Lipid accumulation using Oil Red O dye was measured in 3T3-L1 murine adipocytes to examine the anti-obesity effect of four types of germinated rice, including germinated brown rice (GBR), germinated waxy brown rice (GWBR), germinated black rice (GB-R), and germinated waxy black rice (GWB-R). GBR methanol extract exhibited the highest suppression of lipid accumulation in the 3T3-L1 cell line and also the anti-obesity effect of GBR on high fat-induced-obese mice. The mice were divided into three groups and were administered: ND, a normal diet; HFD control, a high fat diet; and GBR, a high fat diet plus 0.15% GBR methanol extract for 7 weeks. GBR administration significantly decreased body weight gain and lipid accumulation in the liver and epididymal adipose tissue as compared to the HFD control group. In addition, serum triglycerides (TGs) and total cholesterol (TC) levels were significantly decreased by following GBR administration compared with those in the HFD control group, whereas the high-density lipoprotein (HDL) cholesterol level increased. Furthermore, the mRNA levels of adipogenic transcriptional factors, such as CCAAT enhancer binding protein (C/EBP)-α, sterol regulatory element-binding protein (SREBP)-1c, and peroxisome proliferator activated receptors (PPAR)-γ, and related genes (aP2, FAS), decreased significantly. Taken together, GBR administration suppressed body weight gain and lipid accumulation in the liver and epididymal adipocytes, and improved serum lipid profiles, in part, by controlling adipogenesis through a reduction in transcriptional factors. These results suggest that GBR is a potential agent against obesity.

Key words: germinated brown rice; 3T3-L1; high fat-induced obese mice; anti-obesity

Obesity is a chronic metabolic disorder caused by an imbalance between energy intake and energy expenditure. It is a serious risk factor for hypertension, cardiovascular disease, type-2 diabetes, and cancer. Additionally, the medical costs of fat-related diseases have increased, and the effort to decrease body fat percentages has become a worldwide interest. Under the guidelines of the US Food and Drug Administration, botanical drugs can be developed faster and more cheaply than conventional single-entity pharmaceuticals. Thus there are many botanicals that might provide safe, natural, and cost-effective alternatives to synthetic drugs. Recent studies have found that natural bioactive compounds, including (−)-epigallocatechin-3-gallate (EGCG), quercetin, resveratrol, and curcumin, can be used to treat obesity in 3T3-L1 adipocytes and in an obese mouse model.

Brown rice is added to various beverages and teas and is sold as a type of “germinated brown rice” (GBR). GBR utilizes physiologically active substances that are present in bran. The soaking process improves texture, and the seed nutrients become easier to digest and absorb following germination. It is known that the GBR shows excellent assimilation and increases useful compounds when it is germinated. This included various vitamins, arabinoxylan, and γ-aminobutyric acid (GABA). Additionally, various components, including phytic acid and calcium, can be separated due to the increase in phytases when plant seeds are germinated. Then absorption of the minerals into the body increases.

In this study, we examined the anti-adipogenic effects of four germinated rices using Oil Red O dye in 3T3-L1 murine adipocytes. Furthermore, we investigated the anti-obesity effects of 7 weeks of GBR administration on body weight gain, serum lipid profiles, histological changes in adipose tissue and the liver, and expression of the genes associated with adipogenesis in high fat-induced-obese mice.

Materials and Methods

Sample preparation and reagents. Germinated brown rice (GBR), germinated waxy brown rice (GWBR), germinated black rice (GB-R), and germinated waxy black rice (GWB-R) were cultivated and processed in Hamyang-gun, Korea. The four rices were extracted with 5 volumes of methanol and then filtered and evaporated under partial vacuum conditions, dried, and stored at −20 °C until assay. For analysis, the samples were dissolved in DMSO (final concentration 0.1%).

Cell culture and differentiation. A 3T3-L1 mouse preadipocyte cell line (#CL-173) was obtained from the American Type Culture
Collection (Rockville, MD). Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% calf serum (CS) and 1% penicillin-streptomycin. The cells were incubated at 37°C in a 5% CO₂ humidified incubator. To induce differentiation, 2 d post-confluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with DMEM media (0.5 mM IBMX, 0.1 μM DEX, and 1 μg/mL of insulin added to DMEM containing 10% fetal bovine serum (FBS) culture medium). On day 2, the DMEM medium was replaced with DMEM containing 10% FBS and 1 μg/mL of insulin (INS) medium. On day 4, the INS medium was replaced with DMEM containing only 10% FBS. This was changed every 2 d until day 8.

Cell viability. Methylthiazol tetrazolium (MTT) assay was done to investigate the proliferation of 3T3-L1 preadipocytes. 3T3-L1 adipocytes were seeded into a 96-well plate at a density of 1 x 10⁴ cells/well and maintained with DMEM containing 10% CS. After 24 h of incubation, the medium was changed to the same medium containing 0.1 mg/mL of sample. After 48 h of incubation, the medium was replaced with serum-free medium containing 10 μL of MTT solution (5 mg/mL). The plate was wrapped with foil and incubated for 4 h at 37°C. The supernatant was removed, and 100 μL of DMSO was added to each well. The amount of MTT formazan product was determined using a microplate reader at an absorbance of 595 nm (Model 680 microplate reader, Bio-Rad, Hercules, CA).

Oil Red O staining. Lipid accumulation in mature 3T3-L1 adipocytes was determined by Oil Red O staining. For this assay, 3T3-L1 preadipocytes were seeded into a 96-well plate at a density of 1 x 10⁴ cells/well and maintained with DMEM containing 10% CS. After 48 h of incubation, the medium was changed to the same medium containing 0.1 mg/mL of sample. After 48 h of incubation, the medium was replaced with serum-free medium containing 10 μL of MTT solution (5 mg/mL). The plate was wrapped with foil and incubated for 4 h at 37°C. The supernatant was removed, and 100 μL of DMSO was added to each well. The amount of MTT formazan product was determined using a microplate reader at an absorbance of 595 nm (Model 680 microplate reader, Bio-Rad, Hercules, CA).

Animals and diet. Four-weeks-old male C57BL/6J mice ( Orient Bio, Seongnam, Korea) were individually housed in standard cages (33 x 23 x 12 cm, five mice/cage), and placed in a room where the temperature was maintained at 22 ± 0.5°C, the relative humidity was 50 ± 5%, and lighting was on a 12-h light/dark cycle. After a 1-week adaptation period, the mice were divided into three dietary groups of 10 mice each: a normal diet (ND) group, a high fat diet (HFD) group, and a 0.15% GBR methanol extract-administered group (HGM). The compositions of the diet are described in Table 1. The animals were maintained on the feeding program for 7 weeks, and body weight and food intake were recorded every week throughout the experimental period. The care and treatment of the animals conformed to the Korea University Guidelines for the Ethical Treatment of Laboratory Animals.

Preparation of blood, adipose tissues, and liver. After 7 weeks, the mice were fasted for 12 h and sacrificed, and tissues were collected for analysis. Blood was extracted from the inferior vena cava of each mouse using a syringe and a test tube. The serum was separated from the blood by centrifuging the sample at 3,000 rpm at 4°C for 15 min. The epididymal adipose tissue and liver were removed, weighed, and stored at −80°C until analysis.

Biochemical analysis. The serum levels of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides (TG), alanine transaminase (ALT), and aspartate transaminase (AST) were measured using commercial kits (Asanpharm, Seoul, Korea).

### Table 1. Compositions of Experimental Diets (g/kg)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>ND</th>
<th>HFD</th>
<th>HGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>397.486</td>
<td>284.986</td>
<td>183.486</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Dextrose</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mineral mixture⁽¹⁾</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture⁽²⁾</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>TBHQ⁽³⁾</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>l-Cystine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>GBR</td>
<td>—</td>
<td>—</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

1) AIN mineral mix containing (g/kg): calcium phosphate dibasic 500, sodium chloride 74, potassium citrate 220, potassium sulphate 52, magnesium oxide 24, manganese carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, and chromium potassium sulphate 0.55.

2) AIN vitamin mix containing (g/kg): thiamin HCl 0.6, riboflavin 0.6, pyridoxine HCl 0.7, nicacin 3, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B12 (0.1% tritation in mannitol) 1, dry vitamin A palmitate (500,000 U/g) 0.8, dry vitamin E acetate (800 U/g) 10, vitamin D3 tritration (400,000 U/g) 0.25, and menadione sodium bisulphate complex 0.15.

3) TBHQ, tertiary butylhydroquinone

Epididymal adipose tissue and liver histology. Slices of epididymal adipose tissue and liver were immediately fixed in 10% (v/v) formalin and embedded in paraffin wax. Sections (3 μm thick) were cut, and each section was stained with hematoxylin and eosin (H & E). All the sections were examined by optical microscopy (Olympus BX 50, Olympus Optical, Tokyo) and were printed at a final magnification of 100X. The average size of the adipocytes was measured using image analysis software (Image-Pro Plus ver 3.01, Media Cybernetics, Atlanta, GA). The area of lipid accumulation in the liver was measured in randomly selected liver cells (50 cells) by the image analysis software.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) analysis. Four μg of total RNA was reverse transcribed using M-RT reverse transcriptase (Chromogen, Seoul, Korea). The primer sets for each target gene, CCAAT enhancer binding protein (C/EBP-α), peroxisome proliferator-activated receptor (PPAR)-γ, sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthetase (FAS), and aP2, were designed with the Primer3 Out software. RT-PCR reactions were conducted using a 2X Premix Solution (Chromogen) under the following conditions: 95°C for 5 min (1 cycle), 95°C for 30 s, 52°C for 30 s, and 72°C for 30 s (40 cycles). The PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide (0.5 μg/mL), visualized as a single compact band of expected size under UV light, and documented with a gel documentation system.

Statistical analysis. Data are presented as mean ± SD (n = 10). Differences between the means of individual groups were assessed by one-way analysis of variance and Tukey’s multiple range test using the SAS statistical software package (SAS, Cary, NC). Differences were considered significant at p < 0.05.

### Results

Cell viability

The cells were treated with methanol extracts (0.1 mg/mL) from the four types of rice, and cell
viability was measured by MTT assay to test whether the four types of rice had cytotoxic effects on 3T3-L1 adipocytes. As shown in Fig. 1, treatment with the four types of germinated rice had no inhibitory effect on cell survival.

Inhibitory effects of various cereals on lipid accumulation in 3T3-L1 adipocytes

Cells were pretreated with the four types of germinated rice at 0.1 mg/mL for 24 h, and intracellular lipid droplets were stained with Oil Red O and quantified to examine the effects of the various germinated rice on lipid accumulation. Lipid accumulation was gradually enhanced during adipocyte differentiation. As positive control, caffeine was also examined, at a concentration of 0.1 mg/mL. As shown in Fig. 2, the GBR- and GWBR-treated cells showed reduced Oil Red O staining, by 49% and 27%, as compared to the control cells. The greatest effect was observed in the GBR treated cells, indicating that GBR significantly suppressed lipid accumulation. Hence, we selected GBR and performed further animal studies.

### Table 2. Gene-Specific Primers Used in RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5' → 3')</th>
</tr>
</thead>
</table>
| LPL      | Forward: ACTGTTCATCTCATTCCTGG  
               Reverse: TCTCATACATCCTCCGCTTAC |
| HSL      | Forward: ACTCAGAACGAGGACGCTA  
               Reverse: TAGGTCCAGAAGGATGTG |
| ATGL     | Forward: ATTTATCCGGTTGTACGTG |
| C/EBP-α  | Forward: GGAGGTCCACAGAAGCTGATTCC  
               Reverse: AGAGGTCCACAGAAGCTGATTCC |
| PPAR-γ   | Forward: GGCACTAAGGGGTCCTCAACCT  
               Reverse: GCCAGTGCTGACGCTATG |
| SREBP-1c  | Forward: ACCATATGAGTCTGCAGGTGT  
               Reverse: TCTCCAGTTTTGAGGAAATC |
| FAS      | Forward: GCCAGAGCGGTGCTACACA  
               Reverse: AGCACATCTCGAAGGCTACACA |
| aP2      | Forward: ATTTATCCGGTTGTACGTG  
               Reverse: TGTCATGACGCTGCTATG |
| β-Actin  | Forward: AGCTCAGTAAACGCTCCCCCTAGA  
               Reverse: DGCTCAAGTCCTCCGCGCTAGA |

### Table 3. Effects of Germinated Brown Rice (GBR) on Body Weight Gain and Food Intake

<table>
<thead>
<tr>
<th>Groups</th>
<th>ND</th>
<th>HFD</th>
<th>HGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>18.89 ± 0.63</td>
<td>18.71 ± 0.53</td>
<td>18.21 ± 0.61</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>29.53 ± 1.75b</td>
<td>35.81 ± 2.80b</td>
<td>29.42 ± 1.75b</td>
</tr>
<tr>
<td>Cumulative BW gain (g/7 weeks)</td>
<td>10.64 ± 1.06b</td>
<td>17.10 ± 3.80b</td>
<td>11.20 ± 3.53b</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>3.59 ± 0.50a</td>
<td>2.84 ± 0.83b</td>
<td>2.49 ± 0.70b</td>
</tr>
</tbody>
</table>

1) ND, normal diet group; HFD, high fat diet group; HGM, high fat diet + 0.15% GBR methanol extract group. Results are presented as mean ± SD. Different letters indicate significant difference (p < 0.05), as determined by Tukey’s multiple range test.

Changes in body weight and food intake

Body weight gain increased significantly, by 1.6-fold, in the HFD control group as compared to the ND group (Table 3). GBR administration for 7 weeks significantly decreased mean body weight gain in the HFD fed mice as compared to the HFD control group. Throughout the experiment, food intake was lower in the HFD control group than in the ND control group, but there was no significant difference in food take between the HFD control group and the HGM group.

Serum lipid profiles

The effects of HFD and GBR administration on serum lipid profiles are shown in Table 4. The serum TG and TC levels in the HFD control group were significantly higher than in the ND control group, but GBR administration significantly reduced the TG and TC levels, by 26% and 23%, respectively, as compared to the HFD group. However, the serum HDL cholesterol level was significantly higher (16%) in the HGM group than in the HFD control group. The toxicity of GBR administration was determined by measuring serum AST and ALT levels. Serum AST levels increased significantly following HFD feeding, whereas GBR administration

### Fig. 1. Effects of Various Germinated Cereals on Viability of 3T3-L1 Adipocytes.

Differentiated 3T3-L1 adipocytes were treated with methanol extracts from four types of germinated rice (0.1 mg/mL), and intracellular lipid accumulation was measured by Oil Red O staining. Results are presented as mean ± SD for at least three independent experiments. Different letters indicate significant difference (p < 0.05), as determined by Tukey’s multiple range test.

### Fig. 2. Effects of Various Germinated Cereals on Lipid Accumulation in 3T3-L1 Adipocytes.

Differentiated 3T3-L1 adipocytes were treated with methanol extracts from four types of germinated rice (0.1 mg/mL), and intracellular lipid accumulation was measured by Oil Red O staining. Results are presented as mean ± SD for at least three independent experiments. Different letters indicate significant difference (p < 0.05), as determined by Tukey’s multiple range test.
The characteristics of people with obesity is the development different among the groups. One of the most common group (Fig. 4A), liver weights were not significantly in the HFD control group than that in the ND control group. Furthermore, a histological examination of epididymal adipose tissue revealed that the HFD control group. Additionally, a histological examination of epididymal adipose tissue was reduced following compared with the ND control group (Fig. 3A), the weight of epididymal adipose tissue in the HFD group increased significantly by 1.9-fold, compared with the ND control group.

The weight of epididymal adipose tissue in the HFD group had dramatically more epididymal adipocytes, whereas the size of adipocytes in the mice administrated with GBR was clearly smaller than that of the mice fed HFD (Fig. 4B), the 7 weeks of GBR administration resulted in dramatically fewer hepatic lipid droplets than in the HFD group. As shown in Fig. 4C, hepatic lipid accumulation decreased significantly in the HGM group as compared to the HFD control group.

### Adipogenesis-related gene mRNA expression

We performed RT-PCR to examine the effects of GBR on the gene expression of adipogenic transcription factors and adipocytokines in adipose tissue and liver of HFD-induced obese mice. GBR significantly attenuated adipogenic transcription factors C/EBP-α and PPAR-γ mRNA levels in the adipose tissue (Fig. 5A). We also examined the expression of the PPAR-γ target gene, aP2, an adipocyte phenotype gene. aP2 mRNA levels also clearly decreased in the GBR group as compared to the HFD control group. In addition, PPAR-γ and SREBP-1c mRNA expression in the liver was clearly suppressed by GBR administration (Fig. 5B). The expression of FAS, an important fatty-acid synthesis enzyme, was also significantly higher in the HFD control group than in the ND control group, the GBR administrated group showed significantly reduced FAS expression, by 49%, as compared to the HFD control group. These results indicate that GBR administration inhibited lipogenesis through FAS expression by suppressing the expression of adipogenic transcription factor in the liver.

### Lipase mRNA expression

To examine the effect of GBR administration on lipolysis, lipase mRNA expression in the adipose tissue was measured by RT-PCR. As shown in Fig. 6, lipoprotein lipase (LPL) mRNA expression in the HFD control group was significantly increased, as compared to the ND control group. In addition, hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) mRNA expression was highly decreased in the HFD control group as compared to the ND control group. However, in the adipose tissue of GBR administrated group, the mRNA expression of HSL and ATGL was increased.
Fig. 4. Histological Changes in Liver in High Fat Diet (HFD)-Induced Obese Mice.
A, Weight of liver; B, Histology of liver; C, Area of lipid accumulation. All sections were stained with hematoxylin and eosin (H & E), ×100. Different letters indicate significant difference (p < 0.05), as determined by Tukey’s multiple range test.

Fig. 5. Adipogenic Gene mRNA Expression in Epididymal Adipose Tissue and Liver of High Fat Diet (HFD)-Induced Obese Mice.
A, mRNA Expression in adipose tissue; B, mRNA expression in the liver. mRNA expression of the adipogenic gene was measured by RT-PCR. Different letters indicate significant difference (p < 0.05), as determined by Tukey’s multiple range test.
Effective anti-obesity treatments. High fat-fed rodents used to search for induced obese mice model. Various animal models that were treated with methanol extracts from four types of germinated rice for 24 h during the differentiation of 3T3-L1 preadipocytes to adipocytes, and lipid accumulation was measured with Oil Red O. Lipid accumulation in the adipocytes is determined by the balance between lipogenesis and lipolysis. The GBR treatment significantly reduced Oil Red O staining, indicating that GBR suppressed lipid accumulation. Hence, we examined the anti-obesity effects of four types of germinated rice, GBR, GWBR, GB-R, and GWB-R in 3T3-L1 adipocytes. The cells were treated with methanol extracts from four types of germinated rice for 24 h during the differentiation of 3T3-L1 preadipocytes to adipocytes, and lipid accumulation was measured with Oil Red O. Lipid accumulation in the adipocytes is determined by the balance between lipogenesis and lipolysis. The GBR treatment significantly reduced Oil Red O staining, indicating that GBR suppressed lipid accumulation. Hence, we selected GBR and performed further animal studies in an HFD-induced obese mouse model. Various animal models that stimulate obesity in humans have been used to search for effective anti-obesity treatments. High fat-fed rodents appear to be the best model of visceral obesity syndrome, because obesity pathogenesis to that in humans, including insulin resistance, and the lack of aberrant obesity gene expression.

Reducing body weight and body fat are important in preventing obesity. GBR significantly decreased body weight gain, the weight and size of epididymal adipose tissue, and hepatic lipid accumulation as compared to the HFD control group. Obesity is characterized by an increase in fat cell numbers, fat cell size, or a combination of the two. A histological examination of adipose tissue showed that the HFD control group had dramatically increased adipocyte tissue size, whereas GBR administration clearly decreased the size of adipocytes. LPL is important enzyme involved in TG accumulation, which hydrolyzes TG-rich lipoproteins such as very-low-density lipoproteins (VLDL) and chylomicrons, and provides substrates for fatty acid uptake into adipose tissue. It is increased in the adipose tissue of obese humans and rodents. Lipolysis, the coordinated catabolism of TG stored in lipid droplets, provides fatty acids, diglycerides (DG), and monoglycerides (MG). HSL and ATGL mRNAs are predominantly expressed in adipocytes of humans and mice, and coordinately catabolize stored TGs in adipose tissue. HSL is the primary DG lipase. It catalyzes the rate-limiting step in DG hydrolysis. ATGL is an important lipase responsible for hydrolyzing the first fatty acid from TG. In the present study, LPL mRNA levels were significantly decreased by GBR administration, whereas in the adipose tissue of the GBR administrated group, HSL and ATGL mRNA expression was significantly increased. These results indicate that GBR administration suppressed the weight and size of epididymal adipose tissue by downregulating LPL and upregulating HSL and ATGL.

Hepatic lipid accumulation generally occurs as a result of an increase in the supply of circulating fatty acids to the liver, but can also result from increased endogenous hepatic fatty acid synthesis. After 7 week administration, the weight and size of adipocytes as well as lipid accumulation in the liver decreased significantly in the HGM group, as compared to the HFD control group. This suggests that GBR has an anti-obesity effect by reducing lipid accumulation in the liver and adipose tissue of obese mice. Additionally, GBR markedly reduced serum TG and TC levels, but increased serum HDL cholesterol levels. These data are in agreement with previous studies, which indicate that weight loss is associated with increased HDL cholesterol levels. Several studies have found that the GABA, vitamin E, and oryzanol in germinated brown rice effectively decreased the triglyceride and the LDL-cholesterol level, whereas, it increased HDL-cholesterol in hypercholesterolaemic animals, although the mechanism is not yet clear. We speculate that the GABA, vitamin E, and oryzanol in germinated brown rice are active components that improve lipid metabolism in high fat diet-induced obese mice, but it is necessary to verify the roles of these compounds through further study on anti-obesity and the suppression of hyperlipidemia.

We performed RT-PCR to analyze the genes known to be involved in lipogenesis to determine the molecular mechanism of GBR-mediated lipogenesis. C/EBP-α, PPAR-γ, and SREBP-1c are transcription factors important in adipogenesis.
mainly found in adipose tissue, are key adipogenesis and lipogenesis transcription factors.\textsuperscript{38,41} In the present study, GBR administration significantly suppressed the expression of these transcription factors in adipose tissue.

The role of SREBP-1c has been clearly established in the liver. It is a key regulator of adipogenesis, and it plays a dominant role in fat tissue development.\textsuperscript{38,41} It is the central regulator of adipogenesis, and it plays a dominant role in fat tissue development.\textsuperscript{38,41} It is known that hepatic PPAR-\(\gamma\) expression increases in some obese and diabetic mice.\textsuperscript{57,42} Reduced PPAR-\(\gamma\) in the liver suppresses the onset of obesity and fatty liver.\textsuperscript{33,43} In this study, PPAR-\(\gamma\) mRNA expression in the liver and adipose tissue decreased significantly in the HGM group as compared to the HFD control group. Furthermore, the mRNA level of PPAR-\(\gamma\) target gene aP2\textsuperscript{38} was significantly suppressed in adipose tissue by GBR. Thus reduced lipid accumulation in the liver and adipose tissue due to GBR was related to the reduction in PPAR-\(\gamma\). Taken together, these results suggest that GBR suppressed lipogenesis and adipogenesis by reducing transcription factors.

In conclusion, GBR administration significantly suppressed lipid accumulation in 3T3-L1 adipocytes and body weight gain, the weight and size of adipose tissue, hepatic lipid accumulation, and the serum levels of TG in 3T3-L1 adipocytes and acetyl CoA carboxylase (ACC), the role of SREBP-1c in adipocytes is unclear.\textsuperscript{41} The SREBP-1c level decreased significantly (51%) following GBR administration, in this study. In addition, FAS expression clearly decreased in the GBR group as compared to the HFD control group. PPAR-\(\gamma\) is the central regulator of adipogenesis, and it plays a dominant role in fat tissue development.\textsuperscript{38,41} It is known that hepatic PPAR-\(\gamma\) expression increases in some obese and diabetic mice.\textsuperscript{57,42} Reduced PPAR-\(\gamma\) in the liver suppresses the onset of obesity and fatty liver.\textsuperscript{33,43} In this study, PPAR-\(\gamma\) mRNA expression in the liver and adipose tissue decreased significantly in the HGM group as compared to the HFD control group. Furthermore, the mRNA level of PPAR-\(\gamma\) target gene aP2\textsuperscript{38} was significantly suppressed in adipose tissue by GBR. Thus reduced lipid accumulation in the liver and adipose tissue due to GBR was related to the reduction in PPAR-\(\gamma\). Taken together, these results suggest that GBR suppressed lipogenesis and adipogenesis by reducing transcription factors.

Acknowledgments

This work was supported by the Grant of the Korean Research Foundation in the Korean Ministry of Education, Science and Technology (code 2008-0060892).

References