries. In this study, a rat model fed with high fat diet was employed to investigate the effects of different intensity of exercise prescription on both weight loss and tissue injury after exercise and to find a proper exercise prescription for losing weight with minimal tissues injuries. We inferred that 3% weight bearing might be the proper exercise prescription for high fat diet-fed rats in our experimental setting.

Keywords: High fat diet-fed model, weight loss, injury, exercise prescription

Introduction

In recent years, the growth of sedentary lifestyle and unhealthy eating habits have contributed to a simultaneous increase in diabetes and obesity throughout the world. Obesity has become a major health problem worldwide and affects almost all of the major cardiovascular risk factors [1] including hypertension [2], type-2 diabetes mellitus (T2DM) [3], and hyperlipidemia [4]. With increasing rates of overweight and obesity worldwide, efforts to promote and maintain weight loss have become important aspects of chronic disease prevention and management.

There are many popular weight loss methods involving taking drugs and performing surgery [5], but they may cause harm to the body and are thus rarely accepted. Exercise is widely used as a kind of “drug” to lose weight and has not shown any side effects such as those resulting from medicine [6]. However, participation in high intensity exercise entails an inherent risk of sports-related injuries [7, 8], and this is heightened at various stages of growth and maturation.

Molecular exercise physiology is the study of exercise physiology using molecular diagnostic techniques [9]. It is a sub-discipline of exercise physiology and can be seen as a complement or extension of classical exercise physiology rather than a replacement. Molecular diagnostic techniques employ molecular biologic methods to detect changes in the structure or expression level of DNA, RNA and protein in patients to make a diagnosis [10, 11]. Detection of the changes in exercise-related genes and tissue morphology through molecular diagnostic technology will help us to understand the individual's adaptability to exercise training [12] and therefore develop a personalized exercise prescription to make exercise more scientific. In this study, we investigated the effects of different intensity exercise prescriptions on tissue injury and exercise-related gene expression in rats fed with a high fat diet to obtain a personalized exercise prescription for weight loss.

Materials and methods

Establishment of high fat diet-fed model

A number of SD male rats of SPF grade weighing 220-250 g were purchased and randomly

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Table 1. Primer sequences used in the study

Gene	Primer sequence
<i>ACE</i>	F: GACGGAAGCATCACCAAGGAGAAC R: GTGCCTCGTGGAAGTGGAACTG
<i>ACTN3</i>	F: TACCCAGCCGTGACCAGACAC R: TGCCAGCCGCCCTACTTCC
<i>AMPD1</i>	F: GAAGCAGCAGCCAGCAGAGATG R: GAACTTCAGACGGCGGTGAGTG
<i>CLSTN2</i>	F: CCACCAACGACACCTTGATGCC R: GGGTGCCAGTTGCGGTAACG
<i>CYP7A1</i>	F: GAGGGATTGAAGCACAAGAACC R: ATGCCAGAGAATAGCGAGGT
<i>ASBT</i>	F: GTAGGGGATCACAATCGTTCCT R: GTGACATGGACCTCAGTGTAGC
<i>TRPV1</i>	F: GGTTCACTCCTGACGGCAAG R: GCCTGGGTCCTCGTTGATG
<i>β-actin</i>	F: ACGTCAGGTCATCACTATCG R: GGCATAGAGGTCTTTACGGATG

divided into two groups: normal diet control group (ND, n = 8) and high fat diet group (HFD, n = 8). Ears were marked and numbered and initial body weight was recorded. All experimental rats were adaptively fed in the same environment for about one week before formal initiation of exercise and regularly fed once a day. The clean drinking water and the bedding was changed once a week. The rats in ND groups were fed with normal food, and in HFD groups were fed with the same amount of high fat food. When there were significant differences in body weight and blood lipids between the ND group and HFD group, the establishment of HFD model was successful, which made the subsequent formal exercise experiment reliable and effective. The research protocol was reviewed and approved by the Ethics Committee in Wuhan University School of Basic Medical Sciences.

The exercise prescription

The exercise prescription was free swimming without interference, which was divided into five experimental stages: non-weight-bearing, 1% weight-bearing, 2% weight-bearing, 3% weight-bearing, and 4% weight-bearing. Each experimental session was for a total of 10 days including 7 days in the exercise session, 1 day in the blood sampling session, and 2 days in the recuperation recovery session. The starting exercise time was set as 10 min and increased

by 5 min every day. A blood sample was collected from day 7 followed by a rest to restore physical fitness for 2 days, and then rats entered the next exercise stage. All the experimental rats completed five stages of exercise in sequence. Due to individual differences, if the rats showed exhaustion in advance in the experiment, the subsequent exercise time was set at 70% of the exhaustion time and remained there until the end of the whole stage. The whole experimental process lasted 48 days.

H&E staining

After completing all exercise plans, according to the weighed body weight, the rats were intraperitoneally injected with an appropriate anesthetic, and immediately sacrificed. Part of the heart tissue, liver, and aortic arch close to the apex were immediately placed in 4% paraformaldehyde for fixation, embedding and sectioning. H&E staining was performed according to standard protocol. Briefly, following deparaffinization and rehydration, sections were stained with hematoxylin solution at 37°C for 5 min, immersed five times in a solution of 1% HCl and 70% ethanol and subsequently rinsed with distilled water. Sections were then stained with eosin solution at 37°C for 3 min, dehydrated with alcohol and immersed in xylene. High-definition morphologic pictures were used to observe morphologic differences. The remaining aliquot tubes were placed in enzyme-free EP tubes previously added with RNA preservation solution and stored in a -80°C cryogenic refrigerator.

ELISA

The level of triglycerides (TG) in blood was determined by using ELISA kit (Renjiebio, Products-26535135) according to instructions.

Quantitative real-time PCR

The total RNA of heart or liver tissues was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The total RNA was then digested using RQ1 RNase-free DNase (Promega, Madison, WI, USA), and reverse transcribed using the TransScript First-Strand cDNA Synthesis Super Mix (TransGen Biotech, Beijing, China), according to the protocols provided by the manufacturer. RT-PCR was performed using the Step One Plus machine (Life

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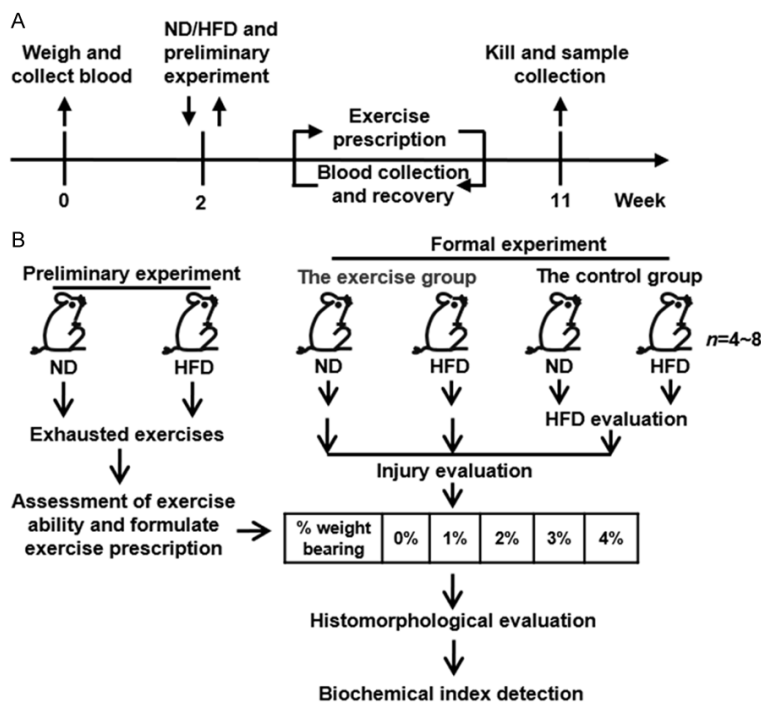


Figure 1. Workflow of the study. A. Timeline of study. B. Preliminary experiment to obtain the limit of exercise prescription (left part) and formal experiments to find a suitable exercise prescription (right part).

Technology, Carlsbad, CA, USA) with SYBR® Green Real time PCR Master Mix (TOYOBO, Shanghai, China), according to the protocol provided by the manufacturer. The primers used in this study are shown in **Table 1**.

Statistical analyses

Differences between different groups were determined by the Student *t* test in GraphPad Prism software. All results shown in the manuscript are representative of at least 2 independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Results

Evaluation of high fat diet-fed model

A preliminary experiment was first conducted to assess exercise ability of these rats and formulate an exercise prescription for formal experiments. The combination of strength training and aerobic endurance training is the key to exercise. Therefore, we set the exercise time gradient and weight-bearing intensity to determine the upper limit of exercise capacity. The exhaustive assessment criteria: phenome-

na such as the head of the rat sinking into the water surface without intermittent expulsion of bubbles, the limbs sinking into the basin bottom while no longer swimming, touching the rat no longer swimming or the body being vertical to the horizontal plane. According to preliminary experiments we found that 5% body weight bearing and exercise time of 40 min could be used as average exhaustion criteria. Based on these preliminary data, 0-4% weight bearing was used to develop the exercise prescription in both the ND group and HFD group (**Figure 1A** and **1B**).

The weights of rats from the ND group and HFD group were recorded after two weeks' feeding. The average weight of rats in ND group was 288.8 g and in the HFD group was

350.7 g. The difference between means of these two groups was 61.98 ± 12.61 , indicating that the weight of rats in HFD group were significantly heavier than that in the ND group (**Figure 2A**). Besides, the histomorphologic examination of the myocardium of both ND- and HFD-fed rats showed a normal arrangement of myocardial fibers, clear transverse striations, no obvious connective tissue hyperplasia, no inflammatory infiltration, and no vasodilatation (**Figure 2B**). The structure of the hepatic lobule was clear in the ND group and was destroyed in the diet control group (**Figure 2C**). The morphology of hepatic cords was normal and the sinusoidal cavity of hepatic blood was obvious in the ND group. However, the morphology of hepatic cords of rats in the HFD group was abnormal and the sinusoidal cavity of hepatic blood collapsed (**Figure 2D**). Regarding the morphology of hepatocytes, the rats in ND group were normal and in the HFD group showed accumulated lipid droplets (vacuoles) around hepatocytes. The hepatocytes were steatotic, increased in size, significantly swollen and the cytoplasm was lightly stained. A large number of inflammatory cells was easily observed (**Figure 2E**). Taken together, the establishment of the high fat diet-fed model was successful.

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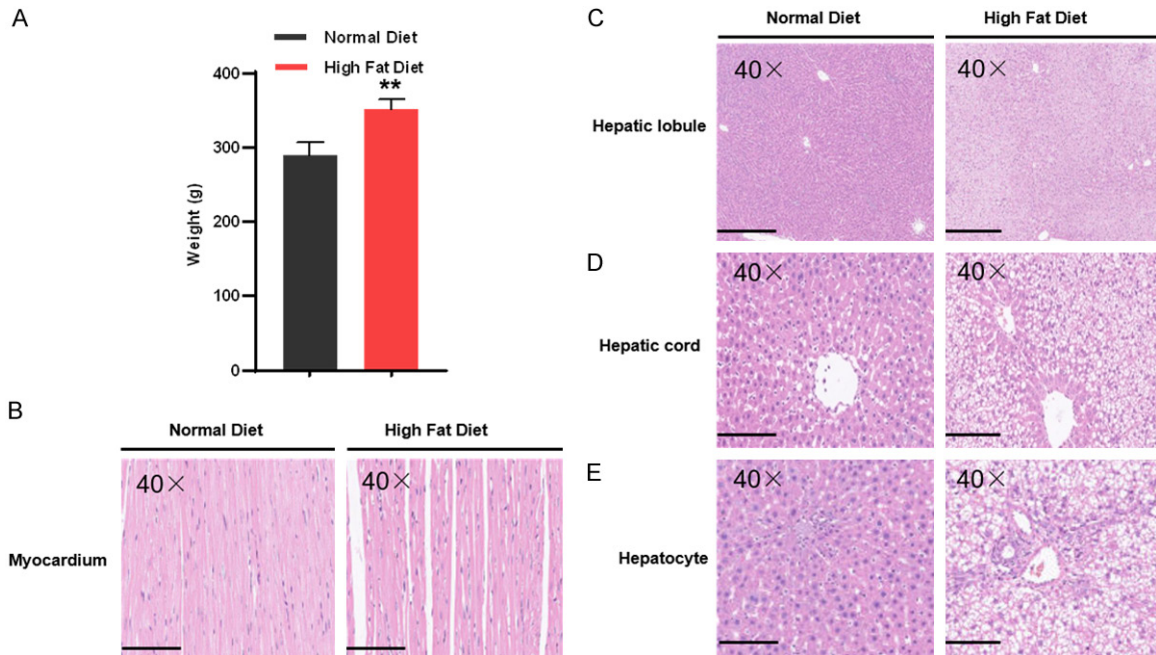


Figure 2. Evaluation of high fat diet-fed model. (A) Weights of rats from normal diet and high fat diet group. $**P = 0.004$. The statistical differences between different groups were determined by the Student t test. (B-E) H&E staining (40 \times) of Myocardium (B), Hepatic lobule (C), Hepatic cords (D) and Hepatocytes (E) of rats from normal diet and high fat diet group.

Establishment and evaluation of exercise prescription

According to the upper limit of exercise derived from the preliminary experiment, we performed the exercise prescription in the formal experiment. The protocol was carried out by free swimming without interference, which was divided into five experimental stages: no weight-bearing, 1%, 2%, 3% and 4% weight-bearing. After going through these five stages, the rats were sacrificed and the histomorphologic examination of heart and liver tissues was performed. For the myocardium, the rats in all four groups showed normal arrangement of myocardial fibers, clear transverse striations, no obvious connective tissue hyperplasia, no inflammatory infiltrate, and no vasodilatation (**Figure 3A** upper panel). Regarding hepatocytes, in the ND group, the control rats' hepatocytes were normal; the exercise rats' hepatocytes showed mild liver injury with clear structure of hepatic lobule, basically normal arrangement of hepatic cords and morphology of hepatic sinusoids, and locally edematous hepatocytes (**Figure 3A** left lower panel). In the HFD group, the injury level of control rats was similar with that of ND control ones. However, the exercised rats

showed severe liver injury with unclear structure of hepatic lobule, disorganized hepatic cords and morphology, collapsed hepatic sinusoid cavity, and locally edematous hepatocytes (**Figure 3A** right lower panel).

The degree of injury of each group was determined by SUZUKIS scoring criteria based on high-resolution images. Similar to abovementioned results, the liver injury score of the ND control group was lower than that of the other three groups, in which the liver injury score of the HD exercise group was greater than that of the other three groups. Notably, both the ND exercise and HFD control group showed similar liver injury (**Figure 3B**) indicating that improper exercise or diet can damage the liver.

Determination of proper exercise prescription

Given that both improper exercise and diet can damage the liver, we next investigated the proper exercise prescription for weight loss with minimal damage to the liver. From the curve of rat weights, we found that the tipping point was under 2% weight bearing and 3% weight bearing for significant weight loss of rats from all four groups (**Figure 4A**). The blood

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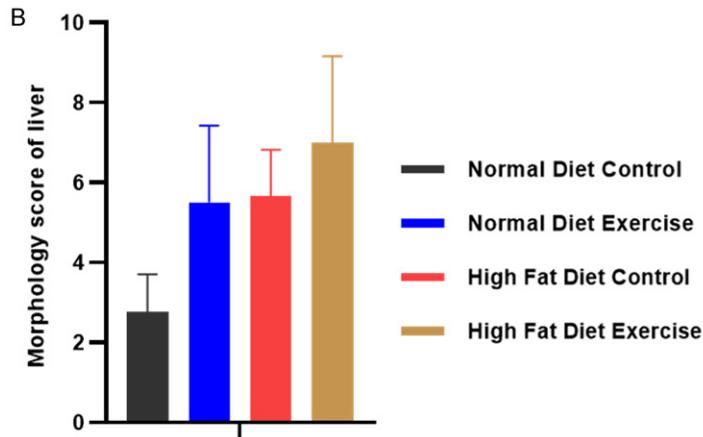
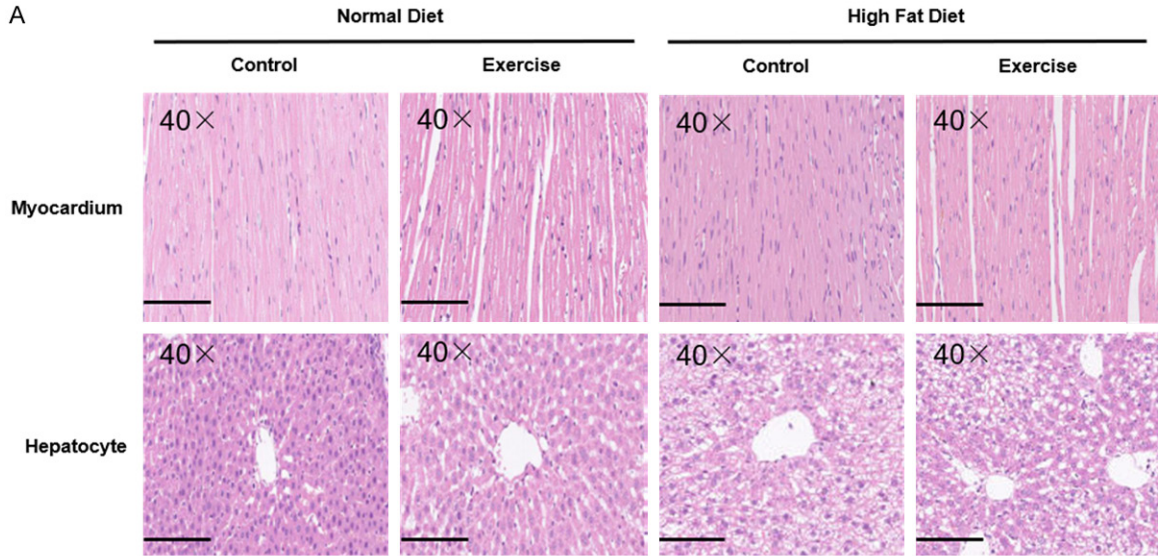


Figure 3. Evaluation of exercise prescription. A. H&E staining (40×) of myocardium (upper panel) and hepatocytes (lower panel) of rats from normal diet and high fat diet group with or without exercise prescription. B. The SUZUKIS scoring criteria based on high-resolution images indicating the degree of injury of each group.

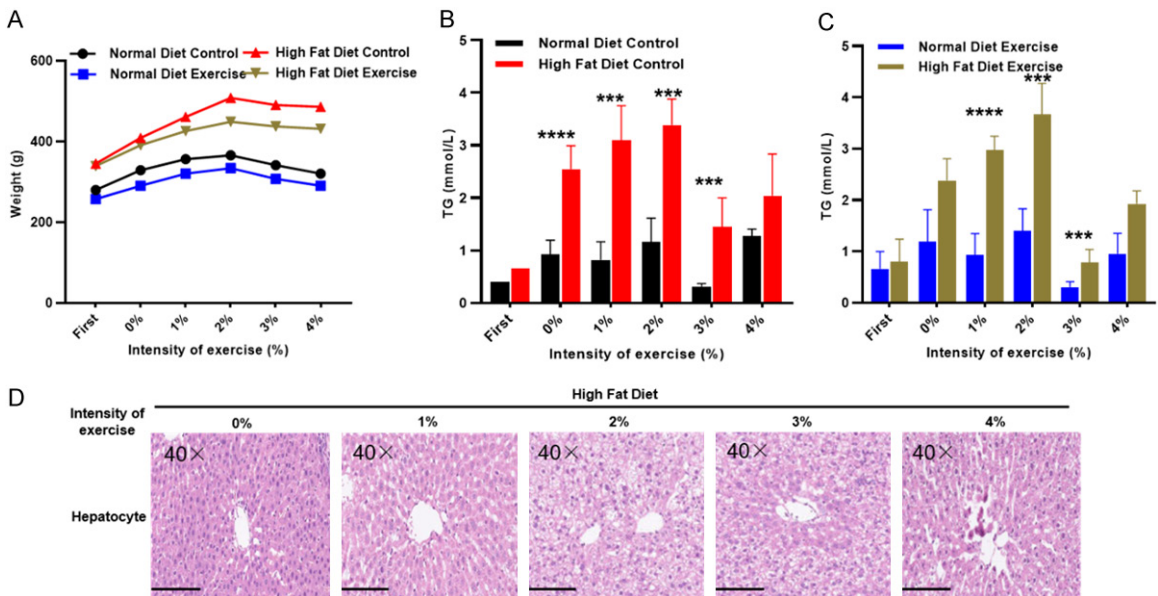


Figure 4. Determination of proper exercise prescription. A. Weight change of rats from all four groups after different intensities of exercise prescription. B. The blood triglyceride (TG) level of control rats from the normal diet and high fat diet group were tested at corresponding same time points with exercise group. C. Blood triglyceride (TG) level

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of rats from normal diet and high fat diet groups with indicated exercise prescriptions. D. H&E staining (40×) of hepatocytes of rats from high fat diet group after different intensities of exercise prescription. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Statistical differences between different groups were determined by the Student t test.

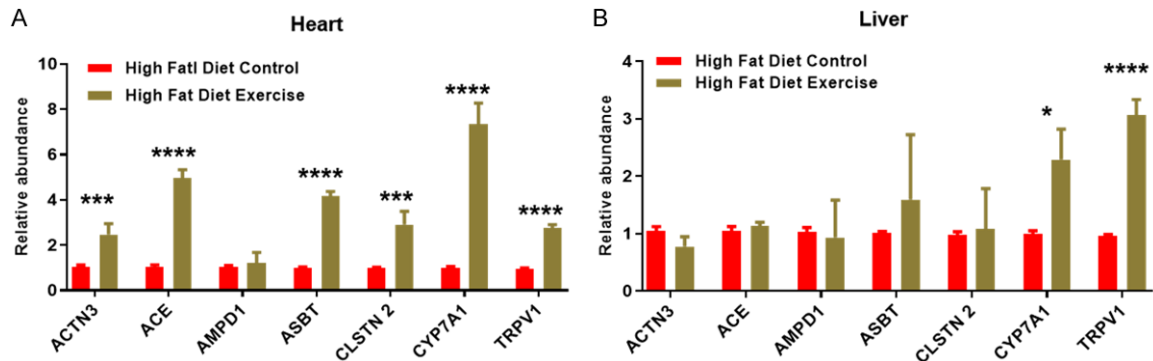


Figure 5. Molecular biologic approach to examine the expression level of sport-related genes. A. The expression level of sport-related genes in heart tissues. p value for ACTN3, ACE, ASBT, CLSTN2, CYP7A1 and TRPV1 were 0.007, 0.00006, 0.00001, 0.0005, 0.00002 and 0.00005. B. The expression level of sport-related genes in liver tissues. p value for CYP7A1 and TRPV1 were 0.014 and 0.00002. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Statistical differences between different groups were determined by the Student t test.

triglyceride (TG) level of these rats was evaluated and we also found that 3% weight bearing could significantly reduce the level of blood TG in both the control and exercise groups (Figure 4B and 4C). Consistently, the histomorphologic examination of liver tissues was also conducted to validate the results. From the histomorphologic examination data, 2% weight bearing rats showed mild liver injury. We found that the extent of injury brought by 3% weight-bearing was similar to 2% weight-bearing (Figure 4D).

To find the proper exercise prescription, histomorphologic examination is not an accepted approach due to its time-consuming nature and subjective judgment of staining results. We next chose a molecular biologic approach to examine the expression level of exercise-related genes [13] including ACTN3 [14, 15], ACE [16, 17], AMPD1 [18] and CLSTN2 [19] and metabolism-related genes including ASBT [20], CYP7A1 [21, 22] and TRPV1 [23]. The expression level of exercise and metabolism-related genes from the rats' hearts were significantly upregulated in the exercise group under 3% weight bearing exercise intensity (Figure 5A). However, the change in those genes was not that significant in liver, which showed a dramatic change in histomorphologic data (Figure 5B). From these data, we inferred that 3% weight bearing might be the proper exercise prescrip-

tion for both weight loss and minimizing tissue injury in our experimental setting.

Discussion

We employed molecular diagnostic techniques to detect changes in exercise-related genes and tissue morphology to investigate the effects of different exercise intensities on tissue injury in rats fed with high fat diet and therefore to obtain a personalized exercise prescription for scientific weight loss. We found that 3% weight bearing might be the proper exercise prescription for both losing weight and minimal tissue injury.

There are two major limitations to our study. First, the evaluation approach was developed and tested on physical training of rats in different experimental conditions. In this context, the exercise training programs and aerobic-anaerobic transition assessment proposed for animal models varied extensively, depending on the species, gender, age, type of stimulus, type of exercise, type of method, and also specific objectives of the program. Second, the evaluation approach presented here was performed in heart or liver tissues which were obtained invasively. Unfortunately, a perfect sample that is obtained non-invasively from any organ of a human after exercise without

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ethical concern does not exist. For this reason, researchers need to decide on the best sample for their purposes considering ethical concerns, possible interventions, relevance for humans and cost. A less invasive method involves using a microbiopsy needle which allows researchers to obtain muscle samples weighing up to about 50 mg, which are enough for RNA extraction to perform qPCR.

Should we use genetic tests to determine the likely response to a training program prior to deciding the final exercise prescription? This is a strategy like personalized medicine applied to sport and exercise and there is no simple answer to this question. We could employ this strategy to obtain a scientific personalized exercise prescription for better weight loss. However, the danger of misusing the emerging genetic knowledge and tools is a concern.

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Disclosure of conflict of interest

None.

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