



ARTICLE

Effects of Supplemented Shengmai San (SMS) on Blood Biochemistry and HSP72 Expression in Peripheral Blood of Flying-saucer Athletes before and after High Temperature Training

Jian Shen^{1,2*} Feng Xu² Bin Jin¹ Rongquan Zhang²

1. Zhejiang College of Sports, Hangzhou, Zhejiang, 311231, China

2. Zhejiang PRC Shoot Archery & Bicycle Management Centre, Changxing, 313100, China

ARTICLE INFO

Article history

Received: 21 August 2020

Accepted: 27 August 2020

Published Online: 31 August 2020

Keywords:

Supplemented Shengmai San

Flying saucer athletes

Training of high temperature

HSP72

ABSTRACT

Objective: The purpose of this paper is to study the effects of supplemented Shengmai San on the expression of heat shock protein 72 (HSP72) and blood biochemistry in peripheral blood of flying-saucer athletes before and after high temperature training, so as to provide experimental basis for enhancing thermal endurance and preventing heat disease of flying-saucer athletes with anti-heat disease drugs. **Methods:** 24 flying-saucer athletes from Zhejiang province were selected and divided into two groups, including control group and traditional Chinese medicine group with 12 people in each group. Among them, those who did not drink supplemented Shengmai San were in the control group, while those who drank it were in the traditional Chinese medicine group. During summer training, high temperature training task was carried out for 2 weeks. Flying-saucer athletes in traditional Chinese medicine group were given supplemented Shengmai San before training every morning and afternoon. Peripheral venous blood of each group was extracted before experiment and 2 weeks later and lymphocyte HSP72 content, blood routine and biochemical indexes were determined respectively. **Results:** Before the experiment, there were no abnormal phenomenon blood routine, biochemical test value and HSP72 expression in the control group and the traditional Chinese medicine group. After comparison, there was no significant difference ($P>0.05$). After the high temperature training, the expression of HSP72 in the Chinese medicine group was significantly higher than that before the training and control group ($P<0.05$). There were significant differences in white blood cell count before and after the experiment ($P<0.05$). **Conclusion:** Taking supplemented Shengmai San can significantly increase expression of HSP72 in up-regulation of flying saucer athletes after thermal stimulation, thus enhancing thermal tolerance and preventing thermal diseases.

*Corresponding Author:

Jian Shen,

Zhejiang College of Sports, Hangzhou, 311231, China; Zhejiang PRC Shoot Archer & Bicycle Management Centre, Changxing, 313100, China;

Email: 419411722@qq.com

Capital projects: Supported by the Key Program of Zhejiang Sports Bureau (2017[397]-09)

1. Introduction

High temperature is a kind of common stressor faced by athletes during training. In high temperature environment, the metabolic and physiological changes in body can lead to the decline of the body's ability to exercise, accelerate the development of fatigue, even affecting the body's health and endangering life safety. Therefore, how to enhance high temperature tolerance is one of the important subjects of sports training. If the body is under high temperature environment and high humidity for a long time, the thermal adaptation level will be weakened or lost.

If no effective measures are taken in time to prevent it, it will lead to a higher proportion of heat generation, heat absorption and heat transmission from thermal environment, water and electrolyte metabolism disorder and internal environment imbalance. Thus, a series of adverse reactions and symptoms of dysfunction of central nervous system and cardiovascular system, such as accelerated heart rate and increased peripheral resistance, are called heat diseases.

Heat disease is the third leading cause of sudden exercise death among American high school students^[1]. Heat disease often occurs in summer training of flying saucer athletes, which often reduces training quality, affects the improvement of competitive performance, even threatening their physical and mental health. Therefore, in summer training, it is of great practical significance to strengthen the monitoring of athletes' health, improve their heat tolerance and thus prevent the occurrence of heat diseases.

Thermal adaptation refers to the adaptability to thermal load generated by the body after being in thermal environment for a period of time. The level of thermal adaptation is a direct reflection of the body adaptability to thermal environment. At present, there is still no unified evaluation standard for thermal adaptation level of body under high temperature environment. Heat shock protein is necessary for the survival of body under normal or high temperature environment, which is most closely related to cell protection. Among them, heat shock protein 72 (HSP72) has the closest relationship with heat stress and is the most sensitive heat stress protein to heat and exercise stimulation. It is easy to be induced to produce, reflecting the degree of heat stress of the body to a certain extent^[2].

Some studies^[3] believe that the expression of HSP72 is decreased in patients with heat diseases, while appropriate up-regulation of HSP72 can improve thermal adaptation level of the body^[4]. This study observed and studied the effects of supplemented Shengmai San on HSP72 expression and blood biochemical changes in peripheral blood

of flying-saucer athletes during summer training, so as to conduct evaluation and discussion, provide reference for enhancing thermal adaptation level of flying-saucer athletes through drugs, improving their thermal tolerance, preventing heat diseases and promoting their competitive level.

2. Objects and Methods

2.1 Research Object and Main Reagents

Twenty-four flying-saucer athletes were selected from Zhejiang PRC Shoot Archery & Bicycle Management Centre. They were in good health without hobbies of smoking or drinking, or family history of genetic disease. They were aged from 16 to 24 with an average age of 20.21 ± 1.55 . The training duration is 3 ~ 10 years. Twenty-four participants were divided into the control group and the Chinese medicine group by random number table method with 12 members in each group. The age, training years and other counting data of the members in the two groups were tested by homogeneity of variance, which were no significant differences ($P > 0.05$) with comparability.

Oral rehydration salts (ORS) is a regular drink for the summer training of flying-saucer athletes. The supplemented Shengmai San consists of ginseng, radix ophiopogonis, schisandra chinensis, provided by the affiliated sports hospital of Zhejiang College of Sports. Lymphocyte separation solution is provided by Shanghai Qiantu biotechnology Co., LTD.

2.2 Experimental Methods

For 2 weeks, the two groups had the same training intensity. They were trained in high temperature environment ($30 - 36^{\circ}\text{C}$) at the same time every day. They played five groups consecutively every day with one group of 30 bullets and each group intermittently for 20mins. During the training, the control group drank oral rehydration salts granule (each bag contains 2.6 grams of sodium chloride, 1.5 grams of potassium chloride, 2.9 grams of sodium citrate and 13.5 grams of anhydrous glucose) as needed and did not take supplemented Shengmai San. The flying-saucer athletes in the Chinese medicine group drank 1 bag (200ml) of supplemented Shengmai San and oral rehydration salt infusion above during the training.

2.3 Laboratory Inspection

Blood tests were performed on both groups of athletes before the high temperature training and after two weeks. The blood was drawn and placed in two anticoagulant

tubes containing EDTA sodium. One of the tubes was sent to the laboratory of Zhejiang Institute of Sports Science for blood routine and biochemical tests. Another tube of blood naturally coagulated at room temperature for 10 ~ 20 minutes and then after centrifuge for about 20 minutes (2000 ~ 3000 RPM), the supernatant was carefully collected and sent to the laboratory of Shanghai Jianglai Biotechnology Co., LTD.

Blood indicators include conventional indicators, such as red blood cells, hemoglobin, white blood cell, platelet count, blood biochemical indicators reflecting body's metabolism situation, such as CK (myocardial kinase), Bun (urea nitrogen) and other indicators reflecting body's electrolyte metabolism, such as Na⁺ (sodium), K⁺ (potassium), Ca²⁺ (calcium ion), Cl⁻ (chloride ion).

The expression level of HSP72 in peripheral blood was determined by double antibody sandwich method. Operation steps: sample addition, enzyme addition, warm breeding, liquid preparation, washing, color development, termination and determination. Human heat shock protein 72 (HSP-72) ELISA kit was provided by Shanghai Jianglai Biotechnology Co., LTD.

2.4 Statistical Analysis

All data are expressed as $\bar{x} \pm S$ and SPSS 19.0 software is used for statistical analysis. One-way analysis of variance LSD method is used to compare the mean values of the two groups before and after training.

3. Laboratory Test Results

3.1 Comparison of Blood Routine between Two Groups before and after High Temperature Training

Table 1. Comparison of blood routine before and after high temperature training ($\bar{x} \pm s$, n= 12)

Groups		Red blood cell(10 ¹² /L)	Hemoglobin(g/L)	White blood cell(10 ⁹ /L)	Blood platelet(10 ⁹ /L)
Traditional Chinese medicine group	Before training	5.11±1.08	144.75±10.71	5.64±1.40	215.35±47.06
	After training	4.98±0.39	143.19±9.56	6.90±1.72***	213.32±46.82
Control group	Before training	5.13±0.28	145.66±10.42	5.82±1.31	211.45±46.63
	After training	5.02±0.37	141.41±10.26	5.78±1.37	210.56±47.27

Note: compared with control group after training, P<0.05; compared with the traditional Chinese medicine group before training, P<0.05.

Before and after high temperature training, red blood cell, hemoglobin, white blood cell and platelet count showed no significant differences between two groups

(P>0.05). There were significant differences in white blood cell count between traditional Chinese medicine group and control group before and after high temperature training (P< 0.05), as shown in table 1.

3.2 Comparison of Blood Biochemical Indexes between two Groups before and after High Temperature Training

Table 2. Comparison of blood biochemistry between two groups before and after high temperature training ($\bar{x} \pm s$, n= 12)

Groups		CK(U/L)	Bun(m-mol/L)	Na ⁺ (m-mol/L)	K ⁺ (m-mol/L)	Ca ²⁺ (m-mol/L)	Cl ⁻ (m-mol/L)
Traditional Chinese medicine group	Before training	81.82±42.49	5.17±1.09	142.47±1.92	4.21±0.43	2.32±0.21	101.02±2.12
	After training	90.24±38.36	5.03±0.96	139.98±1.62	3.98±0.33	2.24±0.25	99.03±2.06
Control group	Before training	85.32±45.37	5.15±1.12	141.27±1.54	4.12±0.34	2.38±0.17	100.24±2.25
	After training	88.46±39.24	5.01±1.08	140.26±1.66	4.06±0.24	2.26±0.36	98.65±1.62

Notes: Before and after high temperature training, there were no significant differences in the measured values of blood CK, Bun, Na⁺, K⁺, Ca²⁺ and Cl⁻ biochemical indexes (P>0.05) between groups, as shown in table 2.

3.3 Comparison of HSP72 Expression in Peripheral Blood between Two Groups before and After High Temperature Training

Table 3. Comparison of HSP 72 expression before and after training in two groups ($\bar{x} \pm s$, n= 12)

Groups	Before training	After training
Traditional Chinese medicine group	872.18±129.08	1512.36±264.42
Control group	880.85±159.27	1052.87±128.75

Note: Compared with before training, P< 0.05; compared with control group after training, P< 0.05. Before high temperature training, there was no significant difference in HSP72 expression between two groups (P> 0.05). After high temperature training, HSP72 expression in both groups was significantly higher than that before the training (P< 0.05) and HSP72 expression in traditional Chinese medicine group was significantly higher than that in the control group (P< 0.05), as shown in Table 3.

4. Discussion

With the aggravation of global greenhouse effect and El Nino phenomenon, global temperature generally rises, which shows characteristics of high temperature. At present, many important international and domestic competitions, such as Summer Olympic Games, FIFA World Cup

and Tour of France, are generally held in summer months in northern hemisphere, usually in high temperature environment^[1]. The 2017 Tianjin National Games flying-saucer event was also held in August of that year.

Flying saucer is an outdoor sport with complex technical movements and high degree of precision. It requires athletes to have strong psychological quality and anti-interference ability, as well as a high level of stability, consistency, flexible coordination ability and continuity. Zhejiang flying-saucer team is affiliated to Zhejiang PRC Shoot Archery & Bicycle Management Centre, which is located in Changxing County, Huzhou City, Zhejiang Province. Changxing county is located in the north of Zhejiang low hills to the west of the Taihu lake plain transition area, belongs to the subtropical maritime climate. There are year-round sunshine, abundant rainfall, rain heat in the same season. Summer is a long time with high temperature and high humidity, especially in July and August every year. The highest temperature is even as high as 39°C above.

Training in such a high temperature, high humidity environment, athletes must have a higher level of thermal adaptation. Heat cramp, heat syncope and other heat diseases are particularly common in the summer training of flying-saucer athletes. The occurrence of mild heat disease may affect normal training plan of athletes, while severe heat disease may terminate training and even threaten the life of athletes. Therefore, in order to avoid occurrence of heat diseases, it is particularly important to fully understand causes of heat diseases and take active preventive measures. It is of great practical significance to strengthen monitoring of athletes' health and prevent heat diseases.

Shengmai San was first published in Zhang Yuansu's *Medicine Origin in the Jin Dynasty*. It is composed of ginseng, radix ophiopogonis and schisandra chinensis with the effect of "supplementing qi, promoting production of body fluid, recollecting Yin and stopping sweat". It is mainly used to treat warm and summer heat, Yin injury caused by consumption of qi, lung injury caused by prolonged cough and qi and Yin deficiency. In high temperature and humidity environment, flying-saucer athletes tend to lose concentration, which is an important factor affecting the performance of flying-saucer athletes. The Ginseng in Shengmai San can benefit qi, calm mind and promote intelligence. Taking Shengmai San can help athletes improve their concentration, so as to increase their hit rate.

It was found that there were no significant differences in blood routine and biochemical indexes between two groups before training. After training, there were no differ-

ences in CK, Bun, Ca²⁺, Na⁺, Cl⁻, K⁺ between two groups (P>0.05); White blood cell count increased in the traditional Chinese medicine group, which was significantly different from that before self training and control group (P<0.05). This may be related to the high temperature stress^[5], which also suggests that the body has a tendency to increase the stress of high temperature after taking the supplemented Shengmai San.

After high temperature training, the expression of HSP72 in the flying-saucer athletes in the traditional Chinese medicine group and control group increased compared with that before the training, among which the Chinese medicine group increased greatly (P<0.05), suggesting that taking supplemented Shengmai San can significantly improve the expression of HSP72 in athletes after high temperature training. HSP72 is a defensive stress protein^[7] and appropriate up-regulation of its expression can enhance the thermal adaptation level of the body.

Although the mechanism of effect of supplemented Shengmai San on HSP72 expression needs to be further explored, but in the 1990s, researchers have found that Shengmai San can reduce the mortality in the elderly animals in high temperature environment^[7] and scholars^[8] experiments confirm that supplemented Shengmai San can not only obviously prolong the survival of mice under high temperature condition, but also enhance fatigue resistance ability under high temperature, which suggests supplemented Shengmai San may prevent or delay the effect of heat disease. Modern pharmacological studies^[9] have also shown that Shengmai San has the functions of anti-oxidation, improving myocardial function, anti-arrhythmia and improving body's ability to tolerate high temperature.

During this study, the flying-saucer athletes in the traditional Chinese medicine group did not suffer from sports heatstroke, while 5 athletes in control group showed symptoms of sports heatstroke. This result also indicates that supplemented Shengmai San has a certain effect on preventing or delaying the occurrence of sports heat stroke. Therefore, the application of supplemented Shengmai San can provide reference for improving the heat adaptation level, preventing heat disease and improving the sports ability under high temperature.

References

- [1] Jiexiu Zhao, Li Zhang, Yingli Lu. Research progress on physiological and biochemical monitoring of exercise training at high altitude (hypoxia) and high temperature[J]. Chinese Journal of Sports Medicine, 2016, 35(12): 1165-1171.
- [2] Shuqiang Cui, Lili Lai, Jiexiu Zhao, et al. Changes

- of human plasma free HSP72 concentration before and after acute high-intensity exercise stress under different humidity and high temperature[J]. *Chinese Journal of Sports Medicine*, 2013, 32(1): 20-23+49.
- [3] Argaud L, Ferry T, Le QH, Short and longterm outcomes of heatstroke following the 2003 heat wave in Lyon, France[J]. *ArchIntern Med*, 2007, 167(20): 2177-2183.
- [4] Lee WC, Wen HC, Chang CP, Heatshock protein 72 over expression protects against hyperthermia,circulatory shock,and cerebral is chemia during heatstroke[J]. *JApplPhysiol*, 2006, 100(6): 2073-2082.
- [5] Jianhong Yang, Xiaojuan Chen, Zhongzhi Tang. Effect of sunstroke decoction on HSP72 expression and blood biochemistry in peripheral blood of soldiers before and after high temperature training[J]. *Journal of Local Surgery*, 2011, 20(1): 36-38.
- [6] Yanmei Lu. Expression of heat shock protein 70 under heat stress and exercise conditions[J]. *Science and Technology Information*, 2010(14): 197-198.
- [7] Sanlin Zhang. Research status of shengmai San[J]. *Chinese patent medicine*, 1991, 13(5): 34.
- [8] Min Li, Faji Zhao, Junsheng Guo. Research on the mechanism of enhanced high-temperature tolerance of supplemented Shengmai San[J]. *Chinese Public Health*,1997, 13(11): 667-668.
- [9] Gengcun Tian, Aixiang Wang. Preparation of Shengmai San[J]. *Chinese Patent Medicine*, 2000, 22(2).