HYPOLIPIDEMIC AND ANTIOXIDANT EFFICACY OF GIANT EMBRYO BROWN RICE IN HIGH-FAT-FED GUINEA PIGS

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Abstract

To investigate the antihyperlipidemic effects of giant embryo rice 'Shangshida No. 5' on high-fat-fed guinea pigs, forty-eight male guinea pigs were randomly assigned and fed the following diets for 12 weeks: normal control (NC), normal control supplemented with giant embryo brown rice (NC-GB), high fat (HF), and high-fat supplemented with giant embryo brown rice (HF-GB). The body weights, lipid profiles, superoxide dismutase (SOD) activities, malondialdehyde (MDA) levels and liver cellular structures were monitored. The results showed that the HF guinea pigs exhibited significantly ($P < 0.05$) higher levels of plasma triglyceride, total cholesterol, low-density lipoprotein cholesterol, and hepatic triglyceride than NC group. However, diet supplementation with giant embryo brown rice partially counteracted the high fat-induced hyperlipidemia. In addition, diet supplementation with giant embryo brown rice markedly enhanced plasma SOD activities, reduced MDA levels and alleviated liver cellular structures damage in high-fat-fed guinea pigs ($P < 0.05$). Besides, 'Shangshida No. 5' is enriched in trace elements, unsaturated fatty acids, dietary fiber, total protein, and total lipids compared with control rice 'Chao2-10'. These findings illustrated that 'Shangshida No. 5' is useful as a functional food because of its hypolipidemic effects and antioxidant activity.

Key words: Antioxidant, Giant embryo brown rice, Guinea pigs, High cholesterol diet, Hypolipidemic

INTRODUCTION

Hyperlipidemia, also known as dyslipidemia, is a representative chronic disease caused by an imbalance in energy intake and consumption associated with a lack of physical exercise, and hyperlipidemia is regarded as a serious risk factor for cardiovascular disease (Braunwald, 1997; Nelson, 2013). Hyperlipidemia is characterized by excessive levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), and reduced levels of high-density lipoprotein cholesterol (HDLC) in the blood and liver. Diet is considered a key cause of hyperlipidemia.

Therefore, the incorporation of functional foods into the diet is desirable for the amelioration or retardation of hyperlipidemia by improving the lipid profile *in vivo*.

In recent years, with the increasing cost of medical care, functional foods are popular in the world due to their hypocholesterolemic, and superior effects in anti-diabetic and anti-obesity (Mermel, 2004; Siro *et al.*, 2008; Ejtahed *et al.*, 2012; Albarracin *et al.*, 2016). In particular, functional foods enriched in bioactive components, including γ -aminobutyric acid

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(GABA) and antioxidants, such as vitamin E and unsaturated fatty acids, are receiving increasing attention due to their good preventive or therapeutic effects on hyperlipidemia; such foods include germinated giant embryonic rice, which is enriched in GABA (Lee *et al.*, 2007); foods with high vitamin E contents (Jialal and Grundy, 1992); seed mixtures and fish oils, which are enriched in unsaturated fatty acids (Makni *et al.*, 2010; Shiba *et al.*, 2011); and green tea, which is enriched in polyphenols.

With increasing requirement for functional foods, nowadays, rice breeders are committed to developing rice cultivars with more functional properties. Various functional rice cultivars, such as high-amylose rice, pigmented rice and golden rice enriched in beta-carotene (Paine *et al.*, 2005; Min *et al.*, 2012; Zhu *et al.*, 2012), have been developed. Giant embryo rice, in particular, is the most representative of all of these cultivars; it has high contents of nutrients, including crude lipids, crude proteins, vitamin E, GABA, and some mineral elements, such as Ca and Mg (Zhang *et al.*, 2005; Zhang *et al.*, 2007; Jeng *et al.*, 2012; Wang *et al.*, 2013). ‘Shangshida No. 5’ giant embryo rice was isolated in our research group from the progeny regenerated from calluses of ‘Chao2-10’ rice (Wang *et al.*, 2013), a local high-yielding cultivar. Additionally, our previous results also suggested that the contents of vitamin E and GABA of ‘Shangshida No. 5’ are significantly higher than those of ‘Chao2-10’ (Wang *et al.*, 2013; Zhao *et al.*, 2017). Moreover, in this study, we preferentially analyzed the contents of unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid), vitamin B₁ (VB₁), dietary fiber, total protein and total lipids in ‘Shangshida No. 5’.

Previous studies have reported the hypolipidemic effects of germinated giant embryonic rice (Lee *et al.*, 2007), giant embryo brown rice (Chung *et al.*, 2014a), high-amylose rice, and green-kerneled rice in hyperlipidemic model rats or mice

(Lee *et al.*, 2013). Previous evidence have shown that there are many differences between lipoprotein metabolism in rats and humans (Speijer *et al.*, 1991). Cholesteryl ester transfer protein (CETP) is absent in rats, which is critical in lipid exchange in humans and intravascular lipoprotein particle processing (Speijer *et al.*, 1991). Like rats, mice are also not suitable for human lipoprotein metabolism. Furthermore, cholesterol is carried mostly as high-density lipoprotein (HDL) particles in both rats and mice, unlike that as low-density lipoprotein (LDL) particles in humans (Bergen and Mersmann, 2005). However, lipoprotein metabolism in guinea pigs has a startling likeness to that in humans, including the majority of cholesterol being carried as LDL, higher LDL to HDL ratio, and possessing plasma CETP (Fernandez and Volek, 2006). In this study, we used guinea pigs for the first time as the hyperlipidemic model to explore the possible preventive effects of giant embryo brown rice on hyperlipidemia by analyzing the body weight, lipid profile and oxidative stress in the guinea pigs.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Shanghai Jiaotong University.

Materials: Giant embryo rice ‘Shangshida No. 5’ (*O. sativa L. japonica*) and the normal embryo control rice ‘Chao2-10’ have were reported previously (Wang *et al.*, 2013). All rice plants were grown in the paddy field of Shanghai Normal University. Rice seeds from both cultivars were collected at 45 days after flowering.

Nutritional component analysis of ‘Shangshida No. 5’ and ‘Chao 2-10’ brown rice: Giant embryo rice ‘Shangshida No.5’ brown rice(1000 g) and normal embryo control rice ‘Chao 2-10’ brown rice (1000 g) were sent to the Rice Product Quality Inspection and Supervision Testing Center at the Ministry of Agriculture for determining the abundance of multi-trace elements, oleic acid, linoleic acid, linolenic acid, Vitamin B₁, and dietary fiber (Table 1).

Table 1. Nutritional components of ‘Shangshida No. 5’ and ‘Chao 2-10’ brown rice

Content tested	Unit	‘Chao 2-10’ (Control)	‘Shangshida No. 5’
Phosphorus	mg/100 g	291±3.20	400±13.45*
Iron	mg/kg	17.8±0.71	20.2±5.23
Zinc	mg/kg	26.7±2.21	37.8±1.51*
Calcium	mg/kg	252.1±5.13	256.7±7.03
Selenium	mg/kg	0.07±0.02	0.08±0.01
Potassium	mg/kg	2914.1±39.78	3717.6±53.20*
Copper	mg/kg	3.0±0.13	3.3±1.20
Oleic acid	mg/100 g	752.50±1.50	1236.00±44.00**
Linoleic acid	mg/100 g	852.00±90.00	1123.00±157.00*
Linolenic acid	mg/100 g	38.10±7.10	50.90±9.90*
Vitamin B ₁	μg/100 g	313.00±13.00	335.50±14.50
Dietary fiber	%	1.31±0.49	1.60±0.50*
Total protein	%	9.73±0.93	10.40±0.61
Total fat	%	2.44±0.17	3.28±0.07*

Values are means ± SD (n=3); *P<0.05; ** P<0.01

Table 2. Compositions of basal diet

Feed ingredients	Amount (g/100 g)
Moisture ¹	11.78
Protein ¹	17.6
Carbohydrates ¹	43.3
Fat ¹	3.2
Fiber ¹	14.82
Mineral mix ²	8.2
Vitamin mix ²	1.1

¹Compositions of feed ingredients were provided by alfalfa meal (35%), soybean meal (15%), corn meal (25%) and wheat meal (25%) and met NRC and GB 14924.5-2001 specified requirements for guinea pigs

²Mineral and vitamin mix adjusted to meet NRC and GB 14924.5-2001 requirements for guinea pigs

Animals and diets: Forty-eight male guinea pigs (5 weeks old, weighing ~250 g) were purchased from Songjiang Songlian Experimental Animal Farm. The animals were fed normal control diets for one week for acclimatization to the experimental animal cages. After acclimation, the animals were randomly divided into 4

groups (n=12) and fed with different diet: normal control diet (NC), normal control diet supplemented with 30% giant embryo rice (NC-GB), high-fat diet (HF), and high-fat diet supplemented with 30% giant embryo rice (HF-GB), respectively. The composition of the experimental diet is presented in Table 2 and Table 3. The animals were allowed free access to the diet and water during the experimental period for 12 weeks. Body weight was measured weekly. After 12 weeks, the male guinea pigs were anesthetized with diethyl ether. Blood samples and the body organs (liver, heart, spleen, and kidney) were operated following the previous report (Lee *et al.*, 2013).

Measurements of plasma and hepatic lipids:

The plasma TG, TC, HDLC, and LDLC as well as the hepatic TG and hepatic TC were measured using commercial kits (Nanking Jiancheng Biological Engineering Research Institute). The hepatic lipids were extracted according to the Technical Standards for Testing and Assessment of Health Food (Ministry of Health of the People’s Republic of

Table 3. Composition of the experimental diets (g/100 g)

Dietary groups	NC	NC-GB	HF	HF-GB
Basal diet ¹	100	70	84.67	54.67
Lard	0	-	15	15
Cholesterol	0	-	0.33	0.33
GBR	0	30	-	30

GBR- giant embryo brown rice; HF- high-fat diet; HF-GB- high-fat diet + giant embryo brown rice; NC- normal control diet; NC-GB- normal control diet + giant embryo brown rice

¹Compositions of basal diet are listed in Table 2

China, 2003). The liver tissues were homogenized, dissolved in 1×PBS, and centrifuged at 4°C at 12000 g to obtain liver homogenates with a mass fraction of 10%.

Plasma SOD and MDA analysis: The plasma superoxide dismutase (SOD) activity and malondialdehyde (MDA) level were measured according to the procedures provided with the detection kits (Nanking Jiancheng Biological Engineering Research Institute, Nanjing, China).

Transmission electron microscopy (TEM) analysis: The livers from the experimental guinea pigs were cut into 1 mm³ cubes and were fixed in a 4% paraformaldehyde solution for 1 h. Then the samples were rinsed. Above operation was performed at 4°C. Afterwards, the samples were incubated in SOD enzyme incubation medium for 1 h at room temperature. Next, the liver tissues were rinsed using sodium cacodylate buffer for 30 min each time for two times. After that, the tissues were fixed in 1% osmium tetroxide. Then the samples were transferred to sodium cacodylate buffer for 30 min each for twice. Afterwards, the tissue samples were dehydrated using a graded alcohol and were embedded in Epon 812 and ultrathin sections were cut into 0.5µm.

Statistical analysis: GraphPad Prism 5.0 (Graph Software, San Diego, CA, USA) was used to statistically analyze. The differences among multiple groups were compared by one-way ANOVA followed by the LSD test.

RESULTS

Nutritional contents of giant embryo brown rice: Higher contents of multiple trace elements, oleic acid, linoleic acid, linolenic acid, dietary fiber, and total lipids were detected in ‘Shangshida No.5’ than in ‘Chao 2-10’ (Table 1). In addition, previous reports have suggested that GABA and vitamin E levels were obviously higher in ‘Shangshida No. 5’ (Wang *et al.*, 2013; Zhao *et al.*, 2017). The above data indicate that ‘Shangshida No. 5’ is a superb rice cultivar with high-quality.

Body weight and organ weight: Guinea pigs used in the study exhibited similar body weights at the beginning and end of the experimental period (Table 4). Unlike previous reports, enriching the diet with giant embryo brown rice considerably decreased the body weight gain in the experimental mice (Chung *et al.*, 2014a). This discrepancy may be caused by the different hyperlipidemic animal models. In addition, the HF group showed significantly higher heart and kidney weights than were seen in NC group; however, the liver and spleen weights did not have obvious difference between groups (Table 4).

Plasma and hepatic lipids: The levels of plasma TG, TC and LDLC concentrations as well as the hepatic TG and TC were significantly higher in the HF group guinea pigs compared with the NC group (Table 5), which indicated that high-fat feeding led to increased lipid levels in

Table 4. Effects of the rice-supplemented diets on body weight and organ weights in guinea pigs fed a high-fat diet

Dietary groups	NC	NC-GB	HF	HF-GB
Initial weight (g)	256.14±22.88	254.08±7.53	270.4±10.04	271±7.69
Final weight (g)	430.67±29.44	440.33±15.51	478.86±13.32	455.65±9.15
Weight gain (g)	174.53±11.07	186.25±7.13	208.46±5.05	184.65±3.07
Liver ¹	2.99±0.45	2.52±0.25	3.1±0.34	3.32±0.65
Kidney ¹	0.98±0.24 ^{##}	0.72±0.1	0.64±0.1 ^{**}	0.73±0.17 [*]
Spleen ¹	0.11±0.02	0.11±0.01	0.12±0.03	0.15±0.07
Heart ¹	0.43±0.23 [#]	0.26±0.04	0.28±0.03 [*]	0.3±0.04

¹Organ weights, g/100 g body weight

Values are means ± SD (n=12); *P<0.05, **P<0.01, compared with the NC group; #P<0.05, ##P<0.01, compared with the HF group; HF- high-fat diet; HF-GB- high-fat diet + giant embryo brown rice; NC- normal control diet; NC-GB- normal control diet + giant embryo brown rice

Table 5. Effects of the rice-supplemented diets on plasma and hepatic lipids in guinea pigs fed a high-fat diet (mmol/L)

	Dietary groups	NC	NC-GB	HF	HF-GB
Plasma	TG (mmol/L)	1.04±0.36 ^{##}	1.12±0.31 [#]	1.69±0.26 ^{**}	1.09±0.16 ^{##}
	TC (mmol/L)	2.09±0.33 ^{##}	2.14±0.33 ^{##}	3.63±0.64 ^{**}	3.29±0.92 [*]
	HDLC (mmol/L)	1.24±0.03	1.26±0.08	1.24±0.05	1.29±0.08
	LDLC (mmol/L)	0.38±0.18	0.40±0.09	0.55±0.17 [*]	0.32±0.09 [#]
Liver	TG (mmol/g)	0.66±0.08 [#]	0.6±0.45 [#]	1.67±0.81 [*]	0.91±0.13 [#]
	TC (mmol/g)	1.16±0.07 [#]	1.32±0.21	1.63±0.51 [*]	1.28±0.1 [#]

Values are means ± SD (n=12); *P<0.05, **P<0.01, compared with the NC group; #P<0.05, #P<0.01, compared with the HF group; HDLC- high-density lipoprotein cholesterol; LDLC- low-density lipoprotein cholesterol; TC- total cholesterol; TG- triglyceride; HF- high-fat diet; HF-GB- high-fat diet + giant embryo brown rice; NC- normal control diet; NC-GB- normal control diet + giant embryo brown rice

the guinea pigs. However, plasma TG and LDLC concentrations as well as hepatic TG and TC levels decreased in the HF-GB group guinea pigs (Table 5), which suggested that diet supplementation with giant embryo brown rice significantly decreased these lipid levels. The plasma TC level was also lower in the HF-GB group guinea pigs than in the HF group guinea pigs (Table 5), although the difference was not significant. However, the plasma HDLC levels were similar with the NC group, NC-GB group, HF group, and HF-GB group guinea pigs (Table 5). In addition, we also found that the plasma and hepatic lipid levels in the guinea pigs were

similar between the NC group and the NC-GB group guinea pigs, which indicated that diets supplemented with giant embryo brown rice did not decrease these levels further than what was seen with the normal control diet group.

Plasma SOD activity and MDA levels: The plasma antioxidant enzyme SOD activity in guinea pigs fed HF diets was lower than that in guinea pigs fed the NC diet and the HF supplemented with giant embryo rice (Table 6). In addition, plasma MDA concentrations, an indicator of the oxidative stress in the body, were significantly higher in guinea pigs fed HF

Table 6. Effects of the rice-supplemented diets on plasma SOD activities and MDA levels in guinea pigs fed a high-fat diet (mmol/L)

Dietary groups	SOD (U/mL)	MDA (nmol/mL)
NC	85.60±9.33 ^{##}	4.31±1.13 [#]
NC-GB	91.70±15.01 ^{##}	4.19±0.85 [#]
HF	43.60±6.56 ^{**}	6.99±1.46 [*]
HF-GB	70.15±12.68 [#]	4.02±1.35 ^{##}

Values are means ± SD (n=12), *P<0.05, **P<0.01 compared with the NC group; # P<0.05, ##P<0.01, compared with the HF group; SOD- superoxide dismutase; MDA- malondialdehyde; HF- high-fat diet; HF-GB- high-fat diet + giant embryo brown rice; NC- normal control diet; NC-GB- normal control diet + giant embryo brown rice

diets alone (Table 6). Diet supplementation with giant embryo brown rice, however, considerably decreased the plasma MDA levels. The results of the SOD activities and MDA levels suggested that diet supplementation with giant embryo brown rice markedly increased the antioxidant levels and decreased oxidative stress in the guinea pigs.

TEM analysis of guinea pigs liver cells:

Previous reported showed that lipid peroxidation may alter the fluidity of biological membranes, which make the biological membranes ruptured and lead to the release of cell and organelle contents (Marnett, 1999; Alshatwi *et al.*, 2011). To illustrate whether the giant embryo brown rice have the role of relieving the liver cell damage, the liver cellular structures in different groups by TEM were compared. Results found that liver cell in HF group show an obvious morphological structure alteration compared with control group, such as turbid ultrastructure of mitochondria and endoplasmic reticulum, more lipid droplet accumulation, however the degenerative changes can hardly be found in HF-GB group (Fig. 1), the result in accordance with the MDA levels.

DISCUSSION

It has been reported that giant embryo rice has notable hypolipidemic effects by regulating

lipid metabolism in high cholesterol-fed rats (Lee *et al.*, 2007) and in high-fat-fed mice (Chung *et al.*, 2014b). In the previous studies, the hyperlipidemic models were usually rats or mice, but guinea pigs have never been used. Previous evidence has shown that guinea pigs, but not rats or mice, are a good model for human lipoprotein metabolism (Bergen and Mersmann, 2005; Fernandez and Volek, 2006). Giant embryo rice enriched with GABA significantly elevated the HDLC concentration in high cholesterol-fed rats (Lee *et al.*, 2007) and in high-fat-fed mice (Chung *et al.*, 2014b). However, in our study, the HDLC concentrations in the HF group and HF-GB group guinea pigs were similar (Table 5). The above discrepancy may be caused by different animal models, which have different lipid metabolism pathways.

Besides the foods enriched in GABA, the foods with high vitamin E, oleic acid, linoleic acid and linolenic acid also been reported to prevent hyperlipidemia (Makni *et al.*, 2010; Shiba *et al.*, 2011), and which also are main antioxidants. Our previous studies suggested that the vitamin E content in 'Shangshida No. 5' giant embryo brown rice is significantly elevated (Wang *et al.*, 2013). In this study, we found for the first time that the levels of unsaturated fatty acid, oleic acid, linoleic acid, and linolenic acid are higher in 'Shangshida No. 5' than those in 'Chao 2-10' (Table 1). In addition, a higher content in dietary fiber was also detected in 'Shangshida No. 5' (Table 1). Reports have shown that dietary fiber can decrease TG and TC levels and regulate lipid and glucose metabolism, as a result of its ability to resist digestion, and then leading to decreased absorption of fats (Lattimer and Haub, 2010). Therefore, GABA, vitamin E, oleic acid, linoleic acid, linolenic acid, and dietary fiber may be the main active components responsible for the hypolipidemic effects and antioxidant effect of

Fig. 1. Phenotypic analysis of guinea pigs liver cells. A and B- NC group; C and D- HF group; E and F- HF+GB group. ER: endoplasmic reticulum; LD: lipid droplet; Mi: mitochondria; N: nucleus; Nu: nucleolus. Magnification for all picture are 6000X

‘Shangshida No. 5’. However, the specific bioactive component responsible for the physiological properties of ‘Shangshida No. 5’ rice remains unclear. Further investigation on the specific composition and bioactive compounds of ‘Shangshida No. 5’ functional rice are needed to better understand its therapeutic potential against HF diet-induced hyperlipidemia.

In conclusion, we found that dietary supplementation with ‘Shangshida No. 5’ giant embryo brown rice markedly reduced the plasma TG and LDLC concentrations and hepatic TG and TC levels in high-fat-fed guinea pigs, and diets supplemented with giant embryo brown rice had no side effects on normal guinea pigs. Meanwhile, diet supplementation with giant embryo brown rice markedly increased the antioxidant levels and decreased oxidative stress in high-fat-fed guinea pigs. Results also suggested that dietary supplementation with giant embryo brown rice relieve the liver cell

damage in high-fat-fed guinea pigs, which indicated that giant embryo brown rice has the effect of protecting the liver by reducing oxidative stress. In addition, we also found that unsaturated fatty acids contents, e.g. oleic acid, linoleic acid, and linolenic acid, and dietary fiber are higher in ‘Shangshida No. 5’ than in ‘Chao 2-10’. The above analysis suggests that ‘Shangshida No. 5’ giant embryo brown rice is beneficial as excellent functional foods for its preventive action against HF diet-induced hyperlipidemia.

Conflict of interest: The Authors declare that they have no conflicts of interest to disclose.

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