Effects of Dietary Wakame (*Undaria pinnatifida*) Sporophyll on Plasma and Liver Lipid Levels in Rats

Masashi KAWASAKI,*2,3 Tomoka KIKUCHI,*2 Ryoko KIKUCHI,*2 Hiromi TAKAHASHI*2 and Risa TANAKA*2

川崎雅志, 菊池朋可, 菊池涼子, 高橋裕美, 田中理沙

The effects of dietary wakame (*Undaria pinnatifida*) sporophyll on plasma and liver lipid levels were studied in Wistar rats. Rats were fed the 10% powdered wakame sporophyll with cholesterol-free (Experiment 1) and cholesterol-supplemented (Experiment 2) diets. In the rats fed the cholesterol-free diet, plasma total cholesterol and very-low-density lipoprotein plus low-density lipoprotein-cholesterol concentrations were not affected by the ingestion of wakame sporophyll, whereas the high-density lipoprotein (HDL)-cholesterol concentration was significantly increased. Thus, the atherogenic index was significantly reduced and the HDL-cholesterol ratio was significantly enhanced by wakame sporophyll ingestion. There were no significant differences in the liver cholesterol and triglyceride contents between the control and the wakame sporophyll-fed groups which had been allowed the cholesterol-free diet. On the other hand, wakame sporophyll significantly decreased the liver cholesterol and triglyceride contents in the group allowed a cholesterol-supplemented diet. These results suggest that wakame sporophyll ingestion increases plasma HDL-cholesterol concentration, and decreases the degree of cholesterol-loaded fatty liver with the decrease in cholesterol and triglyceride contents.

**Keywords:** wakame sporophyll, plasma lipid, liver lipid

**Introduction**

Dietary seafoods and their ingredients have been well documented to bring beneficial effects to the lipid metabolism. We have investigated the ability of dietary sea squirt to reduce serum lipid concentrations such as total cholesterol, very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL)-cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA), and to enhance steroid excretion into feces. Dietary black sea cucumber and oyster have been reported to decrease the serum total cholesterol concentration and elevate the ratio of high-density lipoprotein (HDL)-cholesterol concentration to total cholesterol concentration, and to decrease liver cholesterol content. We have also reported that dietary fish oil, rich in n-3 polynsaturated fatty acids, decreased serum lipid concentrations in tumor-bearing rats used as a model of endogenous hyperlipidemia.

Wakame (*Undaria pinnatifida*) is a seaweed found along the coast of Japan. The edible fraction of wakame includes its leaves, stipe and center vain, and sporophyll. The wakame sporophyll is a unique organ rich in fucoidan and alginic acid, some of the polysaccharides, and the cut pieces of the wakame sporophyll are very viscous. The wakame sporophyll is called *mekabu* in Japanese.

In the present study, we examined the effects of the wakame sporophyll on plasma and liver lipid levels in rats. Rats were fed wakame sporophyll not only in a cholesterol-free diet but also in a cholesterol-supplemented diet used as a model of endogenous hypercholesterolemia and fatty liver.

**Materials and Methods**

Animals and diets. Male Wistar rats (3 wk old, Charles River Japan Inc., Kanagawa, Japan) were individually housed in stainless steel cages with wire bottoms 21 by 15 by 17 centimeters in an air-conditioned room at a temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12-h light cycle (0800-2000). They were fed a stock pellet diet (MF; Oriental Yeast Co., Tokyo, Japan) followed by a basal diet for 4 d.

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*2 Food and Nutrition Major, Science of Living Department.

*3 Corresponding author.

Abbreviations: EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.
Table 1. Composition of experimental diets (g / 100g).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experiment 1</th>
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<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Wakame sporophyll</td>
<td>Control</td>
<td>Wakame sporophyll</td>
</tr>
<tr>
<td>Casein 1</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>α-Cornstarch 1</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Cornstarch 1</td>
<td>39.75</td>
<td>29.75</td>
<td>39.125</td>
<td>29.125</td>
</tr>
<tr>
<td>Sucrose 2</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose powder 1</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Soybean oil 1</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mineral mixture (AIN9G composition) 1</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture (AIN93 composition) 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline bitartrate 3</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Cystine 3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Wakame sporophyll dry powder 4</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol 3</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium cholate 3</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

1 Oriental Yeast Co., Tokyo, Japan.
2 Nissin Sugar Manufacturing Co., Tokyo, Japan.
3 Wako Pure Chemical Industries, Osaka, Japan.
4 Yanagisawa Store, Iwate, Japan.

Subsequently, the rats were divided into four groups with similar body weights, and in Experiment 1, the rats were fed the basal or an experimental diet containing 10% of wakame sporophyll in the form of a dry powder. In Experiment 2, the rats were fed the basal or experimental diet supplemented with cholesterol. The wakame sporophyll was purchased from a market, and was freeze-dried and powdered. The composition of the experimental diets is shown in Table 1. Rats were maintained for another 28 d. Water and diet were available at all times. Animals were deprived of their diet at 0900 on the 28th d but allowed free access to water until sacrifice, which was performed 4 h later. Blood was collected from the heart and left to clot at room temperature to obtain plasma. The liver was quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. The plasma and liver were stored at -80°C until analyzed. Aliquots of the liver were also preserved in methanol and stored at 4°C until lipid content analyses.

Animal experiment was carried out in accordance with the standards relating to the care and management, etc. of experimental animals (Notification No. 6, March 27, 1980 of the Prime Minister’s Office, Japan).

Lipid analyses. Plasma total cholesterol, HDL-cholesterol, triacylglyceride, phospholipid, and NEFA concentrations were determined by an enzymatic method using a Cholesterol E-test Wako, HDL-Cholesterol E-test, Triglyceride E-test, Phospholipid C-test, and NEFA-C test, respectively. All test kits were obtained from Wako Pure Chemical Industries, Osaka, Japan. The difference between total cholesterol concentration and HDL-cholesterol concentration was regarded as (VLDL+LDL)-cholesterol concentration. The ratio of (VLDL+LDL)-cholesterol concentration to HDL-cholesterol concentration was designated as the atherogenic index. The ratio of HDL-cholesterol concentration to total cholesterol concentration was estimated as the HDL-cholesterol ratio.

Total lipids from the liver were extracted according to the procedure described by Folch et al.9 After portions of the chloroform phase had been dried under nitrogen, cholesterol, triglyceride, and phospholipid contents were determined.

Plasma thiobarbituric acid-reactive substance (TBARS) value was determined using a commercial kit purchased from Wako Pure Chemical Industries. Liver TBARS value was measured using the method described by Mihara et al.12

Statistical analysis. Results were expressed as the mean ± standard error. Statistical analysis was carried out by unpaired Student's t-test using the SPSS program. Differences were considered significant at p < 0.05.

RESULTS

Table 2 shows initial body weight, food intake, and body weight gain for the duration of 28 d of experimental feeding, and relative liver weight at the end of experimental feeding. The food intake and body weight gain were not significantly different between the control and the wakame sporophyll-fed
Table 2. Effects of dietary wakame sporophyll on food intake, body weight gain, and relative weight of liver in rats fed cholesterol-free (Experiment 1) and cholesterol-supplemented (Experiment 2) diets.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control (g)</th>
<th>Wakame sporophyll (g)</th>
<th>Control (g)</th>
<th>Wakame sporophyll (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>73.3 ± 0.9</td>
<td>73.3 ± 3.2</td>
<td>73.3 ± 3.4</td>
<td>73.3 ± 1.6</td>
</tr>
<tr>
<td>Food intake (g/28 d)</td>
<td>486.3 ± 26.1</td>
<td>497.1 ± 5.0</td>
<td>504.3 ± 6.1</td>
<td>497.4 ± 15.6</td>
</tr>
<tr>
<td>Body weight gain (g/28 d)</td>
<td>219.7 ± 3.7</td>
<td>211.2 ± 2.6</td>
<td>224.5 ± 3.6</td>
<td>209.0 ± 9.0</td>
</tr>
<tr>
<td>Liver weight (g/100 g body wt)</td>
<td>4.21 ± 0.22</td>
<td>3.94 ± 0.10</td>
<td>6.01 ± 0.12</td>
<td>5.07 ± 0.14</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for five rats.

* Significantly different from the control group at p < 0.05 by Student’s t-test.

groups both in the cholesterol-free and cholesterol-supplemented diets. Relative liver weight was not affected by the wakame sporophyll in the cholesterol-free diet, though the ingestion of wakame sporophyll significantly reduced the relative liver weight in the cholesterol-supplemented diet group.

Plasma cholesterol concentrations are shown in Figure 1. In Experiment 1 (C vs. W), the plasma total cholesterol and VLDL+LDL-cholesterol concentrations were not affected by the ingestion of wakame sporophyll, whereas HDL-cholesterol concentration was significantly increased. Thus, the atherogenic index was significantly reduced and the HDL-cholesterol ratio was significantly enhanced by the wakame sporophyll. In Experiment 2 (CC vs. CW), the plasma total cholesterol, and HDL- and VLDL+LDL-cholesterol concentrations were not significantly different between the control and the wakame sporophyll-fed groups, though wakame sporophyll ingestion tended to decrease the total cholesterol and VLDL+LDL-cholesterol concentrations.

Figure 2 shows the plasma triglyceride, phospholipid, and NEFA concentrations. There were no significant differences in the plasma triglyceride, phospholipid, and NEFA concentrations due to wakame sporophyll ingestion, though it tended to decrease the plasma triglyceride and NEFA concentrations in both the cholesterol-free and cholesterol-supplemented diet groups.

Liver lipid contents are shown in Figure 3. There were no significant differences in the liver cholesterol, triglyceride, and

![Graphs showing data](image-url)

Fig. 1. Effects of dietary wakame sporophyll on plasma cholesterol concentrations, atherogenic index, and high-density lipoprotein (HDL)-cholesterol ratio in rats fed cholesterol-free and cholesterol-supplemented diets. Each value represents the mean for five rats. Vertical bars indicate standard errors. * Significantly different from the basal diet (Control) group at p < 0.05 by Student’s t-test. C, cholesterol-free-basal diet group; W, cholesterol-free-wakame sporophyll diet group; CC, cholesterol-supplemented-basal diet group; CW, cholesterol-supplemented-wakame sporophyll diet group.
phospholipid contents between the control and the wakame sporophyll-fed groups allowed the cholesterol-free diet. On the other hand, the ingestion of wakame sporophyll significantly decreased liver cholesterol and triglyceride contents. The liver phospholipid content was significantly enhanced by the wakame sporophyll.

Plasma and liver TBARS are shown in Table 3. There was no significant difference in the plasma TBARS value between the control and the wakame sporophyll-fed groups both in the cholesterol-free and cholesterol-supplemented diets. The ingestion of wakame sporophyll significantly suppressed the liver TBARS in the cholesterol-free diet group. In the cholesterol-supplemented rats, no significant difference was seen in the liver TBARS value between the control and the wakame sporophyll-fed groups.

DISCUSSION

This study was performed to evaluate the effects of dietary wakame sporophyll on plasma and liver lipid levels in rats. The feeding of 10% wakame sporophyll in a dry powder form significantly increased the plasma HDL-cholesterol concentration and HDL-cholesterol ratio in the rats fed a cholesterol-free diet. The mechanism of this plasma HDL-cholesterol lifting action might be the specificity of the wakame sporophyll protein. It is well known that the difference of a diet’s amino acid composition changes the total cholesterol or lipoprotein cholesterol concentration in the plasma or serum. The amino acid composition of dietary proteins has also been suggested as a possible determinant responsible for their effects on plasma or serum cholesterol concentration. Krichevsky has proposed that the lysine to arginine ratio of dietary protein may be related to their cholesterolemic action. The lower lysine to arginine ratio of fish protein, as compared to casein, was seen to increase the ratio of serum HDL-cholesterol concentration to total cholesterol concentration. The lysine to arginine ratio of the wakame protein (1.12) is lower than that of the casein (2.18). Thus, there is a possibility that the wakame sporophyll protein might be related to the plasma HDL-cholesterol lifting action seen in the present study.

Dietary wakame sporophyll significantly reduced the liver lipid (cholesterol and triglyceride) contents, and decreased the degree of fatty liver caused by the ingestion of the cholesterol-supplemented diet. The wakame sporophyll contains a variety of sterols, such as fucosterol and 24-methylene cholesterol, whose content levels in the wakame sporophyll are higher than those in other seaweeds. In the present study, a high amount of exogenous cholesterol in the liver was seen in the group fed the cholesterol-supplemented diet. These plant sterols inhibit cholesterol absorption. Thus, cholesterol absorption was inhibited by these plant sterols found in the wakame sporophyll, and the cholesterol content might be decreased in the present study.

Another possible mechanism of the liver cholesterol lowering effect of the wakame sporophyll might be the action of bile acid. The wakame sporophyll is rich in fucoidan and alginic acid, viscous polysaccharides, which bind bile acid and promote bile acid excretion. The bile acid content might be reduced by wakame sporophyll containing these
polysaccharides, and bile acid biosynthesis might be promoted to supplement the lack of bile acid content. The enhancement of bile acid biosynthesis from cholesterol might decrease content of liver cholesterol.

The mechanism of the liver triglyceride lowering action of wakame sporophyll might involve the decrease in fatty acid and triglyceride syntheses. Wakame sporophyll oil contains a high ratio of n-3 polyunsaturated fatty acids, especially, eicosapentaenoic acid (EPA). The EPA-inhibited synthesis of triglyceride in cultured rat hepatocytes, as well as a fish oil diet rich in EPA, have been reported to decrease liver triglyceride content and suppress fatty acid synthesis. In the present study, a large amount of endogenous triglyceride in the liver was seen in the rats fed the cholesterol-supplemented diet. n-3 Polyunsaturated fatty acid decreased the fatty acid content and slowed the esterification to the triglyceride, thus decreasing the triglyceride content in the present study.

The liver TBARS value was significantly reduced by dietary wakame sporophyll in the rats fed the cholesterol-free diet. The TBARS value is an index of lipid peroxidation in plasma or tissues. Polyunsaturated fatty acids are easily oxidized. The ratio of polyunsaturated fatty acids to all fatty acids in seafoods is higher than that in other animal foods. Wakame sporophyll oil contains a high level of polyunsaturated fatty acids. Though, the liver lipid peroxidation might have been suppressed by the ingestion of wakame sporophyll, our results showing a significant decrease in the liver TBARS value might have been caused by constituents other than this foodstuff in wakame sporophyll in the present study.

In conclusion, in the present study we discovered the plasma HDL-cholesterol lifting effect of a cholesterol-free diet, and the liver lipid (cholesterol and triglyceride) lowering effects of a cholesterol-supplemented diet of wakame sporophyll powder fed to rats. It is possible that dietary wakame sporophyll may have some beneficial affects on lipid metabolism. It remains unknown what constituent elements of the wakame sporophyll exert these beneficial actions related to

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Table 3. Effects of dietary wakame sporophyll on thiobarbituric acid-reactive substance (TBARS) values of plasma and liver in rats fed cholesterol-free (Experiment 1) and cholesterol-supplemented (Experiment 2) diets.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Wakame sporophyll</td>
</tr>
<tr>
<td>TBARS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (µmol/L)</td>
<td>2.00 ± 0.23</td>
<td>2.23 ± 0.25</td>
</tr>
<tr>
<td>Liver (µmol/g liver)</td>
<td>129.3 ± 7.3</td>
<td>102.2 ± 4.5 *</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for five rats.
* Significantly different from the control group at $p < 0.05$ by Student's $t$-test.
Effects of Dietary Wakame (Undaria pinnatifida) Sporophyll on Plasma and Liver Lipid Levels in Rats (Masashi KAWASAKI, Tomoka IKUCHI, Ryoko IKUCHI, Hiromi TAKAHASHI and Riyo TANAKA)

liver metabolism. Further studies are needed to clarify this point.

REFERENCES

和文要旨
血漿および肝臓脂質レベルに対する食餌メカブの作用をウィスタラットにおいて検討した。ラットには10%のメカブ乾燥粉末をコレステロール無添加食（実験1）およびコレステロール添加食（実験2）とともに与えた。コレステロール無添加食を摂取したラットにおいて、血漿総コレステロールおよび超低密度リポタンパク質 + 低密度リポタンパク質+低密度リポタンパク質コレステロール濃度にメカブの摂取による影響がみられなかったのに対し、血漿高密度リポタンパク質（HDL）コレステロール濃度が有意に上昇した。このため、メカブの摂取により動脈硬化指数が有意に低下し、HDL コレステロール比が有意に上昇した。肝臓コレステロールおよびトリグリセリド含量が、コレステロール無添加食摂取群には対照群とメカブ摂取群との間に有意な差はみられなかったのに対し、コレステロール添加食摂取時にはメカブ摂取によって有意に減少した。これらの結果より、メカブの摂取は血漿 HDL コレステロール濃度を有意に上昇させ、また、コレステロールおよびトリグリセリド含量を減少させることにより、コレステロール添加食摂取による脂肪肝を軽減させることが示唆された。