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Korean red ginseng (*Panax ginseng*) inhibits obesity and improves lipid metabolism in high fat diet-fed castrated mice

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ABSTRACT

Ethnopharmacological relevance: Korean red ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) has been historically used as a traditional drug for the prevention and treatment of most ageing-related diseases, such as obesity, dyslipidemia, diabetes, and cardiovascular disease. Elderly men with testosterone deficiency are strongly associated with many of the aforementioned metabolic diseases. The aim of this study was to determine the effects of ginseng on obesity and lipid metabolism in a mouse model of testosterone deficiency (castrated C57BL/6J mice).

Materials and methods: The effects of ginseng extract (GE) on obesity and lipid metabolism in high-fat diet (HFD)-fed castrated C57BL/6J mice were examined using hematoxylin and eosin staining, serum lipid analysis, and quantitative real-time polymerase chain reaction (PCR). The effects of GE, ginsenosides, and testosterone on adipogenesis were measured using Oil Red O staining, XTT assay, and real-time PCR.

Results: Compared with HFD mice, mice receiving HFD supplemented with GE (HFD-GE) for 8 weeks showed decreased body weight, adipose tissue mass, and adipocyte size without affecting food intake. Serum levels of triglycerides and total cholesterol were lowered in HFD-GE mice than in HFD mice. GE also markedly reduced HFD-induced hepatic lipid accumulation. Concomitantly, HFD-GE decreased mRNA expression of adipogenesis-related genes (SREBP-1C, PPARγ, FAS, SCD1, and ACC1) in visceral adipose tissues compared with HFD alone. Consistent with the in vivo data, GE and major active ginsenosides (Rb1 and Rg1) decreased lipid accumulation and mRNA expression of PPARγ, C/EBPα, and SCD1 in 3T3-L1 adipocytes compared with control. Similarly, testosterone also decreased lipid accumulation and mRNA levels of PPARγ, C/EBPα, and SCD1. These inhibitory effects were further increased by co-treatment of GE or ginsenosides with testosterone.

Conclusions: Our results demonstrate that ginseng can inhibit obesity and dyslipidemia in HFD-fed castrated mice, possibly by inhibiting adipogenic gene expression. In addition, our results indicate that ginseng may act like testosterone to inhibit adipogenesis, suggesting that ginseng may be able to prevent obesity, hyperlipidemia, and hepatic steatosis in men with testosterone deficiency.

1. Introduction

Obesity is the result of energy imbalance caused by an increased ratio of caloric intake to energy expenditure. The prevalence of obesity has rapidly increased in adult male men, and the related metabolic disorders of dyslipidemia, atherosclerosis, and type 2 diabetes have become global health problems.

Herbs have long been used to treat illness and improve health. In fact, herbs still account for about 80% of medical treatments in the developing countries, and approximately one-third of pharmaceutical drugs are derived from plant sources (Winslow and Kroll, 1998; Bent and Ko, 2004; Kang et al., 2010). Ginseng, in particular, has widely been used as a traditional herbal medicine in Korea, China, and Japan for more than 2,000 years (Yun, 2001; Park et al., 2012). Numerous studies have described the pharmacological effects of ginseng on the immune, central nervous, endocrine, and cardiovascular systems (Gillis, 1997; Attele et al., 1999; Lu et al., 2009). Ginseng has also been traditionally used in the prevention and treatment of most ageing-related metabolic syndrome, such as obesity, diabetes, and hyperlipidemia (School, 1986; Yin et al., 2008). Recently, ginseng is reported to induce weight loss in animal models of obesity and can effectively regulate signaling pathways and genes that play a role in obesity (Attele et al., 2002; Kim et al., 2005; Karu et al., 2007; Mollah et al., 2009; Lee et al., 2009, 2012). In addition, ginseng significantly inhibits visceral

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adipocyte hypertrophy (Lee et al., 2013, 2014, 2016), which is closely associated with metabolic syndromes including insulin resistance and hepatic steatosis (Jeong and Yoon, 2009; Lee et al., 2014). Our previous study demonstrated that ginseng decreases adipose tissue mass and prevents obesity in diet-induced and ovariectomized obese mice (Lee et al., 2013, 2014, 2016).

Obesity in the ageing men is associated with lowered testosterone levels (Michalakis et al., 2013; Fui et al., 2014; Traish, 2014; Kelly and Jones, 2015). Recent studies have shown that obese men have lower testosterone levels compared with lean men, and testosterone therapy in both ageing and hypogonadal men with testosterone deficiency results in weight loss and a lower risk of metabolic syndrome (Yassin and Doros, 2013; Francomano et al., 2014). Thus, we hypothesized that Korean red ginseng is able to inhibit weight gain and regulate obesity in castrated male mice, an animal model of men with testosterone deficiency, by regulating the expression of adipogenesis-associated genes.

In this study, we fed castrated mice a high-fat diet (HFD) supplemented with Korean red ginseng extract (GE). We found that treatment with GE decreases body weight gain, adipose tissue mass, adipocyte size, and inhibits dyslipidemia and hepatic steatosis. Concomitantly, adipogenesis-related gene expression was lowered by GE in HFD mice. Similarly, treatment with GE and ginsenosides inhibits lipid accumulation and adipogenic gene expression in 3T3-L1 adipocytes. In addition, testosterone also decreased lipid accumulation and mRNA levels of adipogenesis-ssociated genes. These inhibitory effects were more increased by co-treatment of GE or ginsenosides with testosterone. Our findings suggest that ginseng can regulate obesity and lipid metabolism in testosterone deficiency.

2. Materials and methods

2.1. GE preparation

The GE was prepared from 6-year-old *Panax ginseng* C. A. Meyer (Korea Ginseng Corporation, Seoul, Korea). A voucher specimen was deposited at the laboratory of Korea Ginseng Corporation and the batch number of ginseng used in our study is 6510100112048. Briefly, red ginseng was boiled in distilled water for 24 h at 95 °C. The aqueous extracts were filtered and freeze-dried under vacuum to produce GE powder.

For analysis of the quality of GE, GE powder (100 g) was placed into a 1-L flask with a refluxing condenser and extracted twice with 500 ml of water-saturated 1-butanol for 1 h at 80 °C. The extracted solution was passed through Whatman filter paper (No. 41) after cooling. The process was repeated twice. The residue and filter paper were washed with 100 ml of water-saturated 1-butanol, and then the filtrate was washed twice with 100 ml of water in a 2-L separating funnel. The butanol layer was then evaporated to dryness. The concentrate was extracted to remove any traces of fat with 100 ml of diethyl ether for 30 min at 36 °C in a flask with a refluxing condenser, after which the ether solution was decanted. The quality control of GE was analyzed by the HPLC/ELSD system and the HPLC profile of GE was described previously (Lee et al., 2014). The contents of ginsenosides in GE were reported previously and GE contained ginsenosides Rb1 and Rg1 (5 mg/g) as major active compounds (Lee et al., 2016).

2.2. Animal treatments

For all experiments, 8-week-old male wild-type C57BL/6 J mice were housed and bred at Mokwon University with a standard 12-h light/dark cycle. Prior to the administration of the special diets, the mice were given standard rodent chow and water ad libitum. The mice were castrated and then divided into three groups (n = 8/group). The first group received a low-fat diet (LFD, 10% kcal fat, Research Diets, Brunswick, NJ, USA) for 8 weeks. The second group received an HFD (45% kcal fat, Research Diets). The third group received an HFD supplemented with 5% GE (HFD-GE). For the HFD-GE preparation, 50 g GE powder was mixed with 1 kg HFD (Lee et al., 2013). Body weight was measured three times a week by a person blinded to each treatment group. Food intake was determined by estimating the amount of food consumed by the mice throughout the treatment period. Cages were inspected for food spillage, but only a little spillage was noticed and collected to measure food intake. After an 8-h fast on the last day of the study, the animals were sacrificed by cervical dislocation. Blood was collected from the retro-orbital sinus into tubes, and serum was separated and stored at −80 °C until analysis. Visceral (VSC) fat pads were removed, weighed, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Portions of the VSC fat pads and liver tissues were prepared for histology. Serum triglyceride levels were measured using an automatic blood chemical analyzer (CIBA Corning, Oberlin, OH). Levels of total cholesterol were measured using SICDIA NEFAZYME (Shinyang Chemical, Seoul, Korea). All animal experiments were approved by the Institutional Animal Care and Use Committees of Mokwon University and were carried out in accordance with the National Research Council Guidelines.

2.3. Histological analysis

The liver and VSC adipose tissues were fixed in 10% phosphate-buffered formalin for 1 day and processed for paraffin sections. Tissue sections (5 μm) were cut and stained with hematoxylin and eosin for examination by microscopy. To quantify adipocyte size, the stained sections were analyzed using the Image-Pro Plus analysis system (Media Cybernetics, Bethesda, MD, USA).

2.4. In vitro cytotoxicity test

Murine 3T3-L1 cells (ATCC, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (Invitrogen, Carlsbad, CA, USA) in a 37 °C incubator with a humidified atmosphere containing 5% CO_2. Cells were plated on a 96 well plate at a density of 1×10^4 cells/well and incubated for 24 h at 37 °C with culture medium in the presence of dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), and testosterone (100 nM). Cell viability was detected by 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl] − 2H-tetrazolium-5-carboxanilide disodium salt (XTT) assays using a Cell Proliferation Kit II (Roche, Basel, Switzerland).

2.5. 3T3-L1 differentiation and analysis of triglyceride content

3T3-L1 cells grown in DMEM containing 10% bovine calf serum were maintained at confluence for 2 days, after which the medium was replaced with DMEM containing 0.5 mM 1-methyl-3-isobutyl-xanthine, 1 μM dexamethasone, and 1 μg/ml insulin, and 10% fetal bovine serum (Invitrogen) (day 0). The cultures were incubated for 2 days to induce adipocyte differentiation, and then the medium was replaced with DMEM containing 10% fetal bovine serum for the remainder of the differentiation process. The cells were treated with dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), and testosterone (100 nM) on days 0–2 only, and the medium was changed every other day. Dimethyl sulfoxide was used as a solvent vehicle. On day 8, the cells were fixed in 10% formalin for 1 h and stained with Oil Red O for 2 h. For quantitative analysis, the Oil Red O stain was eluted by adding isopropanol and quantified by measuring absorbance at 520 nm.

2.6. Quantitative real-time polymerase chain reaction (PCR)

Total cellular RNA from VSC adipose tissues and 3T3-L1 cells was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY, USA) according to the manufacturer's instructions. Total cellular RNA (2 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase to generate an antisense cDNA template. The genes of

Table 1
Sequences of primers used for quantitative real-time PCR assays.

Genes	Gene bank	Primer sequences
Mouse		
ACC1	NM_133360	Forward: 5′-GGGCTACCTCTAATGGTCTT-3′ Reverse: 5′-CTACCTGATGGTAAATGGGA-3′
β-actin	NM_007393.5	Forward: 5′- TGGAATCCTGTGGCATCCATGAAA -3′ Reverse: 5′- TAAAACGCAGCTCAGTAACAGTCCG -3′
C/EBPα	NM_007678	Forward: 5′-AGACATCAGCGCCTACATCG-3′ Reverse: 5′-TGCAGGTGCATGGTGGTC-3′
FAS	NM_007988	Forward: 5′-CTTGGGTGCTGACTACAACC-3′ Reverse: 5′-GCCCTCCCGTACACTCACTC-3′
PPARγ	NM_013124	Forward: 5′- ATTCTGGCCCACCAACTTCGG -3′ Reverse: 5′- TGGAAGCCTGATGCTTTATCCCCA -3′
SCD1	NM_009127.4	Forward: 5′- CGGAAATGAACGAGAGAAGG -3′ Reverse: 5′- CCGAAGAGGCAGGTGTAGAG -3′
SREBP-1c	BC056922	Forward: 5′- CTTCTGGAGACATCGCAAAC -3′ Reverse: 5′- GGTAGACAACAGCCGCATC -3′
Human		
β-actin	NM_001101.3	Forward: 5′- GCAAGAGAGGCATCCTCACC -3′ Reverse: 5′- CGTAGATGGGCACAGTGTGG -3′
C/EBPα	NM_004364	Forward: 5′-TGAGCAGCCACCTGCAGAG-3′ Reverse: 5′-GCCAGGAACTCGTCGTT-3′
FAS	NM_004104	Forward: 5′-AGCTGCCAGAGTCGGAGAAC-3′ Reverse: 5′-TGTAGCCACGAGTGTCTCG-3′
PPARγ	NM_005037	Forward: 5′-GCAGGAGCAGAGCAAAGAGGTG-3′ Reverse: 5′-AAATATTGCCAAGTCGCTGTCATC-3′
SCD1	NM_005063.4	Forward: 5′- CATAATTCCCGACGTGGCTTT -3′ Reverse: 5′- AGGTTTGTAGTACCTCCTCTGGAACA -3′

interest were amplified from the synthesized cDNA using *Accu*Power® GreenStar™ qPCR PreMix (Bioneer, Deajeon, Korea) on an Excycler™ 96 Real Time Quantitative Thermal Block machine (Bioneer). The PCR primers used for gene expression analysis are shown in Table 1. PCR was performed using the following conditions: denaturing at 95 ˚C for 5 min followed by 50 cycles of 95 ˚C for 10 s, 60 ˚C for 40 s, and 72 ˚C for 10 s. Transcript concentrations were calculated as copies per μl using a standard curve, and the relative expression levels were calculated as the ratio of target gene cDNA to β-actin cDNA.

2.7. Statistical analysis

All values are expressed as mean ± standard deviation (SD). Groups were compared by analysis of variance followed by Tukey's multiple comparison test; $p < 0.05$ was considered significant.

3. Results

3.1. Effects of GE on body weight gain, VSC adipose tissue mass, and adipocyte size in HFD-fed castrated mice

Body weight gain and VSC adipose tissue mass were measured in male castrated C57BL/6J mice on an LFD, HFD, and HFD-GE for 8 weeks. After 8 weeks of treatment, HFD obese mice had 166% greater body weight gains compared with LFD mice (16.14 ± 1.42 g vs. 6.06 ± 0.41 g, respectively) ($p < 0.05$; Fig. 1A). In contrast, HFD-GE mice had 47% (8.50 ± 1.1.51 g) lower body weights compared to HFD mice ($p < 0.05$). As shown in Fig. 1B, VSC adipose tissue mass in HFD-GE mice was reduced in comparison to that of HFD mice. VSC adipose tissue weight was decreased by 31% after GE treatment ($p < 0.05$). Histological analysis revealed that GE treatment decreased mean VSC adipocyte size in HFD-fed mice. The VSC adipocyte size was decreased by 19% in HFD-GE mice (8143 ± 259 μm^2) compared with that of HFD mice (9993 ± 259 μm^2; $p < 0.05$; Fig. 1C and D). In addition, HFD mice had higher food consumption compared to HFD mice, but there were no significant differences in food consumption profiles between HFD-GE and HFD mice throughout the study (Fig. 1E).

3.2. Effects of GE on serum lipid levels in HFD-fed castrated mice

Compared to LFD mice, HFD mice had increased serum levels of triglycerides and total cholesterol ($p < 0.05$; Fig. 2). However, HFD-GE mice exhibited a significant reduction in serum concentrations of triglycerides and total cholesterol compared with HFD mice ($p < 0.05$).

3.3. Effects of GE on hepatic steatosis in HFD-fed castrated mice

Hepatic lipid accumulation was evaluated by hematoxylin and eosin staining of liver sections. Our results showed that hepatic lipid accumulation was evident in HFD mice (Fig. 3). However, treatment with GE considerably lowered hepatic lipid accumulation in HFD mice. Furthermore, triglyceride droplets were almost abolished in HFD-GE mice compared with HFD mice.

3.4. Effects of GE on adipogenesis-related gene expression in VSC adipose tissues of HFD-fed castrated mice

Expression patterns of genes involved in adipogenesis were investigated in VSC adipose tissues of the castrated mice. Administration of HFD increased mRNA levels of sterol regulatory element-binding protein 1c (SREBP-1c), peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα), fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), and acetyl-CoA carboxylase 1 (ACC1) in VSC adipose tissue compared with LFD ($p < 0.05$; Fig. 4). However, treatment with GE decreased SREBP-1c, PPARγ, FAS, SCD1, and ACC1 in HFD mice compared with HFD alone ($p < 0.05$).

3.5. Inhibition of lipid accumulation by GE, ginsenosides, and testosterone in 3T3-L1 adipocytes

We then examined the ability of GE, major active ginsenosides (Rb1 and Rg1), and testosterone to prevent lipid accumulation in 3T3-L1 cells. After incubation in differentiation medium, lipid droplets were markedly increased in 3T3-L1 cells (control), as shown by the increase in Oil Red O staining, compared with undifferentiated cells (ND) (Fig. 5A). However, GE (10 μg/ml) and ginsenosides Rb1 and Rg1 (10 μM each) significantly decreased lipid accumulation by 54%, 43%, and 35%, respectively, compared with control ($p < 0.05$; Fig. 5B). Testosterone (100 nM) also decreased fat accumulation by 26% in these cells compared with control. Combination of GE, Rb1, or Rg1 with testosterone further decreased triglyceride droplets by 47%, 38%, or 26%, respectively, compared with testosterone alone ($p < 0.05$). Inhibition of lipid accumulation by GE, Rb1, Rg1, and testosterone was not due to cytotoxic effects, because XTT assays showed that all treatments had no effects on cell viability (Fig. 5C).

3.6. Effects of GE, ginsenosides, and testosterone on adipogenic gene expression in 3T3-L1 adipocytes

Adipogenesis gene expression was increased in differentiated control cells compared with ND cells (Fig. 6). In contrast, treatment with GE significantly decreased mRNA levels of PPARγ, C/EBPα, FAS, and SCD1 compared with control ($p < 0.05$). Rb1 and Rg1 decreased PPARγ, C/EBPα, and SCD1 mRNA levels. Testosterone also decreased mRNA levels of PPARγ, C/EBPα, and SCD1. Co-treatment of GE or ginsenosides with testosterone further decreased mRNA levels of these genes compared with testosterone alone ($p < 0.05$).

4. Discussion

In many model systems, GE made from the root, rootlet, berry, and

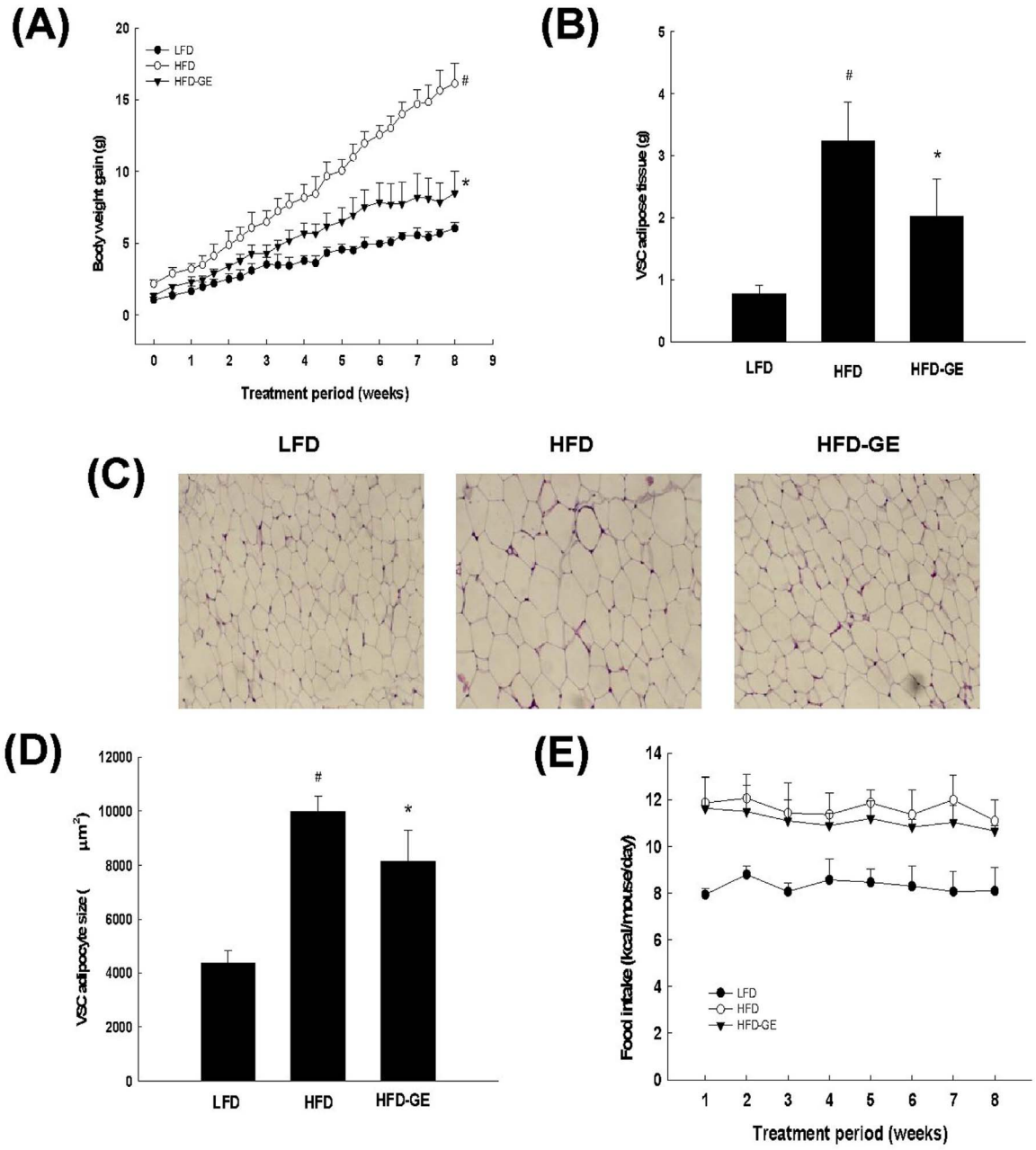

Fig. 1. Regulation of body weight gain, visceral (VSC) adipose tissue mass, VSC adipocyte size, and food intake by ginseng extract (GE) in high-fat diet (HFD)-fed castrated mice. Adult castrated mice (n = 8/group) were fed a low-fat diet (LFD), HFD, or HFD supplemented with GE (HFD-GE) for 8 weeks. (A) Body weight gain at the end of the treatment period. (B) VSC adipose tissue weights. (C) Histology of VSC adipose tissue. Representative hematoxylin-eosin-stained VSC adipose tissue sections (original magnification × 100). (D) VSC adipocyte size. The size of adipocytes in a fixed area (1000,000 μm^2) was measured. (E) Effects of GE on food intake. All values are expressed as mean ± SD. # $p < 0.05$ versus LFD group. * $p < 0.05$ versus HFD group.

leaf of *Panax ginseng*, *Panax quinquefolium* and *Panax notoginseng* has historically demonstrated antiobesity, antihyperglycemic, insulin sensitization, lipid-lowering effects (School, 1986; Yin et al., 2008). Based on reports showing that GE regulates obesity (Lee et al., 2013, 2014, 2016) and that low testosterone leads to adiposity and obesity (Fui et al., 2014; Kelly and Jones, 2015), we examined the effects of ginseng on obesity and lipid metabolism in testosterone-deficient castrated mice. Our results indicate that GE can prevent obesity and dyslipidemia in HFD-fed castrated mice, and these effects are mediated in part by inhibiting the expression of adipogenesis-related genes.

We found that HFD-GE mice exhibited significantly decreased body weight gain and VSC adipose tissue mass by 47% and 31%, respectively, compared with HFD mice. Previous studies have shown that ginseng root extract significantly decreased body weight and adipose tissue mass in other animal models of obesity, such as Otsuka Long-Evans Tokushima fatty rats, *ob/ob* mice, and HFD-fed Sprague-Dawley rats (Mollah et al., 2009; Lee et al., 2009, 2012). In addition, our previous results demonstrated that Korean red ginseng decreases fat mass and inhibits obesity in HFD-fed obese C57BL/6J mice (Lee et al., 2013). However, this study is the first to provide evidence that supports the ability of ginseng to inhibit obesity in testosterone-deficient obese mice.

Obesity is the result of increased adipocyte size (hypertrophy) and number (hyperplasia). In our study, VSC adipocytes were smaller in HFD-GE mice than in HFD mice. These results indicate that GE effectively inhibits VSC adipocyte hypertrophy in HFD-fed castrated mice. It is known that VSC obesity due to adipocyte hypertrophy indicates adipose tissue dysfunction and is closely associated with various metabolic syndromes, including insulin resistance, type 2 diabetes,

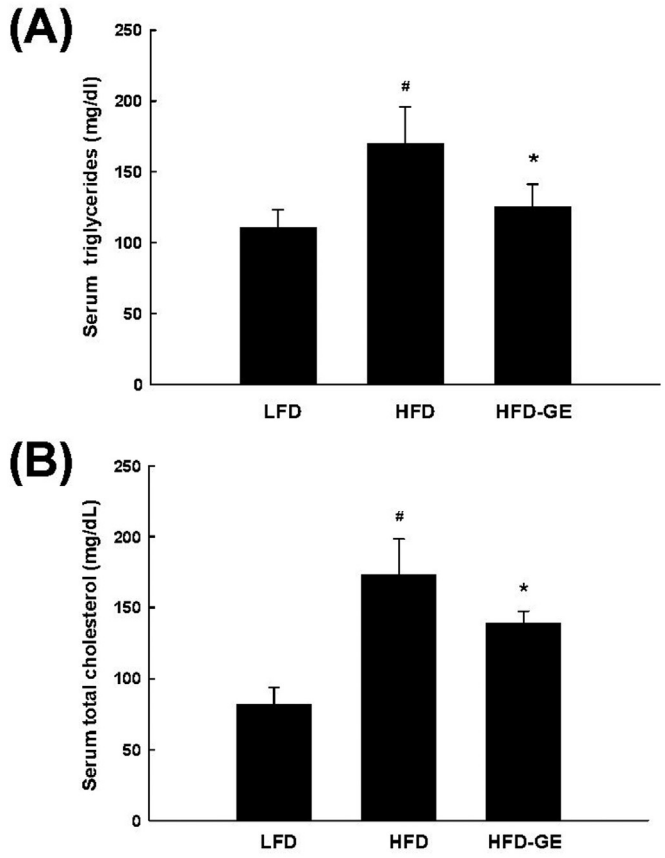

Fig. 2. Changes in circulating triglycerides and total cholesterol by ginseng extract (GE) in high-fat diet (HFD)-fed castrated mice. Adult castrated mice (n = 8/group) were fed a low-fat diet (LFD), HFD, or HFD supplemented with GE (HFD-GE) for 8 weeks. Serum concentrations of triglycerides (A) and total cholesterol (B) were measured and all values are expressed as mean ± SD. # $p < 0.05$ versus LFD group. * $p < 0.05$ versus HFD group.

dyslipidemia, atherosclerosis, and nonalcoholic fatty liver disease (Jeong and Yoon, 2009; Laforest et al., 2015; Oh et al., 2015; Kim et al., 2017), suggesting that ginseng may reduce metabolic disease by inhibiting adipocyte hypertrophy.

Consistent with the effects of GE on VSC adipocyte size, GE inhibited hepatic lipid accumulation in HFD-fed castrated mice. The

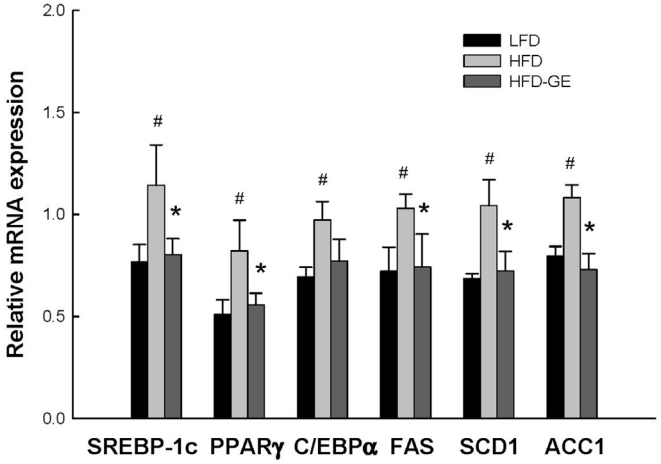

Fig. 4. Effects of ginseng extract (GE) on adipogenesis-associated gene expression in high-fat diet (HFD)-fed castrated mice. Adult castrated mice (n = 8/group) were fed a low-fat diet (LFD), HFD, or HFD supplemented with GE (HFD-GE) for 8 weeks. RNA was extracted from visceral adipose tissues. Relative mRNA levels of SREBP-1c, PPARγ, C/EBPα, FAS, SCD1, and ACC1 are expressed as mean ± SD using β-actin as a reference gene. # $p < 0.05$ versus LFD group. * $p < 0.05$ versus HFD group.

present data are supported by our previous reports showing that ginseng treatment ameliorates hepatic steatosis in obese animal models such as female *db/db* mice and HFD-fed ovariectomized mice (Lee et al., 2014, 2016). These results demonstrate that ginseng is able to inhibit hepatic steatosis in obese castrated mice and may be useful in alleviating nonalcoholic fatty liver disease in obese patients with testosterone deficiency.

Lipids stored in adipose tissue are known to largely derive from circulating triglycerides. Similar to changes in adipose tissue mass, serum triglycerides and total cholesterol were significantly increased in castrated mice on a HFD for 8 weeks compared with mice on an LFD. However, GE treatment significantly decreased HFD-induced increases of circulating total cholesterol and triglycerides, as evidenced by the present study and others (Ji and Gong, 2007; Song et al., 2012; Kim et al., 2016), indicating that ginseng effectively regulates lipid metabolism in obese castrated mice. In this respect, ginseng suggests the potential to be used for treating testosterone-deficient men with hypertriglyceridemia and hypercholesterolemia.

The expansion of adipose tissue and adipocyte hypertrophy are

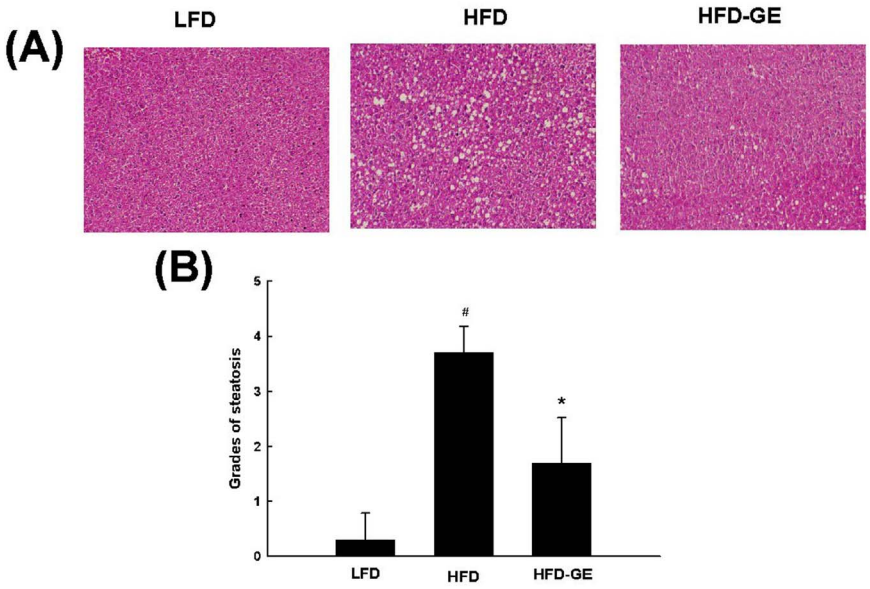

Fig. 3. Inhibition of hepatic lipid accumulation by ginseng extract (GE) in high-fat diet (HFD)-fed castrated mice. Adult castrated mice (n = 8/group) were fed a low-fat diet (LFD), HFD, or HFD supplemented with GE (HFD-GE) for 8 weeks. (A) Representative hematoxylin and eosin-stained liver sections are shown (original magnification ×100). (B) Histological analysis of hepatic lipid accumulation. Steatosis was graded as follows: 0, no steatosis; 1, mild; 2, moderate; 3, severe; 4, very severe. # $p < 0.05$ versus LFD group. * $p < 0.05$ versus HFD group.

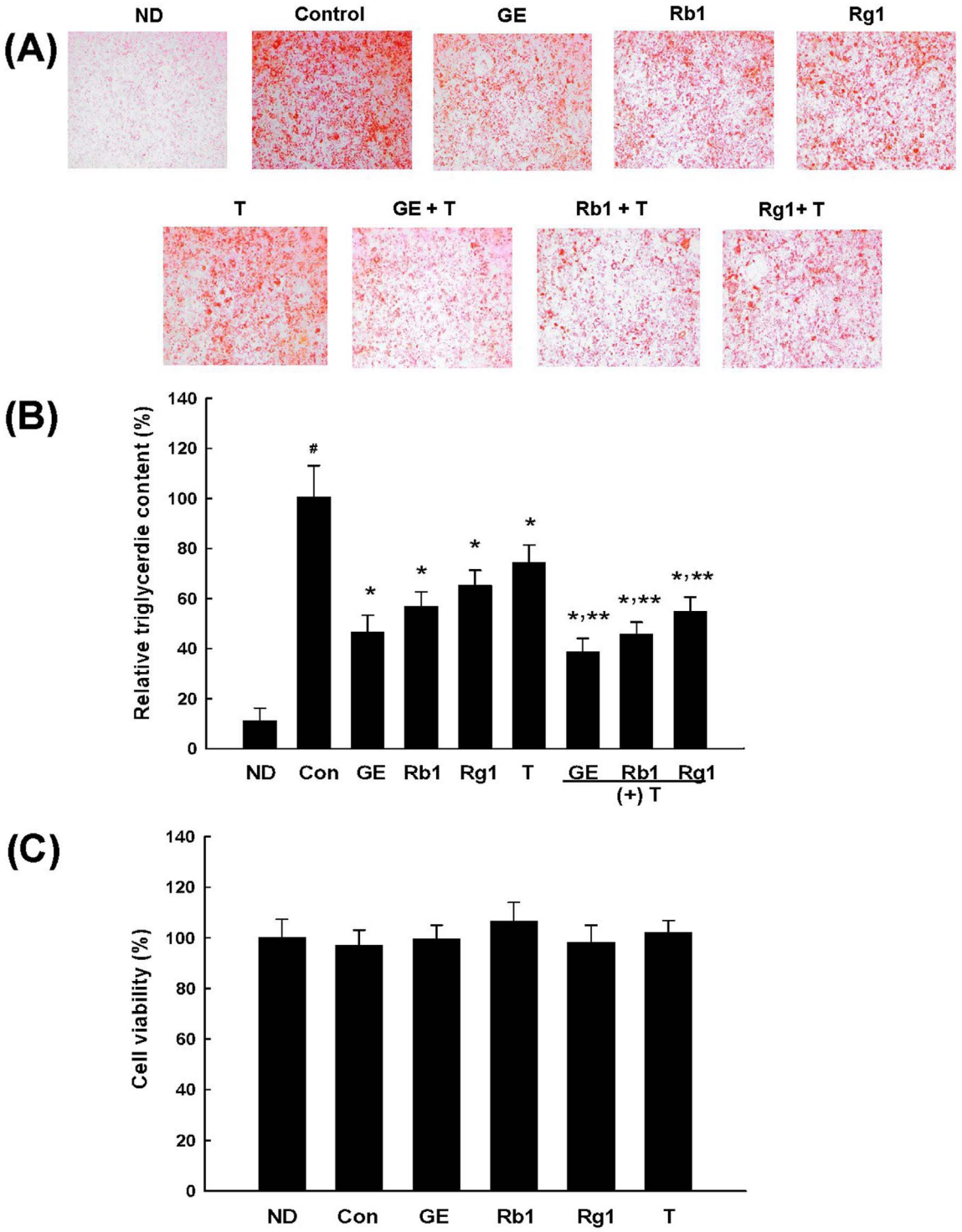

Fig. 5. Effects of ginseng extract (GE), ginsenosides, and testosterone on lipid accumulation in 3T3-L1 cells. (A) The 3T3-L1 preadipocytes were differentiated into mature adipocytes and then treated with dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), or testosterone (100 nM) on days 0–2 only. At day 8 post-induction, the cells were fixed, and neutral lipids were stained with Oil Red O (representative cells are shown). (B) Quantitative analysis of triglyceride content. (C) The effect of ALS on 3T3-L1 cell viability by XTT assays. All values are expressed as mean ± SD. # $p < 0.05$ versus ND. * $p < 0.05$ versus control. ** $p < 0.05$ versus testosterone.

deeply associated with adipogenesis. Adipogenesis is a complex process involving adipocyte proliferation, lipid accumulation, morphological changes, and lipogenic gene expression (Rosen and Spiegelman, 2000). SREBP-1c, PPARγ and C/EBPα are major transcription factors in the early stage of adipocyte differentiation (Ericsson et al., 1997; Gregoire et al., 1998). Their expression activates the target genes FAS, SCD1, and ACC1, which play roles in lipogenesis (Zhang et al., 2001; Strable and Ntambi, 2010; Lee and Sung, 2016). Based on ability of GE to decrease body weight and adipocyte size, we hypothesized that GE inhibits obesity by regulating the expression of adipogenesis-associated genes. Our results showed that GE negatively regulates the expression of SREBP-1c, PPARγ, FAS, SCD1, and ACC1 in the VSC adipose tissue of castrated mice. Our data are supported by previous studies demonstrating that Korean red ginseng downregulates PPARγ and adipocyte

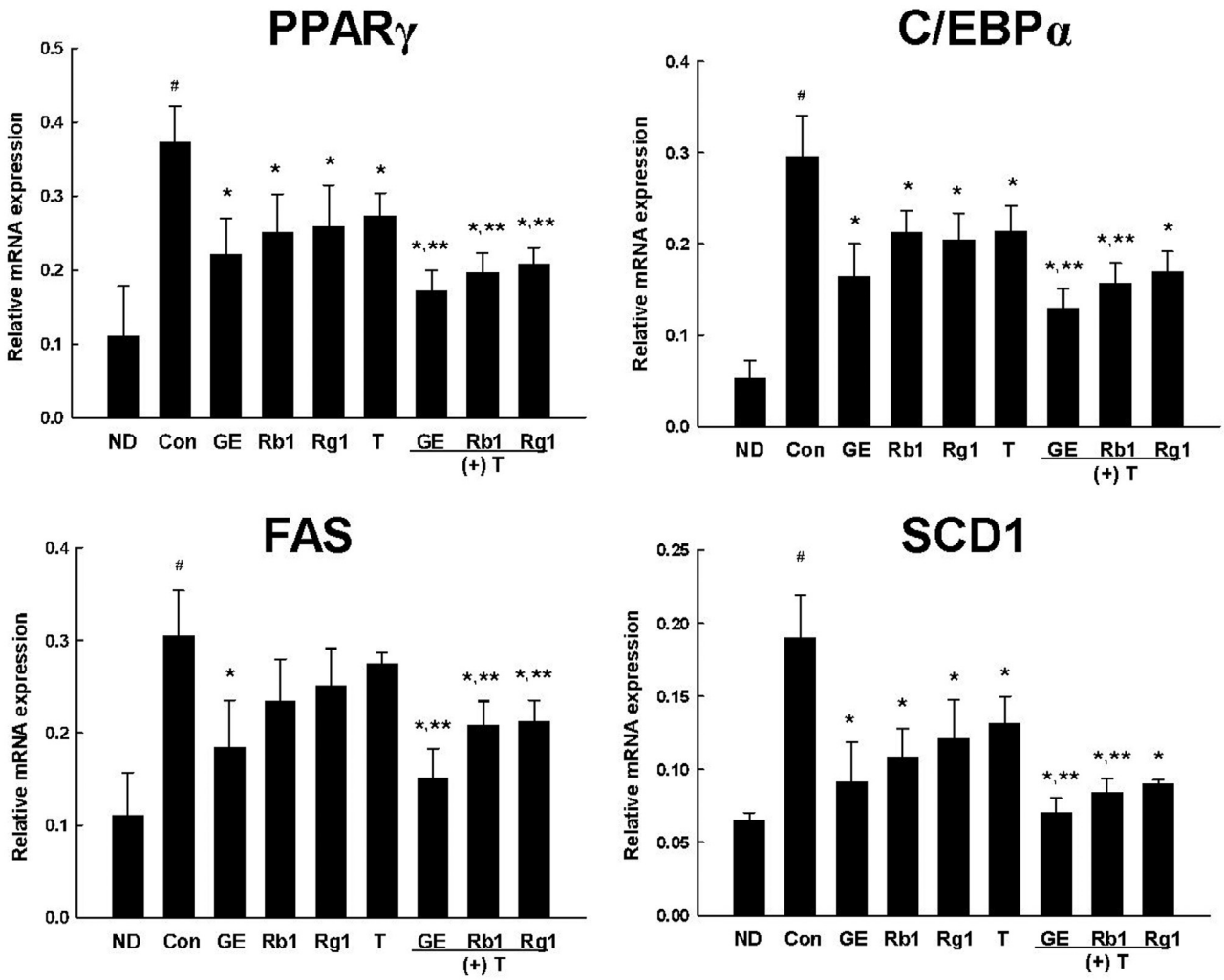

Fig. 6. Effects of ginseng extract (GE), ginsenosides, and testosterone on adipogenesis gene expression in 3T3-L1 cells. The 3T3-L1 preadipocytes were differentiated into mature adipocytes and then treated with dimethyl sulfoxide (5 μl/ml), GE (10 μg/ml), Rb1 (10 μM), Rg1 (10 μM), or testosterone (100 nM) on days 0–2 only. Relative mRNA levels of PPARγ, C/EBPα, FAS, and SCD1 are expressed as mean ± SD using β-actin as a reference gene. # $p < 0.05$ versus ND. * $p < 0.05$ versus control. ** $p < 0.05$ versus testosterone.

protein 2 (aP2) expression in white adipose tissue of HFD-fed obese Sprague-Dawley rats (Jung et al., 2015). These results support our hypothesis that decreased body weight gain, adipose tissue mass, and adipocyte size following ginseng treatment are due to the down-regulation of adipogenesis-related genes.

Adipose tissue is composed of diverse cell populations, such as preadipocytes, adipocytes, adipose stromal cells, endothelial cells, and inflammatory cells. Of the cell populations, preadipocytes and adipocytes are predominant constituents, and 3T3-L1 cells have thus been used for examining fat metabolism as a cell model of obesity (Hong et al., 2011; Woo et al., 2016). After adipocyte differentiation, 3T3-L1 control cells had significant lipid droplets. However, GE and ginsenosides (Rb1 and Rg1) decreased lipid accumulation in 3T3-L1 adipocytes, as shown by the decreased Oil Red O stains. In parallel with the reduced lipid accumulation, treatment with GE decreased PPARγ, C/EBPα, FAS, and SCD1 expression in 3T3-L1 cells. Rb1 and Rg1 also decreased PPARγ, C/EBPα, and SCD1 expression. Our findings are supported by several studies reporting individual ginsenosides Rb1, Rc, and Rf that stimulate anti-adipogenic activities by suppressing PPARγ, C/EBPα, and aP2 in 3T3-L1 cells (Oh et al., 2012; Siraj et al., 2015; Yang and Kim, 2015). The major active metabolites of ginseng are ginsenosides, which comprise approximately 3–6% of ginseng components and contain most of the pharmacological actions of ginseng (Attele et al., 1999; Huang, 1999). Similarly, testosterone decreased lipid accumulation and PPARγ, C/EBPα, and SCD1 expression. Our results are supported by a study describing that testosterone inhibits adipogenic differentiation and decreases C/EBPα and PPARγ2 mRNA and protein expression in 3T3-L1 cells (Singh et al., 2006). Co-treatment of GE or ginsenosides with testosterone further decreased lipid accumulation and adipogenesis-related gene expression compared with testosterone alone. Taken together, our in vitro results indicate that the effects of ginseng and testosterone on adipogenesis may be mediated by changes in the expression of adipogenesis-related genes, providing the first evidence that ginseng may act like testosterone to inhibit adipogenesis.

5. Conclusion

In conclusion, the results of our study demonstrate that ginseng can prevent obesity and dyslipidemia in HFD-fed castrated mice and suggest that these processes are mediated through the inhibition of adipogenesis-related gene expression. In addition, our results show that ginseng may act like testosterone to inhibit adipogenesis, suggesting that ginseng may be useful for the prevention and treatment of obesity and related disorders in men with testosterone deficiency. Further studies will be necessary not only to examine the effects of individual ginsenosides on adipogenesis and obesity in testosterone deficiency, but also to determine the interaction of ginseng and testosterone in the

regulation of obesity and related metabolic diseases.

Conflict of interest

None declared.

Author contributions

Soon Shik Shin (ssshin@deu.ac.kr) and Michung Yoon (yoon60@mokwon.ac.kr) designed and performed the experiments, analyzed the data, and wrote the paper.

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References

Attele, A.S., Wu, J.A., Yuan, C.S., 1999. Ginseng pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol. 58, 1685–1693.
Attele, A.S., Zhou, Y.P., Xie, J.T., Wu, J.A., Zhang, L., Dey, L., Pugh, W., Rue, P.A., Polonsky, K.S., Yuan, C.S., 2002. Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. Diabetes 51, 1851–1858.
Bent, S., Ko, R., 2004. Commonly used herbal medicines in the United States: a review. Am. J. Med. 116, 478–485.
Ericsson, J., Jackson, S.M., Kim, J.B., Spiegelman, B.M., Edwards, P.A., 1997. Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. J. Biol. Chem. 272, 7298–7305.
Francomano, D., Lenzi, A., Aversa, A., 2014. Effects of five-year treatment with testosterone undecanoate on metabolic and hormonal parameters in ageing men with metabolic syndrome. Int. J. Endocrinol. 2014, 527470.
Fui, M.N., Dupuis, P., Grossmann, M., 2014. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J. Androl. 16, 223–231.
Gillis, C.N., 1997. *Panax ginseng* pharmacology: a nitric oxide link? Biochem. Pharmacol. 54, 1–8.
Gregoire, F.M., Smas, C.M., Sul, H.S., 1998. Understanding adipocyte differentiation. Physiol. Rev. 78, 783–809.
Hong, Y., Kim, M.Y., Yoon, M., 2011. The anti-angiogenic herbal extracts Ob-X from Morus alba, Melissa officinalis, and Artemisia capillaris suppresses adipogenesis in 3T3-L1 adipocytes. Pharm. Biol. 49, 775–783.
Huang, K.C., 1999. The Pharmacology of Chinese Herbs. CRC Press, Boca Raton.
Jeong, S., Yoon, M., 2009. Fenofibrate inhibits adipocyte hypertrophy and insulin resistance by activating adipose PPARalpha in high fat diet-induced obese mice. Exp. Mol. Med. 41, 397–405.
Ji, W., Gong, B.Q., 2007. Hypolipidemic effects and mechanisms of Panax notoginseng on lipid profile in hyperlipidemic rats. J. Ethnopharmacol. 113, 318–324.
Jung, S., Lee, M.S., Shin, Y., Kim, C.T., Kim, I.H., Kim, Y., 2015. High hydrostatic pressure extract of red ginseng attenuates inflammation in rats with high-fat diet induced obesity. Prev. Nutr. Food Sci. 20, 253–259.
Kang, K.S., Yamabe, N., Kim, H.Y., Park, J.H., Yokozawa, T., 2010. Effects of heat-processed ginseng and its active component ginsenoside 20(S)-Rg3 on the progression of renal damage and dysfunction in type 2 diabetic Otsuka Long-Evans Tokushima Fatty rats. Biol. Pharm. Bull. 33, 1077–1081.
Karu, N., Reifen, R., Kerem, Z., 2007. Weight gain reduction in mice fed Panax ginseng saponin, a pancreatic lipase inhibitor. J. Agric. Food Chem. 55, 2824–2828.
Kelly, D.M., Jones, T.H., 2015. Testosterone and obesity. Obes. Rev. 16, 581–606.
Kim, J.H., Hahm, D.H., Yang, D.C., Kim, J.H., Lee, H.J., Shim, I., 2005. Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. J. Pharmacol. Sci. 97, 124–131.
Kim, J., Lee, H., Lim, J., Oh, J., Shin, S.S., Yoon, M., 2017. The angiogenesis inhibitor ALS-L1023 from lemon-balm leaves attenuates high-fat diet-induced nonalcoholic fatty liver disease through regulating the visceral adipose-tissue function. Int. J. Mol. Sci. 18, E846.
Kim, M.H., Lee, E.J., Cheon, J.M., Nam, K.J., Oh, T.H., Kim, K.S., 2016. Antioxidant and hepatoprotective effects of fermented red ginseng against high fat diet-induced hyperlipidemia in rats. Lab. Anim. Res. 32, 217–223.
Laforest, S., Labrecque, J., Michaud, A., Cianflone, K., Tchernof, A., 2015. Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. Crit. Rev. Clin. Lab. Sci. 52, 301–313.
Lee, H., Choi, J., Shin, S.S., Yoon, M., 2016. Effects of Korean red ginseng (*Panax ginseng*) on obesity and adipose inflammation in ovariectomized mice. J. Ethnopharmacol. 178, 229–237.
Lee, H., Kim, M., Shin, S.S., Yoon, M., 2014. Ginseng treatment reverses obesity and related disorders by inhibiting angiogenesis in female db/db mice. J. Ethnopharmacol. 155, 1342–1352.
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.
Lee, H.J., Lee, Y.H., Park, S.K., Kang, E.S., Kim, H.J., Lee, Y.C., Choi, C.S., Park, S.E., Ahn, C.W., Cha, B.S., Lee, K.W., Kim, K.S., Lim, S.K., Lee, H.C., 2009. Korean red ginseng (*Panax ginseng*) improves insulin sensitivity and attenuates the development of diabetes in Otsuka Long-Evans Tokushima fatty rats. Metabolism 58, 1170–1177.
Lee, M., Sung, S.H., 2016. Platyphylloside isolated from *Betula platyphylla* inhibit adipocyte differentiation and induce lipolysis via regulating adipokines including PPARγ in 3T3-L1 cells. Pharmacogn. Mag. 12, 276–281.
Lee, S.H., Lee, H.J., Lee, Y.H., Lee, B.W., Cha, B.S., Kang, E.S., Ahn, C.W., Park, J.S., Kim, H.J., Lee, E.Y., Lee, H.C., 2012. Korean red ginseng (*Panax ginseng*) improves insulin sensitivity in high fat fed Sprague-Dawley rats. Phytother. Res. 26, 142–147.
Lu, J.M., Yao, Q., Chen, C., 2009. Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr. Vasc. Pharmacol. 7, 293–302.
Michalakis, K., Goulis, D.G., Vazaiou, A., Mintziori, G., Polymeris, A., Abrahamian-Michalakis, A., 2013. Obesity in the ageing man. Metabolism 62, 1341–1349.
Mollah, M.L., Kim, G.S., Moon, H.K., Chung, S.K., Cheon, Y.P., Kim, J.K., Kim, K.S., 2009. Antiobesity effects of wild ginseng (*Panax ginseng* C.A. Meyer) mediated by PPAR-gamma, GLUT4 and LPL in *ob/ob* mice. Phytother. Res. 23, 220–225.
Oh, J., Lee, H., Lim, H., Woo, S., Shin, S.S., Yoon, M., 2015. The herbal composition GGEx18 from *Laminaria japonica, Rheum palmatum,* and *Ephedra sinica* inhibits visceral obesity and insulin resistance by upregulating visceral adipose genes involved in fatty acid oxidation. Pharm. Biol. 53, 301–312.
Oh, J., Lee, H., Park, D., Ahn, J., Shin, S.S., 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 Altern. Med. 2012, 265023.
Park, H.J., Kim, D.H., Park, S.J., Kim, J.M., Ryu, J.H., 2012. Ginseng in traditional herbal prescriptions. J. Ginseng Res. 36, 225–241.
Rosen, E.D., Spiegelman, B.M., 2000. Molecular regulation of adipogenesis. Annu. Rev. Cell Dev. Biol. 16, 145–171.
School, J.N.M., 1986. Renshen. Chinese Materia Medica Dictionary 1. Shanghai Scientific & Technical Publishers, Shanghai, China.
Singh, R., Artaza, J.N., Taylor, W.E., Braga, M., Yuan, X., Gonzalez-Cadavid, N.F., Bhasin, S., 2006. Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. Endocrinology 147, 141–154.
Siraj, F.M., Natarajan, S., Huq, M.A., Kim, Y.J., Yang, D.C., 2015. Structural investigation of ginsenoside Rf with PPARγ major transcriptional factor of adipogenesis and its impact on adipocyte. J. Ginseng Res. 39, 141–147.
Song, Y.B., An, Y.R., Kim, S.J., Park, H.W., Jung, J.W., Kyung, J.S., Hwang, S.Y., Kim, Y.S., 2012. Lipid metabolic effect of Korean red ginseng extract in mice fed on a high-fat diet. J. Sci. Food Agric. 92, 388–396.
Strable, M.S., Ntambi, J.M., 2010. Genetic control of de novo lipogenesis: role in diet-induced obesity. Crit. Rev. Biochem. Mol. Biol. 45, 199–214.
Traish, A.M., 2014. Testosterone and weight loss: the evidence. Curr. Opin. Endocrinol. Diabetes Obes. 21, 313–322.
Winslow, L.C., Kroll, D.J., 1998. Herbs as medicines. Arch. Intern. Med. 158, 2192–2199.
Woo, S., Yoon, M., Kim, J., Hong, Y., Kim, M.Y., Shin, S.S., Yoon, M., 2016. The anti-angiogenic herbal extract from Melissa officinalis inhibits adipogenesis in 3T3-L1 adipocytes and suppresses adipocyte hypertrophy in high fat diet-induced obese C57BL/6J mice. J. Ethnopharmacol. 178, 238–250.
Yang, J.W., Kim, S.S., 2015. Ginsenoside Rc promotes anti-adipogenic activity on 3T3-L1 adipocytes by down-regulating C/EBPα and PPARγ. Molecules 20, 1293–1303.
Yassin, A., Doros, G., 2013. Testosterone therapy in hypogonadal men results in sustained and clinically meaningful weight loss. Clin. Obes. 3, 73–83.
Yin, J., Zhang, H., Ye, J., 2008. Traditional Chinese medicine in treatment of metabolic syndrome. Endocr. Metab. Immune Disord. Drug Targets 8, 99–111.
Yun, T.K., 2001. Brief introduction of *Panax ginseng* C.A. Meyer. J. Korean Med. Sci. 16, S3–S5.
Zhang, L., Ge, L., Tran, T., Stenn, K., Prouty, S.M., 2001. Isolation and characterization of the human stearoyl-CoA desaturase gene promoter: requirement of a conserved CCAAT cis-element. Biochem. J. 357, 183–193.