Anti-obesity Effect of Dioscorea nipponica Makino with Lipase-inhibitory Activity in Rodents

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In the process of screening for pancreatic lipase inhibitors, which could be used as an anti-obesity measure, the methanol extract of Dioscorea nipponica Makino powder (DP) appeared to have potent inhibitory activity against porcine pancreatic lipase with an IC50 value of 5–10 μg/ml, where the enzyme activity was assayed by using 4-methylumbelliferyl oleate as a substrate. Further purification of active components present in the herb generated dioscin that belongs to the saponin family. Dioscin and its aglycone, diosgenin, both suppressed the time-dependent increase of blood triacylglycerol level when orally injected with corn oil to mice, suggesting their inhibitory potential against fat absorption. Sprague-Dawley rats fed on a high-fat diet containing 5% Dioscorea nipponica Makino and 40% beef tallow gained significantly less body weight and adipose tissue than control animals fed on a high-fat diet alone during an 8-week experimental period (P < 0.05).

Key words: obesity; Dioscorea nipponica; lipase inhibition; dioscin; diosgenin

Obesity is a chronic, stigmatized and costly disease that is rarely curable and is increasing in prevalence throughout most of the world. The inhibition of dietary fat absorption has been reported to be one of the effective ways of managing obesity. For instance, the application of a strong lipase inhibitor such as Orlistat, a hydrogenated derivative of lipostatin derived from Streptomyces tozitricini, has proven to be used successfully for the treatment of obesity. In our preliminary study, about 200 medicinal herbs were tested for their inhibitory activity against porcine pancreatic lipase by using an assay system containing 4-methylumbelliferyl oleate as a substrate. The methanol extract of Dioscorea nipponica Makino showed the strongest inhibitory effect against pancreatic lipase. Dioscorea nipponica Makino, a perennial herb growing in mountainous areas of the Korean peninsula, has long been used as a folk medicine for asthma, rheumatoid arthritis, bronchitis, and other diseases in this country. In the present study, the active component from the herb was purified, and its effect on the elevation of plasma triacylglycerol level in mice caused by the oral administration of a lipid emulsion was examined. The effects of the powder on the body weight gain, amount of adipose tissue, and blood parameters in rats fed on a high-fat diet for 8 weeks were also investigated.

Materials and Methods

Materials. Porcine pancreatic lipase (type VI-S), 4-methylumbelliferyl oleate (4-MU oleate), and cellulose were obtained from Sigma (St. Louis, MO, U.S.A.). Assay kits for triacylglycerol, and total-, HDL-, and LDL-cholesterol were also purchased from Sigma. Beef tallow was from Samwoo (Seoul, Korea). Vitamin and mineral mixtures were from Teklad (Madison, WI, U.S.A.). All other chemicals were of reagent grade. The root of Dioscorea nipponica Makino was obtained from Uisung Medicinal Farm (Uisung, Korea), freeze-dried, powdered, and kept at −20°C. This study was approved by the committee of Andong National University for the care and use of laboratory animals.

Animals and diet composition. Male ICR mice (8-week old) and male Sprague Dawley (SD) rats (7-week old) were obtained from Korea Experimental Animal Center (Umsung, Korea), and housed for 1 week under a 12-hr light:dark cycle (light 0700–1900 h) in a temperature- and humidity-controlled room (22 ± 1°C). The animals were given free access to the diet and water. The ICR mice were given standard feed (Purina, Korea), and the SD rats were fed on a standard feed for 1 week and then on experimental diet whose composition is shown in Table 1.
The reaction mixture consisted of 0.1 ml of lipase was measured by using 4-MU oleate as a sub-
inhibitory activity of each sample against pancreatic

Measurement of the lipase-inhibitory activity. The inhibitory activity of each sample against pancreatic lipase was measured by using 4-MU oleate as a substrate. The reaction mixture consisted of 0.1 ml of 0.1 mM 4-MU oleate, 0.04 ml of a McIlvane buffer (0.1 M citrate-Na2 HPO4, pH 7.4) and 0.01 ml of a sample solution. Adding 0.05 ml of lipase, all in a final volume of 0.2 ml, started the reaction. After incubating at 37°C for 20 min, 1 ml of 0.1 N HCl and 2 ml of 0.1 M sodium citrate were each added. The amount of 4-MU released by the lipase was measured fluorometrically at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. The inhibitory activity (%) was calculated as (1 – A/B) × 100, where A and B represent the activities of the enzyme with and without sample. IC50, the concentration of a tested compound giving 50% inhibition of the enzyme activity, was evaluated from the least-square regression line of the plot of the logarithm of concentration vs. the inhibitory activity.

Purification and characterization of the inhibitor(s). Three kilograms of Dioscorea nipponica root were extracted three times with five liters each of methanol. After filtration, the extract was evaporated in vacuo to give 115.1 g of a dry sample. The evaporated sample (25 g) was loaded into a silica gel column (90 × 6 cm, silica gel 900 g; Merck 7734, Darmstadt, Germany), and eluted with chloroform/methanol/water (7/3/1). From 60 min after starting the elution, eight fractions of 21 each in volume were collected. The 5th fraction was further fractionated by silica gel chromatography eluted with ethylacetate/methanol (98/2 → 95/5). Thirty fractions of 500 ml each were collected. The fractions from 35th to 50th showed strong inhibitory activity against pancreatic lipase. One of those fractions was dioscin as analyzed by IR spectroscopy (Perkin-Elmer 283B, Shelton, CT, U.S.A.) and NMR spectroscopy (Bruker AMX 300, Rheinstetten, Germany).

Measurement of the plasma triacylglycerol level after the oral administration of a lipid emulsion to rats. Lipid emulsions were prepared with 6 ml of corn oil, 80 mg of choleric acid, 2 mg of cholesteryl oleate, and 6 ml of saline in the absence or presence of dioscin or diosgenin. After male ICR mice weighing 40 g had been fasted overnight, they were orally administered with 200 µl of a lipid emulsion. Blood samples were taken from the tail vein at 0, 0.5, 1, 2, 3 and 4 hr after administering the lipid emulsion with or without dioscin or diosgenin. The plasma triacylglycerol level was determined with a Triglyceride E-test kit (Sigma).

Evaluation of the body and several adipose tissue weights, plasma triacylglycerol and total cholesterol in rats fed on the high-fat diet for 8 weeks. After adaptation to the high-fat diet and laboratory conditions for 1 week, male Sprague-Dawley rats (7-week old, weighing 220–240 g) were divided into three groups, each group matched for its body weight. The control group was given only the high-fat diet; the other group was given the high-fat diet with Dioscorea nipponica root powder mixed in at a concentration of 2% or 5% (Table 1). The body weight was measured once weekly. After 8 weeks of the Dioscorea nipponica powder treatment, the animals were killed; adipose tissues from the subcutaneous, perirenal, inguinal, and epididymal areas were collected and their weights measured. Feces were collected for 3 days immediately before sacrificing the rats, dried, and powdered. The fecal fat content was measured by the Soxhlet extraction method. Blood was taken by heart puncture just prior to killing. The blood samples were centrifuged, and plasma was separated and frozen at −80°C until needed for the assay. The plasma triglyceride (TG), total cholesterol (TC), HDL-cholesterol, VLDL-cholesterol, aspartate aminotransferase (GPT) activities were measured by kits from Sigma. The atherogenic index (AI) was calculated as follows: AI = (total cholesterol − HDL cholesterol)/HDL cholesterol.

HPLC analysis of dioscin. The root of Dioscorea nipponica was dried for 7 days at room temperature and then powdered in a homogenizer. Two grams of the powder was extracted with 100 ml of methanol by a sonic vibrator (Branson 3210R-DTH, Branson, U.S.A.) at 50°C for 8 hr. Dioscin, prosapogenin A and prosapogenin C in the root extract of Dioscorea nipponica were determined by a HPLC system comprising an SCL-10A system controller, LC-10AD pump and SPD-10A UV detector (Shimadzu, Japan). The analytical column was a Nova-Pak C18 type (Waters, U.S.A.). The mobile phase for HPLC consisted of 60% acetonitrile (v/v) with a flow rate of 1 ml/min. The column temperature was maintained at

### Table 1. Composition of the Experimental Diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>2% DN</th>
<th>5% DN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg of diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Casein</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Starch</td>
<td>100</td>
<td>84.3</td>
<td>60.8</td>
</tr>
<tr>
<td>Sugar</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>45.7</td>
<td>39.2</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Dioscorea nipponica powder</td>
<td>0</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

Dioscorea nipponica powder

Component Control 2

Methionine 3 3 3

Choline 2 2 2

Mineral mixture 35 35 35

Vitamin mixture 10 10 10

Cellulose 50 45.7 39.2

Sugar 100 100 100

Starch 100 84.3 60.8

Casein 300 300 300

Beef tallow 400 400 400

Table 1. Composition of the Experimental Diets

The analytical column was a Nova-Pak C18 type (Waters, U.S.A.). The mobile phase for HPLC consisted of 60% acetonitrile (v/v) with a flow rate of 1 ml/min. The column temperature was maintained at
Fig. 1. Inhibition of Porcine Pancreatic Lipase (PL) by the Dioscorea nipponica (DN) Extract.

The inhibitory activity of the methanol extract of DN and of Orlistat against porcine pancreatic lipase was measured at concentrations of 1, 10, 100, and 1000 μg/ml under these analytical conditions, respectively.

**Statistical analyses.** Data are expressed as the mean ± s.d. and were analyzed by one-way ANOVA and Duncan’s multiple-range test; a P-value of < 0.05 is considered significant.

**Results**

**Inhibitory activity of dioscin and its derivatives against pancreatic lipase**

The methanol extract of DN root inhibited the lipase activity in a dose-dependent manner in the assay system with 4-MU oleate as a substrate (Fig. 1). The lipase activity was inhibited by 50% at the concentration of 10 μg/ml of the methanol extract of the herb. The inhibitor(s) from the methanol extract of the herb was purified by sequential silica gel chromatography, using chloroform-Methanol-Water (7:3:1, lower layer) and then ethylacetate-Methanol mixtures (gradient) as the eluent. The purified inhibitor was identified as dioscin by IR, and 1H- and 13C-NMR spectroscopic analyses.

Mp 289–292°C; [α]D 25 = −110.5° (c, 0.3 in MeOH); IR (KBr, cm⁻¹): 3420, 1645, 1100–1000, 920, 863, 835 (900 > 920, 25(R)-spiroketal), 810; 1H-NMR (300 MHz, pyridine-d₅) δ: 0.68 (d, J = 5.2 Hz), 0.82 (s), 1.05 (s), 1.12 (d, J = 6.9 Hz), 1.60 (d, J = 6.9 Hz), 1.74 (d, J = 6.2 Hz), 4.92 (d, J = 6.7 Hz), 5.32 (br d, J = 4.6 Hz), 5.80 (s), 6.34 (s); 13C-NMR (75.5 MHz, pyridine-d₅) δ: 37.4, 29.9, 78.0, 38.8, 140.7, 121.5, 32.0, 31.6, 50.6, 37.0, 20.9, 39.8, 40.3, 56.5, 32.2, 80.9, 62.8, 16.2, 19.3, 41.8, 14.8, 109.0, 31.7, 29.1, 30.4, 66.7, 17.1, 100.1, 79.5, 76.5, 77.7, 77.6, 61.8, 101.7, 72.1, 72.2, 73.9, 70.1, 18.2, 102.7, 72.4, 72.5, 73.6, 69.2, 18.3.

Dioscin and its derivatives had been isolated from DN in the previous study.8 We therefore examined the inhibitory activities of dioscin and its family against the lipase. Dioscin, diosgenin, gracillin, prosapogenin A (diosgenin-3-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside) and C (diosgenin-3-O-β-D-glucopyranoside) each showed strong inhibitory activity against porcine pancreatic lipase, with respective IC₅₀ values of 20.0, 28.0, 28.9, 1.8 and 42.2 μg/ml that caused 50% inhibition of the enzyme (Table 2).

Table 2. Lipase Inhibitory Activity of the Saponins Isolated from Dioscorea nipponica

<table>
<thead>
<tr>
<th></th>
<th>Dioscin</th>
<th>Diosgenin</th>
<th>Gracillin</th>
<th>Pro-sapogenin A</th>
<th>Pro-sapogenin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (μg/ml)</td>
<td>20.0</td>
<td>28.0</td>
<td>28.9</td>
<td>1.8</td>
<td>42.2</td>
</tr>
</tbody>
</table>

**Effect of dioscin and diosgenin on the plasma triacylglycerol level after orally administering a lipid emulsion to mice**

Figures 2 and 3 show the time-course characteristics of the plasma triacylglycerol concentration when a corn oil suspension with or without dioscin and diosgenin was orally administered to mice. At 2, 3, and 4 hr after orally administering the lipid emulsion, the elevation of the plasma triacylglycerol concentration has been significantly reduced by dioscin (100 mg/kg bw) and diosgenin (100 mg/kg bw) in the mice. The
peak plasma triacylglycerol concentration was also reduced by dioscin and diosgenin.

**Effects of the Dioscorea nipponica powder on the food consumption, and body and adipose tissue weights of rats fed on a high-fat diet**

The mean food consumption per week per rat was not significantly different between the high-fat group and high-fat plus 2% or 5% DN diet group (Fig. 4). The change in body weight of the groups during the experimental period of 8 weeks is shown in Fig. 5.

Feeding the high-fat diet plus 5% DN powder significantly suppressed the body weight gain when compared to the control group fed on the high-fat diet alone during the experimental period. The amounts of subcutaneous, perirenal, and epididymal fat were significantly lower in the group fed on the diet containing 5% DN powder than in the control group (Table 3).

**Fecal fat excretion**

Fecal samples from rats fed on the control and experimental diets containing 2% or 5% DN powder were collected for 3 days prior to sacrifice. The fecal fat excretion of the experimental groups fed with the 2% and 5% DN powder were significantly higher than that of the control group, suggesting that the DN powder at the 2% or 5% level prevented dietary fat absorption in the rats (Table 4).

**Effect of the Dioscorea nipponica intake on the plasma parameters after 8 weeks of treating lean rats fed on the high-fat diet**

The plasma concentrations of TG, cholesterol, VLDL-cholesterol, and LDL-cholesterol were significantly lower in the group fed on the diet containing 2% or 5% DN powder, than in the control group fed on the high-fat diet alone. Furthermore, the DN powder increased the level of HDL-cholesterol, leading to an improvement in the atherogenic index (Table 5).

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**Table 3.** Effect of *Dioscorea nipponica* Powder (DN) on the Body Weight Gain and Body Fat in Rats Fed on the High-fat Diet for 8 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain (g)</th>
<th>Subcutaneous fat (g)</th>
<th>Epididymal fat (g)</th>
<th>Inguinal fat (g)</th>
<th>Perirenal fat (g)</th>
<th>Total body fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>192.7 ± 31.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 2.0&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>6.8 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.6 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% DN</td>
<td>161.8 ± 18.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.4 ± 1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.8 ± 1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.0 ± 1.2&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.6 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.4 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% DN</td>
<td>140.0 ± 20.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 ± 1.1&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.6 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.6 ± 5.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values not sharing a common subscript within the same column are significantly different from each other (P<0.05). N.S represents ‘not significant’.
quantification of dioscin in Dioscorea nipponica

The content of dioscin in the root extract of Dioscorea nipponica was about 2.7% (w/w), with prosapogenin A and prosapogenin C being below the detection limit under the HPLC conditions used.

Discussion

Obesity is associated with the development of type 2 diabetes, coronary heart disease, an increased incidence of certain forms of cancer, respiratory complications, and osteoarthritis of the large and small joints. The strategy for preventing and/or treating obesity includes the suppression of dietary intake, increased thermogenesis, and inhibition of adipocyte differentiation. A drug for inhibiting the absorption of energy nutrients, in particular, fat, has been successfully launched on the market. Orlistat, a hydrogenated derivative of a bacterial lipase inhibitor, is known to inhibit pancreatic lipase, thus decreasing TG digestion and body weight gain. Dioscin and diosgenin, the saponins present in DN, showed a strong inhibitory effect on pancreatic lipase and suppressed the time-dependent increase of plasma TG concentration in mice that had been orally injected with corn oil, although its effect was lower than that of Orlistat, a prescription drug. Prosapogenin A, diosgenin 3-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, has good potential to suppress dietary TG digestion and absorption as it showed the strongest inhibitory activity against pancreatic lipase among the compounds tested; however, its effect on the plasma TG increase after corn oil load could not be evaluated due to the limited amount of the compound. Considering relative abundance as well as lipase inhibitory activity, dioscin seems to have been major compound exerting the lipase inhibitory action in Dioscorea nipponica. Prosapogenin A and C, which were formerly known to be present in the herb, could not be detected under our HPLC conditions. However, we do not exclude the possibility that prosapogenenin A in the herbal extract might have contributed to the lipase inhibition and exerted anti-obesity activity as it showed the strongest lipase inhibitory activity among the saponins tested.

Since the in vitro study demonstrated strong lipase inhibitory activity of the extract of Dioscorea nipponica and its components such as disocin, we continued to evaluate the effect of the DN powder on obesity in rats induced by feeding a high-fat diet. The inclusion of the DN powder at the 5% level in the diet significantly suppressed the body weight increase of rats fed on a high-fat diet containing 40% beef tallow for 8 weeks. However, the use of the herb powder at the 2% level was only marginally effective in suppressing the body weight gain, although its level was enough to affect the blood parameters toward a reduced atherogenic risk (Table 5). The inhibition of body weight gain did not depend upon a decreased food or energy intake, because there was no significant difference in diet intake between the control and experimental groups, but was caused by preventing and/or delaying of fat absorption. As shown in Table 4, the intake of DN powder increased the excretion of fat through the feces. This observation further supports the assumption that the weight reduction from the herb intake was caused by the decreased fat absorption. The administration of the DN powder at the 5% level in the diet also significantly reduced the amount of fat in the subcutaneous, inguinal, and perirenal tissues in the rats, this being consistent with the reduced body weight gain of experimental group. Long-term feeding of the DN powder to rats caused significant changes in the blood parameters, including decreased levels of plasma triacylglycerol, and total, LDL- and VLDL-cholesterol, but an increased HDL-cholesterol level. All blood parameters in the rats fed on the diet containing the DN powder at the 2% and 5% levels indicated an improvement in the atherogenic index (Table 5). It seems that the saponins present in DN were responsible for preventing high-fat diet-induced obesity, because they were effective in inhibiting the triacylglycerol absorption by the mice injected with corn oil. Han and co-workers have also found that saponins prevented the high-fat diet-induced increase in parametrial adipose tissue weight by inhibiting the
intestinal absorption of dietary fat via inhibition of the pancreatic lipase activity.  

It has been reported that herb saponins caused hemolysis and cytotoxicity. In particular, dioscin and diosgenin have been shown to be cytotoxic against several cancer cell lines. No sign of sickness and erythrocyte hemolysis in the groups of experimental rats was apparent for 8 weeks in this study.

In conclusion, the DN powder was effective for preventing both the body and adipose tissue weight gains in rodents induced by a high-fat diet.

Acknowledgments

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References