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Molecular Medicine Reports, 3(3): 449-453

2010-05

http://hdl.handle.net/2115/45370

article

MMR3-3_449-453.pdf

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Effects of sea squirt (Halocynthia roretzi) lipids on white adipose tissue weight and blood glucose in diabetic/obese KK-A'y mice

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Received January 5, 2010; Accepted February 22, 2010

DOI: 10.3892/mmr_00000278

Abstract. Lipids extracted from Halocynthia roretzi contain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, as well as carotenoids. The aim of the present study was to evaluate the effect of H. roretzi lipids on white adipose tissue (WaT) weight and high blood glucose levels in diabetic/obese KK-A'y mice. H. roretzi lipids were fed to the diabetic/obese KK-A'y mice for 5 weeks. In the mice treated with the H. roretzi lipids compared to control mice, WaT weight was reduced, blood glucose levels and leptin mRNA expression in the epididymal WAT were significantly decreased, serum leptin levels also tended to decrease, and serum adiponectin levels tended to increase. These results demonstrate that H. roretzi lipids have beneficial health effects on diabetic/obese KK-A'y mice.

Introduction

Halocynthia roretzi (Maboya) is an edible marine invertebrate sea squirt with an orange-colored mantle covered by a hard tunic that is eaten in Japan and Korea. H. roretzi contains various functional components such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), carotenoids, taurine and plasmalogens (1-3). In a previous study, Kawasaki reported that hexane extracts from H. roretzi muscle decreased total cholesterol, triglycerides, phospholipids and NEFA in the serum of rats (4). We also reported that acetylene carotenoids such as alloxanthin and diatoxanthin contained in H. roretzi lipids suppress the secretion of proinflammatory cytokines such as interleukin-6 and interleukin-1β from macrophage-like RAW 264.7 cells stimulated by lipopolysaccharides (5). In addition, halocynthiaxanthin and fucoxanthinol isolated from H. roretzi have been reported to induce apoptosis in human leukemia and colon cancer cells (6).

Materials and methods

Materials. H. roretzi was obtained from Kamiiso Fisheries Cooperative Association (Hokkaido, Japan). Sucrose and L-cystine used in animal diets were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Choline bitartrate and tetra-butylhydroquinone were purchased from Sigma-aldrich® (St. Louis, MO, USA). Soybean oil was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other ingredients for the animal diets were obtained from Clea Japan, Inc. (Tokyo, Japan).

Extraction of H. roretzi lipids. The entire body of H. roretzi including the tunic was cut into small pieces, and crude lipids were extracted with ethanol for 4 days at room temperature under shade. The extraction was repeated twice. The pooled extracts were concentrated by evaporation, and water soluble components were removed from crude lipids using chloroform-methanol-water (10/5/3, v/v/v). H. roretzi lipids were obtained from the chloroform layer.

Animals. This animal experiment was approved by the Ethics Committee of Experimental Animal Care of Hokkaido University. KK-A'y diabetic/obese mice (4-week-old, male) were obtained from Clea Japan, Inc. The mice (n=6) were individually housed at 23±1°C and 45-60% humidity under a 12-h light/dark cycle. A diet prescribed by the American Institute of Nutrition (AIN-93G) (8) was used as the control diet. The composition of the control diet consisted of 10.0% soybean oil, 37.4% cornstarch, 12.4% dextrised cornstarch, 20.7% casein, 9.4% sucrose, 5.0% cellulose, 3.5% AIN-93G mineral mix, 1.0% AIN-93G vitamin mix, 0.3% L-cystine,

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Key words: Halocynthia roretzi, type 2 diabetes, hyperglycemia, blood glucose level, serum adipocytokine secretion
Results and Discussion

The yield of lipids obtained from the entire body of *H. roretzi* was 5.1% yield per dry weight. The lipid composition was 49.1% neutral lipids, 38.4% polar lipids containing carotenoids and 12.5% others. The fatty acid composition of *H. roretzi* lipids was mainly 11.4% palmitic acid, 27.2% EPA and 13.8% DHA, respectively. In addition, alloxanthin, halocynthia roretzi and diatoxanthin were detected in *H. roretzi* lipids using high performance liquid chromatography as previously reported (2,5).

To investigate the effects of *H. roretzi* lipids on WAT weight and blood glucose level, KK-*A* diabetic/obese mice were fed a diet containing 5% *H. roretzi* lipids + 5% soybean oil for 5 weeks. Control mice were fed a diet containing 10% soybean oil. No significant difference in food intake and body weight gain was noticed between the two groups during the experimental period (Table 1). However, the WAT weight to body weight ratio was significantly lower in the mice fed the diet containing 5% *H. roretzi* lipids compared to the control mice (Fig. 1). In addition, the 5% *H. roretzi* lipid diet significantly decreased serum LDL-cholesterol levels compared to the control (Fig. 2). Triglyceride, free fatty acids and total cholesterol levels did not differ between the two groups. Kawasaki reported that hexane extracts from *H. roretzi* decreased VLDL and LDL-cholesterol in Wistar rats (4). *H. roretzi* lipids extracted with ethanol had the same effect on the LDL-cholesterol of the diabetic/obese KK-*A* mice.

Blood glucose levels were also significantly lower in the mice fed the diet containing 5% *H. roretzi* lipids compared to the control mice after 33 days of feeding and 12 h of fasting (Fig. 3). Furthermore, serum insulin levels in mice fed the diet containing 5% *H. roretzi* lipids tended to decrease compared to the control mice (Fig. 3). To evaluate the insulin sensitivity of the KK-*A* mice fed the diet containing 5% *H. roretzi* lipids, we performed a glucose tolerance test. After administration of glucose (2 mg/g of body weight), the blood glucose concentrations of the mice fed the diet containing 5% *H. roretzi* lipids were significantly low at all time intervals as compared to those of the control mice, although the reduction in the blood glucose levels of the 5% *H. roretzi* lipid-fed mice was not significantly different from that of the control mice (Fig. 4). KK-*A* mice are known to have hyperglycemia, hyperinsulinemia and hyperleptinemia, and exhibit insulin resistance along with type 2 diabetes. Our results suggest that *H. roretzi* lipids are effective for preventing or improving hyperglycemia through the attenuation of WAT weight gain in KK-*A* mice.

### Table I. Effect of *H. roretzi* lipids on the growth parameters of KK-*A* mice.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th><em>H. roretzi</em> lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>37.3±1.2</td>
<td>38.8±1.0</td>
</tr>
<tr>
<td>Change in body weight (g)</td>
<td>10.3±0.8</td>
<td>11.4±0.7</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>3.9±0.4</td>
<td>4.4±0.2</td>
</tr>
</tbody>
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Values are expressed as the mean ± SE (n=6).

### Statistical analysis.

Data were expressed as the mean ± standard error (SE). Statistical significance was determined between the two groups using Welch's t-test. A significant difference was defined at P<0.05 or P<0.01.
Adiponectin up-regulates insulin signaling by activating PPARα and AMP kinase (9). In hypertrophied adipose tissue observed in obese mice, dysregulation of adipocytokine secretion occurs and induces insulin sensitivity. Adiponectin, in particular, is known to be reduced in obese states (10). In this study, the serum adiponectin and adiponectin mRNA expression levels in mesenteric WAT in the 5% H. roretzi lipid diet-fed mice tended to increase, although not significantly, as compared to the control mice (Figs. 5 and 6A). In addition, serum leptin levels tended to decrease and leptin mRNA
expression levels in epididymal WAT were significantly lower in mice fed the diet containing 5% *H. roretzi* lipids than in the control mice (Figs. 5 and 6B). Serum leptin is known to be correlated positively to fat mass (11). Therefore, the results in the 5% *H. roretzi* lipid group suggest that the serum leptin levels were dependent on the suppression of WAT accumulation in the KK-*A*′ mice.

The fatty acid composition of *H. roretzi* lipids was 27.2% EPA and 13.8% DHA. These n-3 polyunsaturated fatty acids have been reported to suppress fat accumulation in WAT and to decrease high blood glucose levels in genetically induced diabetic/obese mice (12) and high fat-induced obese mice (13). However, the EPA and DHA concentrations in the 5% *H. roretzi* lipid diet were 0.48 and 0.25%, which is lower than in previous reports (12,13). In addition, we did not observe a suppressive effect on WAT weight gain and blood glucose levels in KK-*A*′ mice fed a diet containing 7% fish.

Figure 4. Glucose tolerance test in KK-*A*′ mice fed a diet containing *H. roretzi* lipids. Mice were fasted for 4 h after 4 weeks of feeding and were then orally administered D-glucose solution (2 mg/g of body weight). After 0, 15, 30, 60, 90, 120 and 150 min of administration, the blood glucose levels were measured using the Glutest Neo Sensor. Blood glucose levels >600 mg/dl were calculated as 600 mg/dl, which was the maximum value of the Glutest Neo Sensor. Values are expressed as the mean ± SE (n=6). *P<0.05 vs. control. **P<0.01 vs. control.

Figure 5. Effects of *H. roretzi* lipids on serum adiponectin and leptin levels in KK-*A*′ mice after 5 weeks of feeding with the control or 5% *H. roretzi* lipid diet. Blood samples were collected after 12 h of fasting, and adipocytokine levels were measured by ELISA. Values are expressed as the mean ± SE (n=6).

A Mesenteric WAT

B Epididymal WAT

Figure 6. Effects of *H. roretzi* lipids on adiponectin and leptin mRNA expression levels in the mesenteric (A) and epididymal (B) WAT of KK-*A*′ mice after 5 weeks of feeding with the control or 5% *H. roretzi* lipid diet. Values are expressed as the mean ± SE (n=6). *P<0.01 vs. control.
oil (14). Therefore, the suppressive effects of 5% \( H. \) \( \text{roretzi} \) lipids on WAT weight gain and hyperglycemia in the KK-A\(^{y}\) mice depended not only on EPA and DHA, but also on other compounds. Furthermore, it is possible that n-3 PUFAs and other compounds had a combined effect. Thus, further investigation is required to clarify the mechanisms of \( H. \) \( \text{roretzi} \) lipids.

In conclusion, the present study indicates that \( H. \) \( \text{roretzi} \) lipids suppress fat accumulation in WAT and reduce high blood glucose levels in diabetic/obese KK-A\(^{y}\) mice. Furthermore, a decrease in serum LDL-cholesterol level was observed in KK-A\(^{y}\) mice fed a diet containing 5% \( H. \) \( \text{roretzi} \) lipids.

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