Effects of Bioactive Components of Sea Cucumber on the Serum, Liver Lipid Profile and Lipid Absorption

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Several studies had indicated that the whole body of sea cucumber had beneficial effects on lipid metabolism. However, little information has been known on the individual functions of its bioactive components, and this study was undertaken to compare the different effects on improving lipid metabolism. The rats were assigned to seven groups: control, whole sea cucumber, saponins, polysaccharides, collagen peptides, dregs and non-saponin residues. After 28 d of feeding, the serum total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and hepatic lipid concentrations were examined. The results indicated that a dietary saponin supplement significantly suppressed adipose accumulation, and reduced serum and hepatic lipids. Saponin proved to be more effective than the other isolated components, so is considered to be the main lipid-lowering component in sea cucumber. The possible mechanism by which saponins improved lipid metabolism was also investigated. The saponins of sea cucumber suppressed and delayed TG and TC absorption which could be related to the pancreatic lipase inhibiting effect of saponins. This may be an important mechanism to explain its lipid-lowering effect on rats.

Key words: sea cucumber; lipid metabolism; obesity; lipid absorption; pancreatic lipase

The sea cucumber is well known as a traditional and valuable seafood in Asian countries, especially being consumed in China, Japan and Korea. It belongs to the Holothuroidea class, Echinodermata phylum in its biological classification.1) The sea cucumber has now attracted considerable attention because of its beneficial health effects. Such bioactive substances as saponins, cerebrosides, polysaccharides, and collagen peptides have been found and isolated from sea cucumber.2–4) and the beneficial physiological functions of sea cucumber may be based on those substances. We have already reported that Cucumaria frondosa improved the disturbed lipid metabolism induced by a high-cholesterol diet, and Japanese researchers have also indicated that Holothuria atra reduce the serum total cholesterol level in rats.5) Although several reports have shown that sea cucumber improved the lipid metabolism in animals, most of them focused on the whole body of the sea cucumber. Further work is therefore needed to elucidate which component in sea cucumber is responsible for each beneficial effect and how it acts.

An initial study was therefore undertaken to compare the effects on body weight, adipose tissue weight, serum and hepatic lipid of saponins, polysaccharides, collagen peptides, dregs and residues without saponins in sea cucumber. The results indicated saponin to be the main lipid-lowering component. A further study was based on these results to elucidate the possible mechanism by which saponins improved lipid the metabolism.

Inhibiting the digestion and absorption of dietary fat is a key to control body weight and dyslipidemia. Orlistat, a pancreatic lipase inhibitor, could reduce obesity and hyperlipidemia by inhibiting fat absorption and increasing fat excretion into the feces.6,7) Previous studies have indicated that several saponins derived from plants, like green tea, ginseng and Platycodi radix, reduced fat absorption by inhibiting the pancreatic lipase activity.8–10) We presumed that the saponins of sea cucumber may have the same potential for suppressing dietary fat absorption, so a second study was undertaken to clarify whether the saponins of sea cucumber improved lipid metabolism by inhibiting dietary fat absorption. The serum lipid profiles were observed in a lipid emulsion supplemented with saponin, and the inhibitory effect of saponins on pancreatic lipase was also determined.

Materials and Methods

Materials. The sea cucumber, Cucumaria frondosa, was obtained from Zhou-Shan Fishery Company (Zhejiang Province, China) and stored at −40 °C before being used. AIN-93G was purchased from Dyets, and male Sprague-Dawley (SD) rats (160–170 g) were purchased from Vital River Laboratory Animal Technology Co. (Beijing City, China). The enzymatic reagent kits to detect the concentrations of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) were purchased from Biosino (Beijing City, China). All other chemicals and solvents were of reagent grade.

Preparation of the saponins of sea cucumber. The body wall of sea cucumber (Cucumaria frondosa) was air-dried and ground into powder. Saponins were extracted according to the method of Dong et al.1) A 100-g amount of the powder was extracted three times with refluxing 60% ethanol at 70 °C. The filtrates were combined and
subjected to vacuum evaporation (Heidolph, Germany) to remove the ethanol. The concentrated fraction was then collected and partitioned between water and n-butanol. The n-butanol extract was evaporated to dryness, containing crude saponins with a weight of 1.89 g. The purity of the yielded saponins was 64%, as determined by the vanillin-perchloric acid colorimetric method. A 6% amount of protein was contained in the crude saponins, as determined by a DC protein assay kit (BioRad).

Preparation of the non-saponin residue in sea cucumber. After extracting the saponins with 60% ethanol, the insoluble powder was combined and heated to dryness, containing non-saponin residues with a weight of 91.5 g.

Preparation of the collagen peptides and polysaccharides of sea cucumber. The insoluble sea cucumber powder collected by the foregoing method was immersed in a 50 mmol/L sodium acetate buffer containing papain and incubated at 60°C for 24 h. The mixture was centrifuged, and the clear supernatant was precipitated with 3 vol of n-butanol. The concentrated fraction was then collected and partitioned in vacuo to remove the ethanol and then lyophilized to give crude polysaccharides, containing collagen peptides (48.2 g).

Preparation of the dregs of sea cucumber. The remaining mixture after enzymatic hydrolysis was collected as the dregs (22.1 g) which were precipitated with ethanol, evaporated to dryness, containing non-saponin residues with a weight of 91.5 g.

Acute treatment of sea cucumber saponins to rats. To clarify the possible mechanism by which saponins exhibited the most effective lipid-lowering effect, we designed an experiment to determine the effect of saponins on the lipid absorption. Six-week-old SD rats were randomly assigned to two groups: the control group and saponin-treated group, with 6 rats in each group. After 10 h of fasting, the rats were orally administered with 2 mL/kg of body weight of a lipid emulsion which had been prepared according to the method of Han et al. with 6 mL of corn oil, 80 mg of cholic acid, 2 g of cholesteryl oleate and 6 mL of saline in the absence or presence of saponins to a final concentration of 500 mg/kg of body weight. Blood samples were taken from the tail vein 0 h, 0.5 h, 1 h, 2 h, 3 h, 4 h and 5 h after administering the lipid emulsion with or without saponins. The serum TG and TC levels were determined by using a commercial enzymatic reagent kit.

Preparation of the blood and liver samples. At the end of the feeding period, the rats were decapitated under light anaesthesia with diethyl ether after overnight fasting. Serum was separated from whole blood by centrifugation at 3,000 × g for 10 min at 4°C. The liver and white adipose tissues were excised, washed with ice-cold isotonic saline and then frozen in liquid nitrogen.

Measurement of the lipid concentrations in the serum. The TC, HDL-C, and TG concentrations in the serum were determined by enzymatic reagent kits according to the manufacturer’s protocols. Lipids were extracted from the liver with chloroform-methanol 2:1 (by vol.) as previously described. The hepatic TG and TC concentrations were also determined by using commercial kits, and the hepatic phospholipid level was measured by the method of Bartlett.

Measurement of the fecal lipids. Feces were collected during the last 3 d of the experimental period. After lyophilizing for 24 h, the feces were stored at −20°C until needed for analysis. Total fecal lipids were extracted from the lyophilized feces with chloroform-methanol 2:1 (by vol.) as previously described. Neutral sterol of the feces was determined by the method of Miettinen et al.

Measurement of the pancreatic lipase activity in vitro. The lipase activity was determined by measuring the rate of release of oleic acid from triolein as reported by Han et al. Briefly, a mixture of triolein (80 mg), phosphatidylcholine (10 mg) and taurocholic acid (5 mg) in 9 mL of a 0.1 mol/L Tris–HCl buffer (pH 7.0), plus 0.1 mol/L of NaCl was sonicated for 5 min. This sonicated substrate suspension (0.1 mL) was incubated with 20 units of pancreatic lipase (porcine from Sigma) and 0.1 mL of various concentrations of the saponin solutions for 30 min at 37°C in a final volume of 0.25 mL. The assay was conducted with three replicates for each treatment.

Statistical analyses. All statistical analyses were performed by using SPSS software, the values in the tables and figures being
expressed as the mean ± standard error of the mean. One-way ANOVA with Tukey’s post hoc test was used to compare the group means with the control. Statistical significance was accepted at p < 0.05.

Results and Discussion

Effects of sea cucumber on the body weight and adipose tissue weight

We examined the food intake, body weight and adipose tissue weight of rats fed with the basal diet and sea cucumber-containing diets. The initial body weight was similar among each group (data not shown), but after feeding for 28 d, the body weight gain of the saponin-treated rats was markedly lower than that of the rats fed with the basal diet (p < 0.01). The polysaccharide-treated rats also exhibited a modest but not significant decrease in body weight gain. No significant difference in the average food intake was apparent among those groups throughout the experiment (Table 1).

The perirenal AT weight of the groups fed with sea cucumber tended to be less than that of the control group, especially with the rats treated with whole sea cucumber, saponins, and dregs. However, supplementation with whole sea cucumber and its bioactive components did not affect the epidymal AT weight (Table 1). These results therefore confirmed that some components in sea cucumber indicated a weight loss potential against obesity, especially the saponins. This anti-obese effect did not depend on any decreased food or energy intake because no significant difference was apparent.

Effects of sea cucumber on the serum lipids

The important role of serum lipids in atherosclerosis and coronary heart disease has been proved by many scientists. Growing evidence has demonstrated the cardiovascular disease risk to be positively associated with TC and TG, and inversely associated with HDL-

Table 1. Effects of Sea Cucumber on the Growth Parameters of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Food intake (g/d)</th>
<th>BW gain (g)</th>
<th>Perirenal AT (g/100 g BW)</th>
<th>Epidymal AT (g/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.3 ± 0.31</td>
<td>155 ± 3.4</td>
<td>1.35 ± 0.12</td>
<td>1.17 ± 0.07</td>
</tr>
<tr>
<td>Whole sea cucumber</td>
<td>18.1 ± 0.33</td>
<td>150 ± 3.0</td>
<td>1.02 ± 0.07**</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>Saponins</td>
<td>17.9 ± 0.32</td>
<td>131 ± 2.2**</td>
<td>0.97 ± 0.05**</td>
<td>1.01 ± 0.07</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>18.5 ± 0.31</td>
<td>142 ± 2.5</td>
<td>1.27 ± 0.05</td>
<td>1.23 ± 0.07</td>
</tr>
<tr>
<td>Collagen peptides</td>
<td>18.3 ± 0.33</td>
<td>161 ± 3.8</td>
<td>1.13 ± 0.08</td>
<td>1.03 ± 0.12</td>
</tr>
<tr>
<td>Dregs</td>
<td>18.4 ± 0.31</td>
<td>165 ± 4.0</td>
<td>1.01 ± 0.07*</td>
<td>1.07 ± 0.08</td>
</tr>
<tr>
<td>Non-saponin residues</td>
<td>18.2 ± 0.39</td>
<td>140 ± 4.2</td>
<td>1.19 ± 0.10</td>
<td>1.03 ± 0.07</td>
</tr>
</tbody>
</table>

Male rats were fed on a diet with or without sea cucumber for 28 d. Values are presented as the mean ± SEM (n = 6). *p < 0.05, **p < 0.01 vs. the control.

Table 2. Effects of Sea Cucumber on the Serum Lipid Concentrations in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.45 ± 0.04</td>
<td>1.19 ± 0.26</td>
<td>0.78 ± 0.05</td>
<td>0.84 ± 0.09</td>
</tr>
<tr>
<td>Whole sea cucumber</td>
<td>1.25 ± 0.06</td>
<td>0.65 ± 0.11*</td>
<td>0.79 ± 0.04</td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>Saponins</td>
<td>1.05 ± 0.09**</td>
<td>0.58 ± 0.13**</td>
<td>0.70 ± 0.03</td>
<td>0.49 ± 0.04*</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>1.07 ± 0.09*</td>
<td>0.53 ± 0.12**</td>
<td>0.75 ± 0.06</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Collagen peptides</td>
<td>1.30 ± 0.08</td>
<td>0.65 ± 0.08*</td>
<td>0.73 ± 0.06</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Dregs</td>
<td>1.29 ± 0.07</td>
<td>0.92 ± 0.08</td>
<td>0.73 ± 0.04</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Non-saponin residues</td>
<td>1.39 ± 0.09</td>
<td>0.67 ± 0.10*</td>
<td>0.75 ± 0.05</td>
<td>0.83 ± 0.11</td>
</tr>
</tbody>
</table>

Male rats were fed on a diet with or without sea cucumber for 28 d. Values are presented as the mean ± SEM (n = 6). *p < 0.05, **p < 0.01 vs. the control.

The serum lipid level was therefore determined at the end of the experiment. Compared with the control group, both the saponin and polysaccharide treatment significantly reduced the serum TC concentration (especially p < 0.01 and p < 0.05), although the other components did not alter the serum TC level. However, a more dramatic effect on lowering serum TG was found in this experiment. Serum TG was decreased to 0.65 ± 0.11 mmol/L in the whole sea cucumber group (a 45.4% decrease, p < 0.05), saponins group (a 51.3% decrease, p < 0.01), and polysaccharide group (a 55.5% decrease, p < 0.01). Although none of the sea cucumber treatments changed the serum HDL-C concentration, the atherogenic index (AI) was significantly decreased by the saponin treatment (Table 2), suggesting that saponins may be beneficial to preventing atherosclerosis. Taken together, these results suggest saponins and polysaccharides to be the two main components responsible for the anti-atherosclerosis effect of sea cucumber, being even more effective than whole sea cucumber.

Our observation is consistent with some previous reports. Tanaka et al. have reported that 5% black sea cucumber supplementation significantly reduced the serum TC concentration, and increased the ratio of HDL-C and TC in rats. According to the study of Gao et al., dietary sea cucumber (Cucumaria frondosa) improved the disturbed lipid metabolism induced by a high-cholesterol diet, with a significant decrease of the TC and low-density lipoprotein-cholesterol (LDL-C) contents in the serum. The present findings, therefore, strengthen the hypolipidemic effect of sea cucumber, even by supplementing with a much smaller proportion (0.5% in our study).

Effects of sea cucumber on the hepatic lipid concentration in rats

Table 3 shows that supplementation with collagen peptides and the non-saponin residues led to a significant increase in the hepatic TC content. Sea cucumber also...
exhibited a suppressive effect on the hepatic TG accumulation. The results show that the hepatic TG concentration in the rats fed with whole sea cucumber and saponins was significantly reduced to 8.53 ± 0.54 mg/g (p < 0.05) and 8.57 ± 0.61 mg/g (p < 0.05) of liver. Other components also contributed to the TG level in the liver, but without statistical significance, while the hepatic phospholipid content remained unchanged in those groups.

The liver plays a central role in lipid transport and metabolism, and is likely to contribute to the onset and progression of several chronic diseases, including atherosclerosis, diabetes, and obesity.19,20) TG present in the liver is stored as lipid droplets or secreted into the blood as lipoprotein.21) In the fasting condition, the source of serum TG is mainly from lipoprotein secreted by the liver, making the hepatic TG content positively associated with the serum lipid level. Our results revealed that saponins and whole sea cucumber significantly decreased the hepatic TG concentration which may partly explain the hypolipidemic effect. It is interesting that the saponins from sea cucumber exerted a stronger effect than even whole sea cucumber. Collagen peptides were found to significantly increase the hepatic TC content as described in Table 3. The opposite effect on lipid metabolism of collagen peptide may explain why the effect of saponin alone was even stronger than that of the whole body.

**Effect of saponin on the serum lipid levels after orally administering the lipid emulsion**

It seems that saponin in sea cucumber was the most effective component for lowering the lipid level. Inhibiting the digestion and absorption of dietary fat is a key to controlling the body weight and adipose weight.22) We first examined serial changes in both the serum TG and TC concentrations after treating rats with the lipid emulsion with or without saponins to test whether the saponins from sea cucumber could change the dietary lipid absorption. The results show that the serum TG level of the control increased from 0 h to 2 h, before subsequently decreasing. The serum TC concentration was significantly lower than that of the control group in the presence of saponins, suggesting that the saponins in sea cucumber may have reduced the fat absorption. In contrast, the serum TC level of the control showed no obvious change after administering the oral lipid emulsion (Fig. 2). However, the serum TC concentration was significantly decreased by the saponin supplement 1 h, 2 h, 3 h and 5 h after administration, suggesting that the saponins in sea cucumber also suppressed the cholesterol absorption.

**Effect of saponin on fecal lipid excretion**

To further clarify whether saponin was associated with the absorption inhibitory effect, lipids in the feces were also determined in this study. We hypothesized that saponins could increase the fecal lipid excretion; however, Table 4 shows that saponins failed to induce the fecal excretion of total lipids. This may have resulted from the low dose (0.09 g/kg of diet) used in the chronic treatment which was not sufficient to block lipid absorption.

In contrast, the fecal neutral sterol excretion, a measure of unabsorbed exogenous and endogenous cholesterol, was significantly higher by the saponin group than that by the control (54.9% increase, p < 0.01). The results are in good agreement with our previous observation that the saponin supplement suppressed dietary cholesterol absorption, consequently resulting in the increased excretion of fecal cholesterol, and this could partly have contributed to its lipid-lowering effect.

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**Table 3. Effects of Sea Cucumber on the Hepatic Lipid Concentrations in Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/g)</th>
<th>TG (mg/g)</th>
<th>Phospholipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.06 ± 0.12</td>
<td>10.55 ± 0.52</td>
<td>32.8 ± 1.34</td>
</tr>
<tr>
<td>Whole sea cucumber</td>
<td>2.94 ± 0.09</td>
<td>8.53 ± 0.54*</td>
<td>34.2 ± 0.96</td>
</tr>
<tr>
<td>Saponins</td>
<td>2.78 ± 0.11</td>
<td>8.57 ± 0.61*</td>
<td>33.8 ± 1.10</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>3.03 ± 0.10</td>
<td>9.63 ± 0.87</td>
<td>34.1 ± 1.18</td>
</tr>
<tr>
<td>Collagen peptides</td>
<td>3.72 ± 0.15*</td>
<td>9.14 ± 0.75</td>
<td>32.9 ± 1.07</td>
</tr>
<tr>
<td>Dregs</td>
<td>3.07 ± 0.09</td>
<td>9.11 ± 0.72</td>
<td>32.5 ± 1.51</td>
</tr>
<tr>
<td>Non-saponin residues</td>
<td>3.43 ± 0.13*</td>
<td>9.43 ± 0.83</td>
<td>33.2 ± 1.62</td>
</tr>
</tbody>
</table>

Male rats were fed on a diet with or without sea cucumber for 28 d. Values are presented as the mean ± SEM (n = 6). * p < 0.05; ** p < 0.01 vs. the control.

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**Table 4. Effects of Saponins on the Fecal Lipid Concentrations in Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipid (g/d)</th>
<th>Neutral sterol (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.27 ± 0.06</td>
<td>6.17 ± 1.09</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.31 ± 0.09</td>
<td>9.56 ± 1.74*</td>
</tr>
</tbody>
</table>

Male rats were fed on a diet with or without saponins of sea cucumber for 28 d. Values are presented as the mean ± SEM (n = 6). ** p < 0.01 vs. the control.
Effect of the saponins of sea cucumber on the pancreatic lipase activity in vitro

Pancreatic lipase is the most critical enzyme involved in the fat absorption process. It is well known that dietary fat cannot be directly absorbed from the intestines unless it is hydrolyzed by pancreatic lipase.\textsuperscript{23} The saponins of sea cucumber were proved in this study to suppress and delay lipid absorption in rats. We based a lipase inhibitory activity assay on these findings to examine the potential effect of saponins.

Figure 3 shows that saponins inhibited the pancreatic lipase activity by using an emulsified triolein system with lecithin. Saponins also exerted a dose-dependent inhibitory effect. The relative activity of 95% of the control value was measured with a saponin concentration of 2.5 mg/mL in the pancreatic lipase assay.

Substantial evidence has shown that saponins extracted from plants such as tea saponins,\textsuperscript{8} ginseng saponins,\textsuperscript{9} and Platycodi radix saponins\textsuperscript{10} could prevent obesity or hyperlipidemia mainly through their pancreatic lipase inhibitory action. The saponins of sea cucumber have a unique structure different from plant saponins. Most of the sea cucumber saponins can be sulfated at the level of the sole xylosem, whereas plant saponins cannot be sulfated. Despite the different structure, a significant inhibitory effect on pancreatic lipase activity by the saponins of sea cucumber was also apparent in vitro. This effect on the pancreatic lipase inhibitory was related to the reduced digestion and absorption of dietary fat, and could be an important mechanism for its hypolipidemic and weight-control effects.

This is the first report comparing the lipid-lowering effect of different components in sea cucumber. The results indicate that saponin was the most effective substance in sea cucumber for its hypolipidemic effect. The underlying mechanism may be related to its effect on pancreatic lipase inhibitory. More studies are still needed to explore the potential effect and details of the mechanism of saponins. The results of this study indicate that the saponins of sea cucumber may be a promising dietary supplement for preventing obesity and hyperlipidemia.

Acknowledgments

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