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Aged Ginseng (*Panax ginseng* Meyer) Reduces Blood Glucose Levels and Improves Lipid Metabolism in High Fat Diet-fed Mice

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Abstract Aged ginseng is unpeeled ginseng root that has been dried and heat-treated in an oven at 80°C for 14 days. The effects of aged ginseng, in comparison with white and red ginseng, on the lipid and glucose metabolism in high fat-fed mice were investigated. C57BL/6N mice were randomly divided into six dietary groups of normal control, high fat, and high fat supplemented with white, red, aged four-year old, and aged five-year old ginseng. After 8 weeks, ginseng counteracted high fat diet-induced body weight gain, hyperlipidemia, and hyperglycemia via a mechanism involving modulation of hepatic lipogenesis, adipokine production, and glucose-regulating enzyme activities. Aged ginseng showed greater antihyperlipidemic and antihyperglycemic activities than white ginseng and exhibited physiological effects similar to red ginseng, perhaps due to a relatively high ginsenoside content. Aged ginseng can be beneficial as a functional food.

Keywords: aged ginseng, red, lipid, glucose

Introduction

Ginseng (*Panax ginseng* Meyer), a slow-growing perennial herb native to Korea and China that has long been recognized for medicinal properties. For thousands of years, the fleshy root has been used in traditional oriental medicine to improve health, increase vitality and resistance to stress, and enhance the immune system (1). Therapeutic effects against the metabolic disorders diabetes, dyslipidemia, and obesity have been widely investigated. Studies have shown that extracts from ginseng root exert anti-obesity and anti-diabetic effects, and have hypolipidemic and hypotensive activities, and antioxidative properties (2-5). Ginseng saponins, known as ginsenosides, are the main bioactive compounds responsible for the pharmacological activities of ginseng and amounts vary depending on the age of plants (6). It was reported that the ginsenoside content increases with the age of the ginseng root, reaching a maximum yield at 5 years (7).

A number of ginseng products, including white, red, and black ginseng, are available in the Asian market. White ginseng is produced by air-drying peeled ginseng root to a moisture content of 12% or less, while red ginseng is prepared by steaming unpeeled ginseng root at 98-100°C for 2-3 h and subsequent sun-drying (2,8). Compared with white ginseng, red ginseng has been found to exert greater pharmacological effects (9) due to larger amounts of ginsenosides and phenolics (8). During the steaming process, ginseng starch is gelatinized, resulting in an increase in the saponin content of red ginseng (6). Black ginseng, on the other hand, is produced via 9 cycles of steaming and drying at high temperature, at which point the ginseng turns black (3). In Korea, unprocessed raw *Panax ginseng* Meyer roots are also widely sold in markets with 4 and 5-year old roots as the most popular due to superior functional properties. A recent study of ginseng revealed that aging can increase the bioactive compound content and the antioxidant activity (10). Unpeeled ginseng roots were dried and heat-treated in an oven at 80°C with 70% relative humidity for 14 days (10). Aged ginseng is easier and cheaper to produce than both red and black ginsengs. Previous studies have shown that (3,6) prolonged heat treatment can increase the ginsenoside content and the biological activity of ginseng.

With rapidly increasing global incidences of metabolic diseases, particularly dyslipidemia and diabetes, there is a growing market demand for natural products and functional foods that have strong antihyperlipidemic and antihyperglycemic properties. This study was carried out to evaluate the effects of aged ginseng, in comparison with white and red ginseng, on body weights, lipid profiles, and glucose levels in mice under high fat diet conditions. Hormones and enzymes associated with the lipid and glucose metabolism were also analyzed.

Materials and Methods

Materials and chemicals Four year old and 5 year old fresh ginseng plants were obtained from Punggi Ginseng Cooperative Association (Yeongju, Korea) in December of 2013. Plants were thoroughly washed with distilled water for 5 times. Then they were placed in plastic food containers with lids (15x20x10 cm) (Lock and Lock Co., Ltd., Seoul, Korea) to prevent drying, and aged in an oven (SW 90D; Sang Woo Scientific Co., Bucheon, Korea) at 80°C with 70% relative humidity for 14 days. Aging conditions used in this study was based on previous results regarding optimum aging processing conditions (10). Four-year old white and red ginseng was purchased from a local market in Yeongju, Korea. All ginseng samples were grown in Yeongju, Korea and were obtained in October of 2012. Ginseng samples were ground into powder (HMF-3260S; Hanil Electric, Seoul, Korea) and passed through a 100 mesh sieve (particle size 149 μm) prior to vacuum freeze drying (Freezone 6, Model 77530; Labconco Co., Kansas City, MO, USA). Ginseng samples generally have similar proximate compositions (Table 1). All other chemicals used were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Determination of the ginsenoside content Ginsenoside contents were measured using reverse-phase (RP)-HPLC following the method described Jochum *et al*. (11). Briefly, 50 mg of freeze-dried ginseng powder was subjected to extraction with methanol (Sigma-Aldrich) and 30 μL of the resulting extract was injected into the RP-HPLC device equipped with a C_{18} reversed-phase column and guard column (Chrompack Standard Columns; Varian Inc., Lake Forest, CA, USA). The mobile phase consisted of water (A), acetonitrile (Sigma-Aldrich) (B), and a phosphate buffer solution (Sigma-Aldrich) (C) under gradient elution with concentrations adjusted as: 0-15 min for 0% A, 19% B, and 81% C; 15-24.5 min for 0% A, 21% B, and 79% C; 24.5-29 min for 0% A, 26.3% B, and 73.7% C; 29-43 min for 0% A, 27% B, and 73% C; 43-47 min for 0% A, 34% B, and 66% C; 47-54 min for 0% A, 36% B, and 64% C; 54-55 min for 0% A, 43% B, and 57% C; 55-64 min for 15% A, 85% B, and 0% C, and 64-74 min for 0% A, 19% B, and 81% C. The flow rate was 1.15 mL/min and the absorbance was measured (Waters-Alliance 2695 HPLC system; Waters Corporation, Milford, MA, USA) at 203 nm. Ginsenosides were quantified using standard curves of the individual Rb_1, Rb_2, Rb_3, Re, Rf, Rg_1, Rg_2, and Rg_3 Ginsenosides. Concentrations of Rb_1, Rb_2, Rb_3, and Rg_3 were added to obtain the total amount of protopanaxadiols and concentrations of Re, Rf, Rg_1, and Rg_2 were added to determine the total amount of protopanaxtriols. Results, expressed as mg/g of dry weight (DW) for aged ginseng are shown in Table 1. All ginseng samples contained marginal amounts of protopanaxadiol ginsenosides (0.01-0.09 mg/g) relative to protopanaxatriol ginsenosides (2.38-22.46 mg/g).

Animals and diet Forty eight male C57BL/6N mice (4 weeks old, approximately 12 g each) were purchased from Orient Inc. (Seoul, Korea). Animals were housed individually in stainless steel cages in a room maintained at 25±2°C with 50% relative humidity and a 12/12 h light/dark cycle. Mice were fed a pelletized commercial chow diet (Samyang Feed, Wonju, Korea) for a week upon arrival, then randomly divided into 6 dietary groups (n=8). The composition of the experimental diet (Table 2) was based on the AIN-76 semisynthetic diet. The first and second groups were fed a normal control diet (NC group) and a high fat diet (HF group), respectively. The other 4 groups were fed a high fat diet supplemented with white ginseng (HF-WG group), red ginseng (HF-RG group), aged 4-year old ginseng powder (HF-AG4 group), or aged 5-year old ginseng powder (HF-AG5 group). Animals were fed with experimental diets for 8 weeks and allowed free access to food and water. At the end of the experimental period, mice were anaesthetized using ketamine-HCl (Ketamine Hydrochloride 50 mg/mL; Yuhan, Seoul, Korea) following a 12 h fast. Blood samples were drawn from the inferior vena cava into a heparin-coated tube (BD Microtainer; BD Inc, Franklin Lakes, NJ, USA**)** and centrifuged (5145R; Eppendorf, Hamburg, Germany) at 1,000x*g* for 15 min at 4°C to obtain plasma. The liver, heart, kidneys, and epididymal, perirenal, and inguinal adipose tissues were removed, rinsed with PBS buffer (pH 7.4), weighed (WTB 2000; RADWAG, Radom, Poland) and stored at −70°C freezer (MDF-U73V; Sanyo Electric Co., Osaka, Japan) until analysis. The study protocol was approved by the Ethics Committee of Kyungpook National University for animal studies. All institutional and national guidelines for the care and use of laboratory animals were followed.

Table 1. Proximate compositions and ginsenoside contents of ginseng

	WG[1)]	RG	AG4	AG5
Composition (% dry basis)				
Moisture	15.3	13.1	13.3	15.2
Crude protein	10.0	10.5	10.9	10.6
Crude fat	0.4	0.6	0.5	0.6
Ash	3.5	3.2	3.6	3.8
Carbohydrate	70.8	72.6	71.7	69.8
Ginsenoside (mg/g of DW)[2)]				
Protopanaxadiols	0.01±0.00[a]	0.08±0.01[c]	0.09±0.01[c]	0.03±0.00[b]
Protopanaxatriols	2.38±0.05[a]	15.29±0.03[b]	22.46±0.07[d]	18.52±0.06[c]

[1)]WG, white ginseng; RG, red ginseng; AG4, aged four-year old ginseng; AG5, aged five-year old ginseng.

[2)]Values are means±SD (n=3). Means in the same row followed by different letters are significantly different at p<0.05.

Analysis of triglyceride and cholesterol concentrations and glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels in plasma Concentrations of plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were determined using commercial kits (Asan Pharmaceutical, Seoul, Korea). Plasma GOT and GPT levels were also measured using commercial kits (Sigma-Aldrich).

Table 2. Compositions of experimental diets (%)

	NC[1)]	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Casein	20.0	20.0	20.0	20.0	20.0	20.0
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	50.0	50.0	46.0	46.0	46.0	46.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Corn oil	5.0	3.0	3.0	3.0	3.0	3.0
Cholinbitartrate	0.2	0.2	0.2	0.2	0.2	0.2
Mineral mixture[2)]	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[3)]	1.0	1.0	1.0	1.0	1.0	1.0
Corn starch	15.0					
Lard		17.0	17.0	17.0	17.0	17.0
WG			4.0			
RG				4.0		
AG4					4.0	
AG5						4.0
Total (%)	100.0	100.0	100.0	100.0	100.0	100.0

[1)]NC, normal control diet; HF, high fat diet; HF-WG, high fat + white ginseng; HF-RG, high fat + red ginseng; HF-AG4, high fat + aged four-year old ginseng; HF-AG5, high fat + aged five year old ginseng.
[2)]AIN-76 mineral mixture
[3)]AIN-76 vitamin mixture

Measurement of blood glucose, hepatic glycogen, and plasma insulin levels Blood glucose levels were measured using Accu-Chek Active Blood Glucose Test Strips (Roche Diagnostics, Berlin, Germany). Glycogen levels were measured following the method of Seifter *et al.* (12). Liver tissue was mixed with 30% KOH, heated at 100°C (Wisebath; Wisd Laboratory Instruments, Wertheim, Germany) for 30 min, then 95% ethanol was added. The mixture was incubated (B145S; LG Electronics, Seoul, Korea) overnight at 4°C, then mixed with distilled water and 0.2% anthrone (Sigma-Aldrich) (in 95% H_2SO_4). The absorbance was measured (Sunrise; Tecan, Salzburg, Austria) at 620 nm and results were calculated based on a standard calibration curve of glucose. Insulin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (TMB Mouse Insulin ELISA kit; Shibayagi, Gunma, Japan).

Determination of plasma adipokine concentrations Concentrations of adiponectin, resistin, and tumor necrosis factor (TNF)-α were determined using ELISA kits (Shibayagi). Leptin concentrations were measured using an enzyme immunoassay (EIA) kit (Spi-Bio, Montigny le Bretonneux, France).

Measurement of hepatic lipid and glucose-regulating enzyme activities An hepatic enzyme source was prepared based on homogenization of liver tissue in a buffer solution containing triethanolamine (Sigma-Aldrich), EDTA (Sigma-Aldrich), and dithiothreitol (Sigma-Aldrich) centrifuged (WX Ultra 80, Thermo Scientific, Waltham, MA, USA) at 1,000x*g* at 4°C for 15 min followed by Hulcher and Oleson (13). The pellet was removed and the supernatant was centrifuged (WX Ultra 80; Thermo Scientific) at 10,000x*g* at 4°C for 15 min. The resulting precipitate served as a mitochondrial fraction and the supernatant was further centrifuged (WX Ultra 80; Thermo Scientific) at 105,000x*g* at 4°C for 1 h. The resulting precipitate and supernatant served as microsome and cytosol fractions, respectively.

For analysis of lipid-regulating enzymes, fatty acid synthase (FAS) activities were determined using a spectrophotometric method (14). Malonyl CoA (Sigma-Aldrich) was added to an assay mixture and the change in absorbance (DU-800 Beckman spectrophotometer; Beckman Coulter, Inc., Fullerton, CA, USA) at 340 nm at 30°C was recorded (15). Malic enzyme (ME) activities were measured following the method of Ochoa (15). The absorbance of the assay mixture was measured at 340 nm at 27°C. Glucose-6-phosphate dehydrogenase (G6PD) activities were determined based on reduction of 6 mM $NADP^+$ by G6PD in the presence of glucose-6-phosphate (16). Enzyme activities were measured based on monitoring an increase in absorption of a reaction mixture at 340 nm at 37°C. Enzyme activities were expressed as nmol of reduced NADPH/min/mg of protein.

For analysis of glucose-regulating enzymes, glucokinase (GK) activities were measured based on the method of Davidson and Arion (17). A reaction mixture was incubated (Wisebath; Wisd Laboratory Instruments) at 37°C for 10 min and a change in absorbance (DU-800 Beckman spectrophotometer; Beckman) at 340 nm was recorded. Phosphoenolpyruvate carboxykinase (PEPCK) activities were determined following the method of Bentle and Lardy (18). The absorbance of an assay mixture was measured at 340 nm (DU-800 Beckman spectrophotometer; Beckman). Glucose-6-phosphatase (G6pase) activities were determined following the method of Alegre *et al*. (19). A reaction mixture was incubated (Wisebath; Wisd Laboratory Instruments) at 37°C for 4 min and a change in absorbance (DU-800 Beckman spectrophotometer; Beckman) at 340 nm was recorded. Enzyme activities were expressed as nmol/min/mg of protein.

Statistical analysis All data were presented as a mean±standard deviation (SD). Data were evaluated using a one-way analysis of variance (ANOVA) with the Statistical Package for Social Sciences software program version 19.0 (SPSS Inc., Chicago, IL, USA). Differences between means were assessed using Tukey's test. Statistical significance was considered at $p<0.05$.

Results and Discussion

Body weight gain and organ weight Initial body weights did not significantly ($p<0.05$) differ among mice in different groups (Table 3). At the end of the experimental period, mice fed a high fat diet exhibited increase in final body weight relative to mice fed a control diet. However, diet supplementation with ginseng significantly ($p<0.05$) decreased high fat diet-induced body weight gain, compared with controls. HF group mice showed the highest feed intake and feed efficiency ratio (FER). The liver weight was also highest in HF group mice. Weights of the heart and kidneys were higher in HF and ginseng-fed group mice than in control group mice. High fat feeding also resulted in a significantly different in the weights of white adipose tissues. On the other hand, all ginseng-fed group mice exhibited significantly ($p<0.05$) lower adipose tissue weights than HF group mice. Thus, ginseng suppressed body weight gain and exerted a reduction effect on body fat amounts in high fat-fed mice. Feeding of red ginseng extracts has also been shown to decrease the body weight and adipose tissue mass in high fat diet-induced obese mice (4). The weight-lowering effect of ginseng may have been due to contained bioactive saponins. Studies in the past revealed that ginseng saponins could reduce the body weight and body fat amounts in mice and rats under high fat diet conditions (20,21).

Plasma lipid profiles and blood glucose levels Significant ($p<0.05$) elevations in levels of plasma triglyceride and total cholesterol were observed in HF mice relative to NC group mice (Table 4). High dietary fat intake has been shown to alter cholesterol and triglyceride levels in both humans and rodents (22). However, addition of ginseng powder to the high fat diet significantly reduced lipid levels in mice. Aged ginseng-fed mice showed triglyceride and total cholesterol levels similar to normal control group mice. Moreover, HF-AG4 mice exhibited the highest HDL-cholesterol level among treatment groups. HDL exerts anti-atherogenic properties and high levels of HDL-cholesterol denote a low risk of coronary heart disease (23). The total cholesterol/HDL-cholesterol ratio and the atherogenic index, which are both indicators of a coronary heart disease risk, significantly ($p<0.05$) increased in HF mice, compared with controls, and significantly ($p<0.05$) decreased in ginseng-fed group mice. Higher blood glucose concentrations were also observed in HF mice, relative to control mice. On the other hand, ginseng-fed group mice showed significantly ($p<0.05$) lower glucose levels than mice fed a high fat diet alone. In particular, aged ginseng reduced the blood glucose concentration to a normal level. HF-AG4 and HF-AG5 group mice exhibited higher glycogen levels than mice of other groups. Plasma insulin levels were significantly ($p<0.05$) lower in ginseng-fed group mice, especially HF-RG, HF-AG4, and HF-AG5 group mice, than in HF mice. Plasma GOT and GPT levels significantly ($p<0.05$) increased in HF mice relative to control group mice. However, diet supplementation with ginseng counteracted this elevation for plasma enzymes. GOT and GPT are specific markers of liver damage resulting from oxidative stress (24). Reduced levels of plasma enzymes observed in ginseng-fed group mice relative to HF mice indicated decreased hepatic oxidative stress in these groups under high fat diet conditions.

Previous studies have also shown that ginseng powders and extracts can reduce plasma cholesterol, triglyceride, and blood glucose levels in both humans and laboratory animals (25). Extracts from red ginseng significantly reduced blood glucose levels in diabetic mice

Table 3. Body weight gain and weights of organs and adipose tissues in mice fed with a high fat diet supplemented with ginseng powder

	NC[1)]	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Initial weight (g)	18.08±0.79^{a}[2)]	18.08±0.84^{a}	18.08±2.37^{a}	18.12±0.90^{a}	18.05±1.04^{a}	18.02±0.38^{a}
Final weight (g)	25.65±2.26^{a}	35.86±3.30^{c}	30.95±2.40^{b}	29.96±2.34^{b}	29.10±1.32^{b}	29.80±0.47^{b}
Weight gain (g/day)	0.12±0.00^{a}	0.28±0.05^{c}	0.21±0.02^{b}	0.19±0.02^{b}	0.18±0.02^{b}	0.19±0.01^{b}
Feed intake (g/day)	3.21±0.76^{a}	3.78±0.82^{c}	3.48±0.65^{b}	3.51±0.79^{bc}	3.53±1.01^{bc}	3.49±0.62^{b}
FER	0.04±0.00^{a}	0.08±0.00^{c}	0.06±0.00^{c}	0.05±0.00^{ab}	0.05±0.00^{ab}	0.05±0.00^{ab}
Organ weight (g)						
Liver	0.96±0.05^{a}	1.96±0.05^{c}	0.99±0.08^{a}	1.11±0.11^{b}	1.05±0.05^{ab}	1.04±0.05^{ab}
Heart	0.12±0.00^{a}	0.14±0.00^{b}	0.13±0.00^{b}	0.13±0.00^{b}	0.13±0.00^{b}	0.14±0.00^{b}
Kidney	0.31±0.05^{a}	0.36±0.02^{b}	0.37±0.02^{b}	0.37±0.02^{b}	0.37±0.02^{b}	0.40±0.02^{b}
White adipose tissue weight (g)						
Epididymal	1.37±0.08^{a}	1.95±0.11^{c}	1.44±0.16^{b}	1.29±0.19^{b}	1.19±0.14^{b}	1.34±0.22^{b}
Perirenal	0.35±0.02^{a}	1.31±0.08^{d}	0.69±0.02^{c}	0.59±0.08^{b}	0.52±0.05^{b}	0.55±0.05^{b}
Inguinal	0.06±0.02^{a}	0.49±0.02^{e}	0.25±0.02^{cd}	0.28±0.05^{d}	0.18±0.02^{b}	0.21±0.05^{bc}

[1)]NC, normal control diet; HF, high fat diet; HF-WG, high fat+white ginseng; HF-RG, high fat+red ginseng; HF-AG4, high fat+aged four year old ginseng; HF-AG5, high fat+aged five year old ginseng; FER, food efficiency ratio=body weight gain/feed intake.

[2)]Values are means±SD ($n=8$). Means in the same row followed by different letters are significantly different at $p<0.05$.

Table 4. Plasma lipid profiles and blood glucose levels in mice fed with a high fat diet supplemented with ginseng powder

	NC[1)]	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Triglyceride (mg/dL)	124.18±5.37^{a}[2)]	175.20±7.60^{d}	147.34±17.11^{cd}	140.57±8.65^{bc}	130.68±6.39^{ab}	131.33±11.93^{ab}
Total cholesterol (mg/dL)	154.68±9.13^{a}	227.27±6.59^{d}	194.09±6.36^{c}	180.91±7.72^{b}	156.72±6.02^{a}	157.78±5.71^{a}
HDL-cholesterol (mg/dL)	101.21±14.00^{ab}	78.99±19.17^{a}	112.21±17.56^{bc}	115.81±19.77^{bc}	137.80±15.98^{c}	122.80±63.30^{bc}
Total cholesterol/HDL ratio	1.68±0.33^{a}	2.70±0.48^{b}	1.72±0.33^{a}	1.54±0.25^{a}	1.56±0.22^{a}	1.43±0.25^{a}
AI	0.68±0.33^{a}	1.70±0.48^{b}	0.72±0.33^{a}	0.54±0.25^{a}	0.56±0.22^{a}	0.43±0.25^{a}
Blood glucose (mg/dL)	87.23±4.97^{a}	102.50±7.07^{c}	94.50±4.94^{b}	92.54±5.85^{b}	88.13±4.55^{a}	87.67±5.45^{a}
Hepatic glycogen (mg/g liver)	5.25±0.93^{a}	5.04±0.93^{a}	5.32±0.87^{a}	5.34±0.53^{a}	5.95±1.44^{ab}	6.29±1.24^{b}
Plasma insulin (ng/mL)	4.10±0.50^{a}	6.97±1.97^{c}	5.22±1.86^{b}	4.94±0.48^{ab}	4.33±1.35^{ab}	4.42±1.92^{ab}
Plasma GOT (karman/mL)	24.72±7.44^{a}	88.09±6.10^{b}	39.41±6.42^{a}	37.86±11.88^{a}	31.68±6.27^{a}	30.91±6.84^{a}
Plasma GPT (karman/mL)	18.29±4.43^{a}	63.77±5.79^{b}	28.45±3.50^{a}	24.58±4.27^{a}	21.68±7.40^{a}	20.71±4.27^{a}

[1)]NC, normal control diet; HF, high fat diet; HF-WG, high fat+white ginseng; HF-RG, high fat+red ginseng; HF-AG4, high fat+aged four year old ginseng; HF-AG5, high fat+aged five year old ginseng; AI, atherogenic index=(total cholesterol–HDL-cholesterol)/HDL-cholesterol; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

[2)]Values are means±SD (n=8). Means in the same row followed by different letters are significantly different at p<0.05.

(2). The pharmacological activities of ginseng can be attributed to bioactive saponins or ginsenosides. Karu *et al*. (20) suggested that anti-obesity and hypolipidemic effects of ginseng saponins may be mediated by inhibition of pancreatic lipase activity. Red ginseng has been reported to have a greater pharmacological activity than white ginseng due to a higher saponin content (8,9). The amount of saponin increases during heat treatment of the ginseng root (6). In this study, red ginseng showed a significantly (p<0.05) higher ginsenoside content than white ginseng (Table 1). Aged ginseng also exhibited greater antihyperlipidemic and antihyperglycemic activities than white ginseng in this study. Furthermore, physiological effects of white ginseng were similar to red ginseng, and aged ginseng-fed mice showed significantly (p<0.05) lower blood glucose and plasma total cholesterol levels than red ginseng-fed group mice.

The strong physiological activities of aged ginseng may be related to relatively high ginsenoside contents, particularly protopanaxatriol ginsenosides. A previous study on aged ginseng revealed that an aging process at 80°C for 14 days substantially increased the amount of bioactive ginsenosides and phenolics and significantly enhanced antioxidant activities in ginseng roots (10). In particular, Rg_2 was the most abundant ginsenoside in aged ginseng and Re was also present in relatively high amounts. Ginsenosides Rg_2 and Re have been previously shown to exert antihyperlipidemic, anti-diabetic, and antioxidant activities (26,27).

AG4 and AG5 showed generally similar effects on body weights, lipid levels, and glucose concentrations in high fat-fed mice, which was unexpected since the ginsenoside content reportedly increases with the age of the ginseng root. Functional properties of aged ginseng using roots of different ages as raw materials warrant further investigation. Nevertheless, the age of ginseng roots, whether cultivated for 4 or 5 years, is not an important factor in the physiological properties of aged ginseng. The typical daily recommended intake of ginseng powder in the human diet is 3 g. In this study, mice consumed approximately 0.14 g (4% of the 3.5 g average daily feed intake) of ginseng powder daily, which was lower than the recommended dosage for human consumption. This study provides the first evidence of antihyperlipidemic, antihyperglycemic, and body fat-lowering effects of aged ginseng, suggesting that ginseng is useful as a functional food for prevention and management of high fat diet-induced hyperlipidemia, hyperglycemia, and body weight gain. Aged ginseng is easier and cheaper to produce than red ginseng. With physiological effects comparable to red ginseng, aged ginseng is a promising alternative to the more expensive and difficult to produce red ginseng.

Plasma adipokine concentrations High fat-fed mice showed significantly (p<0.05) higher levels of leptin, resistin, and TNF-α than control group mice (Table 5). However, diet supplementation with ginseng powders, particularly red and aged ginseng, significantly (p<0.05) reduced concentrations of these adipokines. Moreover, the adiponectin level was significantly (p<0.05) higher in HF-RG, HF-AG4, and HF-AG5 group mice than in HF mice. Kim *et al*. (21) reported a decrease in the serum leptin level in high fat-fed rats treated with crude saponin from red ginseng. Adipokines are protein hormones produced by adipose tissues that regulate the lipid and glucose metabolism (28). Expression and release of leptin, resistin, and TNF-α adipokines were reported to be associated with the progression of obesity (29). Furthermore, leptin level has been shown to be positively correlated with glycemia (28). Adiponectin concentrations, on the other hand, is negatively correlated with obesity and exerts hepatoprotective and anti-atherosclerotic properties (30). Hence, elevation in plasma adiponectin concentrations and reduction in leptin, resistin, and TNF-α levels in mice fed red ginseng and aged ginseng relative to mice fed with a high fat diet alone may have been partly responsible for decreases in the plasma triglyceride, total cholesterol, and blood glucose levels in these mice.

Activities of hepatic lipid-regulating and glucose-regulating enzymes Activities of the lipogenic enzymes FAS, ME, and G6PD increased with a HF mice (Table 6). On the other hand, feeding of

Table 5. Plasma adipokine concentrations in mice fed a high fat diet supplemented with ginseng powder

Groups	Adiponectin (μg/mL)	Leptin (ng/mL)	Resistin (ng/mL)	TNF-α (ng/mL)
NC[1)]	1.35±0.06[ab2)]	0.37±0.06[a]	26.57±2.57[a]	0.93±0.03[c]
HF	1.28±0.03[a]	0.66±0.11[b]	31.79±0.85[b]	1.03±0.03[d]
HF-WG	1.35±0.06[ab]	0.38±0.11[a]	28.32±1.53[ab]	0.74±0.00[b]
HF-RG	1.36±0.03[b]	0.33±0.06[a]	26.76±2.43[a]	0.63±0.00[a]
HF-AG4	1.37±0.03[b]	0.29±0.08[a]	25.43±2.88[a]	0.62±0.03[a]
HF-AG5	1.38±0.08[b]	0.27±0.06[a]	25.91±3.05[a]	0.62±0.03[a]

[1)]NC, normal control diet; HF, high fat diet; HF-WG, high fat + white ginseng; HF-RG, high fat+red ginseng; HF-AG4, high fat+aged four year old ginseng; HF-AG5, high fat+aged five year old ginseng.
[2)]Values are means±SD (*n*=8). Means in the same column followed by different letters are significantly different at $p<0.05$.

Table 6. Activities of hepatic lipid-regulating and glucose-regulating enzymes in mice fed a high fat diet supplemented with ginseng powder

	NC[1)]	HF	HF-WG	HF-RG	HF-AG4	HF-AG5
Lipid-regulating enzymes (nmol/min/mg of protein)						
FAS	4.18±0.54[a2)]	5.97±0.28[b]	4.19±0.28[a]	4.29±1.10[a]	4.01±0.14[a]	4.09±0.38[a]
ME	43.78±3.56[b]	78.25±3.05[c]	43.13±3.05[b]	38.55±1.50[b]	29.06±0.14[a]	29.68±0.63[a]
G6PD	4.13±0.34[b]	5.59±0.62[d]	4.61±0.28[c]	4.18±0.19[b]	3.61±0.16[a]	3.60±0.05[a]
Glucose-regulating enzymes (nmol/min/mg of protein)						
GK	0.89±0.08[a]	0.99±0.17[a]	1.12±0.11[a]	1.48±0.37[b]	1.72±0.07[bc]	1.86±0.04[c]
G6pase	92.81±10.04[a]	115.38±5.03[b]	101.43±4.58[a]	98.46±4.36[a]	100.39±2.26[a]	101.55±1.31[a]
PEPCK	5.00±0.28[b]	7.40±0.85[c]	3.21±0.14[a]	3.04±0.14[a]	2.95±0.03[a]	3.06±0.06[a]

[1)]NC, normal control diet; HF, high fat diet; HF-WG, high fat+white ginseng; HF-RG, high fat+red ginseng; HF-AG4, high fat+aged four year old ginseng; HF-AG5, high fat+aged five year old ginseng.
[2)]Values are means±SD (*n*=8). Means in the same row followed by different letters are significantly different at $p<0.05$.

ginseng counteracted the increase in enzyme activities. In particular, aged ginseng-fed mice exhibited the lowest ME and G6PD activities among all treatment group mice. Similarly, activities of the glucose-regulating enzymes G6pase and PEPCK significantly ($p<0.05$) increased with a high fat diet, but decreased with addition of the ginseng to the diet. In addition, the GK activity was highest in aged ginseng-fed group mice.

Aside from modulation of adipokine production, suppression of lipogenesis via inhibition of lipogenic enzyme activities and regulation of glucose-regulating enzyme activities may also be associated with the antihyperlipidemic and antihyperglycemic effects of ginseng. FAS, ME, and G6PD enzymes are involved in biosynthesis of fatty acids and cholesterol, and a decrease in activities of these enzymes could limit the availability of fatty acids necessary for synthesis of triglycerides (31,32). The PEPCK and G6pase enzymes, on the other hand, are involved in regulation of gluconeogenesis and hepatic glucose output and an increase in the activities of these enzymes could result in an elevation of the glucose level (33,34). The GK enzyme is involved in glucose homeostasis and an increased activity has been associated with increased glycogen production and decreased blood glucose levels (35). Thus, reductions in the activities of FAS, ME, and G6PD in ginseng-fed group mice, particularly HF-AG4 and HF-AG5 mice, relative to HF mice, could have been partly responsible for decreases in triglyceride and cholesterol levels in these animals. Moreover, the relative increase in the GK enzyme activity and reductions in the activities of PEPCK and G6pase enzymes could have caused an increase in the hepatic glycogen level and a decrease in the blood glucose level in mice fed a ginseng supplemented diet.

In conclusion, dietary supplementation with aged ginseng can suppress body weight gain and improve the lipid and glucose metabolism in mice under high fat diet conditions via a mechanism involving inhibition of hepatic lipogenesis, regulation of adipokine production, and modulation of glucose-regulating enzyme activities. Aged ginseng exhibited greater antihyperlipidemic and antihyperglycemic activities than white ginseng and exerted physiological effects similar to red ginseng, which could partly be attributed to a relatively high ginsenoside content. Aged ginseng may be useful as a functional food with a therapeutic potential against high fat diet-induced hyperlipidemia and hyperglycemia.

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