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Anti-obesity Effects of Protopanaxatriol-type Ginsenosides Isolated from American Ginseng Leaves in Mice Fed a High-fat Diet

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ABSTRACT

The protopanaxatriol-type of ginsenosides isolated from the leaves of American ginseng mainly contained Rg1 and Re by High Performance Liquid Chromatography. Then isolation and purification of ginsenoside Rg1 and ginsenoside Re were carried out by repeated crystallization and silica gel column chromatography. And the purity of both ginsenoside Rg1 and ginsenoside Re were higher than 95%. *In vitro* experiment, assayed the effect of ginsenoside Rg1, ginsenoside Re and the protopanaxatriol-type of ginsenosides isolated from the leaves of American ginseng on porcine pancreatic lipase activity. It was determined by measuring released free fatty acids after incubating in substrate emulsions containing bile salts and phosphatidylcholine by water bath heating at 37°C. The absorbance of extreme colouration solutions on pancrestic lipase activity was measured at 480 nm. Both the protopanaxatriol-type of ginsenosides and ginsenoside Rg1 had no effect on inhibiting the pancreatic lipase activity, while ginsenoside Re had little effect. In order to clarify whether the protopanaxatriol-type of ginsenosides isolated from American ginseng leaves had anti-obesity effect *in vivo* or not, we examined the anti-obesity activity by testing the saponins preventing the obesity induced by feeding a high-fat diet to mice for 8 weeks. Body weight, food intake, organ weight, adipose tissue weight, serum parameters and liver lipids were measured and analyzed. The results demonstrated that the protopanaxatriol-type of ginsenosides isolated from American ginseng leaves had no inhibition activity of pancreatic lipase *in vitro*, however, it played an important role in preventing obesity, fatty liver and hypertriglyceridemia in mice fed with a high-fat diet during the long-term experiment.

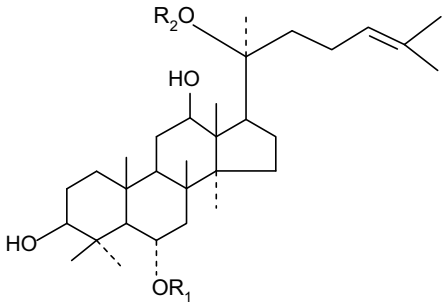
Keywords: adipose tissue, body weight, pancreatic lipase activity, serum lipids

INTRODUCTION

The aim of the present study was to clarify anti-obesity effect of the protopanaxatriol-type of ginsenosides isolated from American ginseng leaves *in vitro* and *in vivo*. This work studied the effect of ginsenoside Rg1, ginsenoside Re and the protopanaxatriol-type of ginsenosides isolated from the leaves of American ginseng on porcine pancreatic lipase activity *in vitro* experiment. The result of *in vitro* pancreatic lipase experiments showed little inhibitory activity. With the intention of clarifying its role in anti-obesity effects *in vitro*, we further studied the anti-obesity activity by testing the saponins preventing the obesity induced by feeding a high-fat diet to mice for 8 weeks.

Panax quinquefolius L. belonging to the Araliaceae family, commonly known as American ginseng and often by the Chinese name of Huaqishen or Xiyangshen, is commonly used in medicine. American ginseng grown in the United States and Canada, is a more popular herbal, nutritional supplement used throughout the world (Nocerino *et al*. 2000; Harkey *et al*. 2001; Bent and Ko 2004). Because of its deficiency, American ginseng has been cultivated, even though its market export value is considerably less than the value of wild ginseng. It also cultivated beyond its range in places such as China. Roots and leaves of American ginseng are being traditionally used for medicinal purposes by human (Xie *et al*. 2004; Popovich *et al*. 2011).

Although American ginseng has not been used longer than *Panax ginseng*, it is already a medicinal plant known worldwide for its tonics. It has many phamacological activities including anticarcinogenic effects (Yun TK *et al*. 1996), reducing stress, lowering high blood sugar, adjusting immunity (Vuksan *et al*. 2000) and anti-hyperglycemic and thermogenic activity (Xie *et al*. 2004). Ginsenosides, known as the principal bioactive components of ginseng,



Protopanaxatriol type ginsenosides

Re R1:glc(2-1)rha; R2:glc

Rg1 R1:glc; R2:glc

Fig. 1 Chemical structures of protopanaxatriol-type of ginsenosides. glc: D-glucopyranosyl; rha: L-rhamnopyranosyl.

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have been widely used for health foods and traditional medicine. Ginsenosides can be divided into two groups according to their sapogenins with a dammarane skeleton, protopanaxatriol and protopanaxadiol groups except the oleanolic acid group including Ro. It can be seen from **Fig. 1** that the sugar moieties of protopanaxatriol-type of ginsenosides attached to the 6-position of the dammarane-type triterpene contain Rg1 and Re. It is possible that different ginsenosides with different structures would show different pharmacology activities.

Ginsenoside Rg1 is panaxatriol with two sugars, which is one of the important active principles of ginseng and shares many pharmacological effects of this drug, such as facilitating learning and memory and alleviating many ailments associated with aging. It was reported that Rg1 had anti-aging nootropic effects (Liu 1996). Rg1 administration led to marked enhancement of the number of dividing cells in the hippocampus of adult mice (Shen and Zhang 2004). Liu and Zhang used methods of fluorescence flow cytometry and Western blot analysis, and found that Rg1 enhanced immune function in old rats (Liu and Zhang 1996). Ginsenoside Rg1 could significantly inhibit tumor necrosis factor-alpha-induced human arterial vascular smooth muscle cell proliferation (Zhang and Wang 2006).

The cytoprotective effects of the ginsenoside protopanaxatriol on hydrogen peroxide- induced endothelial cell injury and cell death were explored (Kwok *et al*. 2009). They found that pretreatment of human umbilical vein endothelial cells with ginsenoside protopanaxatriol for 24 h was able to protect the cells against hydrogen peroxide-induced injury.

Ginsenoside Re is another active component of protopanaxatriol-type of American ginseng and ginseng. Re was shown to decrease in both fed and fasting serum insulin levels in mice and increase glucose tolerance of ob/ob mice significantly (Xie *et al*. 2005). Ginsenoside Re protected cardiomyocytes from oxidant injury induced by both exogenous and endogenous oxidants, and it may be mostly attributed to scavenging H_2O_2 and hydroxyl radicals (Xie *et al*. 2006).

Although dietary fat intake is the main factor inducing metabolic disorders, such as diet-induced obesity and hyperlipemia, it cannot be digested without enzyme in gastrointestinal.

Pancreatic lipase is the most important enzyme of the human lipases for digesting fats and is responsible for the hydrolysis of 50–70% of total dietary fats (Mukherjee *et al*. 2003). Theoretically, obesity, especially diet-induced obesity, can be controlled by pancreatic lipase inhibitors. Currently, orlistat, a positive drug, is effective for the treatment of human obesity as a gastrointestinal lipase inhibitor. However, it has certain unpleasant gastrointestinal side effects like oily stools, oily spotting and flatulence among others (Hadváry *et al*. 1988; Hauptman *et al*. 1992; Drent *et al*. 1995; Sjöström *et al*. 1998). So many researchers carried out experiments of pancreatic lipase activity *in vitro* to screen lipase inhibitors. Han LK *et al*. investigated the anti-obesity effects of pancreatic lipase inhibitor: teasaponin, Platycodi Radix saponin and chikusetsusaponins (Han *et al*. 1999, 2001, 2002, 2005; Xu *et al*. 2005). Nakai *et al*. also did *in vitro* experiments of oolong tea polyphenols on pancreatic lipase inhibition activity (Nakai *et al*. 2005). The inhibitory effect of other saponins has been studied (Kimura *et al*. 2006; Yoshizumi *et al*. 2006).

There are few reports about anti-obesity of ginsenosides from American ginseng so far. In our previous study, we investigated the effects of pancreatic lipase inhibition activity *in vitro* and anti-obesity in mice fed with a high-fat diet about crude ginsenosides from stems and leaves of American ginseng. And the results showed that it could suppress plasma lipids and parametrial adiposetissue weight compared to those of high-fat diet group (Liu *et al*. 2008).

MATERIALS AND METHODS

Materials

The standards of ginsenosides Rg1, Re, Rb1, Rc, Rb2, Rb3 and Rd (purity > 99.5%) were isolated and purified from ginseng or American ginseng in Chinese Herbal Medicine Laboratory of Jilin Agricultural University (Changchun, China). The chromatographic grade acetonitrile and methanol were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Shimadzu 2010 LC (including a LC-20AT pump, an online vacuum deaerator, a SPD-20A detector, a CTO-10AS VP column oven and LC-solution) was purchased from Shimadzu Co. (Shimadzu, Japan). The analytical column employed was packed with reverse-phase C-18 (VP-ODS)(150 mm × 4.6 mm, 5 μm) and also purchased from Shimadzu Company. The leaves of American ginseng were obtained from Fusong of Jilin province in China.

Triolein, taurocholic acid sodium salt hydrate, TES buffer and porcine pancreatic lipase were purchased from Sigma (St. Louis, MO). Phosphatidylcholine was purchased from Wako Pure Chemical Industries, Ltd (Wako, Japan). Lipid assay kits (TG, TC, HDL-C and LDL-C) were purchased from Biosino Bio-technology and Science Inc. (Beijing, China). Other chemicals were of reagent grade.

Diet

Normal diet consisting of 5% fat, 53% carbohydrate, 23% protein, with total calorific value 25 kJ/kg and high-fat diet consisting of 22% fat, 48% carbohydrate, and 20% protein with total calorific value 44.3 kJ/kg were supplied from the stoyer center of the Experimental Animal Holding (Jilin, China). The diets were stored at -30°C and prepared freshly each day to avoid auto-oxidation of lipids.

Animals

Female Kunming mice, 3 weeks of age, were obtained from the Experimental Animal Holding of Jilin University, and housed individually in plastic cages in a 12/12 h light/dark cycle in a temperature-and humidity-controlled room for 1 week. The animals were allowed free access to food and water and the healthy ones were used. The mice were treated according to the ethical guidelines of the Experimental Animal Holding of Jilin University.

Preparation of ginsenoside Re, Rg1 and protopanaxatriol-type of ginsenosides from American ginseng leaves

1. Preparation of protopanaxatriol-type of ginsenosides

The air-dried leaves of American ginseng were obtained from Fusong of Jilin province in China. Firstly, the crude saponins were extracted according to our previously reported procedures (Liu *et al*. 2008): 1000 g of dried leaves of American ginseng was powdered and extracted with 100 L of distilled water for 3 hrs under reflux. The aqueous solution was filtrated and evaporated to obtain 300 g of aqueous extract. The aqueous extract was mixed in 10 L of 95% ethanol at room temperature for 8 hrs, and the ethanol solution was evaporated under vacuum to obtain 200 g of ethanol extracts. The ethanol extract was dissolved in water at a concentration of 100 g/L and then fractionated on a macroporous adsorption resin D101 column with water and 80% ethanol. The ethanol extract crude saponins were obtained from the 80% ethanol fractions. The yield was about 70 g.

The powder of the above crude ginsenoside extract 50 g was dissolved in water, absorbed on AB-8 macroporous adsorption resin, and eluted by different content rations of ethanol. According to the method of the previous report (Zhao *et al*. 2004) with a modification, the protopanaxatriol-type of ginsenosides gained were as follows. The aqueous nonsaponins were washed with 20% of ethanol of the resin weight, the protopanaxatriol-type of ginsenosides was enriched with 30% of ethanol of the resin weight. Then the fractions of eluted with 30% of ethanol were evaporated, dissolved in the chromatographic grade methanol, and analyzed by

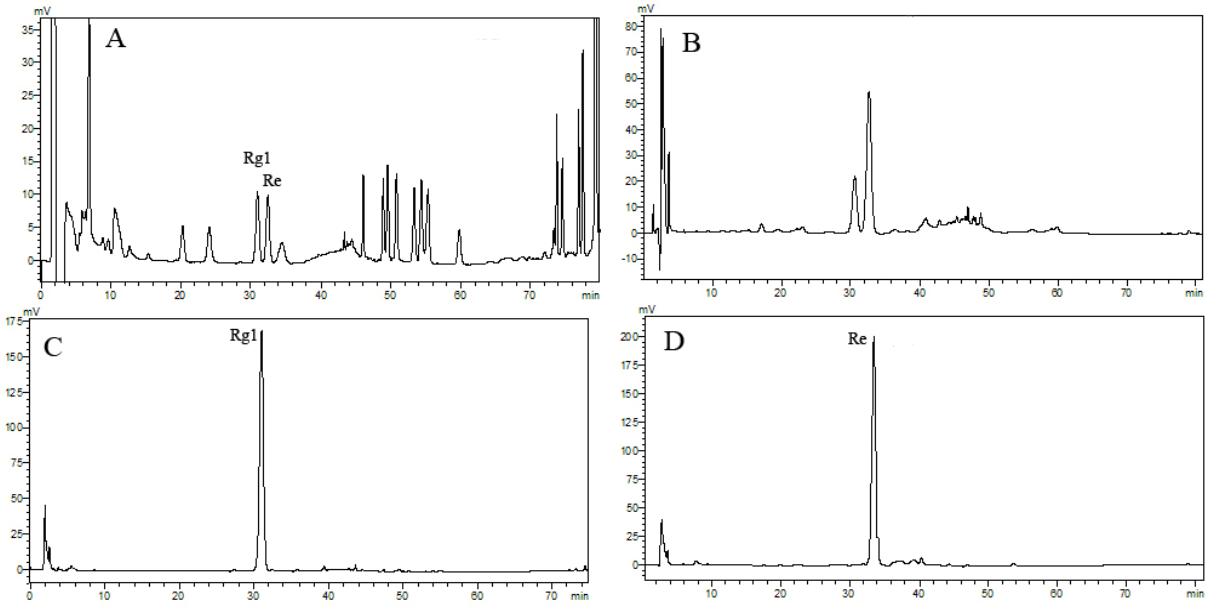

Fig. 2 HPLC chromatograms of mixed ginsenoside standards (**A**), protopanaxatriol-type of ginsenosides (**B**), ginsenoside Rg1 (**C**) and ginsenoside Re (**D**) from the leaves of American ginseng.

High Performance Liquid Chromatography (HPLC) later. With this method, we gained 10.25 g of the protopanaxatriol-type of ginsenosides. The yields of the protopanaxatriol-type of ginsenosides reached higher than 1.4%.

2. Conditions of High Performance Liquid Chromatography

The assays of analyzing and determining the concentration of ginsenosides by TLC and HPLC were carried out. Gradient elution was performed using water (A) and acetonitrile (B). Initial conditions were 82% A, linearly changed to 81% A at 20 min and 78% A at 32 min. And then 68% A at 39 min, 66.5% A at 56 min and 65% A at 60 min. Flow rate was 1.0 mL/min, and UV absorption was measured at wavelength of 203 nm. Column oven and the sample injection volume were 25°C and 20 μL, respectively.

The chromatogram for the standard ginsenosides, protopanaxatriol-type of ginsenosides, ginsenoside Rg1 and ginsenoside Re from the leaves of American ginseng were shown in **Fig. 2**. Retention time of all ginsenosides was confirmed by authentic standards. The protopanaxatriol-type of ginsenosides contained ginsenosides Rg1 and Re.

3. Preparation of ginsenoside Re and ginsenoside Rg1

A small amount of methanol was added to a flask containing the above protopanaxatriol-type of ginsenosides (5 g) isolated from American ginseng leaves. After that, the contents of the flask were heated using a hot water bath until the above protopanaxatriol-type of ginsenosides dissolves. Next, the solution was cooled. Slower watering led to a higher purity product like snowflakes. A more pure solid precipitates was gained, and impurities were dissolved in solvent. Vacuum filtration was used to isolate the crystals, and removed much of the solvent. This was repeated several times, and the final residue (2.23 g) was obtained. That is compound 1. It was dissolved in the chromatographic grade methanol, and analyzed by HPLC.

The supernatant was evaporated and then subjected to the classical silica gel column chromatography. All procedures of ginsenoside purification were carried out at room temperature. And then eluted with 8: 1 chloroform: methyl alcohol according to TLC. Each fraction was collected with 50 mL bottle and analyzed by TLC. The fractions, which contained mostly ginsenoside Rg1, were merged according to the standard Rg1 on the Thin-layer board and then evaporated. Compound 2 was thus obtained (0.94 g). A small amount of compound 2 was taken and dissolved in the chromatographic grade methanol, and then analyzed by HPLC.

4. Determination of compound 1 and compound 2

Compound 1 was analyzed by liquid chromatography-electrospray ionization- mass spectrometry (LC–ESI-MS). Mass chromatogram at the m/z 945 for the $[M-H]^-$ ion and the m/z 969 for the $[M+Na]^+$ ion suggested its molecular mass was 946. It was consistent with ginsenoside Re. 799$[M-H-162]^-$ was the fraction peak after missing a rhamnose. 783$[M-H-162]^-$ was the fraction peak after missing a glucose. 637$[M-H-146-162]^-$ was the fraction peak after missing a rhamnose and a glucose. 475$[M-H-146-162-162]^-$ was the fraction peak of the aglycon. The molecular mass of the aglycon was 476. The aglycon belonged to protopanaxatriol-type. The conditions of TLC were as follows: silica gel G as stationary phase, three mobile phases (4: 1: 5 *n*-butanol -ethyl acetate-water; 65: 35: 10 chloroform- methyl alcohol-water; 3: 1: 1 ethyl acetate- glacial acetic acid-water), coloration by spraying sulfuric acid (10%). Analyzed by TLC, we found compound 1 had the same location and colour as the standard Re on the TLC plate. Analyzing it by HPLC, we also found compound 1 had the same retention time as the standard Re (**Fig. 2**). Therefore, we defined compound 1 as ginsenoside Re.

The molecular mass of compound was 800 according to m/z 823$[M+Na]^+$, 799$[M-H]^-$, 637$[M-H-162]^-$ and 475$[M-H-162-162]^-$. It was consistent with ginsenoside Rg1. The molecular mass of the aglycon was 476. The aglycon belonged to protopanaxatriol-type. Analyzing it by TLC with the above three mobile phases, we found compound 2 had the same location and colour as the standard Rg1 on the TLC plate. Compound 2 had the same retention time as the standard Rg1 by HPLC (**Fig. 2**). We defined compound 2 as ginsenoside Rg1.

In vitro experiments to investigate the effect of ginsenoside Rg1, Re and protopanaxatriol-type of ginsenosides on pancreatic lipase activity

Lipase activity was determined by measuring the rate of release of oleic acid from triolein. Briefly, a suspension of triolein 80 mg, phosphatidylcholine 10 mg and taurocholic acid 5 mg in 9 ml of 0.1 M TES buffer (pH 7.0) containing 0.1 M NaCl was sonicated for 10 min. This sonicated substrate suspension 10 μL was incubated with 50 μL (10 units) of pancreatic lipase and 100 μL of various concentrations of chondroitin sulfate solution for 30 min at 37°C in a final volume of 250 μL. The amount of released oleic acid was determined by previous method (Zapf *et al*. 1981) with a slight modification (Tsujita and Okud 1983). The incubation mixture was added to 3 mL aliquots of a 1: 1 (v/v) mixture of chloroform and *n*-heptane containing 2% (v/v) methanol and extracted

by shaking the tubes horizontally for 10 min in a shaker. The mixture was centrifuged at 2000 × *g* for 10 min, and the upper aqueous phase was removed by suction. 1 mL of copper reagent was then added to the lower organic phase. The tube was shaken for 10 min, the mixture was centrifuged at 2000 × *g* for 10 min, and 0.5 mL of the upper organic phase, which contained copper salts of the extracted free fatty acids, was treated with 0.5 mL of 0.1% (w/v) bathocuproine in chloroform containing 0.05%(w/v) 3(2)-*t*-butyl-4-hydroxyanisole. The absorbance was then measured at 480 nm. In addition, pancreatic lipase activity was determined using gum Arabic as emulsifier: 45 mg of gum Arabic instead of phophatidylcholine was used and the enzyme activity was assayed as described above. Lipase activity was expressed as moles of oleic acid released per liter of reaction mixture per hour.

Animal experiments to estimate body weights, organ weights, food intake, adipose tissue, serum parameters and liver parameters in mice fed with a high-fat diet

1. Estimation of body weights and food intake in high-fat-diet mice

After adaptation to the normal diet and the new life condition for 1 week, the healthy mice were divided into five groups according to the weight in the present experiments and fed with different diets. The normal group was constantly fed normal diet. The feeds were freshly prepared each day to avoid auto-oxidation of its fat component at room temperature. Each rat was weighed once a week and the weight was recorded. The total amount of food intake by each group was recorded every day.

2. Estimation of organ weights, adipose tissue, serum parameters and liver parameters in mice

After 8 weeks of consuming the indicated experimental diets, blood was taken from each mouse by the artery and vein of fossa orbitalis. Then the blood was centrifugalized at 4°C and the serum of each mouse was obtained and frozen at -80°C until analysis. Serum triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C) and LDL-Cholesterol (LDL-C) were determined using the lipid assay kits, respectively. After taking blood, the mice were killed by cervical fracture. The heart, liver, spleen, kidney and adipose tissue (peritoneal plus periuterine and perirenal adipose tissue) were quickly removed and weighed. The liver tissues were stored at -80°C until analysis. The liver TG and TC concentrations were measured by the following method: 0.5 g of the liver tissue was homogenized in 4.5 mL of Krebs Ringer phosphate buffer (pH 7.4). Then 0.2 mL of the homogenate was extracted with 4 mL chloroform/methanol (2: 1, v/v) and the extract was concentrated under a nitrogen stream. The residue was analyzed using TG E-Test and TC E-Test kits.

Statistical analysis of data

The values were expressed as means ± standard errors (s.e.). Statistical analysis was calculated using SPSS software. Student's *t*-test, Fisher's Protected LSD test and Scheffe's test were used to analyze the data. The criterion for statistical significance was $P < 0.05$, and extreme significance was $P < 0.01$.

RESULTS AND DISCUSSION

Effects of protopanaxatriol-type of ginsenosides, ginsenoside Rg1 and ginsenoside Re on pancreatic lipase activity (*in vitro*)

As shown in **Table 1**, the protopanaxatriol-type of ginsenosides did not inhibit pancreatic lipase activity at the concentrations of 0.5 mg/mL in the assay system using triolein emulsified with phosphatidylcholine *in vitro*. It was the same as ginsenoside Rg1. Ginsenoside Re had little effect on pancreatic lipase inhibition activity, and it inhibited hydrolysis of about 4.6% of trioein. Our previous study showed crude saponins, ginsenoside Re and ginsenoside Rg1 had little inhibitory effect of pancreatic lipase (Liu *et al*. 2008).

Table 1 Effects of ginsenosides isolated from American ginseng leaves on pancreatic lipase activity.

Addition	Lipase activity of control(%) mean ± SE (*n*=5)
None	100 ± 0
PTG (0.5 mg/mL)	105.34 ± 0.29**
Re (0.5 mg/mL)	95.42 ± 0.50**
Rg1 (0.5 mg/mL)	141.87 ± 0.45**
Orlistat (0.008 mg/mL)	1.87 ± 0.44**

PTG: protopanaxatriol-type of ginsenosides.
Lipase activity was measured using porcine pancreatic lipase and triolein with lecithin as substrate. Inhibiting effect was shown as the lowering of pancreatic lipase activity (%) against the lipase activity of control (0 mg/mL of additions). Values are expressed as means ± Standard Error (SE) of five experiments. The mean difference is significant from control (0 mg/mL of additions) (*$P<0.05$; **$P<0.01$).

Effects of protopanaxatriol-type of ginsenosides on food consumption, body weights and organ weights in mice fed with a high-fat diet for 8 weeks

The total food consumption data of three groups were as follows [values expressed in g/wk/group, mean ± S.E.]: the normal group (270.0 ± 21.9); the HF group (316.6 ± 16.2) and the HF plus 0.05% PTG group (411.7 ± 33.3). The effects of body weight gain of mice fed with different diets with or without ginsenosides are shown in **Fig. 3**. The body weight of the three groups of mice all displayed increasing tendency from the first week to the eighth week. Compared with the HF group, the normal group had no significant difference until the fourth week, and more and more significance was observed from the fourth week to the eighth week. Ginsenosides could only slightly suppress body weight gain. This evidence is consistent with our previous observations in which body weight of mice had not been affected significantly (Liu *et al*. 2008). It is different from the report that consumption of 3% ginseng extract-amended diet for 3 weeks reduced the body-weight gain of mice fed high-fat diet (Karu *et al*. 2007). The body weight was gradually increased with time, and the body weight was higher for the high-fat diet fed rats than for the N diet fed rats. However, the gain of body weight was lower in the rats treated with ginsenosides of red ginseng compared with the rats that were fed the HF diet. After about 7 weeks, ginsenosides of red ginseng could significantly reduce body weights of mice fed with high-fat diets (Kim *et al*. 2005, 2009).

As shown in **Table 2**, the results of the organ weights containing the liver weight, the spleen weight and the kidney weight about the mice were analyzed deliberatively after treating with different diets. Making the HF group the controlled group, the contradistinction of the other two groups was different on the different organ weights. The liver weights of the normal group showed extremely significant difference, while the spleen and the kidney weights showed no significant difference. The weights of the spleen and the kidney showed no difference in other two groups.

Effects of protopanaxatriol-type of ginsenosides on adipose tissue weights in mice fed with a high-fat diet for 8 weeks

Feeding a high-fat diet for 8 weeks caused significant increases in perirenal and parametrial plus peritoneal adipose tissue weights, compared to the normal diet group. Furthermore, the perirenal adipose tissue weights were much lower than the parametrial plus peritoneal adipose tissue weights in all groups. Although the groups consuming high-fat diets with protopanaxatriol-type of ginsenosides did not reach the level of the normal group on the adipose tissue weights, it had much more reduction effects on the adipose tissue weights (**Fig. 4**). Crude saponins of American ginseng suppressed parametrial adipose tissue weight compared to those of high-fat diet group (Liu *et al*. 2008). Although it is

Table 2 Effects of PTG isolated from American ginseng leaves on organ weights in mice fed with a high-fat diet for 8 weeks.

Group	Final body weight (g/mouse) mean ± SE	Liver (g/mouse) mean ± SE	Spleen (g/mouse) mean ± SE	Kidney (g/mouse) mean ± SE
Normal	34.35 ± 0.312**	1.58 ± 0.060**	0.13 ± 0.014	0.37 ± 0.020
HF	37.83 ± 0.329	2.31 ± 0.074	0.12 ± 0.030	0.41 ± 0.012
HF + 0.05% PTG	37.91 ± 0.566	1.81 ± 0.082**	0.10 ± 0.009	0.42 ± 0.041

Normal: fed with normal diet; HF plus 0.05%PTG: High-fat diet containing 0.05% (w/w) PTG. Values are expressed as means ± SE (n=8). The mean difference is significant from high-fat diet-treated group (*P<0.05; **P<0.01).

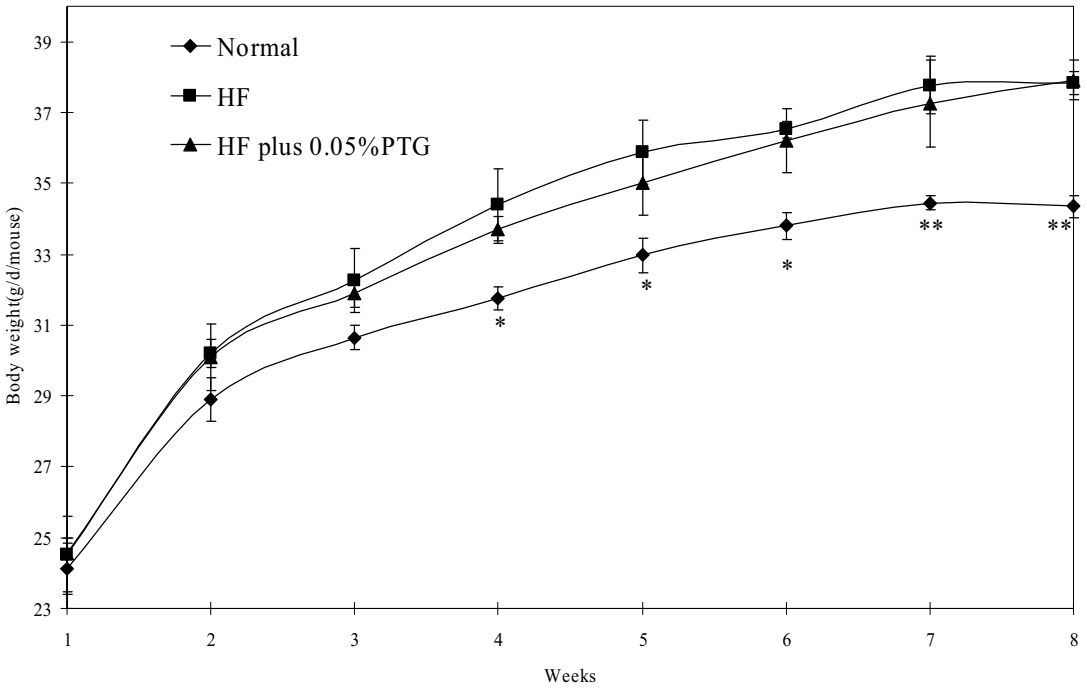

Fig. 3 Effects of PTG isolated from American ginseng leaves on body weight in mice fed with a high-fat diet for 8 weeks. Values are expressed as mean ± SE. (n=8). The mean difference is significant from high-fat diet-treated group (*P<0.05; **P<0.01).

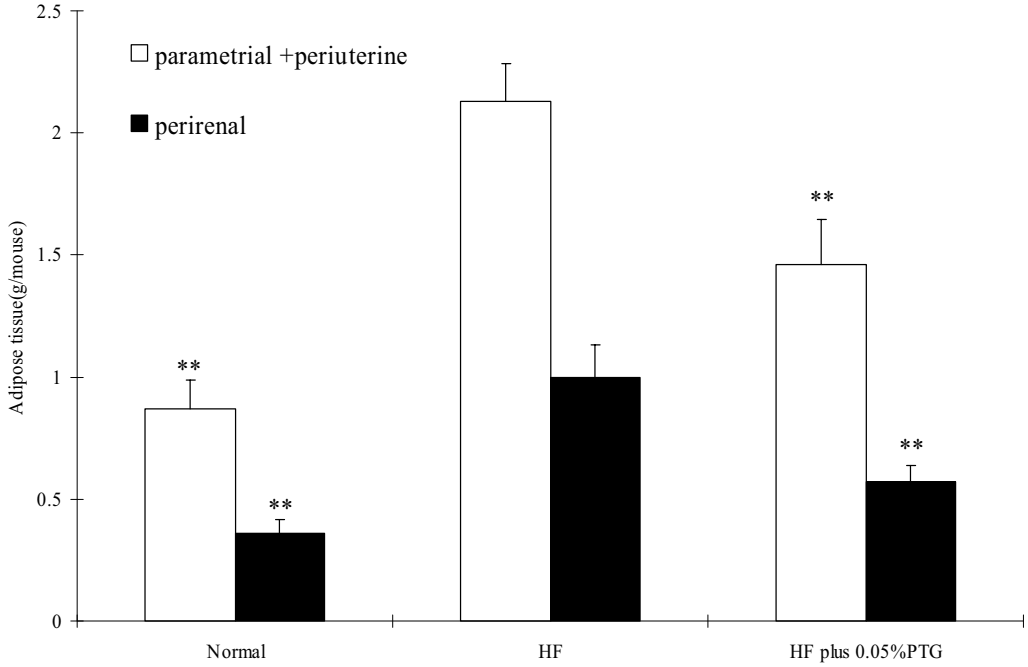

Fig. 4 Effects of PTG isolated from American ginseng leaves on parametrial and perirenal adipose tissue weight in mice fed with a high-fat diet for 8 weeks. Values are expressed as means ±SE (n=8). The mean difference is significant from high-fat diet-treated group (*P<0.05; **P<0.01).

also similar to the crude saponin of red ginseng (Kim *et al*. 2005), it is different from Kim *et al*.'s report, which protopanaxatriol-type of ginsenosides isolated from red ginseng could not reduce peritoneal and perirenal fat mass (Kim *et al*. 2009).

Table 3 Effects of PTG isolated from American ginseng leaves on parameters of serum after 8 weeks of treatment in mice fed with a high-fat diet.

Group	TG (mmol/L) mean ± SE	TC (mmol/L) mean ± SE	HDL-C (mmol/L) mean ± SE	LDL-C (mmol/L) mean ± SE
Normal	1.27 ± 0.084**	2.00 ± 0.102**	1.65 ± 0.039**	0.16 ± 0.024**
HF	2.76 ± 0.182	3.64 ± 0.208	2.49 ± 0.259	0.37 ± 0.058
HF + 0.05% PTG	1.99 ± 0.100**	3.49 ± 0.232	2.58 ± 0.246	0.12 ± 0.014**

TG: Triglycerides; TC: Total Cholesterol; HDL-C: HDL-Cholesterol; LDL-C: LDL-Cholesterol.
Values are expressed as means ± SE (n=8). The mean difference is significant from high-fat diet-treated group ($^{*}P<0.05$; $^{**}P<0.01$).

Effects of protopanaxatriol-type of ginsenosides on serum parameters and liver lipids in mice fed with a high-fat diet for 8 weeks

Table 3 showed the data of the serum parameters containing TG, TC, HDL-C and LDL-C of the six group mice. The HF group exhibited a significant increase in serum TG, TC and LDL-C. Compared with the HF group, the normal group showed an extremely significant decrease in serum TG, TC, HDL-C and LDL-C concentrations. And the group fed with high-fat diet with protopanaxatriol-type of ginsenosides showed an extremely significant decrease only in serum TG and LDL-C concentrations. It is the same as Karu *et al*.'s results (Karu *et al*. 2007). They found that high-fat diets with 3% ginseng extract did not affect plasma total cholesterol concentrations of mice. Unlike cholesterol, plasma triacylglycerol concentration was reduced when high-fat diet amended with ginseng extract was consumed.

However, another report showed that protopanaxatriol-type of ginsenosides isolated from red ginseng could reduce total cholesterol concentrations of mice, and the effect was much higer than protopanaxadiol type of ginsenosides isolated from red ginseng (Kim *et al*. 2009).

As shown in **Table 4**, liver of the mice of the HF group fed with a high-fat diet accumulated high content of triacylglycerol (17.18 ± 1.105 μmol/gliver) and total cholesterol (10.01 ± 0.485 μmol/gliver). The concentration of liver TG was 1.79-fold than that of the normal group; its liver TC was 1.6-fold than that of the normal group. High-fat diets containing 0.05% protopanaxatriol-type of ginsenosides significantly prevented the accumulation of hepatic total cholesterol caused by a high-fat diet, compared with a high-fat diet alone.

To some extent, decreasing appetite is one path of anti-obesity. Some gut peptides can decrease appetite. Leptin controls appetite through the hypothalamus (Taylor and McClanahan 2009). Inhibition of fat absorption is another path of anti-obesity. The inhibition of pancreatic and gastric lipases can prevent hydrolysis of triglycerides, thereby reduce triglyceride absorption and the amount of free fatty acids and monoglycerides in the intestine in order to fight obesity (Bernbäck *et al*. 1990). However, appetite was not decreased, and pancreatic lipase activity was not inhibited *in vitro* in our study. It was said that 20(S)-ginsenoside Rg2, 20(S)-ginsenoside Rh1, 20(R)-ginsenoside Rh1, ginsenoside F1, 3-oxo-ginsenoside Rh1 and protopanaxatriol were the metabolites of ginsenoside Re in rat (Chen *et al.* 2009). Ginsenoside Rh1 is famous for its strong pharmacological activity (Loadman *et al*. 2000). Therefore, we ratiocinate that the metabolites of protopanaxatriol-type ginsenosides may own the ability to inhibit panceatic lipase activity. They may reduce triglyceride absorption and the amount of free fatty acids and monoglycerides in the intestine in order to fight obesity.

Adipose tissue lipolysis can decrease fat stores, and is the catabolic process leading to the breakdown of triglycerides stored in fat cells and release of fatty acids and glycerol. Growing hormone and parathyroid hormone stimulates lipolysis in human adipocytes (Langin 2006; Jocken and Blaak 2008). Ethyl alcohol extract and polyphenol fraction of *Salix matsudana* leaves could enhance norepinephrine-induced lipolysis. The polyphenol fractions completely inhibited the incorporation of palmitic acid into brush border membrane vesicles (Han *et al*. 2003). The anti-obesity of protopanaxatriol-type of ginsenosides might also accelerate fat mobilization via enhancing norepinephrine-induced lipolysis, or inhibit the incorporation of palmitic acid into brush border membrane vesicles.

Table 4 Effects of PTG isolated from American ginseng leaves on hepatic triacylglycerol and total cholester in mice fed with a high-fat diet for 8 weeks.

Group	Triacylglycerol (μmol/gliver) Mean ± SE	Total cholester (μmol/gliver) Mean ± SE
Normal	9.53 ± 0.626**	6.11 ± 0.511**
HF	17.18 ± 1.105	10.01 ± 0.485
HF + 0.05%PTG	18.64 ± 1.459	7.47 ± 0.580**

TG: Triglycerides; TC: total Cholesterol.
Values are expressed as means ± SE (n=8). The mean difference is significant from high-fat diet-treated group ($^{*}P<0.05$; $^{**}P<0.01$).

CONCLUSIONS

The results of *in vitro* pancreatic lipase experiments showed that both the protopanaxatriol-type of ginsenosides and ginsenoside Rg1 had no effect on inhibiting the pancreatic lipase activity, while ginsenoside Re had little effect. However, protopanaxatriol-type of ginsenosides isolated from American ginseng leaves had anti-obesity effect *in vivo*. After feeding a high-fat diet to mice for 8 weeks, we found the group fed with high-fat diet with protopanaxatriol-type of ginsenosides showed an extremely significant decrease in serum TG and LDL-C concentrations. It also significantly prevented the accumulation of hepatic total cholesterol and had much more reduction effects on the adipose tissue weights in mice fed with a high-fat diet. Therefore, more studies are needed to further investigate and illuminate their anti-obesity mechanisms, and further studies are necessary to study the lipase activity and anti-obesity of other ginsenosides.

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REFERENCES

Bent S, Ko R (2004) Commonly used herbal medicines in the United States: A review. *The American Journal of Medicine* **116 (7)**, 478-485

Bernbäck S, Bläckberg L, Hernell O (1990) The complete digestion of milk triacylglycerol *in vitro* requires gastric lipase, pancreatic colipase dependent lipase, and bile salt-stimulated lipase. *The Journal of Clinical Investigation* **85 (4)**, 1221-1226

Chen G, Yang M, Guo D (2009) Metabolic study of ginsenoside Re in rats. *China Journal of Chinese materia medica* **34 (12)**, 1540-1543

Drent ML, Larsson I, William-Olsson T, Quaade F, Czubayko F, von Bergmann K, Strobel W, Sjöström L, van der Veen EA (1995) Orlistat (RO 18-0647), a lipase inhibitor, in the treatment of human obesity: A multiple dose study. *International Journal of Obesity and Related Metabolic Disorders* **19**, 221-226

Hadváry P, Lengsfeld H, Wolfer H (1988) Inhibition of pancreatic lipase *in vitro* by the covalent inhibitor tetrahydrolipstatin. *The Biochemical Journal* **256**, 357-361

Han LK, Kimura Y, Kawashima M, Takaku T, Taniyama T, Hayashi T, Zheng YN, Okuda H (2001) Anti-obesity effects in rodents of dietary tea-

saponin, a lipase inhibitor. *Journal of Obesity and Related Metabolic Disorders* **25**, 1459-1464

Han LK, Sumiyoshi M, Zhang J, Liu MX, Zhang XF, Zheng YN, Okuda H, Kimura Y (2003) Anti-obesity action of Salix matsudana leaves (Part 1). Anti-obesity action by polyphenols of Salix matsudana in high fat-diet treated rodent animals. *Phytotherapy Research* **17 (10)**, 1188-1194

Han LK, Takaku T, Li J, Kimura Y, Okuda H (1999) Anti-obesity action of oolong tea. *International Journal of Obesity and Related Metabolic Disorders* **23**, 98-105

Han LK, Zheng YN, Xu BJ, Okuda H, Kimura Y (2002) Saponins from *Platycodi radix* ameliorate high fat diet-induced obesity in mice. *Nutrition Journal* **132**, 2241-2245

Han LK, Zheng YN, Yoshikawa M, Okuda H, Kimura Y (2005) Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicus* rhizomes. *BMC Complementary and Alternative Medicine* **5**, 9-18

Harkey MR, Henderson GL, Gershwin ME, Stern JS, Hackman RM (2001) Variability in commercial ginseng products: An analysis of 25 preparations. *The American Journal of Clinical Nutrition* **73 (6)**, 1101-1106

Hauptman JB, Jeunet FS, Hartmann D (1992) Initial studies in humans with the novel gastrointestinal lipase inhibitor Ro 18-0647 (tetrahydrolipstatin). *The American Journal of Clinical Nutrition* **55**, 309-313

Jocken JW, Blaak EE (2008) Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiology and Behavior* **94 (2)**, 219-230

Karu N, Reifen R, Kerem Z (2007) Weight gain reduction in mice fed *Panax ginseng* saponin, a pancreatic lipase inhibitor. *Journal of Agricultural and Food Chemistry* **55**, 2824-2828

Kim JH, Hahm DH, Yang DC, Kim JH, Lee HJ, Shim I (2005) Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. *Journal of Pharmacological Sciences* **97**, 124-131

Kim JH, Kang SA, Han SM, Shim I (2009) Comparison of the antiobesity effects of the protopanaxadiol- and protopanaxatriol-type saponins of red ginseng. *Phytotherapy Research* **23**, 78-85

Kimura H, Ogawa S, Jisaka M, Kimura Y, Katsube T, Yokota K (2006) Identification of novel saponins from edible seeds of Japanese horse chestnut (*Aesculus turbinata* Blume) after treatment with wooden ashes and their nutraceutical activity. *Journal of Pharmaceutical and Biomedical Analysis* **41**, 1657-1665

Kwok HH, Ng WY, Yang MS, Mak NK, Wong RN, Yue PY (2010) The ginsenoside protopanaxatriol protects endothelial cells from hydrogen peroxide-induced cell injury and cell death by modulating intracellular redox status. *Free Radical Biology and Medicine* **48 (3)**, 437-445

Langin D (2006) Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacological Research* **53 (6)**, 482-491

Liu M (1996) Studies on the anti-aging and nootropic effects of ginsenoside Rg1 and its mechanisms of actions. *Sheng li ke xue jin zhan [Progress in Physiology]* **27 (2)**, 139-142

Liu M, Zhang JT (1996) Studies on the mechanisms of immunoregulatory effects of ginsenoside Rg1 in aged rats. *Acta Pharmaceutica Sinica* **31 (2)**, 95-100

Liu WC, Zheng YN, Han LK, Wang HM, Saito M, Ling MS, Kimura Y, Feng YX (2008) Saponins (ginsenosides) from stems and leaves of *Panax quinquefolium* prevented high-fat diet-induced obesity in mice. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* **15**, 1140-1145

Loadman PM, Phillips RM, Lim LE, Bibby MC (2000) Pharmacological properties of a new aziridinylbenzoquinone, RH1 (2,5-diaziridinyl-3-(hydroxylmethyl)-6-methyl-1,4-benzoquinone), in mice. *Biochemical Pharmacology* **59 (7)**, 831-837

Mukherjee M (2003) Human digestive and metabolic lipases - a brief review. *Journal of Molecular Catalysis. B, Enzymatic* **22**, 369-376

Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, Mitsunaga T, Hashimoto F, Kiso Y (2005) Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. *Journal of Agricultural and Food Chemistry* **53**, 4593-4459

Nocerino E, Amato M, Izzo AA (2000) The aphrodisiac and adaptogenic properties of ginseng. *Fitoterapia* **71**, S1-5

Popovich DG, Yeo SY, Zhang W (2011) Ginseng (*Panax quinquefolius*) and licorice (*Glycyrrhiza uralensis*) root extract combinations increase hepatocarcinoma cell (Hep-G2) viability. *Evidence-based Complementary and Alternative Medicine* **2011**, 408273

Shen LH, Zhang JT (2004) Ginsenoside Rg1 promotes proliferation of hippocampal progenitor cells. *Neurological Research* **26 (4)**, 422-428

Sjöström L, Rissanen A, Andersen T, Boldrin M, Golay A, Koppeschaar HP, Krempf M (1998) Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *Lancet* **352**, 167-172

Taylor PJ, McClanahan KK (2009) Treatment of the obese patient. *Journal of Pediatric and Adolescent Gynecology* **22 (5)**, e174-e182

Tsujita T, Okuda H (1983) Carboxylestrase in rat and human sera and their relationship to serum aryl acylamidases and cholinesterase. *European Journal of Biochemistry (FEBS)* **133**, 215-220

Vuksan V, Sievenpiper JL, Koo VY, Francis T, Beljan-Zdravkovic U, Xu Z, Vidgen E (2000) American ginseng (*Panax quinquefolius* L.) reduces postprandial glycemia in nondiabetic subjects and subjects with type 2 diabetes mellitus. *Archives of Internal Medicine* **160**, 1009-1013

Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, A Rue P, Bell GI, Yuan CS (2005) Anti-diabetic effect of ginsenoside Re in ob/ob mice. *Biochimica et Biophysica Acta* **1740 (3)**, 319-325

Xie JT, Mehendale SR, Wang A, Han AH, Wu JA, Osinski J, Yuan CS (2004) American ginseng leaf: Ginsenoside analysis and hypoglycemic activity. *Pharmacological Research: The Official Journal of the Italian Pharmacological Society* **49 (2)**, 113-117

Xie JT, Shao ZH, Vanden Hoek TL, Chang WT, Li J, Mehendale S, Wang CZ, Hsu CW, Becker LB, Yin JJ, Yuan CS (2006) Antioxidant effects of ginsenoside Re in cardiomyocytes. *European Journal of Pharmacology* **532 (3)**, 201-207

Xu BJ, Han LK, Zheng YN, Lee JH, Sung CK (2005) *In vitro* inhibitory effect of triterpenoidal saponins from Platycodi Radix on pancreatic lipase. *Archives of Pharmacal Research* **28**, 180-185

Yoshizumi K, Hirano K, Ando H, Hirai Y, Ida Y, Tsuji T, Tanaka T, Satouchi K, Terao J (2006) Lupane type saponins from leaves of *Acanthopanax sessiliflorus* and their inhibitory activity on pancreatic lipase. *Journal of Agricultural and Food Chemistry* **54**, 335-341

Yun TK, Lee YS, Kwon HY, Choi KJ (1996) Saponin contents and anticarcinogenic effects of ginseng depending on types and ages in mice. *Acta Pharmacologica Sinica* **17**, 293-298

Zapf J, Schoenle E, Waldvoge M, Sand M, Foresch ER (1981) Effects of trypsin treatment of rat adipocyte on biological effects and binding of insulin and insulin-like growth factors: Further evidence for the action of insulin-like growth factors through the insulin receptor. *European Journal of Biochemistry (FEBS)* **133**, 605-609

Zhang HS, Wang SQ (2006) Ginsenoside Rg1 inhibits tumor necrosis factor-alpha (TNF-alpha)-induced human arterial smooth muscle cells (HASMCs) proliferation. J*ournal of Cellular Biochemistry* **98 (6)**, 1471-81

Zhao Y, Chen B, Luo XB, Zhan GF, Yao SZ (2004) Study on separation and purification process of Panaxdiol-type ginsenosides and panaxtriol type ginsenosides with macroporous adsorption resin. *Natural Product Research and Development* **16**, 235-238