beneficial effects by intake of euphausia pacifica on high-fat diet-induced obesity

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obesity is a major health problem showing increased incidence in developed and developing countries. we examined the effect of euphausia pacifica (e. pacifica) (pacific krill) on high-fat diet (hfd)-induced obesity in c57bl/6 mice. no significant differences were observed in average food intake between the hfd and hfd with e. pacifica group, or the low-fat diet (lfd) and lfd with e. pacifica group for 18 weeks. the increased ratio of body weight in the hfd containing e. pacifica group was significantly reduced, being 10% lower than that with hfd group in the 18th week (hfd, 298.6±18.8% vs. hfd with e. pacifica, 267.8±16.2%; p<0.05), while the ratio for the lfd containing e. pacifica group was reduced by 4% compared with lfd group (lfd, 244.2±11.6% vs. lfd with e. pacifica, 234.1±18.0%). there were no effects of e. pacifica on total cholesterol levels in serum and liver, whereas the supplement of e. pacifica tended to decrease triglyceride levels in the hfd groups. the leptin level in serum was significantly decreased in the hfd group (p<0.01) by e. pacifica. the adipocyte area (1926±175 μm²) in the hfd containing e. pacifica group was significantly reduced by 20% (p<0.001) compared with the hfd group. these results suggested that e. pacifica supplementation in the diet is beneficial for the prevention of hfd-induced obesity.

key words euphausia pacifica; obesity prevention; leptin; triglyceride; high-fat diet

obesity is a major health problem,5 and its incidence has increased by the over-intake of calorie-rich diets and exercise-lacking lifestyles in developed and developing countries.5,6 the prevalence varies from 12 to 41%.4 metabolic syndrome is a cluster of metabolic abnormalities consisting essentially of obesity, especially abdominal obesity. furthermore, metabolic syndrome is a risk factor for type 2 diabetes,5 cardiovascular,6 dyslipidemia and hypertension.7,8 thus metabolic syndrome is a major health problem that must be resolved. although it is important to improve physical activity and dietary habits to reduce obesity, it is really difficult to reduce body weight. current anti-obesity drugs have safety issues,9,10 so a safe anti-obesity drug is strongly required. on the other hand, if some foods show an anti-obesity effect, it is considered that they should be non-toxic and safe for humans.

euphausia pacifica (e. pacifica) (pacific krill) (fig. 1) is the dominant euphausid species in the subarctic north pacific and also broadly distributes in far eastern sea area.13 it plays an important role in the marine food chain and many predators depend on this species for food.1,2,13 e. pacifica has been studied for morphology and ecology,12 whereas there are few reports on the physiological and pharmacological effects. e. pacifica contains mineral (4.0%), lipid (1.8%), protein (19.1%), vitamin (0.8%) and so on and is used in some rice crackers, pasta relish, rice and some noodles and seasonings in japan. however, most e. pacifica is not an effective use, because only feed for cultured fish. in our previous study, the water-soluble extract of e. pacifica prevented triglyceride accumulation in adipocytes by suppression of peroxisome proliferator-activated receptor gamma (pparγ) and ccaat enhancer binding protein alpha (cebpα) relating with adipogenesis regulation in vitro.14

in this study, the effect of e. pacifica on high-fat diet-induced obesity was examined in c57bl/6 mice in vivo, which are known as a good model of diet-induced obesity.15 furthermore, the effects of e. pacifica on total cholesterol and triglyceride levels in serum and liver, serum leptin level and adipocyte area as indications of obesity were investigated. the application of e. pacifica as a pharmaceutical agent and functional food for the improvement of obesity was clarified.

materials and methods

e. pacifica extract distilled water was added to e. pacifica (e. pacifica: water=3:1, w/w), then homogenized for 5 min by blender. the supernatant was collected after centrifugation of homogenate at 8000 rpm for 30 min, and was filtered by filter paper (5a, advantec, tokyo, japan). after this water-soluble extract was freeze-dried, it was crushed. the crushed powder was used in the experimental diets.

mice and diets male c57bl/6 mice (14–19 g), aged

fig. 1. picture of e. pacifica
4 weeks, were purchased from Charles River Laboratories (Tokyo, Japan). The animals were housed singly (a mouse per cage) in a temperature-controlled environment (23±1°C) with 12 h light/dark cycle. Animal experiments were approved by the institutional animal care and use committee at Iwate Medical University. The animals were maintained on a normal pellet diet for 3 d, then randomly divided into four groups (5 mice/group): low-fat diet (LFD), LFD with 1% *E. pacifica*, high-fat diet (HFD) and HFD with 1% *E. pacifica*. The LFD (D12450B: Rodent Diet with 10 kcal% Fat) and HFD (D12451: Rodent Diet with 45 kcal% Fat) were purchased from Research Diets, Inc. (New Brunswick, NJ, U.S.A.); their compositions are listed in Table 1. The animals were maintained on these diets for 18 weeks. Body weight and food intake were measured every three days. Feed was removed 24 h before sacrifice. Blood samples were collected from the heart in each mouse. Serum was separated from the blood by centrifugation (3000 rpm, 15 min) at 4°C. After centrifugation, the water layer was removed and then the chloroform layer was evaporated. Ten percent Tween 80 was added to this pellet and was suspended. Total cholesterol and triglyceride in the serum and liver suspension were measured by commercial kits (cholesterol; Total Cholesterol E-Test Wako, triglyceride; Triglyceride E-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum leptin was determined by commercial kit (ELISA Mouse Leptin kit, Morinaga Institute of Biochemical Science, Inc., Kanagawa, Japan).

### Histology

The gonadal fat depots were fixed in 10% formalin (Wako, Osaka, Japan) for 3 h and embedded in paraffin (Wako, Osaka, Japan). Paraffin embedded samples were sectioned at 6µm thickness with a microtome (Leica, Tokyo, Japan). Sections were subjected to standard hematoxylin and eosin staining. The adipocyte area was measured with ImageJ software (http://rsbweb.nih.gov/ij/).

### Statistical Analysis

All values are presented as mean±S.D. Each group was compared with the other group by ANOVA and Student–Newman–Keuls post hoc test.

### RESULTS

#### Change of Body Weight and Food Intake

The effects of *E. pacifica* on body weight gain are shown in Fig. 2. The ratio of body weight was significantly 115% higher in the HFD group compared to the LFD group at 57 d (*p*<0.05), and significantly 119–123% higher from 69 to 125 d (*p*<0.01) (Fig. 2A). Both LFD and HFD-induced weight gain were reduced by feeding with diets containing *E. pacifica*, in particular, body weight gain in the HFD containing *E. pacifica* group was significantly reduced. At the end point of the experimental period, the increased ratio of body weight in the HFD containing *E. pacifica* group was reduced by 10% compared with the HFD group (HFD, 298.6±18.8% vs. HFD with *E. pacifica*, 280.7±19.7%).

#### Measurement of Cholesterol, Triglyceride and Leptin

Hepatic lipids were extracted from the liver according to the method of Folch et al.¹⁶ In brief, the liver was homogenized in 10 volumes (w/v) of 10 mM phosphate buffer (pH 7.8). Each homogenate was mixed for 60 s with 5.0 mL of chloroform–methanol (2 : 1, v/v) and then was centrifuged (3000 rpm, 15 min) at 4°C. After centrifugation, the water layer was measured by ANOVA and post hoc test.

Table 1. Composition of Low-Fat and High-Fat Diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g% kcal</th>
<th>g% kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>19 20</td>
<td>24 20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>67 70</td>
<td>41 35</td>
</tr>
<tr>
<td>Fat</td>
<td>4 10</td>
<td>24 45</td>
</tr>
<tr>
<td>Total</td>
<td>90 100</td>
<td>89 100</td>
</tr>
<tr>
<td>kcal/g</td>
<td>3.8 4.7</td>
<td>3.8 4.7</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g kcal</th>
<th>g kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, 80 Mesh</td>
<td>200 800</td>
<td>200 800</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3 12</td>
<td>3 12</td>
</tr>
<tr>
<td>Corn starch</td>
<td>315 1260</td>
<td>72.8 291</td>
</tr>
<tr>
<td>Maltodextrin 10</td>
<td>35 140</td>
<td>100 400</td>
</tr>
<tr>
<td>Sucrose</td>
<td>350 1400</td>
<td>172.8 691</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50 0</td>
<td>50 0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>25 225</td>
<td>25 225</td>
</tr>
<tr>
<td>Lard</td>
<td>20 180</td>
<td>177.5 1598</td>
</tr>
<tr>
<td>Mineral Mix S10026</td>
<td>10 0</td>
<td>10 0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>13 0</td>
<td>13 0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.5 0</td>
<td>5.5 0</td>
</tr>
<tr>
<td>Potassium citrate, 1 H2O</td>
<td>16.5 0</td>
<td>16.5 0</td>
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<tr>
<td>Vitamin mix V10001</td>
<td>10 40</td>
<td>10 40</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2 0</td>
<td>2 0</td>
</tr>
<tr>
<td>FD&amp;C Yellow Dye #5</td>
<td>0.05 0</td>
<td>0 0</td>
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<tr>
<td>FD&amp;C Red Dye #40</td>
<td>0 0</td>
<td>0.05 0</td>
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</table>
267.8 ± 16.2%; p < 0.05), while the ratio in the LFD containing E. pacifica group was reduced by 4% of the LFD group level (LFD, 244.2 ± 11.6% vs. LFD with E. pacifica, 234.1 ± 18.0%) (Fig. 2B). In liver weight and the ratio to body weight, no significant differences were observed between the HFD and HFD with E. pacifica group, or the LFD and LFD with E. pacifica group. As shown in Fig. 3A, no significant differences were observed in average food intake between the HFD and HFD with E. pacifica group. Thus, it did not affect the cumulative energy intake in HFD and LFD groups (Fig. 3B).

Lipid Levels in Serum and Liver The effects of E. pacifica on total cholesterol and triglyceride in serum are shown in Fig. 4. The total cholesterol level in the HFD group was higher than that of the LFD group (Fig. 4A). There were no significant effects of combined E. pacifica on total cholesterol level in serum, while E. pacifica showed a tendency to decrease the triglyceride level in serum of the HFD group by 11% (Fig. 4B). There were no significant effects of E. pacifica on liver total cholesterol level (Fig. 5A). In contrast, the triglyceride level in the liver of the HFD group was significantly 2.3 times higher than that of the LFD group (LFD, 13.6 ± 2.2 mg/g of liver vs. HFD, 30.8 ± 9.9 mg/g of liver; p < 0.05), and combined E. pacifica decreased the triglyceride level in the liver of the HFD group by 21% (Fig. 5B).

Leptin in the Serum The effects of E. pacifica on the leptin in the serum are shown in Table 2. The serum leptin level in the HFD group was significantly 2.5 times higher than that in the LFD group (p < 0.001). This level in the HFD containing E. pacifica group was significantly reduced by 16% compared with the HFD group (p < 0.01), while the level in the combined E. pacifica group was reduced by 11% compared...
studies in which HFD induced higher increased body weight (10–15%), and it was indicated that feeding with HFD induced obesity in mice. In this study, feeding with *E. pacifica* significantly reduced the ratio of body weight in the HFD group (*p*<0.05), namely *E. pacifica* seemed to have anti-obesity effect, while *E. pacifica* did not significantly reduce the ratio in mice fed LFD, that is a normal diet. *E. pacifica* does not affect normal growth, but reduces the effects of HFD. In addition, the cumulative energy intake values between the HFD and the HFD with *E. pacifica* group were the same. Thus it is considered that the reduction in the increased ratio of body weight by combined *E. pacifica* was not due to a decrease of food intake, but was connected to absorption inhibition, metabolic promotion and so on. It was suggested that the feeding of *E. pacifica* is beneficial for the suppression of diet-induced obesity.

Cholesterol and triglyceride levels in the serum are known as indicators of obesity. Furthermore, it has been reported that nonalcoholic fatty liver disease (NAFLD) is strongly associated with metabolic syndrome. The pathogenesis of NAFLD is often explained by triglyceride deposition in hepatocytes mainly due to insulin resistance and liver inflammation and oxidative stress. Triglyceride accumulation in hepatocytes is the basic initiator of NAFLD. However, the hepatic triglyceride level has an impact on metabolic syndrome. The effects of *E. pacifica* on total cholesterol and triglyceride in serum and liver were examined. There were no effects of *E. pacifica* on total cholesterol level in the serum and liver, whereas *E. pacifica* decreased hepatic triglyceride levels in the HFD group. It was reported that anti-obesity drugs decreased the triglyceride level in the liver of an obese mouse HFD group by 19%, and in the serum by 17%. Combined *E. pacifica* showed the same suppression rate of triglyceride level in the liver and serum. Therefore, it was suggested that the decrease of liver triglyceride level by intake of *E. pacifica* may contribute to suppressing ratio of body weight.

Leptin is one of the hormones secreted from fat cells and inhibits food intake by stimulating the satiety center in the hypothalamus. Obesity promotes leptin secretion by increase in fat cells and produces hyperleptinemia, namely leptin resistance. Most obese people have leptin resistance and have high leptin levels in serum. Although it was reported that extraction of *E. pacifica* by ethanol is beneficial for the prevention of obesity due to secretomotory of leptin, it has not been confirmed that this extract induces suppression of body weight gain in vivo. In contrast, *E. pacifica* significantly decreased serum leptin level in the HFD group in this study. Therefore, this result indicated that the suppression of increased ratio of body weight by *E. pacifica* was not due to the secretomotory effect of leptin. The detailed mechanism for leptin should be examined in a future study.

Adipose tissue is the site of safe storage of fat and is indispensable for normal metabolic function. It was reported that the water-soluble extract of *E. pacifica* prevented triglyceride accumulation in adipocytes by suppression of two master regulators of adipocyte differentiation. Obesity is a major health problem and a cause of metabolic syndrome. We examined the effects of *E. pacifica* on HFD-induced obesity in C57BL/6 mice to investigate the potential of *E. pacifica* for the improvement of obesity.

It was revealed that the increased ratio of body weight in the HFD group was significantly 10% higher than that in the LFD group at 8 weeks. This result was supported by previous
extract of *E. pacifica* may contribute to diet-induced obesity.

In conclusion, we have demonstrated that intake of *E. pacifica* significantly reduced the increased ratio of body weight with decreased triglyceride levels in obese mice. Furthermore, the serum leptin level was decreased by combined *E. pacifica* in obese mice. These results suggested that feeding of *E. pacifica* is beneficial for the prevention of HFD-induced obesity. *E. pacifica* is mainly fished in the seacoast of north-eastern Japan which was greatly damaged by a massive earthquake (11th, March, 2011). We hope that this study will be helpful for the recovery of this area.

**Conflict of Interest**  The authors declare that they have no conflict of interest.

**REFERENCES**