

Antidiabetic Effects of *Panax ginseng* Berry Extract and the Identification of an Effective Component

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We evaluated antihyperglycemic 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 12, 150 mg/kg extract-treated *ob/ob* mice became normoglycemic (137 ± 6.7 mg/dl) and had significantly improved glucose tolerance. The overall glucose excursion during the 2-h intraperitoneal glucose tolerance test decreased by 46% ($P < 0.01$) compared with vehicle-treated *ob/ob* mice. The improvement in blood glucose levels in the extract-treated *ob/ob* mice was associated with a significant reduction in serum insulin levels in fed and fasting mice. A hyperinsulinemic-euglycemic clamp study revealed a more than twofold increase in the rate of insulin-stimulated glucose disposal in treated *ob/ob* mice (112 ± 19.1 vs. 52 ± 11.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for the vehicle group, $P < 0.01$). In addition, the extract-treated *ob/ob* mice lost a significant amount of weight (from 51.7 ± 1.9 g on day 0 to 45.7 ± 1.2 on day 12, $P < 0.01$ vs. 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 ($P < 0.01$). Treatment with the extract also significantly reduced plasma cholesterol levels in *ob/ob* mice. Additional studies demonstrated that ginsenoside Re plays a significant role in antihyperglycemic action. This antidiabetic 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. *Diabetes* 51: 1851–1858, 2002

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HPLC, high-performance liquid chromatography; IP, intraperitoneal; IPGTT, intraperitoneal glucose tolerance test.

Diabetes is a major health problem, affecting ~5% of the total population in the U.S. and 3% of the population worldwide. Over 90% of patients with diabetes have type 2 diabetes; the remainder have type 1 diabetes. 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 blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes (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). Although insulin resistance is independently associated with obesity, which exists in 80% of type 2 diabetic patients in the West (10,13), it is more severe in obese patients (14).

Historical records reveal that in traditional medical systems, a disease similar 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 antihyperglycemic activity. However, most in vivo animal studies have used type 1, not type 2, diabetic models. In addition, these previous studies have not investigated the mechanisms responsible for the antidiabetic effects of *Panax ginseng*, 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.

The root of ginseng is a commonly used herbal medicine. Whether *Panax ginseng* berry exhibits significantly more potent antihyperglycemic activity than the root has not been explored. The identification of compounds from ginseng with antihyperglycemic activity may also provide an opportunity to develop a new class of antidiabetic agent.

This study sought to determine whether *Panax ginseng* berry extract normalizes hyperglycemia and reduces body weight in an animal model with type 2 diabetes. We used the *ob/ob* mouse model, which exhibits profound obesity and insulin resistance (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, these 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 only, plays an important role in antihyperglycemic activity.

RESEARCH DESIGN AND METHODS

***Panax ginseng* berry extract analysis.** *Panax ginseng* berry extract from one batch was obtained from Jian Pharmaceutical (Changchun, China). Fresh berry was first mixed with 75% EtOH. The seeds were removed, and the pulp was collected and refrigerated. The pulp was filtered and refrigerated again, and EtOH evaporated. Distilled water was added to the solution and filtered. The solution was further mixed with 1-BuOH, the water layer was removed, and 1-BuOH evaporated. Finally, the extract solution was lyophilized. Constituents of the extract were analyzed in our laboratory using high-performance liquid chromatography (HPLC).

We also prepared *Panax ginseng* root extract using the same extraction procedure for the berry extract preparation. The root was obtained from Shanghai Pharmaceutical (Shanghai, China). Figure 1 compares six major ginsenoside concentrations of *Panax ginseng* root extract and *Panax ginseng* berry extract using the same HPLC assay. Chemical structure of ginsenoside Re is also shown in the figure.

In some experiments, we evaluated the effects of ginsenoside Re, which was obtained from Shanghai Pharmaceutical. HPLC analysis was performed in our laboratory to confirm that ginsenoside 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). Mice were housed in environmentally controlled conditions with a 12-h light/dark cycle and had free access to standard rodent pellet food (Zeigler Brothers, Gardners, PA), except when fasted before some experiments. Adult animals at 10–18 weeks of age were used.

Drug preparation and administration. *Panax ginseng* berry extract was dissolved in polyvinylpyrrolidone, or PVP-10 (Sigma, St. Louis, MO), and then evaporated. Before each experiment, the dried extract was dissolved in distilled water and injected at an intraperitoneal (IP) dose of 50 or 150 mg/kg. Ginsenoside Re, at a dose of 5, 10, or 20 mg/kg, was also dissolved in PVP-10 solution for IP administration. The 150 mg/kg berry extract contains ~20 mg/kg ginsenoside Re. Control treated animals were injected with an equimolar solution of PVP-10. No detectable irritation or restlessness was observed after 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 A.M. (for fed) or at 1:00 P.M. after a 4-h fast (starting from 9:00 A.M.) 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, Angelholm, Sweden), and serum insulin levels were assayed with a Unitor Sensitive Insulin ELISA Kit (Crystal Chemicals, Chicago).

An intraperitoneal glucose tolerance test (IPGTT) was performed on day 0 and day 12. On test days, animals fasted for 4 h (from 9:00 A.M.) and then

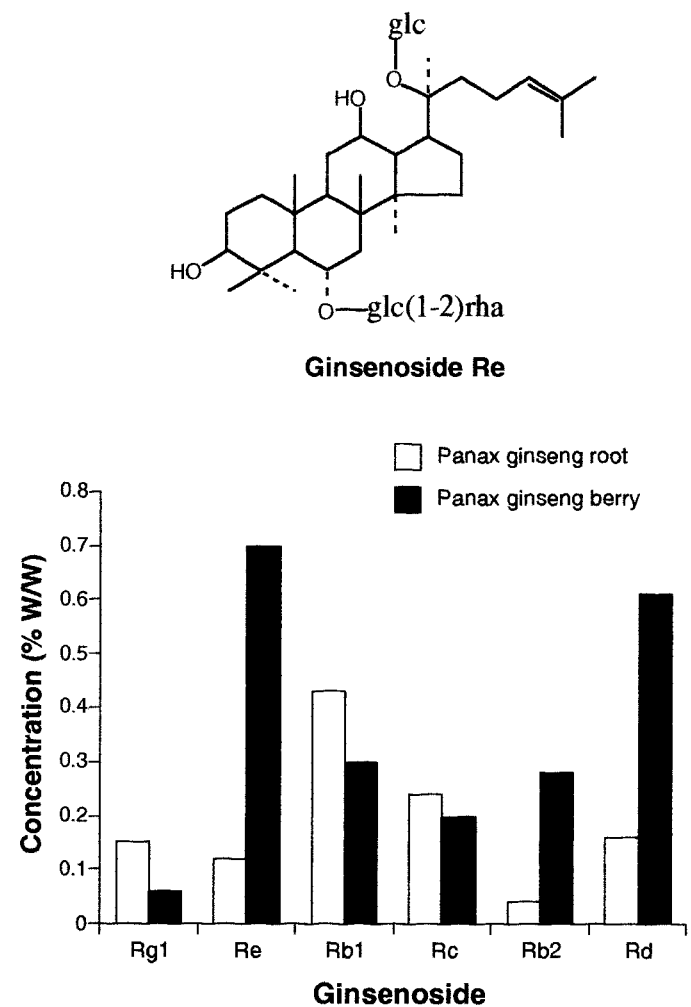


FIG. 1. Percentage weight of six ginsenosides in fresh *Panax ginseng* berry versus fresh *Panax ginseng* root using HPLC analysis. Concentrations of ginsenoside Re, Rb2, and Rd in the berry were significantly higher than in the root. Chemical structure of ginsenoside Re is also shown.

received IP administration of glucose (2 g/kg). Blood glucose levels were determined from tail blood samples at 0 (before glucose administration), 30, 60, and 120 min after glucose administration.

Hyperinsulinemic-euglycemic clamp. For the glucose clamp study, animals received daily *Panax ginseng* berry extract or vehicle for 12 consecutive days. On day 10, animals 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 ~4 days (from day 10 and day 14) while they continued to receive the treatment on days 11 and 12 and no treatment afterward. Clamp studies were performed on day 14. The 120-min hyperinsulinemic-euglycemic clamps ($6 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for lean mice and $10 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for *ob/ob* mice) were performed on 4-h-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. A two-channel microdialysis syringe pump (CMA/Microdialysis, Acton, MA) was used to control the rate of infusion. Blood samples (~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 (Nalgene Nunc International, Rochester, NY) that had a food chamber that only permits the insertion of the head and a deck to collect spilled food pellets without contamination. Food intake was

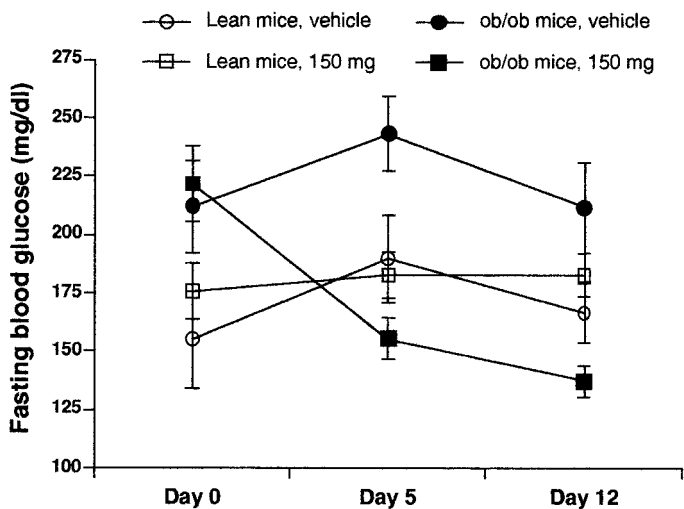


FIG. 2. Effect of *Panax ginseng* berry extract on fasting blood glucose concentrations in adult lean littermates ($n = 5$) and *ob/ob* mice ($n = 6$). On day 0, glucose levels were higher in *ob/ob* mice than in lean mice. Glucose levels decreased significantly in 150 mg/kg *Panax ginseng* berry extract-treated *ob/ob* mice on day 5 ($P < 0.01$ vs. vehicle-treated mice) and day 12 ($P < 0.01$ vs. vehicle-treated mice). 150 mg/kg = 150 mg/kg *Panax ginseng* berry extract.

determined by measuring the difference between the preweighed standard food and the weight of the food and spilled food every 24 h.

Body temperature and energy expenditure. The body temperatures of the mice were measured with a thermocouple probe (Physitemp, Clifton, NJ). On day 0, 5, and 12 at 3:00 P.M., the thermocouple probe was inserted ~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 h at 25°C. Airflow was controlled and measured using a mass flow meter (Flow Control [R-1]; Applied Electrochemical, Pittsburgh, PA). Gas composition of incoming outdoor air and exhaust gas was measured using an infrared gas analyzer for CO₂ (Infrared Analyzer 864; Beckman Instruments, Fullerton, CA) and an electrochemical O₂ detector (Ametek S-3A; Applied Electrochemical). Gas analyzers were calibrated daily using cylinders of primary gas standard mixtures with known concentrations of CO₂, O₂, and N₂. Each animal was placed in a respiration chamber and allowed to equilibrate for 1 h. Oxygen consumption and CO₂ production were monitored every 5 min during the second hour with 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 means \pm SE. Statistical significance between the vehicle-treated versus drug-treated mice and between before drug-treated versus after drug-treated mice were determined by Student's *t* test and 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 h of fasting in C57BL/6J *ob/ob* mice and their lean littermates were measured on day 0, 5, and 12 after daily administration of *Panax ginseng* berry extract or vehicle. As shown in Fig. 2, *ob/ob* mice had significantly higher fasting blood glucose levels than lean controls on day 0 (222 ± 16.2 vs. 176 ± 12.1 mg/dl, $P < 0.01$). On day 5, blood glucose concentrations decreased significantly in *ob/ob* mice treated with 150 mg/kg *Panax ginseng* berry extract (156 ± 9.0 mg/dl, $P < 0.01$ vs. 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, $P < 0.01$ vs. 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 vs. 167 ± 12.8 mg/dl of vehicle-treated mice) on either of those days.

Effects of *Panax ginseng* berry extract on the glucose tolerance test. Glucose tolerance was evaluated by IPGTT before and 12 days after treatment in *ob/ob* and lean mice with the extract or vehicle. As shown in Fig. 3, on day 0, *ob/ob* mice demonstrated basal hyperglycemia, and this hyperglycemia was exacerbated by the IP glucose load and failed to return to the fasting level after 120 min, indicating glucose intolerance. After 12 days of treatment with 50 mg/kg (Fig. 3B) and 150 mg/kg (Fig. 3C) *Panax ginseng* berry extract, the glucose tolerance of the *ob/ob* mice dose dependently improved compared with the vehicle-treated group (Fig. 3A). On day 12, the blood glucose levels at 120 min after glucose administration approached baseline (fasting) levels in 150 mg/kg extract-treated *ob/ob* mice. The difference in area under the curves 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 $623 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ on day 0 to $334 \text{ mg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ on 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. As shown in Fig. 4A, the *ob/ob* mice were profoundly hyperinsulinemic under fed conditions 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% ($P < 0.01$ vs. vehicle-treated mice). Similar to the decline in fasting glucose level, the fasting insulin levels of *ob/ob* mice treated with the extract reduced by ~40% on both day 5 and day 12 compared with vehicle-treated mice ($P < 0.01$; Fig. 5B). In addition, treatment with *Panax ginseng* berry extract also improved the insulin secretory response to glucose load at 30 min of the IPGTT in *ob/ob* mice. The percentage increase of insulin levels at 30 min over 0 min was $6.6 \pm 1.0\%$ on day 0 and $45 \pm 17.9\%$ on day 12 ($P < 0.05$).

Effects of *Panax ginseng* berry extract on insulin-stimulated glucose disposal. To further elucidate the mechanisms of the antihyperglycemic 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 minute to maintain blood glucose levels at ~6.6 mmol. Figure 5 shows clamped blood glucose levels (A) and exogenous glucose infusion rates (B). Glucose infusion rate for untreated *ob/ob* mice was only ~18% of that in lean controls, indicating a severe peripheral insulin resistance. After 12 days of 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. $52 \pm 11.8 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for the vehicle-treated group, $n = 4$, $P < 0.01$), although this rate

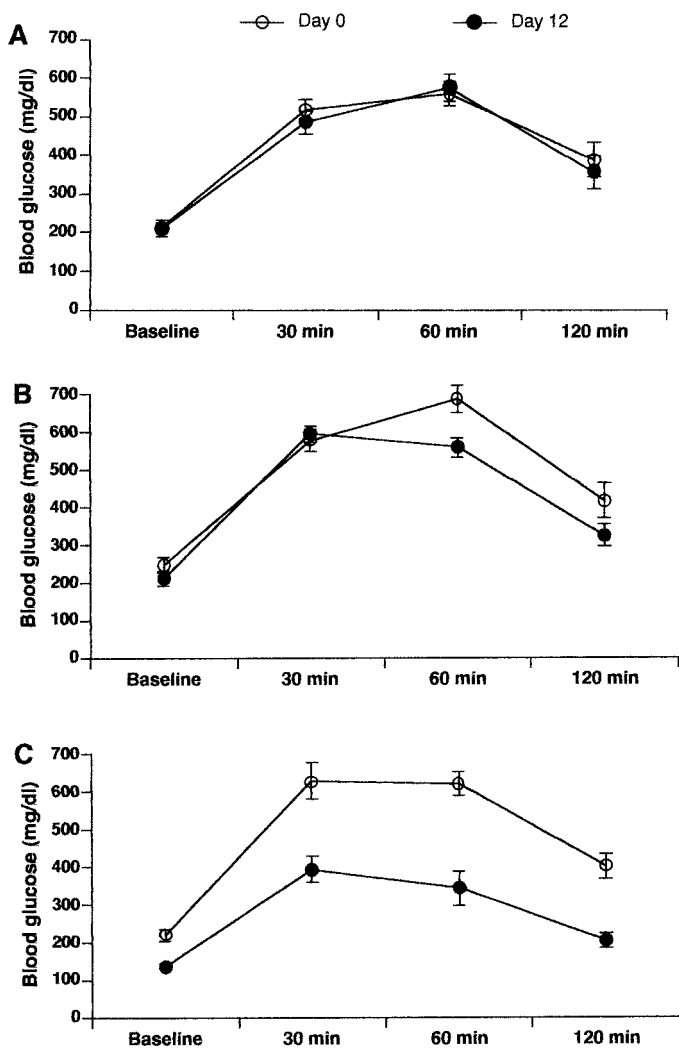


FIG. 3. IPGTT in *ob/ob* mice before and after a 12-day treatment with *Panax ginseng* berry extract. A: Vehicle-treated group ($n = 5$). B: 50 mg/kg extract-treated group ($n = 5$). C: 150 mg/kg extract-treated group ($n = 6$), which has a significantly higher rate of glucose disposal at 30, 60, and 120 min ($P < 0.01$ vs. vehicle-treated mice).

was only 28% of the rate for lean mice. 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 that of their lean littermates. Figure 6A shows the effects of *Panax ginseng* berry extract on body weight changes in *ob/ob* mice. 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 gain 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 to 48.3 ± 1.5 g on day 5 to 45.7 ± 1.2 g on day 12; $P < 0.05$ and $P < 0.01$ vs. day 5 and day 12 vehicle-treated *ob/ob* mice, respectively). After 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 (Fig. 6B).

The body weight of lean mice in the vehicle-treated group also showed a tendency to increase from $27.1 \pm$

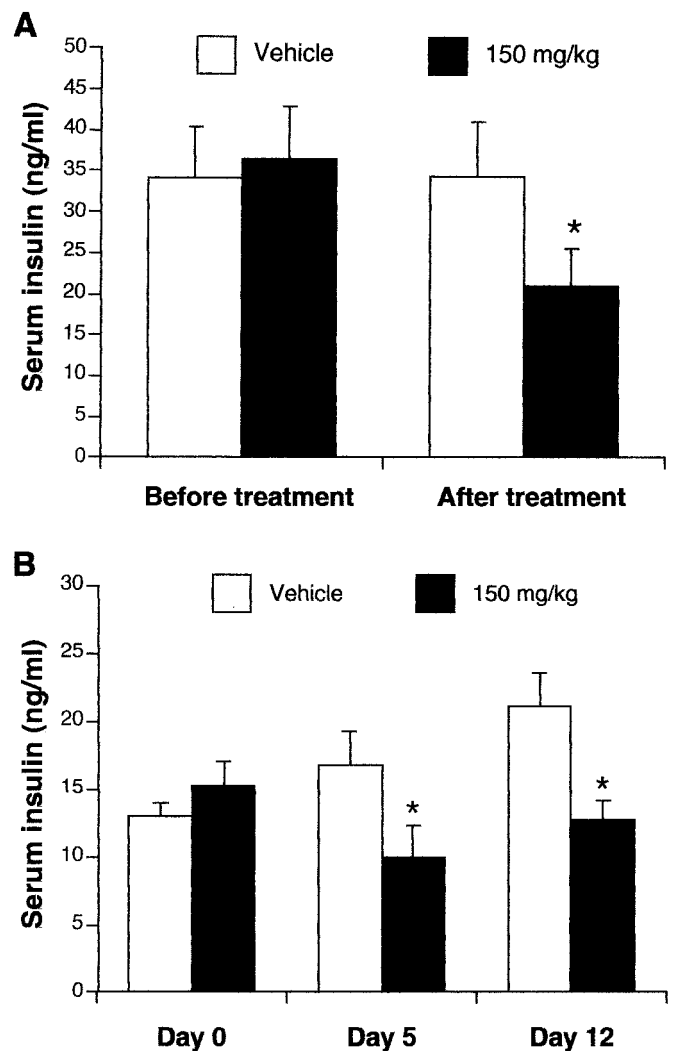


FIG. 4. Effects of *Panax ginseng* berry extract on serum insulin concentrations in fed and fasting *ob/ob* mice. A: Serum insulin levels reduced significantly under the fed state after a 12-day extract treatment ($n = 5$ per group). B: Fasting serum insulin levels also reduced significantly after 5- and 12-day extract treatment ($n = 5$ per group). * $P < 0.01$ vs. vehicle-treated mice.

1.2 g on day 0 to 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 treatment and body temperature and energy expenditure before and after treatment.

During a 12-day observation in *ob/ob* mice, the mean daily food intake of the vehicle group ($n = 6$) and the 150 mg/kg extract-treated group ($n = 8$) was 88.7 ± 2.5 g \cdot kg $^{-1} \cdot$ day $^{-1}$ and 75.0 ± 2.2 g \cdot kg $^{-1} \cdot$ day $^{-1}$, 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 ($35.6 \pm 0.2^\circ\text{C}$, $n = 14$) compared with their lean litter-

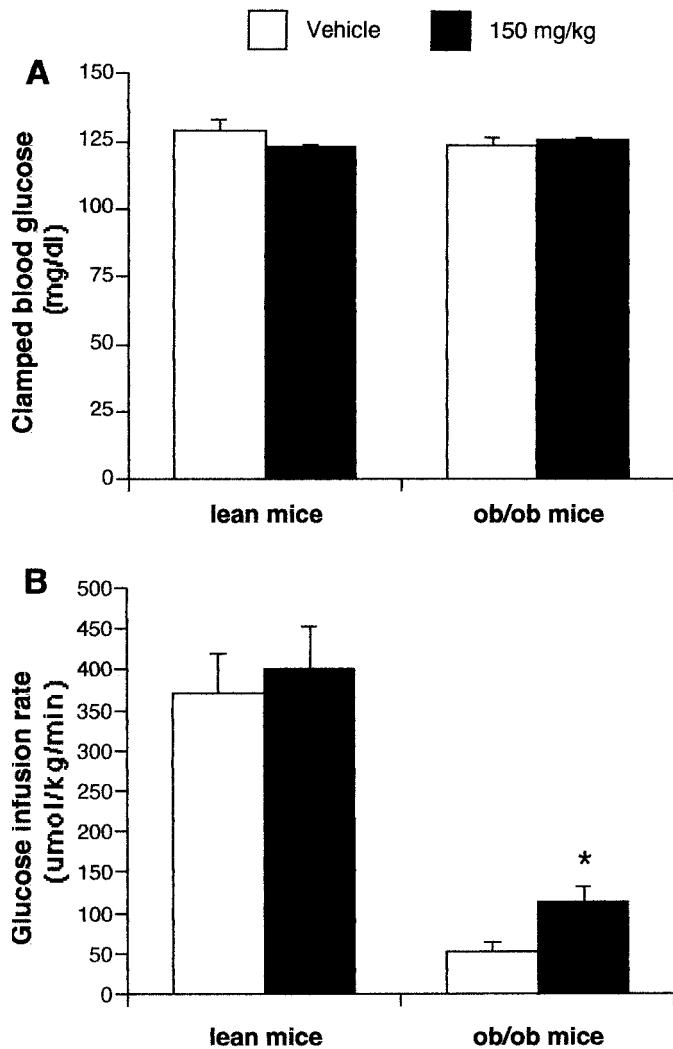


FIG. 5. Effect of *Panax ginseng* berry extract on whole-body glucose disposal during hyperinsulinemic-euglycemic clamps in *ob/ob* mice and lean littermates. Clamped blood glucose levels (A) and steady-state glucose infusion rates (B) obtained from the average rates of 120-min hyperinsulinemic-euglycemic clamps were determined on day 14 after a 12-day treatment with 150 mg/kg extract. Data were obtained from four separate experiments. * $P < 0.01$ vs. vehicle-treated mice.

mates ($36.9 \pm 0.2^\circ\text{C}$, $n = 14$, $P < 0.01$). After a 12-day treatment with 150 mg/kg extract, body temperature in *ob/ob* mice ($n = 6$) significantly increased from $35.6 \pm 0.1^\circ\text{C}$ (day 0) to $36.6 \pm 0.1^\circ\text{C}$ (day 12, $P < 0.01$).

Energy expenditure values were obtained in *ob/ob* mice treated with vehicle or 150 mg/kg *Panax ginseng* berry extract. After a 12-day treatment, there was a significant increase in energy expenditure in the extract-treated group ($n = 6$) compared with the vehicle-treated group ($n = 5$) (19.3 ± 1.0 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 concentrations in 150 mg/kg extract-treated *ob/ob* mice after 12 days of treatment were significantly lower (117 ± 18.3 mg/dl) than those in the vehicle-treated animals (169 ± 12.4 mg/dl, $n = 6$, $P < 0.05$).

Antihyperglycemic, but not anti-obese, activities of ginsenoside Re. Blood glucose levels after 4 h of fasting

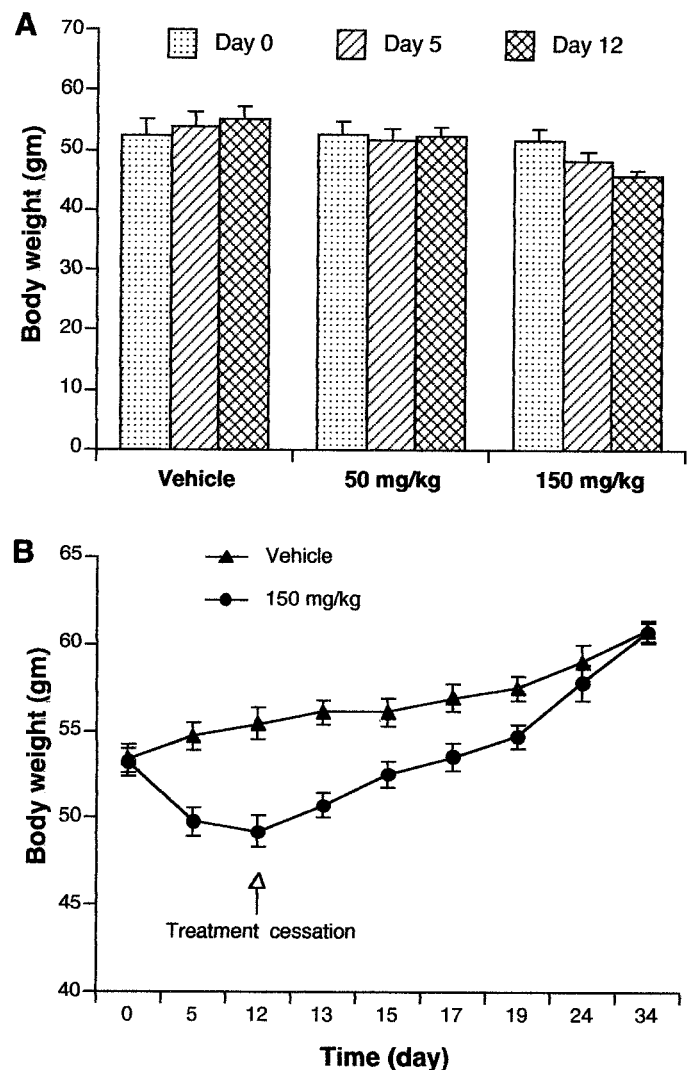


FIG. 6. Effects of *Panax ginseng* berry extract on body weight in *ob/ob* mice. A: The tendency of increased body weight from day 0 to day 12 in vehicle-treated mice. The 50 mg/kg of extract ceased body weight increase. After 12 days of treatment with 150 mg/kg extract, body weight reduced significantly ($P < 0.01$ vs. vehicle-treated mice). B: After the cessation of 150 mg/kg extract treatment ($n = 6$), *ob/ob* mice gradually regained body weight similar to vehicle-treated mice ($n = 5$). Note the ordinate scale starts from 40 g in B. 50 mg/kg = 50 mg/kg *Panax ginseng* berry extract. 150 mg/kg = 150 mg/kg *Panax ginseng* berry extract.

were measured on day 0, 5, and 12 after daily administration of ginsenoside Re. Figure 7 shows the dose-dependent effects of ginsenoside Re on fasting blood glucose in *ob/ob* mice. Fasting blood glucose concentrations decreased significantly after treatment with 20 mg/kg ginsenoside Re on day 5 (188 ± 9.2 mg/dl) and day 12 (180 ± 10.8 mg/dl) (both $P < 0.01$ vs. the vehicle-treated group on day 5 [234 ± 13.7 mg/dl] and day 12 [239 ± 13.3 mg/dl]). Fasting blood glucose concentrations did not change sizably in lean mice after treatment with ginsenoside Re.

Figure 8 shows glucose tolerance evaluated by IPGTT before and 12 days after ginsenoside Re administration. Figure 8A shows that with 20 mg/kg ginsenoside Re treatment in lean mice, glucose tolerance is not affected statistically. Compared with vehicle-treated *ob/ob* mice (Fig. 8B), 20 mg/kg ginsenoside Re treatment (Fig. 8C) significantly decreased blood glucose levels in *ob/ob* mice

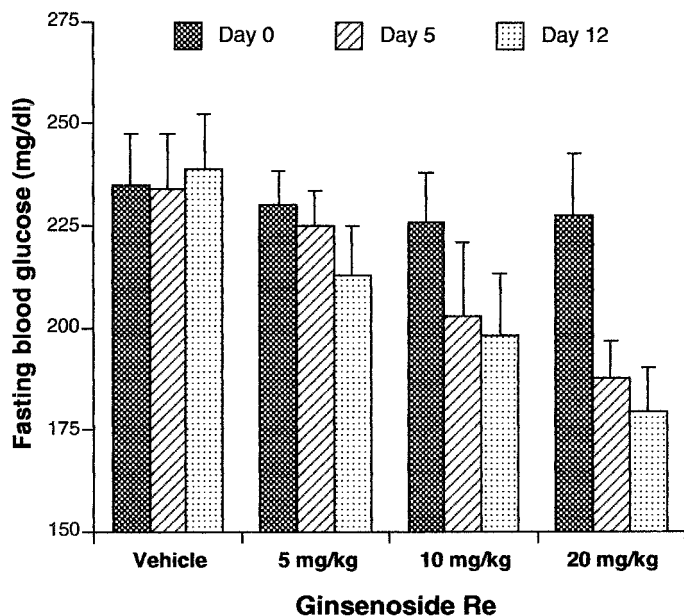


FIG. 7. Dose-dependent effect of ginsenoside Re on fasting blood glucose concentrations in adult *ob/ob* mice. Vehicle group, $n = 6$; 5-mg/kg group, $n = 6$; 10-mg/kg group, $n = 6$; 20-mg/kg group, $n = 11$. Compared with the vehicle group, fasting glucose levels decreased significantly after 20 mg/kg ginsenoside Re treatment on day 5 and day 12 (both $P < 0.01$).

at 60 and 120 min after glucose administration (both $P < 0.01$).

In contrast to both the antidiabetic and anti-obese effects of *Panax ginseng* berry extract, after a 12-day treatment with 20 mg/kg ginsenoside Re, body weight did not change significantly in *ob/ob* mice. Body weight in the 20 mg/kg ginsenoside Re 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 g on day 12. In addition, there were no significant changes in daily food intake and energy expenditure values after treatment with 20 mg/kg ginsenoside Re.

DISCUSSION

The present study was undertaken to investigate antihyperglycemic 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 demonstrate that *Panax ginseng* berry extract significantly improves glucose homeostasis. The fasting blood glucose levels started to decrease after 5 days of treatment and became completely normal by day 12. More importantly, the IPGTT results normalized after treatment. Dose-dependent antidiabetic 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.

The antidiabetic effect of ginseng root has recently been demonstrated. For instance, Kimura et al. (25) observed a

notable fall in blood glucose levels 6 h after a single 90-mg/kg ginseng root extract IP dose in genetically obese diabetic KK-CA^y mice. Vuksan et al. (27) demonstrated that 3 g American ginseng root given 40 min before the test meal significantly lowered blood glucose in nondiabetic subjects and type 2 diabetic patients. However, when studying a chronic disease such as diabetes, it is more pertinent to test the maintenance of lower blood glucose levels with long-term treatment rather than the acute hypoglycemic effect after a single dose. In this study, we measured fasting blood glucose 5 and 12 days after treatment. Unlike the short-term treatment study, we found that these compounds progressively reduced blood glucose levels in *ob/ob* mice.

Prospective studies of populations at high risk for type 2 diabetes suggest that in most patients, the initial inherited defect 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 6 weeks of age, insulin resistance and hyperinsulinemia are 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 in both the fed and fasting states, indicating an improvement in peripheral insulin action. The insulin-sensitizing effect of the extract was further supported by our hyperinsulinemic-euglycemic clamp study.

Another possible action site for ginseng berry to exert its postprandial hypoglycemic effect is in the gastrointestinal tract. We previously 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. Chung et al. (36) recently showed that the antidiabetic effect of ginseng root could be attributed to blocking intestinal glucose absorption and inhibiting hepatic glucose-6-phosphatase activity.

Insulin resistance is 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, insulin resistance in obese type 2 diabetic patients is significantly worse than that in nonobese diabetic individuals (14). Therapeutic agents with both antidiabetic and anti-obese effects are therefore particularly beneficial. Our results show that *ob/ob* mice treated with *Panax ginseng* berry extract underwent a dose- and time-dependent reduction in body weight. Past studies have shown that insulin sensitivity in type 2 diabetic patients improves with weight loss (37), possibly because of an improvement in insulin-stimulated glucose transport into muscle (38). A similar mechanism may operate in the extract-treated *ob/ob* mice to improve insulin resistance. The extract may exert its antidiabetic effect through actions that improve insulin sensitivity and the balance between food intake and energy expenditure. Because the antidiabetic 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

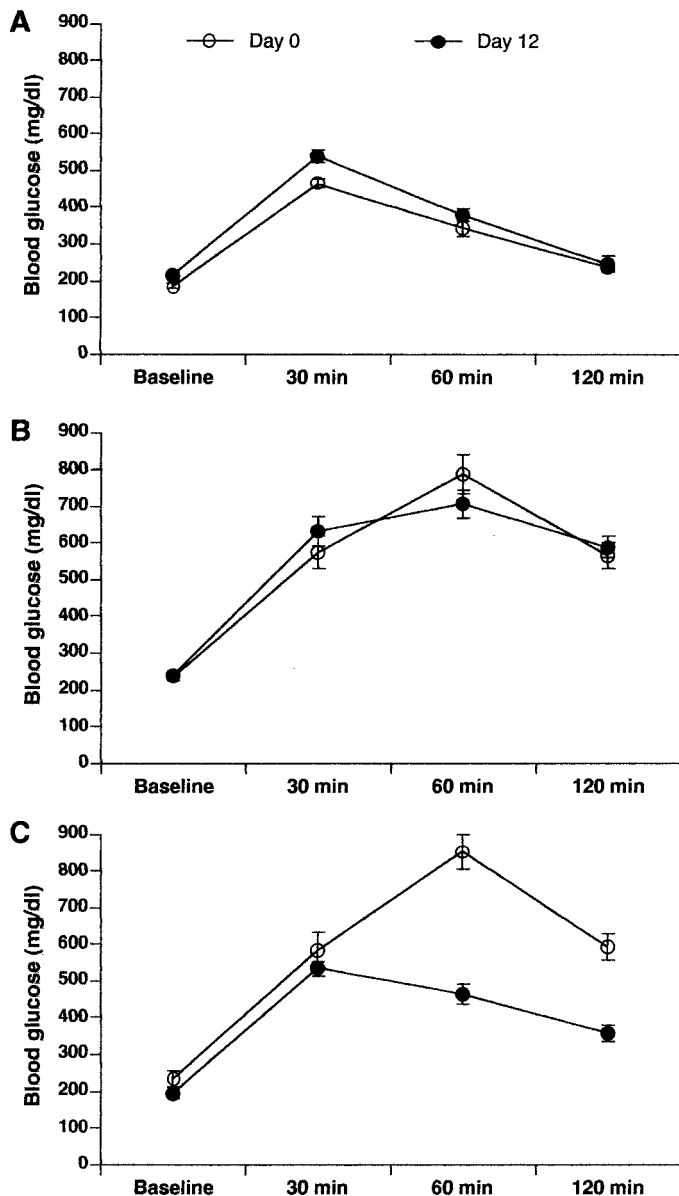


FIG. 8. IPGTT in lean and *ob/ob* mice before and after a 12-day treatment with ginsenoside Re. A: 20 mg/kg ginsenoside Re treatment in lean mice ($n = 6$). B: Vehicle-treated *ob/ob* mice ($n = 6$). C: 20 mg/kg ginsenoside Re treatment in *ob/ob* mice ($n = 6$), with a significantly higher rate of glucose disposal at 60 and 120 min compared with the vehicle group (both $P < 0.01$ vs. vehicle-treated mice).

ob/ob mice. Future studies are required to identify compound(s) in the extract with anti-obese action.

The weight loss we observed after the extract treatment resulted in a 15% reduction in food intake and a 35% increased energy expenditure. Our results further support the latter by demonstrating a significantly higher body temperature in the extract-treated *ob/ob* mice, along with an increase in oxygen consumption. In patients with type 2 diabetes, calorie restriction, independent of weight loss, can improve insulin sensitivity (39). Obesity, hyperphagia, hypothermia, and reduced energy expenditure in *ob/ob* mice is due to a lack of leptin, which, in lean mice, signals hypothalamic centers on fat stores (28,40). Whether the extract improve these defects by restoring hypothalamic control awaits further study. Past studies have shown that

leptin also has a tendency to reduce body weight gain in lean mice (40,41). 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 significantly reduces plasma cholesterol levels in *ob/ob* mice. Reduction of cholesterol levels by the extract may have an important clinical significance, since hyperlipidemia is often associated with type 2 diabetic patients.

The profile and concentrations of ginsenosides vary between ginseng root and berry, and this difference may contribute to the significant antihyperglycemic and/or anti-obese effects observed in our study. *Panax ginseng* berry contains a much higher concentration of ginsenoside Re than the root. We observed that ginsenoside Re has a significant antihyperglycemic activity without affecting body weight in *ob/ob* mice. Food intake and energy expenditure did not significantly change with ginsenoside Re. 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. Our results support overall in vivo antihyperglycemic and anti-obese activity of the extract that may prove to be of clinical importance in improving the management of type 2 diabetes. In addition, the identification of a significant antihyperglycemic activity in ginsenoside Re may provide an opportunity to develop a novel class of antidiabetic agent.

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