Effects of Korean red ginseng supplementation on muscle glucose uptake in high-fat fed rats

Hyun Lyung Jung, Ho Youl Kang*

Department of Physical Education, Kyungpook National University, Daegu, Republic of Korea

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[ABSTRACT] It has been recognized that ginseng has anti-diabetic effects in skeletal muscle, but the mechanism has not been intensively investigated. The aim of this study was to investigate the effects of Korean red ginseng (Panax ginseng) supplementation on muscle glucose uptake in high-fat fed rats. Sixteen rats were randomly divided into two groups: a control group (CON, n = 8) and a Korean red ginseng group (KRG, n = 8). The KRG group ingested RG extract (1 g ·kg⁻¹, 6 days/week) mixed in water for two weeks. After the two-week treatment, plasma lipid profiles, and glucose and insulin concentrations were measured. The triglyceride (TG) and glucose transporter 4 (GLUT-4) contents were measured in the skeletal muscle and liver. The rate of glucose transport was determined under a submaximal insulin concentration during muscle incubation. Plasma FFA concentrations were significantly decreased in KRG (P < 0.05). Liver and muscle triglyceride concentrations were also decreased in the KRG treatment group (P < 0.05) compared to the CON group. In addition, resting plasma insulin and glucose levels were significantly lower after Korean red ginseng treatment (P < 0.05). However, muscle glucose uptake was not affected by Korean red ginseng treatment, as evidenced by the rate of glucose transport in the epitocrhealis muscle under submaximal insulin concentrations. These results suggest that while KRG supplementation could improve whole body insulin resistance and plasma lipid profiles, it is unlikely to have an effect on the insulin resistance of skeletal muscle, which is the major tissue responsible for plasma glucose handling.

[KEY WORDS] Korean red ginseng, plasma lipids, muscle glucose uptake, insulin resistance

1 Introduction

Ginseng roots are used as a remedy for lifestyle-related diseases such as arteriosclerosis hyperlipidemia, hypertension, and non-insulin-dependent diabetes mellitus in China, Korea, Japan, and Europe, and a number of studies have investigated the pharmacological properties of ginseng roots [1-3]. The most widely used ginseng species is Panax ginseng C. A. Mey. (Araliaceae), also called Korean red ginseng. Tonics containing Panax ginseng root extract have been used to treat diabetes for many years, and no adverse effects have been reported [4-6]. Animal studies support that the roots of P. ginseng and those of other ginseng species, including Panax quinquefolius L. (American ginseng), possess anti-hyperglycemic activity [7-8]. Ginseng therapy has been shown to decrease fasting blood glucose levels and body weight in non-insulin-dependent diabetic patients [2]. Furthermore, American ginseng has been shown to attenuate postprandial glycemia in healthy individuals [9-11].

Type 2 diabetes mellitus is a complex metabolic disease, and its pathogenesis involves abnormalities in both peripheral insulin action and insulin secretion by pancreatic β-cells. One of the major defects involved in the development of impaired glucose tolerance and Type 2 diabetes is decreased glucose transport to skeletal muscles, a primary insulin-sensitive tissue, due to insulin resistance [12]. The other feature of type 2 diabetes is that glucose fails to stimulate an adequate release of insulin from pancreatic β-cells [13]. Eventually, the pancreatic β-cells fail to compensate for the raised glucose to achieve homeostasis, resulting in overt hyperglycemia.

Oral administration of P. ginseng root has been shown to improve fructose-rich, chow-induced, insulin resistance in rats [14]. Furthermore, the plasma glucose-lowering action of
P. ginseng root appears to be dependent on an increase in insulin secretion induced by activation of muscarinic M3 receptors in pancreatic β-cells through ACh released from cholinergic nerve terminals [15]. These results suggest that P. ginseng root can potentially be used to treat disorders of glucose homeostasis. However, it is not clear whether P. ginseng root has anti-diabetic effects in skeletal muscle, even though skeletal muscle is the major tissue responsible for plasma glucose handling. Thus, we investigated the effects of Korean red ginseng (KRG) on the insulin resistance of skeletal muscle in rats fed a high-fat diet.

2 Experimental

2.1 Experimental animals

Three-week-old male Wistar rats were divided into two groups and were raised individually in separate (20.7 cm × 17 cm) cages. The rats were kept at a constant temperature of 21 °C, and exposure to light was as follows: 12 hours (08:00–20:00) of darkness, and 12 h of light (20:00–08:00). After a one-week environmental adaptation period, subjects were randomly assigned to the control (CON) group or Korean red ginseng (KRG) group.

2.2 Experimental procedures

The high fat diet comprised 40% beef tallow high fat feed (#101556 AIN-76A; Dyets Inc., Bethlehem, PA, USA), and food and water were provided ad libitum. Six-year-old fresh ginseng was steamed at 95 °C for 3 h, and subsequently dried at 55 °C for 96 h. The moisture content of the dried ginseng root was extracted with hot water under reflux at 95 °C three times, and then filtered. Total crude extracts were concentrated to 65°Bx with hot water under reflux at 95 °C.

3 Material and Methods

3.1 Plasma lipid concentrations

Total cholesterol (TC) and triglyceride (TG) levels were determined by an enzymatic method (Elitech, See, France). Free fatty acid (FFA) levels were measured using Noma’s method [16].

3.2 Plasma glucose, insulin and HOMA-IR measurements

Plasma glucose levels were measured using a YSI 1500 blood and lactate analyzer (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Insulin was measured with a radioimmunoassay method using a commercial diagnostic kit (Lincoplex Research Inc., St. Louis, MO, USA). HOMA-IR was calculated as the product of fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels [17]: HOMA-IR = FPGL× FPI (μIU·L⁻¹)/2430.

3.3 Skeletal muscle and liver TG

The soleus muscle was homogenized in buffer solution (50 mmol·L⁻¹ potassium fluoride + 1 mmol·L⁻¹ EDTA, pH 7.4) at a 1 : 20 dilution, and the liver was homogenized with buffer solution (50 mmol·L⁻¹ potassium phosphate + 1 mmol·L⁻¹ EDTA, pH 7.4) at a 1 : 10 dilution. The homogenized sample was mixed with SDS (100 mmol·L⁻¹), ethanol and n-heptane in order, and then centrifuged at 1 000 × g for 10 min. The supernatant was transferred to a tube, dried for 12 h, and then the remaining liquid was completely evaporated using nitrogen gas. TG in the tube was dissolved with isopropanol, and then the concentration of TG was measured using a diagnostic reagent (Elitech, See, France).

3.4 Rate of glucose transport in skeletal muscle

The glucose transport rate in skeletal muscle was measured using methyl [³H]-D-glucose and D-[¹⁴C]mannitol [18]. Thin portions (20–30 mg) of the left epitrochleas muscle were extracted for use, and after the removal of muscular glucose, the muscle was cleaned once with 18 mmol·L⁻¹ mannitol + 0.1% BSA + 2 mmol·L⁻¹ pyruvate containing KHB for 10 min. After the initial cleaning, the muscle was again incubated in 16 mmol·L⁻¹ mannitol + 0.1% BSA + 4 mmol·L⁻¹ glucose + KHB with/without insulin (1000 μIU·mL⁻¹) [19] for 30 min, cultured, and then cleaned for another 10 minutes. Afterwards, the muscle was once again incubated at 30 °C in 4 mmol·L⁻¹ [³H]-MG (2.2 μCi·mL⁻¹) and 16 mmol·L⁻¹ [¹⁴C] mannitol (0.2 μCi·mL⁻¹) containing 1.5 mL KHB. After culturing, the soleus muscle was fixed with liquid nitrogen and was immediately weighed. The weighed muscle was placed in 1 mol·L⁻¹ NaOH, and then put in a 50–60 °C water bath and shaken for 60 min⁻¹. Afterwards, the mixture was centrifuged at 1 600 r·min for 4 min. The supernatant was removed, and then, after adding Opti-Phase Hisafe 2, a liquid scintillation counter was used to measure radioactivity (Wallac 1409 DSA; PerkinElmer Inc., Waltham, MA, USA) for 10 min.

3.5 Determination of GLUT-4 in skeletal muscle

Portions of gastrocnemius muscle were homogenized for 15 seconds in ice-cold HES buffer (20 mmol·L⁻¹ HEPES, 1 mmol·L⁻¹ EDTA, and 250 mmol·L⁻¹ sucrose), using a motor driven homogenizer (Art-Micera D-8 Model, Art Labortechnik, Müllheim, Germany). Sample homogenates and standards were diluted by 1 : 2 with 2 × Laemmli sample buffer (S3401, Sigma-Aldrich, St. Louis, MO, USA) and incubated for 20 min. Muscle homogenates, which contained 50 μg of protein, were then subjected to SDS-polyacrylamide-gel-electrophoresis under reducing conditions of a 10% resolving gel. Resolved proteins were transferred to a nitrocellulose membrane (BioRad, Hercules, CA). The electrophoregrams were scanned with a densitometer (Amersham, Buckinghamshire, UK) and quantified with an ImageMaster VDS system (Pharmacia, Uppsala, Sweden).
CA, USA) and blocked for 60 min with 5% non-fat milk. Membranes were incubated with GLUT-4 antiserum (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted with 1:10 000 in a T-TBS/5% dry milk, for 90 min. Membranes were washed with T-TBS and incubated with secondary antibody (ZYMED Laboratories; at a dilution of 1:10 000 with T-TBS/1% non-fat milk) for 60 min at room temperature, and washed with T-TBS. The GLUT-4 protein was visualized by Hyperfilm (Eastman Kodak, Rochester, NY, USA) using the Western blot luminal reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

3.6 Statistical analysis
The mean and standard error (mean ± SE) of each measurement was calculated, and statistical analysis was performed using the statistics program, SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The independent sample t-test was used to determine if differences between groups after treatment were significant. Statistical significance was defined as \( P < 0.05 \).

4 Results
4.1 Weight
There was no statistical difference in weight between the CON and KRG groups (Fig. 1).

![Fig. 1 Change in body weight over a period of two weeks (means ± SE, n = 8). CON: Control group; KRG: Korean red ginseng group](image)

4.2 Blood plasma lipid profiles
Blood plasma lipid profiles after two weeks of red ginseng treatment are presented in Table 1. TC, TG, and FFA concentrations were significantly lower in the KRG group than the CON group.

4.3 Plasma glucose and insulin
The mean plasma glucose concentration of the KRG group (7.8 ± 0.5 mmol·L\(^{-1}\), \( P < 0.05 \)) was significantly lower than that of the CON group (8.7 ± 0.5 mmol·L\(^{-1}\)), and the mean insulin concentration of the KRG group (2.1 ± 0.4 μIU·L\(^{-1}\), \( P < 0.05 \)) was significantly lower than that of the CON group (3.2 ± 0.5 μIU·L\(^{-1}\)) (Fig. 2). HOMA-IR was significantly lower in the KRG (0.12 ± 0.00, \( P < 0.05 \)) group than the CON group (0.21 ± 0.00) (Fig. 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>KRG</th>
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<tbody>
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<td>FFA (mmol·L(^{-1}))</td>
<td>0.73 ± 0.07</td>
<td>0.54 ± 0.08*</td>
</tr>
<tr>
<td>TC (mmol·L(^{-1}))</td>
<td>3.7 ± 0.2</td>
<td>3.4 ± 0.2*</td>
</tr>
<tr>
<td>TG (mmol·L(^{-1}))</td>
<td>0.63 ± 0.05</td>
<td>0.54 ± 0.02*</td>
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* \( P < 0.05 \); CON: Control group; KRG: Korean red ginseng group; FFA: free fatty acids; TC: total cholesterol; TG: Triglycerides.

![Fig. 2 Plasma glucose (A) and insulin (B) concentrations (means ± SE, n = 8). *P < 0.05; CON: control group; KRG: Korean red ginseng group](image)

4.4 TG in the liver and muscle
The TG content in the liver and soleus muscles of the KRG group (12.5 ± 0.4, 6.7 ± 0.2 μmol·gww\(^{-1}\), \( P < 0.05 \)) was significantly lower than the CON group (17.0 ± 1.4, 8.0 ± 0.4 μmol·gww\(^{-1}\), respectively) (Fig. 4).

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* \( P < 0.05 \); CON: Control group; KRG: Korean red ginseng group; FFA: free fatty acids; TC: total cholesterol; TG: Triglycerides.

![Fig. 4 Triglyceride content in the liver (A) and soleus muscle (B) (means ± SE, n = 8). *P < 0.05; CON: control group; KRG: Korean red ginseng group](image)

4.5 Glucose transport rate in skeletal muscle
The glucose transport rate of skeletal muscle was not significantly different between the KRG and CON groups at a submaximal insulin concentration, or when insulin was absent (Fig. 5).
4.6 GLUT-4 contents in muscle

The GLUT-4 content of skeletal muscle was not significantly different between the KRG [RQ: (34.9 ± 0.6)%, WQ: (19.4 ± 0.9)%) and CON [RQ: (33.8 ± 1.2)%, WQ: (20.9 ± 1.4)%) groups (Fig. 6).

6 Discussion

The beneficial effects of red ginseng (RG) supplementation on fasting glucose levels are well documented [20-21]. The finding here that RG supplementation lowered the fasting glucose levels of rats is consistent with previous studies [22-24]. Abnormal elevations in blood glucose levels are averted predominantly through the actions of insulin. The beta cells of the pancreas sense fluctuations in blood glucose and respond by adjusting insulin secretion appropriately. Insulin resistance, as the name implies, is a condition in which a greater than normal insulin concentration is required to control upward fluctuations in blood glucose [25]. In the present study, KRG rats had significantly lower fasting glucose and insulin levels than CON rats. Also, when HOMA-IR index was calculated, as a marker of insulin sensitivity, the value of HOMA-IR in KRG rats was significantly enhanced in the comparison with that in CON rats.

In the fasted condition, blood glucose concentration is primarily a function of liver glucose output, which is dependent on gluconeogenesis and glycogen hydrolysis [26]. Therefore, the lower fasting insulin level following RG supplementation may have resulted from enhanced liver insulin sensitivity, reducing the need to lower the fasting glucose concentration. In the current study, it was observed that the hepatic triglyceride content in KRG-supplemented rats was significantly lower than that of CON rats. The triglyceride content in the liver is strongly and positively related to both liver and whole body insulin resistance [27-28]. These results suggest that KRG supplementation could reduce plasma glucose and insulin levels through the attenuation of hepatic insulin resistance, as evidenced by the lower liver triglyceride content in the KRG group than the CON group.

Skeletal muscle is the predominant tissue responsible for glucose disposal, and is therefore an important tissue to examine when assessing insulin sensitivity [29]. Insulin sensitivity was therefore assessed by incubating muscle in a solution with a submaximal insulin concentration, which allowed us to evaluate skeletal muscle insulin resistance. It was found that KRG supplementation did not affect the glucose transport rate, a marker of muscle insulin sensitivity, of skeletal muscle. Also, GLUT-4 protein levels were similar between the KRG and CON groups, in which GLUT-4 is the major glucose transporter protein in skeletal muscle, and the muscle GLUT-4 protein levels are significantly related to the rate of glucose transport during muscle incubation [30]. However, it was reported that KRG supplementation for 12-18 weeks significantly increased GLUT-4 at adipocyte tissue [31] and skeletal muscle membrane [4]. It was assumed that the reason for the difference between this study and the two previous studies [4, 31] in the results of GLUT-4 could be due to the duration of KRG intake. Thus, a future investigation is needed with various dosages and duration for GLUT-4 in muscle.

The majority of the previous studies reported that ginseng supplementation in humans, rats, and mice significantly improved insulin sensitivity based on OGTT [4, 20, 22, 32]. However, OGTT measures whole body insulin resistance, and not the insulin sensitivity of skeletal muscle, despite the importance of skeletal muscle in insulin resistance. It is therefore recommended that a muscle incubation be performed to evaluate the changes in muscle insulin sensitivity when investigating the effects of putative anti-diabetic compounds. It is reported for the first time that two-week KRG supplementation of rats fed a high-fat diet did not affect the insulin resistance of skeletal muscle. However, the long-term effect of KRG supplementation on the insulin resistance of skeletal muscle was not determined. A longer follow-up period is required in future studies.

In the present study, the hypolipidemic effects of RG in
rats consuming a high-fat diet were also evaluated. High blood cholesterol level is a major risk factor for the development of coronary heart disease (CHD) [33] and high serum TG levels are associated with higher risks of CHD [34-35]. In this study, rats that consumed KRG had lower concentrations of plasma TC and TG than rats that ate a high-fat control diet. Previous studies reported that ginseng significantly reduced serum triglyceride levels in rats and humans [3, 4, 36]. Similar to the effects of RG on serum cholesterol levels, it was assumed that the RG-induced decrease in serum triglyceride levels was, in part, due to the RG-induced reduction in lipid absorption by the small intestine. The serum cholesterol content in the body is balanced by dietary cholesterol absorption, cholesterol synthesis in the body, and biliary cholesterol excretion. The cholesterol-lowering effect of saponins extracted from ginseng are due to an increase in bile acid excretion [37], which occurs through interference with the absorption of cholesterol [38]. Potter [39] also indicated that saponins might alter the absorption of cholesterol and bile acids. One of the possible mechanisms for this effect is that saponins can form micelles with bile acid [40]. KRG treatment resulted in a significant decrease in plasma triglyceride levels. Furthermore, the hepatic triglyceride content of KRG rats was significantly lower than that of high-fat fed controls. Thus, because plasma triglyceride levels are determined by hepatic triglyceride synthesis, release from the liver, and activity of lipoprotein lipase, it was hypothesized that RG affects these processes.

Many previous studies have suggested that an increase in circulating FFA is a key factor mediating insulin resistance [41, 42]. It was found in this study that plasma FFA levels were significantly decreased in RG-supplemented rats compared to control rats. This result suggests that short-term KRG supplementation could improve lipid accumulation and the insulin sensitivity of the liver and whole body, independently of an effect on FFA levels.

In conclusion, KRG supplementation significantly improved plasma glucose and lipid profiles in rats fed a high-fat diet. However, KRG supplementation for two weeks did not affect the rate of glucose transport of skeletal muscle under a submaximal insulin concentration. Therefore, these results suggest that while KRG supplementation could improve whole body insulin resistance and plasma lipid profiles, it is unlikely to have an effect on the insulin resistance of skeletal muscle, which is the major tissue responsible for plasma glucose handling.

References


