Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease worldwide and affects 20% to 30% of the general population. The prevalence of NAFLD has grown proportionally with the rise in unfavorable food intake, patterns, sedentary lifestyle, obesity, and metabolic syndrome. NAFLD is a condition ranging from benign hepatic lipid accumulation to steatosis combined with inflammation. NAFLD can progress to non-alcoholic steatohepatitis (NASH), characterized by inflammation, apoptosis, and ballooning degeneration. NAFLD carries a 20%-50% risk for progressive fibrosis, 30% risk for hepatic cirrhosis, and 5% risk for hepatic carcinoma. NAFLD may be considered the hepatic event in the metabolic syndrome and is therefore linked with common metabolic syndrome risk factors such as obesity, insulin resistance, hypertension, and dyslipidemia. The progression of NAFLD is also partly linked to unhealthy dietary pattern. Therefore, dietary therapy is important and could be considered as the first choice treatment or at least as important as medical therapy.

Oysters (Crassostrea gigas) contain many nutrients and are widely consumed in Japan. The main components of oyster-extract include zinc, glycogen, and taurine, and oyster-extract powder is currently being marketed as a supplement. The nutritional benefits of oyster-extract for health maintenance and lifestyle-related diseases have been reported. Oysters contain many nutrients and are widely consumed in Japan. The main components of oyster-extract include zinc, glycogen, and taurine, and oyster-extract powder is currently being marketed as a supplement. The nutritional benefits of oyster-extract for health maintenance and lifestyle-related diseases have been reported.
the hepatic lipid accumulation in rats fed high-fat diet. The aim of this study was to investigate the effect of dietary oyster-extract on serum and liver lipid contents in rats fed high-fat diet and examine the mechanisms underlying these effects.

Materials and Methods

1. Materials

Oyster-extract was obtained from Japan Clinic Co. Lt. (Kyoto, Japan). Casein, dextrinized cornstarch, cornstarch, sucrose, cellulose, American Institute of Nutrition (AIN)-93G mineral mixture, and AIN-93 vitamin mixture were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). All other reagents were purchased from commercial sources and were reagent grade.

2. Animals care and experimental diets

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai University and followed the “Guide for the Care and Use of Experimental Animals” issued by the Prime Minister’s Office of Japan. Male 4-week-old Wistar rats obtained from Japan SLC, Inc. (Shizuoka, Japan) were kept in an air-conditioned room (temperature, 21-22°C; humidity, 55-65%; lights on, 08:00-20:00), with free access to tap water and feed. Twelve rats were divided into the following 2 dietary groups of 6 rats each: control diet (AIN-93G modified high-fat diet) and oyster-extract diet (OE diet). Table 1 presents the composition of the experimental diets.

Table 1 Composition of the experimental diets

<table>
<thead>
<tr>
<th>Groups</th>
<th>control</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrinized corn starch g/kg</td>
<td>92.1</td>
<td>92.1</td>
</tr>
<tr>
<td>Corn starch</td>
<td>277.386</td>
<td>241.286</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellulose</td>
<td>230</td>
<td>216.8</td>
</tr>
<tr>
<td>Casein</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>AIN-93G mineral mixture</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-93 vitamin mixture</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Lard</td>
<td>130</td>
<td>129.3</td>
</tr>
<tr>
<td>Oyster-extract</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>

*Oyster-extract provided by Japan Clinic Co., Ltd. (Kyoto, Japan). The composition of oyster-extract was as follows (% w/w): carbohydrate, 61.0; protein, 22.4; fat, 1.2; moisture, 3.5; and ash, 11.9.

3. Analysis of lipid components

Serum trilacylglycerol (TAG), cholesterol, phospholipid (PL), high-density lipoprotein (HDL)-cholesterol, and non-HDL-cholesterol contents were determined using a commercial service (Japan Medical Laboratory, Osaka, Japan).

Food consumption and body weight were recorded daily. After feeding for 28 days, rats were weighed and sacrificed under isoflurane (Intervet K.K.; Osaka, Japan) anesthesia for 09:00-11:00. Blood was collected from the abdominal aorta without the use of anti-coagulant, and serum was obtained by centrifugation at 2,000×g for 15 min. The liver and abdominal white adipose tissue (WAT) from the epididymis, mesentery, perinephria, and retroperitoneum were removed rapidly, then weighed, rinsed with saline, and frozen in liquid nitrogen, followed by storage at −80°C until analysis. Feces were collected from each group every 24 hours for 7 days before being sacrificed, dried to a constant weight, and ground to a fine powder using a conventional mill. Rats were not deprived of food prior to being sacrificed because food deprivation leads to a significant down regulation of the genes involved in fatty acid synthesis and cholesterol metabolism.

3. Analysis of lipid components

Serum trilacylglycerol (TAG), cholesterol, phospholipid (PL), high-density lipoprotein (HDL)-cholesterol, and non-HDL-cholesterol contents were determined using a commercial service (Japan Medical Laboratory, Osaka, Japan).

Total liver lipids were extracted using the chloroform/methanol/water method as described previously. Each total lipid sample was dilute to 10 mL with 2-propanol, and the TAG content was determined using an enzymatic assay kit (Triglyceride-E-Test Wako, Wako Pure Chemical Industries; Osaka, Japan). The cholesterol content in liver was analyzed using a gas–liquid chromatograph (GC) system (GC-14B; Shimadzu Co.) with an SE-30 column (Shinwa Chemical Industries, Kyoto, Japan) and 5α-cholestan-3-yl as an internal standard. The phospholipid contents were determined by phosphorus analysis.

Fecal neutral steroids content was determined by GC as described above. The acidic steroids content in feces was measured using an enzymatic assay commercial kit (Total Bile Acid Test Kit; Wako Pure Chemical Industries Ltd.) according to the manufacturer’s method.

4. Analysis of mRNA expression level

Total RNA was extracted from liver using TRIzol® RNA Isolation Reagents (Thermo Fisher Scientific K.K., Kanagawa, Japan). Then, cDNA was synthesized from total RNA using a GoScript™ Reverse Transcription System (Promega KK., Tokyo, Japan). Real-time quantitative PCR analysis was performed with an automated sequence detection system (Thermal Cycler Dice® Real Time System).
Single; Takara Bio Inc., Shiga, Japan) using GoTaq® qPCR Master mix (Promega KK.). The primer sequences used for the detection of the ATP-binding cassette A1 (ABC) A1, ABCG5, ABCG8, acyl-coenzyme A cholesterol acyltransferase-1 (ACAT-1), cholesterol 7α-hydroxylase (CYP7A1), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), low-density lipoprotein receptor (LDLR), scavenger receptor class B type I (SR-BI), sterol regulatory element binding factor (SREBF)-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were as follows: forward: 5'-CCCGGCCGAGTAGAAAGG-3' and reverse: 5'-AGGGCGATGCAAACAAAGAC-3'; for ABCA1, forward: 5'-ATGGCCTGTACCAGAAGTGG-3' and reverse: 5'-GGATACAAGCCCAGAGTCCA-3'; for ABCG5, forward: 5'-TTCTGCTGCAACGCTCTCTA-3' and reverse: 5'-GGAGGAACGACATCTTGGAA-3'; for ABCG8, forward: 5'-AAGTACGCCATCGGCTCTTA-3' and reverse: 5'-TCACCACCCAGTCTGGTATA-3'; for ACAT-1, forward: 5'-CCCAGACCCTTTGACTTTCA-3' and reverse: 5'-GATCCGAAGGGCATGTAGAA-3'; for CYP7A1, forward: 5'-AGCATAACAGCCAAGCAGCA-3' and reverse: 5'-ACCGCCATGAGGTACGTAAG-3'; for HMGR, forward: 5'-ACCGCCATGAGGTACGTAAG-3' and reverse: 5'-CGGCGCTGTAGATCTTTCTC-3'; for LDLR, forward: 5'-AAGAAGGCCTCTTACTTCTC-3' and reverse: 5'-GTCTAGCCCCACGTTATGTT-3'; for SRB1, forward: 5'-CACCTGTGGAGCAGTCTCAA-3' and reverse: 5'-TGCCAGAGTGTTGTCCCTCAG-3'; for SREBF-2, forward: 5'-ATGACTCTTACCACGCGCAAG-3' and reverse: 5'-TACTCACACACAGCATCACC-3'; for GAPDH, which were designed using Primer3Plus (http://primer3plus.com/). Results were quantified using a comparative method and were expressed as a relative level after normalization to the GAPDH expression level.

5. Statistical analysis

Data represent means and standard error of mean (SEM). The statistical significance of differences was evaluated using Student’s t-test and means were considered significantly different at p < 0.05 and p < 0.01. Analyses were performed using GraphPad Prism6 software (GraphPad Software, California, USA).

Results and Discussion

Oyster-extract has been reported the various beneficial effects7, 8). Our previous studies suggested that dietary oyster-extract recovered proximal tubular epithelial cell function in p-aminophenol-induced nephrotoxicity and suppressed of the initiator action of the carcinogen in animal experiment9, 10). The present study examined the effects of dietary oyster-extract on hepatic cholesterol metabolism in normal rats fed high-fat diet.

Table 2 shows the growth parameters, organ weights,
and serum lipid contents in rats fed OE diet. The growth parameters including the initial body weight, final body weight, body weight gain, food intake, water intake, and food efficiency were not found to be significantly different between the experimental groups. There were also no significant differences in relative liver, epididymal, mesenteric, perirenal, and retroperitoneal WAT weights between the groups. The serum cholesterol, PL, and HDL-cholesterol contents in the rats fed OE diet tended to be higher, but not significantly, than that in the rats fed control diet \((p = 0.15, 0.14, \text{and } 0.15, \text{respectively})\). Previous study reported that basal diet contained taurine, supplemented at level of 0.5% (w/w), enhanced the serum HDL-cholesterol content in normal rats\(^{16}\). In the OE diet, taurine was contained about 0.3% (w/w) because oyster-extract contained 5.6% (w/w) taurine\(^{17}\). Therefore, the increasing tendency of HDL-cholesterol content in the OE group might be caused by the taurine. No significant differences were observed in the serum TAG and non-HDL-cholesterol contents between the two groups. Fig. 1 shows the hepatic TAG, cholesterol, and PL contents in rats fed OE diet. The hepatic TAG and PL contents were not found to be significantly different between the experimental groups. Interestingly, OE diet significantly decreased the hepatic cholesterol content compared with control diet. Dietary oyster-extract could decrease the hepatic cholesterol content in rats fed high-fat diet. Two main mechanisms have been proposed to explain the decreases in cholesterol levels in the serum and liver that are associated with dietary food components. One is an increase in excretion of steroids, including neutral and acidic steroids; the other is a change in cholesterol metabolism in the hepatic tissue, which is maintained through a balance of excretion, biosynthesis, storage, catabolism, and uptake of steroids. To test the first mechanism, we measured the fecal neutral and acidic excretions in rats fed OE diet. Fig. 2 shows the fecal neutral and acidic steroids contents in rats fed OE diet. The fecal neutral and acidic steroids contents in rats fed OE diet. The fecal neutral and acidic steroids contents in hepatic TAG, cholesterol, and PL contents in rats fed OE diet. The hepatic TAG and PL contents were not found to be significantly different between the experimental groups. Interestingly, OE diet significantly decreased the hepatic cholesterol content compared with control diet. Dietary oyster-extract could decrease the hepatic cholesterol content in rats fed high-fat diet. Two main mechanisms have been proposed to explain the decreases in cholesterol levels in the serum and liver that are associated with dietary food components. One is an increase in excretion of steroids, including neutral and acidic steroids; the other is a change in cholesterol metabolism in the hepatic tissue, which is maintained through a balance of excretion, biosynthesis, storage, catabolism, and uptake of steroids. To test the first mechanism, we measured the fecal neutral and acidic excretions in rats fed OE diet. Fig. 2 shows the fecal neutral and acidic steroids contents in rats fed OE diet. The fecal neutral and acidic steroids contents in rats fed OE diet. The fecal neutral and acidic steroids contents in rats fed OE diet.
tents in the OE group were significantly higher than that in the control diet. Previous study showed that diet containing 3% (w/w) taurine decrease the serum and liver cholesterol contents in part due to enhance the fecal bile acid excretion\(^\text{18}\). Taurine in the OE diet might affect the fecal steroids excretion, however, it was possible to consider that a components other than taurine enhances the fecal steroids excretions because taurine was contained about 0.3% (w/w) in OE diet. Further investigation is required to clarify which component in the oyster-extract is enhanced the fecal steroids excretions. Consequently, it has been suggested that the decreases in hepatic cholesterol in rats fed OE diet could be attributable to the suppression of neutral and acidic steroids absorption in intestine through the enhancement of fecal neutral and acidic steroids excretions.

To test the second proposed mechanism, we analyzed hepatic mRNA expression levels of genes encoding proteins involved in cholesterol metabolism. Fig. 3 shows the hepatic mRNA expression levels of genes related to cholesterol metabolism in rats fed OE diet. ABCA1 is a transporter involved in the production of HDL. CYP7A1 is a rate-limiting enzyme involved in the conversion of cholesterol to \(7\alpha\)-hydroxylated bile acid, ABCG5 and ABCD8 are heterodimer forms play major roles in the excretion of cholesterol into bile, SR-B1 is responsible for the selective uptake of HDL, and LDLR is required for LDL uptake into cells\(^\text{19}\). There were no differences in the expression levels of ABCA1, ABCG5, ABCG8, CYP7A1, SR-B1, and LDLR between the groups. HMGCR is a rate-limiting enzyme for cholesterol synthesis and is regulated via a negative feedback mechanism mediated by steroids\(^\text{20}\). The OE diet significantly reduced the hepatic HMGCR expression level compared with the control diet. The expression level of ACAT-1, which catalyzes the storage of excess cholesterol in cells, was significantly lower in rats fed the OE diet than in rats fed the control diet, suggesting that the ACAT-1 expression level was affected by the reduction of hepatic cholesterol content in the OE group. These results suggested that the reduction of hepatic cholesterol contents in partly due to a reduction in cholesterol synthesis and accumulation as a results of the suppression of HMGCR and ACAT-1 mRNA expression levels.

The maintenance of cholesterol homeostasis is also regulated by transcription factors and nuclear receptors. For example, sterol regulatory element binding protein (SREBP)-2 mainly activates the transcription of cholesterol synthesis-related genes, such as HMGCR and LDLR\(^\text{21}\). In this study, the OE diet significantly decreased the SREBF-2 expression level compared with the control diet (Fig. 3). This result suggests that oyster-extract decreases HMGCR expression level through the suppression of the SREBF-2 pathway.

The major components of the oyster-extract were minerals, carbohydrates, protein, and amino acids\(^\text{7-10}\). Although taurine was a convincing component as a bioactive component, it was not determined which components of the oyster-extract affected the cholesterol metabolism in rats. Therefore, future studies will determine which components of the oyster-extract are responsible for the improvement of cholesterol metabolism.

Conclusions

Dietary oyster-extract led to decreased hepatic cholesterol content through the enhancement of fecal steroids excretions and the suppression of HMGCR and ACAT-1 expression levels due to the suppression of the SREBF-2 pathway. Therefore, the oyster-extract could be prevented the hepatic cholesterol accumulation under high-fat diet conditions.

Acknowledgements

We would like to thank Kazumasa Miyauchi of Kansai University for him help with animal care and lipid analyses.

References

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