

Effects of Tao-Hong-Si-Wu-Tang, A Traditional Chinese Herbal Medicine Formula on Glucose and Lipid Metabolism During Endurance Exercise in Male Rats

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Abstract: Tao-Hong-Si-Wu-Tang (THSWT) is a famous traditional Chinese herbal medicine formula which has traditionally been used in China for about 1000 years. The previous studies have shown that THSWT had anti-fatigue effects but the mechanisms are scanty. The present study was designed to evaluate anti-fatigue mechanisms of THSWT through determining the change of glucose and lipid metabolism. The rats were randomly divided into four groups: Sedentary Control group (SC); Exercise Control group (EC); Sedentary plus THSWT Treated group (STT) and Exercise plus THSWT Treated group (ETT). Rats in the STT and ETT groups received 20 mL kg⁻¹ BW of THSWT solutions. After 28 days, rats in the EC and ETT groups were forced to swim for 90 min with a load (5% BW). The results showed that THSWT could improve blood glucose levels of rats during exercise as well as increase the liver and muscle glycogen contents while decreasing the plasma Free-Fatty Acid (FFA) levels. The anti-fatigue mechanisms of THSWT might be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

Key words: Tao-Hong-Si-Wu-Tang, exercise, blood glucose, glycogen, free-fatty acid, China

INTRODUCTION

Chinese herbal medicine, a major modality in Traditional Chinese Medicine (TCM) and practiced for thousands of years in China and other Asian countries is used for treating various diseases (Xiong *et al.*, 2010). Herbal formulations are the common form of administration in Chinese herbal practice and herbal formulas are well documented in ancient and modern literature (Zhang *et al.*, 2009). Tao-Hong-Si-Wu-Tang (THSWT) is a famous TCM formula which mainly consists of six plant materials: Shu Di Huang (*Rehmannia glutinosa* Liboschitz), Bai Shao (*Paeonia lactiflora* Pallas), Dang Gui (*Angelica sinensis* (Oliv.) Diels), Chuan Xiong (*Ligusticum chuanxiong* Hort.), Tao Ren (*Prunus persica* (L.) Batsch.) and Hong Hua (*Carthamus tinctorius* L.) (Ju *et al.*, 2007).

THSWT has long been employed clinically to promote blood circulation to relieve women's irregular menses disorder and is also used to treat immunological disorders, Migraine and Cardiovascular Diseases (CVDs) such as hypertension and angina (Huang, 2006). Furthermore, it can increase blood flow of the microcirculation thereby regulating diabetic neuropathies and glucocorticoid-induced avascular necrosis of the femoral head. The previous studies also have shown that

THSWT could extend exhaustive swimming time of mice and had anti-fatigue effects. However, anti-fatigue mechanisms of THSWT are scanty. Hence, studies were carried out in rat subjected to swimming exercise to evaluate anti-fatigue mechanisms of THSWT through determining the change of glucose and lipid metabolism.

MATERIALS AND METHODS

Plant materials: Shu Di Huang (*Rehmannia glutinosa* Liboschitz), Bai Shao (*Paeonia lactiflora* Pallas), Dang Gui (*Angelica sinensis* (Oliv.) Diels), Chuan Xiong (*Ligusticum chuanxiong* Hort.), Tao Ren (*Prunus persica* (L.) Batsch.) and Hong Hua (*Carthamus tinctorius* L.) were purchased from the local herbal shop (Chengdu, China) and identified by Dr. MF Li, Botanist of Sichuan University (Chengdu, China) and all the voucher specimens were deposited at the Institute of Chinese Medical Sciences, Sichuan University.

Main reagents: The kits of liver and muscle glycogen were purchased from the Jianchen Bioengineering Institute (Nanjing, China). The kit of Free-Fatty Acid (FFA) was purchased from the Huaying biotechnology Research Institute (Beijing, China). Other chemicals and biochemicals were of analytical grade and were purchased

from Sigma Chem. Co. (St. Louis, MO, USA) and Changsheng Pharmaceutical Co. (Chengdu, China) unless otherwise indicated. The freshly prepared redistilled water was used in the present study.

Animals: Male Wistar rats weighing 140-180 g were obtained from Sichuan Research Animal Center (Chengdu, China). Rats were housed in a room with alternating 12 h periods of light and dark, ambient temperature of $24\pm 1^\circ\text{C}$ and humidity of $55\pm 5\%$. All animals were allowed free access to distilled water and fed on a commercial diet (purchased from Sichuan Research Animal Center, Chengdu, China). The animals received humane care according to the guideline of Guidebook for the Care and Use of Laboratory Animals. The study protocol was approved by the animal research ethics committee at Sichuan University (Chengdu, China).

Preparation of THSWT solutions: THSWT solutions were prepared by decocting the dried prescription of herbs with boiling water. After the first decoction, the duration of which was about 30 min, the suspension was filtered and water was added for the second decoction, the duration of which was about 20 min. The filtered and mixed suspension from two decoction was condensed to the concentration of 1 g dried herb weight mL^{-1} solution and then stored at -20°C before administration. The ingredients of 63 g THSWT include 15 g of Shu Di Huang, 10 g of Bai Shao, 15 g of Dang Gui, 8 g of Chuan Xiong, 9 g of Tao Ren and 6 g of Hong Hua.

Exercise training protocol: After being adaptively fed for a week, the rats were divided into 4 groups of 12 rats each.

Sedentary Control group (SC): The rats were allowed to free access to a normal diet and treated with isotonic saline solution for 28 days.

Exercise Control group (EC): The rats were allowed to free access to a normal diet and treated with isotonic saline solution for 28 days. About 30 min after the last treatment, the rats were forced to swim for 90 min with a load (5% BW).

Sedentary plus THSWT Treated group (STT): The rats were allowed to free access to a normal diet and treated with 20 mL kg^{-1} BW of THSWT solutions for 28 days.

Exercise plus THSWT Treated group (ETT): The rats were allowed to free access to a normal diet and treated with 20 mL kg^{-1} BW of THSWT solutions for 28 days. About 30 min after the last treatment, the rats were forced to swim for 90 min with a load (5% BW).

The forced swimming of rat was performed as described before with slight modifications (Nozawa *et al.*, 2009). Briefly, the rats were placed in an acrylic plastic pool ($90\times 45\times 45\text{ cm}$) filled with fresh water (approximately 37 cm deep). Water temperature was maintained at $25\pm 1^\circ\text{C}$. The rats were loaded with a steel washer weighing approximately 5% of their body weight attached to the tails.

Biochemical assays: After forced swimming for 90 min, the rats were taken out and sacrificed by cervical dislocation. Blood was collected in polystyrene tubes without the anticoagulant. Serum was immediately separated by centrifugation for the assay of Free-Fatty Acid (FFA). Then, the liver and gastrocnemius muscle was removed quickly and homogenates were prepared for the assay of glycogen.

Method of measurement: Plasma glucose was measured using a Kyoto blood sugar test meter and test strip (Arkray, Inc. Kyoto, Japan). FFA and glycogen were determined according to the procedures recommended by the manufacturer of the kits.

Statistical analysis: The data are expressed as means \pm SD. Statistical comparisons were compared by One-way Analysis of Variance (ANOVA). The results were considered statistically significant if the p values were 0.05 or less.

RESULTS AND DISCUSSION

Effects of THSWT on the blood glucose levels of rats: The effect of THSWT on the blood glucose levels of rats are shown in Fig. 1. The blood glucose levels were not

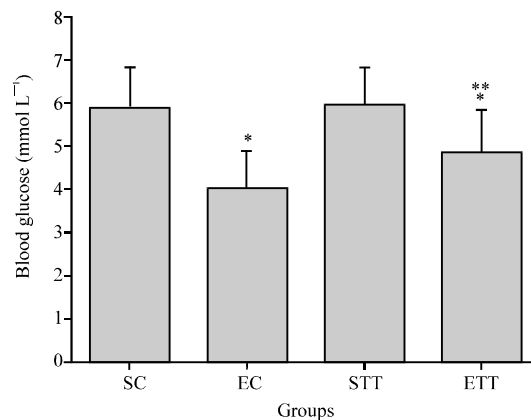


Fig. 1: Effects of THSWT on the blood glucose levels of rats. Values are mean \pm SD, * $p<0.05$ compared with SC group. ** $p<0.05$ compared with EC group

significantly different the SC and STT group ($p>0.05$). Compared with the SC group, the EC and ETT groups showed significant decreases in blood glucose levels ($p<0.05$). However, the ETT group showed a significantly higher level of blood glucose than the EC group ($p<0.05$).

Effects of THSWT on the tissue glycogen contents of rats:

The effect of THSWT on the tissue glycogen contents are shown in Fig. 2 and 3. As shown in Fig. 2, compared with the SC group, the STT group showed significant increases in liver glycogen contents while the EC group showed significant decreases ($p<0.05$). Although, the ETT group was also decreased, no significant difference was observed ($p>0.05$). However, the liver glycogen contents of the ETT groups were significantly higher than that of the EC group ($p<0.05$). As

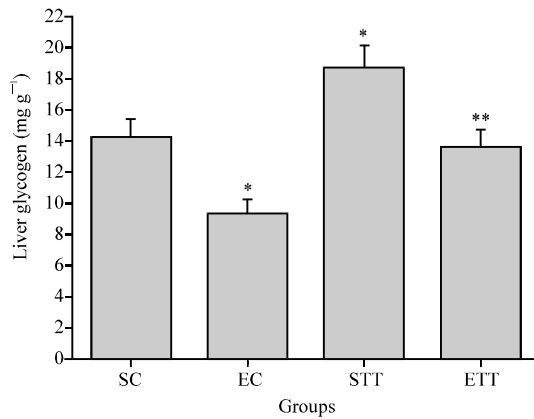


Fig. 2: Effects of THSWT on the liver glycogen contents of rats. Values are mean±SD, * $p<0.05$ compared with SC group. ** $p<0.05$ compared with EC group

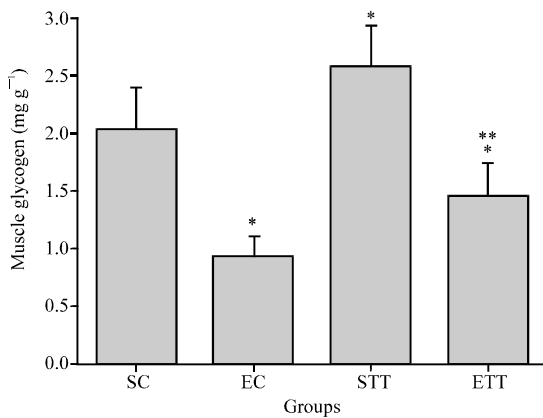


Fig. 3: Effects of THSWT on the muscle glycogen contents of rats. Values are mean±SD, * $p<0.05$ compared with SC group. ** $p<0.05$ compared with EC group

shown in Fig. 3, compared with the SC group, the STT group showed significant increases in muscle glycogen contents while the EC and ETT groups showed significant decreases ($p<0.05$). However, the muscle glycogen contents of the ETT groups were significantly higher than that of the EC group ($p<0.05$).

Effects of THSWT on the plasma free-fatty acid levels of rats:

The effect of THSWT on the plasma Free-Fatty Acid (FFA) levels are shown in Fig. 4. The plasma FFA levels were not significantly different the SC and STT group ($p>0.05$). Compared with the SC group, the EC groups showed significant increases in blood glucose levels ($p<0.05$). Although, the ETT group was also increased, no significant difference was observed ($p>0.05$). However, the ETT group showed a significantly lower level of blood glucose than the EC group ($p<0.05$).

It is well recognized that the homeostasis of blood glucose plays an important role in prolonging endurance period during exercise. Blood glucose is used to produce energy as well as to maintain brain functions for endurance exercise. Hypoglycemia can suppress the active functioning of the brain and this often leads to the inability to continue exercise (Wang *et al.*, 2006). Thus, blood glucose is an important biomarker which illustrate the speed and degree of fatigue development. In the present study, the data showed that THSWT significantly improved blood glucose levels of rats during exercise and which is certainly related to improvements in exercise activity and resistance to fatigue.

Glycogen, a carbohydrate is an energy source stored in the liver and skeletal muscle (Baldwin *et al.*, 1975). Liver glycogen is broken down to provide glucose for all tissues whereas the breakdown of muscle glycogen results in lactate formation (Virk *et al.*, 1999). Energy for

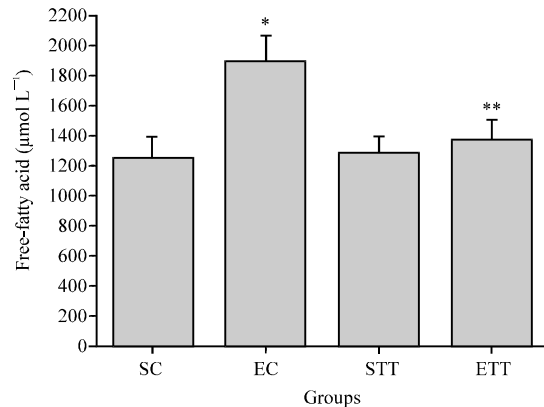


Fig. 4: Effects of THSWT on the plasma FFA levels of rats. Values are mean±SD, * $p<0.05$ compared with SC group. ** $p<0.05$ compared with EC group

exercise is derived initially from the breakdown of glycogen in muscle and later from circulating glucose released by the liver as a result of glycogenolysis (Greenberg *et al.*, 2006; Suh *et al.*, 2007). Thus, the amount of glycogen which reflects the source of the energy would be more suitable marker of physical fatigue. In the present study, the data showed THSWT significantly increased liver and muscle glycogen contents of rats during exercise which indicated that THSWT possessed the glycogen sparing action. The anti-fatigue mechanisms of THSWT might be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

Exercise can cause depletion of glycogen reserves when glycogen and blood glucose concentrations are low, the intensity of exercise must be reduced to a level that can be supported by the body's limited ability to convert body fat into energy (Klein *et al.*, 1994). Enhanced availability and use of FFA are considered to reduce glucose use which in turn spares glycogen and suppresses lactate production and results in an increase in endurance (Tarnopolsky *et al.*, 2007; Zarins *et al.*, 2009).

CONCLUSION

In the present study, the data showed THSWT significantly decreased plasma FFA levels of rats during exercise which indicated that the lipid metabolic effects of THSWT on exercise appear to be caused by the increase in fatty acid utilization as an energy source with sparing of glycogen. The glycogen thus saved could become an available energy source for the later stages of exercise thus delaying the onset of fatigue.

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