Pre-germinated brown rice prevented high fat diet induced hyperlipidemia through ameliorating lipid synthesis and metabolism in C57BL/6J mice

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Pre-germinated brown rice (PGBR) can ameliorate hyperlipidemia, but the action mechanism is not clear. We focus the mechanisms of PGBR prevented hyperlipidemia. Six-week-old mice were divided into: standard-regular diet (SRD), high-fat diet (HFD) and HFD with PGBR (HFD + PGBR) groups for 16 weeks. The HFD group has higher concentrations of TG, TC, HDL and Non-HDL in the blood, and a higher atherosclerosis index (AI). The TG levels in the liver, and TG, bile acid levels in the feces were enhanced; and the total adipocytokines level in adipose tissue was reduced. The HFD group had higher protein expressions of SREBP-1, SCD-1, FAS, LDLR, and CYP7α1 in the liver. Moreover, the greater expressions of SREBP-1, SCD-1, FAS and the less expressions of PPAR-α and adiponectin were in adipose tissue. In the HFD + PGBR group, the PGBR regulated the levels of TG, TC, HDL, Non-HDL, AI and adipocytokines. PGBR increased more cholesterol and bile acid exhaust in feces. The SREBP-1, SCD-1, FAS, HMGR, LDLR, CYP7α1 and PPAR-α proteins in the liver; and the SREBP-1, SCD-1, FAS, PPAR-α and adiponectin proteins in adipose tissue were reversed by PGBR. Taken together, PGBR can improve lipid synthesis and metabolism, and we suggest PGBR is a recommendable food for controlling hyperlipidemia.

Key Words: pre-germinated brown rice, hyperlipidemia, lipid synthesis and metabolism

Metabolic syndrome, such as hyperlipidemia and type II diabetes mellitus, is a chronic disease caused by an imbalance energy intake and consumption. In addition, hyperlipidemia is characterized by excessive amounts of cholesterol, triglycerides and lipoproteins in the blood. These increased levels of lipids and lipoproteins are regarded as serious risk factors for cardiovascular disease, atherosclerosis and acute pancreatitis.1–3 Diet is an important environmental factor that may induce hyperlipidemia. The modern diets are full of high fat, high sugar and refined grains products that lead to this disease.4 Providing well-balanced diet is a beneficial way to ameliorate hyperlipidemia.5

Pre-germinated brown rice (PGBR) is created by soaking brown rice kernels in water to slightly germinate which has richer dietary fiber and more functional compounds such as γ-aminobutyric acid (GABA) and γ-oryzanol than white rice. PGBR is comprised of the endosperm, aleurone layer, bran layer and germ are considered to have a physical shape delays the digestion and absorption of carbohydrate.6 Each 100 g PGBR contained 69.9 g carbohydrates, 2.9 g fats, 7.5 g proteins, 5.6 g dietary fiber, 16.5 mg GABA, and 3,664 μg γ-oryzanol.7 Many studies have demonstrated that PGBR improves levels of blood glucose, lipids and peroxidation in human8–10 and have recommend replacing the principle food white rice with PGBR to reduce metabolic syndrome.11

Previously, we showed that PGBR lowered body weight, blood pressure, blood glucose and improved glucose tolerance in mice with high-fat diet (HFD)-induced hyperglycemia. Moreover, PGBR could prevent hyperglycemia through improving insulin levels, insulin receptor, and glucose transporters and enhancing glucose metabolism.12 In this study, we investigate the effect of long-term consumption of PGBR on the prevention of HFD-induced hyperlipidemia and its mechanism on the levels of lipids, lipoproteins, adipocytokines and the enzymes involved in the synthesis and metabolism of lipid.

Materials and Methods

Animals and experiments. Six-week-old male C57BL/6J strain mice were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) and housed under constant temperature and illumination (light between 7:30 and 19:30). Water and standard regular diet (SRD) were made available ad libitum. Each 100 g SRD contained 6 g fats (15% energy), 58 g carbohydrates (65% energy), and 18 g proteins (20% energy). After an acclimatization period, the mice were randomly divided into three groups. Group 1 (n = 8) was fed the SRD and group 2 (n = 8) was fed high-fat diet (HFD) for 16 weeks. The HFD was made from SRD with adding lard oil and cholesterol. It contained 60% energy from fat, 21.4% energy from carbohydrates and 18.6% energy from proteins. In group 3 (n = 8), we replaced the source of carbohydrates in HFD, which was, with PGBR (Asia RICE Biotech, Inc, Taiwan) and fed it to the mice for 16 weeks. The nutrient compositions of diets we used in this experiment are referred in Shen et al.12 For all groups, body weight and feces were measured and collected. The feces were stored at −80°C until analysis. After feeding the respective diets for 16 weeks, bloods were collected for biochemical assay, and the excised liver and gonadal adipose tissues from all mice were stored at −80°C until analysis. This study was approved by the Animal Care and Use Committee of Meiho University.

Measurement of biochemical parameters. Blood samples were collected to measure triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) and Non-HDL which were performed using a HITACHI Clinical Analyzer 7070.
Analysis of TG and TC in liver. The extraction method of hepatic lipids was described by Bligh and Dyer.(12) Total lipids were extracted from 30-40 mg of homogenized liver tissue, and then dissolved in isopropanol. Hepatic TG and TC were analyzed by using triglyceride and cholesterol assay kits (BioAssay Systems, Hayward, CA), respectively.

Analysis of TG, TC and bile acid in feces. Following previously described procedures,(13) after lyophilized, homogenized, and then re-lyophilized, the fecal TG were extracted at 60°C for 90 min with a buffer [10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% TritonX-100, and 80% isopropranol]. And fecal TC and bile acid were extracted at 65°C for 4 h with 90% ethanol. The TG, TC and bile acid concentrations were respectively analyzed by triglyceride, cholesterol and total bile acid assay kits (BioAssay Systems).

Analysis of adipocytokines in adipose tissue. Following previously described procedures,(13) the adipose tissues were rinsed with phosphate-buffered saline (PBS) and weighed, cut into small pieces and then transferred into a 12-well plate. Serum-free DMEM was added to the wells and incubated with fat tissue at 37°C in a CO₂ incubator with gentle rocking. After 3 h, the conditioned media were collected and centrifuged at 4°C for 10 min.

Results

Effect of PGBR in body weight and weight gain. Compared with SRD group, the HFD group gained more weight. However, in the HFD + PGBR group, weight was gained but more slowly. PGBR could inhibit HFD induced weight gain. Comparing the food intake per day, all groups had no significant difference (Data was obtained from our previous study:(7) SRD group: 22.6 ± 8.4 g, in HFD group: 25.6 ± 7.6 g and in HFD + PGBR group: 26.2 ± 7.8 g).

Effect of PGBR in biochemical parameters of blood. After 16 weeks, the TG, TC and Non-HDL levels of HFD group (119.1 ± 9.5, 94.1 ± 9.1 and 37.5 ± 2.8 mg/dl) were significantly higher than those of SRD group (71.1 ± 9.4, 54.1 ± 5.1 and 19.9 ± 2.9 mg/dl). The HDL of HFD group (56.5 ± 6.3 mg/dl) was also higher than that of SRD group (34.1 ± 5.4 mg/dl). In the HFD + PGBR group, the TG, TC, Non-HDL and HDL were lower than HFD group (66.7 ± 3.1, 70.5 ± 2.9, 24.6 ± 6.5 and 45.9 ± 3.6 mg/dl). Compared with the HFD group, blood lipids were reduced in the HFD + PGBR group. Comparing the atherosclerosis index (AI), the HFD group was higher than SRD and HFD + PGBR group. PGBR obviously decreased the AI induced by HFD (Table 1).

Table 1. The effects of PGBR on lipids and AI levels of mice fed HFD

| Measurement | SRD (n = 8) | HFD (n = 8) | HFD + PGBR (n = 8) |
|-------------|-------------|-------------|-------------------|
| TG (mg/dl)  | 71.1 ± 9.4  | 119.1 ± 9.5* | 66.7 ± 3.1*       |
| TC (mg/dl)  | 54.1 ± 5.1  | 94.1 ± 9.1*  | 70.5 ± 2.9*       |
| HDL (mg/dl) | 34.1 ± 5.4  | 56.5 ± 6.3*  | 45.9 ± 3.6*       |
| Non-HDL (mg/dl) | 19.9 ± 2.9 | 37.5 ± 2.8*  | 24.6 ± 6.5*       |
| AI          | 0.50 ± 0.04 | 0.66 ± 0.02* | 0.54 ± 0.16*      |

SRD, standard regular diet; HFD, high fat diet; HFD + PGBR, high fat diet with pre-germinated brown rice; AI, atherosclerosis index = (TC-HDL)/HDL. *p<0.05 vs SRD; **p<0.05 vs HFD.

Effect of PGBR in biochemical parameters of liver. The liver weight and TG of HFD group were significantly higher compared with SRD group. In the HFD + PGBR group, the TG was similar with SRD group. However, PGBR did not influence the increased liver weight (Table 2). TC levels were not significantly different between the groups.

Table 2. The effects of PGBR in hepatic lipids levels of mice fed HFD

| Measurement | SRD (n = 8) | HFD (n = 8) | HFD + PGBR (n = 8) |
|-------------|-------------|-------------|-------------------|
| Liver weight (g) | 0.9 ± 0.1 | 1.29 ± 0.1* | 1.32 ± 0.1*       |
| TG (mmol/g)  | 23.6 ± 3.4  | 37.4 ± 6.9* | 24.2 ± 6.9*       |
| TC (μg/g)    | 588.1 ± 77.6| 525.8 ± 69.1| 516.9 ± 167.8     |

SRD, standard regular diet; HFD, high fat diet; HFD + PGBR, high fat diet with pre-germinated brown rice. *p<0.05 vs SRD; **p<0.05 vs HFD.
Effect of PGBR in biochemical parameters of feces. The HFD group had higher TG and bile acid levels than the SRD group in feces, but not TC. In the HFD + PGBR group, the TG, TC and bile acid levels were found to be higher than HFD group in feces, suggesting that PGBR exerted a significant lipid absorption reducing effect in hyperlipidemic mice (Table 3).

Effect of PGBR in biochemical parameters of feces.

| Measurement     | SRD (n = 8) | HFD (n = 8) | HFD + PGBR (n = 8) |
|-----------------|-------------|-------------|-------------------|
| TG (mmol/g)     | 5.3 ± 0.2   | 6.2 ± 0.2*  | 11.8 ± 1.4*       |
| TC (mg/g)       | 1,544.8 ± 150.2 | 1,592.8 ± 49.6 | 2,032.7 ± 205.8* |
| Bile acid (mmol/100 g) | 6.7 ± 0.4   | 10.6 ± 0.8*  | 14.4 ± 1.1*       |

SRD, standard regular diet; HFD, high fat diet; HFD + PGBR, high fat diet with pre-germinated brown rice.

The proteins expressions of lipid synthesis and metabolism in liver. Western blot analysis of the liver isolated from HFD group showed higher levels of SREBP-1 (56%), SCD-1 (130%), FAS (320%), LDLR (31%) and CYP7α1 (74%) proteins, compared with the SRD group. But the protein level of HMGCR and PPARα had no obvious change in SRD and HFD group. In the HFD + PGBR group, there were significant reduction in SREBP-1 (48%), SCD-1 (34%), FAS (57%), HMGCR (78%), and increase in LDLR (50%), CYP7α1 (66%) and PPARα (75%) protein levels compared with the HFD group (Fig. 1).

The proteins expressions of lipid synthesis and metabolism in adipose tissue. Compared with adipose tissue of SRD group, the greater protein expressions of the SREBP-1 (137%), SCD-1 (217%), and FAS (71%), the lower protein expressions of the PPAR-α (30%) and adiponectin (53%) were found in HFD group. In the HFD + PGBR group, PGBR significantly reduced the protein expressions of SREBP-1 (27%), SCD-1 (63%), FAS (48%), and increased PPAR-α (33%), adiponectin (33%) compared with the HFD group (Fig. 2).

Effect of PGBR on adipocytokines of adipose tissue. The level of total adipocytokines of adipose tissue in HFD group (n = 8; 163.8 ± 35.1 μg/g adipose tissue) was lower than that in SRD group (n = 8; 255.3 ± 26.4 μg/g adipose tissue) (p<0.05). In HFD + PGBR group (n = 8), the level of total adipocytokines was 233.9 ± 32.7 μg/g adipose tissue. PGBR significantly reversed the decreased adipocytokines induced by HFD (p<0.05).

Discussion

In our previous study, we had proved PGBR as a staple carbohydrate markedly controlled the weight gain, blood pressure, decreased fasting blood glucose, HbA1c and enhanced insulin levels in hyperglycemic mice. Based on our results, PGBR ameliorated hyperglycemia through recovering the protein expressions of insulin receptor (IR), insulin receptor substrate-2 (IRS-2), phosphatidylinositol-3-kinase (PI3K), serine/threonine kinase PI3K-linked protein kinase B (Akt/PKB), glucose transporter-1 (GLUT-1), GLUT-4, AMP-activated protein kinase (AMPK), glucokinase (GCK), PPARγ and glucogen synthase kinase (GSK). PGBR was able to increase insulin receptor sensitivity, glucose
uptake, and glycolysis, glycogenesis.\(^{17}\) The current study shows that PGBR exhibited beneficial effects on lipid synthesis and metabolism in hyperlipidemic C57BL/6J mice. First, we found that blood lipids levels, such as TG, TC, HDL and Non-HDL, in mice fed HFD were significantly reduced by PGBR. Compared with HFD group, the HDL level was not enhanced by PGBR, but the AI, the most reliable indicator of an increased risk in atherosclerosis,\(^{16}\) was reduced in HFD + PGBR group (Table 1). Based on these results, we suggest that PGBR, when used as a staple carbohydrate, can effectively prevent hyperglycemia and hyperlipidemia and reduce the risk of atherosclerosis. How does PGBR possess this effect? Some reports demonstrated that the basic polysaccharides of brown rice could inhibit lipase activity, promote fecal excretion, and reduce the TG level.\(^{7}\) Rice bran had also been shown to inhibit the pancreatic lipase activity, and suppress the visceral fat accumulation.\(^{18,19}\) In addition, PGBR contained various functional compounds such as GABA and \(\gamma\)-oryzanol, which regulated blood pressure, affected nervous system as neurotransmitter, and potentiated insulin secretion from the pancreases, bile acid excretion from feces. GABA and \(\gamma\)-oryzanol also had been proved to improve levels of blood glucose, lipids and peroxidation.\(^{9,8,10,11}\) According to our previous study,\(^{17}\) the HFD and HFD + PGBR had the higher ratio of calories, crude fat, cholesterol and lower carbohydrate than SRD. The rations of dietary fiber of HFD + PGBR were higher than HFD, but, we suspected that the dietary fiber was not a major role in the regulation of metabolic syndrome. The GABA and \(\gamma\)-oryzanol concentrations were higher in PGBR compared with SRD and HFD.\(^{17}\) We considered that the GABA and \(\gamma\)-oryzanol of PGBR helped to reduce tissue constituent, even out of shape. Although the hepatic TG, but not TC, was excluded by PGBR, PGBR did not reverse the bloated liver. The same results were found in Matsumoto et al.\(^{13}\) and Miura et al.\(^{19}\) We suggested that PGBR could decrease the HFD-induced fat accumulation in the liver, but not inhibit hepatic weight changes in this study.

Abnormal lipid production or lipid consumption caused hyperlipidemia.\(^{22–26}\) As we known, SREBP-1 is a transcriptional activator and regulates the cholesterol and fatty acid synthesis and metabolism.\(^{27}\) SCD-1 is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of monounsaturated fatty acids from saturated fatty acids that are either synthesized or derived from the diet.\(^{27,28}\) FAS is a multi-enzyme protein that catalyzes fatty acid synthesis and participates in metabolism of energy.\(^{27,29}\) HMGR is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol.\(^{30}\) On the other hand, LDLR in the liver plays an important role in plasma lipoprotein metabolism in vivo. The activity of HMGR might affect LDLR activity. Cholesterol homeostasis is maintained by coordinated regulation of endogenous synthesis and exogenous uptake of lipoprotein cholesterol by LDLRs.\(^{30,31}\) CYP7a1 is the rate-limiting enzyme in the synthesis of bile acid from cholesterol. CYP7a1 increases the production of bile acids and reduces the level of cholesterol in hepatocytes.\(^{32}\) PPAR\(\alpha\) is a transcription factor and a regulator of lipid metabolism in the liver, and activated PPAR\(\alpha\) inhibits HMGR and enhances adiponectin expression.\(^{33}\) In the results, we found the protein level of SREBP-1, SCD-1, FAS, LDLR and CYP7a1 were enhanced by HFD. The protein levels of HMGR and PPAR\(\alpha\) had no obvious change between SRD and HFD group. However, in HFD + PGBR group, we found the level of SREBP-1, SCD-1, FAS, LDLR and CYP7a1 were enhanced by HFD. The protein levels of HMGR and PPAR\(\alpha\) were increased. PGBR not only repressed the lipids absorption through excreting bile acid, but also reduced lipids synthesis and enhanced lipid metabolism through ameliorating above protein expressions. Imam et al.\(^{34}\) had demonstrated that germinated brown rice (GBR) improved hypercholesterolemia through ameliorating LDLR. We suggested that the PGBR had the same mechanisms on lipid-lowering effect. On the other hand, the high levels insulin activated the SREBP-1 and SCD-1 expressions, and high levels SREBP-1 could enhance uptake, and glycolysis, glycogenesis.\(^{17}\) The current study shows that PGBR exhibited beneficial effects on lipid synthesis and metabolism in hyperlipidemic C57BL/6J mice. First, we found that blood lipids levels, such as TG, TC, HDL and Non-HDL, in mice fed HFD were significantly reduced by PGBR. Compared with HFD group, the HDL level was not enhanced by PGBR, but the AI, the most reliable indicator of an increased risk in atherosclerosis,\(^{16}\) was reduced in HFD + PGBR group (Table 1). Based on these results, we suggest that PGBR, when used as a staple carbohydrate, can effectively prevent hyperglycemia and hyperlipidemia and reduce the risk of atherosclerosis. How does PGBR possess this effect? Some reports demonstrated that the basic polysaccharides of brown rice could inhibit lipase activity, promote fecal excretion, and reduce the TG level.\(^{7}\) Rice bran had also been shown to inhibit the pancreatic lipase activity, and suppress the visceral fat accumulation.\(^{18,19}\) In addition, PGBR contained various functional compounds such as GABA and \(\gamma\)-oryzanol, which regulated blood pressure, affected nervous system as neurotransmitter, and potentiated insulin secretion from the pancreases, bile acid excretion from feces. GABA and \(\gamma\)-oryzanol also had been proved to improve levels of blood glucose, lipids and peroxidation.\(^{9,8,10,11}\) According to our previous study,\(^{17}\) the HFD and HFD + PGBR had the higher ratio of calories, crude fat, cholesterol and lower carbohydrate than SRD. The rations of dietary fiber of HFD + PGBR were higher than HFD, but, we suspected that the dietary fiber was not a major role in the regulation of metabolic syndrome. The GABA and \(\gamma\)-oryzanol concentrations were higher in PGBR compared with SRD and HFD.\(^{17}\) We considered that the GABA and \(\gamma\)-oryzanol of PGBR helped to reduce tissue constituent, even out of shape. Although the hepatic TG, but not TC, was excluded by PGBR, PGBR did not reverse the bloated liver. The same results were found in Matsumoto et al.\(^{13}\) and Miura et al.\(^{19}\) We suggested that PGBR could decrease the HFD-induced fat accumulation in the liver, but not inhibit hepatic weight changes in this study.

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PPAR-γ expression. We had proved that PGBR increased insulin levels and PPAR-γ to ameliorate hyperglycemia, and PGBR also inhibited HFD-induced SREBP-1 and SCD-1 expressions to ameliorate hyperlipidemia. This may suggest that PGBR in hepatic lipid metabolism is mediated through SREBP-1-dependent and PPAR-independent mechanism. Hatori et al. proved PPARα activity could be induced by circadian food intake that altered liver metabolism, and improved nutrient utilization and energy expenditure. Even though the mice were fed high calories diet, circadian food intake could prevent metabolic syndrome. In this study, the mice ate the diet ad libitum but not circadian, and PPARα expression was enhanced by PGBR. Taken together, PGBR is the PPARs regulator that improves glucose and lipid synthesis and metabolism.

When individuals become obese, in which lead to adipocytes enlarge, adipose tissue affects many systemic variations. Adipose tissue produces hormones and cytokines that influence feeding behavior, glucose metabolism, lipid metabolism, inflammation, coagulation, and blood pressure factors that play a crucial role in insulin resistance, type 2 diabetes, and obesity-related cardiovascular disease. The cytokines from adipose tissue are called adipocytokines. Some adipocytokines, such as tumor necrosis factor α (TNF-α), are upregulated by obese, and known to cause insulin resistance. On the other hand, adiponectin is proved to increase the insulin sensitivity, lipid catabolism, and control energy metabolism, and is downregulated by obesity. The decreased production of adiponectin is considered to reflect adipose tissue dysfunction. In the results, the protein expressions of SREBP-1, FAS and SCD-1 were increased, and PPARα and adiponectin were reduced in adipose tissue by HFD. The total adipocytokines level of HFD group was lower than that of SRD group in adipose tissue. On the other hand, in HFD + PGBR group, PGBR downregulated SREBP-1, FAS and SCD-1, and upregulated PPARα and adiponectin. PGBR obviously enhanced the adiponectin level in adipose tissue. According to the results, we suggested that PGBR could ameliorate the HFD-induced lipid catabolism, adipocyte growth, and accelerate lipid metabolism.

Conclusions

PGBR lowered blood glucose and blood pressure and improved glucose tolerance in mice with HFD-induced hyperglycemia. From the results of the present study, we conclude that PGBR ameliorates hyperlipidemia by improving the excretion of bile acid through dietary fiber and γ-oryzanol, inhibiting SREBP-1, SCD-1, FAS, HMGCR, increasing LDLR, CYP7a1 and recovering adiponectin through regulating PPARs. PGBR decreases lipid absorption, lipid synthesis, and increases lipid metabolism (Fig. 3). Taken together, these findings suggest that using PGBR as a staple food can help to control HFD-induced metabolic syndrome.

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Abbreviations

AI atherosclerosis index
CYP7a1 cholesterol-7α-hydroxylase
FAS fatty acid synthase
HDL high density lipoprotein
HFD fed high-fat diet
HMGCR 3-hydroxy-3-methylglutaryl-CoA reductase
LDLR low-density lipoprotein receptor
PGBR pre-germinated brown rice
PPARα peroxisome proliferator-activated receptor-α
SCD-1 stearoyl-CoA desaturase 1
SRD standard regular diet
SREBP-1 sterol regulatory element-binding protein-1
TC total cholesterol
TG triglyceride

Conflict of Interest

No potential conflicts of interest were disclosed.
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