Anti-Diabetic and Anti-Obese Effects of Ginseng: from Root to Berry

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Abstract

We investigated anti-hyperglycemic and anti-obese effects of *Panax ginseng* berry extract and its major constituent, ginsenoside Re, in obese diabetic C57BL/6J *ob/ob* mice and their lean littermates. Animals received daily intraperitoneal injections of *Panax ginseng* berry extract for 12 days. On Day 5, 150 mg/kg extract-treated *ob/ob* mice had significantly lower fasting blood glucose levels compared to vehicle-treated mice (156±9.0 mg/dl vs. 243±15.8 mg/dl, $P<0.01$). On Day 12, the extract-treated *ob/ob* mice became normoglycemic (137±6.7 mg/dl) and had significantly improved glucose tolerance. The overall glucose excursion during the two-hour intraperitoneal glucose tolerance test (IPGTT), calculated as area under the curve (AUC), decreased by 46% ($P<0.01$) compared to vehicle-treated *ob/ob* mice. Glucose levels of lean mice were not significantly affected by the extract. The improvement in blood glucose levels in 150 mg/kg extract-treated *ob/ob* mice was associated with significant reduction in serum insulin levels of fed and fasting mice. Consistent with an improvement in insulin sensitivity, hyperinsulinemic euglycemic clamp study revealed a more than 2-fold increase in the rate of insulin-stimulated glucose disposal in treated *ob/ob* mice (112±19.1 vs. 52±11.8 μmol/kg/min for the vehicle group, $P<0.01$). In addition, 150 mg/kg extract-treated *ob/ob* mice, but not the lean mice, lost significant weight (from 51.7±1.9 g on Day 0 to 45.7±1.2 on Day 12, $P<0.01$ compared to vehicle-treated *ob/ob* mice), associated with a significant reduction in food intake ($P<0.05$) and a very significant increase in energy expenditure ($P<0.01$) and body temperature ($P<0.01$). A 12-day treatment with 150 mg/kg *Panax ginseng* berry extract also significantly reduced plasma cholesterol

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levels in *ob/ob* mice. Additional studies demonstrated that ginsenoside Re, a major constituent of the ginseng berry, but not from the root, plays a significant role in anti-hyperglycemic action. This anti-diabetic effect of ginsenoside Re was not associated with body weight changes, suggesting that other constituents in the extract have distinct pharmacological mechanisms on energy metabolism. The identification of a significant anti-hyperglycemic activity in ginsenoside Re may provide an opportunity to develop a novel class of anti-diabetic agent.

Introduction

Diabetes mellitus is a major health problem, affecting approximately 5% of the total population in the U.S. and 3% of the population world-wide. Over 90% of diabetics belong to type 2, or non-insulin-dependent diabetes mellitus (NIDDM); the remainder falls into the category of type 1, or insulin-dependent diabetes mellitus (IDDM). Although the two types of diabetes have distinct pathogeneses, hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are the most common features. Epidemiological studies (1-3) and clinical trials (4,5) strongly support the notion that hyperglycemia is the principal cause of complications. Effective control of the blood glucose level is the key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients (6,7). Thus, sustained reductions in hyperglycemia will decrease the risk of developing microvascular complications, and most likely reduce the risk of macrovascular complications (8).

The ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load (9,10). Patients with type 2 diabetes exhibit a marked reduction in insulin-mediated glucose disposal (11,12). While insulin resistance is independently associated with obesity, which accompanies 80% of type 2 diabetic patients in the West (10,13), insulin resistance is more severe in these obese patients (14).

Currently available drugs for type 2 diabetes have a number of limitations such as adverse effects and high rates of secondary failure. This has led to the search for alternative therapies that may have a similar degree of efficacy without the troublesome side effects associated with the conventional drug treatment. The identification of compounds from medicinal plants with anti-hyperglycemic activity may also provide an opportunity to develop a new class of anti-diabetic agent.

Historical records reveal that in traditional medical systems, a disease corresponding to type 2

diabetes was treated with plant extracts (15). For example, the root of *Panax ginseng* or Asian ginseng has been used clinically to treat type 2 diabetes (16,17) and has also been used as a tonic, often taken for years without evidence of adverse effects or toxicity (18,19). Results of *in vitro* (20,21) and *in vivo* (22-25) animal studies and clinical trials (26,27) support the claim that the root of *Panax ginseng* and the root of other ginseng species (e.g., *Panax quinquefolius* or American ginseng) possess anti-hyperglycemic activity. However, most *in vivo* animal studies have utilized type 1, not type 2, diabetic models. In addition, these previous studies have not investigated the mechanisms responsible for its anti-diabetic effects which are yet unknown.

The active components of ginseng are considered to be ginsenosides, a group of steroidal saponins (17,19). Ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry. Different parts of the plant contain distinct ginsenoside profiles (19), and these parts may have different pharmacological activities. Whether *Panax ginseng* berry exhibits significantly more potent anti-hyperglycemic activity than the root has not been explored.

We recently observed that, compared to *Panax ginseng* root, the berry extract possessed significantly stronger activities in normalizing hyperglycemia and reducing body weight in an animal model with type 2 diabetes. We used the *ob/ob* mouse model, which exhibits profound obesity and type 2 diabetes (28). In *ob/ob* mice, mutation of the obese gene leads to morbid obesity and metabolic abnormalities such as hyperglycemia, glucose intolerance, and hyperinsulinemia that phenotypically resemble human type 2 diabetes. In addition, *ob/ob* mice exhibit reduced metabolism and body temperature. We also explored the mechanisms responsible for glucose homeostasis by measuring *in vivo* insulin-stimulated glucose disposal, body weight regulation, and energy expenditure changes. Finally, we examined whether ginsenoside Re, a major constituent from the berry but not from the root, plays an important role in anti-hyperglycemic activity.

Research Design and Methods

Panax ginseng berry extract analysis

Panax ginseng berry organic solvent extract, from one batch of ginseng, was obtained from Jian Pharmaceutical Company, China. The constituents of the extract were analyzed in our laboratory using high performance liquid chromatography (HPLC). The high pressure gradient HPLC system was manufactured by Shimadzu Corp. (Kyoto, Japan). Chromatography was

performed on a Phenomenex, Prodigy C_{18} 5 μm 150 × 3.2 mm analytical column protected by guard column Phenomenex C_8 30 × 3.2 mm. 20 mg dried powder of the extract was dissolved in 1,000 μl 90% MeOH, and 20 μl of solution was injected into the system. Separations were obtained by linear gradient elution, using eluents A (water) and B (acetonitrile) according to the following profile: 0-60 min, A 90-60%, B 10-40%, curve = 1; 60-70 min, A 60%, B 40%. Flow rate was 0.6 ml/min at 22°C. The UV detector range was 0.01 AUFS and 202 nm wavelength.

We also prepared *Panax ginseng* root extract using the same extraction procedure for the berry extract preparation. The root was obtained from Shanghai Pharmaceutical Company, China. Six major ginsenoside concentrations of *Panax ginseng* root extract and *Panax ginseng* berry extract were compared using the same HPLC assay. In some experiments, we evaluated the effects of ginsenoside Re which was obtained from Shanghai Pharmaceutical Company, China. HPLC analysis was performed in our laboratory to confirm that ginsenosides Re had a purity of >99%.

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Chicago. Male C57BL/6J *ob/ob* mice and their lean littermates (+/?) were obtained from Jackson Laboratory (Bar Harbor, ME). Adult animals at 10-18 weeks of age were used. Mice were housed in environmentally controlled conditions with a 12-h light/dark cycle and had free access to standard rodent pellet food (Zeigler Bros., Gardners, PA), except when fasted before some experiments.

Drug preparation and administration

250 mg of ginseng berry extract was dissolved in 50 ml MeOH as solution A. 1,500 mg polyvinylpyrrolidone or PVP-10 (Sigma Chemicals, St. Louis, MO) was dissolved in 50 ml MeOH as solution B. After mixing A and B, the mixture was evaporated under N_2 to yield 250 mg dried extract at 50°C. Before each experiment, the dried extract was dissolved in distilled deionized water and vortexed for 2 min at room temperature. The solution was injected intraperitoneally (IP) once a day at a dose of 50 or 150 mg/kg body weight. Ginsenoside Re, at a dose of 5, 10 or 20 mg/kg, was also dissolved in PVP-10 solution for daily IP administration. The extract or ginsenoside Re were administered for 12 days. 150 mg/kg *Panax ginseng* berry extract contains approximately 20 mg/kg ginsenoside Re. Control treated animals were injected an equimolar solution of PVP-10. No detectable irritation or restlessness was observed following

each drug or vehicle administration. No noticeable adverse effects (i.e., respiratory distress, abnormal locomotion, and catalepsy) were observed in any animals after the drug or vehicle treatment.

Measurement of blood glucose and serum insulin levels, and intraperitoneal glucose tolerance test

Fed or fasting blood glucose and serum insulin levels were measured in tail blood samples obtained either at 9 : 00 AM (for fed) or at 1 : 00 PM following a 4 hr fasting (starting from 9 : 00 AM) on Day 0 (before treatment), Day 5 (during treatment), and Day 12 (last day of treatment). Blood glucose levels were determined with a Glucose Analyzer (Hemocue AB, Angelholm, Sweden), and serum insulin levels were assayed with a Unitra Sensitive Insulin ELISA Kit (Crystal Chem, Chicago, IL).

Intraperitoneal glucose tolerance test (IPGTT) was performed on Day 0 and Day 12. On the days of the test, animals fasted for 4 hr (starting from 9 : 00 AM) followed by an IP administration of glucose (2 g/kg). Blood glucose levels were determined in tail blood samples at 0 (prior to glucose administration), and 30, 60 and 120 min after glucose administration.

Hyperinsulinemic-euglycemic clamp

For the glucose clamp study, animals at Day 10 of treatment were catheterized in the right internal jugular vein under general anesthesia. The catheter was externalized through an incision in the skin flap at the vertex of the head. The catheterized animals were allowed to recover for 3-5 days under continuous treatment of the extract or vehicle before the clamp studies. 120-min hyperinsulinemic-euglycemic clamps (6 mU/kg/min for lean mice, and 10 mU/kg/min for the *ob/ob* mice) were performed on 4 hr fasted mice by maintaining blood glucose concentrations at 6.6 mmol using a variable rate of 20% glucose infusion as previously described (29). During the clamps, mice were awake and unrestrained. Both glucose and insulin (porcine regular insulin, Eli Lilly, Indianapolis, IN) were administered into the same catheter implanted in the jugular vein through a T-connector. A two-channel micro-dialysis syringe pump (CMA/Microdialysis, Acton, MA) was used to control the rate of infusion. Blood samples (approximately 5 μl each) were collected from the tail every 15 min during the clamp to measure the glucose levels and adjust the rates of glucose infusion. The average glucose infusion rate in the second half of the clamp was taken as the rate of whole body glucose disposal.

Monitoring of food consumption

Animals were individually housed in a specially designed Metabolic Cage (Nalge Nunc International, Rochester, NY), which has a food chamber that only permits the insertion of the head. The cage also has a deck to collect spilled food pellets without contamination. Food intake was determined by measuring the difference between the pre-weighed standard chow and the weight of chow and spill every 24 hours.

Body temperature and energy expenditure

Mice body temperature was measured with a thermocouple probe (Physitemp, Clifton, NJ). On Day 0, Day 5, and Day 12 at 3 : 00 PM, the thermocouple probe was inserted approximately 1 cm into the rectum to obtain body temperature.

Oxygen consumption measurements were made in an Oxymax chamber with an air flow rate of 0.18 l/min for 2 hr at 25°C. Airflow was controlled and measured using a mass flow meter (Flow control [R-1], Applied Electrochemical Inc., Pittsburgh, PA). Gas composition of incoming outdoor air and exhaust gas were measured using an infrared gas analyzer for CO_2 (Infrared Analyzer 864, Beckman Instruments, Fullerton, CA), and an electrochemical O_2 detector (Ametek S-3A, Applied Electrochemical Inc., Pittsburgh, PA). Gas analyzers were calibrated daily using cylinders of primary gas standard mixtures with known concentrations of CO_2, O_2 and N_2. Each animal was placed in a respiration chamber and was allowed to equilibrate for 1 hr. Oxygen consumption and CO_2 production were monitored every 5 min during the 2nd hr with the use of a computer-controlled open circuit calorimetry system. Values for energy expenditure (30) were calculated every 5 min. Instruments were interfaced with a computer for calculations.

Statistical Analysis

Data are expressed as mean±SE. Statistical significance between the vehicle-treated vs. drug-treated mice, and between prior to drug-treated vs. after drug-treated mice were determined by Students *t* test and analysis of variance (ANOVA) for repeated measures, with $P<0.05$ considered statistically significant.

Results

Effects of Panax ginseng berry extract on fasting blood glucose levels

Blood glucose levels after 4 hr fasting in C57BL/6J *ob/ob* mice and their lean littermates were measured on Day 0, Day 5, and Day 12 after daily administration of *Panax ginseng* berry extract or vehicle. We observed that *ob/ob* mice had significantly higher fasting blood glucose levels compared to lean controls (222±16.2 vs. 176±12.1 mg/dl, $P<0.01$) on Day 0. On Day 5, blood glucose concentrations of *ob/ob* mice treated with 150 mg/kg *Panax ginseng* berry extract decreased significantly (156±9.0, $P<0.01$ compared to vehicle-treated mice 243±15.8 mg/dl). On Day 12, *ob/ob* mice treated with the extract were normoglycemic (137±6.7 mg/dl, $P<0.01$ compared to vehicle treated mice 211±19.6 mg/dl), and there was no significant difference in the levels between *ob/ob* mice and lean littermates (167±12.8 mg/dl). The blood glucose concentrations of lean mice were not affected significantly in response to treatment with the extract (182±9.2 mg/dl vs. 167±12.8 mg/dl of vehicle-treated mice) on either of those days.

Effects of Panax ginseng berry extract on glucose tolerance test

Glucose tolerance was evaluated by IPGGT, prior to and 12 days after treatment in *ob/ob* and lean mice with the extract or vehicle. On Day 0, *ob/ob* mice demonstrated basal hyperglycemia, and this hyperglycemia was exacerbated by the IP glucose load, and failed to return to fasting level after 120 min, indicating glucose intolerance. After 12 days of treatment with *Panax ginseng* berry extract 50 mg/kg and 150 mg/kg, the glucose tolerance of the *ob/ob* mice dose-dependently improved, compared to the vehicle-treated group. On Day 12, the blood glucose levels at 120 min following glucose administration approached baseline (fasting) levels in 150 mg/kg extract treated *ob/ob* mice. The difference in area under the curves (AUC) of blood glucose between Day 0 and Day 12 in the 150 mg/kg extract-treated *ob/ob* mice group was 46%. This was a significant improvement in glucose exposure from 623 mg/mlmin of Day 0 to 334 mg/mlmin of Day 12 ($P<0.01$). In contrast, the glucose tolerance of lean control mice was unaffected by both the vehicle and the 150 mg/kg extract.

Effects of Panax ginseng berry extract on serum insulin levels

In parallel with the improvement of blood glucose concentrations, there was a significant reduction in both fed and fasting serum insulin levels in animals treated with 150 mg/kg *Panax*

ginseng berry extract. The *ob/ob* mice were profoundly hyperinsulinemic under the fed condition before treatment (36±6.6 ng/ml on Day 0; the average value for lean control was 2.1±0.4 ng/ml), and 12-day treatment with the extract reduced fed serum insulin by 40% ($P < 0.01$ compared to vehicle-treated mice, n = 5). Similar to the decline in fasting glucose level, the fasting insulin levels of *ob/ob* mice treated with the extract reduced by approximately 40% on both Day 5 and Day 12, compared to vehicle-treated mice ($P<0.01$, n = 5). In addition, treatment with *Panax ginseng* berry extract also improved the insulin secretory response to glucose load at 30 min of the IPGTT in the *ob/ob* mice. The percentage increase of insulin levels at 30 min over 0 min was 6.6±1.0% on Day 0 and 45±17.9% on Day 12 ($P<0.05$).

Effects of Panax ginseng berry extract on insulin stimulated glucose disposal

To further elucidate the mechanisms of the anti-hyperglycemic effect of *Panax* ginseng berry extract, we measured body-wide insulin-stimulated glucose disposal rate with the hyperinsulinemic euglycemic clamp. The rate of glucose disposal by the animals during the insulin stimulation was inferred from the amount of glucose infused per min to maintain blood glucose level at approximately 6.6 mmol. We observed clamped blood glucose levels and exogenous glucose infusion rates. Glucose infusion rate for untreated *ob/ob* mice was only approximately 18% of that in lean controls, indicating a severe peripheral insulin resistance. After a 12-day treatment with 150 mg/kg extract, the rate of insulin-stimulated glucose disposal in *ob/ob* mice was more than double relative to the vehicle-treated *ob/ob* mice (112±19.1 vs. 52±11.8 μmol/kg/min for the vehicle-treated group, n = 4, $P<0.01$). Again, the extract did not affect the rate of glucose disposal in lean control mice (400±53.8 vs. 370±51.4, n = 4, $P<0.01$).

Effects of Panax ginseng berry extract on body weight changes

The average body weight of adult *ob/ob* mice is almost twice as great of their lean littermates. The effects of *Panax ginseng* berry extract on body weight changes in *ob/ob* mice were observed. The body weight of animals in the vehicle-treated group showed a tendency to increase from Day 0 to Day 12. During a 12-day treatment with extract at 50 mg/kg, body weight increase ceased. However, after a 12-day treatment with extract at 150 mg/kg, body weight reduced significantly from 51.7±1.9 g on Day 0, 48.3±1.5 g on Day 5, to 45.7±1.2 on Day 12 ($P<0.05$ and $P<0.01$ compared to Day 5 and Day 12 vehicle-treated *ob/ob* mice, respectively). Following the cessation of treatment, *ob/ob* mice gradually regained weight, and their body weight approached

that of vehicle treated *ob/ob* mice after 22 days.

The body weight of lean mice in the vehicle-treated group also showed a tendency to increase from 27.1±1.2 g on Day 0, 27.8±1.9 g on Day 5, to 28.9±1.0 g on Day 12. However, during a 12-day treatment with extract at 150 mg/kg, body weight increase in lean mice ceased, i.e., 26.5±1.5 g on Day 0, 26.9±1.4 g on Day 5, and 26.5±1.0 g on Day 12.

Effects of Panax ginseng berry extract on food consumption and energy expenditure

To understand the mechanisms of body weight reduction associated with *Panax ginseng* berry extract treatment, we measured daily food consumption during the treatment, and body temperature and the energy expenditure before and after the treatment.

During a 12-day observation in *ob/ob* mice, the mean daily food intake of vehicle group (n = 6) and 150 mg/kg extract-treated group (n = 8) was 88.7±2.5 g/kg/day and 75.0±2.2 g/kg/day, respectively. There was a significant difference in daily food intake between the vehicle group and 150 mg/kg extract-treated group ($P<0.05$).

As expected, *ob/ob* mice were significantly hypothermic (35.6±0.2°C, n = 14) compared to their lean littermates (36.9±0.2°C, n = 14, $P<0.01$). After 12-day treatment with 150 mg/kg extract, body temperature in *ob/ob* mice (n = 6) significantly increased from 35.6±0.1°C (Day 0) to 36.6±0.1°C (Day 12, $P<0.01$).

Energy expenditure values were obtained in *ob/ob* mice treated with vehicle or *Panax ginseng* berry extract 150 mg/kg. After the 12-day treatment, there was a significant increase in energy expenditure of the extract-treated group (n = 6) compared to the vehicle-treated group (n = 5) (19.3±1.0 cal/min vs. 12.6±0.4 cal/min, $P<0.01$).

Effects of Panax ginseng berry extract on plasma cholesterol level changes

Panax ginseng berry extract also significantly reduced plasma cholesterol levels in *ob/ob* mice. Plasma cholesterol concentration of 150 mg/kg extract-treated *ob/ob* mice after 12 days treatment was significantly lower (117±18.3 mg/dl) compared to the vehicle-treated animals (169 ±12.4 mg/dl, n = 6, $P<0.05$).

Anti-hyperglycemic, but not anti-obese activities of ginsenoside Re

Blood glucose levels after 4 hr fasting were measured on Day 0, Day 5, and Day 12 after daily administration of ginsenoside Re. Dose-dependent effects of ginsenoside Re on fasting blood

glucose in *ob/ob* mice have been showed. Fasting blood glucose concentrations decreased significantly after treatment with 20 mg/kg ginsenoside Re on Day 5 of 188±9.2 mg/dl and Day 12 of 180±10.8 mg/dl (both $P<0.01$ compared to vehicle-treated group on Day 5 of 234±13.7 mg/dl and Day 12 of 239±13.3 mg/dl). Fasting blood glucose concentrations did not change sizably in lean mice after treatment with ginsenoside Re.

Glucose tolerance evaluated by IPGGT was obtained, prior to and 12 days after ginsenoside Re administration. With 20 mg/kg ginsenoside Re treatment in lean mice, and the glucose tolerance was not statistically affected. Compared to vehicle-treated *ob/ob* mice, 20 mg/kg ginsenoside Re treatment significantly decreased the blood glucose levels at 60 min and 120 min following glucose administration (both $P<0.01$).

In contrast to both anti-diabetic and anti-obese effects of *Panax ginseng* berry extract, after 12-day treatment with ginsenoside Re 20 mg/kg, body weight did not change significantly in *ob/ob* mice. Body weight in ginsenoside Re 20 mg/kg group (n = 6) was 53.1±1.4 g on Day 0, 52.9± 1.5 g on Day 5, and 54.7 ± 1.7 on Day 12.

Discussion

The present study was undertaken to investigate anti-hyperglycemic effects of *Panax ginseng* berry extract and ginsenoside Re in a type 2 diabetic *ob/ob* mouse model. In this obese, insulin resistant mouse model, obesity is due to a mutation in the obese gene that codes for leptin. Animals that are homozygous for the mutation exhibit morbid obesity and metabolic abnormalities that resemble type 2 diabetes in humans. The heterozygous littermates are lean and normoglycemic. Our results clearly demonstrated that extract of *Panax ginseng* berry significantly improved the glucose homeostasis of this mouse model of type 2 diabetes. The fasting blood glucose levels started to decrease after 5 days of treatment with 150 mg/kg of the extract, and became completely normal by Day 12. More importantly, the IPGTT results normalized after treatment. Dose-dependent anti-diabetic effects of ginsenoside Re were also observed in *ob/ob* mice. Our results showed that lean littermate controls were not sensitive to the glucose lowering effects caused by the extract and ginsenoside Re. This is the first report demonstrating that ginseng berry and ginsenoside Re can be used to treat diabetes. In our most recent study, similar results were observed in *db/db* mice (data to be published separately).

The anti-diabetic effect of ginseng root has recently been demonstrated experimentally by

several groups. For instance, Kimura et al. (25) observed a notable fall in blood glucose levels 6 hours after a single 90 mg/kg ginseng root extract IP dose in genetically obese diabetic KK-CA^y mice. Vuksan et al. demonstrated that 3 g of American ginseng root, given 40 minutes prior to the test meal, significantly lowered the blood glucose in non-diabetic subjects and type 2 diabetic patients (27). However, differences between these studies and ours make it difficult to have a direct comparison. When studying a chronic disease, like diabetes, it is more pertinent to test the maintenance of lower blood glucose levels with long-term treatment rather than acute hypoglycemic effect after a single dose. In this study, we measured fasting blood glucose 5 and 12 days after treatment with *Panax ginseng* berry extract and ginsenoside Re. Unlike the short-term treatment study, we found that these compounds progressively reduced blood glucose levels in *ob/ob* mice. The treatment effects could be seen on Day 5, and became more evident on Day 12.

Prospective studies of populations at high risk for type 2 diabetes suggest that in most patients, the initial inherited lesion is insulin resistance (31,32). Insulin-stimulated *in vivo* glucose disposal is markedly reduced in patients with type 2 diabetes (12). In *ob/ob* mice by six weeks of age, insulin resistance and hyperinsulinemia is well developed (33). In association with normalization of blood glucose levels, treatment with *Panax ginseng* berry extract in *ob/ob* mice also significantly reduced serum insulin concentration at both fed and fasting states, indicating an improvement of peripheral insulin action. The insulin sensitizing effect of the berry extract was further supported by our hyperinsulinemic euglycemic clamp study. Improvement in peripheral insulin sensitivity will increase tissue glucose uptake and lower blood glucose levels.

Another possible action site for ginseng berry to exert its hypoglycemic effect is in the gastrointestinal tract. Gastric vagal afferents are the primary neuroanatomical link between the stomach and the brainstem. In a previous study, we reported that ginseng root extract, via gastric vagal afferents, inhibited brainstem neuronal activity (34). Others have reported that gastric secretion *in vitro* was inhibited by ginseng (35). These results suggest that ginseng may slow the digestion of food, and decrease the rate of carbohydrate absorption into portal circulation.

Insulin resistance is very often accompanied by obesity. Obesity not only increases the chance of developing type 2 diabetes, it is independently associated with insulin resistance and other morbidity (10). Thus, the insulin resistance in obese patients with type 2 diabetes is significantly worse than the insulin resistance of non-obese diabetic individuals (14). Therapeutic agents with both anti-diabetic and anti-obese effects are, therefore, particularly beneficial. Our results show

that *ob/ob* mice treated with 50 and 150 mg/kg of *Panax ginseng* berry extract underwent a dose and time-dependent reduction in body weight. In this study, we observed, for the first time, that *Panax ginseng* berry has an anti-obese effect in addition to an anti-diabetic effect. Past studies have shown that insulin sensitivity in type 2 diabetes patients improves with weight loss (36), possibly due to an improvement in insulin-stimulated glucose transport into muscle (37). A similar mechanism may operate in *Panax ginseng* berry extract-treated *ob/ob* mice to improve insulin resistance. The extract could exert its anti-diabetic effect through actions which involve improvement of insulin sensitivity and the balance between food intake and energy expenditure. Since anti-diabetic effect of ginsenoside Re was achieved without an anti-obese effect, it is possible that weight reduction induced by *Panax ginseng* berry extract was not solely responsible for the hypoglycemic effect in *ob/ob* mice. Future studies are required to identify compound(s) in the extract with anti-obese action.

The weight loss we observed after treatment with *Panax ginseng* berry extract resulted from a 15% reduction in food intake and a 35% increased energy expenditure. Our results further support the latter by demonstrating a significant higher body temperature in the extract-treated *ob/ob* mice along with an increase in oxygen consumption. Kelley et al. (38) reported that in patients with type 2 diabetes, calorie restriction, independent of weight loss, can improve insulin sensitivity. The obesity, hyperphagia, hypothermia, and reduced energy expenditure of *ob/ob* mice is due to a lack of leptin, which, in lean mice signals hypothalamic centers on the status of fat stores (28,39). Whether *Panax ginseng* berry extract improve these defects by restoring hypothalamic control awaits further study. Past studies have shown that leptin also had a tendency to reduce body weight gain in lean mice (39,40). We also observed a relative body weight reduction in extract-treated lean mice in our study.

In this study, we observed that *Panax ginseng* berry extract also significantly reduced plasma cholesterol levels in *ob/ob* mice. The mechanisms of action of this effect require further investigation. However, reduction of cholesterol level by the extract may have an important clinical significance, since hyperlipidemia is often associated with type 2 diabetic patients.

Based on previous ginseng root anti-hyperglycemic studies, it appears that ginsenosides, the active components present in both root and berry, play a therapeutic role. The profile and concentrations of ginsenosides vary between the root and berry, and this difference may contribute to the significant anti-hyperglycemic and/or anti-obese effects observed in our study. Our data demonstrated that *Panax ginseng* berry contains a much higher concentration of

ginsenoside Re compared to the root. Interestingly, we observed that ginsenoside Re had a significant anti-hyperglycemic activity without affecting body weight in *ob/ob* mice. This suggests that other constituents in *Panax ginseng* extract have distinct pharmacological mechanisms that affect energy metabolism.

In summary, the present study demonstrated that administration of *Panax* ginseng berry significantly improved systemic insulin sensitivity and glucose homeostasis in *ob/ob* mice. Improvements in insulin sensitivity were associated with normalization of insulin-mediated glucose utilization and reduced body weight. Loss of body weight was accompanied by a reduction in food intake, and perhaps more importantly, by an increase in energy expenditure. Our results support overall *in vivo* anti-hyperglycemic and anti-obese activity of *Panax ginseng* berry extract that may prove to be of clinical importance in improving the management of type 2 diabetes. In addition, the identification of a significant anti-hyperglycemic activity in ginsenoside Re may provide an opportunity to develop a novel class of anti-diabetic agent.

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