Biosci. Biotechnol. Biochem., **71** (4), 893–899, 2007

Effects of Astaxanthin in Obese Mice Fed a High-Fat Diet

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Received September 25, 2006; Accepted January 29, 2007; Online Publication, April 7, 2007
[doi:10.1271/bbb.60521]

Astaxanthin is a natural antioxidant carotenoid that occurs in a wide variety of living organisms. We investigated the effects of astaxanthin supplementation in obese mice fed a high-fat diet. Astaxanthin inhibited the increases in body weight and weight of adipose tissue that result from feeding a high-fat diet. In addition, astaxanthin reduced liver weight, liver triglyceride, plasma triglyceride, and total cholesterol. These results suggest that astaxanthin might be of value in reducing the likelihood of obesity and metabolic syndrome in affluent societies.

Key words: astaxanthin; high-fat diet; obesity

Obesity is a chronic, stigmatized, and costly disease that is rarely curable and is increasing in prevalence throughout most of the world. It is an abnormal condition in which lipid accumulates in adipose tissue. Recently, it has become well known that obesity is caused by various environmental and genetic factors.[1] One of the main environmental factors causing obesity is consumption of a high-fat diet, which is common today.[2,3] Obesity is a risk factor for various diseases, including diabetes, hyperlipidemia, and hypertension. Therefore, it is very important to prevent obesity for a healthy life.

Astaxanthin, a red carotenoid pigment, is a biological antioxidant that occurs naturally in a wide variety of living organisms. It has many highly potent pharmacological effects, including antioxidant,[4–6] anti-tumor and anti-cancer,[7] anti-diabetic,[8] and anti-inflammation activities.[9,10]

The chronic effects of astaxanthin as an anti-obesity agent, However, have not been demonstrated. In the present study, we investigated the effect of astaxanthin in mice fed a high-fat diet.

Materials and Methods

Astaxanthin. The astaxanthin used in this study was AstaREAL 50 F, supplied by Fuji Chemical Industry Toyama, Japan.

Animals. Female ddY mice (SLC, Tokyo, Japan) were used. They were housed in standard cages (21.5 × 32 × 14 cm, five mice/cage) under controlled conditions of temperature (24 ± 1 °C), humidity (50 ± 2%), and lighting (lights on from 08:00 to 20:00).

Animal studies were performed according to the regulations of our laboratory in line with the 1980 guideline Notification No. 6 of the Prime Minister's Office of Japan.

Experiment 1: High-fat feeding test. Female ddY mice (4 weeks old) were used. After receiving a standard laboratory diet (Oriental Yeast Tokyo, Japan) and water *ad libitum* for 1 week, they were divided into five groups matched for body weight. One group, the normal diet group, the basic composition of the experimental diet was as follows (g/100 g food): beef tallow 4%, casein 14%, alpha-corn starch 15.5%, beta-corn starch 46.5692%, sugar 10%, cellulose 5%, vitamin mixture (AIN-93G) 1%, mineral mixture (AIN-93G) 3.5%, L-cystine 0.18%, choline hydrogen tartrate 0.25%, and *t*-butylhydroquinone 0.0008%. The other four groups were fed a high-fat diet or a high-fat diet plus astaxanthin. High-fat diets shared the following basic composition: beef tallow 40%, casein 14%, alpha-corn starch 15.5%, beta-corn starch 10.5692%, sugar 10%, cellulose 5%, vitamin mixture (AIN-93G) 1%, mineral mixture (AIN-93G) 3.5%, L-cystine 0.18%, choline hydrogen tartrate 0.25%, and *t*-butylhydroquinone 0.0008% (w/w per 100 g diet). The mice were given either the vehicle (olive oil) or astaxanthin in doses of 1.2, 6, and 30 mg/kg body weight by stomach intubation for 60 d. Samples were administrated in a volume of 200 µl. The mice were housed in standard cages (five mice per cage). The total amount of food intake by each group was recorded every 3 d. The intake of food showed the total intake of each group for 60 d. The body

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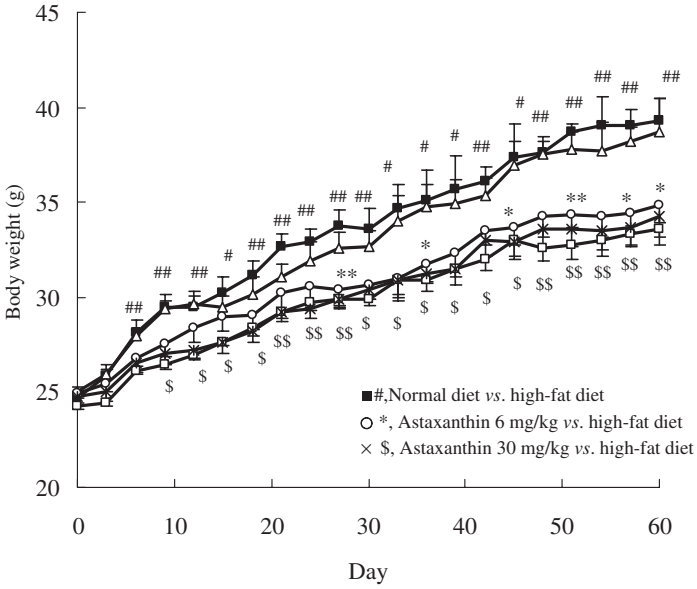

Fig. 1. Body Weight of Mice Administered Vehicle or Astaxanthin at 1.2, 6, or 30 mg/kg wt. during the Experimental Period.
□, normal diet; ■, high-fat diet; △, high-fat diet + astaxanthin 1.2 mg/kg; ○, high-fat diet + astaxanthin 6 mg/kg; ×, high-fat diet + astaxanthin 30 mg/kg. Values are means ± SE (n = 8 per group) #, $P < 0.05$; ##, $P < 0.01$ *vs.* normal diet. *, $P < 0.05$; **, $P < 0.01$; 6 mg/kg *vs.* high-fat diet. $, $p < 0.05$; $$, $p < 0.01$; 30 mg/kg *vs.* high-fat diet.

weight of each mouse was recorded every 3 d. After 60 d, the mice were killed by anesthesia. Blood samples were collected from the heart. The concentrations of triglyceride and total cholesterol in the plasma were determined by enzymatic colorimetric tests with commercial kits (triglyceride E-test, cholesterol E-test) from Wako Pure Chemical Industries, Tokyo, Japan.

Parametrial adipose tissue and organs (liver, kidney, spleen, and heart) were quickly removed and weighed. The liver was stored at −20 °C until analysis. The liver triglycerides were extracted by the procedure of Folch *et al.*[11] and were determined with a commercial kit.

Experiment 2: Lipid-loading test. Female ddY mice were housed for 1 week under the conditions described above. They were provided a normal diet (MR stock, Nihon nousan, Tokyo, Japan) and water *ad libitum*. After the mice had been deprived of food overnight, they were orally administered 0.3 ml of olive oil with or without astaxanthin at a dose of 30 mg/kg (each group, n = 10). Blood samples were taken from the tail 0, 1, 2, 3, 4, 5, 6, 7, and 8 h after administration. The concentrations of triglyceride in the plasma were determined by enzymatic colorimetric tests with commercial kits (triglyceride E-test) from Wako Pure Chemical Industries, Tokyo, Japan.

Experiment 3: Respiratory exchange ratio. Female ddY mice were housed for 1 week under the conditions described above. They were provided a normal diet (MR stock, Nihon nousan, Tokyo, Japan) and water *ad libitum*. Twenty mice were divided into two groups (n = 10 per group). The mice were given either vehicle

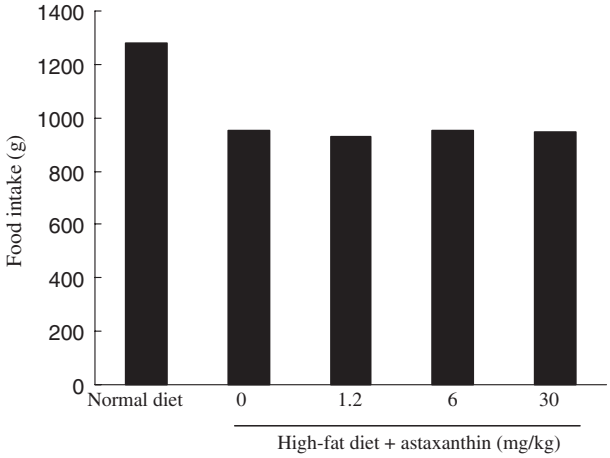

Fig. 2. Total Food Intake over 60 d.
The mice were housed in standard cages (five mice per cage). The total amount of food intake by each group was recorded every 3 d. The intake of food shown is the total intake for 60 d for each group.

(olive oil) or astaxanthin in doses of 30 mg/kg body weight by stomach intubation at every day for 4 weeks. Four weeks later the mice were prohibited access to food 12 h before administration of samples to avoid the effect of the components in the diet or of digestion and absorption on respiratory gas. The mice were administered vehicle (olive oil) or astaxanthin in doses of 30 mg/kg body weight by stomach intubation. Eight h later, the respiratory gases were measured for 1 h. The instruments used for measurement of the respiratory quotient in mice consisted of acrylic metabolic chambers, gas analyzers (model LE 405 Gas Analyzer Panlab Technology for Bioresearch, Madrid, Spain), and a

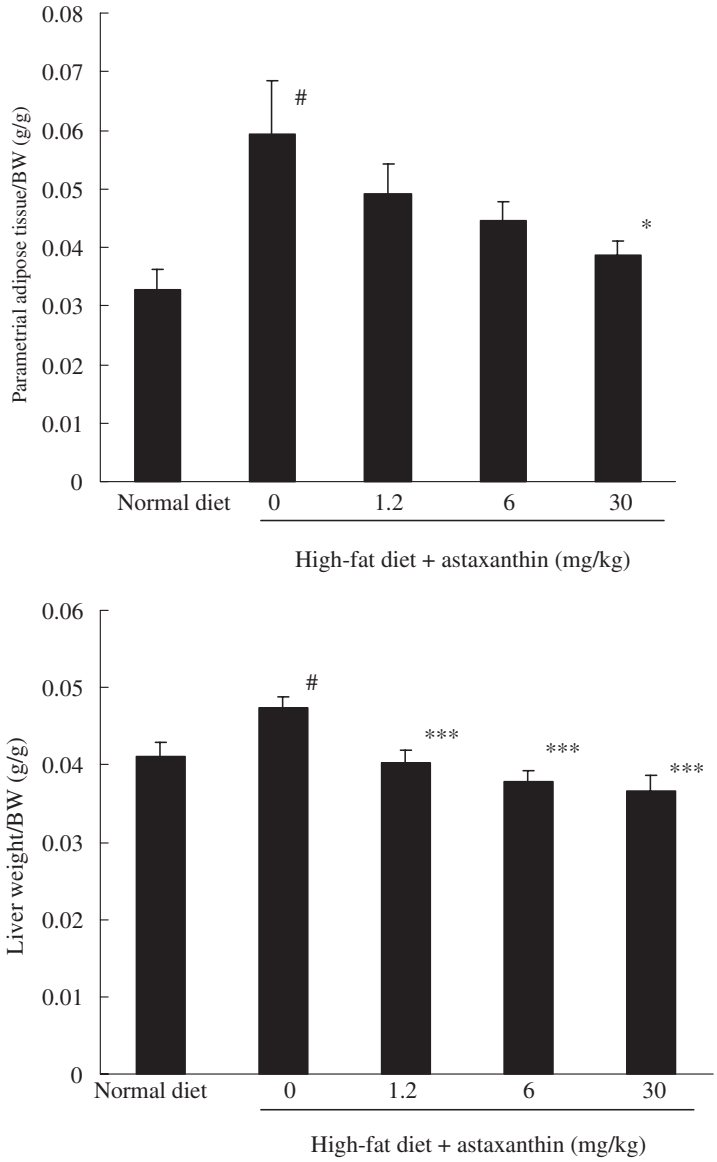

Fig. 3. Effects of Astaxanthin on Parametrial Adipose Tissue and Liver Weight in Mice Fed a High-Fat Diet for 60 d. Values are means ± SE (n = 8 per group) #, $P < 0.05$ *vs.* normal diet; ***, $P < 0.005$ *vs.* high-fat diet.

switching system (model LE 400 Air Supply and Switching, Panlab Technology for Bioresearch).

Statistical analysis. All values are expressed as mean ± SE. Data were analyzed by one-way ANOVA, and differences among the means of groups were analyzed by the Tukey-Kramer multiple comparison test. Differences were considered significant at $p < 0.05$.

Results

Change in body weight (Experiment 1)

Figure 1 shows the changes in body weight of the groups during the experiments. Feeding a high-fat diet containing 40% beef tallow caused a marked increase in body weight as compared to feeding a normal diet. However, feeding a high-fat diet plus astaxanthin at levels of 6 mg/kg or 30 mg/kg significantly reduced the body weight gain induced by the high-fat diet (Fig. 1). Food intake during the experiments was weighed. Feeding a high-fat diet caused a marked decrease in food intake as compared to feeding a normal diet, but astaxanthin did not affect food intake (Fig. 2).

Periepididymal tissue and liver weight

The mice fed a high-fat diet containing 40% beef tallow for 60 d had a significantly higher adipose tissue weight than the mice fed the normal diet. In the high-fat diet plus astaxanthin 30 mg/kg group, adipose tissue weight was significantly lower than in the mice fed the high-fat diet alone (Fig. 3). In the high-fat diet plus astaxanthin 1.2 mg/kg and 6 mg/kg groups, the adipose tissue weight tended to be lower than in the group receiving the high-fat diet alone, but not significantly.

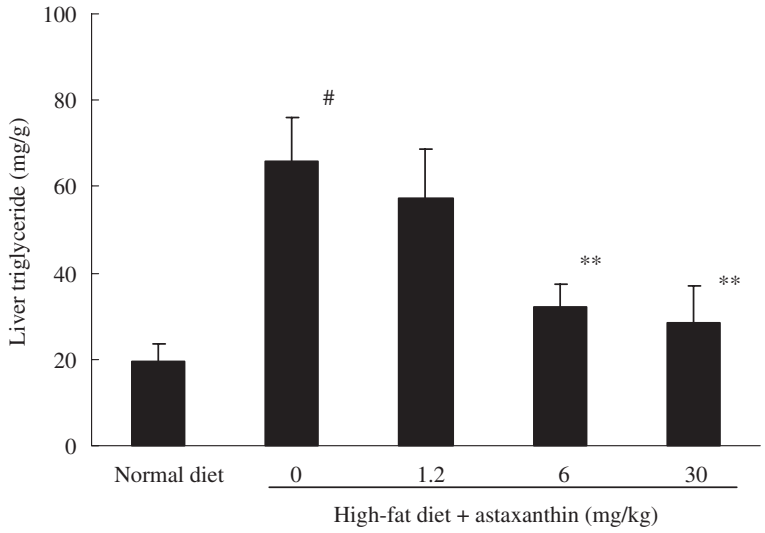

Fig. 4. Effects of Astaxanthin on Liver Triglyceride in Mice Fed a High-Fat Diet for 60 d.
Values are means ± SE (n = 8 per group) #, $P < 0.05$; *vs.* normal diet. ***, $P < 0.005$ *vs.* high-fat diet.

The high-fat diet plus astaxanthin also significantly suppressed the increase in liver weight induced by the high-fat diet alone. The weights of the kidney, spleen, and heart did not differ significantly among groups (data not shown).

Liver lipid content

The lipid levels in the liver when astaxanthin was administered are shown in Fig. 4. The liver triglyceride level was higher in the high-fat diet group than the normal diet group. In the high-fat diet plus astaxanthin 6 mg/kg and 30 mg/kg groups, liver lipids were lower than in the group fed the high-fat diet alone.

Plasma lipids

The plasma triglyceride level was higher in the high-fat diet alone group than in the normal diet group. However, values in the high-fat diet plus astaxanthin 6 mg/kg and 30 mg/kg groups were lower than in the group receiving the high-fat diet alone (Fig. 5). In the high-fat diet plus astaxanthin 1.2 mg/kg group, the triglyceride level tended to be lower than in the group fed the high-fat diet alone, but not significantly. The plasma total cholesterol level was higher in the high-fat diet alone group than in the normal diet group. However, values in the high-fat diet plus astaxanthin 30 mg/kg group were lower than in the high-fat diet alone group.

Lipid-loading test (Experiment 2)

In the lipid-loading test, plasma triglyceride concentration gradually increased until 4 h after sample (olive oil or olive oil plus astaxanthin) administration, and then it gradually decreased. The plasma triglyceride level was not reduced in the astaxanthin group more than in the vehicle group (Fig. 6).

Respiratory exchange ratio (Experiment 3)

Figure 7 shows the changes in metabolic rate in mice administrated vehicle and astaxanthin 30 mg/kg every day after 4 weeks. The vehicle group maintained its respiratory quotient at 0.9–0.95. The respiratory quotient was lower in the astaxanthin 30 mg/kg group than in the mice of the vehicle group (Fig. 7).

Discussion

It is generally accepted that diets high in fat contribute to obesity in both humans[12] and animal models.[13] High dietary fat intake is widely accepted to be associated with a higher risk for obesity and chronic diseases such as cardiovascular disease, some types of cancer,[14] diabetes, hyperlipidemia, and hypertension. For example, high fat consumption is related to the prevalence of metabolic syndrome in affluent societies.[15]

In the present study increases in the body weight and weight of adipose tissue were prevented in mice fed a high-fat diet plus astaxanthin without changing the energy intake, except in the normal diet group. At the end point of the experiments, astaxanthin inhibition of elevation in the weights of the body and of adipose tissue appeared to be dose-dependent (Figs. 1, 2). In addition, astaxanthin reduced liver weight (Fig. 3), liver triglyceride (Fig. 4), plasma triglyceride, and total cholesterol (Fig. 5). These results indicate that astaxanthin prevents the obesity and fatty liver induced by feeding a high-fat diet.

Reducing energy intake and increasing energy expenditure are fundamental to the treatment of obesity.

In terms of energy intake, for the purpose of clarifying the mechanism of the body weight reduction observed in this study, the effect of astaxanthin on the absorption of triglyceride in the intestines was investigated. Astaxan-

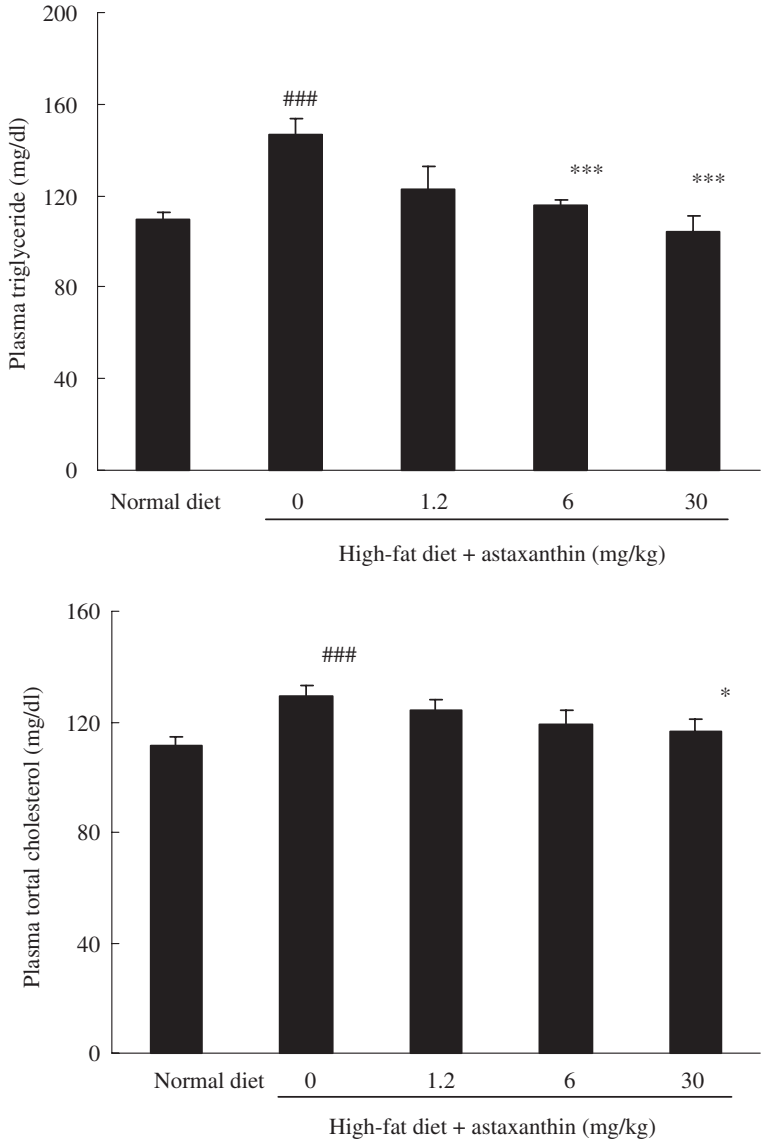

Fig. 5. Effects of Astaxanthin on Plasma Triglyceride and Cholesterol in Mice Fed a High-Fat Diet for 60 d.
Values are means ± SE (n = 8 per group) ###, $P < 0.005$ *vs.* normal diet. *, $P < 0.05$; ***, $P < 0.005$ *vs.* high-fat diet.

thin did not decrease the plasma triglyceride gain caused by olive oil administration (Fig. 6). It is well known that dietary fat is not absorbed from the intestine unless it has been subjected to the action of pancreatic lipase. In strategies to prevent obesity, one of the key steps is inhibition of the digestion and absorption of dietary fat. We tested astaxanthin for inhibitory action against pancreatic lipase *in vitro* and found that astaxanthin did not inhibit pancreatic lipase activity (data not shown). Thus, our findings indicate that astaxanthin does not affect energy intake.

Astaxanthin might affect energy expenditure, however. Because thermogenesis and fat oxidation are to a large extent under the control of the sympathetic nervous system, approaches that mimic or interfere with the sympathetic nervous system and its neurotransmitter norepinephrine offer a rational approach to obesity management.[16,17] For example, capsaicin has been reported to increase catecholamine secretion and energy expenditure and to suppress body fat accumulation during long-term treatment in experimental animal studies.[18–20] Oral administration of capsaicin resulted in increased endurance time during prolonged work. These increases were associated with enhanced lipolysis and sparing of glycogen, which results in delaying complete glycogen depletion through an increase in circulating catecholamine. In our previous study, astaxanthin may have had beneficial effects on endurance capacity.[21] Astaxanthin increased the supply of blood free fatty acid in the early phase of exercise. The administration of astaxanthin causes a decrease in the utilization of glucose and an increase in the utilization of fatty acid as an energy source during exercise, which spares glycogen. According to the results of this report, astaxanthin can stimulate an increase in fatty acid utilization.

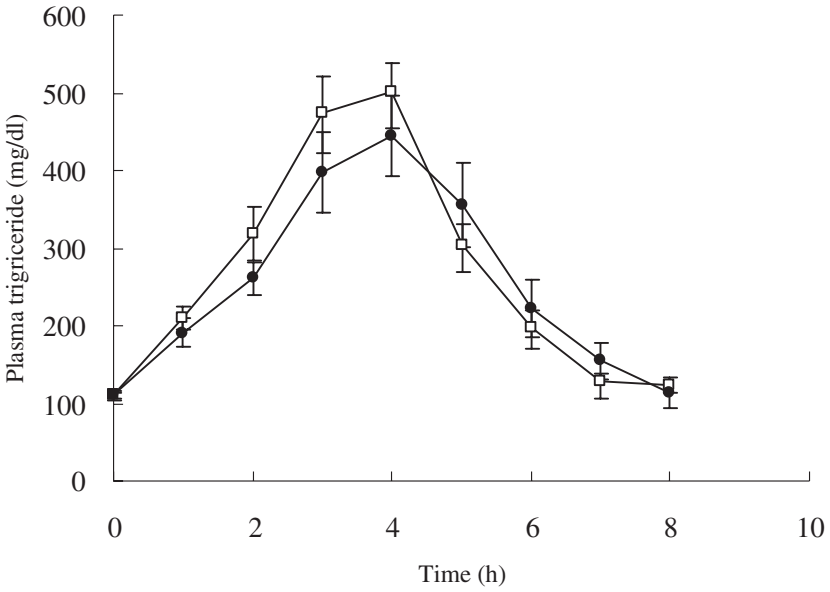

Fig. 6. Effect of Astaxanthin on Plasma Triglyceride Concentration in a Lipid-Loading Test.
●, vehicle; □, astaxanthin 30 mg/kg. Mice (8 weeks old, female, n = 10 per group) were orally administered 0.3 ml of olive oil with or without astaxanthin 30 mg/kg. Plasma triglyceride was measured from 0 to 8 h. Each value represents mean ± SE.

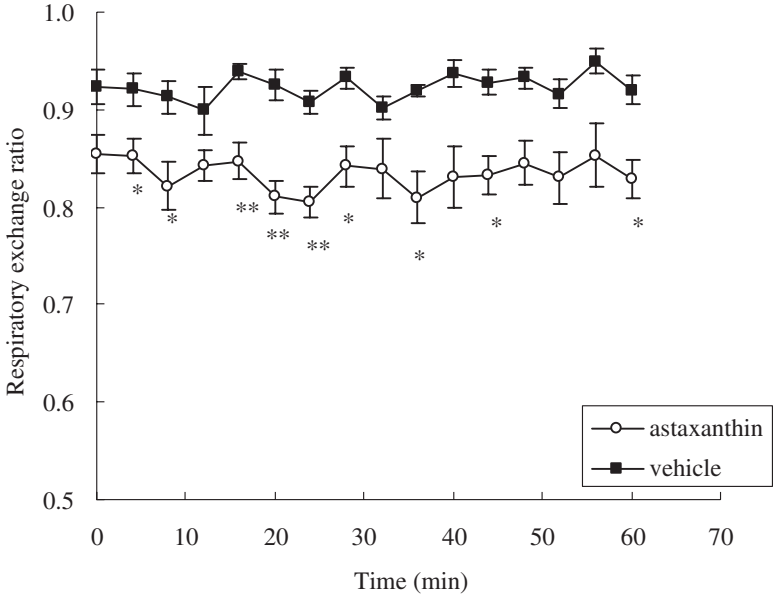

Fig. 7. Effect of Long-Term Astaxanthin Treatment on Respiratory Exchange Ratio.
●, vehicle; □, astaxanthin 30 mg/kg. Each value represents mean ± SE. *, $P < 0.05$; **, $P < 0.01$ *vs.* vehicle. Twenty mice were divided into two groups (n = 10 per group). The mice were given either vehicle (olive oil) or astaxanthin in doses of 30 mg/kg body weight by stomach intubation every day for 4 weeks. Four weeks later the mice were prohibited access to food 12 h before administration of samples to avoid the effect of components in the diet and of digestion and absorption on respiratory gas. The mice were administrated 30 mg/kg of astaxanthin or vehicle (olive oil), and then 8 h later they were rested in a metabolic chamber for 60 min.

In the study we analyzed the respiratory gas of mice administrated vehicle and astaxanthin 30 mg/kg at every day for 4 weeks. Analysis was started at the last administration and for 8 h afterward, because generally the astaxanthin concentration in the blood can be detected at 2 h, reaches a peak at 6–7 h, and exists in the blood till 72 h. The half-life is 21 h. This study indicates that after long-term administration of astaxanthin, the respiratory quotient was lower in the Astaxanthin 30 mg/kg group than in the mice of vehicle group (Fig. 7). Astaxanthin might stimulate an increase in fatty acid utilization in daily life. We must measure uncoupling protein in brown adipose tissue or beta-oxidase enzymes in liver and muscle. Further studies are necessary to elucidate the mechanisms.

In conclusion, we found that astaxanthin inhibits the

elevations in body weight and adipose tissue weight caused by a high-fat diet. In addition, astaxanthin reduced liver weight, liver triglyceride, plasma triglyceride, and total cholesterol. These results indicate that astaxanthin might be of value in preventing obesity and the metabolic syndrome in affluent societies.

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