Effects of Dietary Oyster Extract on Lipid Metabolism, Blood Pressure, and Blood Glucose in SD Rats, Hypertensive Rats, and Diabetic Rats

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Oyster extract was prepared by hydrolysis of oyster protein with proteases, Aloase (a protease from Bacillus subtilis), and Pancitase (a protease from Aspergillus oryzae). Rats were fed a diet containing 20% casein (the control diet) or 15% casein and 5% oyster extract (the oyster extract diet) as the protein source. The oyster extract diet exerted a significant reduction in serum cholesterol and liver triglyceride concentrations as compared with the control diet in Sprague-Dawley (SD) rats fed cholesterol-supplemented diets for 4 weeks. The activities of cytosolic fatty acid synthase and glucose-6-phosphate dehydrogenase were significantly lower in the oyster extract group than in the control group in the liver of SD rats. Hepatic cholesterol and triglyceride concentrations were significantly lower in spontaneously hypertensive (SH) rats and Otsuka Long-Evans Tokushima Fatty (OLETF) rats, type 2 diabetic rats, fed the oyster extract diet, for 4 weeks and 4 months respectively, than in those fed the control diet in the cholesterol-free diet. Blood pressure was significantly lower in the oyster extract group than in the control group at the 2nd and 4th weeks after the beginning of feeding experimental diets in SH rats. These results suggest that oyster extract prepared by hydrolysis of oyster induces triglyceride-lowering activity in the liver through a decrease in hepatic lipogenesis in SD rats, and that it exerts the antihypertensive effect in SH rats.

Key words: oyster extract; cholesterol; triglyceride; lipogenesis; antihypertension

When proteins are hydrolyzed during gastrointestinal digestion and during food processing, biologically active peptides which are inactive within the sequence of parent proteins are liberated. These peptides act as physiological modulators, and they have multiple functional properties such as antihypertensive, hypocholesterolemic, and antioxidative activities, etc.1,2 Soybean protein is known to have hypocholesterolemic and antihypertensive effects.3–6 Soybean peptide, in addition to these functions of soybean protein, inhibits bacterial translocation in cultured C2Bbe cells and suppresses the accumulation of body fat in mice.7,8 Also, soybean peptide has useful physical functional properties, viz., facility of digestion and improvement of solubility as compared with soybean protein. Milk protein-derived peptides are reported to have hypotensive effects.9 Thus, peptides might improve the functions of parent proteins and exhibit new functions. Therefore, several peptides might be used as health-enhancing foods and in pharmaceutical applications.

Epidemiological studies have shown that the population eating seafood every day has low mortality and low risk for cardiovascular and cerebrovascular disease.10,11 Although these effects are ascribed primarily to the reduction of serum triglyceride and cholesterol levels and to the suppression of platelet aggregation by lipid components occurring in fish, eicosapentaenoic and docosahexaenoic acids, there is a possibility that components other than lipids in seafood have favorable effects on lipid metabolism. Recently, we observed that non-lipid fractions of seafood, short-necked clam, squid, shrimp, and octopus, yield decreased cholesterol and triglyceride concentrations of the serum and liver in mice and rats.15–17 Fish protein has been reported to improve lipid metabolism.18,19 Hypertension and diabetes are known to be major factors in the development of coronary heart disease in addition to dyslipidemia. Fish protein has been observed to suppress the incidence...
of hypertension and diabetes.\textsuperscript{20–22} Hence, the fractions other than lipids, mainly the protein fraction, in seafood might play an important role in human health. Wergedahl \textit{et al.}\textsuperscript{23} reported that fish protein hydrolysate exhibits the hypocholesterolemic effects. Blood pressure regulating peptides derived from several seafoods, sardine, bonito, alga, \textit{etc.}, have been discovered.\textsuperscript{24,25} We observed that oyster exerts a hypolipidemic activity, and the effect can be ascribed to both the lipid and the non-lipid fractions.\textsuperscript{26} There is a report that chronic feeding of oyster improves glucose tolerance in alloxan diabetic rats, insulin dependent diabetes mellitus rats.\textsuperscript{27} But there is little information on the nutritional functions of oyster peptide. In the present study, the effects of oyster extract containing peptides obtained by the hydrolysis of oyster protein on lipid metabolism, blood pressure, and the level of blood glucose were investigated in Sprague-Dawley (SD) rats, spontaneously hypertensive (SH) rats, and Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are non-insulin dependent diabetes mellitus model rats, respectively.

\section*{Materials and Methods}

\textit{Preparation of oyster extract.} Oyster meat was boiled to deactivate autolysis enzymes at $75^\circ$C with twice the amount of water. To hydrolyze protein contained in oyster meat to peptides and free amino acids, Aloase (activity, 40,000 U/g, Yakult, Tokyo), an endoprotease from \textit{Bacillus subtilis}, was added at the 0.1\% level of total oyster meat weight and incubated at $60^\circ$C for 6 h. In addition, Pancitase (activity, 20,000 U/g, Yakult), an exoprotease from \textit{Aspergillus oryzae}, was added at the 0.1\% level of total weight and incubated at $50^\circ$C for 4 h. Two proteases were used to shorten effectively the molecular weight of oyster protein. The digest was heated to stop the reaction at $90^\circ$C for 30 min, and it was centrifuged to remove undigested proteins at $1,750 \times g$ for 30 min, and consequently a clear brown supernatant was obtained. This supernatant was denuded of salts by electrodialysis equipment (CH-O type, Asahi Glass Engineering, Chiba, Japan) and concentrated using a calandria-type concentrator under reduced pressure (600 mmHg, 60°C). The concentrate was dried with a spray dryer (FGA-8, Ohkawara Kakokhi, Kanagawa, Japan) and powdered, and then it was fed as oyster extract.

\textit{Chemical analyses.} The chemical composition of oyster extract preparation was determined. The crude protein and lipid contents, which were assayed by the Kjeldahl method and the Soxhlet method with diethyl ether, were 36.1 and 0.1\% respectively. The sugar content, determined by the phenol/sulfonic acid method, was 54.0\%. Moisture, determined as the loss in weight after drying at $105^\circ$C for 24 h, was 4.1\%. Oyster extract contained 3.6\% ash, which was measured by the direct ignition method ($540^\circ$C overnight). The molecular weight pattern of peptides in oyster extract was measured with a Waters HPLC, ProteinPac 60 column (Japan Waters, Tokyo). Oyster extract preparation diluted with 50-fold amount of water and filtrated with 0.25 \textmu m filter was injected into the HPLC column. Analyses were conducted with 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl as the mobile phase at a flow rate of 1.0 ml/min. Nine molecular weight fractions were obtained (Fig. 1), and three quarters of total peptides were approximately 10,000, 13,800, and 15,100 of molecular weight fractions.

\textit{Animals and diets.} Male SD rats and SH rats (Charles River Japan, Kanagawa, Japan) were used in experiments 1 and 2 respectively. OLETF rats, a model of spontaneous non-insulin dependent diabetes mellitus
rats, and Long-Evans Tokushima Otsuka (LETO) rats, the control model of OLETF rats, presented by Otsuka Pharmaceutical (Tokushima, Japan), were employed in experiment 3. The rats were housed individually in stainless cages under a controlled atmosphere (temperature, 22 ± 1°C; humidity, 55 ± 5%; light cycle, 8:00–20:00). The compositions of experimental diets in experiments 2 and 3 are shown in Table 1. Cholesterol and sodium cholate were added at the 0.5% and 0.125% levels respectively, at the expense of cornstarch in experiment 1. The protein content was adjusted to 20% on an isonitrogenous basis.

### Table 1. Composition of the Experimental Diets in Experiments 2 and 3

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>Oyster extract diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein2</td>
<td>217</td>
<td>163</td>
</tr>
<tr>
<td>Oyster extract2</td>
<td>0</td>
<td>139</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture (AIN-93-MX)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture (AIN-93-VX)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>to 1,000</td>
<td>to 1,000</td>
</tr>
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</table>

1Cholesterol and sodium cholate were added at the 0.5% and 0.125% levels respectively, at the expense of cornstarch in experiment 1.

### Experiment 1

Male SD rats (4 weeks old) were fed experimental diets ad libitum for 4 weeks (Table 1). Feces were collected for 2 d at the end of the experiment. After fasting for 6 h, rats were sacrificed by withdrawing blood from the abdominal aorta under sodium pentobarbital anesthesia. The liver was immediately excised and dissected, and a portion was chilled on ice for homogenization, and the rest of the liver was frozen in liquid nitrogen. A portion of the liver was homogenized in 6 volumes of a 0.25M sucrose solution containing 1 mM EDTA in a 10 mM Tris–HCl buffer (pH 7.4). After precipitating the nuclei fraction, the supernatant was centrifuged at 100,000 x g for 60 min to precipitate microsomes, the remaining supernatant being used as the cytosol fraction. The mitochondrial and microsomal pellets were resuspended in a 0.25M sucrose solution containing 1 mM EDTA in a 10 mM Tris–HCl buffer (pH 7.4).

Serum lipids were assayed enzymatically using commercial kits (Cholesterol C-II Test, Triglyceride G-Test, Phospholipid B-Test, Wako Pure Chemical Industries, Osaka, Japan, and HDL-C-2-Daiichi, Daiichi Chemicals, Tokyo). Liver lipids were extracted by the method of Folch et al.28) The concentrations of cholesterol, triglycerides, and phospholipids were measured by the methods of Sperry and Webb,29) Fletcher,30) and Rouser et al.31) respectively. Fecal neutral and acidic steroids were measured by gas-liquid chromatography using an OV-17 (Chromatotec, Tokyo) column32) and an AN-600 (Chromatotec) column33) respectively.

The activities of cytosolic fatty acid synthase (FAS),34) glucose-6-phosphate dehydrogenase (G6PDH),35) the malic enzyme,36) microsomal phosphatidate phosphohydrolase (PAP),37) and mitochondrial carnitine palmitoyltransferase (CPT)38) were determined in the liver. Protein was assayed by the method of Lowry et al.,39) using bovine serum albumin as a standard.

### Experiment 2

Male SH rats (4 weeks old) were fed experimental diets ad libitum for 4 weeks. The composition of the experimental diet is shown in Table 1. Systolic blood pressure in the conscious state was measured using a BP Monitor for Rats and Mice Model MK-2000 (Muromachi Kikai, Tokyo) on the first day of feeding the experimental diet and every week. At the termination of the feeding period, the SH rats were killed by withdrawing blood from the abdominal aorta under sodium pentobarbital after 6 h of starvation, and the livers were collected. Serum and liver lipid concentrations were determined as described above.

### Experiment 3

Male OLETF and LETO rats (5 weeks old) were fed a commercial diet (CE-2, CLEA Japan, Tokyo) for 3 months. When the rats were 4 months old, the level of blood glucose was measured with a commercial kit (Glucose C-II Test, Wako Pure Chemical Industries) after 6 h fasting. OLETF rats were divided to two groups equal body weight and the value of blood glucose, and then they were given an experimental diet. The composition of the experimental diets is shown in Table 1. OLETF rats were fed a control or oyster extract diet, and LETO rats were fed a control diet. The value of blood glucose was measured after 6 h fasting every half month during 1 to 4 months after the beginning of feeding the experimental diets. After feeding experimental diets for 4 months, the rats were anesthetized with sodium pentobarbital, and blood was collected and the liver was excised. The concentrations of serum and liver lipids were measured as described above. Serum free fatty acids were measured using a commercial kit (NEFA C-Test, Wako Pure Chemical Industries). Serum insulin concentration was determined by radioimmunoassay (Insulin “Eiken” Radioimmunoassay Kit, Eiken Chemical, Tokyo).
Values of analyzed by one-way ANOVA and Tukey's procedure.

Statistical analysis. In experiments 1 and 2, data were inspected by Student’s t-test. In experiment 3, data were analyzed by one-way ANOVA and Tukey’s procedure. Values of P < 0.05 were considered statistically significant. Data are reported as means ± SEM.

Results

Experiment 1

There were no differences in body weight gain, food intake, or relative liver weight between the control and oyster extract groups (Table 2).

The concentration of serum cholesterol was 34% lower in rats fed the oyster extract diet than in rats fed the control diet (Table 2). The level of high density lipoprotein (HDL)-cholesterol and the ratio of HDL-cholesterol to total cholesterol were comparable between the two groups. No differences in serum triglyceride and phospholipid concentrations were observed between the control and oyster extract groups.

The levels of hepatic cholesterol and phospholipids were the same between the two groups, but the level of hepatic triglycerides was 42% lower in the oyster extract group than in the control group (Table 2). The activities of FAS, an enzyme related to fatty acid synthesis, and G6PDH, an NADPH-generating enzyme, in liver cytosol were lower in rats fed the oyster extract diet than in those fed the control diet (Table 3). There were no differences in the activities of the malic enzyme, an NADPH-generating enzyme, mitochondrial CPT, the rate-limiting enzyme of mitochondrial β-oxidation, or microsomal PAP, the rate-limiting enzyme of triglyceride synthesis, between the two groups.

Fecal weight was comparable between the two groups (Table 4). Although the oyster extract diet accelerated by 2.6 times fecal acidic steroid excretion as compared with the control diet, it reduced by 26% fecal neutral steroid excretion. Consequently, total steroid excretion was comparable between the two groups.

Experiment 2

No differences in body weight gain, food intake, or liver weight were observed between SH rats fed the control diet and those fed the oyster extract diet (Table 5).

Systolic blood pressure at 2 and 4 weeks after the start of feeding the experimental diet was lower in the oyster extract group than in the control group (Fig. 2).

The concentrations of serum total cholesterol, HDL-cholesterol, and triglycerides in SH rats were identical between the control and oyster extract groups (Table 5). The serum phospholipid level was higher in rats fed the oyster extract diet than in those fed the control diet. Feeding oyster extract induced a reduction in hepatic cholesterol and triglycerides as compared with the control diet. There was no difference in liver phospholipid concentration between the two groups.

Experiment 3

Body weight gain during the feeding period of the experimental diet was the same among LETO rats, OLETF rats fed the control diet, and OLETF rats fed the oyster extract diet (Table 6). Food intake, water intake, and relative liver weight were significantly higher in OLETF rats than in LETO rats, but these parameters were identical between the control and oyster extract
Table 6. Effects of Dietary Oyster Extract on Growth Parameters and Serum and Liver Lipid Levels in LETO and OLETF Rats (Experiment 3)

<table>
<thead>
<tr>
<th></th>
<th>LETO</th>
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<th>OLETF</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Oyster extract</td>
<td>Control</td>
<td>Oyster extract</td>
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<tr>
<td>Growth parameters</td>
<td></td>
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<tr>
<td>Initial body weight (g)</td>
<td>423 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>609 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>623 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Final body weight (g)</td>
<td>538 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>780 ± 25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>735 ± 40&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Body weight gain (g)</td>
<td>116 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171 ± 40</td>
<td>112 ± 46</td>
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<tr>
<td>Food intake (g/day)</td>
<td>19.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.1 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Water intake (g/day)</td>
<td>24.5 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.0 ± 6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5 ± 11.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Relative liver weight</td>
<td>2.67 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.88 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>(g/100 g body weight)</td>
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<tr>
<td>Serum lipids and insulin</td>
<td></td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.23 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.54 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.51 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.78 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.86 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.81 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>HDL-/Total cholesterol (%)</td>
<td>42.1 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.4 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.97 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.04 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.07 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Phospholipids (mmol/l)</td>
<td>2.48 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.81 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Free fatty acids (mEq/l)</td>
<td>0.66 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.94 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Insulin (μU/ml)</td>
<td>24.9 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>294 ± 49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>216 ± 37&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Liver lipids</td>
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<tr>
<td>Cholesterol (μmol/g)</td>
<td>7.79 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.31 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.72 ± 1.41&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Triglycerides (μmol/g)</td>
<td>37.0 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>308 ± 49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>154 ± 27&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Phospholipids (μmol/g)</td>
<td>38.7 ± 1.09</td>
<td>34.4 ± 0.67</td>
<td>35.4 ± 0.96</td>
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</tr>
</tbody>
</table>

Values are means ± SEM of six rats. Different letters in the same column indicate statistical difference, \( P < 0.05 \).

LETO, Long-Evans Tokushima Otsuka rats; OLETF, Otsuka Long-Evans Tokushima Fatty rats.

The value of blood glucose in LETO rats was constant and low during the feeding period (Fig. 3). The blood glucose in OLETF rats fed the control diet or the oyster extract diet was significantly higher than that in LETO rats from 1.5 to 4 months after the initiation of feeding the experimental diets. There was no difference in the value of blood glucose between the two groups in OLETF rats.

The concentrations of serum total cholesterol, HDL-cholesterol, triglycerides, phospholipids, and free fatty acids were significantly higher in OLETF rats than in LETO rats (Table 6). There were no differences in these parameters between OLETF rats fed the control and the oyster extract diets. Although the ratio of HDL-cholesterol to total cholesterol was significantly lower in OLETF rats fed the control and the oyster extract diets as compared with LETO rats fed the control diet, no differences in the two groups of OLETF rats were found. The serum insulin level was higher in OLETF rats than in LETO rats, but oyster extract did not significantly improve the insulin level in OLETF rats.
The hepatic cholesterol level was 36% lower in OLETF rats fed the oyster extract diet than in OLETF rats fed the control diet, and the value in OLETF rats fed the oyster extract diet was comparable to the level in LETO rats fed the control diet (Table 6). The liver triglyceride concentration was markedly higher in OLETF rats fed the control diet than in LETO rats fed that diet. Feeding oyster extract reduced 50% hepatic triglyceride level as compared with feeding the control diet in OLETF rats. There were no differences in liver phospholipid concentration among the three groups.

**Discussion**

Oyster extract prepared by hydrolysis of oyster in the current study contained 54% sugar and 36% peptides or amino acids. A large portion of sugar contained in oyster meat is glycogen, which is structurally and functionally similar to starch. Therefore, it is not thought that glycogen has different effects on lipid metabolism, blood pressure, and blood glucose than starch. In addition, since oyster extract was added to the control diet at the expense of cornstarch in the preparation of the oyster extract diet, the contents of sugar were approximately the same between the control and oyster extract diets. Hence, we think that the effect of the sugar component of oyster extract is negligible.

In the present study, oyster extract was observed to induce a reduction in the hepatic triglyceride level in SD rats, SH rats, and OLETF rats (Tables 2, 5 and 6). Since hepatic FAS and G6PDH activities in SD rats fed oyster extract were lower than those in rats fed the control diet (Table 3), it is thought that the rate of fatty acid synthesis in the liver of rats fed oyster extract is lower than in those fed casein. The activities of CPT, an indicator of mitochondrial β-oxidation, and PAP, the key enzyme in the synthesis of triglycerides, were not appreciably modified by oyster extract. Therefore, the reduction in the hepatic triglyceride level in SD rats fed oyster extract is thought to be induced mainly by a reduction in the activities of fatty acid synthesis in the liver. Because oyster extract decreased liver triglyceride concentration in SH rats and OLETF rats, it is quite possible that oyster extract reduced hepatic fatty acid synthesis in these strains.

Previously we observed that dietary oyster and defatted oyster accelerated neutral and acidic steroid excretion into feces in SD rats fed a cholesterol-supplemented diet, resulting in a decrease in liver cholesterol concentration. But in the present study, oyster extract increased fecal acidic steroid excretion and reduced fecal neutral steroid excretion, and consequently it did not accelerate total steroid excretion. This might be the reason oyster extract did not induce a reduction in the liver cholesterol level. Thus, the effects on fecal neutral steroid excretion and liver cholesterol concentration were different between intact oyster or defatted oyster and oyster extract. This means that oyster extract prepared in the present experiment is structurally and functionally different from the hydrolyzate produced from oyster protein during gastrointestinal digestion. Nagaoka et al. indicated that the soyprotein peptic hydrolyzate exerted hypocholesterolic action through inhibiting cholesterol absorption by direct interaction between cholesterol in mixed bile salt micelles and peptide or protein. Hydrolyzate from oyster protein produced in the gastrointestinal tract might have a higher capacity to bind to cholesterol in mixed bile salt micelles as compared with the oyster extract prepared in the present study, inducing an enhancement of neutral steroid excretion into the feces. Iwami et al. showed that the higher hydrophobicity of protein imparts a higher binding capacity to bile acid and inhibits the reabsorption of bile acid in the small intestine. The oyster extract in the present experiment appears to have a bile acid binding capacity. Although the hepatic cholesterol concentration was the same between the oyster extract group and the control group in SD rats fed a cholesterol-supplemented diet, it was significantly lower in the oyster extract group than in the control group in SH rats and OLETF rats fed a diet free of cholesterol. Dietary cholesterol is known to stimulate bile acid synthesis in the liver. Madani et al. reported that the activity of hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, was lower in rats fed soybean protein than in rats fed casein on a cholesterol-free diet, but the activity was higher in rats fed soybean protein than in those fed casein on a cholesterol-supplemented diet. It appears that oyster extract accelerates bile acid synthesis more than casein in cholesterol-supplemented diets. Hence, more bile acids were secreted into the intestinal...
lumen and fecal output was enhanced. Since increased bile acid concentration in the lumen can accelerate the micellar solubility of cholesterol, it can increase cholesterol absorption in feeding oyster extract, resulting in reduction of fecal neutral steroid excretion. There is a possibility that the supplementation of diets with cholesterol weakens the effect of the reduction of hepatic cholesterol by oyster extract.

Although oyster extract induced the reduction of serum cholesterol concentration in SD rats fed the diet containing cholesterol, it did not affect serum cholesterol level in SH rats and OLETF rats fed diets free of cholesterol. The causes of this difference in the effect of oyster extract on serum cholesterol level are obscure. They might be induced by the presence or absence of cholesterol in the diet and/or by the difference in strains of rats.

In the current study, oyster extract was observed to prevent increases in blood pressure in the state preceding hypertension, approximately 150 mmHg, in SH rats. It is expected that oyster extract induces a reduction in blood pressure in the hypertensive state more than 180 mmHg. Angiotensin I-converting enzyme (ACE) plays a crucial role in the regulation of blood pressure. ACE inhibitory peptides are isolated from many food proteins, and they have antihypertensive activity. These peptides contain 5–13 amino acid residues per molecule, and most of them have a C-terminal sequence of Ala-Pro or Pro-Pro. Since the peptides in oyster extract prepared in the present experiment consisted of relatively high molecular weights, from 5,000 to 15,000, there is a possibility that small peptides released during gastrointestinal digestion exerted ACE inhibitory activity. In fact, some peptides with weak ACE inhibitory activities show strong antihypertensive activities after oral administration. That is, these weak inhibitory peptides might be converted into strong ones by gastrointestinal proteases which are absorbed in intact form to exhibit antihypertensive effects. Peptides in oyster extract might serve as prodrug-type ACE inhibitory peptides. Further investigation is necessary on this point.

There are some reports on the effects of dietary protein and peptide on the development or alleviation of diabetes. The plant protein mixture which was constituted of alfalfa seed, soybean, Brewer’s dried yeast, wheat germ, and dried red lentils was observed to develop diabetes in diabetic model rats. On the other hand, hydrolyzed casein, as compared with the cereal-based diet, was reported to inhibit the onset of insulin-dependent diabetes in diabetes-prone Bio-Breeding rats. In the present experiment, since oyster extract did not influence serum glucose or insulin levels in OLETF rats, the oyster extract prepared in present experiment appears to be inactive for type 2 diabetes. However, oyster extract prepared with a different enzyme (Sumizyme, a protease from aspergillus oryzae, Shin-Nihonkagakukogyo, Tokyo) from those used in the present experiment showed remarkable suppression of the level of blood glucose in OLETF rats (unpublished data). It is possible that certain oyster peptides released from oyster protein can suppress the level of blood glucose.

Hyperlipidemia, hypertension, and diabetes are major risk factors in the development of coronary heart disease. The present study revealed that oyster extract hydrolyzed by proteases improves lipid metabolism and suppresses increases in blood pressure, and it shows promise in the development of a novel physiologically functional food for preventing cardiovascular disease.

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References

10) Crombie, I. K., McLoone, P., Smith, W. C., Thompson,


