A Steroidal Glycoside from *Polygonatum odoratum* (Mill.) Druce. Improves Insulin Resistance but does not Alter Insulin Secretion in 90% Pancreatectomized Rats

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The aim of this study was to investigate the effects of three steroidal glycosides (SG-100, SG-280, and SG-460) obtained from *Polygonatum odoratum* (Mill.) Druce. on insulin secretion, insulin action, and relative glucose uptake in various tissues of 90% pancreatectomized male Sprague-Dawley rats. One of the compounds (30 mg/kg body weight daily) with a 40%-fat diet was orally administered to a group of such rats for 13 weeks. On the day after a hyperglycemic clamp, euglycemic hyperinsulinemic clamp with 1 μCi of [1-14C]2-deoxyglucose per 100 g body weight was used. Serum glucose levels were lowest in the rats receiving SG-100. Insulin secretion from pancreatic β-cells did not change with SG administration. Whole-body glucose disposal rates increased with SG-100 administration by 39%. SG-100 increased the glycogen contents and glycogen synthase activity in the soleus muscle of pancreatectomized rats. Uptake of [1-14C]2-deoxyglucose into the soleus muscle was higher in such rats receiving SG-100 than in rats receiving other compounds. In conclusion, SG-100 has an antihyperglycemic effect by promoting peripheral insulin sensitivity without changing insulin secretion.

**Key words:** *Polygonatum odoratum* (Mill.) Druce.; hyperglycemic clamp; euglycemic hyperinsulinemic clamp; glucose uptake; glycogen synthase

Among the compounds isolated from traditional herb extracts, steroidal glycosides, water-soluble polysaccharides, and flavonoids have been considered candidate antidiabetic agents.¹ ² If herbs or isolated compounds are to be used as hypoglycemic agents in type 2 diabetes mellitus, they should alleviate insulin resistance, increase insulin release by increasing pancreatic β-cell function or both, leading to a decrease in blood glucose levels. Without an understanding of their mechanisms of lowering blood glucose levels, we cannot use these substances for treatment of either type 1 or type 2 diabetes. Few compounds increased pancreatic β-cell mass or insulin sensitivity except for the thiazolidinediones, which were isolated from an herb in Japan.³

About three years ago, the U.S. Food and Drug Administration approved thiazolidinediones as a new class of compounds to be used to increase insulin sensitivity in target tissues such as skeletal muscle, liver, and adipose tissue. These compounds increase peripheral glucose uptake while decreasing gluconeogenesis of the liver in a wide variety of experimental models of type 2 diabetes and in humans.⁴ ⁵ The compounds improve glucose tolerance and insulin sensitivity in both diabetic and glucose-intolerant patients. However, troglitazone, one of this class compounds, can cause liver damage and multiple progenesis in humans, and is no longer prescribed.⁶ ⁷

*Polygonatum odoratum* (Mill.) Druce. (*P. odoratum*) has been traditionally used as an antidiabetic agent in Korea Water and methanol extracts of *P. odoratum* alleviated insulin resistance with increased glucose use by skeletal muscles (our unpublished data). The extract probably contains an insulin sensitizer. The 100%-methanol fraction fractionated by Diaion HP-20 column chromatography from a 70%-methanol extract, increased insulin sensitivity the most among all fractions (our unpublished data). Here, we separated three steroidal glycosides (SG-100, SG-280, and SG-460) from the 100%-methanol fraction, and investigated their effects on insulin action, insulin secretion, and glucose uptake in various tissues of 90% pancreatectomized male Sprague-Dawley rats.

**Materials and Methods**

Isolation of steroidal glycosides. Dry *P. odoratum* purchased from a traditional herb market (Kyung Dong Market, Seoul, Korea) was chopped into small pieces in a blender and the pieces were extracted with 70% methanol for 12 h. The extract was concentrat-
ed and divided into six fractions by Diaion HP-20 column chromatography with a stepwise gradient of water-methanol (7:3, 3:7, and 0:1), and finally with ethyl acetate. The 100%-methanol fraction was chromatographed on silica gel with a stepwise gradient of chloroform-methanol (9:1, 4:1, 1:1, and 1:1). The 4:1 fraction was treated by column chromatography on ODS silica gel with a stepwise gradient of methanol-distilled water (3:1, 2:1, and 1:1) to give SG-100, SG-280, and SG-460. Each fraction was lyophilized and identified as a single steroidal glycoside as follows. Each fraction was spotted on a silica-gel plate for TLC and developed in two different phases (chloroform-methanol-water = 4:1:0.1 and 7:4:1). In HPLC, each fraction was injected into a Lichrosorb-NH2 column with detection by a differential refractometer. The mobile phase was acetonitrile-water-n-butanol (80:20:10). All three fractions were confirmed as steroidal glycosides with an external standard. Lyophilized SG-100, SG-280, and SG-460 were stored at −20°C until being mixed with feed for the following week.

**Experimental animals.** All surgical and experimental procedures were approved by the Animal Care and Use Review Committee at Hoseo University, Korea, in compliance with National Research Council Guide for Animal Care. Male Sprague-Dawley rats weighing 430 ± 56 g (mean ± SD) were anesthetized with ketamine (50 mg/kg body weight) and 90% of the pancreas was removed by the technique of Hosokawa et al. The residual pancreas was anatomically well defined, being the tissue within 2 mm of the common bile duct and extending from the duct to the first part of the duodenum. For the control group for intact rats, 12 male rats underwent a sham operation in which the pancreas was disengaged from the mesentery and gently rubbed between the fingers. Sham-operated rats did not have any signs of diabetes after the operation. The animals were given free access to standard laboratory chow (Sam Yang Co., Kangwon-Do) and distilled water for 1 week after these procedures. They were housed individually in stainless steel cages in a controlled (23°C; 12-h light-dark cycle) environment. Among the 90%-pancreatectomized rats, those with blood glucose levels of less than 9.4 mmol/l at 2 weeks after the pancreatectomy were eliminated.

Each of the remaining fifty pancreatectomized rats was randomly assigned to one of four groups by a random-number table. Three of the groups were to be given lyophilized SG-100, SG-280, or SG-460 in their feed as the concentration of 1000 mg/kg of feed. There were 13 rats in the SG-100 group, 12 rats in the SG-280 group, and 13 rats in the SG-460 group. Daily consumption of the added compound was about 80 mg/kg of body weight, and the experiment lasted for 13 weeks. They were 12 sham-operation control rats and 12 pancreatectomized control rats, all with cellulose instead of a test compound added to their feed. The control-sham group was used to see if steroidal glycoside supplementation could overcome the impaired glucose homeostasis in pancreatectomized rats to reach the levels of these control-sham rats. The semipurified diet for the experimental period was a modification of a published diet. The feed was 40% carbohydrates, 20% proteins, and 40% fats in terms of energy. The sources of the carbohydrates, proteins, and fats were corn starch (Doossan Corn Products Korea Inc., Seoul, Korea), milk casein (Ducksan Pure Chemical Co., Ltd., Ansan-Si, Korea), and shortening from animal fat (Samilp-Wellga Inc., Sungnam-Si, Korea), respectively. Blood was collected from rats starved overnight by cutting of the tail tips of conscious rats weekly at a set time and used in measurement of serum glucose. Food intake and body weight were measured weekly at the same time.

**Hyperglycemic clamp.** Indwelling catheters were inserted into the jugular vein and carotid artery in week 12 of the experiment period. After 5 or 6 days, the clamp was used in evaluation of insulin secretion capacity with the rats being awake and unstressed except for being starved overnight. A continuous infusion of 25% glucose at a rate adjusted so that the serum glucose concentration increased by about 5.6 mmol/l above the baseline and the concentration was maintained between 60 to 90 min. Serum insulin concentrations were assayed at time 0, 60, and 90 min. Serum insulin levels at 60 and 90 min were taken to represent the insulin secretion capacity of rats when serum glucose levels had increased by 5.6 mmol/l. After this test, rats were provided with feed as before and water for 6 h, and then starved for 16 h.

**Euglycemic hyperinsulinemic clamp.** On the day after the hyperglycemic clamp, a euglycemic hyperinsulinemic clamp was used to test rats that, as before, were awake and unstressed except for being starved as described above. Hyperinsulinemia was brought about with a constant infusion of human insulin (12 mU/kg per minute) and euglycemia (5.6 mmol/l) was maintained by a variable rate of infusion of 25% glucose with adjustment every 5 min depending on the serum glucose level of blood collected from the jugular vein. Euglycemia was maintained between 120 to 150 min. The glucose infusion rate was calculated and expressed in terms of milligrams of glucose per kilogram of body weight per minute. The glucose infusion rate is considered the equivalent value of the glucose disposal rate. The glucose disposal rate is an index of whole-body glucose use in response to exogenous insulin.
Infusion of [1-14C]2-deoxyglucose. After 90 min of the euglycemic hyperinsulinemic clamp, [1-14C]2-deoxyglucose was given as an intravenous bolus in the jugular vein, and heparinized saline was then given. The solution injected contained [1-14C]2-deoxyglucose (51.1 mCi/mmol, New England Nuclear, Boston, MA) at the dose of 1 μCi per 100 g body weight, 0.9% NaCl, and 5.1 mM glucose buffered to pH 7.35 with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES).15 Forty-five minutes later, the rats were killed by decapitation. The brain, heart, liver, soleus and quadriceps muscles, and epididymal fat pads were rapidly dissected out, weighed, and frozen in liquid nitrogen. Tissues were stored in -70°C until examined further.

Biochemical measurements. Serum glucose levels were assayed by a glucose analyzer (Beckman, Fullerton, CA). Serum insulin levels were measured with a commercial radioimunoassay kit (Linco Research Inc., St. Charles, MO).

 Supernatants from homogenates of the brain, heart, liver, soleus and quadriceps muscles, and epididymal fat pads were obtained and assayed for 14C as counts per minute. The uptake of [1-14C]2-deoxyglucose was used to express glucose uptake by organs as the percentage of the dose injected per gram of tissue.16

For assays of the glycogen levels in the liver and soleus and quadriceps muscles, these tissues were homogenized and deproteinized with 1.5 N perchloric acid. The glycogen was digested into glucose with α-amylglucosidase in acidic buffer, and the level was expressed as glucose from glycogen in the tissues.17 Triacylglycerol deposited in soleus and quadriceps muscles was extracted with a chloroform-methanol solution (2:1, vol:vol), and the triacylglycerol in the extract was suspended in chroloform18 and assayed with a Trinder kit (Sigma, St. Louis, MO). Glycogen synthase activity was assayed by a modified method of Thomas et al.19 and Rossetti et al.13 After centrifugation of a muscle homogenate, the supernatant was obtained and incubated with a physiologic concentration (0.3 mM) of the substrate uridine 5'-diphosphate-[3H]glucose in the presence or absence 10.0 mM glucose 6-phosphate. Glycogen synthase activity was calculated from the radioactivity in glycogen synthesized during the incubation. Maximum glycogen synthase activity called glucose 6-phosphate dependent activity was assayed in the presence of 10 mM glucose-6-phosphate; its independent activity was assayed in the absence of the glucose-6-phosphate. Total glycogen synthase activity was the sum of the dependent and independent activities, and expressed as nanomoles of uridine 5'-diphosphate-[3H]glucose per milligram protein per minute and also as the fractional velocity, the ratio of independent and total activities as a percentage. Total membranes from soleus and quadriceps muscles were prepared by the method of Walker et al.20 The glucose transporter 4 (GLUT4) protein content of the total membranes was measured by western blotting with rabbit GLUT4 antibodies (Chemicon, Temecula, CA). The muscle standard (an unrelated crude membrane fraction) was run on every gel for comparison of samples from different immunoblots. Quantification was performed with a scanning laser densitometer (BioRad, Richmond, CA). The advanced glycated endproduct of the subcutaneous tissue was measured using fluorescence methods. Subcutaneous tissues were homogenized with phosphate buffered saline, and centrifuged at 10,000 x g for 30 minutes at 4°C. The supernatant was eliminated. Chloroform and methanol (2:1, vol:vol) were added to pellets and kept at 4°C overnight. After removing the organic solvent, the defatted tissue was incubated at 37°C for 48 hours with collagenase type 7 and proteinase K in PBS.21 The defatted tissue was incubated with an equal amount of 0.2 mol/l NaOH overnight at 4°C. After centrifugation at 10,000 x g for 10 minutes at 4°C, half of the supernatant was used for fluorescence determination at excitation 370 nm, emission 440 nm. The rest was used for assaying the content of hydroxyproline, which was determined by colorimetric measurement.

Statistical analysis. All results are expressed as means ± standard deviations. Statistical evaluation was done using the SAS program (version 8.01).22 Normal distribution of the data was checked by univariate analysis. Effects of steroidal glycosides in pancreatectomized rats were examined by one-way ANOVA followed by Tukey’s test for multiple comparisons. Comparison of the effects of pancreatectomy and sham-operation was done by the unpaired two-sample Student’s t-test. Differences with a P < 0.05 were considered to be statistically significant.

Results

Food intake and body weight
Steroidal glycoside consumption and diabetic status did not affect the mean daily caloric intake or glycoside intake during steroidal glycoside administration. The mean daily food intake (±SD) was 32.9 ± 3.4 g for the groups averaged together, which was equivalent to 198.6 ± 20.4 kcal. Before glycoside supplementation, the mean body weight of all rats was 428.9 ± 37.9 g, without much difference among groups (Table 1). Body weights did not change in pancreatectomized rats whatever the supplement, but sham-operated rats gained weight compared with the pancreatectomized rats (P = 0.043).

Serum glucose and insulin levels during experiment

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Table 1. Values before and after Steroidal Glycoside Administration

<table>
<thead>
<tr>
<th></th>
<th>SG-100 (n=13)</th>
<th>SG-280 (n=12)</th>
<th>SG-460 (n=13)</th>
<th>Control-px (n=12)</th>
<th>Control-sham (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
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<tr>
<td>Initial*</td>
<td>428.8 ± 43.3b</td>
<td>448.0 ± 71.4</td>
<td>420.0 ± 43.3</td>
<td>410.0 ± 56.6</td>
<td>437.2 ± 44.3</td>
</tr>
<tr>
<td>Final</td>
<td>423.6 ± 45.0</td>
<td>465.0 ± 63.9</td>
<td>439.1 ± 51.6</td>
<td>441.7 ± 46.0</td>
<td>518.3 ± 46.1*</td>
</tr>
<tr>
<td><strong>Food intake (g/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Initial</td>
<td>33.0 ± 3.6</td>
<td>33.4 ± 3.5</td>
<td>31.9 ± 2.8</td>
<td>32.3 ± 3.6</td>
<td>32.4 ± 3.2</td>
</tr>
<tr>
<td>Final</td>
<td>34.7 ± 3.9</td>
<td>35.8 ± 3.6</td>
<td>33.8 ± 4.1</td>
<td>36.2 ± 3.9</td>
<td>37.2 ± 3.9</td>
</tr>
<tr>
<td><strong>SG intake (mg/day)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>33.0 ± 3.6</td>
<td>33.4 ± 3.5</td>
<td>31.9 ± 2.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Final</td>
<td>34.7 ± 3.9</td>
<td>35.8 ± 3.6</td>
<td>33.8 ± 4.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Serum insulin (pmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>277.6 ± 38.4</td>
<td>271.8 ± 39.2</td>
<td>273.9 ± 37.5</td>
<td>269.6 ± 36.8</td>
<td>355.6 ± 52.6**</td>
</tr>
<tr>
<td>Final</td>
<td>278.5 ± 35.6</td>
<td>287.6 ± 37.1</td>
<td>296.6 ± 32.7</td>
<td>291.2 ± 33.6</td>
<td>676.3 ± 48.6***</td>
</tr>
<tr>
<td><strong>AGE</strong> (arbitrary unit/mg collagen)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>1.73 ± 0.14</td>
<td>1.73 ± 0.11</td>
<td>1.72 ± 0.15</td>
<td>1.72 ± 0.13</td>
<td>1.59 ± 0.11</td>
</tr>
<tr>
<td>Final</td>
<td>1.69 ± 0.12*</td>
<td>1.80 ± 0.13*</td>
<td>1.81 ± 0.13*</td>
<td>1.83 ± 0.12*</td>
<td>1.57 ± 0.12*</td>
</tr>
</tbody>
</table>

Significantly different (Student's t-test) from the control-pancreatectomized group at *P<0.05, **P<0.01, or ***P<0.001.
* Initial: After a two-week recovery from pancreatectomy or a sham operation.
† Mean ± standard deviation.
* Final: At the end of steroidal glycoside administration.
* AGE: Advanced glycated endproduct.
* Values in the same row with different letters are significantly different (ANOVA followed by Tukey's test) at P<0.05.

SG-100 gave lower serum glucose levels than in the control-pancreatectomized group; SG-280 and SG-460 did not change the glucose levels. During this experimental period as well, the glucose level was lower in the control-sham group than in the control-pancreatectomized group. Before glycoside administration, fasting insulin levels with the rats starving were not different among the pancreatectomized rats (273.2 ± 23.5 pmol/l), but they were higher in control-sham rats (355.6 ± 52.6 pmol/l) than in the other rats (P=0.009, Table 1). After the 13-week experiment, serum insulin levels were not affected by steroidal glycosides in the pancreatectomized rats. Advanced glycated endproduct of subcutaneous tissues of the abdomen used as an index of long-term glucose control, after the hyperinsulinemic clamp was lower in the SG-100 group than in the control-pancreatectomized group (P=0.044).

Insulin secretion during hyperglycemic clamp
At the end of the supplementation period, serum insulin levels at the elevated serum glucose level of 5.6 mmol/l above the baseline, called the steady state, were higher than those at the baseline in all groups (Table 2). Insulin secretion at the steady state was not affected by steroidal glycosides, but was less in pancreatectomized rats than in control-sham rats.

Insulin resistance at euglycemic hyperinsulinemic clamp
Table 3 shows whole-body glucose disposal rates during the second clamp, indicating the degree of insulin resistance. Among pancreatectomized rats, the glucose disposal rate decreased in the order of SG-100, SG-460, SG-280, and control groups.

Before the glycoside administration, serum glucose levels were not different among groups of pancreatectomized rats, but were higher in the pancreatectomized group than in the sham group (Fig. 1).
Table 2. Serum Glucose and Insulin Levels during Hyperglycemic Clamp

<table>
<thead>
<tr>
<th></th>
<th>SG-100 (n=13)</th>
<th>SG-280 (n=12)</th>
<th>SG-460 (n=13)</th>
<th>Control-px (n=12)</th>
<th>Control-sham (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.0 ± 0.9b</td>
<td>9.1 ± 0.9b</td>
<td>8.6 ± 1.4e</td>
<td>9.7 ± 1.3c</td>
<td>6.3 ± 1.6e</td>
</tr>
<tr>
<td>Steady state</td>
<td>13.5 ± 1.4c</td>
<td>14.7 ± 2.2</td>
<td>14.2 ± 2.0</td>
<td>15.4 ± 2.5</td>
<td>11.9 ± 1.7c</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>266.2 ± 22.6c</td>
<td>283.6 ± 30.8</td>
<td>272.2 ± 29.2</td>
<td>274.8 ± 31.8</td>
<td>355.4 ± 39.3c</td>
</tr>
<tr>
<td>Steady state</td>
<td>362.8 ± 38.8</td>
<td>371.2 ± 45.2</td>
<td>368.6 ± 39.6</td>
<td>342.8 ± 43.5</td>
<td>654.3 ± 45.7e</td>
</tr>
</tbody>
</table>

* Significantly different (Student’s t-test) from the control-pancreatectomized group at P < 0.01.
* Baseline: Values before hyperglycemic clamp.
* Values in the same row with different letters are significantly different (ANOVA followed by Tukey’s test) at P < 0.05.
* Mean ± standard deviation.

Table 3. Glucose Disposal Rates and Serum Glucose and Insulin Levels during Euglycemic Hyperinsulinemic Clamp

<table>
<thead>
<tr>
<th></th>
<th>SG-100 (n=13)</th>
<th>SG-280 (n=12)</th>
<th>SG-460 (n=13)</th>
<th>Control-px (n=12)</th>
<th>Control-sham (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose disposal rates (mg/kg body weight per min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>27.5 ± 2.7a</td>
<td>18.5 ± 3.4c</td>
<td>24.2 ± 2.1b</td>
<td>16.8 ± 2.3e</td>
<td>29.6 ± 3.6h</td>
</tr>
<tr>
<td>Steady state</td>
<td>8.1 ± 0.8f</td>
<td>9.1 ± 0.7f</td>
<td>8.7 ± 0.9g</td>
<td>9.4 ± 0.8f</td>
<td>6.4 ± 0.8f</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.7 ± 0.6g</td>
<td>5.6 ± 0.9g</td>
<td>5.6 ± 0.7g</td>
<td>5.7 ± 0.8g</td>
<td>5.6 ± 0.5g</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>271.8 ± 45.6e</td>
<td>311.9 ± 49.8</td>
<td>280.7 ± 41.7</td>
<td>279.1 ± 42.9</td>
<td>411.2 ± 57.1e</td>
</tr>
<tr>
<td>Steady state</td>
<td>2610 ± 381</td>
<td>2738 ± 358</td>
<td>2663 ± 374</td>
<td>2658 ± 393</td>
<td>2819 ± 363</td>
</tr>
</tbody>
</table>

* Significantly different (Student’s t-test) from the control-pancreatectomized group at P < 0.01.
* Values in the same row with different letters are significantly different (ANOVA followed by Tukey’s test) at P < 0.05.
* Mean ± standard deviation.

Table 4. Liver and Soleus and Quadriceps Muscle Glycogen, Triacylglycerol, and GLUT4 Protein at the End of Steroidal Glycoside Administration

<table>
<thead>
<tr>
<th></th>
<th>SG-100 (n=13)</th>
<th>SG-280 (n=12)</th>
<th>SG-460 (n=13)</th>
<th>Control-px (n=12)</th>
<th>Control-sham (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg/g tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>48.4 ± 7.8h</td>
<td>42.4 ± 6.4g</td>
<td>45.5 ± 7.2j</td>
<td>41.6 ± 7.3k</td>
<td>52.0 ± 8.4l</td>
</tr>
<tr>
<td>Soleus muscle</td>
<td>5.1 ± 0.7h</td>
<td>4.1 ± 0.6g</td>
<td>4.6 ± 0.8k</td>
<td>4.2 ± 0.5l</td>
<td>5.6 ± 0.9j</td>
</tr>
<tr>
<td>Quadriceps muscle</td>
<td>4.2 ± 0.9h</td>
<td>2.8 ± 0.9j</td>
<td>3.1 ± 0.7l</td>
<td>2.7 ± 0.8k</td>
<td>4.3 ± 1.0i</td>
</tr>
<tr>
<td>Triacylglycerol (mg/g tissue)</td>
<td>8.6 ± 1.2e</td>
<td>8.1 ± 1.2e</td>
<td>8.4 ± 0.9e</td>
<td>8.8 ± 1.3e</td>
<td>9.7 ± 1.1f</td>
</tr>
<tr>
<td>Soleus muscle</td>
<td>14.5 ± 1.3e</td>
<td>14.9 ± 1.1e</td>
<td>14.7 ± 1.4e</td>
<td>15.4 ± 1.1e</td>
<td>14.3 ± 1.6e</td>
</tr>
<tr>
<td>Quadriceps muscle</td>
<td>155.9 ± 19.5c</td>
<td>124.5 ± 17.1b</td>
<td>123.5 ± 14.8a</td>
<td>98.6 ± 13.4b</td>
<td>162.4 ± 18.2c</td>
</tr>
</tbody>
</table>

* Significantly different (Student’s t-test) from the control-pancreatectomized group at P < 0.05.
* Values in the same row with different letters are significantly different (ANOVA followed by Tukey’s test) at P < 0.05.
* Mean ± standard deviation.

SG-100 consumption increased whole-body glucose use, and SG-460 also increased it, but not as much as SG-100. The SG-280 group did not use more glucose than the controls. The rates were higher in control-sham rats than in the other rats.

Values after this clamp, for liver and muscle glycogen, triacylglycerol, and GLUT4 are shown in Table 4. In pancreatectomized rats given SG-100 or SG-460, the liver glycogen contents increased to the level of control-sham rats. Soleus and quadriceps muscle glycogen levels changed in the same pattern as liver glycogen: They were higher in the SG-100 group than in the SG-280 group or the control-pancreatectomized group. Soleus and quadriceps muscle triacylglycerol levels were not significantly different among the groups. Rats given SG-100 had their GLUT4 pro-
tein in soleus and quadriceps muscles increase to the level in the control-sham group. GLUT4 protein contents of the control-sham group were higher than the other groups.

Table 5 shows total glycogen synthase activity and the fraction velocity in soleus and quadriceps muscle tissues after the euglycemic hyperinsulinenemic clamp. Total glycogen synthase activity was lower in the control-pancreatectomized group than in the control-sham group, but there was no difference in activity depending on steroidal glycoside administration. Fraction velocity in the soleus and quadriceps muscles was high in the SG-100 group compared with the other groups given glycosides; it increased to a level close that in the control-sham group.

Uptake of [1-14C]2-deoxyglucose
Uptake of [1-14C]2-deoxyglucose by different organ tissues is shown in Fig. 2. Glucose uptake by the soleus muscle was affected by the diabetic status and by steroidal glycoside administration (P < 0.05). The glucose uptake was higher with SG-100 than with control-pancreatectomized rats, and the control-pancreatectomized group had approximately 26% less glucose uptake by the soleus muscle than the control-sham group. The glucose uptake by the soleus and quadriceps muscles changed in the same pattern as glycogen contents in those muscles. Heart and brain tissues showed little if any difference in glucose uptake with diabetic status and steroidal glycoside administration.

Discussion

Traditional herbs have been used for diabetes treatment in Asian countries, but not in the West. However, recent research reported that 31% of diabetes patients in Canada are taking alternative medications that they considered efficacious, and they spent almost as much money on over-the-counter supplements and alternative medications together as they did on their diabetic medications. It is important to evaluate these remedies, to establish what merit they have, and to identify the mechanisms of their action.

A new paradigm of traditional herbs is emerging in which pharmacological effects of single compounds from herbs can be understood in the light of their polyvalent actions, as seen for ginseng saponins and mushroom glycans with their anti-mutagenic, anti-cancer, anti-inflammatory, anti-diabetic and neurovascular effects.24,25

Pancreatectomized rats, selected here as the experimental model of diabetes, represent a pathophysiology similar to that of mild type 2 diabetes.2,12 Even though total β-cell mass is decreased in pancreatectomized rats, the remaining pancreas has the same proportion of α- and β-cells, and the ratio of serum insulin and glucagons levels is similar to that of sham-operated rats.2,12 Pancreatectomized rats have
near-normal or slightly high blood glucose levels when starved, elevated post-prandial glucose levels, increased insulin resistance, and decreased insulin secretion. However, in our study, fasting serum glucose levels during starvation were higher and insulin levels were lower than those predicted from the results of Rossetti et al. The discrepancies probably arose from several factors, such as selection of animals after surgery, the age of animals, and their diet. In our study, other factors that might decrease insulin resistance were eliminated for more nearly optimum investigation of steroidal glycoside effects. Two weeks after pancreatectomy, pancreatectomized rats with serum glucose levels during starvation of more than 9.4 mmol/l were selected. Eight-month-old rats were used because the proliferation and differentiation of pancreatic β-cells would be slower in rats older than 6 months than in weaning rats. Diabetes becomes less severe when differentiation and proliferation of pancreatic β-cells from the ductal epithelial cells begins in young rats.

Insulin resistance is increased by saturated fat and also by polyunsaturated fat, by an unknown mechanism, but possibly by reduced glucose use in skeletal muscle. High-fat diets can impair intracellular glucose metabolism by suppressing skeletal glycolysis and increasing lipid oxidation, resulting from impairing intracellular insulin action on glucose uptake in skeletal muscle. Our results also suggested that reduced glucose uptake and glucose use in skeletal muscle increased insulin resistance during the consumption of high-fat feed. Insulin resistance that had increased with the high-fat diet was improved by SG-100 administration. SG-100 increased glucose use in skeletal muscles, which also had increased GLUT4, and fraction velocity of glycogen synthase.

Broadhurst et al. reported that cinnamon, hazel, green and black teas, allspice, bay leaves, nutmeg, cloves, mushrooms, and brewer's yeast increases the insulin-dependent use of cellular glucose in rat epididymal adipocytes. The effects of these plant extracts on insulin activity in vitro suggest the possible use of these plants in improving glucose and insulin metabolism in vivo. Treatment with the insulin sensitizer troglitazone produces a rise in the metabolic clearance rate of glucose during a euglycemic hyperinsulinemic clamp in obese rats and Zucker diabetic rats. Troglitazone increases the muscle glycogen content after the clamp, compared with untreated obese rats. Troglitazone may improve insulin sensitivity associated with improvements in intracellular substrate use and energy stores. Another study showed that two months of troglitazone treatment made whole-body peripheral insulin resistance normal, lead to glucose homeostasis, and decreased free fatty acids without detectable changes in plasma insulin levels. In conclusion, as with treatment with troglitazone, SG-100 and SG-460 supplementation had desirable effects on insulin use by increasing the glucose disposal rate in skeletal muscles without altering insulin secretion. SG-100 had stronger effects on the reduction of insulin resistance, and this compound may be a useful as an insulin sensitizer.

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References

Steroidal Glycosides, Insulin Resistance and Insulin Secretion


