Effects of Dietary Fish Oil and Apple Polyphenol on the Concentration Serum Lipids and Excretion of Fecal Bile Acids in Rats

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Summary We studied the effects of fish oil and apple polyphenol combined with a high cholesterol diet in rats, and assessed serum and liver lipids concentrations, serum oxidative stress and fecal bile acid excretion. Young male rats were fed a diet containing the control (Control), apple polyphenol (AP), fish oil (FO) or fish oil + apple polyphenol (FO + AP) for 4 wk. The control diet contained a lard component. Posterior abdominal wall fat and testicle peripheral fat weights decreased in the FO + AP group compared to the AP group. The concentration of total cholesterol in the serum and liver decreased in the FO group and the FO + AP group compared to the Control and the AP groups. The concentration of adiponectin and biological antioxidant potential in the serum increased in the FO group compared to the other groups. The diacron-reactive oxygen metabolites in serum decreased in the FO group and the FO + AP group compared to the Control and the AP groups. The bile acid excretion in feces increased in the AP group, the FO group and the FO + AP group compared to the Control group. These results suggested that the combination of fish oil and apple polyphenol in the diet improved serum and liver lipids, which should assist in the prevention and improvement of metabolic syndrome.

Key Words fish oil, apple pholyphenol, fecal bile acids, rat

In recent years, metabolic syndrome has become a significant public health issue. Visceral fat type obesity is also associated with overlapping risk factors for cerebral and myocardial infarction (1–3). Furthermore, lifestyle-related diseases are a risk factor for arteriosclerosis, and are known to increase oxidative stress (4). Consequently, there has been increased attention on functional foods in the prevention and improvement of metabolic syndrome. Serum lipids such as n-3 fatty acids are known to have a beneficial effect, and can help to prevent of diseases associated with metabolic syndrome and the vascular system (5–10). Furthermore, it has been noted that polyphenol compounds can be used for specified health use, as food components. Among the polyphenol compounds, catechins from green tea, cocoa polyphenols, and flavonoids have been shown to have antioxidant properties (11–13). Coffee polyphenols have also been shown to be involved in the regulation of lipid metabolism (14), while apple polyphenol and naringenin have a cholesterol-lowering effect (15, 16). Black soybean seed coat polyphenols promote energy consumption due to heat production; therefore it is possible that these substances can prevent obesity and hyperglycemia (17). In particular, the physiological function of procyanidins contained in apple polyphenol has attracted significant attention as they have a strong inhibitory lipase activity in the intestinal tract (18). By the addition of apple polyphenol to a diet, lowered serum lipid (15, 19), and visceral fat (20–22) levels have been observed. Such food components have been reported to have beneficial utility in lifestyle-related diseases. Fish oil is composed of polyunsaturated fatty acids, which are easily oxidized in nature, and the process of lipid peroxidation occurs quickly. In contrast, apple polyphenols are potent antioxidants; thus it is conceivable that ingestion of apple polyphenols could control fish oil lipid peroxidation to some extent.

In this study, we decided to study the effect of fish oil and apple polyphenol supplementation on serum lipid concentrations, oxidative stress and fecal bile acid excretion.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats aged 4 wk were purchased from CLEA Japan, Inc. (Shizuoka, Japan). The animals were housed in cages in a ventilated animal room with controlled temperature (23 ± 2 °C) and relative humidity (55 ± 5%) and exposed to a cycle of 12 h of light (7:00 to 19:00). All animal procedures were in accordance with the guidelines of the Management of Laboratory Animals in Chiba Prefectural University of Health Sciences. The animal experiments were approved by the Institutional Animal Care and Use Committee (2015-A002).

Diets. dl-Methionine, choline bitartrate, cholesterol and sodium cholate were manufactured by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Casein, α-cornstarch, vitamin mix, mineral mixture and cellulose

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Table 1. Fatty acid composition of sample lipids used in the experiment (%).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Lard</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>nd</td>
<td>1.2</td>
</tr>
<tr>
<td>14:0</td>
<td>2.1</td>
<td>6.4</td>
</tr>
<tr>
<td>16:0</td>
<td>23.9</td>
<td>6.4</td>
</tr>
<tr>
<td>16:1</td>
<td>4.5</td>
<td>10.0</td>
</tr>
<tr>
<td>18:0</td>
<td>9.7</td>
<td>3.6</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>44.7</td>
<td>15.9</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>11.7</td>
<td>1.2</td>
</tr>
<tr>
<td>18:3 (n-3)</td>
<td>nd</td>
<td>0.9</td>
</tr>
<tr>
<td>18:4 (n-3)</td>
<td>tr</td>
<td>5.0</td>
</tr>
<tr>
<td>20:4 (n-6)</td>
<td>tr</td>
<td>0.7</td>
</tr>
<tr>
<td>20:5 (n-3)</td>
<td>tr</td>
<td>27.2</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>nd</td>
<td>1.9</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
<td>tr</td>
<td>10.5</td>
</tr>
<tr>
<td>Others</td>
<td>3.4</td>
<td>9.1</td>
</tr>
</tbody>
</table>

nd: not detected, tr: trace.

Table 2. Composition of the experimental diets (%).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AP</th>
<th>FO</th>
<th>FO + AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>AIN-93 vitamin mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>AIN-93 mineral mixture</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sucrose</td>
<td>44.25</td>
<td>42.25</td>
<td>44.25</td>
<td>42.25</td>
</tr>
<tr>
<td>Apple extract powder</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lard</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

AP: apple polyphenol, FO: fish oil.

Table 1. Composition of the experimental diets (%).

powder were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Apple polyphenols in apple extract powder were procured from Marine Bio Co., Ltd. (Tokyo, Japan). Apple polyphenols in apple extract powder were adjusted to 1.5%. Two grams of apple extract powder was added to 100 g of feed, which was approximately 1% of the regimen used in a previous study by Osada et al. (15). In other words, the control lard group, apple polyphenol (apple polyphenol: AP) group, fish oil (FO) group and fish oil and apple polyphenol (FO + AP) group comprised the 4 groups. Six rats per group were fed each diet. Each of the experimental diet administration periods was lasted 4 wk. Experimental diets and drinking water were provided ad libitum.

Animal treatment method. After the rats had fasted for 16 h on the last day of the experimental period, they were sacrificed under somnopentyl anesthesia, before collecting blood from the right ventricle of the heart. We immediately removed the liver, posterior abdominal wall fat, and the testicle peripheral fat, and weighed all of these. After exsanguination, we flushed the liver by press-fitting the portal vein with 0.9% saline. The blood was allowed to stand for 1 h and then centrifuged for 15 min at 3,500 rpm. The measurement of serum concentrations of various factors occurred after centrifugation.

Measurement methods. Serum total cholesterol was measured by the cholesterol oxidase-DAS method using a cholesterol E-Test (Wako Pure Chemical Industries, Ltd.), triglycerides were measured using the GPO-DAS method with a triglyceride E-Test (Wako), and free fatty acids were measured using the cholesterol oxidase-DAS method via a free cholesterol E-Test (Wako). Adiponectin was measured using a rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). After extracting the liver lipids with a mixture of chloroform-methanol (CM) (2 : 1, v/v), and drying them under reduced pressure, measurement of total cholesterol and triglycerides was performed using an enzymatic method kit. Oxidative stress in vivo was measured by assessing the degree of oxidation/reduction in the serum, using the Free Radical Electroactive Evaluator from Wismerll Ltd. The d-ROMs test is used to determine the amount of hydroperoxide, which is a metabolic product of active oxygen, and free radicals were measured by assessment of chromogen in the colored solution as follows:

R-OH + Fe²⁺ → R-O⁻ + Fe³⁺ + OH⁻
R-O⁻ + A-NH₂ → R-O⁻ + R-O⁻ + [A-NH₂]²⁺
R-OH + Fe³⁺ + H⁺ → R-O⁻ + Fe²⁺ + H₂O
R-O⁻ + A-NH₂ → R-O⁻ + [A-NH₂]²⁺

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R-O⁻ + A-NH₂ → R-O⁻ + [A-NH₂]²⁺

The measurement methods involve determining the concentration of antioxidants as agents that can reduce iron from the ferric to ferrous form. The addition to the sample is reduced to ferrous iron by the action of antioxidants in the sample, whereby it is decolorized. Measuring the color change was evaluated by using the reducing power of the sample.

FeCl₃ + AT → [FeCl₂-AT]
FeCl₂-AT + BP(e⁻) → FeCl₂ + AT + BP
Feces were collected 3 d before the end of the experimental period, lyophilized and weighed. For fecal bile acid estimation, a dry powder of 10 mg was suspended in 0.2 mL 90% ethanol and incubated for 1 h at 65˚C. After centrifugation, the solvent was evaporated to dryness. The combined washings of the supernatant and precipitate formed the residue, which was dissolved in 0.5 mL 90% ethanol and this comprised the measurement sample. Total bile acids were measured using an enzymatic colorimetric method utilizing a total bile acids test kit (Wako).

Statistical analysis. All results were expressed as the mean±SE, and statistical significance was determined by a one-way analysis of variance using SPSS for Windows, version 20 (Nippon IBM Ltd., Tokyo, Japan). When the F-test was significant, comparisons between the groups were done using Tukey’s multiple range test. The significance level was set at p, 0.05.

RESULTS

Physical parameters of the rats

Final body weight, body weight gain, total food intake and liver weight showed no difference among the groups (Table 3). Posterior abdominal wall fat weight was significantly (p<0.05) lower in the FO+AP group than in the AP group. Testicle peripheral fat weight was significantly (p<0.05) lower in the FO+AP group than in both the Control and the AP groups (Fig. 1).

Serum and liver lipid concentration

The concentration of total cholesterol in serum and liver was significantly (p<0.01) lower in the FO group and the FO+AP group than in the Control and the AP groups. There was no difference among the groups with respect to the concentration of triglycerides in serum or liver. The concentration of serum triglyceride had a tendency to decrease in the FO+AP group compared with the AP group. The concentration of serum free fatty acid was significantly (p<0.01) lower in the FO group and the FO+AP group than in the Control group. The concentration of serum adiponectin was significantly (p<0.01) higher in the FO group than in the Control group, AP group and FO+AP group. The concentration of serum adiponectin in the AP group was significantly (p<0.05) lower than in the other groups (Fig. 2).

Oxidative stress level and antioxidant potential

The BAP value was significantly (p<0.01) higher in the FO group than in the Control group, AP group and FO+AP group. The BAP value in the AP group was significantly (p<0.05) higher than in the Control group. The d-ROMs value was significantly (p<0.01) lower in the FO group and FO+AP group than in the Control group and the AP group (Fig. 3).

Feces weight and fecal bile acid concentration

Feces weight was significantly (p<0.01) higher in the AP group and the FO+AP group than in the Control group and the FO group. The concentration of fecal bile acid was significantly (p<0.05) lower in the Control group than in the other groups (Fig. 4).

DISCUSSION

As there were no differences in the final animal weight, body weight gain, total food intake or liver weight among the groups, the overall growth of all animals was considered to be the same. The posterior
abdominal wall fat weight and testicle peripheral fat weight were significantly lower in the FO+AP group than in the AP group. The testicle peripheral fat weight was also significantly lower in the FO+AP group than in the Control group. Kim et al. (23) have reported a mechanism whereby body fat is not burned upon fish oil intake in mice. Furthermore, Ohta et al. (24) have reported that fat accumulation is inhibited when a high fat diet supplemented with 1% apple polyphenol is ingested by rats. With respect to lipid accumulation weight, we did not observe a combined effect of fish oil and apple polyphenol. However, there was a trend for reduced accumulation of lipid when the combined fish oil and apple polyphenol diet was compared with the intake of fish oil alone.

The concentrations of total cholesterol in serum and
Fish and Apple Polyphenol on Excretion of Fecal Bile Acids in Rats

The concentration of serum adiponectin increased in the FO group compared with the other groups but decreased in the AP group compared with the other groups. In the AP group, there was no increase in serum adiponectin, which was most likely due to the fatty acid composition of lard used in the experimental diet. In the FO+AP group, we observed a reduction in fat accumulation. However, it was not accompanied by a concomitant increase in serum adiponectin. We postulate that this was due to the 1.5% apple polyphenol content. The content of this product was higher than usual, but we did not observe the original physiological effects. Rossi et al. (26) showed comparable results where they reported increased in the serum adiponectin concentration due to fish oil intake. Apple polyphenol intake resulted in a different effect from the study by Azuma et al. (27), where there was a tendency for the concentration of serum plasma adiponectin to increase. The concentration of serum adiponectin was found to be elevated in the fish oil group compared with the apple polyphenol group.

The BAP value was increased in the FO group compared with the other groups. The d-ROMs value was decreased in the FO group and the FO+AP group compared with the Control group and the AP group. When fish oil is ingested, this results in a high BAP value coupled with a low d-ROMs value. Consequently, this results in reduced oxidative stress. During simultaneous intake of fish oil and apple polyphenol, the BAP value decreased. However, the same effect was not observed for fish oil uptake. Furukawa et al. (28) have reported that overproduction of active oxygen species (ROS) from the adipose tissue can enhance oxidative stress throughout the body. Williamson et al. (13) have reported that flavonoids have an important antioxidant activity effect on ROS. In addition, Murdolo et al. (29) have reported that oxidative stress can inhibit the differentiation of precursor fat cells and abnormal secretion of adipocytokines and lipocalin, which can lead to insulin resistance. Reduction of fat accumulation through changes in eating habits is considered to alleviate such oxidative stress.

Feces weight was increased in the AP group and the FO+AP group compared with the Control group and the FO group. It is conceivable that the apple polyphenol has an excretion-promoting effect. After absorption inhibition of lipase (18), we postulated that the lipases are being excreted intact after ingestion of the apple polyphenol. The concentration of fecal bile acid was increased in the other groups compared with the Control group. We have clarified that it is possible to suppress the absorption of excess cholesterol by the simultaneous ingestion of apple polyphenol. The unique physiological properties of fish oil and apple polyphenol are thought to be responsible for this action. Osada et al. (15) have reported that the absorption of lipids is negated, resulting in excretion in the feces due to apple polyphenol intake. Apples contain plant sterols, glycolipids and cerebrosides, resulting in absorption inhibition of cholesterol (30, 31). Furthermore, green tea extract (32) and oolong tea polyphenols (33) are known to have lipase inhibitory activity. Shishikura et al. (34) have reported the possibility that lipid absorption is suppressed by minimal ingestion of polyphenols. Sugiyama et al. (18) also suggest that the small intestine inhibits absorption, which leads to the increased production of fecal bile acid. Garg et al. (35) also reported the same effect in those who were taking fish oil compared with
lard. In this study, the increased excretion of bile acid into the feces is considered to be influenced by apple polyphenol. From these data, we speculate that rather than a synergistic effect of fish oil and apple polyphenol, lipid metabolism is being affected through an improvement in the in vivo oxidative stress due to fish oil. Furthermore, apple polyphenol appears to have an inhibitory effect on lipid absorption. The simultaneous ingestion of fish oil and apple polyphenol had an augmented suppression effect compared with individual ingestion of either dietary supplement.

In conclusion, the results of our study indicate that the simultaneous intake of fish oil and apple polyphenols in the diet can result in the prevention of metabolic syndrome. Such foods are expected to be of use in the improvement in a number of health conditions.

Acknowledgments

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REFERENCES


