

The Hypolipidemic and Hypoglycemic Activities of Fermented Brown Rice Fibers by Regulating PPAR α and ChREBP in the Livers of C57BL/6J Mice

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Abstract

The fermentation of brown rice produces black vinegar that has been suggested with beneficial metabolic effects; however, the mechanisms of actions of fermented brown rice have not been studied. We found that fermented brown rice extracts, especially a fraction of fermented brown rice fibers (FBRF), exhibited hypolipidemic and hypoglycemic activities *in vivo*. The oral administration of FBRF to C57BL/6J mice reduced plasma cholesterol, triglycerides, low-density-lipoprotein cholesterol levels, hepatic lipid accumulation and adipocyte size. The activation and induction of hepatic PPAR α and subsequent regulation of its target gene expressions in fatty acid uptake and oxidation were the major mechanism for reducing plasma and hepatic triglyceride concentrations. In addition, FBRF improved glucose tolerance and insulin sensitivity. FBRF feeding reduced the expressions of hepatic ChREBP, a key transcription factor in gluconeogenesis, and pro-inflammatory cytokines, thus improved insulin resistance. These results demonstrated that FBR, especially FBRF, shows potent hypolipidemic and anti-diabetic activities through regulating the expression of genes associated with lipid, glucose metabolism and inflammatory cytokines.

Keywords: Fermented brown rice; Dietary fiber; Lipid metabolism; Glucose metabolism; Inflammation

Introduction

The mechanism of dyslipidemia causing cardiovascular disease (CVD) has been intensively investigated at the molecular level, providing evidence that an elevated level of low-density-lipoprotein (LDL) cholesterol is the major cause for CVD. Therefore, reducing LDL cholesterol has been a critical strategy in preventing CVD. In this regard, the efficacy study of a cholesterol-lowering treatment showed that an approximately 12% reduction in cholesterol leads to a reduction in the risk of CVD by 19% [1], and this level of reduction could be achieved through appropriate diet interventions. Several hypolipidemic drugs, including statins, are available; however, potent drugs usually have severe adverse effects. For example, statins could cause myopathy, peripheral neuropathy, and hepatotoxicity [2]. Therefore, a great deal of effort has focused on the investigation of the biological function of natural compounds that could be applied to the prevention of CVD by dyslipidemia.

Dietary fiber is a major dietary factor in the prevention of CVD and metabolic syndrome [3]. Epidemiologic studies demonstrated that the intake of high fibers significantly reduces the risk of CVD. It has been reported that the intake of high fiber foods reduced the relative risk of CVD by 33% [4]. Studies also indicated that the consumption of dietary fiber resulted in various beneficial metabolic effects, including the improvement of insulin sensitivity, the regulatory secretion of gut hormones, such as cholecystokinin, and the reduction of pro-inflammatory cytokines [5]. Furthermore, the intake of high-fiber foods is associated with reduced plasma LDL levels [6], lowered body weight [7], and improved glucose, lipid metabolism [8], and immune function [9,10].

Brown rice has nutritional benefits due to its high content of dietary fibers; therefore, the intake of whole grains is recommended. However, high-fiber foods such as soy are often consumed in their fermented form, and the fermented products are known to have biological effects [11]. The bacterial fermentation of indigestible dietary fiber produces several

organic acids and novel modified fibers. Studies have shown that the long-term consumption of fermented insoluble dietary fibers prevents a high-fat diet-induced obesity phenotype in mice [12]. In addition, viscous and fermentable dietary fibers have had hypocholesterolemic effects in hamster experiments [13]. Furthermore, it has been reported that fermented compounds in rice were digested more slowly and improved lipid metabolism.

The fermented brown rice (FBR) is used to produce black vinegar that has health-promoting activities, including preventive effects on esophageal tumorigenesis and colon carcinogenesis [14,15]. It has been previously suggested that FBR could eliminate toxic chemicals, such as polychlorinated-biphenyls and dioxin-like polychlorinated biphenyls, in humans [16]. However, the biological mechanisms and metabolic effects of FBR have not been properly investigated. This study investigates the role of FBR and its fiber (FBRF) and small peptide fraction (FBRP) in regulating lipid and glucose metabolism, and suggests their potential application in the food industry.

Materials and Methods

Preparation and composition analysis of FBR, FRBE, and FBRP

Brown rice (*Oryza sativa* L.) from cultivars Ilpum (Korean

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harvest, 2012) was obtained from NH Agricultural Association Cooperation (Seoul, Korea). Composition of brown rice are shown in Supplemental Table 1. For fermentation, brown rice was soaked for 5 h in distilled water and gelatinized for 20 min at 60°C until the moisture content of the gelatinized brown rice reached 30%. Then, filtered water (1.5 \times volume) was added and mixed completely. After gelatinization, *Aspergillus oryzae* and *Rhizopus delemere* (0.5% w/v, each) were added, and the mixture was fermented for 18 h at 60°C for saccharification and liquefaction with agitation.

Then, the fermented brown rice was sterilized for 1 h at 90°C. This sample was classified as FBR in the study. The FBR was centrifuged at 8000 rpm for 30 min to obtain the supernatant. The crude polysaccharides were precipitated by the addition of four volumes of 95% cold ethanol to the supernatant samples. The precipitate was then dissolved in water and dialyzed using a Spectra/Por2 instrument (molecular weight cut off: 12,000-14,000; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). The high-molecular-weight solution (>1 Kd) was lyophilized to yield the crude polysaccharide fraction (FBRF) from FBR. To remove the starch-based polysaccharides, FBRF (10 g) was suspended in

50 mM ammonium formate buffer (pH 4.9) and hydrolyzed with 10 units of β -Amylase (Sigma Co. St. Louis, MO, USA) and 100 units of glucoamylase (Sigma) for 24 h at 40°C. The residual enzyme activities were then inactivated by boiling at 100°C for 30 min. The enzyme-treated FBRF was centrifuged to recover the supernatants. The FBRF fraction with molecular weight <1 Kd was finally prepared using 80% ethanol precipitation and lyophilized after desalting by dialysis (1.06 g). The total neutral sugar content was determined by means of the phenol-sulfuric acid reaction with galactose as the standard [17]. The uronic acid content was determined by measuring the absorbance at 525 nm using the *m*-hydroxybiphenyl colorimetric procedure with galacturonic acid as the standard [18]. The protein contents were analyzed using Bradford assays [19] with BSA as the standard. The content of Kdo (2-keto-3-deoxy-D-manno-2-octulosonic acid) was determined colorimetrically by the modified thiobarbituric acid method [20]. The sugar composition of the polysaccharide samples was determined by the gas chromatography (GC) analysis of their alditol acetates [21]. The samples were hydrolyzed with 2 M trifluoroacetic acid for 1.5 h at 121°C and converted into the corresponding alditol acetates. The resulting carboxyl-reduced alditol acetates were analyzed through GC (GC ACME-6100, Young-Lin Co., Anyang, Korea) using an SP-2380 capillary column (0.2- μ m film thickness, 0.25-mm i.d. \times 30 m; Supelco, Bellefonte, PA, USA) at 60°C for 1 min, 60 \rightarrow 220°C (30°C/min), 220°C for 12 min, 220 \rightarrow 250°C (8°C/min), and 250°C for 15 min. The molar percentage was calculated from the peak areas and response factors using the flame-ionization detector (FID).

Soluble Polysaccharides	0.4%
Glucose	95.8
Galactose	1.2
Mannose	1.2
Arabinose	1.0
Xylose	0.8
Amino acid	1.2%
glutamic acid	19.2
aspartate	12.5
arginine	10.0
leucine	7.5
serine	6.7
proline	6.7
alanine	6.7
glycine	5.8
phenylalanine	5.0
tyrosine	4.2
threonine	3.3
valine	3.3
lysine	3.3
methionine	2.5
isoleucine	2.5
histidine	2.5
cysteine	1.7
Mineral	0.8%
folate	82.1
potassium	8.7
phosphorus	6.8
sodium	1.8
calcium	0.3
vitamin E	0.1
niacin	0.1
zinc	0.0
manganese	0.0
iron	0.0
vitamin B1	0.0
vitamin B6	0.0

Table 1: Compositions of soluble polysaccharides, amino acids, and minerals in FBRs.

Cell culture

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA; Cat # SH30243.01) with 10% fetal bovine serum (Hyclone, USA; Cat # SH30919.03) and 1% penicillin/streptomycin (Hyclone, USA; Cat # SV30010).

Cell viability test

The viability test was performed according to a previously described method [22]. HepG2 cells were seeded in 24-well plates for 24 h. The cells were then incubated with test materials at varying concentrations. After 24 h incubation, the culture medium was removed, and 1 mL of DMEM with 10% of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added. The mixture was incubated for 3 h. The MTT solution was removed, and the cells were dried for 1 min. Then, 1 mL of dimethyl sulfoxide was added to the cells, and the mixture was incubated for 2 h with mild shaking. The absorbance was measured at 570 nm in a Model 680 microplate reader (Bio-Rad, Hercules, CA, USA; Cat # 168-1000XTU).

Mouse feeding study

All of the animal experiments complied with the approved protocol by the Institutional Animal Care and Use Committees of Korea University (Protocol No KUIACUC-2013-139). The breeding environment of the mice was aseptic, and the lights were turned on and off at 12-h cycles. Eight-week-old male C57BL/6J mice were housed under standard conditions with free access to water and fed a high-fat-diet during the experiments (40% calories from fat, Supplementary Table 2). The test materials were orally administered for 4 weeks in combination with a high-fat diet. There were four groups in the feeding experiment: vehicle-fed control, FBRF, FBRE, and FBR groups. Each group was fed 500 mg/kg body weight of FBR, FBRF, or FBRE in 200 μ L of distilled water or 200 μ L of distilled water as a vehicle for 4 weeks.

The food intake and body weight of the mice were monitored once a week for 4 weeks.

Blood analysis

The mice were anesthetized with avertin (2,2,2-tribromoethanol, 20 mg/mL, Sigma), and the blood was collected retro-orbitally after 12 h overnight fasting at base line and by heart puncture at the end of the feeding experiments. The blood was centrifuged at 3000 rpm for 15 min, and the plasma was collected to measure the glucose, triglyceride, total cholesterol, low-density-lipoprotein (LDL), and high-density-lipoprotein (HDL) concentrations using an automated blood analyzer (cobas C111, Roche, Basel, Switzerland) through an enzymatic method. The plasma samples were stored at -80°C until analysis. The leptin (Millipore, Bedford, MA, USA; Cat. #EZML-82K) and adiponectin (Abcam, Cambridge, UK; Cat. #ab108785) concentrations were quantified with commercially available ELISA kits.

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

After 2 weeks of oral administration, the OGTT was conducted by the oral administration of 200 μL of glucose (1.5 g/kg bodyweight) after 12 h of fasting, and the ITT was conducted by the intraperitoneal injection of 100 μL of insulin (0.75 unit/kg body weight) after 6 h of fasting. The blood glucose levels were quantified with an Auto-check glucometer (Diatech Korea, Seoul, Korea) at different time points.

Histological analysis

The liver and abdominal adipose tissues from the mice were obtained immediately fixed in 4% paraformaldehyde to make paraffin-embedded blocks. The tissue sections were prepared using a microtome and stained with hematoxylin and eosin (H&E) according to a previously described method [23]. The images were acquired with an inverted microscope (Axio Observer D1, Carl-Zeiss AG, Oberkochen, Germany). The adipocyte size was also measured by the AxioVision software (Carl-Zeiss AG, Oberkochen, Germany).

Quantitative PCR (qPCR)

The total RNA from the liver were extracted, and cDNA were synthesized from 2 μg of total RNA using M-MLV Reverse Transcriptase (Mbiotech, Seoul, Korea) and oligo(dT) primers. The expression levels of each gene were quantified using AccuPower[®] 2X Greenstar qPCR Master mix (Bioneer, San Francisco, CA, USA; Cat # K-6252) and IQ5 Real-Time PCR Detection Systems (Bio-Rad, Berkeley, CA, USA; Cat # 170-9780) as described previously [24]. The level of gene expression was normalized to GAPDH and calculated automatically using iQ5 Optical System Software version 2 (Bio-Rad, Hercules, CA, USA).

Rate of fatty acid oxidation and synthesis

HepG2 cells were seeded in 12-well plates. After 24 h, the medium was removed, and the cells were washed with PBS, then lipid-loaded with palmitic acid, oleic acid (40 $\mu\text{mol/L}$) and 0.16% fatty acid-free BSA (w/v) for 24 h. Lipid-loaded cells were treated with FBRF, FBRP and FBR for 24 h. For the fatty acid oxidation experiments, [$1-^{14}\text{C}$] palmitate was added. After 1 h of incubation, the supernatant of each well was collected, and the CO_2 was extracted by mixing with NaOH (Daejung, Siheung, Korea; Cat # 7574-4400) and HCL (Junsei, Tokyo, Korea; Cat # 7647-01-0). The amount of $^{14}\text{CO}_2$ was measured by a liquid scintillation counter. For the fatty acid synthesis experiments, [$1-^{14}\text{C}$] acetic acid was added. One hour later, the fatty acids were extracted and measured as described previously [25].

Results

Composition of FBRs

The composition of FBRs was analyzed by GC. The FBRF contained 0.4% soluble polysaccharides, 1.2% amino acids, and 0.8% minerals. Glucose (95.8%) occupied most of the soluble polysaccharides, followed by galactose (1.2%) and mannose (1.2%). Of the amino acids, glutamic acid (19.2%) had the largest proportion, followed by aspartate, arginine (12.5%), and leucine (10.0%). Finally, folate (82.1%) was identified as the most abundant mineral in FBRs, followed by potassium (8.7%) and phosphorus (6.8%) (Table 1).

FBR administration revealed hypolipidemic effects in mice

In HepG2 cells, FBRs (FBRF, FBRP, and FBR) did not affect cell viability, as assessed with the MTT assay, at concentrations up to 500 $\mu\text{g/mL}$ (Supplemental Figure 1). To investigate the metabolic effects of FBRs (FBRF, FBRP, and FBR) *in vivo*, the FBRs were orally administered to mice for 4 weeks. The body weights of the mice in all groups increased over the 4-week feeding period; however, the weight gained in the FBRF group was significantly lower than that observed in the other groups after 3 weeks of feeding (Figure 1A, Supplemental Figure 2). The liver masses were similar among all of the groups; however, the adipose weights of the FBRF and FBR groups were significantly lower than that of the control group, especially that of the perirenal, abdominal, and total fat (Figure 1B, Supplemental Figure 3). The average adipocyte sizes of all three FBR groups were significantly smaller (56.3%, 55.3% and 56.7%, respectively) than that of the control group (Figure 1C and 1D), suggesting that the intake of FBRs reduces fat accumulation *in vivo*.

After 4 weeks of feeding, the plasma cholesterol concentrations of the FBRF, FBRP, and FBR groups were significantly decreased by 30.3%, 24.7%, and 16.5%, respectively, compared to those of the control group. The triglyceride concentrations of the FBRF and FBR groups were significantly decreased by 44.1% and 38%, respectively, compared to that of the control group, and the LDL-cholesterol concentrations and atherogenic index of the FBRF and FBRP groups were also significantly decreased by 39.3% and 33.6%, respectively, compared to those of the control group. The HDL-cholesterol levels were similar among the groups (Figure 2A).

The hepatic triglycerides of the FBRF, FBRP and FBR groups were significantly decreased by 43.5%, 34.8%, and 33.7%, respectively, compared to that of the control group, and the hepatic cholesterol in the liver of the FBRF group was significantly decreased by 36.2% compared to that of the control group (Figure 2B). These results were confirmed by the histological analysis of the liver with H&E staining, which showed that the hepatic lipid accumulations in the FBRF, FBRP, and FBR groups were markedly reduced (Figure 2C). These results demonstrate that FBRs can regulate body weight and ameliorate the hypercholesterolemic conditions by improving the hepatic and/or plasma lipid profiles, which suggests that they have hypolipidemic effects.

Feeding with FBRs regulates the hepatic gene expression related to lipid metabolism

The hypolipidemic mechanisms of FBRs were investigated through an analysis of the hepatic expression levels of genes involved in lipid metabolism. First, we assessed the rate of fatty acid synthesis and fatty acid oxidation in lipid-loaded HepG2 cells. The rates of fatty acid oxidation were significantly increased in the FBRP and FBR groups, whereas the

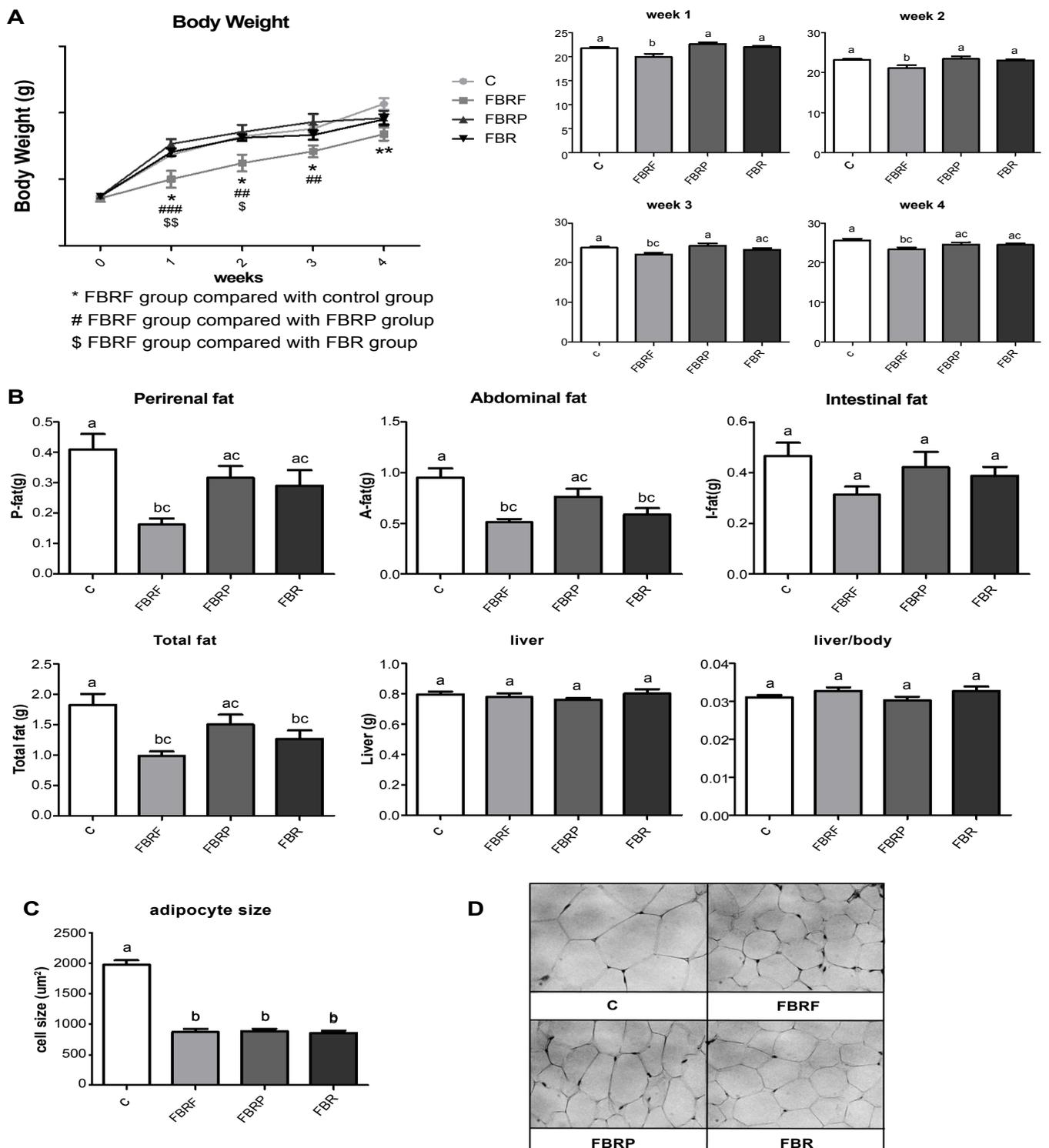


Figure 1: Body weight, organ weight and adipocyte size in mice fed FBRs. (A) Body weight during the feeding period. (B) Liver and adipose weights. (C) Average adipocyte sizes. The size was calculated from the measurement of 30 randomly selected adipose tissues. (D) Representative images of adipocytes in mice fed FBRs. All of the data were analyzed using one-way ANOVA for repeated measures with Tukey's Studentized range test between the groups. A common letter indicates a significant difference ($P < 0.05$).

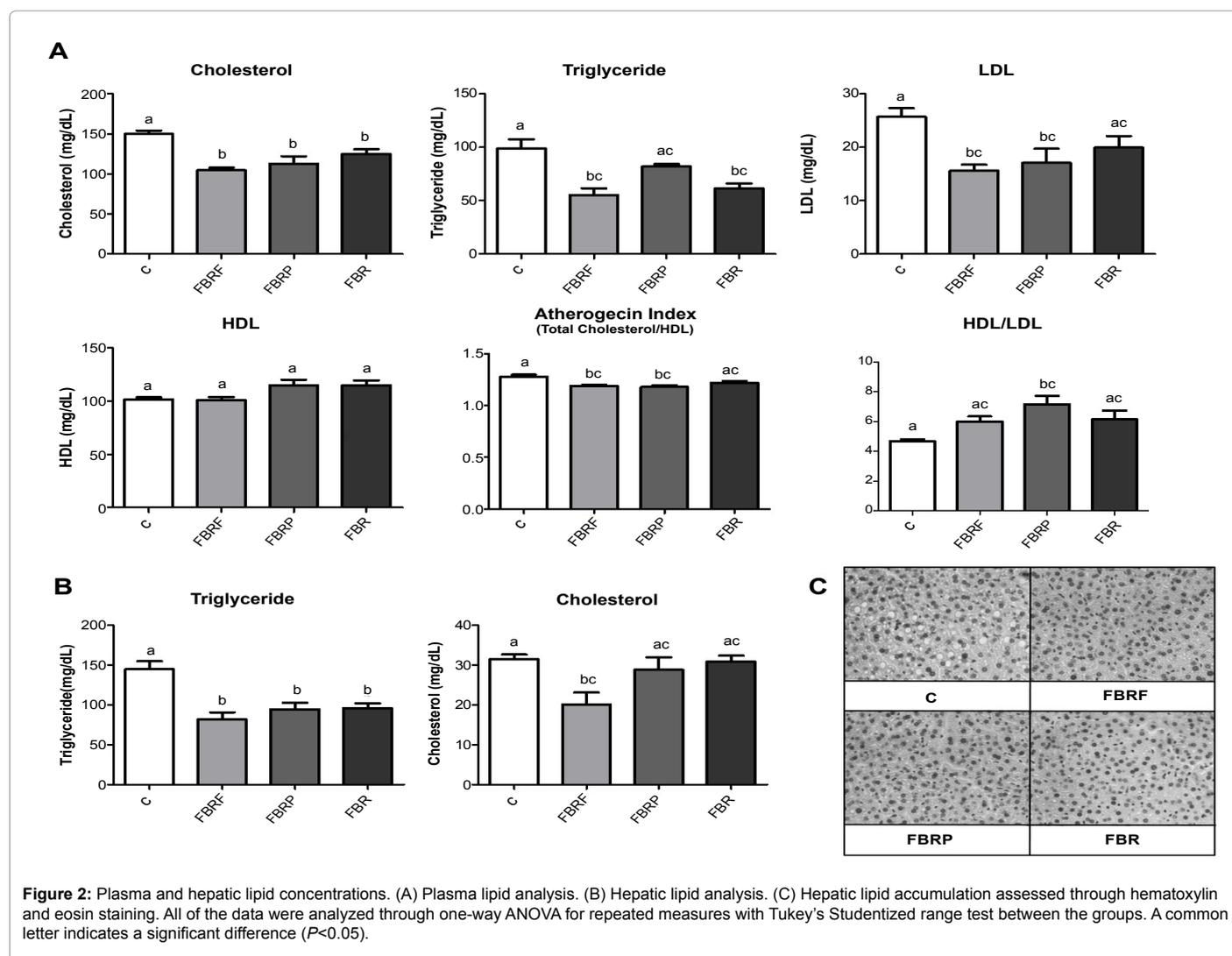


Figure 2: Plasma and hepatic lipid concentrations. (A) Plasma lipid analysis. (B) Hepatic lipid analysis. (C) Hepatic lipid accumulation assessed through hematoxylin and eosin staining. All of the data were analyzed through one-way ANOVA for repeated measures with Tukey's Studentized range test between the groups. A common letter indicates a significant difference ($P < 0.05$).

rates of fatty acid synthesis were similar in all of the groups (Figure 3A). Second, we analyzed the expression of multiple hepatic genes in the PPAR- α pathway, which is a major mechanism for inducing fatty acid oxidation. The expressions of PPAR- α , the key transcription factor in hepatic lipid homeostasis, were significantly increased in the FBRF and FBR groups compared to the control (Figure 3B). Third, the expression of PPAR- α was upregulated, then the expression of PPAR- α target genes were quantified. In fatty acid oxidation, the expression levels of fatty-acid-binding protein 1 (FABP1) and carnitine palmitoyltransferase-1a (CPT1a) were significantly increased in the FBRF group compared to the control. The expression of lipoprotein lipase (LPL) and fatty acid transporter protein 5 (FATP5) was also significantly increased in the FBRF group compared to control (Figure 3D). Although the rates of fatty acid synthesis were similar in all of the groups in the *in vitro* experiment, the expression levels of acetyl-CoA carboxylase-1 (ACC1) and fatty acid synthase (FAS) were significantly decreased in the FBRF, FBRP, and FBR groups compared to the control. Furthermore, the expression of insulin-induced gene 2a (Insig-2a), which delays the posttranslational processing of the sterol regulatory element binding protein (SREBP), was significantly increased only in the FBRF group. Finally, the expression of SREBP1c was significantly reduced in the FBRF and FBR groups (Figure 3C). Regarding cholesterol metabolism,

the expression levels of LDL-receptor (LDLR) and CYP7A1 were increased in the FBRF groups (Figure 3E). These results suggest that the feeding of FBRs regulates PPAR α and its target genes in fatty acid metabolism and reduces the plasma triglyceride concentrations. The administration of FBRs also reduces the LDL cholesterol concentrations via induction of LDLR and CYP7A1 expression.

FBRs feeding improved insulin sensitivity

Adipose tissue is one of the major tissues for plasma glucose homeostasis [26], and adiposity is associated with insulin resistance in metabolic syndrome [27]. The OGTT results showed that the blood glucose concentrations were significantly lower at 15, 30 and 60 min in the FBRF group compared to other groups. The area under the curve (AUC) of the OGTT results for the FBRF group was significantly lower than that of the control group by 27.9% (Figure 4A). The ITT results showed that the blood glucose levels of the FBRF group were lower compared to the levels found in the other groups at 15, 30, and 90 min. In addition, the AUC of the ITT results for the FBRF group was significantly lower than that found in the control group by 15.4% (Figure 4B). These suggest that the glucose and insulin tolerances were improved in the FBR groups, particularly in the FBRF group, compared to the control group.

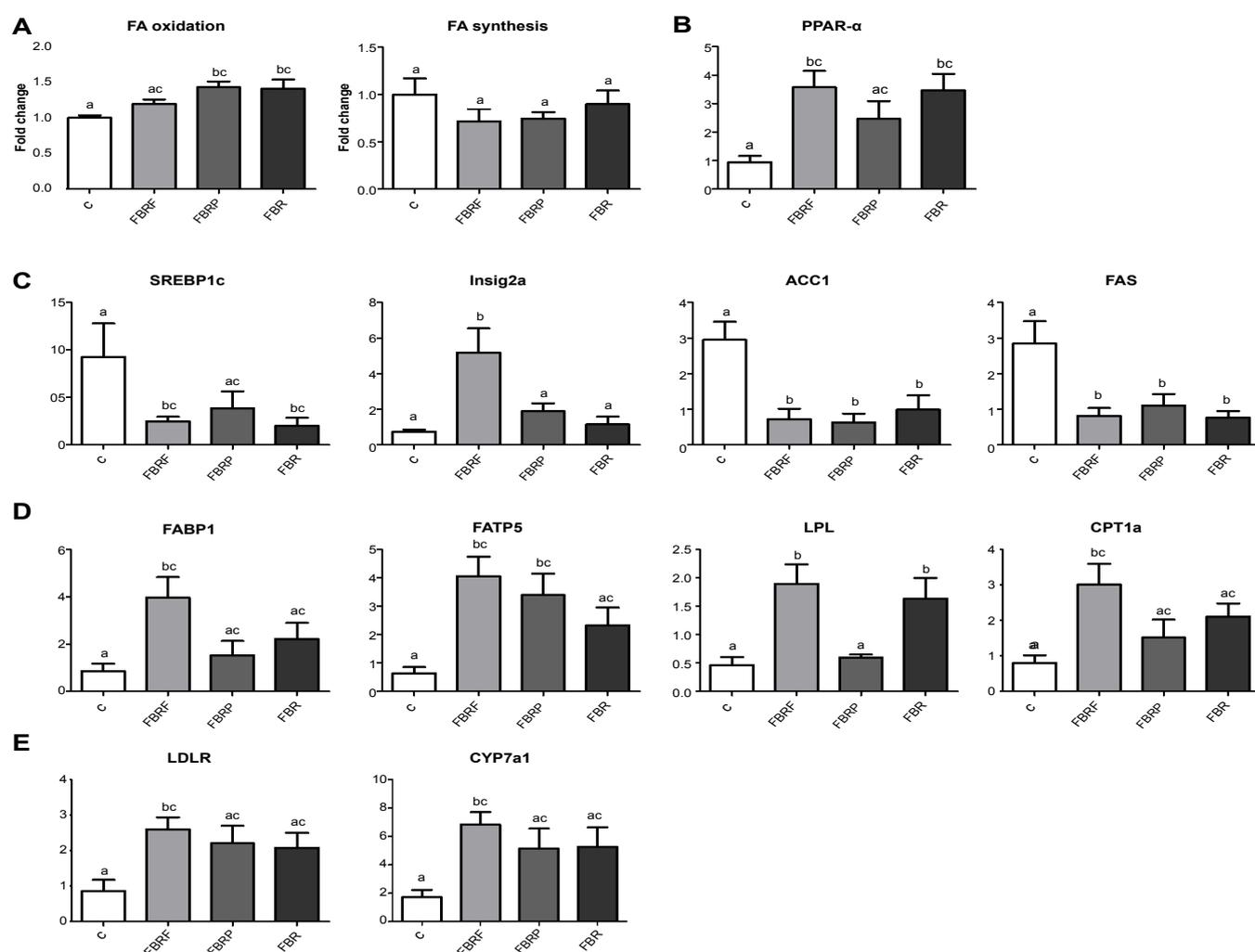


Figure 3: The rate of fatty acid metabolism and the expression of lipid metabolism genes. (A) The rate of fatty acid synthesis and fatty acid oxidation. (B) PPAR- α gene expression. (C) Expression of genes in fatty acid synthesis. (D) Expression of genes in fatty acid oxidation. (E) Expression of genes in cholesterol metabolism. The mRNA was isolated from the livers of each group, and the expression levels of the genes were quantified by qPCR as described in the methods. All of the data were analyzed using one-way ANOVA for repeated measures with Tukey's Studentized range test between the groups. A common letter indicates a significant difference ($P < 0.05$).

LDLR, low-density lipoprotein receptor; CYP7a1, cholesterol 7 α -hydroxylase; ACC1, acetyl-CoA carboxylase 1; FAS, fatty acid synthase; SREBP1c, sterol regulatory element-binding protein; LPL, lipoprotein lipase; FATP5, fatty acid transport protein 5; FABP1, fatty acid-binding protein; CPT1a, carnitine palmitoyltransferase; PPAR- α , peroxisome proliferator-activated receptor α ; FA, fatty acid.

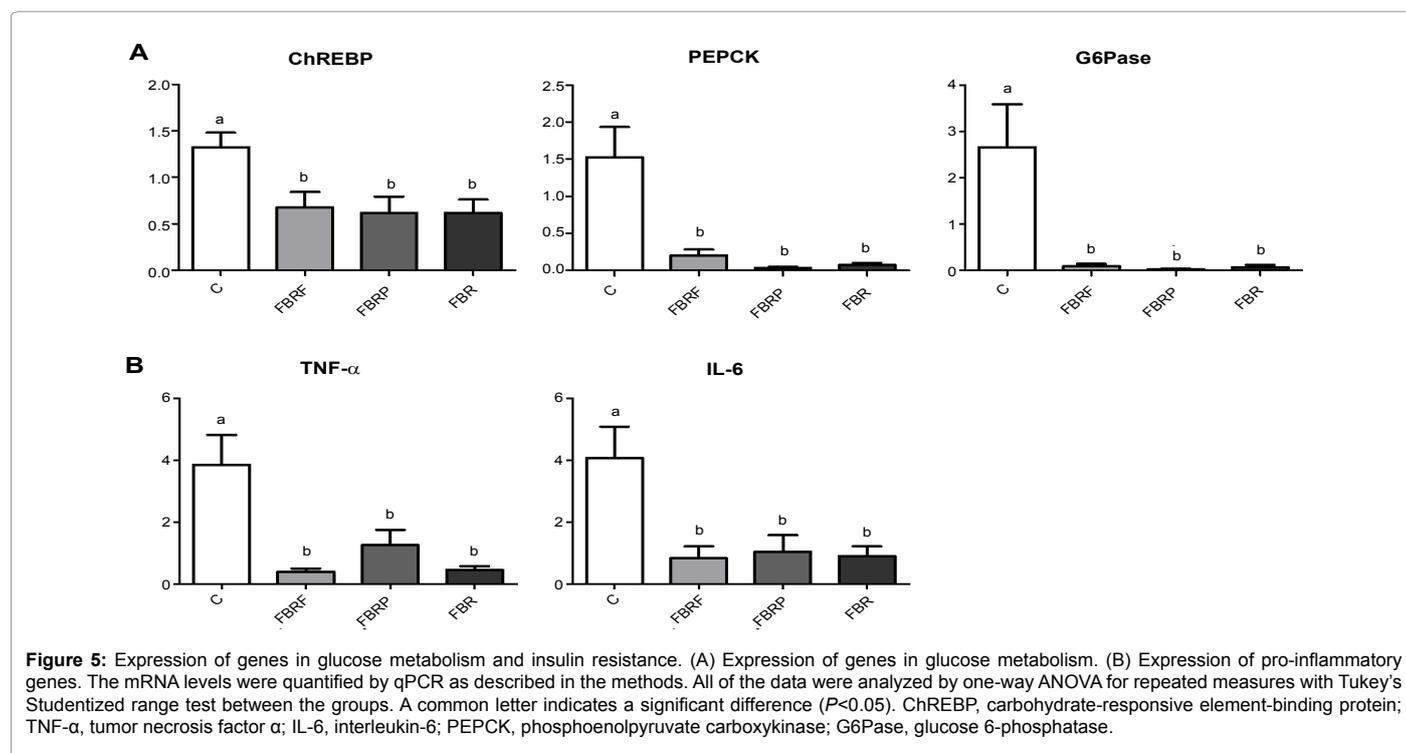
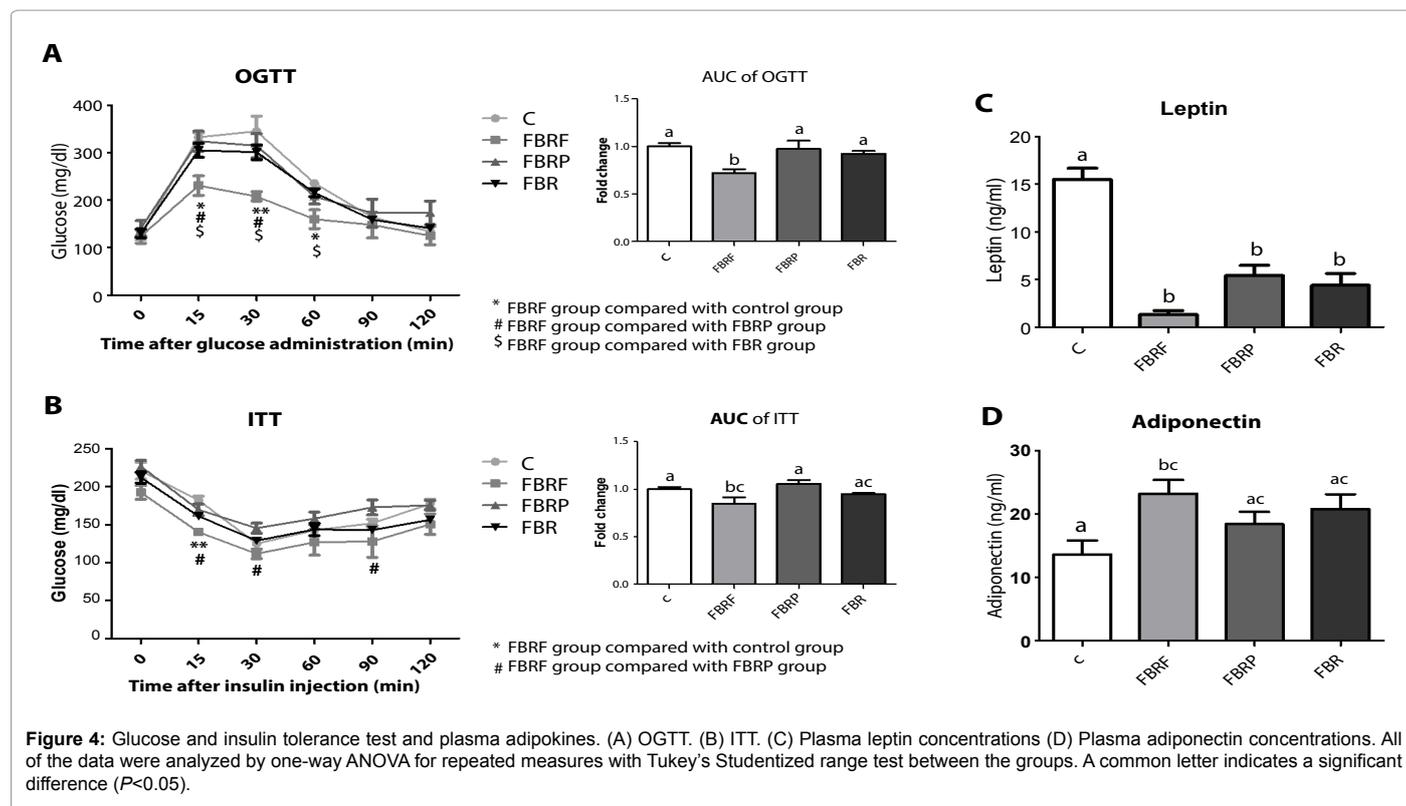
The plasma concentrations of adipokine represent the status of metabolic syndrome. Especially, the plasma leptin and adiponectin concentrations are correlated with insulin resistance [28,29]. The plasma leptin levels of the FBRE, FBRP, and FBR groups were significantly reduced compared to the control group by 91.3%, 65.0%, and 71.7%, respectively (Figure 4C), and the plasma adiponectin concentrations of the FBRE group were significantly increased compared to the control by 70.1%. The levels of adiponectin in the FBRP and FBR groups tended to increase compared to that in the control group (Figure 4D). These demonstrated that intake of FBRs improved glucose and insulin tolerance through regulating plasma adipokines, leptin and adiponectin concentrations.

FBRs regulate the hepatic expression of genes associated with glucose metabolism

Pro-inflammatory cytokines secreted from hepatocytes and

adipocytes are correlated with insulin resistance [30]. The expression levels of tumor necrosis factor α (TNF- α) in the FBRE, FBRP, and FBR groups were significantly reduced by 89.7%, 67.4%, and 88.0%, respectively. The expression levels of interleukin 6 (IL-6) were also decreased significantly in the FBRE, FBRP, and FBR by 79.4%, 74.3%, and 77.8%, respectively, compared to the control group (Figure 5B). These results demonstrated that the feeding of FBRs reduced the expression of pro-inflammatory cytokines and therefore may ameliorate insulin resistance.

Decreased hepatic gluconeogenesis improves glucose control and insulin sensitivity. Notably, the expression levels of carbohydrate-responsive element binding proteins (ChREBP), a key transcription factor of gluconeogenic genes in glycolysis, were significantly decreased in the fermented brown rice groups (FBRE, FBRP, and FBR) by 48.9%, 53.5%, and 53.5%, respectively, compared to the control group. In

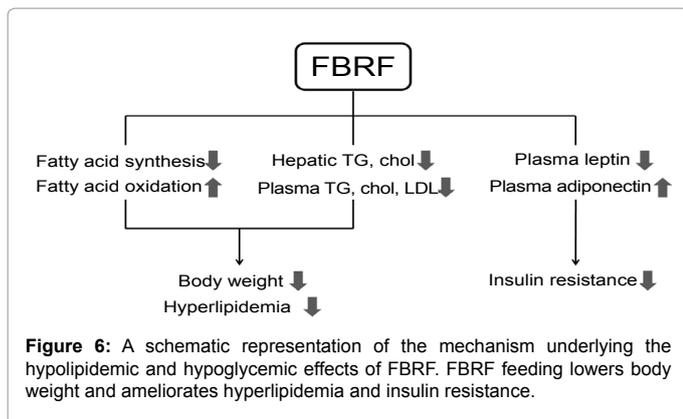


addition, the expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), which are essential enzymes in gluconeogenesis, were significantly decreased in the FBRF, FBRP, and FBR groups (Figure 5A). These results indicated that the intake of FBRs improved glucose tolerance via downregulation of

hepatic ChREBP expression and subsequent inhibited gluconeogenesis via reducing expression of PEPCK and G6Pase (Figure 6).

Discussion

Brown rice fermentation is used to produce traditional black



vinegar in Korea, which has been suggested to have health-promoting activities; however, its biological activities have not been investigated. Studies indicate that microbial fermentation produces novel bioactive metabolites. Modified fibers and small peptides could have novel biological beneficial activities [30]. In this study, we report the hypolipidemic and hypoglycemic activities of FBRs. The analysis of obese C57BL/6J mice fed a high-fat diet showed that the lipid accumulations in both adipose and liver tissues and the body weight were significantly reduced in the FBRF group compared with the controls. In addition, the feeding of FBRs significantly reduced the total cholesterol, LDL cholesterol and triglyceride concentrations compared to the control group, which demonstrated the anti-obesogenic and hypolipidemic activities of FBR, especially the fiber fraction.

In gene expression studies, PPAR- α is the primary molecular target because it is highly expressed in the liver and functions as a key transcription factor that regulates the expression of genes involved in lipid and carbohydrate homeostasis [31]. The pharmacological activation of PPAR- α with synthetic ligands, such as fibrates, ameliorates symptoms in metabolic syndrome that reduces the triglyceride concentrations and controls glucose metabolism through the reduction of body weight [32]. The results from this study revealed that the expressions of PPAR- α and its target genes associated with fatty acid uptake and oxidation and lipoprotein metabolism were significantly regulated; therefore, the rates of fatty acid oxidation were induced in cells stimulated with FBR. The expression levels of PPAR- α in the liver were significantly increased by FBRF and FBR administration compared to the control. In addition, we also assessed the expression levels of genes involved in fatty acid oxidation including LPL, CD36, FATP5, FABP1, CPT1a, and ACOX1. LPL is a lipase that hydrolyzes triglycerides into free fatty acids and is involved in the cellular uptake of lipid materials, such as chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids. The feeding of FBRF and FBR increased the expressions of LPL significantly. FABP5 is a transporter related to fatty acid uptake, and FABP1 is a protein that facilitates the transfer of fatty acid via the transporter. FATP5 expressions were upregulated by FBRF and FBRP administration, whereas FABP1 was only upregulated by FBRF feeding. In the case of ACOX1 and CPT1a, which are the key enzymes in beta-oxidation, only CPT1a was significantly upregulated by FBRF feeding. Additionally, the rates of fatty acid oxidation, measured *in vitro*, were also increased in all groups fed FBRs. These results suggested that feedings with FBRs facilitated fatty acid uptake and degradation by upregulating the expression of FABP1, FATP5, LPL, and Cpt1a, which subsequently resulted in reduced fat deposition and body weight in the FBR groups compared to the control group.

Alternatively, a lipogenic transcription factor and PPAR- α target gene, SREBP1c, was downregulated in the FBR groups, and the SREBP1c-responsive lipogenic genes ACC1 and FAS were downregulated accordingly. These results suggested that PPAR- α activation suppressed hepatic lipogenesis by inhibiting key gene transcription. Insig-2a is an ER protein that forms a complex with precursor SREBP1c and SCAP (a SREBP chaperone). The overexpression of Insig-2a holds the SREBP-1c-SCAP complex in the ER and delays the formation of active nuclear SREBP-1c [33]. The expression of *Ins2a* (a gene for Insig-2a) was induced in FBRF, and this could contribute to the reduction of additional hepatic lipogenesis in the FBRF group.

We assessed the expression levels of cholesterol metabolism genes, including HMGCR, LDLR, and CYP7A1, in the liver. The *Hmgcr* gene encodes the enzyme in the pathway that produces cholesterol. As a negative feedback mechanism, a high intracellular cholesterol level reduces both LDLR and HMGCR expressions while upregulating CYP7A1 to convert cholesterol to bile acids [34]. Our results suggested that the feeding of FBRs promoted the absorption and secretion of cholesterol by upregulating the expression of LDLR and CYP7A1, which explained the reduction in the cholesterol concentrations in the plasma and liver in the FBR groups.

The effects of FBR on glucose metabolism were assessed through the expression of ChREBP, PEPCK, and G6Pase in the liver. ChREBP mediates the activation of not only enzymes of glycolysis but also enzymes associated with lipogenesis and fatty acid synthesis [35]. Downregulated ChREBP expressions were observed in the FBRF, FBRP, and FBR groups, which implied a decreased hepatic glucose output and lipogenesis. PEPCK and G6Pase, two key genes in hepatic gluconeogenesis and also PPAR- α target genes, were downregulated in the livers of the mice fed FBRs. Taken together, these results demonstrated that FBRs improved glucose control and insulin sensitivity by reducing the hepatic glucose output through the inhibition of key genes in gluconeogenesis.

Finally, FBRs reduced the expression of TNF- α and IL-6, pro-inflammatory mediators that directly contribute to vascular injury, insulin resistance, and atherogenesis. IL-6 acts as a pro-inflammatory cytokine in hyperlipidemic status, and TNF- α contributes to the initiation and propagation of atherosclerotic lesion formation [36]. Taken together, the downregulation of these pro-inflammatory genes could improve lipid metabolism and glucose control and prevent the development of chronic disease, such as CVD.

In conclusion, this study demonstrated that the feeding of FBRs, especially FBRF, exhibited significant hypolipidemic effects by improving the plasma lipid profiles and reducing fat deposition and hypoglycemic effects by ameliorating glucose tolerance. In addition, these effects were regulated through the PPAR- α pathway. Thus, the appropriate intake of FBRs may ameliorate symptoms of metabolic syndrome.

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