Effects of Korean white ginseng extracts on obesity in high-fat diet-induced obese mice

Young-Sil Lee · Byung-Yoon Cha · Kohji Yamaguchi · Sun-Sil Choi · Takayuki Yonezawa · Toshiaki Teruya · Kazuo Nagai · Je-Tae Woo

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Abstract The present study examined the anti-obesity effect and mechanism of action of Korean white ginseng extracts (KGE) using high-fat diet (HFD)-induced obese mice. Mice were fed a low-fat diet (LFD), HFD or HFD containing 0.8 and 1.6% (w/w) KGE diet (HFD + 0.8KGE and HFD + 1.6KGE) for 8 weeks. We also examined the effects of KGE on plasma triglyceride (TG) elevation in mice administered with oral lipid emulsion. Body weight gain and white adipose tissue (WAT) weight were significantly decreased in the HFD + 1.6KGE group, compared with the HFD group. The plasma TG levels were also significantly reduced in both HFD + 0.8KGE and HFD + 1.6KGE groups, while leptin levels were significantly decreased in only the HFD + 1.6KGE group, compared with the HFD group. The HFD + 1.6KGE group showed significantly lower mRNA levels of lipogenesis-related genes, including peroxisome proliferator-activated receptor γ 2 (PPAR γ 2),

sterol regulatory element binding protein-1c (SREBP-1c), lipoprotein lipase (LPL), fatty acid synthase (FAS) and diacylglycerol acyltransferase 1 (DGAT1), compared with the HFD group. In addition, a dose of 1000 mg/kg KGE inhibited the elevation of plasma TG levels compared with mice given the lipid emulsion alone. These results suggest that the anti-obesity effects of KGE may be elicited by regulating expression of lipogenesis-related genes in WAT and by delaying intestinal fat absorption.

Keywords Korean white ginseng · High-fat diet-induced obese mice · Lipogenesis-related genes · Intestinal fat absorption

Introduction

Obesity is a serious health problem which has become prevalent in developed countries in recent years. It is a risk factor for metabolic diseases such as insulin resistance, hypertension, arteriosclerosis (Björntorp 1997; Kopelman 2000; Spiegelman and Flier 2001). Therefore, prevention and treatment of obesity are important for achieving a healthy life. Obesity results from an imbalance between energy intake and energy expenditure, and is characterized by increased fat accumulation in adipose tissue and elevated lipid concentrations in blood (Prins and O’Rahilly 1997; Devlin et al. 2000; Fujioka 2002). Enlarged fat mass is associated with an increase in

Y.-S. Lee · B.-Y. Cha · K. Yamaguchi · S.-S. Choi · T. Yonezawa · T. Teruya · K. Nagai · J.-T. Woo
Research Institute for Biological Functions,
Chubu University, 1200 Matsumoto, Kasugai,
Aichi 487-8501, Japan

K. Nagai · J.-T. Woo (✉)
Department of Biological Chemistry, Chubu University,
1200 Matsumoto, Kasugai, Aichi 487-8501, Japan
e-mail: jwoo@isc.chubu.ac.jp

number and size of adipocytes differentiated from preadipocytes in adipose tissue (Choi et al. 2007). Differentiated adipocytes store free fatty acids (FFAs) in the form of triglyceride (TG) in cytoplasm, a synthesizing process known as lipogenesis. Lipogenesis is caused mainly by two different pathways. One of them is *de novo* lipogenesis which synthesize the fatty acids from glucose. It is regulated by various enzymes such as SREBP-1c and FAS (Pai et al. 1998; Weissman 1999). The alternative pathway for lipogenesis is mediated by LPL which is in charge of the release of FFAs liberated from chylomicrons and very-low-density lipoprotein (VLDL)-TG (Eckel 1989). These FFAs may be re-esterified into newly synthesized TG. Thus, LPL plays an important role in controlling lipid accumulation in adipose tissue.

Ginseng has been used in herbal medicine as a general tonic to promote health in Asian countries including Korea, China and Japan for 1,000 years (Yun et al. 2004; Han et al. 2005). Ginseng is to get its distinctive features according to its growing environment, geographical conditions, and the picking times. These differences may give rise to variation of the bioactive compounds in ginseng. The pharmacological properties of ginseng are attributed to ginsenosides, also referred to as steroid saponins that are found in extracts of ginseng. The pharmacological effects of ginseng extracts and ginsenosides have been reported in immunology, cancer, arteriosclerosis, hypertension and diabetes (Attele et al. 1999; Francis et al. 2002; Yoon et al. 2003). It has also been reported that ginseng has an effect on obesity and lipid metabolism. Ginseng extract and isolated ginsenosides displayed anti-hyperglycemic and anti-obesity activities following administration to diabetic rodents such as ob/ob and KKA^y mice (Chung et al. 2001; Attele et al. 2002). Wild ginseng extract and crude saponins were also shown to reduce plasma lipid levels and obesity when administered to rodents fed a high-fat diet (Yoon et al. 2003; Kim et al. 2005; Karu et al. 2007). Although the anti-obesity effects of ginseng and ginsenosides have been reported, the molecular mechanism by which ginseng reduces obesity in mice fed a high-fat diet is still unknown. This study investigated the effect of Korean white ginseng extracts (KGE) on obesity, and analyzed the mechanisms involved in its effects in HFD-induced obese mice.

Materials and methods

Preparation and extraction of Korean white ginseng

Korean ginseng (*Panax ginseng* C.A.Meyer) was purchased from Geumsan Undried Ginseng Center (Geumsan, Korea). The fine roots were panax ginseng-strained, 4–6-year-old, cultivated in Geumsan County, Korea. The roots were washed and dried at 50 °C, and named Korean white ginseng. The Korean white ginseng roots were extracted twice by refluxing with 70% ethanol at 70 °C for 8 h. The extract was then filtered, and the filtrate was concentrated with a vacuum rotary evaporator (Changjin, Korea) under low pressure at 40–60 °C. The yield of the KGE from the dried root was 30–45% and was stored at −20 °C until use. The KGE used in the present study contained 0.40% ginsenoside Rg1, 1.28% ginsenoside Re, 0.19% ginsenoside Rf, 2.15% ginsenoside Rb1, 2.09% ginsenoside Rc, 1.80% ginsenoside Rb2, 0.92% ginsenoside Rd, 0.25% ginsenoside Rg2, 0.07% ginsenoside Rg3, 0.02% ginsenoside Rh1 by reverse-phase preparative high-pressure liquid chromatography (HPLC) system equipped with μ C₁₈-AR-IIcolumn (mobile phase; water and methanol 20:80 (v/v, size; 50 × 500 mm, flow rate of mobile phase; 50 ml/min).

Experimental animals and protocol

4 week-old female ICR mice were purchased from Charles River (Yokohama, Japan) and housed (two per cage) in a 12 h light/12 h dark cycle (23 ± 3 °C) with free access to food and water. After 1 week of acclimatization on a LFD, the mice were assigned to one of the following four diets for 8 weeks: 1: LFD, containing 4% fat (LFD group); 2: HFD, containing 40% fat (HFD group); 3: HFD supplemented with 0.8% KGE (HFD + 0.8KGE group); or 4: HFD supplemented with 1.6% KGE (HFD + 1.6KGE group). Table 1 shows the compositions of the four test diets. Body weights were measured every week for 8 weeks. Food intake was measured on a per-cage basis throughout the study every 2 or 3 days. Food intake (g/mouse/day) was determined by subtracting the remaining food weight from the initial food weight of the previous feeding day and dividing by the number of mice housed in the cage. At the end of

Table 1 Composition of experimental diets (g/kg diet)

Diet composition	LFD	HFD	HFD + 0.8KGE	HFD + 1.6KGE
Casein	140	140	140	140
Beef tallow	40	400	400	400
β corn starch	465.7	105.7	105.7	105.7
α corn starch	155	155	155	155
Sucrose ^a	100	100	100	100
Cellulose	50	50	42	34
Mineral mixture	35	35	35	35
Vitamin mixture	10	10	10	10
L-cystine ^a	1.8	1.8	1.8	1.8
Choline hydrogen tartrate ^a	2.5	2.5	2.5	2.5
<i>t</i> -Butylhydroquinone ^a	0.008	0.008	0.008	0.008
KGE	0	0	8	16

LFD low-fat diet, HFD high-fat diet, KGE Korean white ginseng extracts

^a Sucrose, L-cystine, Choline hydrogen tartrate and *t*-Butylhydroquinone were purchased from Wako Pure Chemical Industries, Ltd. Mineral, vitamin mixture (AIN-76) and other basic ingredients were purchased from Oriental Yeast, Co., Ltd

the experimental period, after 16 h fasting, mice were anesthetized by diethyl ether and blood was collected from the tail vein into a heparin-coated tube, and the plasma was obtained by centrifuging the blood at 10,000g for 10 min at 4 °C for biochemical plasma parameters analyses. Separated plasma was stored at −80 °C until analysis. The liver, kidney and adipose tissues (epididymal and perirenal pads) were immediately excised, rinsed, weighed and frozen in liquid N₂ and stored at −80 °C until use. This experimental design was approved by the Animal Experiment Committee, Chubu University, Japan, and the mice were maintained in accordance with the guidelines.

Biochemical analyses

Plasma levels of glucose, TG and total cholesterol were determined using commercially available enzymatic assay kits (Wako Chemicals, Japan). Plasma concentrations of leptin and adiponectin were measured via immunoassay using a mouse Leptin ELISA Kit (Morinaga, Japan), and Mouse Adiponectin/Acrp30 ELISA Kit (R&D system, USA), respectively, according to the manufacturer's protocol.

Histological analysis

Tissue samples of the epididymal fat pads were fixed with 4% formalin solution and embedded in paraffin.

Sections of 5 μ m were cut and stained with hematoxylin and eosin, viewed with an optical microscope (Olympus, Japan), and photographed at a final magnification of 200X.

RNA isolation and real-time PCR

Total RNA was extracted from frozen adipose tissues using Isogen according to the manufacturer's instructions (Nippon Gene, Japan). Two microgram of total RNA from each sample were reverse-transcribed to cDNA according to the protocol of the reverse transcription system (a3500, Promega, USA). Quantification of gene transcripts for SREBP-1c, FAS, LPL and DGAT1 was completed using gene-specific primers by real-time PCR using the FastStart universal SYBR Green Master PCR kit (Roche, Germany) according to the manufacturer's instructions on an ABI Prism 7700 system (Applied Biosystems, USA). The following primers were used: β -actin, forward, 5'-TGT TAC CAA CTG GGA CGA CA-3', and reverse, 5'-CTC TCAGCTGTG GGT GGT GGT GAA-3'; PPAR γ 2, forward, 5'-GAG CTG ACC CAA TGG TTG CTG-3' and reverse, 5'-GCT TCA ATC GGA TGG TTC TTC 3'; SREBP-1c, forward, 5'-GTG AGC CTG ACA AGC AAT CA-3' and reverse, 5'-ACC AAG CCA GCA AAT ACA CC-3'; FAS, forward, 5'-CTT CGC CAA CTC TAC CAT GG-3', and reverse, 5'-TTC CAC ACC CAT GAG CGA GT-3'; stearoyl-CoA

desaturase-1 (SCD-1), forward, 5'-CCC TCC GGA AAT GAA CGA GAG-3', and reverse, 5'-GCC GGG CTT GTA GTA CCT C-3'; LPL, forward, 5'-GCA TTT GAG AAA GGG CTC TG-3' and reverse, 5'-CTG ACC AGC GGA AGT AGG GAG-3'; DGAT1, forward, 5'-CAG AGC TTC TGC AGT TTG GA-3' and reverse, 5'-CAC AGC TGC ATT GCC ATA GT-3'. β -actin was used as an internal control. Relative mRNA levels were normalized to β -actin mRNA levels and expressed as the values of relative expression relative to that of the HFD group.

Western blot analysis

Epididymal WATs (white adipose tissues) were homogenized in modified RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium dodecylsulfate (SDS), 1 mM phenylmethanesulfonylfluoride (PMSF)). The tissue homogenates were centrifuged (750g, 5 min, 4 °C) to remove nuclei and unbroken cells. The supernatants were further centrifuged (12,000g, 20 min, 4 °C) and the resulting supernatants were used for western blot analysis. The total protein concentrations of whole tissue extracts were determined by Bradford assay (Bio-Rad). Protein samples (30 μ g) were separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, UK). After incubation with 5% (w/v) non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T), the membrane was incubated with the specific antibodies against PPAR γ (Cell Signaling Technology, USA) and SREBP-1 (abCam, USA). Following the incubation with a horseradish peroxidase-conjugated relative IgG secondary antibody (Amersham Biosciences, UK), the immunocomplexes were visualized by enhanced chemiluminescence (Amersham, UK). The exposed films were scanned and the obtained images were subjected to densitometric analysis using Scion Image Release Beta 4.02 software (<http://www.scioncorp.com>). The SREBP-1c and PPAR γ expression levels were expressed relative to those in the HFD group.

Measurement of plasma triglyceride levels after oral administration of lipid emulsion

8 week-old female ICR mice were housed for 1 week as previously described. After overnight fasting

(16 h), they were orally administered 0.5 mL of a lipid emulsion (0.5 mL of olive oil, 40 mg of cholesterol and 1 mg of sodium cholate plus 0.5 mL of saline) or lipid emulsion plus KGE (final concentration, 500 mg/kg or 1,000 mg/kg). Blood samples were taken from the tail vein at 0, 1, 2, 3, 4, 5, 6 and 7 h after administration of the lipid emulsion $\pm$ KGE using a heparin-coated tube, and the plasma was obtained by centrifuging the blood at 10,000g for 10 min at 4 °C. Plasma TG levels were measured using a Wako Triglyceride E-Test kit.

Statistical analysis

Data are expressed as mean $\pm$ standard error of mean (SEM). Comparison between the control and treated groups was analyzed by Student's *t*-tests and one-way analysis of variance (ANOVA) using Origin 7 software (Microcal Software, USA). Difference of $p < 0.05$ were considered statistically significant.

Results

Effect of KGE on body weight and food intake rate

The effect of KGE on body weight gain and food intake rate is shown in Table 2. After 8 weeks, the body weight gain of the HFD group was significantly higher than that of the LFD group ($p < 0.005$). The body weight gain was significantly reduced in the HFD + 1.6KGE group compared with the HFD group ($p < 0.05$). In the HFD + 0.8KGE group, the body weight gain tended to be lower than in the HFD group, although the difference was not significant. The food intake rate of the LFD group was higher than that of the HFD group ($p < 0.05$), but there was no significant difference in food intake between the HFD and HFD + KGE groups.

Effect of KGE on organ and white adipose tissue weights

The effect of KGE on organ and WAT weights are shown in Table 3. The weight of WAT in the HFD group was significantly increased compared with the LFD group ($p < 0.05$). Moreover, mean WAT weight in the HFD + 1.6KGE group was significantly

Table 2 Effect of KGE on body weight and food intake rate in HFD-induced obese mice for 8 weeks

Group	Body weight (g)			Food intake rate (g/mouse/day)
	Initial	Final	Gain	
LFD	23.95 ± 0.34	29.44 ± 0.94	5.76 ± 0.81	4.30 ± 0.52
HFD	24.02 ± 0.32	37.01 ± 1.68	11.90 ± 1.35***	3.44 ± 0.03*
HFD + 0.8KGE	23.97 ± 0.32	33.08 ± 1.74	8.46 ± 1.21	3.40 ± 0.34
HFD + 1.6KGE	23.98 ± 0.33	32.45 ± 0.95	8.28 ± 0.95 [#]	3.62 ± 0.06

LFD low-fat diet, HFD high-fat diet, KGE Korean white ginseng extracts. Data are expressed as mean ± SEM ($n = 8$)

* $p < 0.05$ and *** $p < 0.005$ vs. LFD; [#] $p < 0.05$ vs. HFD

Table 3 Effect of KGE on organ weights in HFD-induced obese mice for 8 weeks (g/100 g body weight)

Group	WAT	Liver	Kidney
LFD	1.60 ± 0.31	5.83 ± 0.19	1.13 ± 0.05
HFD	4.16 ± 0.76*	5.68 ± 0.18	1.01 ± 0.04
HFD + 0.8KGE	3.24 ± 0.76	5.65 ± 0.21	1.08 ± 0.04
HFD + 1.6KGE	2.31 ± 0.27 [#]	5.52 ± 0.24	1.08 ± 0.03

LFD: low-fat diet; HFD: high-fat diet; KGE: Korean white ginseng extracts. Data are expressed as mean ± SEM ($n = 8$).

* $p < 0.05$ vs. LFD; [#] $p < 0.05$ vs. HFD

decreased compared with the HFD group ($p < 0.05$). However, there were no significant differences in kidney and liver weight to body weight ratios among the four groups. In the HFD + 0.8KGE group, WAT weight tended to be lower compared with the HFD group, but not significantly so.

Effect of KGE on plasma biochemical parameters

The effect of KGE supplementation on plasma biochemical parameters in HFD-fed mice are shown in Table 4. Plasma TG levels in the HFD group were increased compared with those in the LFD group ($p < 0.005$). Compared with the HFD group, plasma TG levels of the HFD + 0.8KGE and HFD + 1.6KGE

groups were significantly decreased ($p < 0.05$ and $p < 0.01$, respectively). Total plasma cholesterol and glucose levels in the HFD group were significantly increased compared with the LFD group ($p < 0.01$ and $p < 0.005$, respectively), but there were no significant differences between the HFD and the HFD + KGE groups.

Effect of KGE on plasma adiponectin and leptin levels

The effect of KGE on plasma adiponectin and leptin levels is shown in Fig. 1. Plasma adiponectin levels in the HFD group were significantly decreased compared with those in the LFD group ($p < 0.05$). However, plasma adiponectin levels were not significantly different between the HFD and the HFD + KGE groups. Plasma leptin levels were significantly higher in the HFD versus the LFD group ($p < 0.05$). However, plasma leptin levels were decreased in the HFD + 1.6KGE group compared with the HFD group ($p < 0.05$).

Histological analysis of WAT

The sizes of epididymal adipocyte is shown for each group in Fig. 2. The sizes of adipocyte in the HFD

Table 4 Effect of KGE on plasma biochemical parameters in HFD-induced obese mice for 8 weeks

Group	TG (mg/dL)	T-CHO (mg/dL)	Glucose (mg/dL)
LFD	93.55 ± 7.50	94.74 ± 5.06	96.15 ± 5.65
HFD	150.62 ± 12.64***	135.73 ± 10.58**	120.55 ± 6.01***
HFD + 0.8KGE	100.98 ± 10.4 [#]	118.81 ± 8.66	109.95 ± 4.62
HFD + 1.6KGE	96.37 ± 8.54 ^{##}	117.56 ± 9.26	112.1 ± 4.73

LFD low-fat diet, HFD high-fat diet, KGE Korean white ginseng extracts, TG triglyceride; T-CHO total cholesterol. Data are expressed as mean ± SEM ($n = 8$)

** $p < 0.01$ and *** $p < 0.005$ vs. LFD; [#] $p < 0.05$ and ^{##} $p < 0.01$ vs. HFD

Fig. 1 Effects of KGE on plasma adipokine levels in HFD-induced obese mice. Plasma adiponectin (a) and leptin (b) levels were measured from mice fasted for 16 h before collecting blood as described in the “Materials and methods” section. LFD: low-fat diet; HFD: high-fat diet; KGE: Korean white ginseng extracts. Data are expressed as mean $\pm$ SEM ($n = 4$ –5/group). * $p < 0.05$ vs. LFD, # $p < 0.05$ vs. HFD

Fig. 2 Histology of epididymal adipose tissue of mice fed on experimental diet for 8 weeks. All sections were stained with hematoxylin and eosin; magnification, $\times 200$

group were significantly larger than those of the LFD group. The sizes of adipocytes were considerably decreased in HFD + 1.6KGE group as compared with HFD, but not in HFD + 0.8KGE group.

Fig. 3 Effect of KGE on mRNA levels of lipogenesis-related genes in WAT of HFD-induced obese mice. Mice were fed a LFD, HFD or HFD containing 0.8 and 1.6% (w/w) KGE for 8 weeks. **a** Relative mRNA levels of lipogenesis related genes were measured using a quantitative real-time RT-PCR as described in the “Materials and methods” section. The amount of mRNA was normalized to that of β -actin and expressed relative to the HFD group. **b** Protein levels of SREBP-1 and PPAR γ 2 were determined by western blot as described in the “Materials and methods” section. Protein level was expressed as the fold relative to the HFD group after normalized by β -actin level. LFD: low-fat diet; HFD: high-fat diet; KGE: Korean white ginseng extracts. Data are expressed as mean $\pm$ SEM ($n = 4$ –5/group). * $p < 0.05$ vs. LFD, # $p < 0.05$ and ## $p < 0.01$ vs. HFD

Effect of KGE on expression of lipogenesis-related genes and proteins

To investigate whether the anti-obesity effect of KGE is related to a reduction in lipogenesis in WAT, the levels of lipogenesis-related mRNA and proteins in WAT were analyzed using real-time PCR and western blot. As shown in Fig. 3a, the mRNA levels of SREBP-1c, LPL and DGAT1 in WAT were increased in the HFD group compared with the LFD group ($p < 0.05$). However, expression levels of

these genes in the HFD + 1.6KGE group were significantly reduced compared with the HFD group ($p < 0.05$). Although mRNA levels of PPAR γ 2 and FAS were not significantly different between LFD group and HFD group, they were significantly reduced in the HFD + 1.6KGE group compared with the HFD group ($p < 0.01$). The mRNA levels of SREBP-1c were significantly reduced in the HFD + 0.8KGE group compared with the HFD group ($p < 0.05$). Although the mRNA levels of SCD-1 in the HFD + 1.6KGE group and the mRNA levels of LPL, FAS and DGAT1 tended to be lower in the HFD + 0.8KGE group compared with the HFD group, the difference was not significant. PPAR γ 2 protein expression level in WAT showed a tendency to decrease in the HFD + 1.6KGE group compared with the HFD group, but the difference was not significant. SREBP-1 protein level was reduced in the HFD + 1.6KGE group compared with the HFD group ($p < 0.05$) (Fig. 3b). Although these protein levels tended to be lower in the HFD + 0.8KGE group compared with the HFD group, the difference was not significant.

Effect of KGE on plasma TG levels after oral administration of lipid emulsion

The effect of KGE on plasma TG levels after oral administration of lipid emulsion is shown in Fig. 4. At 1, 2, 3 and 4 h post-administration of a dose of 1,000 mg/kg KGE, mean plasma TG levels were significantly lower than in the control group ($p < 0.05$).

Fig. 4 Effect of KGE on plasma TG levels after an oral administration of lipid emulsion. After fasting 16 h, mice were orally administrated 0.5 mL of lipid emulsion with or without KGE. Plasma TG levels were measured as described in the “Materials and methods” section. Data are expressed as mean $\pm$ SEM ($n = 8$). * $p < 0.05$ vs. group treated with lipid emulsion

Discussion

Dietary fat is one of the most important environmental factors associated with the incidence of obesity and chronic disease such as hypertension, diabetes and hyperlipidemia (Björntorp 1997; Kopelman 2000; Spiegelman and Flier 2001). For 1,000 years, ginseng has been used in herbal medicine as a general tonic to promote health in Oriental societies (Yun et al. 2004; Han et al. 2005). The present study investigated the anti-obesity effect of KGE in HFD-induced obese mice. It has previously been shown that HFD is a good strategy for inducing obesity (Kim et al. 2000). The HFD thus leads to an increase in body weight, adipose tissue weight and hyperlipidemia in animals. Weight reduction occurs when adipose tissue mass decreases as a result of reduced adipocyte differentiation or by decreased adipocyte size (Rosenbaum et al. 1997). In our study, we found that body weight gain, WAT weight, adipocyte size and plasma TG levels were reduced in the HFD + 1.6KGE group, compared with the HFD group, without changing food intake. In the HFD + 0.8KGE group, only the plasma TG levels were significantly decreased without changes in body weight gain or WAT weight. These results suggest that KGE exerts the anti-obesity effect as well as a hypolipidemic effect in HFD-induced obese mice. And they are in accordance with previous reports that the supplementation of ginseng extracts and ginsenosides has anti-obesity and hypolipidemia in HFD-induced obese animals and obese type 2 diabetic animals, in spite of not showing the anti-diabetic effect in other reports (Yamamoto et al. 1983; Chung et al. 2001; Attele et al. 2002; Yoon et al. 2003; Yun et al. 2004; Kim et al. 2005; Karu et al. 2007). We expect that difference would be caused by the differences in dietary fat content, feeding period, kind of experimental and components of ginseng extracts.

Leptin is produced by adipose tissue, and regulates food intake and energy expenditure. Adiponectin is secreted by fat cells, and has insulin-sensitizing properties. These two adipokines, leptin and adiponectin, have been reported to positively or negatively correlate with obesity, respectively (Fried et al. 2000; Ahima 2006). In our study, the plasma leptin levels as well as the adipocytes sizes in the HFD + 1.6KGE group were decreased compared with the HFD group.

This observation suggests that the decreased plasma leptin levels in the HFD + 1.6KGE group may be attributable to reductions in adipose tissue. However, the plasma adiponectin levels were not significantly different between the HFD and HFD + KGE groups.

Differentiation of preadipocytes and the induction of metabolic pathways related to lipid metabolism were associated with the expression of genes controlling lipogenesis and lipolysis (Kim et al. 1998). To clarify the mechanism for the anti-obesity effect of KGE, we focused on the expression levels of several lipogenesis-related genes. PPAR γ is a nuclear receptor that regulates the expression of genes involved in adipocyte differentiation and lipid metabolism. A previous report has demonstrated that PPAR γ 2 expression was significantly increased in WAT of HFD fed obese mice (Vidal-Puig et al. 1996). SREBP-1c is a major transcription factor involved in the activation of lipogenic genes such as FAS and SCD-1 (Huang et al. 2008). The selective deletion of the SREBP-1c isoform results in decreased expression of enzymes involved in fatty acid and TG synthesis (Liang et al. 2002). It also regulates PPAR γ expression and production of endogenous ligand for PPAR γ , resulting in enhancement of the transcriptional activity of PPAR γ (Brown and Goldstein 1997). There are several reports that ginseng regulates the expression of PPAR γ and SREBP-1c. It has been reported that *Panax notoginseng* extracts decrease SREBP-1c mRNA expression level in liver of rat fed high-fat and high-cholesterol diet (Ji and Gong 2007). And, it has been variable effects on expression and activity of PPAR γ . Some studies have reported that ginseng increases the PPAR γ mRNA expression in adipose tissue (Park et al. 2005; Mollah et al. 2009) and in some studies, it decreases. For instance, ginsenoside Rb1, Rg1, Rg3 and Rh2 decreased the lipid accumulation and PPAR γ mRNA expression levels in 3T3-L1 adipocytes (Hwang et al. 2007; Park et al. 2008; Hwang et al. 2009), and ginsenoside Rh2 and Rg3 have been reported as an antagonist for PPAR γ (Hwang et al. 2007; Hwang et al. 2009). In our study, expression levels of mRNA and protein expression both of PPAR γ 2 and SREBP-1 levels were decreased in WAT of KGE fed mice. In addition, FAS mRNA expression was also decreased by KGE. These results may explain the decreased lipogenesis and anti-obesity effect of KGE, at least partially, although the study of PPAR γ ligand activity and effect for

SREBP-1c of KGE remains to be elucidated. LPL is a key enzyme involved in metabolism and transport of lipid (Wang and Eckel 2009). Several reports have shown that reduction of adipose deposition is caused by decreasing LPL mRNA expression levels (Naaz et al. 2003; Yang et al. 2007). DGAT1 is the enzyme which catalyzes the final step in the TG synthesis pathway and considered to play key regulatory role in TG synthesis (Chen and Farese 2000). DGAT1-deficient mice showed a reduced adipose mass in comparison to wild-type mice on a chow diet (Smith et al. 2000; Chen and Farese 2005). As shown in Fig. 3, the mRNA levels of LPL and DGAT1 were markedly decreased in the WAT of the HFD + 1.6KGE group, compared with the HFD group. This finding might explain the decreased fat uptake and an additional control step in the reduced adiposity in HFD + 1.6KGE group. These results suggest that KGE might suppress the accumulation of fat mass by down-regulating the mRNA expression of genes involved in lipogenesis. In addition, we investigated effects of KGE on expression levels and phosphorylation of energy expenditure related to proteins such as AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase 2 (ACC) in WAT and muscle. There was no significant difference in AMPK and ACC phosphorylation between the HFD and HFD + KGE groups (data not shown).

It is well known that dietary fat is not absorbed from the intestine unless it has been subjected to the action of pancreatic lipase during the digestion process. The products, fatty acid and 2-monoacylglycerols, formed by hydrolysis of pancreatic lipase are absorbed in the lumen of the gut (Verger 1984; Hernell et al. 1990; Astrup et al. 1994). Therefore, inhibition of hydrolysis of dietary fat may decrease intestinal absorption of fat, leading to a reduction in obesity and hyperlipidemia. Previous studies report that saponin in *Panax ginseng*, *Panax japonicus rhizomes*, *Platycodon grandiflorum* and teasaponin of *Thea sinensis* exhibited strong inhibitory effects on pancreatic lipase in vitro and elevation of TG levels after lipid emulsion tolerance test in vivo (Han et al. 2001, 2002, 2005; Karu et al. 2007). As shown in Fig. 3, we found that 1,000 mg/kg KGE significantly inhibited the elevation of plasma TG levels after oral administration of a lipid emulsion. This observation suggests that KGE may delay the absorption of dietary fat via the inhibition of pancreatic lipase.

In summary, KGE may prevent the development of obesity and hyperlipidemia in HFD-induced obese mice. These effects of KGE are mediated by delaying the intestinal absorption of dietary fat and regulating the mRNA expression of genes involved in lipogenesis in WAT.

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