REMISSION EFFECT OF SOY ISOFLAVONE ON SPORT FATIGUE AND ITS ACTION MECHANISM

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ABSTRACT

As the internal structure of mice is the most similar to that of human body, we used mice instead of humans for the experimental test and observed the anti-fatigue effect of soy isoflavone on the mice during swimming. Then, we further confirmed the remission effect of soy isoflavone on sports fatigue, which provided a solid theoretical basis for its application in sports. We selected the mice as the research objects and divided them into three groups. Through the swimming experiment, we observed the influences of soy isoflavone on hepatic glycogen content, muscle glycogen content, hepatic malondialdehyde and serum urea and the activity of serum lactic acid dehydrogenase in mice. Under the regulation effect of soy isoflavone, protein consumption of the mice reduced in the process of swimming, while fat increased, thus to provide energy for the body, relieve the sports fatigue and improve the exercise tolerance of the body. In addition, supplement of soy isoflavone could also improve the function of skeletal muscle mitochondria of the mice and reduce the generation of oxidative injury products; consequently, soy isoflavone was beneficial to easing the oxidative stress damage during sports. In conclusion, with a favorable regulating effect on the energy metabolism of mice during movement, soy isoflavone can effectively relieve physical fatigue.

1. Introduction

As our pace of life is speeding up, fatigue is becoming more and more common. For the people who play sports regularly, fatigue can cause a decline in their athletic ability as well as in their resistance. If body fatigue is not eliminated timely, it is likely that over-training syndromes and chronic fatigue syndromes will come up, which can decrease immunity of human body and affect body health. Therefore, research on how to relieve sport-induced fatigue is of great significance (Lombardi et al., 2009; Maes, 2011)

Widely and abundantly distributed in China, soybeans contain rich nutrients and bioactive substances, including soy isoflavone. With the ability to improve body's immunity, soy isoflavone has a variety of medical and health care effects, such as resisting oxidation, reducing blood pressure and regulating body's immune functions. One of the features of sports fatigue is that the body produces apparent oxidative stress, consequently, antioxidant ability of the body decreases and oxidative stress injury appears. In this case, soy isoflavone can play its favorable anti-fatigue role by inhibiting the oxidation (Rietjens et al., 2013; Akaza, 2012; Chang et al., 2016).

In recent years, a lot of experts and scholars analyzed and studied the application of soy isoflavone in resisting fatigue. In 2009, Cavallini et al. (Cavallini et al., 2009) pointed out that the mechanism of fatigue might be associated with the free radicals in human body; they further found that soy isoflavone could remove excess free radicals and increase

the activity of antioxidant enzymes, thus playing the role of oxidation resistance. In 2012, Hunerberg et al. (Flachowsky et al., 2011) found that soy isoflavone could extend exercise time and enhance the vitality of super oxide dismutase in human livers, which indicated its anti-fatigue function. In 2015, Gleason et al. (Gleason et al., 2015) revealed that soy isoflavone was similar with the excretive estrogen of human body in structure; it could improve the mineral content in bones and reduce the risk of osteoporosis.

Taking mice as the research objects, this paper explores the remission effect of soy isoflavone on physical fatigue of mice and its action mechanism, which provides a solid theoretical basis for the application of soy isoflavone in health food and medical domain.

2. Materials and methods

2.1. Experiment subjects

A total of 30 male mice, each of which weighted about 20 g, were selected. All the experimental procedures were performed according to the national health guidelines for management and application of experimental animals. In addition, the experiment received the approval of the National Ethics Committee.

2.2. Experimental groups

The 30 male mice were averagely divided into three experiment groups— normal control group, low-dose soy isoflavone group and high-dose soy isoflavone group. All the three groups of mice were free to drink water and eat for 15 days. Their weights and food intake were recorded every day. During feeding, the mice took 10-minute adaptive swimming for 3 times. After 15 days of feeding, all the experimental mice took loaded swimming (under 2% of their weights) in a glass swimming box (36 °C) for 60 minutes, and the data were collected according to their conditions at the 30th minute and the 60th minute of the swimming. After swimming, the mice were wiped dry; then, their heads were cut off to collect their blood, and the serum was isolated; moreover, their livers

and skeletal muscles of hind legs were separated. Then, the materials were frozen in a refrigerator (-20 °C) for later use.

2.3. Experimental instruments and materials

The experimental instruments included: a microplate reader, a high-speed refrigerated centrifuge, a glass swimming box, a precise torque balance, a spectrophotometer and a vortex mixer.

The experiment materials included: soy isoflavone, blood glucose, serum urea, free fatty acid, lactic dehydrogenase and calcium lactate.

2.4. Determination of biochemical indicators
2.4.1. Determination of blood glucose

First, 10 µl of serum and 1 ml of working liquid were added into a test tube; they were mixed evenly with a vortex mixer. Then, the mixed liquid was kept under 37 °C for heat preservation for 15 min; reagent blank was adjusted to zero; blood glucose was determined by 505 nm colorimetric determination.

2.4.2 Determination of serum urea

(1) First, 20 µl of serum was added into a measurement tube; 20 µl of standard liquid was added into a standard tube; 20 µl of double distilled water was added into a blank tube. Respectively, 250 µl of buffer enzyme liquid was added into each tube and mixed with the former solutions by shaking up.

(2) Then, 1 ml of phenolic color developing agent and 1 ml of alkaline sodium hypochlorite were added into the three tubes in turn. Then, they were shaken up again and placed in 37 °C water bath for 10 to 15 minutes.

(3) After water bath, the test tubes were taken out and placed at the wavelength of 640 nm for colorimetric assay so as to determine serum urea.

2.4.3. Determination of liver glycogen content and muscle glycogen content

(1) The mice livers and muscles were washed with NaCL solution twice and wiped clean with filter paper. Respectively, 1 g of

liver tissue and 1 g of muscle tissue were accurately weighed and added into a test tube.

(2) Then, 3 ml of KOH solution (30%) was added into the tube which was then treated with 25-minute boiling water bath; then, the test tube was taken out and cooled off at room temperature.

(3) After cooling off, the liquid was transferred to a flask (50 ml) to reach a constant volume; then, the liquid was made into glycogen extract.

(4) The glycogen extract was placed in boiling water bath for 10 minutes; then it was taken out and cooled off. The blank tube was adjusted to zero point, and the absorbance was measured at the wavelength of 620 nm to determine liver glycogen content and muscle glycogen content.

2.4.4. Determination of blood lactic acid

(1) First, 20 ml of whole blood was drawn into the bottom of a test tube; 0.5 ml of NaF solution and 1.5 ml of protein precipitating agent were added into the tube; then, after being shaken up, the mixed liquid was centrifuged at 3000 r/min for 15 minutes. After centrifugation, the supernate was extracted.

(2) Then, 0.5 ml of mixed liquid (precipitant-NaF) was added into a blank tube; 0.5 ml of lactic acid standard application liquid was into a standard tube; 0.5 ml of supernate was added into a sample tube of fluid. Then, 0.1 ml of CuSO4 (4%) and 3 ml of concentrated sulfuric acid were added into each tube which was fully shaken up and heated in boiling water bath for 5 minutes; then, they were taken out and cooled off at room temperature.

(3) Respectively, 0.1 ml of para-hydroxydiphenyl (1.5%) was added into each test tube which was shaken up again and placed in water bath (30 °C) for half an hour. Then, the tubes were taken out and heated in boiling water bath for 100 s; afterwards, the tubes were taken out and cooled off. At last, the absorbance was measured at the wavelength of 560 nm to determine the content of blood lactic acid.

2.4.5. Determination of hepatic malondialdehyde content

(1) First, 1 ml of 0.9 NaCL was added into the blank tube; 1 ml of homogenate was added into the sample tube; respectively, 2 ml of TCA-TBA-HCL was added into standard tube, blank tube and sample tube; then, the tubes were plugged and shaken up.

(2) The test tubes were placed in boiling water bath for 15 min; then, they were taken out and cooled off; afterwards, they were centrifuged at the speed of 4000 r/min for 15 min.

(3) Supernate was extracted from the test tubes. The absorbance was measured at the wavelength of 553 nm and the content of liver malondialdehyde was determined.

2.5. Data collection

Respectively, with relaxation edit pulse sequence and diffusion edit pulse sequence, the data of serum samples were collected and used for observation of micromolecule metabolites and lipid metabolites in the serum.

2.6. Statistical analysis

Statistical software SPSS17.0 was used for statistical analysis in the study. Experimental data were expressed in the form of mean ± standard deviation. Variance analysis was applied to between-group test. Least significant difference (LSD) method was applied to the comparison of every two items; $p < 0.05$ means there is statistical significance; when p is greater than 0.05, there is no statistical significance.

3. Results and discussions

3.1. Changes of mice weights

During the experiment, body weight growth and feeding conditions of the mice in each group were satisfactory. Body weight changes of the mice during experiment are shown in Figure 1.
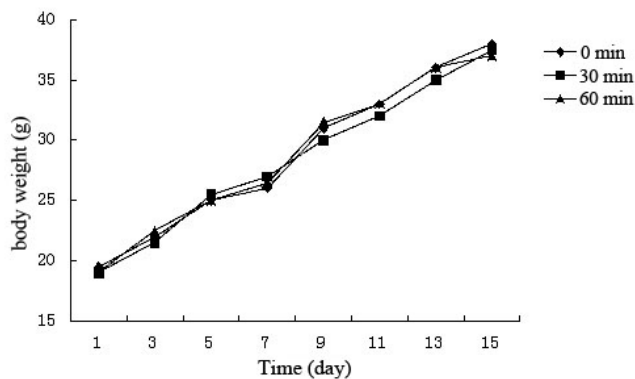
Fig. 1 Line chart of the change in body weights of three groups of mice during experiment

According to Figure 1, the increase in body weights of the mice was not significant after swimming (in comparison with their weights before swimming), which indicated that the growth of the mice was not affected, with quantitative supply of soy isoflavone.

3.2. Influence of soy isoflavone on serum urea of mice

Table 1. Influence of soy isoflavone on serum urea of mice

Experimental groups	Serum urea (mmol/L)
Normal control group	8.5±1.3
Low-dose soy isoflavone group	6.8±0.9
High-dose soy isoflavone group	6.5±1.1

As can be seen from Table 1, either in low-dose soy isoflavone group or high-dose soy isoflavone group, the serum urea was lower than that of normal control group ($p < 0.05$); in addition, the serum urea of mice in low-dose group was close to that in high-dose group of soy isoflavone. The difference was of statistical significance.

3.3. Influence of soy isoflavone on liver glycogen content and muscle glycogen content of mice

In comparison with normal control group, liver glycogen content and muscle glycogen content decreased obviously in the other two experimental groups after swimming. By the 30th minute of the swimming, the decrease range of liver glycogen and muscle glycogen content in the two experimental groups was the greatest; afterwards, the content kept decreasing in a flattening trend, basically remaining unchanged as the level of the 30th minute (as shown in Figure 2 and Figure 3). The differences were of statistical significance.
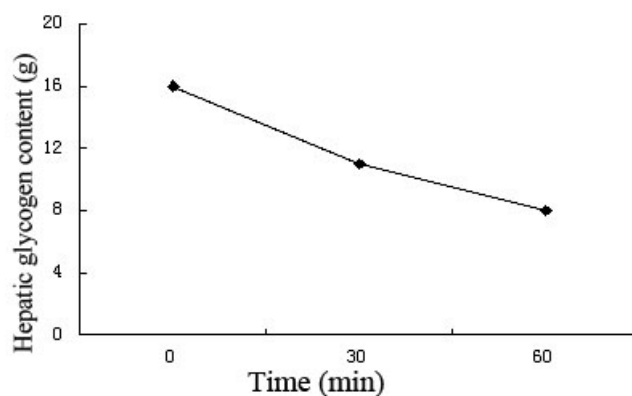

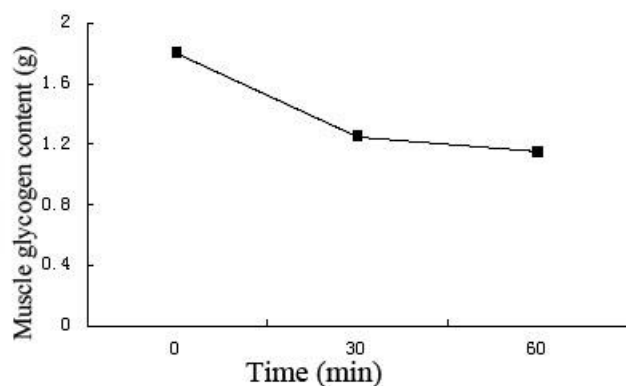**Fig. 2** Influence of soy isoflavone on hepatic glycogen content in mice

Fig. 3 Influence of soy isoflavone on muscle glycogen content in mice

3.4 Influence of soy isoflavone on blood lactic acid content of mice

Table 2. Effects of soy isoflavone on blood lactic acid content of mice

Groups	Blood lactic acid (mg/L)		
	Before swimming	After 30-min swimming	After 60-min swimming
Normal control group	313±122	302±96	296±85
Low-dose soy isoflavone group	258±71	201±72	185±67
High-dose soy isoflavone group	217±125	170±60	156±51

According to Table 2, in comparison with the situation before swimming, blood lactic acid content of the mice in high-dose and lose-dose groups of soy isoflavone was obviously lower than that of the mice in normal control group. As the swimming time increased, the content of blood lactic acid in mice was decreasing, and content of blood lactic acid of the mice in high-dose soy isoflavone group was much lower than that of the normal control group and low-dose group of soy isoflavone, which indicated that a certain amount of soy isoflavone had an influence on blood lactic acid of mice, and the difference was of statistical significance ($p < 0.05$).

3.5. Influence of soy isoflavone on hepatic malondialdehyde content of mice

Table 3. Soy isoflavone effects on mice liver malondialdehyde (MDA) content

Groups	Hepatic MDA content (nmol/g)
Normal control group	3.8±0.8
Low-dose soy isoflavone group	3.2±1.0
High-dose soy isoflavone group	2.8±1.1

According to Table 3, it can be observed that the liver MDA content of mice in low-dose group and high-dose group of soy isoflavone was lower than that of normal control group ($p < 0.05$). With the increase of the dose of soy isoflavone, the content of MDA in liver subsequently decreased faster, which was of statistical significance. The results indicated that the supply of a certain dose of soy isoflavone had an important influence on hepatic MDA content of mice.

Being a kind of complex physiological and chemical change in the body, fatigue can indicate a temporary decline in the original operation ability of the body; moreover, it is a previous sign of injury status that the body has developed into. On account that excessive fatigue has an impact on the physical and mental health of humans, how to relieve sports fatigue with proper remedy remains a problem for us to solve (Liao et al., 2012; Shi et al., 2012).

In this study, with the supply of different dosage of soy isoflavone on mice, we researched and analyzed the change law of serum metabolism and its influence by the 30th minute and the 60th minute of the loaded swimming of the mice (Han et al., 2016; Wang et al., 2013). Through the experiment, we observed the effects of soy isoflavone on the body weights, serum urea, muscle glycogen, liver glycogen, blood lactic acid and hepatic MDA of the mice (Ozden et al., 2013; Wang et al., 2016; Upadhaya et al., 2016). We found that a proper dose of soy isoflavone could delay the exhaustion time of the mice in loaded swimming and enhance their sports endurance. Liver glycogen content and muscle glycogen content are two important indexes reflecting the fatigue degree; therefore, we also observed the changes of the two indexes. The supply of soy isoflavone could slow down the digestion of liver glycogen and muscle glycogen of the mice after swimming, thus to provide more sufficient energy for the movement of the body, which was contributive to easing fatigue (Wang et al., 2016). The activity of serum lactic acid dehydrogenase of mice was obviously higher in high-dose group of soy isoflavone than that of the control group, which showed that soy

isoflavone could enhance the activity of blood lactic acid to some extent; consequently, it was beneficial to the decrease of fatigue and recovery of vitality of the body (Wiest, 2015; Banovic et al., 2010). Liver MDA content of the mice in low-dose soy isoflavone group was lower than that of control group, and the difference was more obvious between the high-dose group and control group. Accordingly, we speculated that with the application of soy isoflavone, the clearing of free radicals in the experiment subjects could be accelerated, thus to relieve fatigue (Yazdanbakhsh et al., 2015; Camblong et al., 2012).

To sum up, a proper dose of soy isoflavone has a relieving effect on sport-induced fatigue to some degree. The study on the action mechanism of soy isoflavone provides a persuasive theoretical basis for the remission of physical fatigue as well as a scientific basis for its application in the fields of health food and medicine.

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