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Rice protein improves oxidative stress by regulating glutathione metabolism and attenuating oxidative damage to lipids and proteins in rats

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ABSTRACT

Aims: To evaluate the effects of rice protein (RP) on glutathione metabolism and oxidative damage.
Main methods: Seven-week-old male Wistar rats were fed diets containing casein and RP without cholesterol for 3 weeks. Plasma and liver lipid levels, hepatic accumulation of total glutathione (T-GSH), oxidized glutathione (GSSG), reduced glutathione (GSH), malondialdehyde (MDA) and protein carbonyl (PCO) were measured. In the liver, the total antioxidative capacity (T-AOC), mRNA levels of glutamate cysteine ligase catalytic subunit (GCLC) and glutamate cysteine ligase modulatory subunit (GCLM), and the activities of hepatic catalase (CAT), total superoxide dismutase (T-SOD), γ-glutamylcysteine synthetase (γ-GCS), glutathione *S*-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GSHPx) were also measured.
Key findings: T-AOC, GCLC and GCLM mRNA levels, antioxidative enzyme activities (T-SOD and CAT) and glutathione metabolism related enzyme activities (γ-GCS, GST, GR and GSHPx) were effectively stimulated by RP feeding compared to casein, and RP significantly reduced the hepatic accumulation of MDA and PCO in rats. These results indicate that lipid-lowering activity was induced by RP feeding.
Significance: The present study demonstrates that RP improves oxidative stress primarily through enzymatic and non-enzymatic antioxidative defense mechanisms, reflected by enhancing the antioxidative status and attenuating the oxidative damage to lipids and proteins. These results suggest that RP can prevent hyperlipidemia in part through modifying glutathione metabolism, and sulfur amino acids may be the main modulator of this antioxidative mechanism.

Introduction

Oxidative stress is one of the major risk factors for developing hyperlipidemia. Diet plays an important role in regulating oxidative stress to prevent hyperlipidemia (Avula and Fernandes, 1999; Coyle et al., 2008; Gorinstein et al., 2005; Lee, 2006). Accordingly, the suppression of oxidative stress may be a useful target for new therapies in preventing hyperlipidemia.

Oxidative stress can be caused by oxidative damage to lipids and proteins. In addition to malondialdehyde (MDA), which is generally used as a marker of lipid peroxidation, the oxidation process may be accelerated by the formation and accumulation of carbonylated protein (Pirinccioglu et al., 2010). Recent studies have shown that protein carbonylation may be involved in various disease states, and protein carbonyls (PCO) may serve as biomarkers of oxidative stress (Dalle-Donne et al., 2003). Thus, lipid peroxidation and protein oxidative damage may indicate a higher risk for developing hyperlipidemia, suggesting that effective dietary antioxidants should be not only reactive oxygen species scavengers but also reactive carbonyl scavengers.

Glutathione (GSH) is an antioxidant that is ubiquitous in mammals, and it plays important roles in several detoxification reactions and in the suppression of lipid peroxidation (Anderson, 1998). The liver represents the major site of GSH metabolism, in which GSH and its related enzymes comprise an antioxidant defense system that protects against oxidative stress. Further, as a non-essential nutrient, GSH can be synthesized in the body from several amino acids (L-cysteine, L-glutamic acid and glycine), suggesting that the amino acid profile of the diet may be a major contributor to GSH metabolism responsible for an antioxidative defense.

Rice is a staple cereal that is widely consumed around the world (Kishine et al., 2008; Ohtsubo and Nakamura, 2007; Tran et al., 2005). There has been a growing emphasis on the improvement of the physiological functions of rice (Tran et al., 2004; Yang et al., 2012a), and an association between rice protein consumption and reduced plasma and liver lipid levels has been extensively demonstrated in some studies (Yang et al., 2007, 2012b; Yang and Kadowaki, 2009). However, the

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precise mechanism by which rice protein affects lipid metabolism has not been fully established. Specifically, there is not yet a comprehensive understanding of the link between the modulation of oxidative status and the consumption of rice protein. Accordingly, evidence describing how rice protein can improve oxidative stress to regulate lipid metabolism is lacking.

The present study, therefore, was conducted to focus on the regulatory effects of rice protein on GSH metabolism and lipid and protein oxidative damage status. The key questions addressed are: (1) whether there is a link between lipid-lowering action and antioxidative stress and the consumption of rice protein and (2) whether rice protein can improve oxidative stress in growing rats fed cholesterol-free diets.

Materials and methods

Protein sources

Rice protein (RP) from *Oryza sativa* L. cv. *Longjing* 26 (Rice Research Institute of Heilongjiang Academy of Agricultural Sciences, Jiamusi, China) and casein (CAS) (Gansu Hualing Industrial Group, Gansu, China) were used as the dietary protein sources in the present study. RP was prepared by the alkaline extraction method (Yang et al., 2011a, 2012b).

Animals and diets

The present experiment was performed in compliance with the Guidelines of the Committee for Animal Experimentation of Harbin Medical University and followed the same protocol used in previous studies (Yang et al., 2011a, 2012b). Seven-week-old male Wistar rats were purchased from the Animal Center of Harbin Medical University (Harbin, China) and individually housed in metabolic cages in a room maintained at 22 ± 2 °C under a 12 h light–dark cycle (07:00–19:00 light). Rats were allowed free access to commercial pellets (Animal Center of Harbin Medical University, Harbin, China) for 3 days. After acclimatization, the rats were randomly divided into two groups of similar body weight. Each group consisted of six rats.

All animals were fed *ad libitum* with experimental diets according to the formula recommended by the American Institute of Nutrition (Reeves et al., 1993). For 3 weeks, growing rats were fed cholesterol-free diets with a dietary protein level of 20% (as crude protein, CP) of CAS and RP, respectively. Diets were completed to 100% with starch. The compositions of the experimental diets are shown in Table 1.

Sample collection

During the feeding period, food consumption and body weight were recorded daily in the morning before replenishing the diets.

Table 1
Composition of the experimental diets (g/kg diet).

	CAS[a]	RP[b]
CAS	228.9	–
RP	–	221.8
Sucrose	100.0	100.0
Cellulose	50.0	50.0
Soybean oil	70.0	70.0
β-Cornstarch	500.6	510.7
Mineral mix[c]	35.0	35.0
Vitamin mix[d]	10.0	10.0
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.014	0.014
L-Cysteine	3.0	–

[a] CAS, casein, crude protein 873.7 g/kg.
[b] RP, rice protein, crude protein 901.7 g/kg.
[c] Mineral mixture, AIN-93G-MX (Nosan Corp., Japan).
[d] Vitamin mixture, AIN-93-VX (Nosan Corp., Japan).

At the end of the feeding period, the rats were deprived of food for 18 h and then sacrificed. Blood was withdrawn from the abdominal vein into a heparinized syringe under anesthesia with sodium pentobarbital (50 mg/kg body weight), immediately cooled on ice and separated by centrifugation at 12,000 ×*g* for 5 min. The plasma obtained was frozen at −20 °C until analysis. After blood collection, the liver was excised immediately, rinsed in saline and weighed after blotting on filter paper. The whole liver was cut into several portions and quickly freeze-clamped in liquid nitrogen and stored at −80 °C until analysis.

Analyses of plasma and liver lipid levels

Plasma free fatty acid (FFA), triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) concentrations were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma non-high-density lipoprotein cholesterol (non-HDL-C) was calculated as non-HDL-C = TC − HDL-C.

The lipids in the liver were extracted and purified as described by Folch et al. (1957) and analyzed as described by Yang et al. (2011a). Samples of liver were extracted with chloroform/methanol (2:1, v/v). Hepatic total cholesterol, triglyceride and free fatty acid levels were measured with a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of malondialdehyde and protein carbonyl

The hepatic malondialdehyde (MDA) and protein carbonyl (PCO) contents were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Estimation of hepatic total antioxidative capacity

The hepatic total antioxidative capacity (T-AOC) was measured with a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurements of reduced glutathione and oxidized glutathione

Total glutathione (T-GSH) and oxidized glutathione (GSSG) in the liver were assayed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Reduced glutathione (GSH) was calculated as GSH = T-GSH − 2 × GSSG.

Analyses of hepatic enzyme activities

The activities of hepatic total superoxide dismutase (T-SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione reductase (GR), glutathione-*S*-transferase (GST) and γ-glutamyl cysteine synthetase (γ-GCS) were determined using kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Quantitative real-time PCR

Total RNA was extracted from rat livers using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was reverse transcribed from 1 μg of total RNA using a PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio. Inc., Otsu, Shiga, Japan). For quantitative real time PCR, cDNA was analyzed with the ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) using SYBR Green (Takara Bio. Inc., Otsu, Shiga, Japan). The primer sequences used were: 5′-ACAGCAACAGGGTGGTGG-AC-3′ (forward) and 5′-TTTGAGGGTGCAGCGAACTT-3′ (reverse) for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH); 5′-CCTCCTCCTCCAAACTCAGAT-A-3′ (forward) and 5′-CCACAAATACCACAT