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Note

Effects of Dietary Nasunin on the Serum Cholesterol Level in Rats

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The effects of nasunin, a major anthocyanin in eggplant, and its aglycone, delphinidin, on the serum cholesterol concentration were determined in rats fed with a cholesterol-enriched diet. The serum total cholesterol and HDL-cholesterol concentrations tended to be decreased and increased, respectively, by feeding nasunin and delphinidin, while the fecal excretion of both cholesterol and bile acids tended to be increased by feeding with the anthocyanins. There was no difference between the nasunin and delphinidin activity to decrease serum total cholesterol, nor to increase serum HDL-cholesterol or the fecal excretion of cholesterol and bile acids. These results suggest that the slightly lower serum total cholesterol concentration in rats fed with nasunin and delphinidin may in part be due to inhibition of the intestinal absorption of both cholesterol and bile acids by these anthocyanins, and that the delphinidin moiety of nasunin mainly contributes to this activity.

Anthocyanin pigments, which are contained in several kinds of vegetables and fruits such as purple radish (*Raphanus sativus* var. *sativus* f comet),[1] red turnip (*Brassica campestris* L.),[2] purple leaves of *Perilla ocimoides* (Japanese name, shiso),[3] grapes,[4-6] red cabbage (*Brassica oleracea*)[7] and eggplant (*Solanum melongena* L.),[8] are consumed as a food constituent of the human diet. The physiological function of anthocyanin, however, has been scarcely reported. Recent results of our work have shown that rubrobrassicin contained in atsumi-kabu (red turnip, *Brassica campestris* L.) had activity for lowering the atherogenic index in rats fed with a cholesterol-enriched diet.[2] Similarly, malvin contained in wild grapes had activity for slightly lowering the serum cholesterol concentration in rats fed with a cholesterol-enriched diet.[9] These findings prompted us to determine the influence of dietary nasunin (delphinidin-3-[4-(*p*-coumaroyl)-rhamnosyl(1→6)glucoside]-5-glucoside), which is the major anthocyanin contained in eggplant, on the serum and liver lipid concentrations in rats. This report deals with the effects of dietary nasunin and its aglycone, delphinidin, which may be released from nasunin during storage and food processing, on the serum and liver lipid concentrations in rats.

Nasunin was prepared from chouja-nasu (little eggplant, *Solanum melongena* L. 'Chouja') as described previously.[10] Delphinidin was prepared by hydrolyzing 1 g of nasunin with 10 ml of 15% HCl. The precipitate obtained after leaving the hydrolysate at 5°C was collected and dissolved in 0.1% HCl in methyl alcohol to produce a precipitate of delphindin after the addition of diethyl ether. The precipitate was collected, dried under vacuum, and used for the animal experiments.

Four-week-old male weanling Wistar-strain rats (Japan SLC, Hamamatsu, Japan), each weighing about 50–60 g, were randomly divided into 3 groups of 6 rats each. The rats were individually housed in stainless-steel cages with screen bottoms, and kept under controlled conditions with a 12-h light-dark cycle (06:00–18:00 light), a temperature range of 22–24°C, and relative humidity of about 55%.

The basal diet (cholesterol-enriched, diet 20CC) contained (by weight) 20% casein, 10% corn oil, 1% Harper's vitamin mixture (Oriental Yeast Co., Tokyo, Japan), 4% Harper's mineral mixture (Oriental Yeast Co.), 2% cellulose, 0.2% choline chloride, 0.5% cholesterol, 0.125% sodium cholate, 5.6% agar (1.9% agar solution in 0.3% acetic acid), and 56.575% sucrose. For the nasunin (N)- or delphinidin (D)-added diet (diet 20CC+N or 20CC+D), 0.1% nasunin or 0.033% delphinidin (equal to 0.1% nasunin on a molar basis), which was dissolved in 5.6 g of agar gel to avoid discoloration, was added to the basal diet at the expense of sucrose. The 0.1% nasunin level was used in this experiment according to the reported cholesterol-lowering action of the phenolic compound, taxifolin, at 0.1% and 0.05% levels.[11] Food and water were provided *ad libitum* for 14 days. The feces of each rat were collected daily during the last 4 days of the experimental period, and freeze-dried prior to extracting lipids. At the end of the feeding period, the rats were anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka, Japan) after 12 h of starvation, and bled by cardiac puncture. The liver of each was immediately removed, weighed and stored at −20°C until the lipid analysis. The serum was separated by centrifuging the blood at 3000 rpm for 15 min. Liver lipids were extracted and purified by the method of Folch *et al.*[12]

Total cholesterol, free cholesterol, HDL-cholesterol, and triacylglycerol in the serum were measured enzymically by using commercial kits (Cholesterol E-Test, Free-cholesterol C-Test, HDL-cholesterol Test and Triglyceride E-Test, respectively, from Wako Pure Chemical Ind., Osaka, Japan). The free fatty acids and phospholipids in the serum were also measured by using commerical kits (NEFA Test and Phospholipid B-Test, respectively, from Wako Pure Chemical Ind.). Total cholesterol in the liver extract was determined by the cholesterol Mono-test kit (Boehringer Mannheim Yamanouchi Co., Tokyo, Japan). Fecal neutral and acidic steroids were extracted with ethanol under reflux as described by Sautier *et al.*,[13] and the neutral steroids were measured by gas-liquid chromatography (glass column (1.5 m × 2 mm i.d.), Uniport HP support (GL Science, Tokyo)), using phenanthrene as an internal standard. The acidic steroids were measured by using a commercial kit (Bile acids Test, Wako Pure Chemical Ind.). The data for each of the three groups were statistically analyzed by Duncan's multiple-range test after an analysis of variance (ANOVA). The data from the control group and from the group fed with anthocyanin were also compared, and the significance of differences between the two groups was determined by Student's *t*-test.

As shown in Table, the ingestion of nasunin and delphinidin did not statistically affect the daily food intake, body-weight gain, liver weight, and serum and liver lipid concentrations when analyzed by ANOVA, although the serum HDL-cholesterol concentration in the rats fed with delphinidin was significantly increased when analyzed by Student's *t*-test. However, nasunin tended to increase serum HDL-cholesterol, and to decrease serum total cholesterol and the atherogenic index. In addition, delphinidin tended to decrease serum total cholesterol and the

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Table Effects of Nasunin and Delphinidin on the Growth, Food Intake, Liver Weight, Serum and Liver Lipid Concentrations, and Fecal Excretion of Lipids in Rats Fed with a Cholesterol-enriched Diet

Diet	20CC	20CC+N	20CC+D
Initial body weight (g)	61.8±0.9	62.1±1.4	60.0±0.6
Body weight gain (g/14 days)	65.5±1.5	64.8±2.2	63.2±2.2
Food intake (g/14 days)	154±1	154±1	153±1
Liver weight (% of body weight)	5.2±0.2	5.3±0.1	5.2±0.1
Serum lipids			
Total cholesterol (mg/dl)	333±36	307±26	296±13
Free cholesterol (mg/dl)	44.7±5.4	36.7±3.0	32.8±1.3
HDL-cholesterol (mg/dl)	38.8±1.4	44.5±2.3	43.1±1.0*
Triacylglycerol (mg/dl)	74.3±3.2	95.9±10.3	89.4±7.0
Phospholipid (mg/dl)	199±15	190±13	185±6
Free fatty acid (mEq/dl)	0.73±0.02	0.83±0.08	0.87±0.05
Atherogenic index**	7.6±1.0	6.6±0.9	5.9±0.3
Liver lipids			
Total cholesterol (mg/g of liver)	37.5±2.0	36.2±2.6	40.5±1.6
Triacylglycerol (mg/g of liver)	37.6±1.7	35.5±1.2	40.0±3.5
Phospholipid (mg/g of liver)	20.0±1.3	19.5±2.0	23.9±1.4
Fecal lipids			
Cholesterol (μmol/4 days)	98.2±11.3	105±8	110±5
Coprostanol (μmol/4 days)	42.2±6.7	42.1±5.0	42.3±4.1
Bile acids (μmol/4 days)	96.5±1.7	110±7.4	112±4.2*

20CC, 20% casein+0.5% cholesterol; N, nasunin; D, delphinidin. Values are means±SE of 5 to 6 rats per group.

* Significantly different from the 20CC group at $p<0.05$ when analyzed by Student's t-test. Values within each line were not significantly different among the three groups when analyzed by ANOVA.

** (Total chol − HDL-chol)/HDL-chol (*i.e.*, (VLDL-chol + LDL-chol)/HDL-chol)).

atherogenic index, besides significantly increasing serum HDL-cholesterol. These results indicate the possibility that the activity for increasing serum HDL-cholesterol may be higher in delphinidin than in nasunin, and that both nasunin and delphinidin may have slight activity for preventing atherosclerosis. On the other hand, when analyzed by Student's t-test, the amount of bile acids excreted from the rats fed with delphinidin was significantly increased and that from the rats fed with nasunin tended to be increased, compared with the figures for the control rats. The amount of excreted cholesterol tended to be increased by feeding with nasunin and delphinidin, but not to a statistically significant degree. It was, therefore, deudced that the slightly lower serum total cholesterol concentration and atherogenic index in the rats fed with nasunin and delphinidin may have mainly been due to inhibition of the intestinal absorption of both bile acids and cholesterol. However, it cannot be excluded that these anthocyanins may have activity for enhancing the catabolism of cholesterol to bile acids and result in an increase in the fecal excretion of bile acids. The colors of feces excreted by the rats fed with nasunin and delphinidin were bluish and reddish, respectively, suggesting that the gastrointestinal absorption of these anthocyanins may have been small, and that the non-absorbed anthocyanins may have brought about the slightly increased excretion of cholesterol and bile acids. There was no distinct difference between nasunin, which has the delphinidin moiety in its chemical structure, and delphinidin in the serum cholesterol-lowering action, nor in the activity to increase the serum HDL-cholesterol and fecal excretion of bile acids, nor to lower the atherogenic index. These facts may suggest that the delphinidin moiety of nasunin mainly contributed to these activities, and further that the other moieties of nasunin such as p-coumaroyl rutinose and glucose scarcely contributed to these activities. The amount of nasunin fed per day to a rat weighing about 100 g was 12 mg, assuming the rat consumed 12 g of diet per day. When converted to human dietary conditions, this figure may be high, since the nasunin content of chouja-nasu (about 25 g) is 12–15 mg. However, this dose is much lower than that previously used for proving the hypocholesterolemic effect of green tea catechin and (−)-epigallocatechin gallate, which were added to a cholesterol-enriched diet at a 0.5–2% concentration.[14,15] It is, therefore, interesting to note that nasunin and delphinidin showed activity for slightly increasing the serum HDL-cholesterol concentration, and for slightly decreasing the serum total cholesterol concentration and atherogenic index in rats fed with the cholesterol-enriched diet, although it may be necessary to examine the dose-activity relationship.

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