Original Article

Optimal exercise intensity and volume to impact rats with Traditional Chinese Medicine phlegm-dampness constitution

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ABSTRACT

Objective: We aim to investigate the effects of different exercise intensities and volumes on Phlegm-dampness constitution (PDC).

Methods: The rats were fed with high-fat food and lived in 75%–85% humidity for 6 weeks to establish the model of PDC. Then PDC rats were screened and intervened by varying exercise intensities for 8 weeks. Weight, constitution scores, blood and liver tissues were collected to detect the concentration of serum total cholesterol (Tch), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), homocysteine (Hcy), blood glucose (GLU), alanine transaminase (ALT) and percentage of lipid droplet area in liver tissue (PLDA).

Results: The weight, Tch, TG, HDL-C, LDL-C, Hcy, GLU, and ALT of rats in moderate-intensity exercise group returned to normal. The rats with high-intensity or low-intensity exercise did not recover as well as moderate-intensity.

Conclusion: Different exercise intensities and volumes have different effects on PDC, moderate-intensity exercise over an 8-week intervention is most appropriate.

Introduction

Constitution of Traditional Chinese Medicine (TCM) define as the inherent characteristics of the organism in life activities, which are determined by innate and acquired factors, including morphological structure, physiological function, and psychological activity. Innate factors include parents' genes, mother's physical status during pregnancy, and acquired factors include living environment, diet, physical exercise of the offspring. Constitutions are divided into nine types in TCM, Calm-quality, Qi-deficiency, Yang-deficiency, Yin-deficiency, Phlegm-dampness, Dampness-heat, Qi-stagnation, Blood-stasis, and Special-qualities. The other 8 constitutions belong to the biased constitutions show susceptibility to different diseases except the Calm-quality. For example, phlegm-dampness constitution (PDC) is more likely to suffer from metabolic system diseases. The formation of PDC is related to excessive intake of “high-fat food” which can impair the function of spleen and stomach. The definition of spleen and stomach in TCM are different from its in modern anatomy, but more of a functional summary of digestive system. In TCM, the spleen and stomach have the function of discharging water and dampness. The high-fat food can product dampness and impair the function of spleen and stomach gradually. Increased dampness condenses into phlegm then forming PDC over time. The classic of TCM named Su Wen stated: “high-fat food produce dampish, and sweet food produce fat”. The main manifestations of PDC are obesity and impairment of spleen and stomach function, the latter main performances are skin grease, feces change, sweat easily, activity ability reducing and mental state is poor.

At present, the establishment of PDC rats model mainly include 3 methods: 1) feed with high-fat diet; 2) gavaged by Chinese herbology formula for purgation; 3) change the living environment (such as increasing air humidity). However, since the latter two methods have a low successfully modeling rate, feeding high-fat diet is used more often.

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Liu successfully established the PDC model by feeding the rats with high-fat feed for 10 weeks. The study showed PDC rats with weight significant increasing, slow movement, abnormal blood lipids and liver function indicators, abnormal oral glucose tolerance and insulin tolerance test, and Hematoxylin-Eosin staining of the liver showed typical manifestations of fatty liver. Shi also used the high-fat feed to establish the PDC rats model, and used the Chinese herbology to eliminate phlegm to verify the model, she found that the high-fat food could establish the PDC model well. At present, regarding the establishment of PDC model, most scholars agree the best method is feeding high-fat feed for more than 6 weeks. Therefore, in our study we fed the rats with high-fat food, combined with adjusting air humidity at 75%–85% to increase the success rate.

At present, many reports about acupuncture and Chinese herbology to regulate the biased constitutions but few about exercise. As a safe and green method to regulate biased constitutions, exercise has the advantage of not being affected by time, space and economy. Recently studies have proved that exercise has a good regulatory effect on metabolic disorders and it is closely related to the intensity and volume of exercise. Guerreiro distributed rats into low-intensity and moderate-intensity swimming group, both of which induced aerobic metabolism; and high-intensity swimming group that induced anaerobic metabolism. The results showed that all levels of intensity of exercise training promoted amelioration of lipid profiles, especially reducing TC and LDL-C. Additional, female rats showed improvement in lipid profiles following low-intensity exercise, while male rats required moderate- and high-intensity exercise to achieve this same benefit. Dotzert found that exercises effectively reduced type 1 diabetes rats’ muscle CD36 protein content and lipid content, combined aerobic and resistance exercise is better than high-intensity aerobic exercise. Maillard reported that high-intensity interval training can decrease total and visceral fat mass in obese rats, but none in moderate-intensity continuous training. Thus, it can be seen that the effects of different exercise intensities and volume on the regulation of metabolic disorders in rats are not consistent. Besides the external symptoms of spleen and stomach injury in the PDC rats, biochemical tests also showed metabolic disorders. However, there are still no reports on the regulation of PDC by exercise from the perspective of TCM constitution. Therefore, based on the research of other scholars, we hypothesize different intensities and amounts of exercise have different regulatory effects on the PDC rats, and moderate intensity exercise intervention may have the best regulatory effect on the PDC.

Materials and methods

Animals and grouping

All methods and procedures were approved by the Animal Center of the Sichuan Academy of Chinese Medical Sciences. Certificate No.: SYXK (C) 2019–036. Eighty 8-week-old Sprague Dawley male rats with an average weight of 201.5 ± 19.7 g were randomly divided into a control group (CG, 10 rats) and an experimental group (EG, 70 rats).

Model establishment

Model establishing method9,10: Rats were modeled after 3 days of adaptive feeding. Rats in CG were fed with normal diet, and the laboratory humidity was controlled at 45%–55%. Rats in EG were fed with high-fat feed, and the environmental humidity was adjusted to 75%–85%. The environmental temperature of the two groups were controlled at 26 ± 1 °C. Normal feed provides 326 kCal heat per 100 g. High-fat feed is composed of 73% common feed, 20% lard, 4% sugar, 2% milk powder, 1% cholesterol, per 100 g provides 453 kCal heat. Both groups of rats drank water freely, and the modeling time lasted for six weeks.

Screening of phlegm-dampness constitution

With reference to “Classification and Judgment Standards of TCM Constitution”4,11 and “Practical TCM Syndrome Animal Modeling”12,13 the inclusion criteria for PDC rats as follows:

1. Greasy hair (yes 1 point, no 0 point);
2. Reduced in activity (yes 1 point, no 0 point);
3. The feces is dry or not formed (yes 1 point, no 0 point);
4. Slowly respond (yes 1 point, no 0 point);
5. Get together (yes 1 point, no 0 point).

After establishing model, rats were weighed and evaluated constitution scores. Rats classified as successfully modeled must satisfy prerequisite and constitution scores ≥ 4.6.11 Forty successfully modeled PDC rats were selected and randomly divided into a model group (MG), or three exercise groups: low-intensity exercise group (LE), moderate-intensity exercise group (ME), and high-intensity exercise group (HE), 10 rats per group.

Exercise intervention methods

The HE, ME and LE groups used treadmill exercise as interventions. The exercise program refers to Cui13 but with slight modifications. The specific exercising program is 1 h per day, 5 days per week, and each exercising session was divided into 4 steps:

1. Warm-up period (5 min);
2. Intermediate period (20 min);
3. Main training period (30 min);
4. Recovery period (5 min).

The treadmill angle set to 0 degree, 8 m/min speed during the warm-up period and recovery period. The intermediate period was divided into two 10-min periods. The speed of first period was increased before the main work period, and the second period speed was decreased after the main work period. According to the report14 the intensity of the LE in the main work period completed at 50% maximal oxygen consumption (VO2max), the rats in ME completed at 65% VO2max, and the HE worked at 80% VO2max. Exercise intervention lasted for 8 weeks. (see Table 1).

Table 1

| Time (weeks) | LE Speed | Volume | ME Speed | Volume | HE Speed | Volume |
|--------------|----------|--------|----------|--------|----------|--------|
| 0–2          | 10 m/min | 300 m  | 15 m/min | 450 m  | 20 m/min | 600 m  |
| 2–4          | 15 m/min | 450 m  | 16 m/min | 540 m  | 24 m/min | 720 m  |
| 4–6          | 15 m/min | 450 m  | 21 m/min | 630 m  | 28 m/min | 840 m  |
| 6–8          | 15 m/min | 450 m  | 24 m/min | 720 m  | 32 m/min | 960 m  |

Remarks: LE = low-intensity exercise group, ME = moderate-intensity exercise group, HE = high-intensity exercise group, m/min = meters/minute.
**Endpoints**

**Weight and constitution scores**
During the intervention, weight and constitution scores of all rats were measured at 10 a.m. each Wednesday. All the rats were fasted for 12 h before the measurements.

**Blood sampling and analysis**
Twenty-four hours following the last training session, rats were anesthetized with 2% pentobarbital sodium. A cut was made through the rat abdominal wall tissues and the inferior vena cava was located. The inferior vena cava was punctured with a 7-gauge venous blood sampling needle, and 5 mL of water was collected in vacutainer tubes containing EDTA. After standing for 30 min, blood samples were separated by centrifugation at 4 °C for 15 min at 2000 g, the serum was extracted, and stored at −70 °C immediately. In this study, total cholesterol (Tch), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), homocysteine (Hcy), blood glucose (GLU), and alanine transaminase (ALT) were determined with diagnostic kits (procedure Tch No. ZC-30654, TG No. ZC-36512, HDL-C No. ZC-31832, LDL-C No. ZC-30441, GLU No. ZC-36154, ALT No. ZC-36427, Hcy No. ZC-34685, Zhuke Biological Technology Co. LTD, Shanghai, China). To eliminate inter-assay variability, all samples from a single subject were analyzed together in each assay. The intra-assay coefficients of variation for Tch, TG, HDL-C, LDL-C, GLU, Hcy, and ALT were 0.7, 1.7, 1.3, 1.9, 1.4, 1.8, and 3.2%, respectively. All samples were analyzed in duplicate. Since blood was only obtained at the time of death, no baseline values are available for the biochemical measures.

**Stained with oil red O**
After sacrificed, a size of 0.5 cm×0.5 cm×0.5 cm tissue was cut from lower edge of the liver right lobe and stored at −70 °C immediately until analysis. The liver tissue was stained using an oil red O kit (Nanjing Jiancheng Bioengineering Institute, China), the operation steps according to the instructions in the manual. After oil red O staining, the lipid droplets in the liver tissues were orange or red, and the nuclei were blue. The images of the microscope field used BA210 digital tri-camera system (Motic china group Co. LTD, Xiamen, Fujian Province, China). Calculated the percentage of lipid droplets area (PLDA = lipid droplets area/microscope field area×100%).

**Statistics**
SPSS20.0 software was used for data analysis. Mean and standard deviation were calculated, all data are expressed as the mean ± SD. One-way analysis of variance was used to compare between groups, and paired sample t-test was used to compare the group before and after intervention. The level of significant difference is set to p < 0.05.

**Results**

**Weight and constitution scores**
All the screened PDC rats and CG rats completed the exercise intervention, a total of 50 rats. Throughout the experiment period, the CG rats were in good mental state and daily activities maintained normally, white and shiny hair, and soft feces. After being fed with high-fat for six weeks, sixty-two rats met the criteria for PDC model, and the model-forming rate was 88.6%. Compared to the CG rats, weight (+32%) and constitution scores (+593%) of exercise groups were significantly higher before intervention, there was no significant difference among the exercise groups (p > 0.05). Compared to the MG, weight (HE -26%, ME -20%, LE -14%) and constitution scores (HE -63%, ME -71%, LE -27%) of all exercise groups decreased after intervention, and the weight of ME group rats returned to normal (compared with the CG, p > 0.05). Compared post-intervention with pre-intervention, MG rats just weight increased, and the constitution scores did not change (p > 0.05). (see Table 2).

**Lipids**
Compared to the CG rats, the Tch (+24%), TG (+83%) and LDL-C (+35%) of the MG were higher, and the HDL-C (-29%) was significant lower. Indicated the PDC rats were dyslipidemia. After 8-week intervention, the blood lipids displayed a significant group effect. Compared to MG rats, the HE rats’ Tch (-27%), TG (-37%), LDL-C (-30%) and HDL-C (+33%), the ME rats’ Tch (-29%), TG (-41%), LDL-C (-26%) and HDL-C (+26%), the LE rats’ Tch (-24%), TG (-26%), LDL-C (-17%) and HDL-C (+18%), all indicators of exercising rats were improved, both HE and ME returned to normal (compared with the CG, p > 0.05), there was no significant difference between ME and HE (p > 0.05). These results indicated that 8 weeks of aerobic exercise intervention improved blood lipids of PDC rats, of which the ME and HE groups had a significant change. (see Table 3).

**Hcy, GLU, ALT and PLDA**
Compared to the CG rats, the MG rats’ Hcy (+7%), GLU (+39%), ALT (+8%), and PLDA (+5713%) were significantly increased, support that PDC rats had abnormal glucose metabolism, fatty liver, and liver injury. Compared to the MG rats, the LE rats’ Hcy (-7%), GLU (-25%), ALT (-14%) and ME rats’ Hcy (-4%), GLU (-28%), ALT (-15%) were reduced, and LE and ME returned to normal (compared with the CG, p > 0.05). The HE rats’ Hcy (-7%) and GLU (-29%) were reduced compared to MG, but have no significant change in ALT (p > 0.05). These results indicated that moderate-intensity and low-intensity exercise are effective in improving abnormal glucose metabolism and reversing liver injury in PDC rats. High-intensity exercise improved abnormal glucose metabolism while had no obvious efficacy on liver injury. In terms of PLDA, each exercise group (HE -59%, ME -58%, LE -49%) had a different degree of reduction compared to MG. The PLDA of all exercise groups were higher than CG group, and did not return to normal. (see Table 4 and Fig. 1).

**Discussion**
Experimental data support aerobic exercise intervention can improve the biased constitutions of phlegm-dampness. However, the efficacy of various exercise intensities on PDC rats was not the same. High and moderate intensity exercise were significantly better than low-intensity exercise in reducing weight and improving PDC symptoms. Lade divided overfed rats into sedentary, moderate intensity endurance training and high intensity interval training(HIIT), the result showed that both moderate endurance training and HIIT protocols included were efficient in reverting or preventing certain metabolic alterations as a consequence of overfeeding rats. Lin carried out aerobic exercise intervention at different frequencies in obese rats, but ensured the amount and intensity of exercise was same in each group. The results showed that there was no significant difference in reducing the weight and improving resting metabolic rate in each exercise group. It indicate that the intensity and amount of exercise are very important to reduce the weight of obese rats. In this study, rats in the high-intensity group also had the largest amount of exercise, and thus the most significant weight loss, conversely, low intensity is the least. This result is consistent with the other scholars.

The abnormal glucose and blood lipids indicated that the rats with phlegm-dampness constitution had metabolic disorders of carbohydrate and fat in their bodies. The results of exercise regulation showed that moderate and high intensity exercise intervention was superior to low intensity exercise, especially in reducing blood lipid. In recent years, scholars have done a lot of studies on the regulation of glucose by exercise. Machado reported that the frequency at which the exercise is
The exercise intervention of varying intensity, the result showed that serum adiponectin concentrations rose faster when the running intensities increased, but there was no significant difference in serum LDL-C and HDL-C concentrations between exercise groups. This study also showed that both high-intensity and moderate-intensity exercise interventions were more effect in overall blood lipids improvements. In addition to the intensity of exercise, the amount of exercise is also important for the regulation of blood lipid but this is not obvious in this experiment. The reason may be that intervention time lasted for 8 weeks and the total amount of exercise in ME group was also very high, and the PDC rats only showed mild dyslipidemia. So the total amount of moderate-intensity exercise was enough to adjust the abnormal blood lipids to the normal state in the PDC rats. Hcy is an independent risk factor for cardiovascular and cerebrovascular diseases. The prevalence of stroke, diabetes, myocardial infarction and other diseases are positively correlated with Hcy concentration. This study provides added data supporting aerobic exercise intervention as a means to reduce Hcy concentration in PDC rats. No significant difference between the various exercise groups was found. This is the same result as Deminie's report.

ALT concentration changes directly reflected the degree of liver damage, and PLDA is an indicator of rats' liver fatosis. In the post-intervention, the degree of liver fatty in the MG rats was the most severe, and ALT concentration increased obviously, indicated the fatty liver and damage of liver cell in PDC rats. Exercise was an important method for the treatment of nonalcoholic fatty liver disease (NAFLD). Li et al. found that exercise reduced the expression of apoptosis proteins C/EBP homologous protein, C-Jun N-terminal kinase and Caspase12 in hepatoctyes by endoplasmic reticulum stress-mediated apoptosis, thereby reducing the degree of liver fatosis. The results of the present study showed that moderate-intensity exercise intervention was the best choice for treating fatty liver and reducing ALT in PDC rats, and high-intensity exercise can increase the degree of liver damage. Zhang and Chen reported that the secretion of IGF-1 from liver tissue increased with exercise, while the activity of Adenosine Monophosphate Activated Protein Kinase (AMPK) was inhibited. Thus, excessive exercise may cause liver damage.

The results of this experiment suggested that aerobic exercise intervention had an improvement on PDC. Different exercise intensities performed has a primary role in glucose intolerance. Yuan et al. also showed that exercise reduced the peak value of postprandial blood glucose significantly, and there were no significant difference between the different exercise intensities. In our experiment, the exercise frequency of the rats in each group was consistent, and the glucose of the rats in each group returned to normal. The conclusion of their study was consistent with the results of this experiment.

As for the improvement of blood lipids, scholars have always believed that exercise is an important intervention for improving blood lipids positively. Pels treated obese rats with moderate(normal 70% VO2max) and high (82% VO2max) intensity continuous aerobic exercise intervention for twelve weeks, the result showed that Tch, HDL-C, and TG concentrations in plasma were not different between the trained groups, but were 33–47% lower compared with the control group. The exercise-induced changes in plasma lipid and lipoprotein concentrations may be a result of a change in preferred substrate utilization in skeletal muscle toward a greater oxidation of lipid. After three months of exercise training combined with diet intervention, Bloomer found that moderate exercise improved the blood triglycerides, cholesterol, malondialdehyde of rats, the effect is better with diet adjustment. Mahmoodi gave the rats exercise intervention of varying intensity, the result showed that serum adiponectin concentrations rose faster when the running intensities increased, but there was no significant difference in serum LDL-C and HDL-C concentrations between exercise groups. This study also showed that both high-intensity and moderate-intensity exercise interventions were more effect in overall blood lipids improvements. In addition to the intensity of exercise, the amount of exercise is also important for the regulation of blood lipid but this is not obvious in this experiment. The reason may be that intervention time lasted for 8 weeks and the total amount of exercise in ME group was also very high, and the PDC rats only showed mild dyslipidemia. So the total amount of moderate-intensity exercise was enough to adjust the abnormal blood lipids to the normal state in the PDC rats. Hcy is an independent risk factor for cardiovascular and cerebrovascular diseases. The prevalence of stroke, diabetes, myocardial infarction and other diseases are positively correlated with Hcy concentration. This study provides added data supporting aerobic exercise intervention as a means to reduce Hcy concentration in PDC rats. No significant difference between the various exercise groups was found. This is the same result as Deminie's report.

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interventions had different targets. High-intensity exercise had more significant effects on weight losing, glucose reducing and lipids improving, but may increase liver damage in PDC rats. Low-intensity exercise was better at reducing blood glucose and reversing liver cell damage but had a lesser effect on improving lipids. Moderate-intensity exercise could integrate the advantages of high-intensity and low-intensity, had the widest range of action, losing weight, reducing blood glucose, improving lipids and reversing liver cell damage. In summary, exercise positively impacted PDC, and moderate-intensity exercise over an 8-week intervention appeared best effect.

In recent years, with the change of people’s diet structure, the proportion of people with PDC is increasing, and the incidence of metabolic diseases is also rising, which seriously damage to people’s health. The results of this study suggest that moderate intensity exercise can improve the PDC, which give a clue for preventing metabolic diseases. However, there are some differences between the metabolic processes of rats and humans. Therefore, the results of this study are only limited to animal experiments. How to apply these results to humans is needed more researches.

Ethical approval statement

We promise that the study was performed according to the international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights. The study protocol was approved by the Animal Center of the Sichuan Academy of Chinese Medical Sciences. Certificate No.: SYXK (C) 2019–036.

Submission statement

The manuscript has not been published and is not under consideration for publication elsewhere.

Authors’ contributions

Yaming Yu and Zhangmeng Xu conceived the study, Zhangmeng Xu, Yong Chen, Jing Zhou, Donghong Feng performed animal experiments, Chenjian Tang, and Tao Li performed data collection and analysis, Zhangmeng Xu and Yaming Yu wrote the manuscript, Zhangmeng Xu and Duo-duo Yu revised the manuscript, all the authors reviewed manuscript to determine the final version.

Conflict of interest

None competing interests of all authors.

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