Progress in Nutrition 2020; Vol. 22, N. 3: e2020033 DOI: 10.23751/pn.v22i3.9368

Original article

Effect of functional activity of walnut protein peptide on fatigue recovery after sports training

Dongsheng Chen[1], *Zhen Ni*[2]*

[1] Physical Education College, Henan Normal University, Xinxiang, Henan 453007, China; [2]* Physical Education and Health College, Nanning Normal University, Nanning, Guangxi 530000, China - E-mail: nij2205@163.com

Summary: Sports fatigue will affect the performance of basketball players. In this study, the possibility of walnut protein peptide as a food supplement for basketball players was studied. Walnut protein peptide was prepared. Forty-eight ICR mice of specific pathogen free (SPF) were divided into a control group (group A) and low, medium and high walnut protein peptide content groups (group B, C and D). The mice swam until they were tired. Then the duration of exhaustive swimming, blood urea nitrogen (BUN), blood lactic acid (BLA) and glycogen of mice were measured. The results demonstrated that the swimming time of group B, C and D was significantly higher compared to group A ($P < 0.05$), BLA was significantly lower than that of group A ($P < 0.05$), BUN was significantly lower than that of group A, and the content of glycogen was significantly higher compared to group A; there were significant differences in BLA, BUN and glycogen between group D and the other groups ($P < 0.05$). The experimental results show that walnut protein peptide can effectively inhibit the production of BLA and BUN, increase glycogen reserve, improve the endurance of mice, and promote fatigue recovery, and this study makes some contributions to the application of walnut protein peptide in supplementary food for basketball players.

Key word: basketball player, walnut protein peptide, sports training, sports fatigue, fatigue recovery

Introduction

Sports training is an essential means to improve the level of athletes. It can help athletes improve their physical functions and be familiar with competitive means, so as to show excellent performance in the competition. Taking basketball players as an example, in order to maintain and improve their competitive level, they need to continuously receive a lot of training. However, in the process of sports training, basketball players will inevitably feel fatigue, which will not only weaken the performance of players, but also increase the risk of sports injury [1]. The intake of nutrition supplement food is helpful to promote the recovery of athletes' fatigue. Wei [2] studied the effect of puerarin on exercise fatigue and found through the study of mice that puerarin could significantly inhibit the increase of hemoglobin, red blood cells and platelets and reduce exercise fatigue. Zamanian et al. [3] studied the effect of troxerutin on male rats and found that troxerutin could significantly improve the level of glucose ($P < 0.05$), reduce creatine kinase (CK) activity ($P < 0.05$), and increase superoxide dismutase (SOD) activity ($P < 0.05$), i.e. having fatigue resistance effect Ronghui et al. [4] analyzed the effect of whey protein powder on athletes and found that hemoglobin (Hb), hematocrit (HCT) and red blood cells (RBC) of athletes were significantly improved, which had the effect of anti fatigue. Hao et al. [5] found that blueberry polysaccharide could increase the lymphocyte proliferation ability and CD4+/CD8+ of mice and enhance the immune ability and anti-fatigue ability of mice.

Peptide is a kind of substance with many biological activities. It is found that the peptide prepared from walnut protein has good anti-fatigue function.

At present, the research on walnut mainly focus on its edible value, the processing of walnut is relatively simple, the deep-processing products are few, and the utilization of walnut protein peptide is also low. Compared with other studies on walnut [6-8], this study mainly analyzed the functional activity of walnut protein peptide and determined its anti-fatigue performance, which is of great significance to promote the development of the walnut protein deep processing industry and walnut industry. In order to determine whether walnut protein peptide can be used as a supplementary food to promote the fatigue recovery of basketball players, this study prepared walnut protein peptide, carried out experiments in mice, and measured indicators such as blood lactate and urea nitrogen to understand the effect of fatigue recovery, providing some theoretical supports for the application of walnut protein peptide in the fatigue recovery of basketball players after sports training.

Walnut Protein Peptide and its Functional Activity

Semen juglandis is rich in substances such as protein and vitamin [9,10], and the protein content is 15% - 22% generally. Walnut dreg is the by-product of walnut oil, which is mostly used in animal feed or discarded, and it also contains high content of protein. It is found that protein is absorbed in the form of peptide in human body. Walnut protein peptide is a biological active peptide with strong hygroscopicity, high concentration and low viscosity. Walnut protein peptide has emulsifying property, i.e., it can transform into emulsified liquid after combining with oil and water. It also has strong oil absorbency and reducing capacity. It has excellent performance in foaming, which makes it more suitable for food processing. The functional activities of peptide include:

1 antioxidant activity [11]: excessive oxyradical may lead to problems such as cancer and aging. Peptide has scavenging effect on hydroxyl radicals [12] and can enhance glutathione peroxidase activity, reduce the content of propylene glycol to protect cells;
2 antihypertensive activity: under the action of angiotensin converting enzyme (ACE), blood vessels will contract [13]; peptide can inhibit ACE, block subsequent reactions, and significantly reduce systolic pressure [14].
3 immune activity [15]: peptide can improve thymus and spleen indexes, proliferate lymphocytes and macrophages, increase the concentration of intestinal digesta IgA, and strengthen immune function;
4 anti-fatigue activity: the generation of fatigue is associated with the consumption of energy substances, accumulation of lactate, free radical oxidation, etc.; during strenuous exercise, human body will produce a large amount of lactic acid, leading to the decrease of pH value, glycogenolysis and reduction of glycogen reserve; biological active peptide can be absorbed well by human body; peptide can reduce the content of blood lactate and improve the glycogen reserve, thus delaying the generation of fatigue and promoting the recovery of fatigue [16].

Materials and methods

Experimental materials

Walnut dreg: Shandong Xiangdaren Food Co., Ltd.

Specific pathogen free (SPF) ICR male mouse: Shanghai Jiake Biotechnology Co., Ltd.

Neutral protease: Anhui Zhonghong Bioengineering Co., Ltd.

Blood lactic acid (BLC), blood urea nitrogen (BUN) and glycogen kits: Wuhan AmyJet Scientific Inc

FE20PH meter: JUOVI Instrument (Shanghai) Co., Ltd.

GL-20G-II centrifuge: Shanghai Anting Scientific Instument Factory

R1005 rotary evaporator: Zhengzhou Dufu Instrument Factory

V-700 vacuum diaphragm pump: Buchi company, Switzerland

B-290 spray dryer: Shanghai Wanjie Technology Co., Ltd.

88-1 magnetic mixer: Shanghai Xiangfan Instrument Co., Ltd.

Analytical balance: Shanghai Hochoice Industry Co., Ltd.

Preparation of walnut protein peptide

1 100 kg of walnut dreg was mixed with water in a ratio of 1:10, and the PH value was adjusted to 10. Then it was processed by two hours of extraction at r oom temperature and filte ed. The filter esidue was remained.
2 Thefilt ate obtained from step (1) was poured into the walnut dreg with the same amount. The H value was adjusted to 10.Then it was pocessed by two hours of extraction at room temperature and filte ed. The filter esidue was remained.
3 After the secondary extraction of the filter esidue obtained from step (1), it was disc arded; after the secondary extraction of the filter esidue obtained fr om step (2), the filter esidue was also discarded. The fil ate was poured into the walnut dr eg with the same amount, the PH value was adjusted to 10, and then it was processed by two hours of extr action at r oom temperature. The filter esidue was remained.
4 After the secondary extraction of the filter esidue obtained from step (3). The filter esidue was discarded, the filt ate was merged, and the PH value was adjusted to 5. Then the fil ate was put aside for 6 h.
5 Ten times of water was added to the sediment obtained from step (4). After even mixing, walnut protein solution was obtained.
6 The liquid obtained in step (5) was heated to 40°C, and the PH value was adjusted to neutral. Then1 kg of neutral protease was added, stirred for 6h, boiled and inactivated for 30 min, and centrifuged. The supe natant was taken.
7 The supernatant was filte ed and processed by 5000Dalton ultrafilt ation membrane. It was concentrated and processed by spray drying at 80°C. Finally the walnut protein peptide powder was obtained.

Mice fatigue test

1 One week after adaptive feeding, the mice were randomly divided into four groups, 12 mice in each group. The four g oups were the control group (group A), the low-dose group (group B, 110 mg/(kg·BW)), the medium-dose group (group C, 220 mg/(kg·B W)) and the high-dose group (group D, 440 mg/(kg·BW)). The solution was prepared with distilled water. The control group was given the same amount of normal drinking water every day. The weight of mice was measured at the same time point everyday, and the food was also giv en at the same time point everyday, for 30 days.
2 Thi ty minutes after the last time of feeding, each group of mice was divided into three subgroups: a, b and c. The blood was colected from the tail tip of mice in gr oup a and put into a constant temperature water tank in a size of 50 × 50 × 50 cm. After swimming for 10 minutes, blood was collected. After resting for 20 minutes, the blood was collected again. 0.48 ml of NaF solution and 1.5 ml of protein precipitant were added to the blood sample and mix ed. After 10 minutes of centr ifugation at 3000 r/min, the supernatant was taken and mixed with 2 μL of sample to be tested, 100 μL of enzyme working liquid and 20 μL of color de veloping agent, followed by 10 min of 37° Cwater bath. Then 200 μL of stop buffer was adde and the absor bance value was detected at the wavelength of 530 nm to obtain the co ntent of blood lactic acid. In group b, blood was collected from the or bit of mice after swimming and centrifuged at 3000 r/min for 15 min. The supernatant was stor ed in a r efrigerator at - 20°C. Then 20 μL of se um, standard liquid and double distilled waterwere added into a determination tube, a standard tube and a blank tube respectively. Then 250 μL of buff enzyme liquid was added into e very tube, followed by 10 min of 37℃ water bath. After water bath, 1 mL of penol chromogenic agent and alkaline sodium h ypochlorite, followed by 10 min of water bath. The abso bance value was

detected at the wavelength of 640 nm to obtain the content of BUN. In group c, each mouse swam by loading sheet lead o n the tail whose weight was 5% that of the mouse. If the mice sank in water for about 8 s, it was co nsidered that the mice wer e exhausted, the mice wer e taken out of water, and the time was recorded. Then the mice wer e killed, and 50 mg of the liver and quadriceps femoris muscle was taken and added with 150 μL of aqueous alkali, followed by 20 min of bath heating with boiling water. After cooling, 4.8 ml of distil led water was added to obtain 1% glycogen detection solution. Then 0.1 ml of the 1% g ycogen detection solution was taken and added with 0.9 ml of distilled water and 2 ml of color de velopment agent, followed by 5 min of bath heating with boiling water. After cooling, the absorbance value was detected at the wav elength of 620 nm to obtain the content of glycogen.

Statistical analysis

The data was recorded in Excel table and analyzed by SPSS v er. 20. The indi ators were expressed by mean ± standard deviation and processed by t test. The difference was considered as significant statically if the value of P was smaller than 0.05.

Results

1 Comparison of mouse growth
It was from Table 1 that the weight of mice increased normally before and after the experiment, and there was no remarkable diffe ence between groups, indicating that the supple - ment of walnut protein peptide had no effect on the nor mal growth of mice and it could be used for the fatigue r ecovery of basketball players after training.

2 Comparison of exhausted swimming time
It was seen fr om Table 2 that the exhaus - tive swimming time of mice in walnut pr o-tein peptide gr oups increased signifi antly, and $P < 0.05$ when co mparing with the co n-trol group; the exhaustive swimming time of group B, C and D were 13.64 ± 0.46, 18.97 ± 0.32 and 24.06 ± 0.92 min, respectively, indicating that the higher the dosage of walnut protein peptide intake, the longer the exhaustive swimming time of mice, and the diffe ence between B and C, B and D, C and D was signifi ant.

3 BLA comparison
Table 3 sho ws the BLA detection results of mice. The e was no signifi ant diffe ence in the BLA between diffe ent groups before swimming. According to the test r esults immediately after swimming, the BLA content of mice increased after 10 minutes of swimming. According to the test r esults after swimming for 20 minutes, the BLA of walnut protein peptide gr oups signifi antly reduced and had a signifi ant diffe ence with group A;

Table 1. Comparison of growth between mice

	Initial weight/g	Final weight/g
A	25.12 ± 1.27	38.64 ± 2.03
B	25.33 ± 1.28	39.07 ± 2.12
C	25.64 ± 1.62	39.32 ± 2.33
D	25.29 ± 1.37	39.24 ± 2.19

Table 2. Comparison of exhausted swimming time

	Exhausted swimming time/min
A	9.87 ± 0.83
B	13.64 ± 0.46*
C	18.97 ± 0.32*#
D	24.06 ± 0.92*#&

* Compared with group A, $P < 0.05$; # Compared with group B, $P < 0.05$; & Compared with group C, $P < 0.05$

Table 3. BLA comparison of mice (mg/L)

	Before swimming	After swimming	20 min after swimming
A	4360.42 ± 1023.66	7302.23 ± 1320.25	7714.24 ± 1085.64
B	4520.23 ± 1204.32	7423.15 ± 1205.87	5215.28 ± 579.36*
C	4425.35 ± 1054.28	7525.24 ± 1108.96	5189.85 ± 562.56*
D	4725.84 ± 1102.52	7701.55 ± 1211.57	4725.54 ± 573.25*#&

there were no remarkable diffe ences between group B and C ($P > 0.05$), but $P < 0.05$ in the comparison of group D with group B and C. It was concluded that walnut protein peptide could inhibit the generation of BLA and was related to the dosage and it could be used for the fatigue recovery of basketball players.

4 BUN comparison

Table 4 shows the detection results of BUN in mice. It was found that the BUN co ntent in walnut protein peptide groups was lower than that in the co ntrol group ($P < 0.05$); in the comparison between group B, C and D, there were signifi ant diffe ences between group B and C, B and D, C and D, which showed that walnut protein peptide could reduce the BUN content, and the higher the dose, the greater the decrease and the mor e conducive to the elimination of fatigue. It indicated that walnut protein peptide was useful to the recovery of fatigue after sports training.

5 Comparison of hepatic and muscle glycogen

Table 5 shows the detection results of hepatic and muscle glycogen of mice. It was found that the glycogen content of walnut protein peptide groups was higher than that of gr oup A; the glycogen content of group D was significantly diffe ent from that of gr oup B and C, and there was no signifi ant diffe ence in the glycogen content between group B, C and D, indicating that walnut protein peptide was effective in incr easing the gl ycogen reserve of mice and was co nducive to fatigue r ecovery after exercise.

Table 4. Comparison of BUN content in mice

	BUN (9 mmol/L)
A	4.69 ± 0.18
B	3.54 ± 0.16*
C	3.19 ± 0.12*#
D	2.87 ± 0.11*#&

Table 5. Comparison of hepatic and muscle glycogen content

	Hepatic glycogen	Muscle glycogen
A	4.78 ± 2.12	2.12 ± 0.43
B	5.16 ± 1.09*	2.33 ± 0.25*
C	5.18 ± 2.11*	2.34 ± 0.31*
D	5.54 ± 0.12*#&	2.33 ± 0.29*

Discussion

According to the experimental results, the swimming time of mice in groups A, B, C and D were 9.87 ± 0.83, 13.64 ± 0.46, 18.97 ± 0.32 and 24.06 ± 0.92 min, respectively. It was found that the swimming time of walnut protein peptide groups was much longer than when comparing with the control group ($P < 0.05$), and the higher the dosage of walnut pr otein peptide, the longer the swimming time, indicating that walnut protein peptide could be used to improve the performance of basketball players.

Glycogen in muscle can decompose into lactic acid, and the lactic acid enters into blood to form blood lactic acid, which will cause muscle ache and discomfort and lead to body fatigue. It was found from the measurement results of BLA in mice that the content of BLA in walnut pr otein peptide groups greatly reduced after sports; in group D, the content of BLA before swimming, after swimming and 20 minutes af ter swimming was 4725.84 ± 1102.52, 7701.55 ± 1211.57 and 4725.54 ± 573.25 mg/L respectively, i.e., BLA was effecti ely inhibited, which indicated that the sup - plementation of high-dose walnut pr otein peptide effectively reduced the accum ulation of lactic acid and could be used to pr omote the fatigue r ecovery of basketball players.

Protein can decompose to pr oduce nitrogen, which is converted into BUN. The higher the c ntent of BUN, the mor e protein decomposed. The dec m- position of a large number of pr oteins will affect th function of the body and c ause fatigue. The detecti n results of BUN showed that the BUN content of group A, B, C and D was 4.69 ± 0.18, 3.54 ± 0.16, 3.19 ± 0.12 and 2.87 ± 0.11 mmol/l, respectively, and the content of BUN of group D was signifi antly lower compared to the other groups, indicating that the intake of walnut protein peptide could effecti ely reduce the production and accumulation of BUN, reduce damages to the body, and promote the fatigue recovery of basketball players.

Glycogen is an important energy substance of the body. Glycogen decomposition can provide energy for the body. When glycogen is insufficien the nor mal blood sugar le vel cannot be maintained, resulting in the lack of energ y and fatigue. According to the r e-sults of glycogen determination in mice, the glycogen content in walnut pr otein peptide gr oups was high, and the hepatic gl ycogen content in group D was the highest, which was much higher than that of the other groups ($P < 0.05$), indicating that the supplement of walnut protein peptide could signifi antly improve the glycogen reserve and enhance the endurance and could be used to realize the anti-fatigue of basketball players.

In conclusion, walnut pr otein peptide c an pro-mote the fatigue r ecovery of mice. The expe imental results provide some references for the subsequent study of the fatigue relief function of walnut pr otein peptide for basketball players. It may be used as a sup-plementary food for basketball players to promote the fatigue recovery of basketball players.

Conclusion

In order to verify the possibility of walnut protein peptide as a supplementar y food for basket ball play-ers, the fatigue r elief effect of walnut p otein peptide in mice was studied. It was found that walnut pr otein peptide had no effect n the nor mal growth of mice and could effecti ely inhibit the accumulation of BLA in mice, effecti ely reduce the production of BUN in mice, and effecti ely improve the glycogen reserve of mice. The experimental results show that walnut pro-tein peptide can effecti ely promote the recovery of fa-tigue after sports training, providing some theoretical bases for its application in the development of supple-mentary food for basketball players.

References

1. Barfield JP, Newsome L, John EB, Sallee D, Frames C, Soangra R, Malone LA. A case report of shoulder fatigue imbalance in wheelchair rugby: implications to pain and in-jury. Spinal Cord 2016; 2: 16002.
2. Wei Z. Research on the Effect of Puerarin on Alleviating Sports Fatigue. Open Biomed Eng J 2015; 9: 288-291.
3. Zamanian M, Hajizadeh MR, Nadimi AE, Shamsizadeh A, Allahtavakoli M. Anti-fatigue effects of t oxerutin on exer-cise endurance capacity, oxidative stress and MMP-9 levels in trained male rats. Fund Clin Pharmacol 2017; 31: 447.
4. Sun RH. The Reasea ch on the Anti-Fatigue Effect of Whey Protein Powder in Basket ball Training. Open Bi - omed Eng J 2015; 9: 330-334.
5. Hao WL, Chen ZB, Zhao R, Bo L. Effect of ycium bar-barum polysaccharide on immune function and anti-fatigue of sub-healthy mice. Chin J Biol 2015; 28: 693-697.
6. Sakai S, Adachi R, Akiyama H, Teshima R, Doi H, Shibata H. Determination of walnut protein in processed foods by enzyme-linked immunosorbent assay: interlaboratory study. J AOAC Int 2010; 93(4): 1255-61.
7. Martínez-García PJ, Crepeau M W, Puiu D, Gonzalez-Ibeas D, Whalen J, Stevens KA, Paul R4, Butterfield TS, Britton MT, Reagan RL, Chakraborty S, Walawage SL, Vasquez-Gross HA, Cardeno C, Famula RA, Pratt K, Ku-ruganti S, Aradhya MK, Leslie CA, Dandekar AM, Salz-berg SL, Wegrzyn JL, Langley CH, Neale DB. The walnut (Juglans regia) genome sequence reveals diversity in genes coding for the biosy nthesis of non-structural polyphenols. Plant J 2016; 87(5):507.
8. Guasch-Ferré M, Jun L, Hu F B , Salas-Salvadó J, Tobias DK. Effects of walnut c nsumption on blood lipids and other cardiovascular risk factors: an updated meta-anal ysis and systematic review of controlled trials. Am J Clin N utr 2018; 108(1):174-187.
9. Zhang Y, Yuan S, Wang X, Feng B, Zhang R, Han J. Analy-sis and reflecti n on development strategy of walnut pr o-cessing industry in China. Trans Chin S oc Agricult Eng 2015; 31: 1-8.
10. Offia lua B. Chemical, Functional and Pasting Properties of Wheat (Triticumspp)-Walnut (Juglansregia) Flour. Food & Nutrition Sciences, 2014; 5(16): 1591-1604.
11. Lu W, Yu W, Sun D. Advances in Application of Marine Bioactive Peptides in Drug Development. Chinese J Org Chem 2017; 37: 1681.
12. Siow HL, Gan CY. Optimization Study in Extracting Anti-Oxidative and A-Amylase Inhibitor Peptides from Cumin Seeds (Cuminum Cy minum): Bioactive Peptides from Cuminum Cyminum. J Food Biochem 2016; 41.
13. Jiang L, Xu H, Li Y. Enzymolysis for pr eparation of ACE inhibitory peptides from walnut protein and studies on its function. J Chin Instit Food Sci Tech 2015; 15(2): 79-85.
14. Ju DT, Ak K, Kuo WW, et al. Bioactive Peptide VHVV Upregulates the L ong-Term Memory-Related Biomarkers in Adult Spontaneously Hypertensive Rats. Int J Mol Sci 2019; 20.
15. Mooney C, Haslam NJ, Holton TA, Pollastri G, Shields DC. PeptideLocator: prediction of bioactiv e peptides in protein sequences. Bioinformatics 2013; 29(9): 1120-1126.
16. Wang L, Zhang HL, Lu R, et al. The de apeptide CMS001 enhances swimming endur ance in mice. Peptides 2008; 29(7): 1176-1182.