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Full Length Research Paper

Effects of corn peptides on exercise tolerance, free radical metabolism in liver and serum glutamic-pyruvic transaminase activity of mice

Fusheng Miao[1]*, Wenqian Yu[1], Yaoguang Wang[1], Meijuan Wang[2], Xiangyan Liu[1] and Fenglin Li[3]

[1]Physical Education College, Liaoning Normal University, Dalian 116029, P.R. China.
[2]Department of National Traditional Sports,Shandong Sport University, Rizhao 276826, P.R. China.
[3]Department of Bioengineering, Jilin Agricultural Science and Technology College, Jilin 132101, P. R. China.

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In order to carry out research on the effects of corn peptides on exercise tolerance, free radical metabolism in liver and serum Glutamic-pyruvic transaminase (GPT) activity of mice, sixty Kun-Ming male mice were randomly divided into two experimental groups, that is , Group Ⅰ (20 mice) and Group Ⅱ (40 mice). Group Ⅰ was a quiet group in which the mice were not trained; Group Ⅱ was a trained group in which the mice were trained. Either group was divided into control group and administered group. The control groups were administered with 1.5 ml distilled water by gavage every morning. The administered groups were administered with 1.50% corn peptides solution at the same dose for 28 days. The trained groups were trained to swim for 4 weeks and then were forced to swim without a load until being exhausted. The time of swimming to exhaustion and the Superoxide dismutase (SOD), Malondialdehyde (MDA) of liver and serum GPT were measured. The results indicated that corn peptides could significantly increase the body exercise tolerance, have a potent function of anti-lipid peroxidation injury and could reduce the body damage caused by endogenous free radicals produced in the movement. In addition, corn peptides had obvious protective effects on the body's liver cell membrane.

Key words: Corn peptide, exercise tolerance, free radicals, glutamic-pyruvic transaminase.

INTRODUCTION

Corn peptides is a mixture composed with peptide molecules and its molecular weight is usually below 2000 (Miyoshi et al., 1995; Wu et al., 2009; Xu et al., 2002; Xu et al., 2003). The peptide molecules are obtained by hydrolyzing corn gluten meal and have low molecular weight but high activity. The absorption of corn peptides is superior to amino acids and protein and it can be completely soluble in water in a wide range of pH, with no turbidity phenomenon and sediment produced.

Meanwhile, corn peptides is extremely stable in heat and its function will not loose, being safe and reliable, having no toxic side effects, so it is a natural food protein (Dai et al., 2008). Modern pharmacological studies have shown that corn peptides has many physiological functions, such as the promotion of ethanol metabolism, anti-oxidation, lowering blood pressure and blood cholesterol, anti-radiation, anti-hepatotoxic and treatment of breast cancer (Yamamoto and Takano, 1999; Baohua and Xiong, 2006; Li et al., 2007; Li et al., 2008; Chen et al., 2008; Guo et al., 2009).

Free radicals are some molecules, atoms, ions or groups which have at least one unpaired electron in the outer orbit. In the organism, free radicals always come from the redox intermediates. On normal physiological conditions, the generation and removal of free radicals remained dynamic balance at low levels (Aruoma, 1994; Xu et al., 2002; Packer et al., 2008). Strenuous exercise can make the body ischemia and hypoxia, enhance the

*Corresponding author. E-mail: fsmunv@sina.com. Tel/Fax.: +86- 0411-84258382

Abbreviations: SOD, Superoxide dismutase; **MDA,** Malondialdehyde; **GPT,** Glutamic-pyruvic transaminase.

Table 1. Animals grouping.

60 male mice	Group Ⅰ (20 mice)	QCG(10 mice)	
		QAG(10 mice)	
	Group Ⅱ (40 mice)	TCG(20 mice)	ITCG(10 mice)
			RTCG(10 mice)
		TAG(20 mice)	ITAG (10 mice)
			RTAG (10 mice)

oxidation effect, make the free radicals rapid increase, triggering a chain reaction and destruction of the cell structure, then cause lipid peroxidation (LPO) and lead to body damage finally (Benderitter et al., 1996). Both exercise and exhaustive exercise can cause an increase in free radicals in liver tissues of the body and cause liver cell damage (Voces et al., 1999; Gul et al., 2006). Therefore, screening natural substances to obtain effective and non-toxic free radical scavengers is becoming a hot study spot in the field of sports biology. In this study, mice swimming model were used, the mice were fed with corn peptides and the time of exhaustive swimming and the liver Superoxide dismutase (SOD), Malondialdehyde (MDA) and serum Glutamic-pyruvic transaminase (GPT) were measured in order to explore the mechanism that this substance can delay the occurrence of exercise-induced fatigue and speed up the recovery process so that it can provide a theoretical basis and experimental support for its wide application in sports medicine.

MATERIALS AND METHODS

Materials

Corn peptides was purchased in Shandong Tianjiu Biotechnology Co.,Ltd., made into 1.50% solution with distilled water before the experiment and conserved in refrigerator at 4℃.

Animals and grouping

Kun-Ming male mice weighing 20 ± 2 g were obtained from the Institute of Experimental Animal Center of Liaoning Normal University. The mice were housed in a room maintained at 22 – 25℃ with a normal day/night cycle. The mice were allowed free access to standard laboratory pellet diet (purchased from Liaoning Research Animal Center, Shenyang, China) and water during the experiment. The mice were allowed to adapt to the laboratory housing for 7days before the experiment began and the mice which could not swim were removed. The mice procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the Anima Ethics Committee of the University.

Sixty male mice were randomly divided into two experimental groups, that is, Group Ⅰ (20 mice) and Group Ⅱ (40 mice). Group Ⅰ was quiet group in which the mice were not trained; Group α was trained group in which the mice were trained. Either group was divided into control group and administered group. As shown in Table 1, the 4 groups were quite control group (QCG, 10 mice), trained control group (TCG, 20 mice), quite administered group (QAG, 10 mice) and trained administered group (TAG, 20 mice). The control groups were administered with 1.5ml distilled water by gavage every morning; the administered groups were administered with 1.50% corn peptides solution at the same dose for 28 days.

The trained groups were trained to swim for 4 weeks. During the first week, the swimming time was 30 min every day; during the following three weeks, the swimming time was respectively 35, 40 and 40 min. The mice were killed after 4 weeks' training. Before being killed the mice were weighed and then were forced to swim without a load until being exhausted. The mice were regarded as being exhausted when they were underwater for over 10 s and the mice could not complete righting reflex when they were put on a flat surface. After exhaustive exercise, Group Ⅱ were divided into two experimental groups, that is, immediate group (20 mice) and recovery group after 24 h (20 mice). As shown in Table 1, the 4 groups were immediate trained control group (ITCG, 10 mice), recovery trained control group (RTCG, 10 mice), immediate trained administered group (ITAG, 10 mice) and recovery trained administered group (RTAG, 10 mice). The mice of immediate group were killed immediately after exhaustive exercise and the mice of recovery group were killed after recovering for 24 h after exhaustive exercise. Meanwhile, the mice of Group Ⅰ were killed.

Sample preparation

Blood were obtained by removing the eyes of mice and immediately centrifuged for 5 min at 7000 r/min at 4℃ to obtain serum and glutamic-pyruvic transaminase (GPT) was measured. Liver was excised from the mice and placed in ice-saline to clear off blood, then the liver were dried with filter paper and weighed. Then the liver was homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The latter was next subjected to high-speed centrifugation at 15000 r/min for 30 min at 4℃. The resulting supernatant was used as such for assaying Superoxide dismutase (SOD) and Malondialdehyde (MDA).

Target detection

SOD and MDA detection kits were purchased from Nanjing Jiancheng Institute of Biology; GPT determination kit was purchased from BIOSINO Biotechnology Co., Ltd. Test methods and formulas were used in strict accordance with the instructions of the kits.

Data analysis

All results are expressed as mean ± S.D. for the mice in each group. To determine the effect of treatment, data were analyzed by using one-way ANOVA repeated measures. P - values of less than 0.05 were regarded as being significant. Significant values were assessed with Duncan's multiple range tests. Data were analyzed by using the statistical package "SPSS 13.0 for Windows"

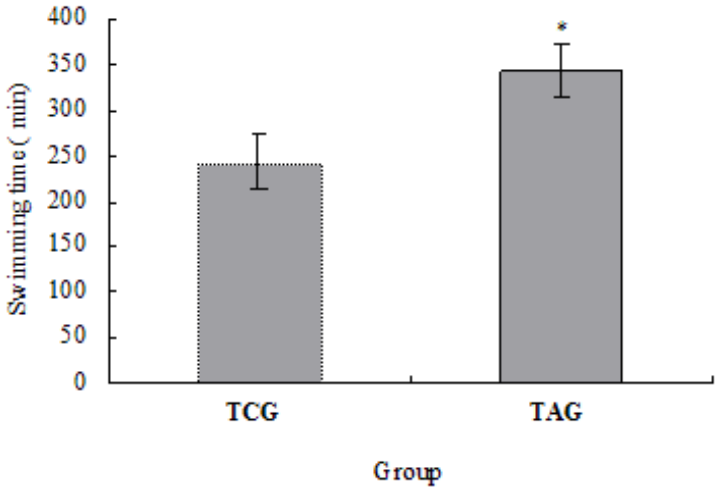

Figure 1. Effects of Corn peptides on the time of swimming to exhaustion of mice (n = 20).
Compare the trained administered group with the trained control group (TAG Vs TCG), * $p < 0.05$.

RESULTS

Effects of corn peptides on the time of swimming to exhaustion of mice

The effects of corn peptides on the time of swimming to exhaustion of mice were shown in Figure 1. As can be seen from Figure 1, time of swimming to exhaustion of trained administered group (TAG) was prolonged obviously and significantly different from that of the trained control group (TCG) ($P < 0.05$).

Effects of corn peptides on the SOD activity and MDA content of mice liver

The effects of corn peptides on the SOD activity and MDA content in mice liver was shown in Table 2. As can be seen from Table 2, the SOD activity of administered groups (QAG, ITAG and RTAG) were significantly increased ($P < 0.05$) as compared with their respective control groups (QCG, ITCG, RTCG) and MDA content were significantly lower ($P < 0.05$). The SOD activity and MDA content of trained control groups (ITCG, RTCG) significantly increased ($P < 0.05$) when compared with the quiet control group (QCG). The SOD activity and MDA content of trained administered groups (ITAG, RTAG) significantly increased ($P < 0.05$) when compared with the quiet administered group (QAG).

Effects of corn peptides on serum GPT activity of mice

The effects of corn peptides on serum GPT activity of mice were shown in Table 3. As can be seen from Table 3, the GPT activity was not significantly different ($P > 0.05$) between the quite administered group (QAG) and the quite control group (QCG). The GPT activity of the immediate trained administered group (ITAG) significantly decreased ($P < 0.05$) when compared with the immediate trained control group (ITCG). The GPT activity of the recovery trained administered group (RTAG) significantly decreased ($P < 0.05$) when compared with the recovery trained control group (RTCG). The GPT activity of the trained control groups (ITCG, RTCG) significantly increased ($P < 0.05$) when compared with the quiet control group (QCG). The GPT activity of the trained administered groups (ITAG, RTAG) significantly increased ($P < 0.05$) when compared with the quite administered group (QAG).

DISCUSSION

The increase in exercise endurance is the most powerful performance for the anti-fatigue ability and the swimming time can reflect the extent of exercise-induced fatigue in animals (Lawrence et al., 1975; Abe et al., 1995; Long et al., 2008). The results of this study showed that the time of swimming to exhaustion of trained administered group (TAG) was significantly longer than that of the trained control group (TCG) ($P < 0.05$), which indicated that corn peptides could significantly increase the body's endurance exercise capacity and delay the fatigue obviously.

Long time and strenuous exercise can make abnormal metabolic changes and varying degrees of metabolic disorders in many organs and make a number of tissue changes in the structure and function, thus the body's

Table 2. Effects of corn peptides on the SOD activity and MDA content in mice liver (n = 10).

Group	SOD(U/mg pro)	MDA(nmol/mg pro)
QCG	275.68 ± 8.64	8.78 ± 1.45
QAG	313.46 ± 10.79*	6.43 ± 0.94 *
ITCG	305.47 ± 7.48#	17.49 ± 0.95#
ITAG	380.86 ± 8.92*&	15.81 ± 1.17*&
RTCG	332.17 ± 10.45#	11.89 ± 1.25#
RTAG	366.32 ± 9.27*&	9.25 ± 0.87*&

Compare the administered group with their respective control group (QAG Vs QCG；ITAG Vs ITCG；RTAG Vs RTCG)，*$p<0.05$； ②Compare the trained control group with quite control group (ITCG Vs QCG; RTCG Vs QCG),#$p<0.05$；③Compare the trained administered group with quite administered group (ITAG Vs QAG；RTAG Vs QAG)，&$p<0.05$.

Table 3. Effects of corn peptides on serum GPT activity of mice (n = 10).

Group	GPT (U/L)
QCG	31.26 ± 4.18
QAG	30.24 ± 2.53
ITCG	75.68 ± 5.19#
ITAG	6234 ± 6.77*&
RTCG	63.94 ± 4.57#
RTAG	46.84 ± 4.56*&

①Compare the administered group with their respective control group (QAG Vs QCG；ITAG Vs ITCG；RTAG Vs RTCG)，*$p<0.05$；②Compare the trained control group with quite control group (ITCG Vs QCG; RTCG Vs QCG),#$p<0.05$；，③Compare the trained administered group with quite administered group (ITAG Vs QAG；RTAG Vs QAG)，&$p<0.05$.

motor function is affected and the exercise capacity decreased (Yu et al., 2006). The liver is the vital organs of the body's metabolism. The effects of exercise on free radical metabolism and antioxidant enzyme in the liver tissue are obvious and the exercise capacity is also closely related to the physiology of the liver (Venditti and Di Meo, 1997; Yu et al., 2006). Superoxide dismutase (SOD) is the main antioxidant enzymes in the body and it has particularly important functions (Shi et al., 2004; Zeng et al., 2006). In the present study, the SOD activity of the trained groups significantly increased ($P < 0.05$) when compared with the quiet groups after having exercised for 28 days, which could be seen as positive adaptation changes in the body to training, in another words, exercise can improve the SOD activity. The SOD activity of the administered groups significantly increased ($P < 0.05$) when compared with the control group, which indicated that corn peptides can improve the activity of antioxidant enzymes in liver tissue, reduce the lipid peroxidation damage caused by free radicals, thereby protecting the integrity of the liver cell membrane and keeping the physiology of the liver operating normally, finally enhances the athletic ability and promotes the recovery process after exercise in mice.

Malondialdehyde (MDA) is a main product in lipid peroxidation of cells and the MDA content in tissue is a common indicator reflected in the number of free radicals (Liang et al., 2008). During strenuous exercise or high-intensity endurance exercise, the generation of oxygen free radicals increased heavily, while the MDA is one of the main products of lipid peroxidation induced by the free radicals. Therefore, determining the MDA content in tissue can evaluate the degree of lipid peroxidation and indirectly assessed the body's antioxidant capacity (Jung et al., 2004; Wang et al., 2008; Lyle et al., 2009). In the present study, the MDA content of the trained groups significantly increased ($P < 0.05$) when compared with the quiet groups after having exercised for 28 days, which indicated that when doing high-intensity endurance exercise, the body is in a strong oxidative stress state and the generation and elimination of free radicals in the body cannot keep balance, leading to the increase of free

radicals and oxidation of the liver tissue. The MDA content of the administered groups significantly decreased ($P < 0.05$) when compared with the control group, which indirectly reflected the strong anti-lipid peroxidation effects of corn peptides, indicating that the corn peptides can help to improve the activity of antioxidant enzymes in exercised body, diminish the exercise-induced lipid peroxidation on a certain degree and have significant effects on getting rid off free radicals. Glutamic-pyruvic transaminase (GPT), also known as alanine aminotransferase (ALT), mainly exists in liver cells, less in other organizations. Determination of serum alanine aminotransferase for liver disease has a high specificity. It is generally believed that when the liver cell necrosis, the cell membrane ruptures or the liver cells degenerate, the enzyme will be released into the blood, making the serum GPT activity increase (Ueda et al., 1982; Nakata et al., 1996; Dong et al., 2003). The results of this study shows that the GPT activity of the trained groups significantly increased ($P < 0.05$) when compared with the quiet groups after having exercised for 28 days, which indicated that when doing exercise, the increase of free radical concentration causes the cell membrane in liver to get injured or break down, then the permeability of the cell membrane increases and makes the GPT leak out. The GPT activity of the trained administered group significantly decreased ($P < 0.05$) when compared with their respective control groups, which indicated that the corn peptides has significantly protective effect on the liver cell membrane and it can reduce the leakage of GPT.

Conclusion

Corn peptides can obviously increase the body's endurance exercise capacity in mice and delay fatigue; corn peptides can help to increase the activity of the antioxidant enzymes in liver tissue, reduce the lipid peroxidation injury in liver tissue caused by free radicals, improve athletic ability and promote the recovery process after exercise in mice; corn peptides has obvious protective effect on the liver cell membrane, it can reduce the leakage of GPT.

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