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Research article

Effects of fermented ginseng root and ginseng berry on obesity and lipid metabolism in mice fed a high-fat diet

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ABSTRACT

Background: Previous studies have shown that both ginseng root and ginseng berry exhibit antiobesity and antidiabetic effects. However, a direct comparison of the efficacy and mechanisms between the root and the berry after oral administration remains to be illuminated.

Methods: In this study, we observed the effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on obesity and lipid metabolism in high-fat diet induced obese mice.

Results: FGR and FGB significantly inhibited the activity of pancreatic lipase *in vitro*. Both FGR and FGB significantly suppressed weight gain and excess food intake and improved hypercholesterolemia and fatty liver, while only FGR significantly attenuated hyperglycemia and insulin resistance. Both FGR and FGB significantly inhibited the mRNA expression of *Ldlr* and *Acs11* while FGR also significantly inhibited expression of *Cebpa* and *Dgat2* in liver. FGR significantly decreased the epididymal fat weight of mice while FGB significantly inhibited the mRNA expression of genes *Cebpa*, *Fas*, *Hsl*, *Il1b*, and *Il6* in adipose tissue.

Conclusion: Saponin from both FGR and FGB had a beneficial effect on high-fat diet-induced obesity. Compared to FGB, FGR exhibited more potent antihyperglycemic and antiobesity effect. However, only FGB significantly inhibited mRNA expression of inflammatory markers such as interleukins 1 β and 6 in adipose tissue.

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1. Introduction

Panax ginseng root and ginseng berry have distinct ginsenoside profiles. The representative ginsenoside in ginseng root is protopanaxadiol (PPD)-type Rb1 while the representative ginsenoside in ginseng berry is protopanaxatriol (PPT)-type Re. Both ginseng root and ginseng berry have been reported to exhibit antiobesity and antidiabetic effects in a murine model [1–4]. Attele et al [1] showed that ginseng berry extract reduced blood glucose levels, food intake, and body weight and increased energy expenditure in ob/ob mice. Dey et al [2] compared the antihyperglycemic and antiobesity effect between ginseng root and ginseng berry and found that ginseng berry exhibited a more potent hypoglycemic activity, and that only ginseng berry showed marked antiobesity effects in ob/ob mice. In their study, however, ginseng root and ginseng berry extracts were administered to mice intraperitoneally, which ignored

the activities of the ginseng components in the gastrointestinal tract. It was reported that ginseng and ginsenosides could work as pancreatic lipase inhibitors and delay the digestion and absorption of lipids [5]. Moreover, ginsenoside bioavailability through intraperitoneal administration is far higher than that through oral administration. In addition, because the mice (ob/ob) used in that study were genetically engineered, the effects of ginseng berry in normal mice fed on a high-fat diet (HFD) is not clear. Therefore, whether ginseng root or ginseng berry exerts a more potent activity remains unclear.

After oral intake, the natural bioactive compounds in herbal medicine can be transformed to their deglycosylated forms in the intestine by microflora [6]. The transformed ginsenosides are more easily absorbed and exhibit a more potent activity. Nevertheless, approximately 20% of individuals cannot efficiently, or even at all, transform ginsenosides [7]. In this study, we fermented ginseng

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root and ginseng berry with molds to transform the ginsenosides. It is worth noting that the molds used do not produce mycotoxin and are considered safe [8].

The aim of this study was to transform ginsenosides in ginseng root and ginseng berry, compare the effects of crude saponin from fermented ginseng root (FGR) and fermented ginseng berry (FGB) on lipid metabolism and obesity and to elucidate their distinct mechanisms in mice fed an HFD.

2. Materials and methods

2.1. Materials

Four-year old Korean ginseng (*Panax ginseng* Meyer, family Araliaceae) roots were purchased from the Nokdu Market (Seoul, Korea). Korean Panax ginseng berry was kindly provided by the Korean Genetic Pharm (Seoul, Korea). *Aspergillus niger* FMB46494 and *Aspergillus oryzae* FMB40247 that do not produce mycotoxin were from the Laboratory of Food Microbiology, Seoul National University. Porcine pancreatic lipase (L3126) and orlistat (O4139) were purchased from Sigma (St Louis, MO, USA), and triolein was purchased from Avention (Inchon, Korea). Ginsenosides Rb1, Rb2, Rd, Rf, Rg1, and compound K (cK) were purchased from Biotech (Nanjing, China). Ginsenoside Re, Rg2, Rg3, F2, Rh1, and Rh2 were purchased from Cogon Biotech (Chengdu, China).

2.2. Fermentation of ginseng root and berry and preparation of crude saponin

Ginseng roots and ginseng berries were fermented with *A. niger* and *A. oryzae*, respectively, as described previously [9]. After fermentation, the culture broth was freeze-dried and extracted with water-saturated *n*-butanol at 80°C. After filtration, the filtrate was mixed with distilled water and stewed overnight. The upper phase was evaporated, and the residue was degreased with diethyl ether. The ginsenoside contents left in the crude saponin samples were determined by HPLC as described previously [9].

2.3. Activity assay of pancreatic lipase

This assay was adapted from a previous study [5]. Briefly, triolein was used as the substrate for pancreatic lipase, and the amount of generated oleic acid was determined with the nonesterified fatty acid (NEFA) assay kit from Wako (Osaka, Japan).

2.4. Animals and diets

Male C57BL/6 mice (age 5 wk), purchased from Central Laboratory Animal (Seoul, Korea), were housed under a 12 h light/12 h dark cycle in a controlled room at a temperature of 23 ± 3°C and a humidity of 50 ± 10%. After acclimating to the facility for 1 wk, the mice were randomly divided into four groups ($n = 10$) and fed a low-fat diet (LFD; 10% of the total calories from fat, Table 1), an HFD (45% of the total calories from fat; Table 1), or an HFD supplemented with crude saponin from the FGR or FGB for 16 wk. All the mice were allowed food and water *ad libitum*. Body weight, fasting blood glucose, and food intake were determined once every 2 wk. Before blood glucose was determined, mice were fasted for 12 h. After consuming an LFD or HFD for 16 wk, the mice underwent 12 h of fasting prior to being anesthetized with Zoletil 50 (Virbac, Carros, France) and Rompun (Ansan, Korea) and then were dissected. Blood samples were collected by heart punctures. Livers and epididymal fat pads were removed and stored at -80°C for subsequent analyses. All procedures relating to the animals and their care were

approved by the Institutional Animal Care and Use Committee of Seoul National University.

2.5. Histopathologic evaluation

After mice were sacrificed, the samples of live and epididymal fat pads were fixed with formalin solution, stained with hematoxylin and eosin, and viewed with an optical microscope.

2.6. Biochemical analyses

Plasma levels of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) as well as activity levels of aspartate transaminase and alanine transaminase were determined with test kits obtained from Asanpharm (Seoul, Korea). The levels of NEFA were determined with the NEFA assay kit. Low-density lipoprotein cholesterol (LDL-C) levels were calculated with the formula $LDL-C = TC - HDL-C - TG \times 0.2$. Plasma insulin levels were determined with the mouse insulin enzyme-linked immunosorbent assay kit from Shibayagi (Shibukawa, Japan), and plasma adiponectin levels were determined with the enzyme-linked immunosorbent assay kit from Cloud-clone (Houston, TX, USA).

2.7. Hepatic lipid analyses

TG and TC levels in the livers of the mice were measured according to the methods described in a previous study [10].

2.8. Real-time polymerase chain reaction

Total RNA was extracted from the liver and adipose tissue with an RNA extraction kit purchased from Takara Bio (Kusatsu, Japan) and RNeasy Lipid Tissue Mini Kit from Qiagen (Venlo, Netherlands), respectively. The concentration of RNA was measured with a Micro Spectrophotometer (Allsheng, Hangzhou, China), and 0.5 µg of total RNA from each sample was reverse-transcribed to cDNA with a cDNA synthesis kit from Takara Bio. Relative quantifications of gene transcripts were completed with SYBR premix from Takara Bio using the Applied Biosystems 7500 system (Thermo Fisher Scientific, Waltham, MA). Relative mRNA levels were normalized to the *Gapdh* mRNA level and expressed as values of relative expression compared to that of the HFD group. The primers used in this study are listed in Table S1.

Table 1
Formula of low-fat diet (LFD) and high fat diet (HFD)

Formula	LFD	HFD
	(10% calorie from fat) g/kg	(45% calorie from fat) g/kg
Casein	210.0	245.0
L-cystine	3.0	3.5
Corn starch	280.0	85
Maltodextrin	50.0	115.0
Sucrose	325.0	200.0
Lard	20.0	195.0
Soybean oil	20.0	30.0
Cellulose	37.15	58.0
Mineral mix, AIN-93G-MX (94046)	35.0	43.0
Calcium phosphate, dibasic	2.0	3.4
Vitamin mix, AIN-93-VX (94047)	15.0	19.0
Choline bitartrate	2.75	3.0
Yellow food color	0.1	0
Blue food color	0	0.1

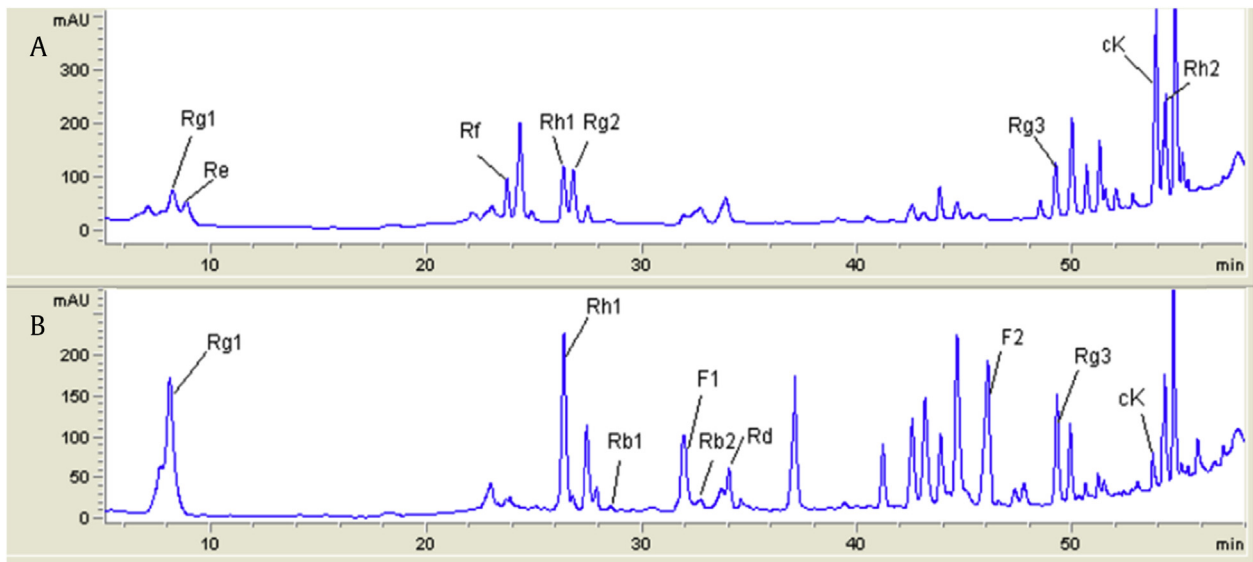


Fig. 1. HPLC chromatograms of the ginsenosides from (A) fermented ginseng root extract and (B) fermented ginseng berry extract. The peaks of various ginsenosides are presented in the chromatograms. HPLC, high-performance liquid chromatography.

Table 2

Ginsenoside profiles of ginseng root, ginseng berry, fermented ginseng root and fermented ginseng berry

	Rb1	Rb2	Rd	Re	Rf	Rg1	Rg2	Rg3	F1	F2	Rh1	Rh2	cK
FGR	—	—	—	3.0	2.5	4.6	1.9	4.9	0.5	1.4	2.7	2.9	9.0
FGB	0.5	2.5	1.3	—	—	19.1	0.6	3.4	4.3	9.2	7.9	—	1.3

Percentage content of various ginsenosides in the butanol fraction (w/w, %); < 0.1%

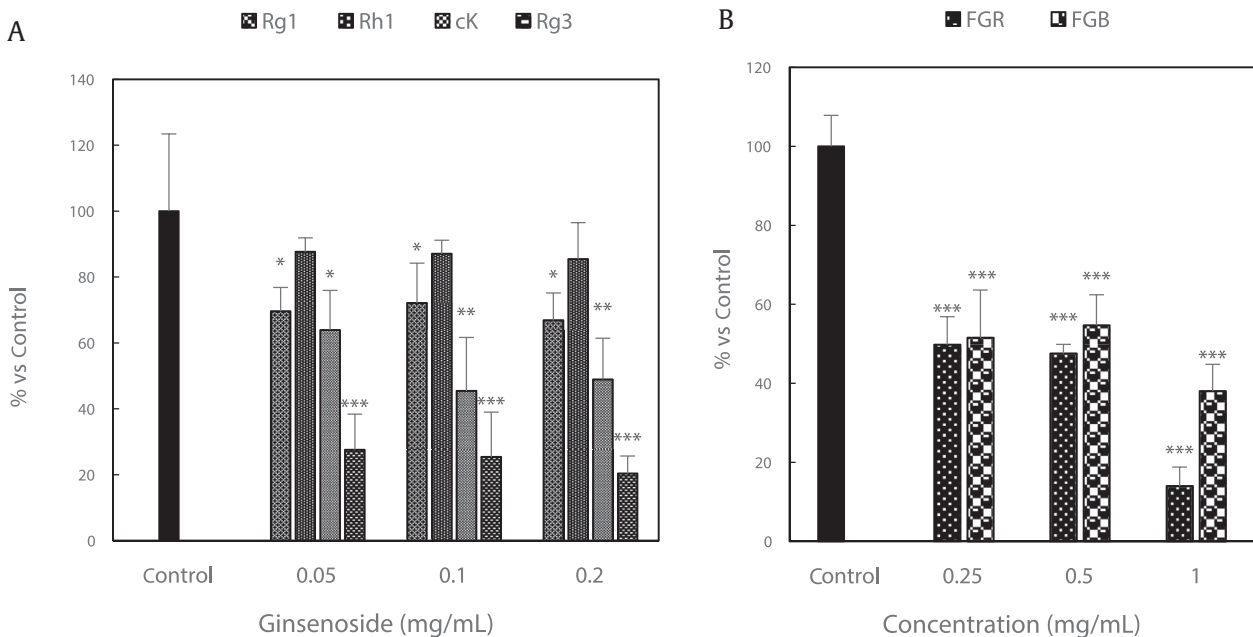


Fig. 2. Effects of ginsenosides Rg1, Rh1, Rg3, cK, fermented ginseng root, and fermented ginseng berry on the activity of pancreatic lipase. (A) Ginsenosides Rg1, Rh1, cK and Rg3. (B) Fermented ginseng root and fermented ginseng berry. The activity of porcine pancreatic lipase was measured using triolein as substrate. Pancreatic lipase activity = (absorbance after treatment/absorbance of control) \times 100%. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (n = 3).

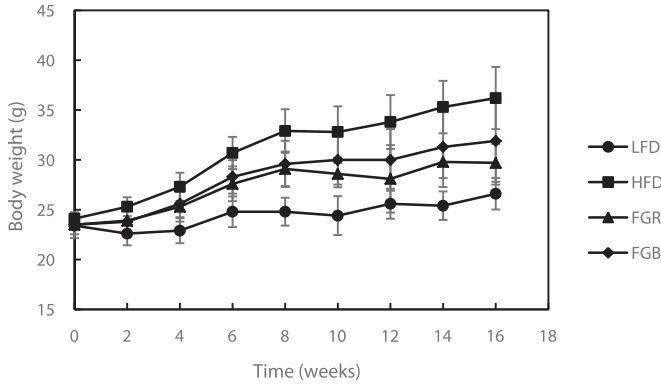


Fig. 3. Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on the body weight of mice fed a high-fat diet (HFD) for 16 weeks. Values are expressed as mean ± standard deviation (n = 10). The body weight of mice in the FGR and FGB groups was significantly lower than that of the HFD group from Week 2 (p < 0.05). LFD = low-fat diet.

Table 3
Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on the weight parameters of mice

	LFD	HFD	FGR	FGB
Original BW (g)	23.0 ± 1.2	24.2 ± 0.6	23.3 ± 1.2	23.3 ± 1.0
Final BW (g)	26.1 ± 1.6 ^a	36.2 ± 4.4 ^c	29.7 ± 1.9 ^b	31.9 ± 4.4 ^b
Food intake (g)	299.0	311.2	260.1	248.4
FER (mg/g)	10.4	31.5	23.0	33.9
Liver (g)	1.0 ± 0.2	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2
Liver/BW (%)	4.0 ± 0.7	3.3 ± 0.8	3.2 ± 0.6	3.2 ± 0.3
EAT (g)	0.6 ± 0.2 ^a	1.8 ± 0.6 ^b	0.9 ± 0.4 ^a	1.6 ± 0.6 ^b
EAT/BW (%)	2.2 ± 0.7 ^a	5.2 ± 1.3 ^b	3.2 ± 1.3 ^a	5.0 ± 1.2 ^b

^{a,b,c} Not sharing a common letter indicates significantly different groups at p < 0.05. (n = 10)
BW, body weight; EAT, epididymal adipose tissue; FER, food effect ratio (= food intake/weight gain); HFD, high-fat diet; LFD, low-fat diet

Table 4
Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on serological parameters of mice

	LFD	HFD	FGR	FGB
TG (mg/dL)	64.9 ± 9.2	55.0 ± 12.1	56.7 ± 18.3	56.5 ± 2.6
TC (mg/dL)	77.3 ± 12.4 ^c	136.3 ± 9.0 ^a	113.3 ± 7.2 ^b	128.2 ± 11.6 ^a
LDL-C (mg/dL)	8.0 ± 5.5 ^c	51.3 ± 6.9 ^a	36.3 ± 7.6 ^b	40.0 ± 10.9 ^b
HDL-C (mg/dL)	58.1 ± 8.4 ^b	75.5 ± 6.8 ^a	57.3 ± 14.6 ^b	74.7 ± 3.9 ^a
NEFA (mEq/L)	1.5 ± 0.2	1.5 ± 0.2	1.7 ± 0.2	1.6 ± 0.3
Insulin (ng/mL)	1.7 ± 0.8	1.9 ± 0.8	1.3 ± 0.1	1.7 ± 0.2
HOMA-IR	11.2 ± 8.0 ^{ab}	13.8 ± 6.3 ^a	5.9 ± 0.8 ^b	8.8 ± 1.4 ^{ab}
Adiponectin (ng/mL)	4.0 ± 1.0	5.5 ± 0.9 ^a	5.8 ± 0.5 ^a	4.4 ± 0.6 ^b
ADP/EAT (ng/g)	6.7	3.1	6.4	2.8

^{a,b,c} Not sharing a common letter indicates significantly different groups at p < 0.05. (n = 6)

The homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as insulin (mU/L) × glucose (mM)/22.5

ADP, adiponectin; EAT, epididymal adipose tissue; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; LDL-C, low-density lipoprotein cholesterol; LFD, low-fat diet; NEFA, nonesterified fatty acid; TC, total cholesterol; TG, triglycerides

2.9. Statistical analysis

The differences among groups were examined with one-way ANOVA followed by Duncan’s multiple range tests. Statistical analyses were done with the SPSS statistical package SPSS 22 (SPSS Inc., Chicago, IL, USA). The significance level of the test results was set at p < 0.05.

3. Results and discussion

3.1. Ginsenoside profile of FGR and FGB: FGR and FGB effects on the activity of pancreatic lipase

Ginsenoside contents in FGR and FGB were measured with HPLC and the ginsenoside profiles are presented in Fig. 1. As shown in Table 2, the main ginsenosides in FGR were the PPD-type, with a

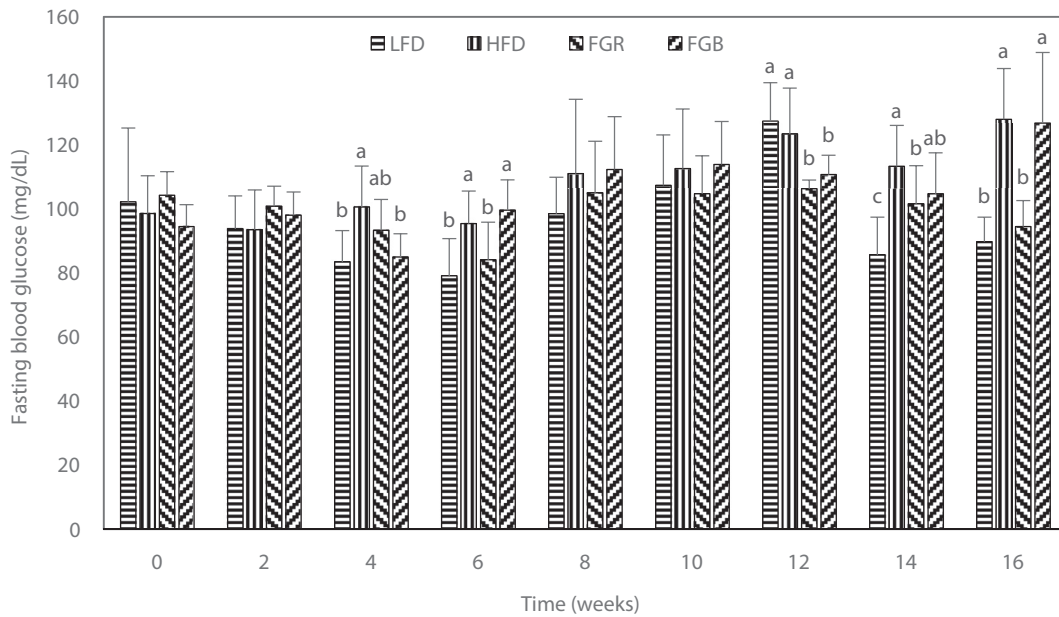


Fig. 4. Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on fasting blood glucose of mice during the 16 weeks (n = 10). Blood glucose was measured after fasting for 12 hours. Blood samples were collected from the tail of mice. ^{a,b,c} Not sharing a common letter indicates significantly different groups at p < 0.05. HFD, high-fat diet; LFD, low-fat diet.

high amount of cK (9.0%) and Rg3 (4.9%). The main ginsenosides in FGB are the PPT-type, with a high amount of ginsenoside Rg1 (19.1%) and Rh1 (7.9%).

Ginsenosides can work as pancreatic lipase inhibitors whereby the digestion and absorption of fats can be suppressed [11]. It was reported that supplementation of ginseng extract increased the fecal weight and fecal lipid content in mice [4,12]. In this research, ginsenoside cK, Rg3, and Rg1 significantly inhibited the activity of pancreatic lipase (Fig. 2A). In addition, the PPD-type ginsenoside such as Rg3 and cK were more effective than the PPT-type such as Rg1 and Rh1. Both FGR and FGB significantly suppressed the activity of pancreatic lipase. Moreover, FGR was more effective than FGB (Fig. 2B). Liu et al [5] reported that the PPD-type ginsenosides more efficiently inhibited the activity of pancreatic lipase than the PPT-type, which was consistent with the present results.

3.2. Effects of FGR and FGB on food intake and body weight

HFDs are considered to cause chronic inflammation in the hypothalamus and passivate leptin signaling, which are mechanisms mediating sustained appetite enhancement [13]. Ginsenoside Rb1 was reported to reduce the levels of inflammatory markers and negative regulators of leptin signaling in the hypothalamus and restore the anorexic effect of leptin in HFD fed mice [14]. Both PPD-type and PPT-type ginsenosides are reported to decrease orexigenic neuropeptide Y and increase anorexigenic cholecystokinin in HFD fed rats [15]. Moreover, many researchers have shown that ginseng extract could improve leptin resistance and diminish excessive energy intake in HFD-induced obese mice or rats [16].

In the present research, mice in the FGR and FGB groups had significantly lower weight gain than the HFD group. In particular, the mice in the FGR group showed the lowest weight gain (Fig. 3). In addition, food intake was also inhibited in the FGR and FGB groups, especially in the FGB group. Food effect ratio was markedly lower in the FGR group than in the HFD group (Table 3).

3.3. Effects of FGR and FGB on blood glucose and lipid profiles

Mice in the FGR group had significantly lower levels of fasting blood glucose starting from Week 12, while the FGB group showed only a sporadic slight hypoglycemic effect on blood glucose (Fig. 4).

The mice in the FGR and FGB groups had similar levels of plasma TG and NEFA with the HFD. For plasma cholesterol, the mice in both the FGR and FGB groups had significantly lower levels of LDL-C, and the mice in the FGR group also had significantly lower levels of TC and HDL-C compared with the mice in the HFD group. HDL-C is considered as the “good cholesterol” and higher HDL-C levels are correlated with cardiovascular health. However, it is the ratio of HDL-C and LDL-C, rather than cholesterol itself, that matters [17]. In this study, both FGR and FGB slightly increased the ratio of HDL-C and LDL-C, though not statistically significantly (data not shown). Moreover, HOMA-IR, a parameter of insulin resistance, was significantly lower in the FGR group. For reference, the ratio of adiponectin release versus epididymal adipose tissue weight in the FGR group was remarkably higher than in the HFD group (Table 4).

3.4. Effects of FGR and FGB on lipid metabolism in liver

Long-term exposure to an HFD can cause nonalcoholic fatty liver disease, a condition wherein large droplets of fat deposited in hepatocytes via the process of steatosis. Some studies have suggested that the kind of lipid rather than the amount of fat determines the susceptibility to the *second hit* of the two-hit theory for the pathogenesis of nonalcoholic fatty liver disease [18]. Excessive cholesterol accumulation disrupts membrane fluidity, promotes cellular dysfunction, and thereby results in the progression fatty liver [19].

In this study, the mice in both the FGR and FGB groups had markedly lower levels of TG and TC contents in the liver (Figs. 5A, 6A). ALT, a liver injury marker, was significantly lower in the FGR

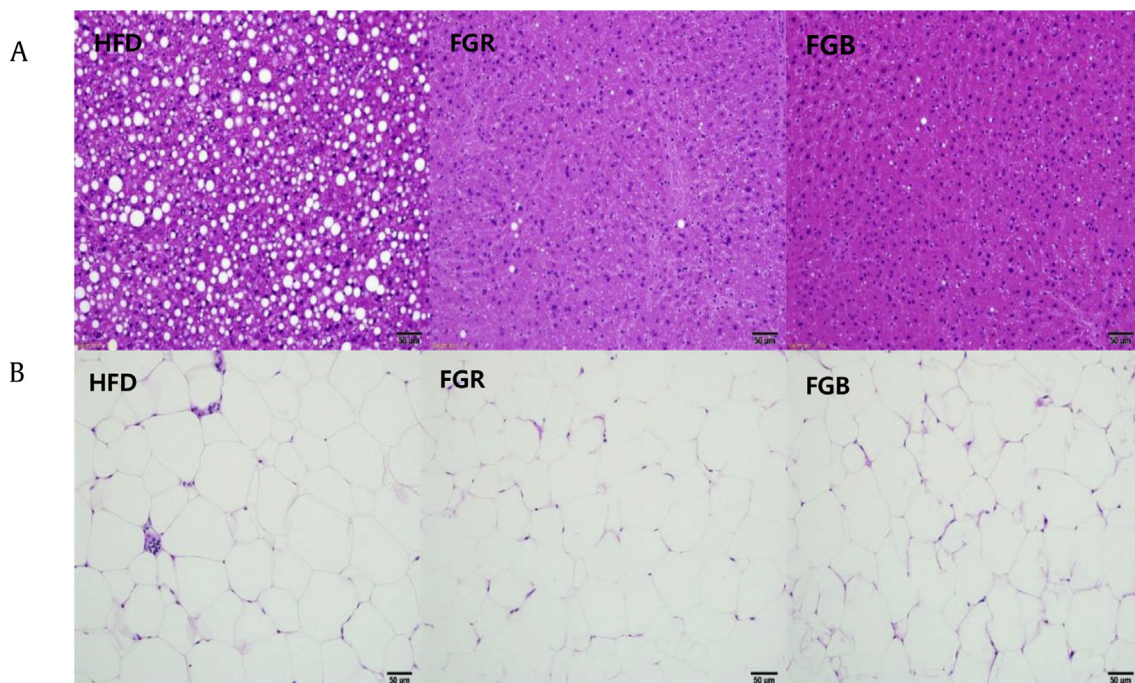


Fig. 5. The hematoxylin and eosin staining of mice fed with high-fat diet for 16 weeks. (A) liver. (B) Epididymal adipose tissue. FGB, fermented ginseng berry; FGR, fermented ginseng root; HFD, high-fat diet.

group than in the HFD group (Fig. 6B). The expression of LDL-R, a receptor mediating the endocytosis of cholesterol-rich LDL, was significantly enhanced in the FGR and FGB groups, which was in line with the decreased plasma LDL-C levels. HMG-CoA reductase, the rate-controlling enzyme in the pathway of cholesterol synthesis, also showed a decreased tendency in the FGR and FGB groups (Fig. 6C).

Obesity is accompanied by increased liver uptake of NEFA, which either undergo oxidation or are esterified with glycerol to produce TG. Recent studies have shown that NEFA could directly cause toxicity by increasing oxidative stress and by activating inflammatory pathways, which aggravate fatty liver disease [20]. In this work, expressions of the gene *Cd36* and *Fatp5*, mediating the liver uptake of NEFA, were repressed in the FGR and FGB groups. In particular, the mRNA level of *Fatp5* in the FGR group was significantly lower than that in the HFD group. Low-density lipoprotein receptor-related protein 1, mediating transfer of fat from chylomicron remnant to the liver, showed a decreasing trend in the FGR

group. ApoB100, a protein used to assemble very-low-density lipoprotein, showed an increasing trend in the FGR and FGB groups. *Fabp1* is involved in the transport and metabolism of long-chain fatty acids, and increased expression levels of *Fabp1* have been observed in obese individuals, which is considered as a compensatory upregulation in an attempt to counter the high metabolic stress associated with obesity [21]. Although not significantly ($p = 0.11$), the mRNA level of *Fabp1* was reduced by 50% in the FGR group compared with the HFD group. *Acs11* plays a critical role in both fatty acid biosynthesis and β -oxidation [22]. Some reports have shown that HFD reduces *Acs11* expression [23,24] while others have shown that HFD induces *Acs11* expression at the mRNA level [25,26]. In this study, the HFD did not significantly increase the mRNA levels of *Acs11*, while FGR and FGB significantly decreased the expression levels of *Acs11*. *Acbp*, which mediates intermembrane acyl-CoA transport and donates acyl-CoA for β -oxidation and TG synthesis, showed an increased tendency in the FGR and FGB groups (Fig. 6D).

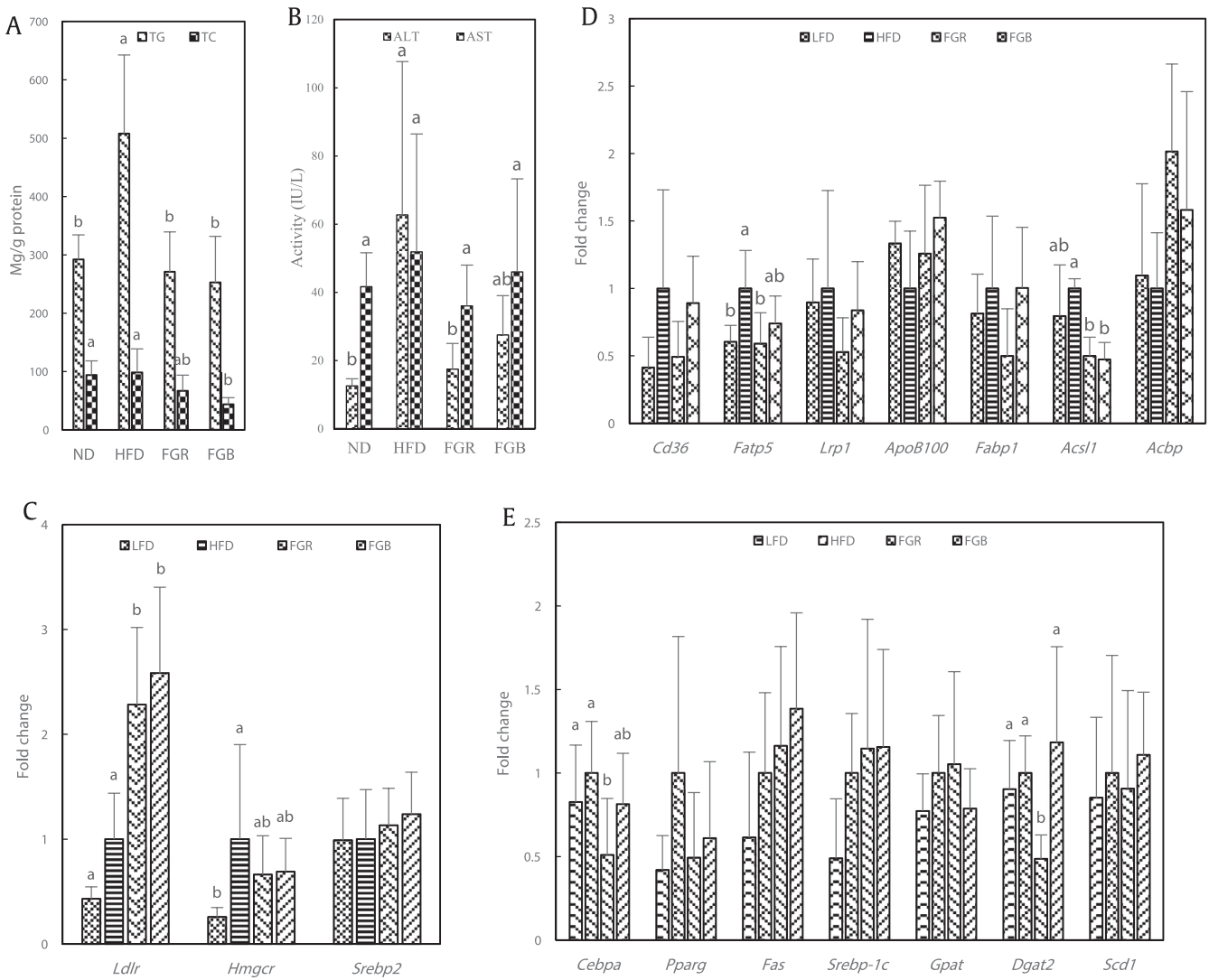


Fig. 6. Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on the liver of mice fed a high-fat diet (HFD) for 16 weeks. (A) Effects on triglycerides (TG) and total cholesterol (TC) levels in the liver ($n = 6$). (B) Effects on alanine transaminase (ALT) and aspartate transaminase (AST) activities in the liver ($n = 6$). (C) Effects on gene expression related to cholesterol metabolism ($n = 4$). (D) Effects on gene expression related to fatty acid uptake and fatty acid channeling ($n = 4$). (E) Effects on gene expression related to lipogenesis and triglyceride synthesis ($n = 4$). ^{a,b,c} Not sharing a common letter indicates significantly different groups at $p < 0.05$.

Hepatic CCAAT/enhancer-binding protein α (C/EBP- α) plays a critical role in the acceleration of lipogenesis in ob/ob mice [27]. It was reported that peroxisome proliferator-activated receptor- γ might be involved in HFD-induced liver steatosis [28]. The expression of these two factors was lower in the FGR group. FGR significantly inhibited the expression of C/EBP- α . In addition, expression of *Dgat2*, gene involved in TG synthesis, was significantly suppressed in the FGR group (Fig. 6E).

Taken together, both FGR and FGB significantly facilitated the mRNA expression of *Ldlr* and *Acs11* while FGR also significantly inhibited expression of *Cebpa* and *Dgat2* in liver. Both FGR and FGB significantly ameliorated fatty liver.

3.5. Effects of FGR and FGB on adipose tissue

The mice in the FGR group had a significantly lower weight of epididymal adipose tissue and smaller adipocyte size (Table 3, Figs. 5B, 7A). The mRNA expression levels of *Cd36* and *Lpl*, mediating the uptake of fatty acid, were significantly increased in the mice fed an HFD. Fatty acid binding protein 4 is extensively used as a marker for differentiated adipocytes, and its blocking has the possibility of treating obesity [29]. The mRNA expression level of *Fabp4* was efficiently reduced in the FGR and FGB groups.

Peroxisome proliferator-activated receptor- γ and C/EBP- α , mediating adipogenesis in adipocytes [30], were down-regulated in the FGR and FGB groups, especially in the FGB groups. The mRNA level of *Fas* in the FGB group was significantly lower than that in the HFD group. It was reported that the expression levels of adipose triglyceride lipase and hormone sensitive lipase were both increased in the subcutaneous adipose tissue of HFD-induced obese mice, which was in line with this study [31]. The mRNA level of ATGL was markedly lower in the FGB group and the mRNA level of hormone sensitive lipase was significantly lower in the FGB group compared with that in the HFD group (Fig. 7A).

Adipocyte hypoxia, due to adipose tissue hypertrophy resulting from obesity, can cause adipocyte necrosis, which leads to macrophage infiltration and proinflammatory cytokine secretion [32]. It was reported that tumor necrosis factor- α and interleukin (IL)-6 hampered the insulin cascade signaling pathway by preventing the phosphorylation of insulin receptors [33]. IL-1 β also induces insulin resistance in adipocytes and its expression is upregulated in the adipose tissue of obese and insulin-resistant mice [34].

In this study, CD68 and Adgre1 (F4/80), used as macrophage markers, were significantly increased in mice fed a HFD and remarkably lower in the mice supplemented with FGR and FGB saponin, especially with FGB saponin. Moreover, lower mRNA

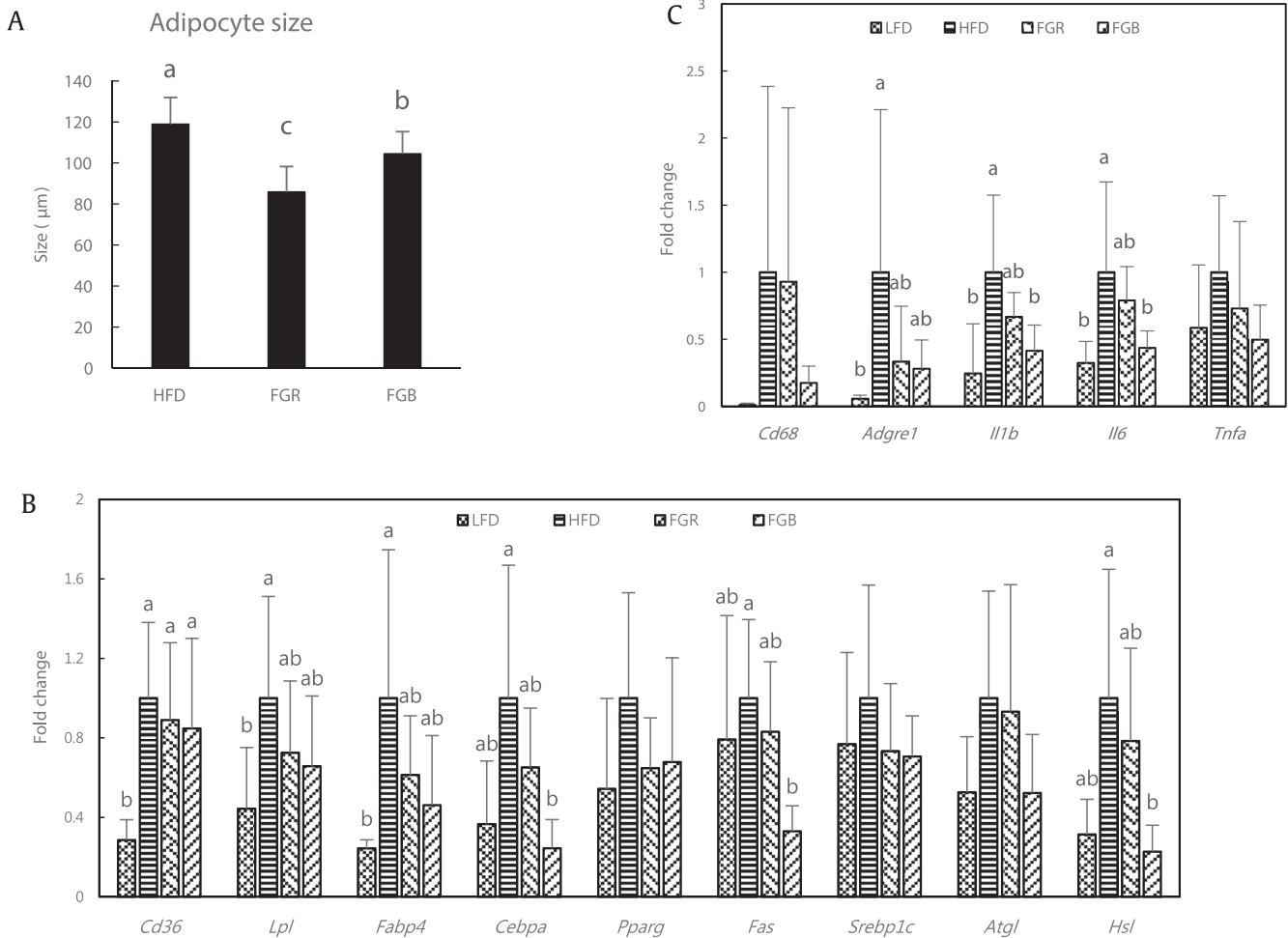


Fig. 7. Effects of fermented ginseng root (FGR) and fermented ginseng berry (FGB) on the adipose tissue of mice fed a high-fat diet (HFD) for 16 weeks. (A) Adipocyte size. (B) Effects on gene expression related to lipid metabolism ($n=4$). (C) Effects on gene expression related to inflammation ($n=4$). ^{a,b,c} Not sharing a common letter indicates significantly different groups at $p < 0.05$. LFD = low-fat diet.

expression levels of cytokines were observed in the adipose tissue of mice in the FGR and FGB groups. FGB supplementation significantly repressed the expression of IL-1 β and IL-6 (Fig. 7B).

Taken together, FGR significantly decreased the epididymal fat weight of mice while FGB significantly inhibited the mRNA expression of *Cebpa*, *Fas*, *Hsl*, *Il1b* and *Il6* in adipose tissue. Gu et al [35] reported that ginsenoside Rh1 ameliorated HFD-induced obesity mice by inhibiting adipocyte differentiation and alleviated adipose inflammation. In fact, ginsenoside Rh1 is a metabolite of ginsenoside Re and is abundant in the FGB in this study.

In summary, FGR and FGB significantly suppressed the weight gain of HFD-fed mice while only FGR effectively attenuated hyperglycemia and insulin resistance. FGR and FGB might improve hypercholesterolemia by facilitating expression of gene *Ldlr* and alleviate fatty liver through inhibiting liver uptake of free fatty acids. Only FGB significantly inhibited inflammatory markers in adipose tissue. Overall, FGR saponin showed a more potent anti-obesity effect on HFD-induced obese mice than FGB saponin, which might be partially attributed to the higher ability of the inhibitory effect of FGR saponin on the activity of pancreatic lipase.

Conflicts of interest

Geun Eog Ji is a professor of Seoul National University and also a president of Bifido Co., Ltd. The other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jgr.2017.04.001>.

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