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Anti-Obese Effects of a Mixture of Fermented Ginseng, *Bifidobacterium longum* BORI, and *Lactobacillus paracasei* CH88 in High-Fat Diet-Fed Mice

고지방 식이를 섭취한 마우스에서 발효인삼, *Bifidobacterium longum* BORI, *Lactobacillus paracasei* CH88 혼합물의 항비만 효과

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Abstract

Anti-Obese effects of a Mixture of Fermented Ginseng, *Bifidobacterium longum* BORI, and *Lactobacillus paracasei* CH88 in High-Fat Diet-Fed Mice

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Ginseng and probiotics have anti-obese effects in mice fed a high-fat diet (HFD). Absorption of ginsenoside and colonization of probiotics occur in the intestine. In this study, the mixture of fermented ginseng and two probiotics, *Bifidobacterium longum* BORI and *Lactobacillus paracasei* CH88, was administered to HFD fed mice for 9 weeks. The mixture significantly suppressed weight gain ($p < 0.05$, $n = 8$) and lipid deposition in the liver and adipose tissues as well as increased the mice' s food intake. The adipocyte size of the adipose tissue was significantly decreased in the mixture group, especially when 0.5% fermented ginseng and 5×10^8 CFU/mL two

probiotics were used ($p < 0.05$, $n = 10$). The expression of TNF-α in adipose tissue was efficiently down-regulated in the mixture group ($p < 0.05$, $n = 4$). The supplement also improved the mice's fasting blood glucose levels ($p < 0.05$, $n = 8$) and total cholesterol feces excretion ($p < 0.05$, $n = 8$). The mixture of fermented ginseng and *B. longum* BORI and *L. paracasei* CH88 could have anti-obese effects and suppress lipid deposit in the liver and adipose tissues.

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key words: Ginseng, *Lactobacteria*, *Bifidobacteria*, Anti-obesity, Lipid metabolism

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1. Introduction

Ginseng has been used as an anti-obesity treatment in Korea and China for many years. The physiological benefits of ginseng and various ginsenosides have been proven by several studies. Jung *et al.* [1] reported that ginseng fed to rats with a HFD resulted in weight loss, reduced adiposity and decreased hepatic triacylglycerol. Meanwhile, because the public' s interest in probiotics has recently increased, much research has been conducted on probiotics. Probiotics are living organisms that confer beneficial impacts on the maintenance of the host health by acting on the gut microbiota. [2] The ingestion of *Lactobacillus* and *Bifidobacterium* has been proven to aid against obesity, inflammation, and related metabolic diseases. [3] *Bifidobacterium longum* BORI and *Lactobacillus paracasei* CH88 were industrially developed and various health benefits have been reported. *B. longum* BORI has novel probiotic features, and the frequency of diarrhea was significantly reduced in rotavirus-infected children with acute colitis [4]. *B. lognum* BORI demonstrated the highest anti-obesity effect on weight loss as well as inhibited lipid deposition in the liver of HFD-fed mice [5]. Thus, both ginseng and probiotics are effective anti-obesity treatments.

In recent years, there has been a growing demand among consumers for probiotics and probiotic foods. Ginseng absorption and probiotics colonization are closely related and occur in the intestine. [6-8] The pharmacological action of ginsenosides occurs via biotransformation, which is deglycosylation by intestinal bacteria [9]. According to Ku [6], FG is a ginseng product that contains a significant amount of biocatalized ginsenoside glycosides via microbial glycosyl hydrolase treatment. When biotransformation occurs, ginsenoside becomes relatively hydrophobic, which improves the bioavailability by the body absorbing it. Meanwhile, probiotics alter the composition of the intestinal microflora via attachment and colonization of the intestinal mucosa, resulting in healthful effects [10]. However, few studies have researched the mechanism of fermented ginseng (FG) and probiotics when consumed together.

Therefore, the purpose of this study was to evaluate whether a FG and probiotics mixture had an anti-obesity effect on mice fed a high-fat diet.

2. Materials and Methods

2.1. Fermented ginseng and probiotics

The FG and probiotics used in this study were in freeze-dried powder form and provided by Bifido Co., Ltd (Hongchun, Korea). The probiotics mix (PM) was prepared by mixing *Bifidobacterium longum* BORI and *Lactobacillus paracasei* CH88 at a ratio of 1:1.

2.2. Ginsenoside analysis of fermented ginseng

To determine the ginsenoside content, the FG was ultrasonically extracted with 80% methanol for 1 h. The pre-treated samples were analyzed using an Ultimate 3000 HPLC system (Thermo Dionex, Sunnyvale, CA, USA) and UV 210 nm detector. A VSD C-18 column (250×4.6 mm, 5 ㎛) was used for all separations. The mobile phase solvent consisted of distilled water (A) and acetonitrile (B). The gradient elution conditions were as follows: 0-50 min (5% B), 50-56 min (95% B), and 56-60 min (5% B). The flow rate and temperature were set to 1 ml/min and 20° C, respectively. Ginsenosides Rb1, Rb2, Rd, Rg1, and F1 were purchased from Biotech (Nanjing, China). Ginsenosides Rc, Re, Rg2, Rg3, Rh1, Rh2, and cK were obtained from Cogon Biotech (Chengdu, China).

2.3. Animals and Diets

In our study, male ICR mice (7 weeks old) were purchased from Central Lab. Animal (Seoul, Korea). The animal breeding environment was adjusted to a dark cycle of 12 h light / 12 h dark at a temperature of 23±1° C and a humidity of 40-60%. After a one-week adaptation period, the mice were randomly divided into the following 5 groups (n = 8); LFD (Low fat diet; 10% fat of total calories), HFD (High fat diet; 60% fat of total calories) or HFD supplemented with FG and PM (FG+PM) were divided into HFD-L (0.25% FG+2.5×10^8 CFU/mL PM), HFD-M (0.5% FG+5×10^8 CFU/mL PM), and HFD-H (1.0% FG+1×10^9 CFU/mL PM).

The mice were fed the experimental diets for 9 weeks was anesthetized with Zoletil (Virbac Lab., Carros, France) and Rompun (Bayer, Leverkusen, Germany) after fasting for 12 h. Cardiac blood, liver tissue, epididymal fat, and mesenteric fat were removed from the specimens and weighed. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC; SNU-161108-7) (Seoul National University, Korea).

2.4. Histological analysis

Morphological analysis of liver and epididymal fat was performed to measure the hepatic fat accumulation and adipocyte size in the adipose tissue (AT). The liver and epididymal fat were fixed in 10% formalin (Wako Pure Chemical Industries, Japan) for one day and stored in paraffin. The hematoxylin and eosin were stained and examined with an optical microscope.

2.5. Biochemical analysis

The plasma triacylglycerol (TAG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were analyzed using a kit purchased from Asanpharm (Seoul, Korea). The low-density lipoprotein cholesterol (LDL-C) was calculated by subtracting HDL-C from TC. The fasting blood glucose (FBG) was measured after 12 h fasting in the 9th week. The plasma insulin was analyzed using a mouse insulin ELISA kit (AKRIN-011T; Shibayagi, Shibukawa, Japan).

2.6. Hepatic lipid analysis

According to Lee [5], we used a modified Folch method [11] for the liver lipid analysis. The liver lipid was measured using a TAG test kit (Asanpharm).

2.7. RNA extraction and quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR)

The total RNAs of the liver and AT were extracted using a TaKaRa MiniBEST Universal RNA Extraction kit (TaKaRa Bio, Kusatsu, Japan) and RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) respectively. The RNA was reverse transcribed to cDNA using a cDNA kit from TaKaRa Bio. An mRNA expression analysis of the cDNA and SYBR premix Ex Taq (TaKaRa Bio) was performed with a StepOne real-time PCR system. The analysis conditions were denaturation at 95° C for 30 seconds, followed by 40 cycles of 95° C for 5 seconds and 60° C for 33 seconds. The data was nomalized for the housekeeping gene GAPDH and compared to the expression levels of the HFD group. Primer sequences used in this study are shown in Table 1.

Table 1. Primer sequences for genes used in real-time PCR

Gene	Forward	Reverse
Gapdh	AGG TCG GTG TGA ACG GAT TTG	TGT AGA CCA TGT AGT TGA GGT CA
Pparr	CCA GAG CAT GGT GCC TTC GCT	CAG CAA CCA TTG GGT CAG CTC
Acc	ATG GGC GGA ATG GTC TCT TTC	TGG GGA CCT TGT CTT CAT CAT
Cpt1	GAT GTT CTT CGT CTG GCT TGA	CTT ATC GTG GTG GTG GGT GT
Ldlr	CCA CTT CCG CTG CAA ATC AT	TCA TGG GAG CCG TCA ACA C
G6p	ACC CTG GTA GCC CTG TCT TT	GGG CTT TCT CTT CTG TGT CG
Gk	CAG GAC AGT GGA GCG TGA AGA C	TTA CAG GGA AGG AGA AGG TGA AGC
Glut2	GGC TAA TTT CAG GAC TGG TT	TTT CTT TGC CCT GAC TTC CT
Tnfa	TCT TCT CAT TCC TGC TTG TGG	GGT CTG GGG CAT AGA ACT GA
Bip	TTC AGC CAA TTA TCA GCA AAC TCT	TTT TCT GAT GTA TCC TCT TCA CCA GT
Chop	CCA CCA CAC CTG AAA GCA GAA	AGG TGA AAG GCA GGG ACT CA
Lpl	ATC CAT GGA TGG ACG GTA ACG	CTG GAT CCC AAT ACT TCG ACC A
Cd36	ACT TGG GAT TGG AGT GGT GAT GT	AGA TGT AGC CAG TGT ATA TGT AGG CTC
Dgat1	CAG AGC TTC TGC AGT TTG GA	CAC AGC TGC ATT GCC ATA GT
Dgat2	CTT CCT GGT GCT AGG AGT GG	GCC AGC CAG GTG AAG TAG AG
Fas	CTT CGC CAA CTC TAC CAT GG	TTC CAC ACC CAT GAG CGA GT

2.8. TC in feces

The collected feces was ground into a powder and the subsequent process was the same as the hepatic lipid analysis. The TC in feces was measured using a TC test kit (Asanpharm).

2.9. Statistical analysis

The results were analyzed using the SPSS statistical package (Chicago, IL, USA) ANOVA test. The statistical differences were examined via Duncan's multiple range tests. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Effect of FG+PM on body weight and food intake

The main ginsenosides of the experimental FG were protopanaxadiol-type Rd (19.1%), Rc (4.5%), and protopanaxatriol-type Re (3.3%). The others were Rb1 (1.4%), Rb2 (0.7%), Rg1 (0.9%), Rg2 (0.7%), and Rg3 (1.0%). (Fig. 1) Table 2 shows the changes in mice body weight and food consumption. The mice' s weight at the 9th week was significantly increased in the HFD group compared to the LFD group. Compared to the control group, the HFD-M group showed a significant decrease in body weight and weight gain.

The HFD group had a significant increased food intake compared to the LFD group, and the experimental groups consumed more calories than that of the control. The food efficiency of the HFD-M group was significantly lower than that of the HFD group.

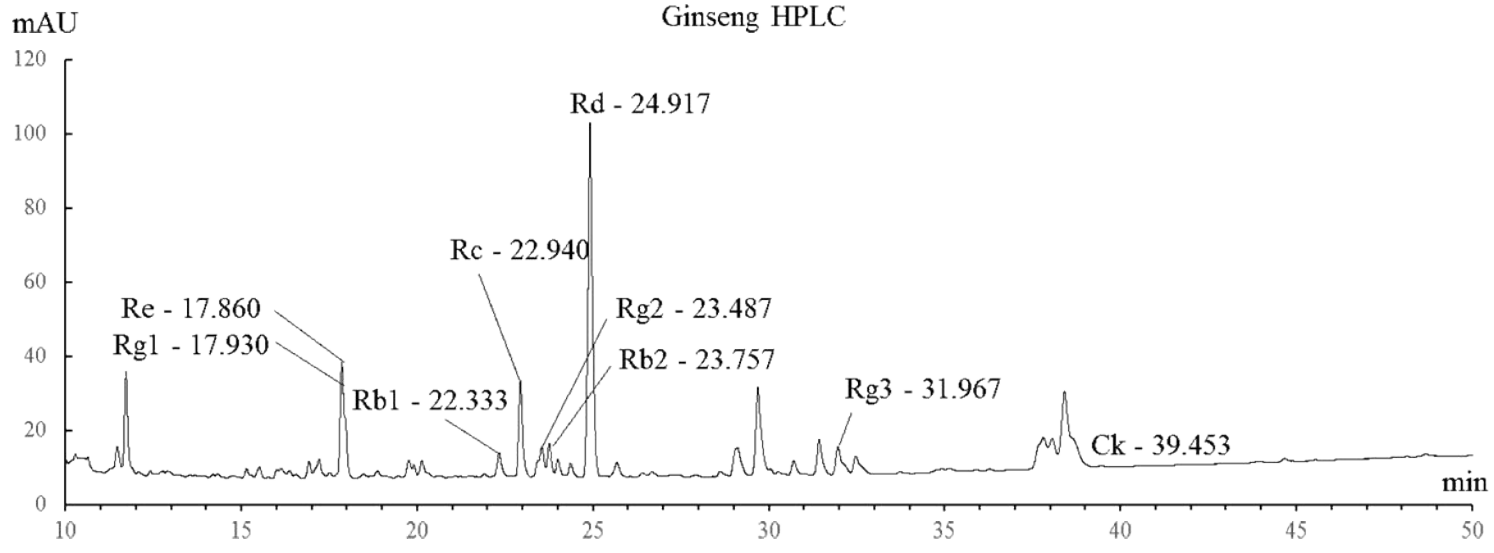


Figure 1. Ginsenoside contents of FG by using HPLC

Table 2. Effects of FG+PM on weight parameters of mice.

	LFD	HFD	HFD-L	HFD-M	HFD-H
initial BW (g)	38.6	38.91.4	37.40.7	38.21.9	37.61.9
final BW (g)	52.15.3^{a}	67.26.2^{c}	65.18.9^{bc}	58.08.6^{ab}	65.07.1^{bc}
BW gain (g)	13.54.4^{a}	28.45.6^{b}	27.88.5^{b}	19.88.1^{a}	27.47.2^{b}
Food intake (kcal)	3589.029.3^{a}	4641.01150.5^{b}	5671.2179.5^{c}	6021.8493.3^{c}	5879.91260.2^{c}
Food efficiency[1]	0.00380.001^{a}	0.00640.002^{b}	0.00490.002^{ab}	0.00340.002^{a}	0.00480.002^{ab}

[1]Food efficiency = food intake(kcal)/BW gain(g).

^{abcd}Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$ ($n = 8$).

3.2. Effect of FG+PM on the weights of the liver, EAT, and MAT

Table 3 shows the liver and the epididymal and mesenteric AT (EAT and MAT) weights. The liver weight was significantly decreased in the HFD-M group compared to the control group. The EAT and MAT weights were significantly increased in the HFD group compared to the LFD group.

Table 3. Effects of FG+PM on organs' weight of mice.

	LFD	HFD	HFD-L	HFD-M	HFD-H
Liver	1.90.24[a]	2.90.77[b]	2.40.66[ab]	2.20.75[a]	2.50.44[ab]
EAT[1]	2.00.65[a]	3.10.60[bc]	3.30.90[c]	2.50.88[ab]	3.00.52[bc]
MAT[2]	0.750.26[a]	1.590.38[b]	1.340.68[b]	1.10.45[ab]	1.60.48[b]

[1]EAT; Epididymal adipose tissue.

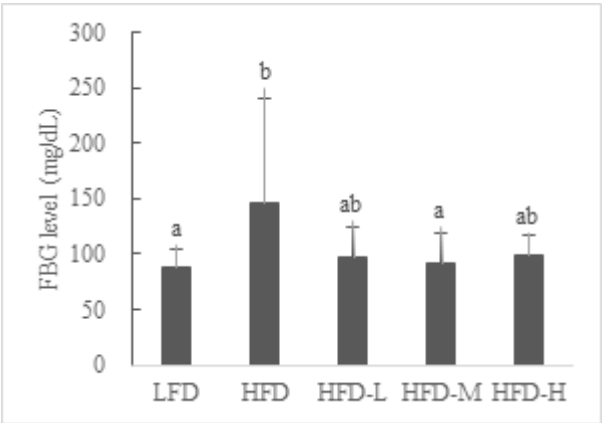
[2]MAT; Mesenteric adipose tissue.

[abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$ ($n = 8$).

3.3. Effect of FG+PM on the fasting blood glucose and insulin levels

The HFD group had a higher FBG level than that of the LFD group. The FBG levels of the experimental groups were lower than the HFD group, and the HFD-M group was significantly lower (Fig. 2A). Although the HFD-M group had the lowest insulin level; however, there was no significant difference (Fig. 2B).

(A)



(B)

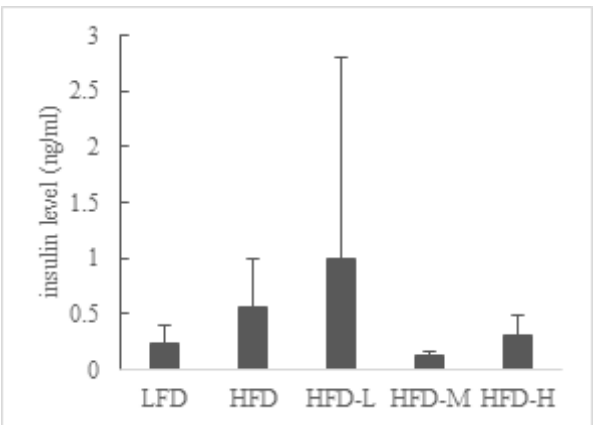


Figure 2. Effects of FG+PM on FBG.

(A) plasma FBG levels (n = 8). (B) plasma insulin levels (n = 7). [abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

3.4. Effect of FG+PM on the plasma lipid profiles

As shown in Table 4, the mice fed the FG+PM had similar plasma TAG levels. Although the plasma TC and LDL-C levels of the HFD-L group were significantly lower than those of the HFD group, the others were significantly higher. The plasma HDL-C level of the experiment groups were lower than that of the HFD group; however, there was no significant difference.

Table 4. Effects on FG+PM on plasma lipid profile.

	LFD	HFD	HFD-L	HFD-M	HFD-H
TAG (mg/dL)	27.39.7	19.49.8	24.510.8	20.24.3	22.28.5
TC (mg/dL)	125.59.4^{c}	109.29.3^{b}	91.211.4^{a}	138.318.7^{d}	123.56.3^{c}
LDL-C (mg/dL)	75.67.3^{c}	62.66.6^{b}	51.07.1^{a}	98.413.6^{d}	82.59.0^{c}
HDL-C (mg/dL)	49.84.1^{b}	46.58.3^{ab}	40.26.3^{a}	39.912.8^{a}	41.09.3^{ab}

^{abcd}Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$. ($n = 8$)

3.5. Effect of FG+PM on the histology of the liver and AT

The lipid accumulations in the liver and AT were determined by H & E staining. The HFD group had higher lipids than the LFD group, and the lowest lipd accumulation of the experimental groups was in the HFD-M group (Fig. 3A). The adipocyte sizes in the EAT were confirmed. (Fig. 3B) The HFD group had a significantly larger adipocyte size than that of the LFD group, and the adipocyte size in the HFD-M and HFD-H groups were significantly smaller than that of the HFD group. (Fig. 4)

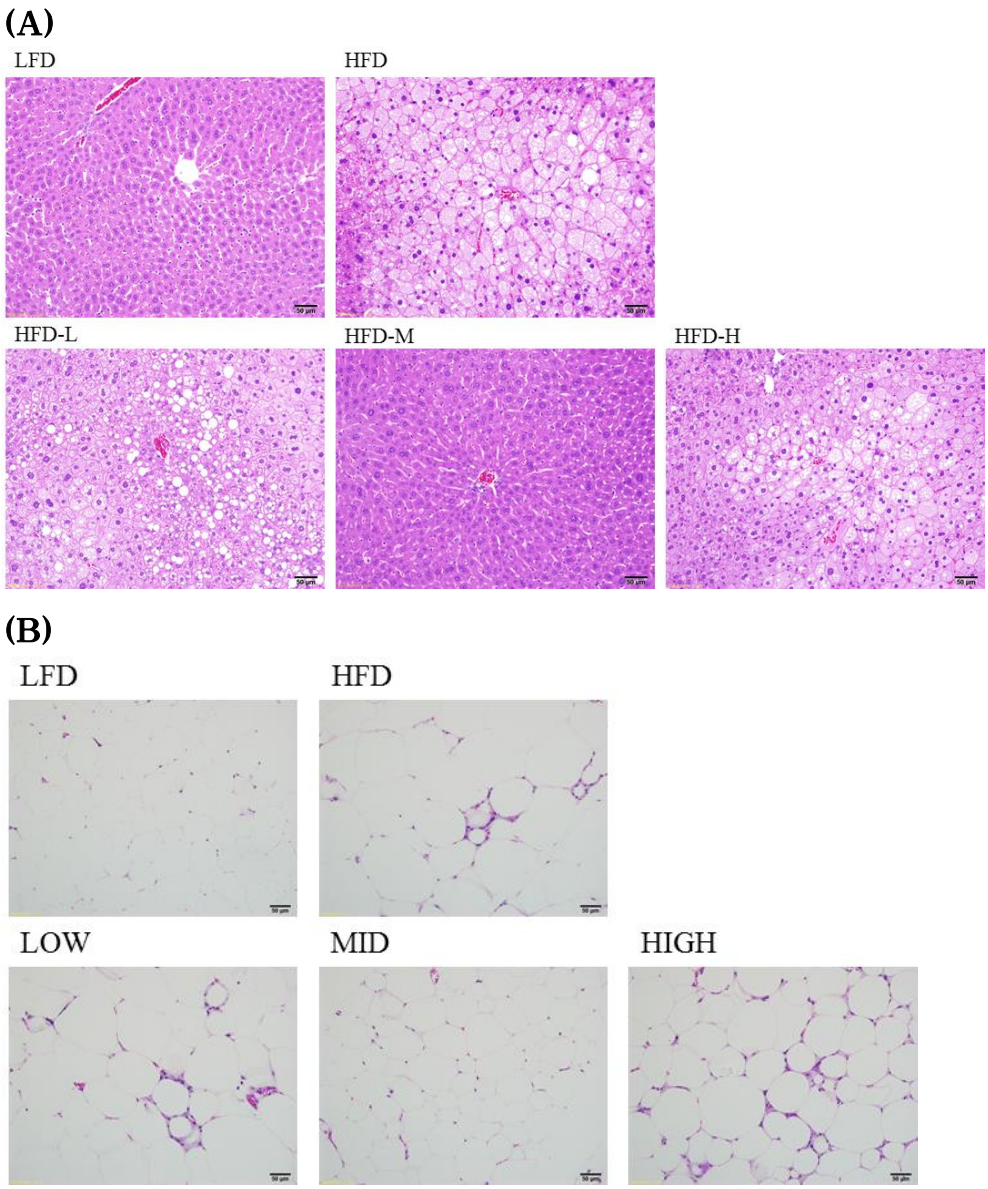


Figure 3. Effects of FG+PM on the histology of liver and adipose tissue.

(A) H&E staining of liver. (B) H&E staining of adipose tissue. [abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

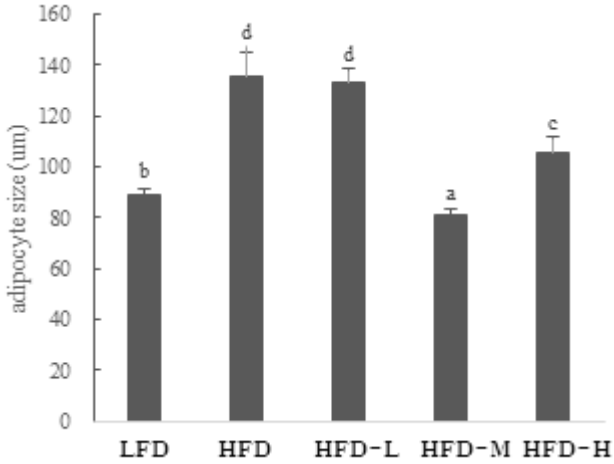


Figure 4. Effects of FG+PM on the adipocyte size of adipose tissue.

[abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

3.6. Effect of FG+PM on the hepatic lipid profiles

The TAG content was lower in the experimental group than the HFD group and the lowest in the HFD-M group. (Fig. 5)

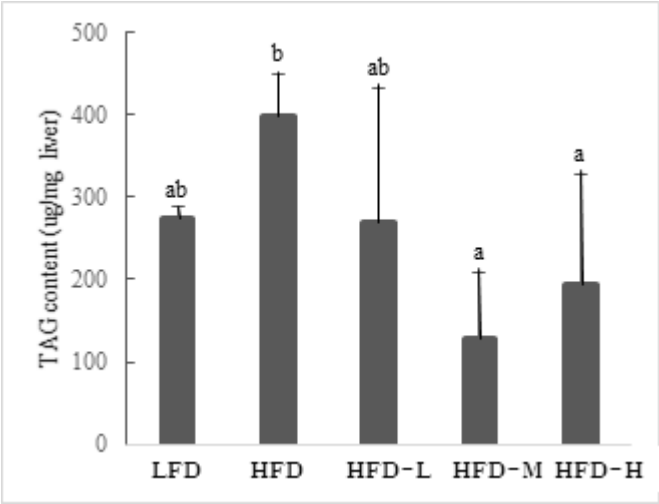
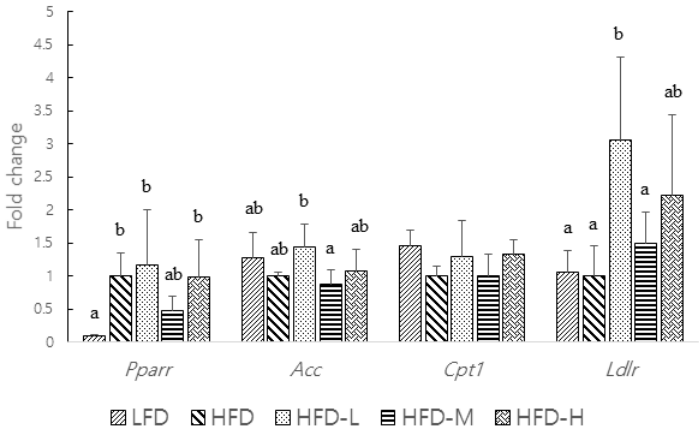


Figure 5. Effects of FG+PM on TAG content in the liver (*n* = 4).
[abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

3.7. Effect of FG+PM on the mRNA expression in liver tissue

Fig. 6A and 6B show the mRNA expression of the lipids and glucose metabolism in the liver. The expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) was significantly higher in the HFD group than the LFD group. The expression of LDL receptor (LDLR) was significantly higher in the HFD-L group compared to the control group. The HFD group had a significantly higher expression of glucokinase (GK) than that of the LFD group.

(A)



(B)

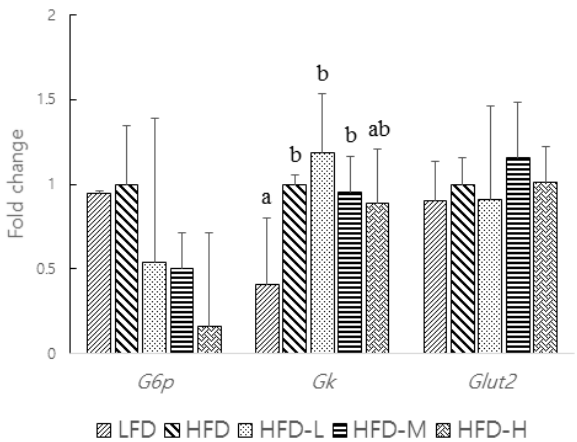


Figure 6. Effects of FG+PM on mRNA expression in liver.

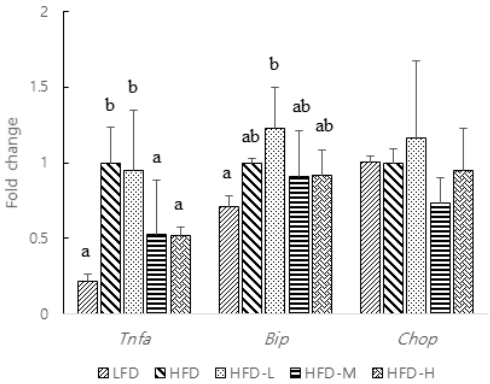
(A) Effects on mRNA expression related to lipid metabolism (n = 4). (B) Effects on mRNA expression related to glucose metabolism (n = 4). ^{abcd}Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

3.8. Effect of FG+PM on the mRNA expression in AT

Fig. 7A shows the mRNA expression of AT inflammation. The expression of tumor necrosis factor (TNF)-α was significantly higher in the HFD group compared to the LFD group and significantly lower in the HFD-M and HFD-H groups compared to the control group.

Fig. 7B shows the mRNA expression of AT lipid metabolism. The expression of PPAR-γ was increased in the HFD-M group compared to the control; however, there was no significant difference. The expression of lipoprotein lipase (LPL) in the HFD-M group was higher compared to the control group but not significant.

(A)



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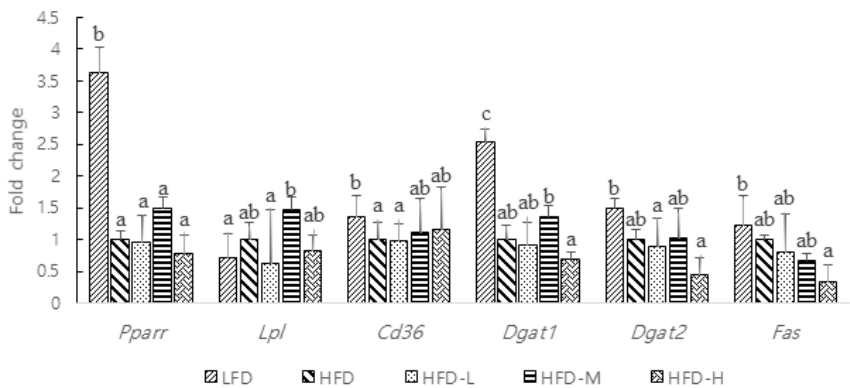

Figure 7. Effects on FG+PM on mRNA expression in adipose tissue.

(A) Effects on mRNA expression related to inflammation (n = 4). (B) Effects on mRNA expression related to lipid metabolism (n = 4). [abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$.

3.9. Effect of FG+PM on the cholesterol in feces

The cholesterol content in the feces was higher in all the FG+PM groups compared to the control group, and the HFD-L and HFD-H groups had a significantly higher cholesterol feces content (Fig. 8).

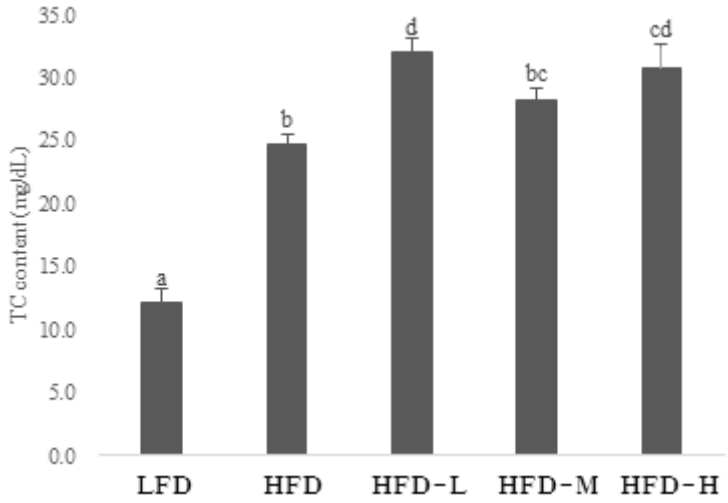

Figure 8. Effect of FG+PM on TC content in feces.

[abcd]Means in the same row not sharing a common letter are significantly different groups at $p < 0.05$. ($n = 8$).

4. Discussion

It has been reported that a HFD induces obesity and impairs leptin and insulin signaling in the hypothalamus, which results in excessive weight gain [12]. Liu *et al.* [13] reported that the ginsenosides Rd, Rb1, Rb2, and Rc are pancreatic lipase inhibitors that prevented obesity by increasing fat excretion. Similarly, probiotics have reduced fat accumulation and weight gain by competing with other probiotic strains in the intestines for nutrients and by reducing the gut microbial diversity [14]. The results of our study are similar to those found in earlier experimental studies. The intake of FG+PM reduced weight gain, especially in the HFD-M group, and significantly suppressed weight gain despite increased food intake.

Obesity is associated with dyslipidemia, which increases TAG and free fatty acids (FFA) and decreases HDL-C. In previous studies, ginseng [15] and probiotics [16] have been reported to improve the lipid profile by inhibiting hyperlipidemia. In the present study, there was no significant difference between TAG and HDL-C. In contrast, the TC was decreased in the HFD groups and increased in the FG+PM groups, with the exception of the HFD-L group. The biosynthesis of cholesterol is regulated

by HMG-CoA reductase (HMGCR). Therefore, most cholesterol in a HFD inhibits HMGCR feed-back and prevents cholesterol biosynthesis. In the present study, FG+PM seemed promoted cholesterol biosynthesis through the positive feed-back of HMGCR. In fact, previous studies have shown that ginsenoside Rb1 improved cholesterol biosynthesis [17]. While it stimulated cholesterol biosynthesis, FG+PM also promoted the excretion of cholesterol into the feces (Fig. 8). Cholesterol can be converted into coprostanol by probiotics and then excreted directly into the feces, which decreases the amount of absorbed cholesterol [18]. However, both the control and the experimental group in this study were within the normal cholesterol range; thus, it was difficult to state that dyslipidemia actually occurred.

Obesity causes hyperglycemia by counteracting FBG homeostasis. Obesity is associated with insulin resistance, and elevated FBG changes lipogenesis and lipolysis. According to the results of this study, while the FBG level was increased in the HFD group, the FG+PM level was lower and effectively inhibited in the HFD-M group. This is consistent with the results that ginseng [15] and probiotics [19] decreased blood sugar. In fact, ginsenoside Rd has been shown to enhance glucose utilization through the PI-3 kinase-dependent pathway [20], and both

ginsenosides Re and Rc have been reported to significantly increase glucose uptake in cells through AMPK activation [21].

Because the liver is one of the major organs responsible for lipid metabolism, damage to the lipid metabolism of the liver leads to an abnormal accumulation of lipids, which is called hepatic steatosis. HFD-induced obesity increases FFA uptake into the liver, which often leads to hepatic fat accumulation resulting in non-alcoholic fatty liver disease (NAFLD) [22]. Ginseng and probiotics are well known as NAFLD ameliorating agents. The results of this study show that the liver weight and hepatic TAG content were effectively suppressed in the FG+PM groups, especially in the HFD-M group. Moreover, the smallest lipid droplet size was observed in the HFD-M group (Fig. 3A).

Excessive energy intake results in the fat storage processes of hypertrophy and hyperplasia, which is controlled by PPAR-γ [23]. The expression of PPAR-γ was decreased by 50% in the HFD-M group compared to the HFD group (p = 0.15). The results of the present study are similar to those found in the earlier experimental studies on ginseng [24] and probiotics [25].

LDLR in the liver is known to uptake LDL-C in the blood, which is involved in blood lipid clearance. LDLR expression was up-regulated in the FG+PM groups and consistent with the decreased plasma LDL-C level in the HFD-L group. The results of our study concur with those found in a previous study [26]. Thus, FG+PM intake may have a role in decreasing the LDL-C concentration in the blood.

NAFLD is strongly associated with hepatic insulin resistance, Type 2 diabetes, and obesity. Although insulin is a major regulator of hepatic lipogenesis, glucose is also known to contribute to the coordinated regulation of carbohydrate and fat metabolism in the liver [27]. The expression of G6P, which is involved in gluconeogenesis, was lower in the experimental groups. Compared to the control group, the expression of G6P in the FG+PM groups was lower, especially in the HFD-H group by 90% (p = 0.1). In contrast, the expression of GK, a glycolytic enzyme, was significantly elevated in the HFD group compared to the LFD group, which has the potential to promote the long-term storage of carbohydrates and TAG.

Taken together, this FG+PM combination effectively inhibited hepatic weight, TAG content, and liver fat accumulation, especially in the HFD-M group.

Obesity is associated with hypertrophy in AT, which can cause adipocytes to move away from blood capillaries and result in adipocyte hypoxia [28]. Dead adipocytes form CLS, and most macrophages that infiltrate the AT are found around the CLS [29]. AT macrophages promote insulin resistance and AT inflammation [30]. As found in previous studies [31, 32], FG+PM significantly suppressed the adipocyte size. In addition, FG+PM significantly inhibited the expression of TNF-α in the FG+PM groups (Fig. 5A), suggesting that FG+PM can inhibit hypertrophy by preventing the excessive formation of CLS in AT. In fact, Kim *et al.* [33] reported that ginsenoside Re inhibited the expression of TNF-α in 3T3-L1.

PPAR-γ is involved in the regulation of genes involved in lipid accumulation and insulin sensitivity [34]. PPAR-γ is also used as a major target in the treatment of insulin resistance with thiazolidinediones (TZDs), PPAR-γ activators. TZDs increase lipid accumulation in AT and decrease the hepatic and muscular fat, ultimately inhibiting the fatty liver [35]. We observed that the HFD-M group had an increased expression of PPAR-γ compared to the control group (p = 0.14), which shows that FG+PM may have a role similarly to TZDs. PPAR-γ activation is associated with the expression of LPL, and fatty acid synthetase (FAS). LPL carries fat and degrades

lipoprotein, which was approximately 50% higher in the HFD-M group compared to the control group (p = 0.15). The upregulation of LPL lowered FFA in the circulation and liver by increasing the fat inflow into AT, which decreased the hepatic lipotoxicity and improved insulin sensitivity [36]. The results of our study correspond well with those found in earlier studies showing that ginsenoside Re, Rg1, and Rb2 increase LPL expression [37], and ginsenoside Rd and Rb1 promoted adipose differentiation and lipid accumulation in 3T3-L1 [38]. FAS expression was suppressed by the consumption of FG+PM, especially in the HFD-H group compared to the control group (p = 0.05). Therefore, FG+PM can improve insulin sensitivity through PPAR-γ activation and inhibit fatty liver development. Thus, FG+PM exhibited anti-obesity effects.

In summary, the combination of fermented ginseng, B. *longum* BORI and L. *paracasei* CH88 efficiently suppressed weight gain, hepatic and adipogenic fat accumulation, and TNF-α secretion in AT. This combination also improved the FBG level and increased cholesterol excretion into the feces. It is suggested that the FG+PM mixture could have an anti-obesity effects in high-fat diet-fed mice.

References

1. Jung S, Lee M-S, Shin Y, Kim C-T, Kim I-H, Kim YS, *et al*. 2014. Anti-obesity and anti-inflammatory effects of high hydrostatic pressure extracts of ginseng in high-fat diet induced obese rats. *J. Funct. Foods* **10**: 169-177.
2. Ku S, Park MS, Ji G, You H. 2016. Review on *Bifidobacterium bifidum* BGN4: functionality and nutraceutical applications as a probiotic microorganism. *Int. J. Mol. Sci.* **17**: E1544.
3. Shen J, Obin MS, Zhao L. 2012. The gut microbiota, obesity and insulin resistance. *Mol. Aspects Med.* **34**: 39-58.
4. Park MS, Kwon B, Ku S, Ji GE. 2017. The efficacy of *Bifidobacterium longum* BORI and *Lactobacillus acidophilus* ad031 probiotic treatment in infants with rotavirus infection. *Nutrients* **9**: E887.
5. Li Z, Jin H, Oh SY, Ji GE. 2016. Anti-obese effects of two lactobacilli and two bifidobacteria on ICR mice fed on a high fat diet. *Biochem. Biophys. Res. Commun.* **480**: 222-227.
6. Ku S. 2016. Finding and producing probiotic glycosylases for the biocatalysis of ginsenosides: a mini review. *Molecules*. **21**: 645.

7. Ku S, You H, Park MS, Ji G. 2016. Whole-cell biocatalysis for producing ginsenoside Rd from Rb1 using *Lactobacillus rhamnosus* GG. *J. Microbiol. Biotechnol.* **26:** 1206-1215.
8. Ku S, You H, Park M, Ji G. 2015. Effects of ascorbic acid on α-l-arabinofuranosidase and α-l-arabinopyranosidase activities from *Bifidobacterium longum* RD47 and its application to whole cell bioconversion of ginsenoside. *J. Korean Soc. Appl. Biol. Chem.* **58:** 857-865.
9. Karikura M, Miyase T, Tanizawa H, Takino Y, Taniyama T, Hayashi T. 1990. Studies on absorption, distribution, excretion and metabolism of ginseng saponins. V. The decomposition products of ginsenoside Rb2 in the large intestine of rats. *Chem. Pharm. Bull.* **38:** 2859-2861.
10. Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S. 1999. Probiotics: mechanisms and established effects. *Int. Dairy J.* **9:** 43-52.
11. Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497-509.
12. Thaler JP, Schwartz MW. 2010. Minireview: inflammation and obesity pathogenesis: the

hypothalamus heats up. *J. Clin. Endocrinol. Metab.* **95:** 4109-4115.

13. Liu W, Zheng Y, Han L, Wang H, Saito M, Ling M, *et al.* 2008. Saponins (ginsenosides) from stems and leaves of *Panax quinquefolium* prevented high-fat diet-induced obesity in mice. *Phytomedicine.* **15:** 1140-1145.

14. Yoo SR, Kim YJ, Park DY, Jung UJ, Jeon SM, Ahn YT, *et al.* 2013. Probiotics *L. plantarum* and *L. curvatus* in combination alter hepatic lipid metabolism and suppress diet-induced obesity. *Obesity.* **21:** 2571-2578.

15. Yun S, Moon S, Ko S, Im B, Chung S. 2004. Wild ginseng prevents the onset of high-fat diet induced hyperglycemia and obesity in ICR mice. *Arch. Pharm. Res.* **27:** 790-796.

16. Zhang X-L, Wu Y-F, Wang Y-S, Wnag X-Z, Piao C-H, Liu J-M, *et al.* 2017. The protective effects of probiotic-fermented soymilk on high-fat diet-induced hyperlipidemia and liver injury. *J. Funct. Foods.* **30:** 220-227.

17. Sakakibara K, Shibata Y, Higashi T, Sanada S, Shoji J. 1975. Effect of ginseng saponins on cholesterol metabolism. I. The level and the synthesis of serum

and liver cholesterol in rats treated with ginsenosides. *Chem. Pharm. Bull.* **23:** 1009-1016.

18. Lye HS, Rusul G, Liong MT. 2010. Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. *J. Dairy Sci.* **93:** 1383-1392.
19. Andersson U, Bränning C, Ahrné S, Molin G, Alenfall J, Önning G, *et al.* 2010. Probiotics lower plasma glucose in the high-fat fed C57BL/6J mouse. *Benef. Microbes.* **1:** 189-196.
20. Park S, Ahn IS, Kwon DY, Ko BS, WK Jun. 2008. Ginsenosides Rb1 and Rg1 suppress triglyceride accumulation in 3T3-L1 adipocytes and enhance β-cell insulin secretion and viability in Min6 cells via PKA-dependent pathways. *Biosci. Biotech. Biochem.* **72:** 2815-2823.
21. Hwang JT, Lee M, Kim M, Kwon DY. 2008. Biological active components found in *Panax ginseng* improve glucose uptake via AMPK signaling pathway. *FASEB J.* **22:** 683.
22. Dowman JK, Tomlinson JW, Newsome PN. 2010. Pathogenesis of non-alcoholic fatty liver disease. *QJM.* **103:** 71-83.
23. Zhang L, Virgous C, Si H. 2017. Ginseng and obesity: observations and understanding in cultured

cells, animals and humans. *J. Nutr. Biochem.* **44:** 1-10.

24. Zhang Y, Yu L, Cai W, Fan S, Feng L, Ji G, Huang C. 2014. Protopanaxatriol, a novel PPARγ antagonist from *Panax ginseng*, alleviates steatosis in mice. *Sci. Rep.* **4:** 7375.

25. Wu C-C, Weng W-L, Lai W-L, Tsai H-P, Liu W-H, Lee M-H, *et al.* 2015. Effect of strain K21 on high-fat diet-fed obese mice. *Evid. Based Complement. Alternat. Med.* **2015:** 391767.

26. Park Y-H, Kim J-G, Shin Y-W, Kim S-H, Whang K-Y. 2007. Effect of dietary inclusion of *Lactobacillus acidophilus* ATCC 43121 on cholesterol metabolism in rats. *J. Microbiol. Biotechnol.* **17:** 655-662.

27. Dentin R, Girard J, Postic C. 2005. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. *Biochimie.* **87:** 81-86.

28. Crandall DL, Goldstein BM, Huggins F, Cervoni P. 1984. Adipocyte blood flow: influence of age, anatomic location, and dietary manipulation. *Am. J. Physiol.* **247:** R46-R51.

29. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, Cinti M. 2008. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J. Lipid Res.* **49:** 1562-1568.

30. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, Defuria J, Jick Z, *et al.* 2007. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes*. **56:** 2910-2918.

31. Lee H, Park D, Yoon M. 2013. Korean red ginseng (*Panax ginseng*) prevents obesity by inhibiting angiogenesis in high fat diet-induced obese C57BL/6J mice. *Food Chem. Toxicol.* **53:** 402-408.

32. Takemura N, Okubo T, Sonoyama K. 2010. *Lactobacillus plantarum* strain no. 14 reduces adipocyte size in mice fed high-fat diet. *Exp. Biol. Med.* **235:** 849-856.

33. K SO. 2007. Ginseng saponin-Re and *Coix lachrymajobi* var. *mayuen* regulate obesity related genes expressions, TNF-alpha, leptin, lipoprotein lipase and resistin in 3T3-L1 adipocytes. *J. Life Sci.* **17:** 1523-1532.

34. Oh J, Lee H, Park D, Ahn J, Shin SS, Yoon M. 2012. Ginseng and its active components ginsenosides inhibit adipogenesis in 3T3-L1 cells by

regulating MMP-2 and MMP-9. *Evid. Based Complement. Alternat. Med.* **2012:** 265023.

35. Ciaraldi TP, Kong AP, Chu NV, Kim DD, Baxi S, Loviscach M, *et al.* 2002. Regulation of glucose transport and insulin signaling by troglitazone or metformin in adipose tissue of type 2 diabetic subjects. *Diabetes.* **51:** 30-36.
36. Choi S-S, Park J, Choi JH. 2014. Revisiting PPARγ as a target for the treatment of metabolic disorders. *BMB Rep.* **47:** 599-608.
37. Masuno H, Kitao H, Okuda H. 1996. Ginsenosides increase secretion of lipoprotein lipase by 3T3-L1 adipocytes. *Biosci. Biotechnol. Biochem.* **60:** 1962-1965.
38. Sekiya K, Okuda H, Hotta Y, Arichi S. 1987. Enhancement of adipose differentiation of mouse 3T3-L1 fibroblasts by ginsenosides. *Phytother. Res.* **1:** 58-60.

국문초록

인삼과 프로바이오틱스는 고지방식을 섭취하는 쥐에서 항비만 효과를 보여준다. 진세노사이드 흡수와 프로바이오틱스 정착은 모두 장 내에서 일어난다는 밀접한 연관성을 지니지만 인삼과 프로바이오틱스를 함께 섭취하였을 때의 항비만 기작을 밝힌 논문은 조사된 바 없다. 이에 본 연구는 고지방식을 섭취한 쥐에게 발효 인삼, *Bifidobacterium longum* BORI, *Lactobacillus paracasei* CH88로 이루어진 혼합물을 투여하여 나타나는 항비만 효과를 확인하였다. 실험 결과, 이 혼합물은 마우스에서 체중 증가 억제 ($p < 0.05$, $n = 8$) 및 간과 지방 조직에서의 지방 축적 억제 효과를 나타내었으며, 식이 섭취량이 증가 되었다. 혼합물은 0.5% 발효 인삼 + 5×10^8 CFU/mL 유산균 농도에서 지방세포의 크기가 유의적으로 감소하였으며 ($p < 0.05$, $n = 10$), 지방 조직의 TNF-α 발현이 유의적으로 억제되었다. ($p < 0.05$, $n = 4$) 이 혼합물 섭취는 마우스의 혈당조절을 개선하였고 ($p < 0.05$, $n = 8$), 변으로 배출되는 콜레스테롤을 증가시켰다. ($p < 0.05$, $n = 8$) 따라서 발효 인삼, *B. longum* BORI, *L. paracasei* CH88의 혼합물은 항비만 효능에 기여하며, 이때 식이의 0.5% 발효 인삼 + 5×10^8 CFU/mL 유산균 혼합물을 섭취한 HFD-M 군에서 가장 효과적으로 나타났다.

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주요어: 인삼, 락토박테리아, 비피도박테리아, 항비만, 지질대사

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