Antihyperlipidemic effects of Korean ginseng in high-fat diet-fed ovariectomized rats

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Abstract Because of decreased estrogen levels, postmenopausal women are at a high risk of developing dyslipidemia, obesity, and other metabolic diseases. The effects of Korean ginseng were investigated to determine whether it can be used as an adjunct supplement for hyperlipidemia. Ovariectomized Sprague-Dawley rats were used to mimic postmenopausal conditions and were randomly divided into five groups ($n=6$): normal control, high-fat diet, high-fat diet+statin, high-fat diet+white ginseng, and high-fat diet + heated ginseng. *In vitro* results revealed that heated ginseng had higher ginsenosides. Meanwhile, *in vivo* results showed that heated ginseng inhibited excessive lipogenesis and promoted lipolysis. It also had a better lipid profile than white ginseng. Finally, plasma adipokine levels indicated a lower risk of developing obesity-induced hyperlipidemia in rats fed heated ginseng. Overall, these results suggest that heated ginseng ameliorates hyperlipidemia and that it can be a suitable alternative to white ginseng.

Keywords: *Panax ginseng*, heated ginseng, high-fat diet, hyperlipidemia, white ginseng

Introduction

Hyperlipidemia is considered to be a metabolic condition and is commonly associated with abnormally high levels of total cholesterol and total triacylglycerol in the serum or plasma. High lipid levels in the plasma can increase the risk of developing cardiovascular diseases such as atherosclerosis. In addition, there are two categories of hyperlipidemia, the first being of genetic origin and the second being induced by other metabolic diseases such as diabetes and obesity (1). The second category, which is associated with aging and lifestyle choices such as physical activities and diet, greatly affects the aging of men and women, particularly perimenopausal women. Menopause is a condition where there is an abrupt decrease in the level of estrogen and other hormones. Although this condition has been said to be commonly associated with an increase in body fat mass, there are other factors that need to be considered and controlled because menopause involves an array of other underlying metabolic disorders (2,3). For example, menopause imposes a great risk for an abnormal atherogenic lipid index and an increased risk of metabolic diseases such as obesity. Although cholesterol-lowering drugs such as statins or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, niacin, bile acid resins, and cholesterol absorption inhibitors are widely available in the market (4) to control lipid profile values, further research is needed to develop safer alternative drugs

because these synthetic drugs, although proven as generally safe and effective, present potential unwanted effects that may be harmful to the body.

Ginseng (*Panax ginseng* C.A. Meyer) is one of the most popular medicinal herbs. It is considered to be one of the most valuable folk medicines in China, Korea, and Japan. Its efficacy and pharmacological effects have been widely and continuously studied in parallel to its increasing demands not only in Eastern but also in Western countries. The active constituents of ginseng, which include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids, have been reported in numerous studies (5). Ginsenosides, the main bioactive component of ginseng, have been found to have antihyperlipidemic, antiobesity, antidiabetes, anticancer, and other pharmacological and therapeutic effects. They are classified according to the type of their aglycone, namely protopanaxadiol (PPD) and protopanaxatriol (PPT) (4). To date, almost 50 ginsenosides have been isolated from ginseng root (6). They are classified by their aglycone component, sugar moiety, or attachment site (7). These minor differences in structure are responsible for their numerous therapeutic activities (8). Moreover, most studies have asserted that ginseng is a herb that can cure almost all ailments and increase non-specific resistance to different pathological conditions (8). It also has a mechanism to restore any abnormality in the body to normal physiological balance. Moreover, ginseng can be classified on the

basis of its country of origin, age, and even method of manufacturing. White ginseng is naturally dried without the use of heat (9). Heated ginseng, on the other hand, can be classified according to the number of stem scars present on the rhizome (10). The more scars it has, the older the ginseng rhizome is. Inoue *et al.* (11) have recently shown that abnormal lipid profiles in hyperlipidemic animals can be lowered by ginseng treatment. Although several studies have explored the potential benefits of ginseng supplementation on metabolic syndrome, ginseng supplementation in relation to menopause-related lipid metabolism has not been examined in detail. Therefore, this study utilized ovariectomized (OVX) female rats, which are a clinical model for pathophysiological conditions associated with menopause, to investigate the effects of heated versus white ginseng supplementation on lipid metabolism and oxidative stress.

Materials and Methods

Standards and chemicals All chemicals and standards used were of HPLC and analytical grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany).

Preparation of heated and white ginseng Three-year-old Korean heated and white ginseng roots (main and fine) were obtained from Punggi Ginseng Cooperative Association (Fig. 1, Yeongju, Korea). The samples were thoroughly washed, placed in a plastic food container (15×20×10 cm, Lock and Lock Co., Ltd., Seoul, Korea) to prevent dryness, and kept in an oven (SW 90D; Sang Woo Scientific Co., Bucheon, Korea) at 80°C with 70% relative humidity for 30 days. Then, the samples were oven dried, pulverized, passed through a 100-mesh sieve, and kept at −20°C until further analyses.

Determination of the total ginsenosides The total ginsenosides were measured based on the procedure of Kim and Park (12), wherein 2 g of freeze-dried ginseng powder was mixed and extracted with 80% methanol for 3 h. The extract was then concentrated using a rotary evaporator (SB-1200; Eyela, Tokyo, Japan). The concentrated extract was mixed with 20 mL solvents (distilled water:n-butanol, V:V, 1:3). Then, the n-butanol layer was separated and further concentrated in a rotary evaporator. The concentrates were dissolved in 2.0 mL methanol, filtered using a 0.45-μm membrane syringe filter, and analyzed using an HPLC equipped with a Capcell Pak C18 column (150×4.6 mm×5 μm, Capcell Pak; Shiseido Co., Ltd., Tokyo, Japan). Water:acetonitrile (V:V, 7:3) was used as the mobile phase; a column temperature of 40°C and flow rate of 1.6 mL/min were also used. The absorbance was measured at 203 nm and ginsenosides Rb₁, Rb₂, Rg₁, and Rg₂ were used as standards. A calibration curve was constructed, and results were expressed as milligrams per gram dry weight of the ginseng sample.

Fig. 1. Photograph of white ginseng (A) and heated ginseng (B).

Table 1. Composition of the experimental diets

Component (g/kg)	Dietary group				
	NC ¹⁾	HF	HF+ST	HF+WG	HF+HG
Corn starch	150.0	84.8	84.8	78.9	78.9
Sucrose	500.0	201.4	201.4	187.3	187.3
Dextrose	-	116.5	116.5	108.3	108.3
Casein	200.0	233.1	233.1	216.8	216.8
Soybean oil	50.0	29.1	29.1	27.1	27.1
Lard	-	206.9	206.9	192.4	192.4
Cellulose	50.0	58.3	58.3	54.2	54.2
Vitamin mix ²⁾	10.0	11.7	11.7	10.9	10.9
Mineral mix ³⁾	35.0	52.4	52.4	48.7	48.7
L-cysteine	3.0	3.5	3.5	3.3	3.3
Choline bitartrate	2.0	2.3	2.3	2.1	2.1
ST	-	-	0.4	-	-
WG	-	-	-	70.0	-
HG	-	-	-	-	70.0
TOTAL (g)	1000.0	1000.0	1000.0	1000.0	1000.0
Total Energy (kcal/g)	3.8	4.6	4.6	4.6	4.6

¹⁾NC, normal diet; HF, high-fat diet; HF+ST, high-fat diet+statin; HF+WG, high-fat diet+white ginseng; HF+HG, high-fat diet+heated ginseng

²⁾AIN-76 vitamin mixture

³⁾AIN-76 mineral mixture

Experimental diet composition Table 1 shows the experimental diet of the laboratory animals based on the commercial semi-synthetic AIN-76A diet (American Institute of Nutrition, New Brunswick, NJ, USA) with slight modifications. The high-fat (HF) diet was based on a 50% calorie high-fat semi-synthetic diet. Based on the total diet, 70 g/kg of aged and white ginseng was used. This value was based on the crude ginseng dosage for humans (1–2 g/day) according to the Expanded German Commission E Monographs (13). The dosage of statin was based on the generally prescribed human dose (10 mg/day) on a daily basis to prevent hyperlipidemia [U.S. Food and Drug Administration (USFDA), 2011].

Experimental animal design Thirty Sprague-Dawley rats aged 9 weeks and weighing 250 g on average were obtained from Central Lab. Animal Inc. (Seoul, Korea). The following experimental animal

design protocol was approved by the Ethics Committee of Kyungpook National University for animal studies (No. 2014-0113). Each rat was housed in a hanging stainless steel cage in a room maintained at $25\pm 2^{\circ}\text{C}$ with 50% relative humidity and a 12/12-h light-dark cycle. Initially, the animals were fed distilled water and commercialized pelletized chow for 7 days *ad libitum*. Bilateral ovariectomy ($n=30$; OVX) was performed under anesthesia (ketamine hydrochloride, 75 mg/kg body weight, intraperitoneal injection; Yuhan Cop., Seoul, Korea). After surgery, the rats were allowed to recover for three weeks. The OVX rats were then randomly divided into five dietary groups ($n=6$): the normal control (NC), HF diet, HF diet+statin (HF+ST), HF diet+white ginseng (HF+WG), and HF diet+heated ginseng (HF+HG). The OVX rats were then fed the modified experimental diets and distilled water *ad libitum* for 8 weeks. Feed intake, feed efficiency ratio, and weight gain were measured. At the end of the experimental period, the rats were sacrificed by anesthetizing via CO_2 inhalation following 12 h fasting.

Blood and tissue collection for biochemical analyses Blood samples were drawn from the abdominal vena cava into pre-treated heparin syringes and immediately centrifuged at $1,000\times g$ for 15 min at 4°C to obtain plasma samples. Internal organs, such as the liver, kidneys, heart, and adipose tissues (abdominal and perirenal), were collected, weighed, and kept at -70°C until further analyses.

Plasma lipid profile The plasma total cholesterol (TC), total triacylglycerol (TG), and HDL cholesterol levels were determined using the instructions on the experimental kits (Asan Pharm. Co., Ltd., Seoul, Korea).

Free fatty acids and phospholipids The plasma free fatty acid (FFA) and phospholipid levels were measured using the procedure of commercially available kits supplied by Enzychrom, BioAssay Systems (Hayward, CA, USA).

Hepatic and adipocyte tissue enzyme extraction Liver and adipose tissue enzymes were prepared using the method of Hulcher and Olson (14). Briefly, the organ tissues were homogenized using 5 mL enzyme buffer composed of 0.1 M triethanolamine, 0.002 M dithiothreitol (DTT), and 0.02 M EDTA. The mixture was then centrifuged (Optima L-100XP; Beckman Coulter, Brea, CA, USA) at $1,000\times g$ for 15 min at 4°C . The supernatant was collected and further centrifuged at $10,000\times g$ for 20 min at 4°C . Thereafter, the precipitate was re-suspended with the enzyme buffer and centrifuged at $10,000\times g$ for 20 min at 4°C . The resulting precipitate was dissolved in an enzyme buffer for carnitoyl transferase (CPT) and β -oxidation (β -Ox) activity assays. Meanwhile, the supernatant was collected and centrifuged at $105,000\times g$ for 60 min at 4°C . The supernatant was collected for glucose-6-phosphate dehydrogenase (G6PD) activity, and the precipitate was re-suspended using the

same buffer for the analysis of fatty acid synthase (FAS) and malic acid dehydrogenase (ME) activities.

Hepatic and adipocyte lipid regulating enzyme activities CPT activities were measured using the mixture used by Beiber *et al.* (15), which contained 116 mM Tris-HCl, 1.1 mM EDTA, 2.5 mM L-carnitine, 0.5 mM 5,5-dithio-2-nitrobenzoic acid, 75 mM palmitoyl CoA, and 0.2% Triton-X. The reaction was initiated by the addition of the tissue sample and by incubating at 25°C for 2 min. The change in absorbance at 412 nm was determined, and the activity was expressed as nmol oxidized CoA/min/mg protein. Meanwhile, the G6PD activities of the samples were analyzed following the method of Pitkänen *et al.* (16) that was based on the reduction of 6.0 mM NADP^+ by G6PD in the presence of glucose-6-phosphate. Changes in the absorbance of the samples at 340 nm at 37°C were determined. Furthermore, the FAS activity was spectro-photometrically determined using Gibson and Hubbard's (17) method. In brief, the assay mixture, which contained 125 mM potassium phosphate buffer (pH 7.0), 10 mM EDTA, 10 mM β -mercaptoethanol, 33 μM acetyl-CoA, 100 μM malonyl-CoA, and 100 μM NADPH reacted with the tissue sample, and the change in absorbance was measured at 340 nm at 30°C . The ME activities were quantified by following the method developed by Ochoa (18), wherein the reaction mixture, composed of 0.4 M triethanolamine, 30 mM malic acid, 0.12 M magnesium chloride, and 3.4 mM NADP^+ , reacted with the tissue sample, and the change in absorbance at 340 nm at 27°C was measured. The activities of G6PD, FAS, and ME were expressed as nmol reduced NADPH/min/mg protein.

β -Oxidation activities β -Ox activities were measured from the final product of NADH by palmitoyl substrates based on the method of Lazarow (19). The assay mixture contained 50 mM Tris-HCl (pH 8.0), 20 mM NAD^+ , 0.33 M DTT, 1.5% bovine serum albumin (BSA), 2% Triton X-100, 10 mM CoA, 1 mM flavin adenine dinucleotide (FAD), 100 mM potassium cyanide, and 5 mM palmitoyl-CoA. The reaction was initiated by the addition of 10 μL tissue sample and by incubation at 37°C for 5 min. The change in absorbance at 340 nm was measured, and the activity was expressed as nmol reduced NADPH/min/mg protein.

Plasma adipokine levels The leptin level was measured using an enzyme immunoassay (EIA) kit (Spi-Bio, Montigny le Bretonneux, France). Resistin, adiponectin, and tumor necrosis factor alpha (TNF- α) levels in the plasma were determined using enzyme-linked immunosorbent assay kits (Shibayagi Co., Gunma, Japan).

Statistical analysis All data are presented as mean $\pm$ SEM. Data were evaluated by one-way ANOVA using Statistical Package for Social Sciences Software Program Version 21 (SPSS Inc., Chicago, IL, USA), and differences between the means were assessed by Tukey's test. Statistical significance was considered at $p<0.05$.

Table 2. Total ginsenoside composition of heated and white ginseng

	PPD ¹⁾ (mg/g dry weight)		PPT (mg/g dry weight)	
	Rb ₁ ²⁾	Rb ₂	Rg ₁	Rg ₂
White ginseng	0.002±0.000 ³⁾	0.003±0.001	0.001±0.000	0.01±0.00
Heated ginseng	0.002±0.000	0.081±0.003*	0.291±0.001*	2.99±0.49*

¹⁾PPD, Protopanaxadiols; PPT, Protopanaxatriols²⁾Rb₁, Rb₂, Rg₁, Rg₂ = ginsenoside varieties³⁾Values are mean±SEM (n=3). Means followed by (*) of the same group are significantly different at *p*<0.05 by an independent *t*-test.**Table 3.** Body weight gain, feed efficiency ratio (FER), and internal organ weights in rats fed experimental diets

Dietary group	Body weight (g)			Feed intake (g/rat/day)	FER ¹⁾	Internal organ weights (g)				
	Initial weight	Final weight	Weight gain			Liver	Heart	Kidneys	Abdominal adipose tissue	Perirenal adipose tissue
NC ²⁾	218.78±4.15 ^{ab3)}	293.21±5.84 ^a	82.79±4.80 ^a	23.57±0.06 ^b	0.05±0.01 ^a	7.50±0.20 ^{ab}	0.77±0.01 ^a	0.76±0.01 ^a	6.92±0.34 ^a	12.04±1.04 ^a
HF	229.41±7.01 ^b	418.60±16.58 ^c	161.71±4.09 ^c	23.42±0.19 ^{ab}	0.11±0.01 ^c	8.26±0.55 ^b	0.82±0.03 ^b	0.85±0.01 ^b	15.52±0.72 ^c	26.75±1.53 ^c
HF+ST	204.71±10.14 ^a	360.62±3.67 ^b	166.24±1.89 ^c	25.35±0.24 ^c	0.11±0.01 ^c	6.67±0.05 ^a	0.73±0.02 ^a	0.74±0.02 ^a	10.90±1.22 ^b	12.08±0.49 ^a
HF+WG	219.34±5.75 ^a	353.54±5.64 ^b	124.90±0.61 ^b	22.49±0.14 ^a	0.09±0.01 ^b	6.48±0.10 ^a	0.74±0.01 ^a	0.75±0.01 ^a	8.71±0.48 ^{ab}	15.02±0.94 ^b
HF+HG	218.22±5.87 ^a	322.59±3.02 ^{ab}	132.74±5.59 ^b	23.55±0.32 ^b	0.09±0.01 ^b	7.35±0.20 ^{ab}	0.77±0.01 ^a	0.74±0.01 ^a	8.71±0.22 ^{ab}	16.37±0.76 ^b

¹⁾FER, food efficiency ratio = body weight gain/feed intake²⁾NC, normal diet; HF, high-fat diet; HF+ST, high-fat diet+statin; HF+WG, high-fat diet+white ginseng; HF+HG, high-fat diet+heated ginseng³⁾Values are mean±SEM (n=6). Means in the same column not sharing a common superscript are significantly different at *p*<0.05.

Results and Discussion

Heated ginseng has higher total ginsenosides than white ginseng

The major PPD and PPT saponins of heated and white ginseng are shown in Table 2. The PPD and PPT aglycone moieties of the ginseng samples greatly differed. PPD-type Rb₁ was the same in both heated and white ginseng, whereas the Rb₂ type was more in heated ginseng (0.081±0.003 mg/g dry wt.) than in white ginseng (0.003±0.001 mg/g dry wt.). Similarly, the PPT-type ginsenosides of heated ginseng Rg₁ and Rg₂ were significantly enriched than those of white ginseng. Overall, heated ginseng had higher ginsenoside amounts than those of white ginseng. In addition, the PPT-type ginsenosides were present in higher amounts than the PPD-type ginsenosides. In general, these ginseng saponins or ginsenosides are said to function together to promote the healing properties of ginseng, such as the lowering of blood glucose, balancing of hormone levels, and stimulation of the endocrine system (20). Therefore, it can be inferred that the higher the ginsenoside content is, the better is the healing quality of the ginseng variety.

Heated ginseng prevents body weight and internal organ weight gain compared with white ginseng in OVX Rats

Hyperlipidemic condition was induced by feeding the animals a saturated fat diet greater than 10%. The present study compared the effects of heated and white ginseng relative to the synthetic drug statin, which is widely used to lower lipid levels in the plasma. The main pharmacologic action of statins is to inhibit the HMG-CoA reductase, which is the main enzyme responsible for cholesterol synthesis, by lowering the total cholesterol and triacylglycerol levels (21). The results revealed

that heated and white ginseng could help suppress body weight gain and reduce the amount of total body fat, thus lowering the risk of developing obesity. Table 3 shows that the OVX rats fed HF+WG and HF+HG had lower body weight gains than those fed an HF diet alone or the commercially lipid-lowering drug statin (HF+ST). However, the total body weight gain and feed intake of the rats fed HF+ST was not significantly different compared with those fed the HF diet. In addition, the HF+ST group showed an increase in feed intake that may have caused greater weight gain than the ginseng groups. Furthermore, Table 3 shows that the organ weights of the OVX rats in the HF group were significantly different compared with those in the other groups. The liver weights in the HF group increased more than 10% relative to the HF+HG and HF+WG groups. Between the two ginseng groups, the liver weights of rats fed heated ginseng were significantly lower than those of the fed white ginseng. Further, the abdominal and perirenal adipose tissue weights of the HF group had increased to about 58.0% relative to the HF+HG and HF+WG groups. However, the weight of adipose tissues between the two ginseng groups was not significantly different. The inclusion of the lipid-lowering statin to the HF diet also lowered the organ weights in general as compared to rats having the HF diet only. Generally, there was a considerable reduction in the organ weights of rats fed ginseng relative to those of the rats in the control group. This further denotes the efficacy of ginseng to reduce adipose tissue mass despite HF diet intake.

Effects of heated and white ginseng on the plasma lipid profile of OVX rats

The HF group had higher TG and TC levels and reduced HDL cholesterol levels than the NC group as shown in Table 4.

However, the inclusion of heated and white ginseng and statin in the HF diet reversed these parameters. TG levels in the HF+HG group were significantly lower than those in the HF+WG group. TC and HDL cholesterol levels in these groups were not significantly different. The HDL cholesterol/total cholesterol ratio (HTR) of the ginseng and statin groups was significantly higher than that of the HF group. An increase above the normal range of serum lipid cholesterol as well as triacylglycerol levels is an abnormal index (=different expression of increase above the normal range) lipid metabolism. Subsequent increases in LDL cholesterol levels in the body are said to be a risk factor for atherosclerosis and other cardiovascular diseases. Meanwhile, HDL cholesterol plays a key role in reversing cholesterol transport, thereby lowering the risk of cholesterol deposition in the arteries (9). With regard to the lipid profile, the rats fed HF+HG and HF+WG diets had lower TC and TG levels than those fed the HF diet, but their HDL cholesterol level was not significantly different from that in the HF diet group. These results suggest that ginseng improves lipid metabolism, thereby preventing hyperlipidemia. Previous studies have reported that ginsenosides, a major component of ginseng, are responsible for its medicinal properties. Ginsenosides lower blood cholesterol levels by increasing cholesterol excretion through bile acid formation (22). Kim and Park (12) have also reported that PPD-type ginsenoside Rb₂ may accelerate serum cholesterol turnover, thereby increasing cholesterol degradation and excretion.

In addition, the atherogenic index (AI) of the ginseng groups was lower than that of the HF group. There was no significant difference in the HTRs and AIs between the commercially available lipid-lowering drug statin and heated and white ginseng. HTRs and AIs of heated ginseng were higher and lower, respectively, than those of the HF diet group. Overall, there was no significant difference in the lipid profile of the OVX rats fed either heated or white ginseng, except that the TG level of the heated ginseng group was lower than that of the white ginseng group. Moreover, plasma FFA and phospholipid levels of the HF group were higher and lower, respectively, than those of the HF+HG and HF+WG groups. These

results indicate the overall low cardiovascular risk incidence caused by heated ginseng intake, in addition to its higher ginsenoside Rb₂ content than white ginseng.

Heated ginseng has higher plasma adipokine levels Plasma adiponectin levels, which have been found to be inversely related to the occurrence of obesity and had antiatherosclerotic activity, were increased in both the HF+HG and HF+WG groups as shown in Table 4. On the other hand, there was a substantial reduction in the levels of leptin and TNF- α in the HF+HG group in comparison with those in the HF group. With respect to resistin levels, however, heated ginseng showed no significant difference in comparison with the HF diet. The decrease in plasma leptin and TNF- α levels and the subsequent elevation in adiponectin levels by ginseng samples are good indicators of their hypolipidemic effect in rats after they were an HF diet. Leptin, which is a regulator of energy homeostasis, is also an adipocyte-derived protein that functions as an adipokine and regulates body energy stores (23). Burguera *et al.* (24) reported that leptin also has the capability of increasing FFA oxidation that contributes to lipid accumulation. They also stated that leptin levels are elevated in obesity and are directly correlated with fat mass. Adiponectin, on the other hand, is said to be a protein hormone that regulates fatty acid catabolism. During obesity, its levels are reduced. This reduction is triggered by the secretion of fatty acids on adipocytes (24). Adiponectin levels in the serum of rats fed powdered ginseng roots were significantly higher than those in the HF diet group. The leptin, resistin, and TNF- α levels of ginseng samples were comparable with those of the synthetic drug, which indicates that ginseng helps in lowering the risk of hyperlipidemia and other metabolic diseases. Finally, the reduced leptin, resistin, and TNF- α levels and higher adiponectin levels of the ginseng samples compared with those of the high HF group may be due to the reduction in body fat mass induced by ginseng consumption, which further leads to a reduction in total cholesterol and triacylglycerol levels in the serum.

Table 4. Plasma lipid profiles and plasma adipokine level of rats fed experimental diets

	NC ¹⁾	HF	HF + ST	HF + WG	HF + HG
TG ²⁾ (mg/dL)	63.15±1.30 ^{bc3)}	80.45±0.37 ^d	60.90±1.30 ^b	67.29±0.99 ^c	54.51±1.35 ^a
TC (mg/dL)	130.64±0.93 ^a	161.82±1.93 ^b	135.48±3.35 ^a	143.54±5.18 ^a	141.39±1.07 ^a
HDL cholesterol (mg/dl)	60.47±6.02 ^b	47.70±3.66 ^a	52.09±2.07 ^a	54.69±3.82 ^a	50.29±1.19 ^a
HTR (%)	46.24±4.38 ^b	29.54±2.63 ^a	38.29±3.48 ^{ab}	36.85±0.61 ^{ab}	40.73±1.84 ^{ab}
AI	1.20±0.22 ^a	2.43±0.29 ^b	1.65±0.25 ^{ab}	1.71±0.04 ^{ab}	1.46±0.11 ^{ab}
FFA (mmol/l)	1.49±0.33 ^{ab}	1.95±0.12 ^{ab}	2.22±0.10 ^b	1.52±0.11 ^{ab}	1.27±0.08 ^a
Phospholipids (mmol/l)	1.31±0.01 ^c	1.21±0.01 ^a	1.30±0.01 ^c	1.26±0.01 ^b	1.31±0.01 ^c
Leptin (ng/mL)	0.17±0.01 ^a	0.53±0.06 ^d	0.33±0.01 ^c	0.25±0.01 ^b	0.36±0.01 ^c
Resistin (ng/mL)	39.28±0.33 ^a	46.14±0.37 ^b	49.77±1.79 ^c	49.50±0.34 ^c	43.72±0.13 ^b
Adiponectin (μg/mL)	1.21±0.05 ^{bc}	0.78±0.01 ^a	0.98±0.06 ^b	1.42±0.09 ^c	0.97±0.02 ^b
TNF- α (ng/mL)	1.06±0.01 ^a	1.42±0.04 ^c	1.16±0.01 ^b	1.13±0.01 ^a	1.27±0.01 ^b

¹⁾NC, normal diet; HF, high-fat diet; HF+ST, high-fat diet+statin; HF+WG, high-fat diet+white ginseng; HF+HG, high-fat diet + heated ginseng

²⁾TC, total cholesterol; TG, total triacylglycerols; HDL/TC ratio (HTR)=(HDL cholesterol/total cholesterol)×100, Atherogenic Index (AI)=(TC HDL cholesterol)/HDL cholesterol; FFA, free fatty acids

³⁾Values are means±SEM (n=6). Means in the same row not sharing a common superscript are significantly different at p<0.05.

Table 5. Lipid-regulating enzymes and β -oxidation activities of rats fed experimental diets

	NC ¹⁾	HF	HF+ST	HF+WG	HF+HG
Hepatic enzyme activity (nmol/min/mg protein)					
FAS ²⁾	3.04±0.22 ^{a3)}	5.49±0.13 ^b	4.88±0.70 ^b	4.60±0.35 ^{ab}	4.59±0.09 ^{ab}
ME	24.72±2.57 ^a	40.71±4.11 ^c	27.50±4.43 ^b	21.75±4.05 ^a	32.48±1.55 ^b
G6PD	9.27±0.60 ^b	26.49±0.13 ^c	11.79±0.88 ^b	6.29±0.57 ^a	5.67±0.52 ^a
CPT	45.40±0.97 ^b	19.92±0.35 ^a	45.14±0.88 ^b	51.38±0.76 ^c	45.40±0.97 ^b
β -oxidation	8.15±1.63 ^c	2.40±0.11 ^a	6.50±0.77 ^b	4.35±0.49 ^{ab}	6.39±0.14 ^b
Adipocyte enzyme activity (nmol/min/mg protein)					
FAS	20.23±4.12 ^a	23.23±1.98 ^a	14.23±0.43 ^a	20.48±1.63 ^a	16.23±0.66 ^a
ME	130.89±4.54 ^a	215.19±3.31 ^b	206.92±0.29 ^b	139.07±7.75 ^a	125.86±2.69 ^a
G6PD	23.31±5.83 ^{ab}	25.82±0.71 ^{ab}	35.64±6.38 ^b	18.31±3.52 ^{ab}	13.73±1.37 ^a
CPT	85.70±3.00 ^b	64.67±4.51 ^a	70.72±2.68 ^a	88.35±2.43 ^b	95.20±1.69 ^b
β -oxidation	13.77±0.79 ^c	3.84±0.14 ^a	6.05±1.37 ^b	5.92±0.46 ^b	6.94±1.63 ^b

¹⁾NC, normal diet; HF, high-fat diet; HF+ST, high-fat diet+statin; HF+WG, high-fat diet+white ginseng; HF+HG, high-fat diet+heated ginseng

²⁾FAS, fatty acid synthase; ME, malic acid dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; CPT, carnitoyl transferase

³⁾Values are means±SEM ($n=6$). Means in the same row not sharing a common superscript are significantly different at $p<0.05$.

Heated ginseng promotes lipolysis and slows down lipogenesis

Table 5 shows that the HF group had higher hepatic and FAS, ME and G6PD values relative to the HF+HG and HF+WG groups, whereas the CPT and β -Ox activities of HF were lower than the HF+HG and HF+WG groups. Between the HF+HG and HF+WG groups, heated ginseng had lower FAS, ME, and G6PD and higher CPT and β -Ox activities than white ginseng. Both ginseng groups had increased lipolysis and decreased lipogenesis on lipid-regulating enzyme activities and were significantly different when compared with the statin-fed rats. Lipogenic enzymes such as FAS, ME, and G6PD are important in the biosynthesis of cholesterol and fatty acids. A decrease in the activity of these enzymes limits the availability of fatty acids needed for triacylglycerol synthesis, thus resulting in a decrease in total triacylglycerol levels in the serum (25). Although there was not much significance in the enzyme activities between heated and white ginseng root samples, they both had significantly decreased enzyme activities relative to the HF group. These strong hypolipidemic activities of the ginseng samples might be due to the presence of various ginsenosides that help modulate lipid metabolism. On the other hand, the hepatic and adipocyte CPT and β -Ox enzyme activities of HF+HG and HF+WG were much higher than those of the HF group. Previous studies have reported that an increase in CPT and β -Ox enzyme activities can increase fatty acid oxidation, thus resulting in a decrease in triacylglycerol accumulation (26). In general, the FAS, ME, and G6PD enzyme activities of both ginseng samples were lower relative to the synthetically treated statin group. These results show that ginseng can be a better alternative to such synthetic drugs in the maintenance treatment for hyperlipidemia as it was earlier reported that ginseng, aside from its fluent action, has no drug tolerance or dependence despite taking it for a longer time period (20).

In conclusion, the results of this study suggest that heated ginseng prevents the progression of menopause-induced hyperlipidemia to a greater extent than white ginseng because of a greater ginsenoside

content of the former. Factors that contribute to the occurrence of hyperlipidemia were lowered by the administration of ginseng in OVX rats fed an HF diet. Overall, these findings strongly suggest that heated ginseng is extremely helpful in alleviating lipid levels or as a therapeutic supplementation in lowering lipid levels and that is comparable with the synthetic drug statin with fewer side effects.

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