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EFFECT OF TRADITIONAL CHINESE MEDICINE ON THE ENERGY METABOLISM OF ATHLETES AFTER RUNNING

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ABSTRACT

Over a long history, some traditional Chinese medicine (TCM) has evolved into health food for humans. Dating back to the Tang Dynasty, in his medical book Dietetic Materia Medica, Meng Xian introduced a lot of TCM diet therapies which were effective in tonifying qi and yin, nourishing spleen and stomach, reinforcing the kidney and lung. Moreover, TCM diet therapies can enhance the capability of athletes. In this study, we observed the effects of TCM intervention on the body function level and recovery ability of exercise rats. We prepared two kinds of TCM decoction (A and B). The experimental rats were divided into quiet group A (QA), movement group A (MA), quiet group B (QB), movement group B (MB), quiet control group (QC) and movement control group (MC). The rats in groups MA and QA took TCM decoction A; the rats in groups MB and QB took TCM decoction B; the rats in groups MC and QC took the same amount of clean water. By observing the influence of TCM intervention on the energy metabolism of the exercise rats, we deduced the action mechanism of TCM intervention in improving the exercise capacity of rats.

1. Introduction

While competitive sports has developed constantly, scientific researchers have attempted to explore the mechanism of over-training from different aspects and the approaches to enhance athletes' endurance to high-intensity training (Scharhag et al., 2013). The ability to carry and use oxygen of an athlete's body is an important factor influencing the athletic ability of the body (Wang, 2012). Therefore, the mechanism of over-training can be explored by studying the changes in the form and functions of red blood cells (Dass et al., 2012; Lombardi et al., 2012). In addition, skeletal muscle is the main tissue that completes competitive activities in sports training; consequently, skeletal muscle is the victim of over-training (Loucks et al., 2011). When an athlete is under the influence of over-training, his/her body might suffer physiological or pathological changes due to a series of overloading training (Artioli et al., 2011). Accordingly, the relevant studies can be performed from the aspect of energy metabolism (Sabatini et al., 2011).

In recent years, more and more researchers have devoted themselves to the studies on the effect of traditional Chinese medicine in enhancing athletic ability. The concept of traditional Chinese medicine is to regulate the balance of Yin and Yang, qi and blood on the whole (Carlsohn et al., 2010), which differs from the concept of modern medicine. In competitive sports, the body function of athletes may be in a declining trend with the increase of exercise load (Zadik et al., 2009). How to improve the recovery ability of body function under overload training is one of the major concerns in the field of competitive sports. Therefore, in this study we confected a TCM decoction of medicinal herbs according to the principle of traditional Chinese medicine (TCM). Through intragastric administration of the TCM decoction, we fed the rats which took long-term exercise training. By observing their red blood cell metabolism, gene expression of metabolic enzyme in skeletal muscle and the change of issue form, we aimed to study the effect of Chinese medicine intervention on the body function level and recovery ability of rats.

2. Materials and methods

2.1. General materials

This study included 66 male rats which were purchased from Nanjing Better Biotechnology Co., Ltd. The rats were divided into two groups (movement group and quiet group), including six subgroups: quiet group A (QA), movement group A (MA), quiet group B (QB), movement group B (MB), quiet control group (QC) and movement control group (MC). Two kinds of TCM decoction (A and B) were prepared according to a certain proportion of ingredients. TCM decoction A consisted of American ginseng, acanthopanax, dodder, fructus schisandrae and etc. TCM decoction B consisted of American ginseng, wolfberry fruit, fructus schisandrae, epimedium, Radix Ophiopogonis, Polygonum multiflorum and etc.

2.2. Experiment methods

2.2.1. Movement mode

The rats took load exercise on a treadmill for seven weeks; the running speed was 28 m/min. In the first week, the initial exercise time was 20 minutes; subsequently, it increased by 5 minutes every day. In the second week, the running speed increased to 32 m/min (increased by 50%) and it increased at this rate until the fifth week when the speed remained unchanged (till the end of the training).

During the first two weeks, the rats took training once a day; in the other five weeks, twice a day (respectively in the morning and evening). If a rat showed any symptoms of severe exhaustion, it should be given mechanical stimulation; if it still could not continue to run or there was an obvious mark in the shape of a soft-shelled turtle in its abdomen after it touched the ground, it was allowed to take a rest for 5 minutes before it continued training.

2.2.2. Blood sampling and test method

Every time after training, blood was collected from each rat by cutting off its tail. A hematology analyzer was used to determine the blood indexes, including hemoglobin (Hb), red blood cell (RBC) count, hematocrit (Hct) and etc. Testosterone was determined using enzyme-linked immunosorbent assay.

2.2.3. Statistical methods

Software SPSS19.0 was used for the statistical analysis on the experimental data which were all expressed in the form of mean±standard deviation. For the T test on the data, $p < 0.05$ means significant difference, while $p < 0.01$ means highly significant difference.

3. Results and discussions

3.1. Effect of TCM intervention on the basic indexes of the exercise rats

As can be seen from Figure 1, after seven-week overload training, in the movement groups, the increase in the weights of the rats was not obvious; however, in the quiet groups, there was a much more evident increase in the weights of the rats. Moreover, the rats in the quiet groups had glossy fur while the rats in the movement groups had matted hair and hair slip. It was observed that the rats in movement groups showed mild fatigue after three-week exercise; in the sixth and seventh weeks, they even showed symptoms of severe fatigue, accompanied by shortness of breath and decrease of movement coordination.

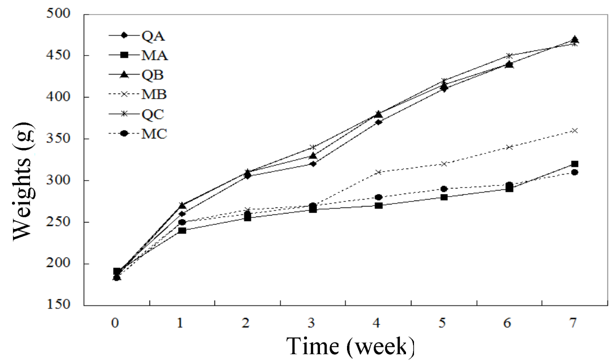

Figure 1 Influence of overload training on the weights of rats

3.2. Effect of TCM intervention on the red blood cell parameters of exercise rats

As shown in Table 1, there was a decrease in Hb concentration, RBC count and Hct of the rats in movement groups, and the level of these indexes was significantly lower than that of quiet groups ($p < 0.05$). Accordingly, it was considered that the overload training method used in this study had an evident effect on the body functions of exercise rats. Hb, RBC and Hct of the exercise rats in MA and MB groups were remarkably higher than those of MC group, suggesting that Chinese medicine intervention could significantly improve the body function of rats. As can be seen in table 1, there was a difference in the serum testosterone of the rats in movement and quiet groups: it was significantly higher in groups A and B than in group C. Based on the comparison between group A and group B, it was found that TCM decoction B was more effective in increasing serum testosterone than TCM decoction A. Both of them could improve the serum testosterone level of exercise rats and give rise to adverse effect on the secretion of testosterone in the rats.

Table 1. Blood cell parameters of different groups

Groups	Concentration of serum testosterone (ng/mL)	Hb(g/L)	RBC($\times 10^{12}$/L)	Hct(×10L/L)
QA	1.86±1.07*	143.84±7.06	7.69±0.50	0.45±0.03
MA	3.23±1.86*#	145.79±14.74*	7.68±0.66*	0.44±0.04*
QB	1.74±1.42*	150.01±7.59	7.96±0.63	0.45±0.05
MB	3.78±2.93*#	148.13±7.34*	7.38±1.08*	0.44±0.04*
QC	1.49±0.48	145.62±6.68	7.76±0.62	0.46±0.05
MC	1.01±0.73	125.15±20.95#	6.38±1.13#	0.40±0.06#

Note: For the comparison between exercise groups and quiet groups, # means $p < 0.05$, ## means $p < 0.01$; for the comparison of groups A, B and C, * means $p < 0.05$, ** means $p < 0.01$.

3.3. Effect of load exercise on the metabolism of free radicals in red blood cells of rats

According to Table 2, after taking load exercise, the malondialdehyde (MDA) level in the red blood cells of the rats in MA and MB groups was significantly higher than that of MC group; MDA level was evidently higher in the movement groups than in the quiet groups; there was no significant difference in either of the comparisons ($p > 0.05$). The activity of catalase (CAT) in movements was significantly lower than that of quiet groups, which suggested that large load exercise led to the decrease of CAT activity in mice. The fact that the CAT activity of MA and MB groups was higher than that of MC group suggested that Chinese medicine could enhance the antioxidant capacity of the body; as for the activity of superoxide dismutase (SOD), there was no significant difference between groups.

Table 2. CAT, MDA and SOD level in the RBC of exercise rats in different groups

Group	CAT(U/mgHb)	MDA(mmol/mL)	SOD(U/mgHb)
QA	3.90±1.92*	1.14±0.46	8891.09±2481.51
MA	3.91±2.31*	2.10±1.32	10556.84±3965.65
QB	2.45±1.37**	2.12±0.83	8293.77±4400.05
MB	4.12±0.42*	2.24±0.87	11084.58±2973.64
QC	5.61±1.95	0.98±0.63	7945.21±2341.24
MC	2.38±0.98#	2.87±0.51##	9820.34±4623.64

Note: For the comparison between exercise groups and quiet groups, # means $p < 0.05$, ## means $p < 0.01$; for the comparison of groups A, B and C, * means $p < 0.05$, ** means $p < 0.01$.

3.4. Effect of Chinese medicine intervention on the gene expression of energy metabolism enzyme in skeletal muscle of exercise rats

Figure 2 shows that the messenger RNA (mRNA) expression quantity of adenosine triphosphate (ATP) inhibitor peptide (IF1) was higher in movement groups than in quiet groups, indicating that exercise training could increase the expression of IF1. In addition, with the application of TCM decoction A and TCM decoction B, there was no such phenomenon as the increase of IF1 gene expression caused by the decrease of load exercise. Chinese medicine intervention resulted in an increasing trend of F0 protein gene expression in the rats of MA group; accordingly, it was speculated that Chinese medicine intervention might promote the increase of F0 protein. The increase of GLUT-4 gene expression caused by long-term load exercise was an adaptative change of exercise, which could improve the energy metabolism ability of the body.

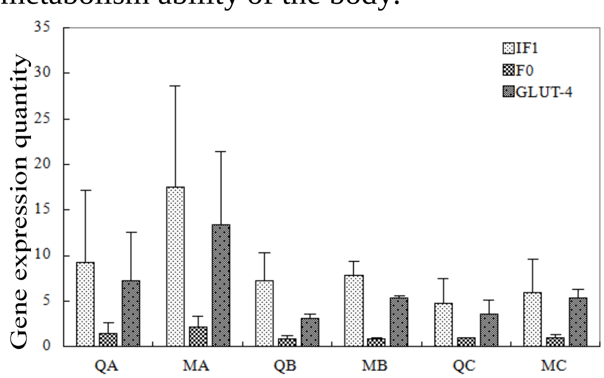

Figure 2 .Effect of Chinese medicine intervention on gene expression of metabolic enzymes in skeletal muscle of exercise rats

3.5. Effect of Chinese medicine intervention on LD content, LDH activity and MDH activity in serum of exercise rats

According to the data in Table 3, there was no significant difference in serum lactic dehydrogenase (LD) content, serum lactate dehydrogenase (LDH) activity and serum malate dehydrogenase (MDH) activity between quiet and movement groups. Under the same condition, serum LD content in group A and group B was lower than in group C ($p < 0.05$), while serum LDH and MDH activity in group A and group B increased evidently in comparison with group C ($p < 0.05$).

3.6. Effect of TCM intervention on the phosphorylation of RBC membrane band 3 protein in exercise rats

Figure 3 shows that due to the long-term load exercise, the phosphorylation of erythrocyte membrane band 3 protein of the rats in MC group was significantly higher than that of QC group. It was pointed out that long-term load exercise led to evident changes in the structure of erythrocyte membrane and seriously affected the normal frame structure of RBC membrane band 3 protein. Furthermore, it had an impact on the structure, function and metabolism of RBC and RBC membrane. Based on the experiment, it proved that TCM intervention was beneficial to improving the structure and function of RBC membrane of rats.

Table 3 Effect of TCM intervention on LD content, LDH activity and MDH activity in the serum of rats

Group	LD(mmoL/L)	LDH(U/mL)	MDH activity (U/mL)
QA	3.01±0.43	2321.05±462.25	0.14±0.03
MA	2.48±0.54*	3434.64±373.56**	0.22±0.04*
QB	3.14±0.51	2325.64±458.31	0.15±0.04
MB	2.48±0.54**	3452.34±375.32*	0.21±0.04*
QC	3.12±1.16	2353.68±275.64	0.14±0.03
MC	3.15±0.61	2551.54±317.84	0.18±0.04

Note: For the comparison of groups A, B and C, * means $p < 0.05$, ** means $p < 0.01$.

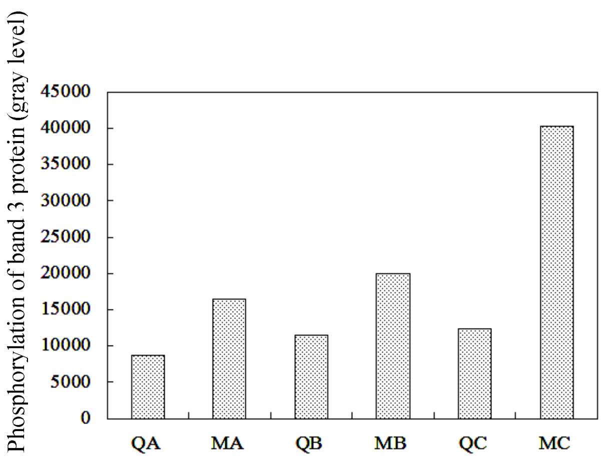

Figure 3 Effect of TCM intervention on the phosphorylation of RBC membrane band 3 protein in exercise rats

As an important approach to examine the effect of training on body functions, treadmill exercise is easy to control, has a similar mode as competitive sports and the mortality of experiment animals is low. A lot of studies (Ke-Tien, 2012; Maughan, 2010; Miccheli et al., 2009) have shown that treadmill exercise could result in a significant decrease in hemoglobin concentration. However, there was a difference in the exercise intensity, exercise frequency and duration of those studies. As the treadmill exercise load was increasing, it was easier for the experimental rats to have exercise-induced anemia or low hemachrome, which was because the rats were highly adaptive (Branth et l., 2008; Corbett, 2008). In this study, due to the long-term load exercise, Hb concentration, RBC count and Hct of the rats in movement groups were lower than those of quiet groups, indicating that long-term load exercise could result in the low hemachrome state of rats, further leading to the decrease in their body function and recovery ability, thus the effect of exercise training was seriously affected.

We found that long-term load exercise was also the reason why MDA in the RBC of the rats in MC group was evidently higher than that of QC group, while CAT activity of MC group was significantly lower than that of QC group, indicating load exercise resulted in the metabolic disorder of free radical in the rats of MC group as well as the increase of generated free radicals which was a major cause of the structure change of RBC membrane. In this case, the supplement of antioxidants can enhance the antioxidant capacity of the body. Quite a few studies have revealed that vitamin E, C and glutathione have a desirable antioxidant effect (Zouhal et al., 2010; Laabes et al., 2008). After seven-week TCM intervention, the MDA level of the exercise rats in groups MA, OA, MB and QB was lower than that of groups MC and QC, indicating that TCM could adjust the metabolic disorder of free radicals caused by long-term load exercise. Although the effect of gastroenteric administration on SOD activity in RBC was insignificant, its effect was reflected in that the CAT activity of the rats in groups MA, OA, MB and QB was significantly higher than that of groups MC and QC.

The cross-linking effect of IF1 with endogenous IF1 is the main factor influencing ATP synthetase. IF1 inhibitor might interfere the rotation of ATP synthase center stem (Perseghin et al., 2009; Kelly et al., 2011). So far, some studies have stated that IF1 could affect the activity of ATP synthetase as IF1 had an inhibitory effect on the influence of F1F0 complex of ATP synthase (Garthe et al., 2011; Erdman et al., 2012; Wong et al., 2012). However, there is no study reporting the influence of exercise training on IF1 yet. In this study, it was observed that the expression quantity of IF1 in exercise groups was higher than that of quiet groups, from which we speculated that exercise training could increase the expression quantity of IF1.

Skeletal muscle is the main movement organ of the body as well as a major part where lactic acid is generated in the movement state (Nosaka et al., 2009). In this study, TCM intervention could reduce the serum lactic acid level in the rats, which indicated that TCM decoction could reduce the generation of lactic acid during exercise and improve the body’s ability to remove lactic acid. TCM intervention could improve the activity of serum LDH either in a quiet state or under long-term exercise load. Serum LDH is a marker enzyme of anaerobic oxidation enzymes. Its activity is usually used to evaluate the anaerobic metabolism ability of skeletal muscle, kidney and myocardium. In the study, we also found that TCM intervention could increase the serum MDH activity of exercise rats. We deduced that the TCM decoction promoted the rise of certain hormones which might have important effects on the change of MDH activity. Another speculation was that the TCM ingredients in this study contained some active substances which had inducing or stimulating effects on the activity of aerobic oxidase. In addition, this study also showed that TCM intervention led to a significant decrease in the phosphorylation level of RBC membrane band 3 protein in exercise rats. In comparison with QC group, the phosphorylation level in groups QA and QB was in a decreasing trend, indicating that TCM could improve the structure and function of RBC membrane of rats.

To sum up, with TCM intervention, we studied the energy metabolism of exercise rats after treadmill exercise, which provides theoretical support for mastering the energy metabolism of athletes in running competition.

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