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Amylopectin is the anti-fatigue ingredient in glutinous rice

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ABSTRACT

The anti-fatigue activities of glutinous rice (GR) and GR amylopectin (GRA) were investigated in mice by determining tissue glycogen, blood lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) after the weight loaded forced swim test (WFST). GR and GRA were given by gavage at various doses of GR (7.5, 15, 30 g/kg body weight) and GRA (3.8, 7.5, 15 g/kg body weight) every day for 7 days, respectively. The results indicated that the hepatic glycogen levels significantly ($P < 0.05$) increased 26–44% and 35–60% and the muscle glycogen levels significantly ($P < 0.05$) increased 36–100% and 67–133% in GR and GRA treatment groups, compared with the negative control group. The GRA treatment groups also had significantly ($P < 0.05$) higher (9.1–20.3%) blood LDH levels. Meanwhile, the blood LDH activities in GR and GRA treatment groups had a significantly positive correlation with the hepatic glycogen levels ($r = 0.978$, $P < 0.01$). Moreover, except of the low-dose GR (7.5 g/kg body weight) supplemented group, mice in all other treatment groups had significantly ($P < 0.05$) lower (13–23%) BUN levels. Compared with the GR treatment groups, GRA treatment groups had similar or even higher anti-fatigue activities, which demonstrated that GRA might play the most important role on the anti-fatigue activities for GR.

1. Introduction

Fatigue is a physiological phenomenon accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental work [1,2]. Physical fatigue might be due to the depletion of tissue glycogen [3] and accumulation of metabolic products, including lactic acid and ammonia in the body [4]. Therefore, recovery from exercise fatigue requires supplying energy resources continuously and promoting elimination of the metabolic products. Regular exercise and a balanced diet are the best ways to reduce fatigue [5]. Natural products, such as polysaccharides, have been widely investigated to postpone or eliminate fatigue [6–8].

Glutinous rice (*Oryza sativa* L.) (GR), characterized by the opaque appearance, low amylose content, soft texture, and resistance to retrogradation, is generally reserved for use in desserts and festival foods, and it also serves as the staple food in upland regions of Southeast Asia [9]. GR has good nutritional value, such as lower fat, higher content of starch, protein, as well as phenolics antioxidants, including kaempferol, gallic acid, and selenium, which can provide antioxidant potential [10]. Moreover, glutinous rice is widely used in traditional Chinese medicine to enhance physical power, replenish qi, invigorate the spleen, and prevent fatigue [10]. Generally, GR is widely used as an industry resource due to its specific property, such as high content of amylopectin, which has a defined crystalline structure composed of tandem linked clusters [11]. And variations of the cluster fine structure are responsible for the variations in the starch properties, tissues, and genetic backgrounds. Many research reports have focused on the genetic basis of GR quality, the properties of gelatinization, retrogradation, pasting and texture of GR starch or flour [11–13]. However, few studies have reported the physiological effects or health function of GR amylopectin (GRA).

The forced swimming test is a behavioral test for rodents that has been used to predict the efficacy of antidepressant treatments, which induces the development of immobility as a reflection of helplessness when subjected to an inescapable situation (a deep water tank) [14]. Many researches have used the forced swimming test to study the anti-fatigue activities of different agents [8,15–17]. In the present study, the anti-fatigue effects of different doses GR and GRA in mice were investigated by measuring the content of tissue glycogen, the activity of blood lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) after the forced swimming test.

2. Materials and methods

2.1. Preparation of GR flour and amylopectin

GR was provided by Huang Guo Grain Co. Ltd. (Henan, China) and was ground in a hammer mill to pass through a 80-mesh screen.

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Then 10 g was suspended in 50 mL 0.085 mol/L NaOH and the GR suspension was stirred at 300 rpm for 3 h at 40 °C and was adjusted to pH 7.0 with 0.1 mol/L HCl. The insoluble residue was obtained by centrifugation at 5000 × *g* for 10 min and washed three times with 0.1% (w/w) NaCl and three times with distilled water. After drying, the starch was passed through a 80-mesh screen. Fractionation of GRA was carried out by following the general procedure of Song and Jane [18]. The starch dispersion (0.8%, w/v in water) was stirred at 300 rpm at 100 °C until starch was gelatinized. The insoluble residue was removed through filtration. The solution was stirred at 300 rpm in a boiling water bath for 2 h to disperse the starch molecules after the pH was adjusted to 6.3 with a phosphate buffer. Thereafter, *n*-butyl alcohol was added (20%, v/v), and the solution was stirred at 100 °C for 1 h, followed by cooling to the room temperature over a period of 24–36 h. Amylose butyl alcohol complex crystals were formed and precipitated during cooling, and was separated by filtration. The amylopectin remaining in the supernatant was recovered by precipitation by adding excess methyl alcohol. The purity of amylopectin was 89.3%.

2.2. Experimental animals

Male ICR (Institute of Cancer Research), SPF grade grown mice (approximately 20 g) were obtained from Spf Experimental Animal Technology Co., Ltd. (Certification of Experimental Animal: No. SCXK (Jing) 2011-0004) (Beijing, China). The mice were housed at the temperature of 20 ± 2 °C with a 12 h light and 12 h dark cycle. The standard chow including wheat bran, corn, wheat flour, vegetable oil, vitamin, and microelement (Beijing Macao Cooperation Feed Co., Ltd., Beijing, China) and water were available *ad libitum*. The experimental procedures were performed in conformity with the international guidelines for care and use of laboratory animals.

Mice were randomly divided into the following experimental groups, with 8 mice in each group. The first group designated as the negative control group (NCG) was administered – distilled water (30 g/kg body weight) by gavage every day for 7 days. The second group designated as the positive control group (PCG) was given American ginseng slice (1 g/kg body weight) (Changxing Scientific Health Care Provisions A Sinop-Us Cooperative Enterprise, Guangdong, China) by gavage every day for 7 days. The third, fourth, and fifth group designated as the GR treatment groups of low, medium, and high doses (GRL, GRM, and GRH) were given by gavage at various doses of GR (7.5, 15, 30 g/kg body weight) every day for 7 days, respectively. The sixth, seventh, and eighth group designated as the GRA treatment groups of low, medium, and high doses (GRAL, GRAM, and GRAH) were given by gavage at various doses of GRA (3.8, 7.5, 15 g/kg body weight) every day for 7 days, respectively.

2.3. Weight loaded forced swimming test

The weight loaded forced swimming test (WFST) was performed as described by Kumar et al. with some modifications [19]. The mice in GR and GRA administered groups and control groups were allowed to swim with constant loads (tagged to the tail base) corresponding to 4% of their body weights. The swimming test was carried out in an adjustable-current water pool (50 cm × 35 cm × 30 cm), filled with water to a depth of 25 cm and maintained at a temperature of 30 ± 2 °C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 s. After the 7-day treatment, the mice were allowed to rest for 60 min and then forced to swim. After 90 min, all the mice were exhausted.

Table 1
Effects of different doses of glutinous rice (GR) and GR amylopectin (GRA) on hepatic glycogen and muscle glycogen levels.

	Amount (g/kg body weight)	Hepatic glycogen (mg/g)[a]	Muscle glycogen (mg/g)
NCG		2.2 ± 0.29e	0.25 ± 0.02f
PCG	1.0	4.3 ± 0.52a	0.68 ± 0.09a
	7.5	2.8 ± 0.29d	0.34 ± 0.04e
GR	15.0	2.8 ± 0.28d	0.48 ± 0.07b
	30.0	3.2 ± 0.32bc	0.50 ± 0.05c
	3.8	3.0 ± 0.40cd	0.41 ± 0.07d
GRA	7.5	3.2 ± 0.39bc	0.46 ± 0.06c
	15.0	3.6 ± 0.29b	0.57 ± 0.07e

[a] Data presented as means ± SE. Different letters indicate significant difference at $P < 0.05$. NCG: negative control group; PCG: positive control group.

2.4. Analysis of biochemical parameters

Animals were sacrificed under mild anesthesia immediately after the WFST. Liver and gastrocnemius muscle were dissected out, frozen in liquid nitrogen, and kept at −80 °C until analysis for the determination of hepatic glycogen and muscle glycogen.

The concentrations of hepatic glycogen and muscle glycogen were determined using commercially available enzyme assay kits (Nanjing Jiancheng Biocompany, Nanjing, China). The blood was collected from the eyes and centrifuged at 3000 × *g* for 15 min at 4 °C for the determination of LDH and BUN levels. The concentrations of LDH and BUN were determined using enzyme assay kits (Leadman Group Co., Ltd., Beijing, China).

2.5. Statistical analysis

All data are expressed as means ± SE. Differences among groups were determined by one-way ANOVA analysis of variance using the Minitab 15 statistical program (Minitab Inc., State College, PA, USA). Pearson correlation coefficients were calculated for investigating relationships of anti-fatigue activities with the expression of hepatic genes and determined by the SPSS16.0 statistical program (IBM Inc., New York, NY, USA). Significance was defined at the 95% confidence level.

3. Results and discussion

In the present study, the tissue glycogen (including hepatic glycogen and muscle glycogen), LDH, and BUN levels were measured to evaluate the anti-fatigue effects of GR and GRA.

3.1. Effects of GR and GRA on tissue glycogen levels of mice

Enhancement of exercise capacity may be accounted by the increased storage of hepatic glycogen and muscle glycogen and a greater potential for fatty acid metabolism [20]. Since tissue glycogen is an important energy storage resource, increasing the glycogen stored in liver and muscle may enhance the endurance capacity of exercise. In this study, mice in the PCG had a significantly ($P < 0.05$) higher hepatic glycogen level (93.3%) and muscle glycogen level (172%) compared with the NCG (Table 1). Compared with the NCG, the hepatic glycogen levels of GRL, GRM, and GRH significantly ($P < 0.05$) increased 26%, 26.9%, and 43.5%, respectively and the muscle glycogen levels increased 36%, 92%, and 100%, respectively. GRA supplements also significantly ($P < 0.05$) increased the hepatic glycogen levels (35–60%) and the muscle glycogen levels (67–133%) compared with the NCG. For GR and GRA supplemented groups, the tissue glycogen levels increased with the increasing GR and GRA concentrations. The data indicated that anti-fatigue activities of GR and GRA might be related to the improvement in the metabolic control of exercise and the activation of energy