Note

Cholesterol-Lowering Effect of Kori-Tofu Protein and Its High-Molecular-Weight Fraction Content

Takahiro Ishiguro,1,2 Seiji Tatsunokuchi,1 Nobuo Mitsui,1 Hisataka Kayahara,1 Hisashi Murasawa,1 Yotaro Konishi,2 and Satoshi Nagaoka3

1Food Research Center, Asahimatsu Foods Co., Ltd., 1008 Dashina, Iida, Nagano 399-2561, Japan
2Faculty of Human Life Science, Osaka City University, 3-3-138 Sagimotocho, Sumiyoshi-ku, Osaka 558-8585, Japan
3Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

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The serum total cholesterol concentration was significantly lower in the kori-tofu feeding group than in the soy protein isolate (SPI) group, except on the 28th day of the experiment. The high-molecular-weight fraction (HMF) content of the kori-tofu protein was significantly higher than that of SPI. This difference in the HMF content may have influenced the cholesterol-lowering effect of the protein.

Key words: kori-tofu; cholesterol; bile acid; high-molecular-weight fraction

Hypercholesterolemia is a major risk factor for arteriosclerosis. Soy protein is widely known to have a cholesterol-lowering effect, and may, therefore, reduce the risk of cardiovascular diseases. One prevalent hypothesis regarding this effect is that the undigested fraction of soy protein binds to bile acid in the intestines which prevents bile acid resorption into the body.1) The prevention of bile acid resorption has a cholesterol-lowering effect, because bile acid is synthesized from cholesterol. Sugano et al. have discovered an undigested fraction in soy protein and named it the high-molecular-weight fraction (HMF).2) They have reported that HMF had high bile-acid-binding capacity and a greater cholesterol-lowering effect on rats than raw soy protein.2) Kori-tofu is a popular food in Japan. It is made from soybean through a process that involves soaking, grinding, heating, coagulating, slow freezing, settling at a subzero temperature, thawing and then drying out in that order. This processing procedure results in the sponge-like porous structure of kori-tofu which gives it its unique texture. We suspected that the process would affect not just the texture but also the cholesterol-lowering property of the protein by increasing its HMF content and bile acid-binding capacity. Several studies on the cholesterol-lowering effect of kori-tofu have been reported. Fujii et al.3) have reported on an experiment conducted using a human subject. The subject’s serum cholesterol level at the end of the kori-tofu diet period was lower than that at the end of a meat diet period.3) Liu et al.4) have reported on the cholesterol-lowering effect of kori-tofu protein and its HMF by illustrating the increased fecal excretion of cholesterol and bile acid by rats.

We investigated in this present study whether the processing method for kori-tofu increased its cholesterol-lowering effect due to the HMF content. The cholesterol-lowering effect of kori-tofu protein was compared with that of a soy protein isolate (SPI) and casein in vivo. A cholesterol-enriched diet was used in order to investigate the hypocholesterolemic effect of the protein. We also compared the content and bile-acid binding capacity of HMFs of SPI and kori-tofu protein in vitro.

The study was approved by the Laboratory Animal Care Committee of Osaka City University, and the rats were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals recommended by Osaka City University.

Male Wistar rats (4 weeks old, Clea Japan) were fed for 28 days on a high-cholesterol diet containing casein, SPI or kori-tofu protein. The chemical composition of casein was as follows (g/kg): protein, 853; ash, 40; moisture, 106; lipids, 1; carbohydrate, 0. The chemical composition of SPI was as follows (g/kg): protein, 722; ash, 26; moisture, 96; lipids, 1; carbohydrate, 155. Kori-tofu protein was prepared from kori-tofu (Asahimatsu Co., Osaka, Japan) by defatting with n-hexane. The chemical composition of kori-tofu protein was as follows (g/kg): protein, 712; ash, 45; moisture, 124; lipids, 4; carbohydrate, 115.

Three experimental diets were prepared from each of the protein sources. The common ingredients of these three diets were as follows (g/100 g): lard, 10.0; cholesterol, 1.0; sodium cholate, 0.3; AIN-96 mineral mix, 5.0; AIN-96 vitamin mix, 1.0; cellulose, 3.0. The specific ingredients of the casein diet were as follows (g/100 g): casein, 23.4; corn starch, 56.3. The specific ingredients of the SPI diet were as follows (g/100 g): SPI, 27.7; corn starch, 52.0. The specific ingredients of the kori-tofu diet were as follows (g/100 g): kori-tofu protein, 28.1; corn starch, 51.6.

The serum total cholesterol level in the tail vein blood was measured every week (on the 7th, 14th, 21st, and 28th days of the experiment) by using a commercial kit (Cholesterol E-test Wako). At the end of the feeding period (28th day), HDL-cholesterol and triglyceride were respectively measured by using commercial kits (HDL-Cholesterol E-test Wako and Triglyceride E-test Wako). The specific ingredients of the casein diet were as follows (g/100 g): casein, 23.4; corn starch, 56.3. The specific ingredients of the SPI diet were as follows (g/100 g): SPI, 27.7; corn starch, 52.0. The specific ingredients of the kori-tofu diet were as follows (g/100 g): kori-tofu protein, 28.1; corn starch, 51.6.

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Table 1. Body Weight Gain, Food Intake, and Serum and Liver Lipid Concentrations in Rats Fed Different Protein Diets

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Diet group</th>
<th>Casein</th>
<th>SPI</th>
<th>Kori-tofu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight gain (g/28 d)</td>
<td>169 ± 4**</td>
<td>153 ± 4**</td>
<td>160 ± 3**</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>15.8 ± 0.3*</td>
<td>16.2 ± 0.3*</td>
<td>15.8 ± 0.4*</td>
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</tr>
<tr>
<td>Serum (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on 7th day</td>
<td>518 ± 42**</td>
<td>465 ± 25*</td>
<td>263 ± 16b</td>
<td></td>
</tr>
<tr>
<td>on 14th day</td>
<td>470 ± 42**</td>
<td>503 ± 33**</td>
<td>272 ± 17a</td>
<td></td>
</tr>
<tr>
<td>on 21st day</td>
<td>485 ± 13**</td>
<td>364 ± 29**</td>
<td>217 ± 14a</td>
<td></td>
</tr>
<tr>
<td>on 28th day</td>
<td>332 ± 18b</td>
<td>202 ± 19b</td>
<td>173 ± 9c</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>17.7 ± 1.1*</td>
<td>19.3 ± 1.1*</td>
<td>27.7 ± 2.8b</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>94.9 ± 10.3*</td>
<td>91.2 ± 5.2*</td>
<td>101.6 ± 10.8a</td>
<td></td>
</tr>
<tr>
<td>Liver (mg/g of liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>78.5 ± 1.0*</td>
<td>76.8 ± 3.1a</td>
<td>65.3 ± 1.1a</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>186 ± 6b</td>
<td>196 ± 7b</td>
<td>171 ± 6b</td>
<td></td>
</tr>
</tbody>
</table>

*The values for HDL-C, triglyceride, and liver total-C are on the 28th day (the end) of the experiment.
Rats were fed experimental diet (casein, SPI, or kori-tofu) for 28 days.
Each values is the mean ± SEM (n = 6).
- Values not sharing a common letter are significantly different (Tukey’s test, p < 0.05, SPSS).

Fig. 1. HMF Contents of SPI and Kori-Tofu Protein.
Each bar represents the mean ± SEM of 3 determinations. (*p < 0.05, Student’s t test).

Fig. 2. Bile Acid-Binding Capacity.
Each bar represents the mean ± SEM of 3 determinations. a,b,c Values not sharing a common letter are significantly different (Tukey’s multiple-comparison test, p < 0.05).

The high-molecular-weight fractions (HMF) of SPI and kori-tofu protein were prepared by using the method of Sugano et al.2) Each protein (2 g) was suspended in 20 mL of 0.1 M Tris–HCl buffer (pH 7.0) and hydrolyzed at 50 °C for 5 h by microbial proteases (protein FN and AC, the respective proteases from Aspergillus oryzae and Bacillus subtilis, 20 mg each; Daiwa Kasei, Osaka, Japan). The digest was heated at 80 °C for 30 min and centrifuged at 3,000 × g for 30 min. The resulting precipitate was washed three times with water, freeze-dried and then weighed. The method for estimating the bile-acid binding capacity was according to the report of Hirose et al.5) HMFs (50 mg derived from SPI and Kori-tofu protein), casein (negative control) and cholestyramine (positive control) were dispersed in 5.0 mL of 0.1 mM sodium taurocholate in a 0.1 M Tris–HCl buffer (pH 7.4). The mixture was shaken at room temperature for 2 h and then centrifuged at 3,000 × g for 30 min. The concentration of the taurocholate in the supernatant (non-bound) was measured by using a commercial kit (Bile Acid Test Wako, Wako Pure Chemicals, Osaka, Japan). The results are expressed in terms of the mean and SEM (n = 6 for each group for the in vivo study, and three determinations for the in vitro study). The statistical significance of differences was evaluated by using Student’s t-test for a 2-group comparison (Fig. 1), and Tukey’s multiple-comparison test for a 3- or more-group comparison (Table 1, Fig. 2).

The serum total cholesterol concentration of the kori-tofu group was lower than that of the casein group throughout the whole experiment (Table 1). The serum total cholesterol concentration of the kori-tofu group was significantly lower than that of the SPI group during the period of the 7th–21st days of the experiment. The serum total cholesterol concentration of the SPI group was significantly lower than that of the casein group after 21 days, but no significant difference in the serum total cholesterol concentrations of the SPI group and kori-tofu group was apparent on the 28th day. This data indicates that the cholesterol-lowering activity of kori-tofu protein was more rapid and stronger than that of SPI, and that SPI required a longer feeding period than kori-tofu to induce the cholesterol-lowering effect.

The serum HDL-cholesterol, triglyceride and liver lipid concentrations were analyzed on the 28th day of the experiment. The serum concentration of HDL-cholesterol was significantly higher in the kori-tofu group than in the casein or SPI group. The serum concentration of triglyceride showed no significant difference among the three groups, but liver total cholesterol tended to be lower in the kori-tofu group than in the other groups. Liver triglyceride was significantly lower in the kori-tofu group than in the SPI group. These results indicate that kori-tofu protein had the effect of increasing serum HDL cholesterol, and decreasing liver triglyceride.

The HMF contents of SPI and kori-tofu protein were analyzed in vitro. The HMF content of kori-tofu protein was significantly higher than that of SPI (Fig. 1). The bile acid-binding capacity of the HMFs, casein (as a negative control) and cholestyramine (as a positive control) were measured (Fig. 2). No significant difference was apparent between the HMFs of kori-tofu protein and SPI. Considering the order of the HMF content of each protein (Fig. 1), kori-tofu protein would have bound a greater amount of bile acid in the body than SPI. The cholesterol-lowering mechanism of the...
soy protein can be explained in terms of the enhanced bile acid extraction due to binding to the HMF. Liu et al. have reported that the fecal excretion of total bile acid increased as a result of feeding with kori-tofu protein and its HMF. The HMF content of kori-tofu protein was significantly higher than that of SPI (Fig. 1). This difference in the HMF content may have influenced the cholesterol-lowering effect of the protein. Kori-tofu is made from soybean by a complex process, and this process might be responsible for the increased content of HMF in kori-tofu protein.

It has been reported that such non-protein soy components as isoflavone and saponin had a cholesterol-lowering effect. The difference in these contents between SPI and kori-tofu, as well as the reason for the different cholesterol-lowering effect between SPI and kori-tofu, are the subjects for a future investigation.

Some recent studies have suggested that a certain soy hydrolyzed peptide had some functions, including cholesterol-lowering, triglyceride-lowering and antioxidation effects. The amount and profile of the peptide created by kori-tofu digestion may differ from that created by SPI digestion, because kori-tofu protein and SPI were digested differently (Fig. 1).

In conclusion, our results show a higher HMF content in kori-tofu than in SPI. The process for making kori-tofu may increase the HMF content. This increase in HMF content endowed the kori-tofu protein diet with a more rapid and stronger cholesterol-lowering effect than the SPI diet. The results indicate that the method used for processing food can alter the cholesterol-lowering effect of proteins.

References