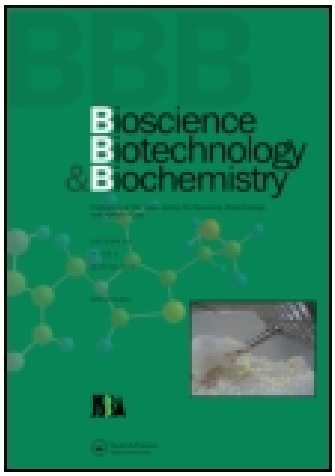


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# Effects of Taxifolin on the Serum Cholesterol Level in Rats

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*Note*

# Effects of Taxifolin on the Serum Cholesterol Level in Rats

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The physiological function of common constituents of food, especially the cholesterol-lowering effect of soybean protein,[1-4] whey protein[5-8] and some phenolic compounds, has recently received much attention. In particular, phenolic compounds such as green tea catechin and gallocatechin, and dietary quercetin have been respectively shown to lower the serum cholesterol and triacylglycerol levels in rats.[9-11] In addition, dietary rutin has been shown to lower the serum cholesterol level in rats with experimental cobalt-induced hyperlipemia.[12] Recent results from our work have shown that the rurbrobrassicin contained in Atsumi-kabu (*Brassica campestris* L.) had activity for lowering the atherogenic index in rats fed with a cholesterol-enriched diet.[13] On the other hand, malvin contained in wild grapes (*Vitis coignetiae* P.) had activity for lowering the serum triacylglycerol and free fatty acid levels in rats fed with a cholesterol-free diet.[14] These findings prompted us to study the influence of other phenolic compounds on the serum lipid levels. In the present study, we examined the effect of taxifolin, which is known to be present in peanuts and white wine in a free form and as its glucosides,[15,16] on the serum and liver lipid levels in rats by comparing with the effects of quercetin, in which the single bond between the 2- and 3-positions of taxifolin is replaced by a double bond. It has been reported that peanuts contain 10 mg of taxifolin/100 g.[15]

Taxifolin (2,3-dihydroquercetin) was prepared by reducing quercetin with sodium hydrosulfite according to the method of Geissman *et al.*[17] The $^{1}H$- and $^{13}C$-NMR spectral data for taxifolin obtained by recrystallization from ethyl acetate–*n*-hexane were in good agreement with those in the literature for trans-2,3-dihydroquercetin.[18,19]

Four-week old male weanling Wistar-strain rats (Japan SLC, Hamamatsu, Japan), each weighing about 60 g, were randomly divided into 4 and 2 groups of 6 rats each for experiments 1 and 2, respectively. The rats were individually housed in stainless-steel cages with screen bottoms, and kept under controlled conditions with a 12 hr light—12 hr dark cycle (06:00—18:00 hr light), a temperature range of 22—24°C, and a relative humidity of about 55%. All the rats were fed with a commercial stock diet (CE-2, Japan CLEA Co., Tokyo) for 3 days to adjust them to the new environment, after which they were fed with a purified diet with or without taxifolin or quercetin.

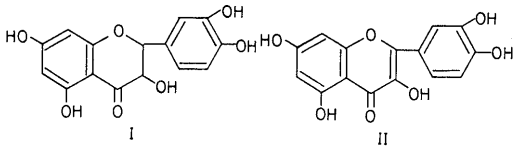


**Fig. 1.** Chemical Structures of Taxifolin (I) and Quercetin (II).

The composition of the experimental diets is given in Table I. For experiment 1, there were four kinds of diets comprising a casein+cholesterol diet (20CC), and three 20CC diets supplemented with 0.1 or 0.05% taxifolin, or 0.1% quercetin. For experiment 2, there were two kinds of diets comprising a casein diet (20C, without cholesterol), and a 20C diet supplemented with 0.1% taxifolin. Taxifolin or quercetin was added to the diets at the expense of α-wheat starch. Food and water were provided *ad libitum* for 21 days. The feces of each rat were collected during the feeding period from day 17 or 18 to day 21 and freeze-dried prior to extracting the lipids. At the end of the feeding period, the rats were anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka, Japan) after 12 hr of starvation, and bled by heart puncture. The liver was excised from each and stored at −20°C until the lipid analysis. The serum was separated by centrifuging the blood at 3000 rpm for 15 min.

Serum total cholesterol and free fatty acid were measured by using commercial kits (Cholesterol C II-Test and NEFA-Test, respectively, Wako Pure Chemical Ind., Osaka, Japan). Serum free cholesterol and triacylglycerol were measured enzymically by using commercial kits from Wako Pure Chemical Ind. (Free-cholesterol C-Test and Triglyceride E-Test, respectively). Serum high-density lipoprotein (HDL)-cholesterol was enzymically measured in the supernatant obtained after heparin-$Mn^{2+}$ precipitation of the other lipoprotein, using a commercial kit (HDL Cholesterol-Test, Wako Pure Chemical Ind.).[20] Liver and fecal lipids were extracted with choloroform–methanol (2:1, v/v) by the method of Folch *et al.*,[21] and cholesterol in the lipid extracts was measured by an enzymic method with a commercial kit (Mono-test Cholesterol, CHO-PAP method, Baehringer Mannheim Yamanouchi Co., Tokyo).[22] Liver total lipid was determined by a gravimetric analysis of the liver lipid extracts.

To determine the fecal bile acids, feces were extracted with 96% ethanol in refluxing apparatus for 2 hr, and the residue remaining after removing the ethanol extract was re-extracted by the same method. The combined ethanol extracts were then evaporated and dissolved in isopropanol for determination by the 3α-hydroxysteroid dehydrogenase

**Table I.** Composition of the Diets (%)

| Ingredients | Experiment 1 | | | | Experiment 2 | |
|---|---|---|---|---|---|---|
| | 20CC | 20CC+0.1%T | 20CC+0.05%T | 20CC+0.1%Q | 20C | 20C+0.1%T |
| Casein | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| α-Wheat starch | 63.275 | 63.175 | 63.225 | 63.175 | 68.9 | 68.8 |
| Corn oil | 10.0 | 10.0 | 10.0 | 10.0 | 5.0 | 5.0 |
| Mineral mixture* | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin mixture* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline chloride | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Cholesterol | 0.5 | 0.5 | 0.5 | 0.5 | — | — |
| Na-cholate | 0.125 | 0.125 | 0.125 | 0.125 | — | — |
| Taxifolin | — | 0.1 | 0.05 | — | — | 0.1 |
| Quercetin | — | — | — | 0.1 | — | — |

20CC, 20% casein +0.5% cholesterol; 20C, 20% casein; T, taxifolin; Q, quercetin.

* Mineral and vitamin mixtures (Harper's mixture: A. E. Harper, *J. Nutr.*, **68**, 405—419 (1959)) were obtained from Oriental Yeast Co.

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**Table II.** Effects of Taxifolin on the Growth, Food Intake, Liver Weight, Serum and Liver Lipid Concentrations, and Fecal Excretion of Lipids in Rats Fed with the Cholesterol-enriched Diet (Experiment 1)

| Group | 20CC | 20CC+0.1%T | 20CC+0.05%T | 20CC+0.1%Q |
|---|---|---|---|---|
| Body weight gain (g/21 days) | 95.0±2.0[a] | 92.6±3.6[a] | 91.0±2.6[a] | 93.7±2.5[a] |
| Food intake (g/21 days) | 232.4±2.3[a] | 231.6±3.8[a] | 231.5±2.5[a] | 228.8±2.0[a] |
| Liver weight (% of body weight) | 5.0±0.1[a] | 4.8±0.1[a] | 5.0±0.1[a] | 4.8±0.3[a] |
| Serum total cholesterol (mg/dl) | 203±15[a] | 160±8[a] | 161±5[a] | 183±9[a] |
| Serum free cholesterol (mg/dl) | 29.0±2.2[a] | 21.9±1.5[a] | 23.6±1.5[a] | 25.6±2.0[a] |
| Serum HDL-cholesterol (mg/dl) | 33.5±1.0[a] | 39.2±1.8[a] | 40.4±2.2[a] | 38.0±1.8[a] |
| Serum triacylglycerol (mg/dl) | 64.8±5.0[a] | 75.2±5.0[a] | 84.6±7.3[a] | 64.4±4.0[a] |
| Serum phospholipid (mg/dl) | 138±6[a] | 137±3[a] | 142±5[a] | 144±7[a] |
| Serum free fatty acid (mEq/dl) | 0.60±0.04[a] | 0.68±0.07[a] | 0.61±0.07[a] | 0.65±0.03[a] |
| Atherogenic index* | 4.7±0.5[a] | 3.4±0.3[b] | 2.9±0.2[b] | 3.9±0.3[ab] |
| Liver total lipids (mg/g of liver) | 179±7[a] | 175±4[a] | 163±8[a] | 175±6[a] |
| Liver total cholesterol (mg/g of liver) | 57.4±2.2[a] | 55.0±2.3[ab] | 49.5±1.4[b] | 52.4±1.2[ab] |
| Liver triacylglycerol (mg/g of liver) | 64.2±2.7[a] | 61.1±1.4[a] | 62.7±1.7[a] | 63.3±1.6[a] |
| Liver phospholipid (mg/g of liver) | 37.6±2.0[a] | 34.2±1.1[a] | 35.3±2.2[a] | 33.6±1.2[a] |
| Fecal total cholesterol (mg/4 days) | 81.4±9.0[a] | 119±10[a] | 103±11[a] | 95.8±7.8[a] |
| Fecal bile acids ($\mu$ mol/4 days) | 86.2±4.0[b] | 112±4[a] | 111±7[a] | 94.8±6.4[ab] |

Values are means±SEM of 5 to 6 rats per group. Values within the same row and not sharing a common superscript letter are significantly different at $p<0.05$. T, taxifolin; Q, quercetin.

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



method with a commercial kit (Bile acids-Test Wako, Wako Pure Chemical Ind.).

The data from experiment 1 were statistically analyzed by Duncan's multiple range test after an analysis of variance (ANOVA). The data from experiment 2 were analyzed by Student's *t*-test, and significant differences of the means were inspected at $p<0.05$.

The effects of taxifolin on lipid levels in the rats fed with the cholesterol-enriched diet are shown in Table II. Ingestion of taxifolin did not affect the daily food intake, body weight gain or liver weight. However, the diets containing taxifolin (diets 20CC+0.1% T and 20CC+0.05% T) compared to the diet without taxifolin (control diet 20CC) tended to decrease the serum total cholesterol level and to increase the serum HDL-cholesterol level. The atherogenic index in those rats fed with the taxifolin-added diet was significantly decreased, compared to that in the rats fed with the control diet. The serum total cholesterol and HDL-cholesterol levels were not affected by the difference in the amount of taxifolin added to the diets. The liver total cholesterol level was significantly decreased in those rats fed with the 0.05% taxifolin diet, compared to that of the control rats. On the other hand, the amount of fecal total cholesterol excreted from the rats fed with the taxifolin-added diet was numerically larger, although not statistically significantly so from that of the control rats. However, when analyzed by Student's *t*-test, the amount of fecal cholesterol was significantly higher in the rats fed with the 0.1% taxifolin diet. The amount of excreted fecal bile acids was significantly larger in the rats fed on the taxifolin-added diet than in those fed on the control diet.

The diet containing quercetin (diet 20CC+0.1% Q) tended to decrease the serum total cholesterol level compared to that with the control diet. However, the other serum lipid levels of the rats fed with the quercetin-added diet were not much different from those of the control rats. The amount of fecal total cholesterol and bile acids was slightly increased by feeding the quercetin-added diet.

The effects of taxifolin on the lipid levels in rats fed with the cholesterol-free diet are shown in Table III. Ingestion of taxifolin did not affect the daily food intake or body weight gain. The serum total cholesterol and HDL-cholesterol levels in those rats fed with the taxifolin-added diet (diet 20C+0.1% T) were significantly decreased compared to those of the rats fed with the control diet (diet 20C). The serum free fatty acid level was significantly increased in the rats fed with the taxifolin-added diet, although the other serum lipid levels were not much different between the control-fed (20C diet) and the taxifolin-fed (20C+0.1% T) rats. The liver lipid levels, and the amount of excreted fecal total cholesterol and bile acids were no different either.

As shown in Table II, taxifolin slightly but significantly increased the fecal excretion of bile acids and cholesterol, respectively, in rats feeding on the cholesterol-enriched diet containing 0.05 or 0.1% taxifolin. This result suggests that the lower atherogenic index and lower serum cholesterol level in the rats fed with the taxifolin-added diet may in part have been due to the inhibition of intestinal absorption of both bile acids and cholesterol. However, it cannot be excluded that taxifolin may have had activity for enhancing the catabolism of cholesterol into bile acid as well as activity for inhibiting the absorption of bile acids and cholesterol.

**Table III.** Effects of Taxifolin on the Growth, Food Intake, Liver weight, Serum and Liver Lipid Concentrations, and Fecal Excretion of Lipids in Rats Fed with the Cholesterol-free Diet (Experiment 2)

| Group | 20C | 20C+0.1%T |
|---|---|---|
| Body weight gain (g/21 days) | 97.8±4.0 | 96.8±4.1 |
| Food intake (g/21 days) | 248.2±8.5 | 255.2±6.6 |
| Liver weight (% of body weight) | 3.8±0.1 | 3.7±0.1 |
| Serum total cholesterol (mg/dl) | 85.0±1.4 | 80.5±1.2* |
| Serum free cholesterol (mg/dl) | 19.0±0.8 | 17.4±0.7 |
| Serum HDL-cholesterol (mg/dl) | 62.7±0.8 | 55.3±2.0* |
| Serum triacylglycerol (mg/dl) | 143±14 | 144±10 |
| Serum phospholipid (mg/dl) | 149±6 | 143±4 |
| Serum free fatty acid (mEq/dl) | 0.43±0.02 | 0.50±0.01* |
| Liver lipids (mg/g of liver) | 49.1±1.5 | 49.9±1.9 |
| Liver total cholesterol (mg/g of liver) | 4.38±0.10 | 4.16±0.16 |
| Liver triacylglycerol (mg/g of liver) | 16.6±0.6 | 18.4±0.8 |
| Fecal total cholesterol (mg/5 days) | 38.0±2.7 | 42.5±3.1 |
| Fecal bile acids ($\mu$mol/5 days) | 15.3±1.7 | 13.7±0.4 |

Values are means±SEM of 5 to 6 rats per group.

* Significantly different from the value for rats fed with the 20C diet at $p<0.05$.

T, taxifolin.

The amount of excreted bile acids and cholesterol was almost equal in the rats fed on the diets containing 0.05 and 0.1% taxifolin. This result suggests the possibility that the amount of taxifolin absorbed from the gastrointestinal tract, and moreover, the extent of inhibition for the absorption of bile acids and cholesterol by taxifolin may have been almost equal regardless of the amount of taxifolin added to the diet. It has already been reported by Brown and Griffiths that the gastrointestinal absorption of taxifolin was small.[23]

On the other hand, quercetin tended to increase the fecal excretion of both bile acids and cholesterol in the rats fed with the cholesterol-enriched diet, but the increase was a little lower with quercetin than with taxifolin. This result indicates that taxifolin may have been more potent

than quercetin in inhibiting the absorption of bile acids and cholesterol from the small intestine. However, further studies are necessary to clarify the mechanism for increasing the fecal excretion of cholesterol and bile acids by feeding taxifolin and quercetin.

The weak but significant serum cholesterol-lowering effect of taxifolin on the rats fed with the cholesterol-free diet indicates the possibility that taxifolin also influenced the endogeneous cholesterol metabolism, and thus reduced the serum cholesterol level. However, the precise mechanism for the action of taxifolin remains to be clarified.

It is well known that green tea catechin and epigallocatechin, which were added to a cholesterol-enriched diet at 0.5—2% concentration, inhibited the increase of plasma cholesterol level in rats.[9] Cho *et al.*, however, have observed that the cholesterol level in rats was not affected by adding 0.2% phenolic compounds, *i.e.*, *p*-coumaric, caffeic or ferulic acids, to a cholesterol-free diet.[24] The present study indicates that taxifolin, a phenolic compound in food, slightly but significantly decreased the atherogenic index and serum cholesterol level, respectively, in those rats fed with the cholesterol-enriched diet, and also slightly decreased the serum cholesterol level in the rats fed with the cholesterol-free diet.

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