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A Vinegar-processed Ginseng Radix (*Ginsam*) Ameliorates Hyperglycemia and Dyslipidemia in C57BL/KsJ *db/db* Mice

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Abstract Having idea to develop more effective anti-diabetic agent from ginseng root, we comprehensively assessed the anti-diabetic activity and mechanisms of *ginsam* in C57BL/KsJ *db/db* mice. The *db/db* mice were divided into 4 groups; diabetic control (DC), *ginsam* at a dose of 300 or 500 mg/kg (GS300 or GS500) and metformin at a dose of 300 mg/kg (MT300). *Ginsam* was orally administered for 8 weeks. GS500 reduced the blood glucose concentration and significantly decreased an insulin resistance index. In addition, GS500 reduced the plasma non-esterified fatty acid, triglyceride, and increased high density lipoprotein-cholesterol as well as decreased the hepatic cholesterol and triglyceride. More interestingly, *ginsam* increased the plasma adiponectin level by 17% compared to diabetic control group. Microarray, quantitative-polymerase chain reaction and enzyme activity results showed that gene and protein expressions associated with glycolysis, gluconeogenesis, and fatty acid oxidation were changed to the way of reducing hepatic glucose production, insulin resistance and enhancing fatty acid β-oxidation. *Ginsam* also increased the phosphorylation of AMP-activated protein kinase and glucose transporter expressions in the liver and skeletal muscle, respectively. These changes in gene expression were considered to be the mechanism by which the *ginsam* exerted the anti-diabetic and anti-dyslipidemic activities in C57BL/KsJ *db/db* mice.

Keywords: *ginsam*, diabetes, *db/db* mouse, adiponectin, AMP-activated protein kinase, glucose transporter, fatty acid oxidation

Introduction

Diabetes mellitus (DM) is one of the main causes of serious diseases in the 21st century. The number of diabetic patients anticipate rising from current estimate to 300 million in 2025 (1). Lifestyle patterns in industrialized societies comprise an increasing ingestion of high-caloric food and decreasing exercise. These factors are emerging as the fundamental causes of this fast-spread 'epidemic' (2). DM is divided into 2 main forms. Type 1 DM (T1DM) is due to an autoimmune-mediated destruction of the insulin-producing pancreatic β-cell islets. On the other hand, type 2 DM (T2DM) is a metabolic disease characterized by the impairment of insulin secretion from the pancreatic β-cell and decreased insulin action in peripheral tissues such as fat, liver, and skeletal muscle tissues (3).

At present, therapy for T2DM relies mainly on several approaches intended to decrease the hyperglycemia itself: sulfonylureas (and related insulin secretagogues) increase insulin secretion from pancreatic islets; metformin plays an important role in the reduction of hepatic glucose production; peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (thiazolidinediones, TZD) enhance an insulin sensitivity in the adipose tissue; acarbose (α-glucosidase inhibitor) interferes with gut glucose absorption; and insulin itself suppresses glucose production and augments glucose uptake into the skeletal muscle and adipose tissue for utilization. Currently available therapeutic agents for T2DM, however, have a number of limitations, such as various adverse effects (hypoglycemia, lactic acidosis, and weight gain, etc) and high rates of secondary failure (4,5). Due to these factors, diabetic patients and healthcare professions are increasingly considering complimentary and alternative approaches, including the use of medicinal herbs with anti-hyperglycemic activities (6).

Ginseng is known to have many pharmacological effects on immune, cardiovascular, central nervous systems, and endocrine system (7,8). Anti-diabetic activities of ginseng extract or its active ingredients were documented in about 80 research papers since 1980 (9-13). However, how and which ingredients exert the anti-diabetic activity of ginseng is yet to be determined. Moreover, anti-diabetic efficacy of ginseng extract is not high enough to be developed as a new therapeutic agent for T2DM. Therefore, to develop anti-diabetic agent with higher efficacy, a vinegar-processed ginseng radix (*ginsam*) was developed. In the present study, we investigated its anti-diabetic activity and mechanisms using T2DM animal model C57BL/KsJ *db/db* mice.

Materials and Methods

***Ginsam* preparation** Ginseng radix of *Panax ginseng* C.A. Meyer was purchased from Geumsan on August of 2006, and botanically identified by Dr. Chang S. Yook at the Department of Oriental Pharmaceutical Science, Kyung Hee University. A voucher specimen of ginseng radix was deposited at the Medicinal Plants Herbarium of the College of Pharmacy, Kyung Hee University, with registration number 111. One kg of ginseng radix was extracted twice at 80°C in 50% ethanol for 4 hr. Vinegar-processed ginseng radix (*ginsam*, GS) sample was prepared as follows; 8 volumes of twice brewed vinegar (pH 2.3, Daesang Corporation) was added to 100 mL of ginseng radix ethanol

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extract, and then incubated at 90°C for 6 hr. Each extract was filtered, and the filtrate was concentrated with a vacuum rotary evaporator under low pressure. The residue was freeze-dried and stored in a deep freezer until use.

Animals and treatment Animal care and experimental procedures followed to the 'guide for the care and use of laboratory animals' (Department of Health, Education, and Welfare, NIH Publication #78-23, 1996). Male, C57BL/KsJ *db/db* mice (5-week-old) were purchased from Orient Bio (Seongnam, Korea). The animals were housed in individual cage with free access to water in a room with a 12/12-hr light/dark cycle (8:00 AM to 8:00 PM), a temperature of 24±1°C, and a humidity of 55±5%. During the acclimatization period, each animal was raised on a standard rodent chow (LabDiet, Orient Bio, Seongnam, Korea) *ad libitum*. The *db/db* mice were acclimatized for 1 week and randomly assigned into the 5 groups as following; diabetic control (DC), 300 mg/kg of *ginsam* (GS300), 500 mg/kg of *ginsam* (GS500), 300 mg/kg of metformin (MT300). *Ginsam* was orally administered once a day for 8 weeks. Body weights were measured weekly, and blood was collected every other week for blood glucose analysis. At the end of the study, blood was withdrawn for determinations of glycated hemoglobin, adiponectin, insulin, and lipid levels. After sacrifice, liver and femoral skeletal muscle tissues were immediately removed, instantly soaked in liquid nitrogen and stored at −70°C for morphological examination and mRNA or protein analyses.

Blood sampling and plasma assay Blood was withdrawn from the orbital venous plexus every other week, using a heparinized capillary tube without anesthesia. The blood samples were placed on ice, centrifuged, and stored at −70°C until assay. The plasma glucose concentration was determined using the glucose oxidase method (YD-Diagnostics, Yongin, Korea). Plasma insulin and adiponectin concentrations were measured according to the protocol described by the manufacturer of the mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi Co., Ltd., Gumma, Japan), and mouse adiponecitn ELISA kit (AdipoGen Inc., Seoul, Korea), respectively. Plasma triglyceride, total cholesterol, high density lipoprotein (HDL)-cholesterol and non-esterified fatty acid (NEFA) were determined using commercially available kits (Asanpharm, Seoul, Korea). Glycated hemoglobin A1c (HbA1c) was measured with a Hemoglobin A1c kit (BioSystems SA, Spain).

Oral glucose tolerance test An oral glucose tolerance test (OGTT) was performed at the end of the treatment. On the test day, animals were fasted for 12 hr, and glucose (1.5 g/kg) was then orally administered. Blood glucose concentration was determined from blood withdrawn from the orbital venous plexus at 0 (at the time of glucose challenge), 30, 60, and 120 min after glucose administration.

Determinations of hepatic triglyceride and cholesterol Liver tissue (150-200 mg) was homogenized using a polytron homogenizer (Ika, Jaya, Malaysia) in chloroform/methanol (2:1) and filtered through Whatman filter paper (0.45 μM). One mL of Milli-Q water was added to the filtrate and samples were vortexed vigorously and then centrifuged at 1,500×g at 4°C. The lower chloroform phase was transferred to a glass tube and solvent was evaporated under nitrogen. Sample remaining in the tube was suspended in 0.5% Triton X-100 by sonication. This solution was then used for measurement of triglyceride (Sigma Diagnostics, St. Louis, MO, USA) or cholesterol (Asanpharm).

Determinations of hepatic enzyme activities Enzyme activities of glucose-6-phosphatase (G6Pase), glucokinase (GK), and glucose-6-phosphate dehydrogenase (G6PD) were measured according to the literatures with slight modifications (14-16).

RNA extraction and RT (reverse transcription)-polymerase chain reaction (PCR) RNA was isolated from the liver using an Easy-Blue Total RNA extraction kit (Intron Biotechnology Inc., Seoul, Korea) (17). Total RNA (1 μg) was reverse transcribed into cDNA using the Moloney murine leukemia virus transcriptase and random hexamers (SeouLin Bioscience Co., Ltd., Seoul, Korea) as primers. Sequence of primers are following: sterol regulatory element binding protein 1 (SREBP1a and c) sense GCG CTA CCG GTC TTC TAT CA, anti-sense TGC TGC CAA AAG ACA AGG G, accession number AF374266; fatty acid synthase (FAS) sense GAT CCT GGA ACG AGA ACA C, anti-sense AGA CTG TGG AAC ACG GTG GT, accession number X13135; PPAR-α sense CCC TGA ACA TCG AGT GTC GA, anti-sense CTT GCC CAG AGA TTT GAG GTC T, accession number NM_011144; CD36 sense TCC TCT GAC ATT TGC AGG TCT ATC, anti-sense GTG AAT CCA GTT ATG GGT TCC AC, accession number L23108; acyl-CoA oxidase (ACOx) sense CAC AGC AGT GGG ATT CCA AAT, anti-sense CTT CCT TGC TCT TCC TGT GAC TC, accession number AF006688; glucose transporter 4 (GLUT4) sense GAT TCT GCT GCC CTT CTG TC, anti-sense ATT GGA CGC TCT CTC TCC AA; cyclophilin (CPN) sense ATG GTC AAC CCC ACC GTG, anti-sense TTA GAG TTG TCC ACA GTC GGA GA; β-actin sense GTC GTA CCA CTG GCA TTG TG, anti-sense GCC ATC TCC TGC TCA AAG TC. The primers were added at a final concentration of 0.5 μM to a 25 μL reaction mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM each dNTP, 5 μL of cDNA, and 2.5 units of *Taq* DNA polymerase (Takara Korea Biomedical Inc., Seoul, Korea). The PCR conditions were denaturation at 94°C for 1 min, annealing at 57.5°C for 1 min, and extension at 72°C for 1 min. The RT-PCR products were electrophoresed in 1% agarose gels under 100 V and stained with 0.5 μg/mL ethidium bromide.

Gene expression profiling For microarray analysis, RNA quality and quantity were determined using the RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA, USA). Fluorescent-labeled cRNA probe was prepared and hybridized from total RNA using amino allyl coupling reactions (AmershamPharmacia, Uppsala, Sweden). DNA chips were then scanned using the GenePix 4000B and analyzed with Genepix Pro 3.0 software (Axon Instruments Inc., Union City, CA, USA) to obtain gene expression

ratios. Transformed data were then normalized using the Lowess procedure (18).

Quantitative (Q)-PCR Q-PCR reaction was performed using 1 μg of total RNA, LightCycler FastStart DNA Master SYBR Green I kit (BMS Korea, Seoul, Korea) in the Light Cycler real-time PCR detection system (Roche, Mannheim, Germany). The primer sets were verified by RT-PCR, Cp values were normalized to a housekeeping genes, β-actin, and fold-change ratios of experimental to control were calculated using the ΔΔCp method (19).

Western blot The skeletal muscle was homogenized in lysis buffer [50 mM Tris buffer, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol] and tissue debris was removed by centrifugation at 10,000×g (5417R; Eppendorf, ON, Canada) and the supernatant was stored in −70°C until use. In the meantime, plasma membrane protein for glucose transporter 4 (GLUT4) was extracted according to the method of Mitsumoto and Klip (20) and concentration of each protein extract was determined by Bio-Rad protein assay reagent (BMS Korea) according to the manufacturer's instruction. Fifty μg of cellular or membrane protein was subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and blotted onto a nitrocellulose membrane following separation on a 10% SDS-PAGE. Blotted membranes were incubated for 1 hr with blocking solution (Tris-buffered saline/Tween 20, TBST) containing 5%(w/v) skim milk at room temperature, followed by incubation overnight at 4°C with a 1:1,000 for p-AMP-activated protein kinase (AMPK) and AMPK antibodies or 1:200 dilution for GLUT4 antibody (Cell Signaling Technology, Santa Cruz, CA, USA). Membranes were washed 4 times with TBST and incubated for 1 hr with blocking solution at room temperature, and then incubated with a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit and anti-rabbit IgG secondary antibody for 1 hr at room temperature. Membranes were washed 4 times in TBST and then developed by ECL (AmershamPharmacia).

Hepatic histology After overnight fasting, the mice were deeply anesthetized with urethane (0.9 mL/100 g BW of 20% solution) and perfused transcardially with 10% buffered formalin. The mice were killed by decapitation, and liver tissue was removed and subsequently embedded in paraffin. Paraffin sections with thickness 5 μm were prepared using a microtome Leica RM2235 (Leica Microsystems Ltd., Seoul, Korea) and these sections were mounted on slides. The tissue section was then deparaffinized and stained with hematoxylin and eosin.

Statistical analysis All data are expressed as means± standard error (SE) and comparisons of data have been done by unpaired Student's *t*-test or analysis of variance (ANOVA), as appropriate. Mean values were considered significantly different when $p<0.05$.

Results and Discussion

Body weight and metabolic parameters Body weight and metabolic parameters are shown in Table 1. There was no significant difference in body weights between DC and GS-treated groups despite of reduction of food intake in the GS groups. MT300 group, however, showed a marginal increase in body weight, compared to the DC group despite of significant reduction in food intake. Fasting plasma glucose levels in the GS-treated groups were significantly decreased by 51 and 61% in a dose dependent manner, compared to the DC group. However, plasma insulin levels in GS- and metformin-treated groups were increased. When homeostasis model assessment of insulin resistance (HOMA-IR) was determined according to the Matthews' method (21), the insulin resistance index of the GS500-treated group was significantly reduced by 32% ($p<0.001$), when compared to the DC group. HbA1c levels in whole blood were also decreased by 2.4% in the GS300 group ($p<0.05$) and 2.7% in the GS500 group ($p<0.05$) compared to that of the DC group. On the other hand, plasma adiponectin levels of the GS-treated mice were

Table 1. Metabolic parameters in GS-treated *db/db* mice

	DC	GS300	GS500	MT300
Body weight (g)	46.9±3.7[1)]	46.5±3.4	45.6±4.7	54.5±2.8
Food intake (g/n, 8 week)	817.2	712.5	664.2	381.5
Water intake (mL/n, 8 week)	694.4	654.5	666.5	653.6
Blood glucose (mM)	29.5±1.1	14.5±1.9***	11.6±0.3***	11.8±2.4***
Insulin (μU)	132.3±23.3	159.3±20.9	228.8±33.4	270.2±8.8*
HOMA-IR	173.4±6.4	102.8±13.4***	118.3±3.1***	141.4±28.9*
HbA1c (%)	8.7±1.6	6.2±0.5*	5.9±0.6*	5.4±1.9*
Adiponectin (μg/mL)	45.7±1.8	53.5±0.2*	53.3±0.8*	41.5±1.5
Plasma lipids[2)]				
NEFA (μEq/L)	1,737.7±105.6	1,563.6±56.4	1,456.5±98.5*	1,551.9±117.8
Triglyceride (mg/dL)	132.2±27.5	109.0±6.9	101.0±7.3	77.3±2.2*
Total cholesterol (mg/dL)	121.8±10.1	130.9±6.4	133.0±2.8	153.3±6.5***
HDL-cholesterol (mg/dL)	73.9±9.8	84.9±4.6	95.6±3.4**	103.4±2.6***

[1)]Values represent the mean±SE (n=5); *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to diabetic control (DC) group.
[2)]Plasma parameter was analyzed in plasma samples obtained from blood of 12 hr fasted mice. Homeostasis model assessment was used to calculate an index of insulin resistance as insulin (μU/mL)×glucose (mM)/22.5. NEFA: Non-esterified fatty acid, HDL-cholesterol: high density lipoprotein-cholesterol.

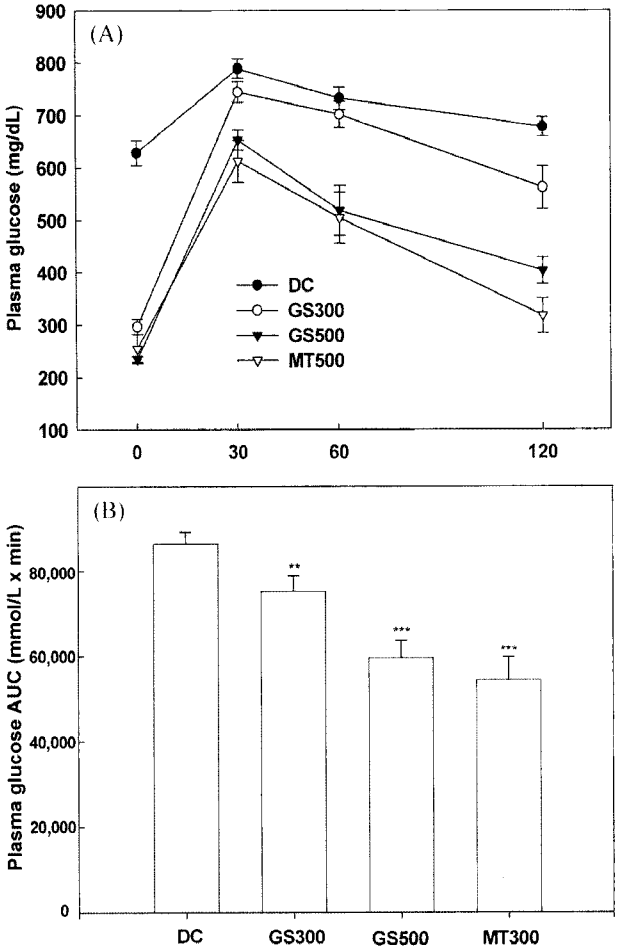

Fig. 1. Plasma glucose responses to an oral glucose challenge (1.5 g/kg) after 12 hr of food deprivation in *db/db* mice (A) and the area under the curve (B). Values are means±SE (n=5). $^{**}p<0.01$, $^{***}p<0.001$ compared to diabetic control (DC) group.

significantly increased by 17% compared to the DC mice ($p<0.05$). In addition, plasma lipid levels of the GS500-treated mice were also significantly improved by 16% reduction in NEFA ($p<0.05$), 24% reduction in triglyceride (TG) and 29% increase in HDL-C ($p<0.01$), compared to the DC mice.

As shown in Table 1, plasma adiponectin level in *ginsam*-treated groups was significantly increased compared to diabetic control group. Adiponectin, also called apM1, GBP28, AdipoQ, and ACRP30, is a hormone secreted by adipocytes that regulates energy homeostasis and glucose and lipid metabolism (22,23). Obesity and T2DM are associated with decreased plasma adiponectin levels, which seem to be associated with an elevated TG content in skeletal muscle and liver (24,25). Elevated TG content in the liver of diabetic control mice was evident in Fig. 2 and *ginsam* lowered the hepatic TG level probably due to fatty acid oxidation, which was stimulated by adiponectin. In the liver, full-length adiponectin is known to activate AMPK (26), thereby reducing enzymes involved in gluconeogenesis and increasing phosphorylation of acetyl-CoA carboxylase (ACC) and fatty acid oxidation. Adiponectin also activates PPAR-α, thereby stimulating fatty acid oxidation and decreasing TG content in the liver (27). These alterations all increase insulin sensitivity *in vivo*.

OGTT To determine the effect of multiple oral administration of GS on glucose tolerance, OGTT was carried out at the end of the experiment (Fig. 1). Glucose challenge dramatically increased the blood glucose levels in the DC group, whereas GS-treated groups significantly suppressed the blood glucose levels from rising during 120 min after glucose challenge (Fig. 1A). When the area under the curve (AUC) was compared between groups, GS300- and GS500-treated groups showed 12 and 32% reduction, respectively, compared to that in the DC group ($p<0.01$ and $p<0.001$, Fig. 1B). Improvement of the glucose intolerance in the GS500-treated group was quite similar to that in the MT300-treated group (37% reduction compared to the DC group, $p<0.001$).

Hepatic gene expression profiles and enzyme activities Among 19,858 genes, we selected 23 hepatic genes functionally significant in diabetes. As shown in Table 2, GS500-treated group showed altered expression of genes responsible for the glycolysis/gluconeogenesis and fatty acid metabolism, including decreased expression of G6Pase (0.8-fold) and increased expressions of GK (1.30-fold) and Acyl CoA oxidase 1, palmitoyl (1.50-fold). To confirm the microarray data, quantitative-PCR for Acyl CoA oxidase 1, palmitoyl and phosphoenolpyruvate carboxykinase (PEPCK) and enzyme activities for G6Pase, GK and G6PD were determined in the liver of *db/db* mice (Table 2). PEPCK gene expression and glucose-6-phosphate enzymatic activity were both significantly reduced (0.5-fold) in the liver of GS500-treated mice, compared to the DC mice. On the other hand, gene expression of Acyl CoA oxidase 1, palmitoyl, and enzyme activities of GK and G6PD were markedly enhanced (1.4-, 1.7- and 3.2-fold) compared to the control mice. PEPCK and G6Pase are the rate-limiting enzymes in gluconeogenetic pathway and enhanced expressions of these enzymes are present and responsible for increased hepatic glucose output in type 2 diabetic patients (28). In the liver, production of glucose-6-phosphate by GK precedes storage of glucose as glycogen, which is stimulated by insulin. In pancreatic β-cells, GK constitutes part of the glucose sensor. Therefore, novel GK activators augment both glucose-induced insulin secretion in β-cells and hepatic glucose metabolism, and as a result lead to improved clearance of glucose from the blood stream (29). As shown in Table 2, GK gene is over-expressed by 1.7-fold compared to diabetic control in the liver of the mice treated with 500 mg/kg of *ginsam*, and hepatic glycogen content was also increased by more than 60% in *ginsam* treated mice, compared to that in diabetic control mice (data not shown).

Morphology, lipid levels, and mRNA expressions of lipogenesis and lipolysis-related genes in the liver TG accumulation was evaluated in liver tissue, since it could be associated with lipotoxicity and insulin resistance (30). Although GS did not reduce liver mass (data not shown), hepatic triglyceride and cholesterol levels in GS-treated groups were decreased compared to diabetic control mice (Fig. 2A and 2B) Next, mRNA expression of genes

Table 2. Differentially expressed genes in the liver of GS500-treated *db/db* mice

GeneBank Acc. No.	Gene name	Fold change		Function
		Array	Q-PCR or Enzyme activity	
Up-regulated				
NM_008493	Leptin	1.2		Adipocytokine
NM_030721	Acyl-Coenzyme A oxidase 3, pristanoyl	1.3		Fatty acid metabolism
NM_015729	Acyl-Coenzyme A oxidase 1, palmitoyl	1.5	1.4	Fatty acid metabolism
BC022940	Acetyl-Coenzyme A carboxylase beta	1.2		Fatty acid biosynthesis
NM_144903	Aldolase 2, B isoform	2.2		Glycolysis
D38379	Pyruvate kinase, muscle	1.5		Glycolysis
NM_013820	Glucokinase	1.3	1.7	Glycolysis
NM_008155	Glucose phosphate isomerase 1	1.2		Glycolysis
NM_011989	Solute carrier family 27 (fatty acid transporter), member 4	1.3		Insulin signaling
NM_010572	Insulin receptor substrate 4	1.3		Insulin signaling
NM_010570	Insulin receptor substrate 1	1.2		Insulin signaling
NM_009204	Solute carrier family 2 (facilitated glucose transporter), member 4	1.2		Insulin signaling
NM_010514	Insulin-like growth factor 2	1.2		Insulin signaling
NM_008062	Glucose-6-phosphate dehydrogenase X-linked	1.4	3.2	PPP
NM_025396	6-Phosphogluconolactonase	1.3		PPP
BC011329	Phosphogluconate dehydrogenase	1.3		PPP
NM_009075	Ribose 5-phosphate isomerase A	1.2		PPP
Down-regulated				
NM_011044	Phosphoenolpyruvate carboxykinase 1, cytosolic	1.1	0.5	Gluconeogenesis
NM_008061	Glucose-6-phosphatase, catalytic	0.8	0.5	Gluconeogenesis
NM_009883	CCAAT/enhancer binding protein (C/EBP), beta	0.8		Insulin signaling
NM_010700	Low density lipoprotein receptor	0.7		Insulin signaling
NM_011949	Mitogen activated protein kinase 1	0.7		Insulin signaling
NM_025683	Ribulose-5-phosphate-3-epimerase	0.7		PPP

involved in lipolysis and lipogenesis was examined in the GS-treated liver tissue. GS-treated mice showed increases in the mRNA levels of PPAR-α, ACox, and CD36 gene, compared to control group (Fig. 2C). However, there was no significant effect on the mRNA levels of SREBP and FAS genes in GS-treated groups (Fig. 2D).

AMPK and GLUT4 expressions in the liver and skeletal muscle Viollet *et al.* (31) evidently reported that activation of AMPK in the liver leads to the stimulation of fatty acid oxidation and inhibitions of lipogenesis and glucose production (31). Moreover, AMPK could mediate some of the effects of the fat cell-derived adiponectin. With increased levels of plasma adiponectin in GS-treated group as shown in Table 1, phosphorylation of AMPK was examined by Western blot. As shown in Fig. 3A, GS stimulated the phosphorylation of AMPK and its degree of activation was comparable to that of MET-treated group, when compared to the control mice. In addition, GS-treated groups over-expressed the mRNA and protein of GLUT4 in the skeletal muscle, indicating facilitation of glucose uptake into the insulin-dependent tissue. Analogous to adiponectin, *ginsam* markedly lowered the plasma glucose level and stimulated β-oxidation probably due to activation of AMPK (Fig. 3A) and over-expression of genes responsible for lipolysis such as PPAR-α, ACoX, and CD36 genes (Fig. 2C). The activation of AMPK suppresses hepatic glucose production and also phosphorylates ACC, which leads to the inhibition of ACC activity and a consequent reduction in the malonyl-CoA content, thereby derepressing carnitine palmitoyltransferase 1 activity and increasing fatty acid oxidation (32). The activation of AMPK has also been shown to stimulate glucose uptake independent of its action on fatty acid in skeletal muscle (33). Insulin resistance in skeletal muscle is associated with down-regulation of GLUT4. The role of GLUT4 down-regulation in pathogenesis of insulin resistance and glucose tolerance has been confirmed in mice with muscle selective ablation of GLUT4 (28). Therefore, the higher mRNA and protein expression of GLUT4 in *ginsam*-treated mice reflects an increase in glucose flux into skeletal muscle in response to the ameliorated insulin resistance (Fig. 3B and 3C).

Ginseng has been used as a tonic and restorative for several thousand years. Ginseng has a glucose lowering effect probably due to increasing insulin secretion, ameliorating insulin resistance or both. However, anti-diabetic efficacy of ginseng is not remarkable and often ambiguous, and thus most of physician would hesitate to recommend the ginseng to diabetic patients as a supplement. Therefore, in a series of investigations to develop anti-diabetic agent with better efficacy, ginseng radix was treated with vinegar. When ginsenoside profile was analyzed by high performance liquid chromatography (HPLC), ginseng radix and *ginsam* showed quite different profiles (34) and therefore differential

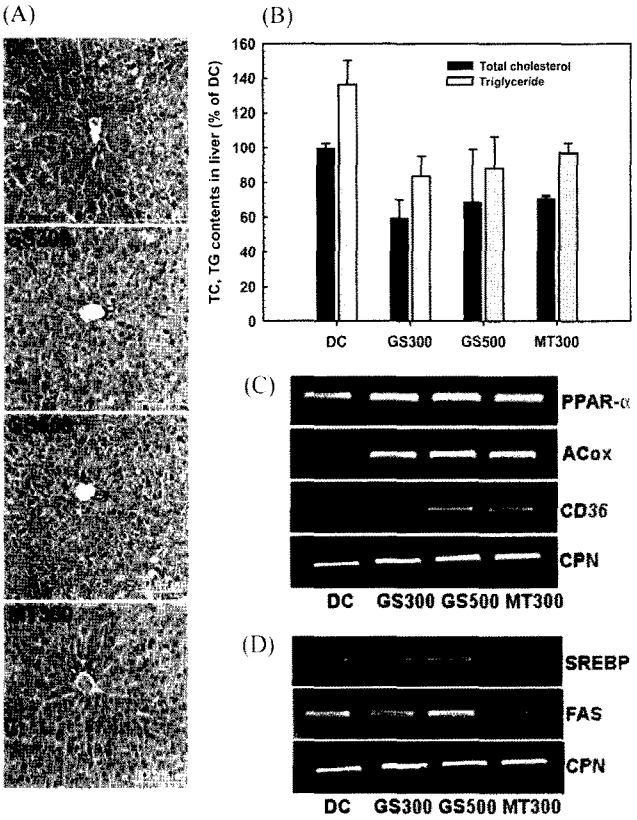

Fig. 2. Effects of *ginsam* on hepatic adiposity. Histological analysis of liver revealed reduced lipid vacuoles in *ginsam*-treated mice. H stainig (200×) (A), triglyceride and total cholesterol concentrations (B), effects of *ginsam* on lipolysis (C), and lipogenesis (D) related genes expression. Values are means±SE. *$p<0.05$ compared to diabetic control (DC) group.

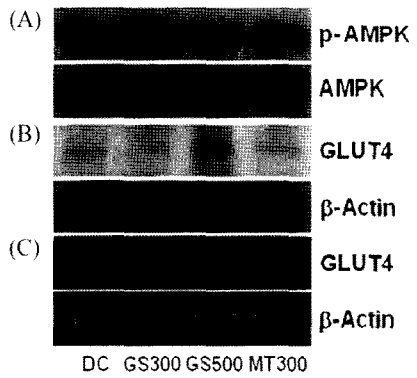

Fig. 3. Effects of ginsam on hepatic AMPK phosphorylation (A), skeletal GLUT4 protein (B), and mRNA expression levels (C).

effects were expected. In the previous study to compare the anti-diabetic efficacy between ginseng radix and *ginsam*, metabolic pre-diabetic syndrome was more significantly improved by *ginsam* rather than ginseng radix (35). The reason for this difference is unclear, but a compelling possibility is that the ginsenoside compositions seem to be responsible. Although many plausible explanations for the differential efficacies of *ginsam* and ginseng radix may exist, the ratio ($Rg_3/Rb_1+Rb_2+Rc+Rd$) or the Rg_3 concentration *per se* might play significant and independent roles. Our experiments showed that Rg_3 significantly increases the glucose-stimulated insulin secretion in HIT-T15 beta cell line and phosphorylates AMPK in a myotubes (36).

In summary, we proposed that *ginsam* increases plasma adiponectin level, increasing AMPK activity, thereby reducing expression of genes involved in gluconeogenesis, and also activation of genes involved in lipolysis, thereby stimulating fatty acid oxidation and decreasing triglyceride content in the liver. These changes in gene expression were considered to be the mechanism by which the *ginsam* exerted the anti-diabetic and anti-dyslipidemic activities in C57BL/KsJ *db/db* mice. *Ginsam* showed potential as a prevention and treatment agent for type 2 diabetic patients with genetic predisposition.

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