Original Article

Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient

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Fucoxanthin has a unique structure including an unusual allenic bond and 5, 6-monoepoxide in its molecule. We found that abdominal white adipose tissue (WAT) weights of rats and mice fed fucoxanthin were significantly lower than those fed a control diet. The daily intake of fucoxanthin in mice also caused a significant reductions of body weight. Clear signals of uncoupling protein 1 (UCP1) and its mRNA were detected by Western and Northern blot analyses in abdominal WAT in mice fed fucoxanthin, although there is little expression of UCP1 in WAT in mice fed a control diet. UCP1 expression in WAT by fucoxanthin intake leads to oxidation of fatty acids and heat production in WAT mitochondria. Substrate oxidation can directly reduce WAT in animals. Fucoxanthin intake also significantly reduced blood glucose and plasma insulin. Furthermore, feeding fucoxanthin significantly increased the level of hepatic docosahexaenoic acid (DHA), a most important n-3 functional polyunsaturated fatty acid in biological systems. These multi-functionalities of fucoxanthin indicate that it is an important bioactive carotenoid that is should be beneficial for the prevention of the metabolic syndrome.

Key Words: fucoxanthin, anti-obesity, anti-diabetic, DHA, metabolic-syndrome

INTRODUCTION

Obesity and diabetes are emerging pandemics in the 21st century. Both are major public health problems throughout the world. On the other hand, n-3 polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have attracted considerable interest in both nutraceutical and pharmaceutical fields.[1] Regular consumption of EPA and DHA lowers the rate of incidence and death from cardiovascular disease.[2] Although the biochemical basics for caridioprotective effects of these n-3 PUFA are unknown, EPA and DHA are effective to reduce the risk of cardiovascular disease induced by obesity and diabetes. EPA and DHA are absorbed directly in the body following the intake of fish oil. However, EPA and DHA in fish oil are much more susceptible to oxidation than other PUFAs in vegetable oils such as linoleic acid and α-linolenic acid. This instability of fish oil is a big problem in its utilization foods and other products.

Fucoxanthin, a specific carotenoid found in brown seaweed, has a unique structure including an allenic bond and a 5,6-monoepoxide in the molecule. It is one of the most abundant carotenoids accounting for >10% of estimated total natural production of carotenoids. In Southeast Asian countries, some seaweeds containing fucoxanthin are often used as a source of food. Among them *Undaria* (Japanese name is Wakame) and *Laminaria* (Japanese name is Konbu) are the most popular edible seaweeds in Japan. Cancer chemoprevention is one of the promising methods for cancer control. Among the chemopreventive agents, carotenoids, especially β-carotene, have been investigated extensively.[3] Hosokawa et al.[4] found that HL-60 human promycelocytic leukemia cells underwent apoptosis with fucoxanthin treatment. Its activity was higher than that of β-carotene. However, there has been little information on other physiological activities of fucoxanthin.

Only recently, we have found that dietary fucoxanthin showed specific functionalities which have not been found in other carotenoids. Fucoxanthin enhanced the amount of DHA in the liver of mice fed soybean oil.[5] This shows the possibility of dietary fucoxanthin induced increase of DHA in biological system without direct fish oil supplementation. In addition, we have reported that abdominal fat weight of rats and mice were significantly reduced by fucoxanthin intake.[6] Blood glucose and plasma insulin of the mice were also decreased by fucoxanthin supplementation.[7]

In the present study, we describe anti-obesity and anti-diabetic effects of fucoxanthin and increased hepatic DHA in animals fed fucoxanthin.

SUBJECTS AND METHODS

Undaria lipids containing fucoxanthin (9.6%) was obtained by extraction of dried powdered seaweed (*Undaria Pinnatifida*) with chloroform/methanol (2:1, v/v). Crude fucoxanthin (fucoxanthin: 78%) was prepared from the *Undaria* lipids according to the procedure described in our

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previous paper.[6] To obtain purified fucoxanthin, crude fucoxanthin was subjected to silica gel column chromatography with acetone:n-hexane (1:1, v/v) as eluent. The purity of fucoxanthin was approximately 93% by HPLC analysis. Fucoxanthinol, a main metabolite of fucoxanthin, was prepared from purified fucoxanthin by hydrolysis with porcine pancreas lipase type II (Sigma, St. Louis, MO, USA) as described in our previous paper.[5] Fucoxanthinol obtained by enzymatic reaction was further purified by silica gel column chromatography using acetone:n-hexane (1:1, v/v). The purity of fucoxanthinol was 99% by HPLC analysis.

Male Wister rats and female KK-Ay mice (5 and 3 weeks of age) were housed at 23±1ºC and at 50% relative humidity with 12-h/12-h light/dark cycle. Animals had free access to drinking water and were fed a diet prepared according to the recommendation of American Institute of Nutrition (AIN-93G).[8] After 1 week of acclimation by feeding the AIN diet, animals were randomly divided into three groups of seven and fed different experimental diets.

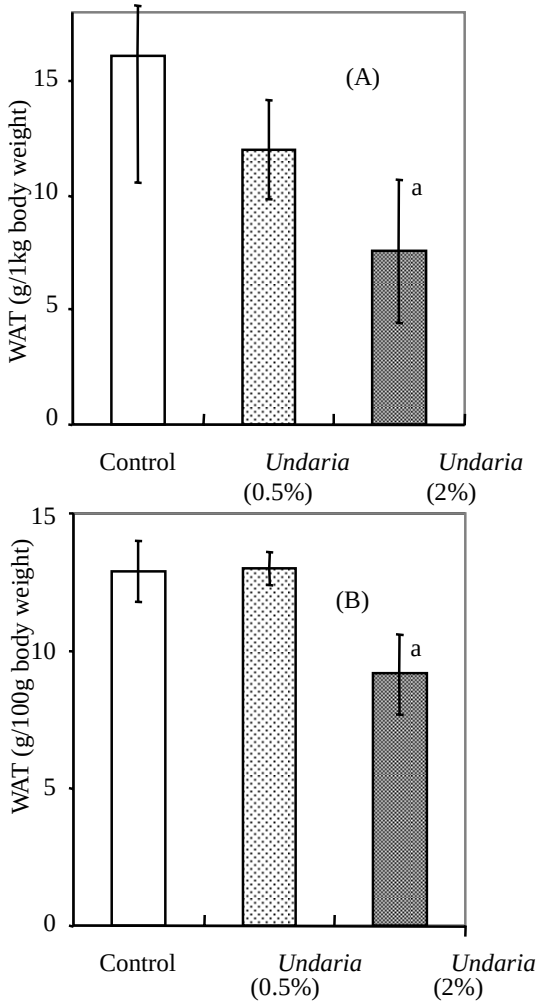

Figure 1. Weight of abdominal WAT of rats (A) and mice (B) fed *Undaria* lipids and the control diet. [a]Significant difference from control (p<0.01). Diets was prepared according to the recommendation of American Institute of Nutrition (AIN-93G). Dietary fat content for rats were 7% soybean oil (control), 6.5% soybean oil + 0.5% *Undaria* lipids, and 5% soybean oil + 2% *Undaria* lipids. Those for mice were 13% soybean oil (control), 12.5% soybean oil + 0.5% *Undaria* lipids, 11% soybean oil + 2% *Undaria* lipids. (Adapted from Meda et al., Biochim Biophys Res Commun 2005; 332: 392-397.)

Dietary fats that were fed to rats consisted of 7% soybean oil (control), 6.5% soybean oil + 0.5% *Undaria* lipids, and 5% soybean oil + 2% *Undaria* lipids respectively. For mice, it consisted of 13% soybean oil (control), 12.5% soybean oil + 0.5% *Undaria* lipids, 11% soybean oil + 2% *Undaria* lipids, 12.6% soybean oil + 0.4% crude fucoxanthin, and 11.2% soybean oil + 1.8% *Undaria* glycolipid fraction. Mice were also fed 13.51% soybean oil (control), 13.41% soybean oil + 0.1% purified fucoxanthin, and 13.31% soybean oil + 0.2% purified fucoxanthin according to their groups.

After the consumption of experimental diets for 4 weeks followed by an overnight fast, animals were killed by exsanguination and their blood was withdrawn via the abdominal artery. Plasma lipid levels were measured with analytical kits (Wako Pure Chemicals, Osaka, Japan). Liver and abdominal white adipose tissue (WAT) were rapidly removed in their entirety, weighed and frozen in liquid nitrogen for Western blot and RNA analyses. Abdominal WAT composed of perirenal and epididymal adipose tissues for rats and perirenal, gonadal, retroperitoneal, and mesenteric adipose tissues for mice. Animal care procedures were approved by the Animal Care and Use Committee of Hokkaido University. Liver lipids were extracted using the Bligh and Dyer method.[9]

Fatty acid composition of the lipids obtained was analyzed using gas-liquid chromatography (GC) after methylation according to the Christopher and Glass method as described by Prevot and Mordret.[10] I individual fatty acids were quantified using 17:0 as an internal standard. Blood glucose levels were determined using a blood glucose monitor, Glutest Neo (Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan). The plasma insulin and leptin concentrations were analyzed using commercial ELISA kits, Mouse Insulin ELISA kit (U-type) (Shibayagi, Gunma, Japan) and Mouse Leptin Assay Kit (L) –IBL (IBL Co., Ltd. Gunma Japan), respectively.

Results are shown as mean ± SD for seven mice. Differences between groups were examined for statistical significance using Dunnett's t-test at p<0.01 or p<0.05.

RESULTS

Undaria lipids, containing 9.6% fucoxanthin, reduced significantly the weights of abdominal WAT of both rats and mice (Fig. 1). Body weights of mice fed 2% *Undaria* lipid was significantly (p<0.05) lower than that of controls, although there were no significant differencein the mean daily dietary intake between the two groups.[6] When purified fucoxanthin was administered to obese KK-Ay mice, the WAT weights of fucoxanthin-fed mice was significantly lower than that of control mice. This result confirmed that fucoxanthin is the active component in the *Undaria* lipids that result in the anti-obesity effect. Furthermore, uncoupling protein 1 (UCP1) expression was found in WAT of *Undaria* lipids-fed mice, although there was little expression in that of control mice.[6] Expressions of UCP1 mRNA was also found in WAT of *Undaria* lipids-fed mice, but little expression in that of the controls. On the other hand, UCP2 expression in WAT decreased by feeding *Undaria* lipids as compared with controls. This suggests that the decrease in WAT weight of *Undaria* lipids-fed mice was not due to thermogenesis

Table 1. Fatty acid composition (wt%) of liver lipids of rats fed the control and *Undaria* lipid diets.

Fatty acid	Control	*Undaria* (0.5%)	*Undaria* (2%)
16:0	23.0 ± 4.0	22.6 ± 1.5	18.9 ± 1.3 [a]
18:0	12.8 ± 2.5	12.2 ± 2.0	17.3 ± 1.8 [b]
16:1n-7	2.2 ± 1.6	3.2 ± 1.0	1.2 ± 0.3
18:1n-7	3.7 ± 0.6	4.8 ± 0.6 [b]	3.0 ± 0.6
18:1n-9	13.1 ± 3.2	13.0 ± 2.3	7.5 ± 1.3 [b]
18:2n-6	20.1 ± 3.4	15.7 ± 1.2 [b]	17.1 ± 1.5
20:3n-6	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
20:4n-6	15.7 ± 3.1	16.2 ± 2.8	20.8 ± 2.7
20:5n-3	0.2 ± 0.1	0.4 ± 0.1 [a]	1.5 ± 0.2 [b]
22:5n-3	0.5 ± 0.3	0.9 ± 0.2	1.8 ± 0.2 [b]
22:6n-3	3.5 ± 0.9	4.5 ± 0.9	5.2 ± 0.6 [b]

[a,b]Significantly different from the control (a:$p<0.05$; b:$p<0.01$).

Table 2. Liver lipid weight and plasma lipid content of rats fed the control and *Undaria* lipid diets.

	Control	*Undaria* (0.5%)	*Undaria* (2%)
Liver lipid weight (mg/100mg liver)	8.6±2.5	7.9±1.5	6.5±1.1[a]
Plasma total cholesterol (mg/dl)	82.6±19.0	73.5±7.0	87.0±10.9
Plasma triacyl-glycerol (mg/dl)	28.0±19.9	26.2±11.9	17.1±11.6
Plasma total lipids (mg/dl)	279 ±80.9	269 ±9.8	272 ±67.2

[a]Significantly different from control ($p<0.05$).

through UCP1 expression in WAT, but through UCP2 expression. UCP1 expression in WAT was also found in crude fucoxanthin-fed mice, but little expression of UCP1 was found in WAT of mice fed *Undaria* glycolipids and control diets. Thus, it is apparent that up-regulation of UCP1 expression in WAT by fucoxanthin induces the decrease in abdominal fat in mouse.

GC analysis showed that *Undaria* lipids containing fucoxanthin significantly increased the proportion of DHA in liver lipids of rats (Table 1) and mice.[5] Proportion of DHA in rats fed a diet containing *Undaria* lipids (2%) was found to be significantly higher than that of rats fed the control diet. In addition, an increase in the proportion of stearic acid (18:0) and arachidonic acid (20:4n–6) were also observed in the group fed *Undaria* lipids. In contrast, the proportion of oleic acid (18:1n–9), and linoleic acid (18:2n–6) in the fatty acid composition of liver lipids were reduced by the feeding of *Undaria* lipids.

Table two shows liver lipid content of the rats fed the control and *Undaria* lipid diets. Liver lipid contents of rats fed *Undaria* lipids were lower than that of rats fed the control diet, while there was no significant difference in the plasma lipids (Table 2). n–3 PUFA such as DHA are known to reduce the activity of hepatic enzymes in fatty acid synthesis and increase hepatic fatty acid β-oxidation. The reduction of liver lipids might be due to DHA increase in the liver.

Undaria lipids fed to rats contained 9.6% fucoxanthin. To clarify the enhancement in total hepatic DHA by fucoxanthin, we used purified fucoxanthin and carried out a quantitative assessment of DHA by using an internal standard.[5] As orally administered fucoxanthin is known to be metabolized to fucoxanthinol and then to amorouciaxanthin A in the mouse, we therefore also examined the effect of fucoxanthinol on hepatic DHA levels.[5] After preparing the control, fucoxanthin and fucoxanthinol containing diets with the same fatty acid composition, mice were fed each diet for 4 week. Throughout the experiment, food intake did not differ among treatment groups. Liver weights and the total amount of liver lipids did not differ among groups. The total amount of hepatic DHA increased in mice fed the 0.1% and 0.2% purified fucoxanthin diets to 1.7 and 1.9 times higher than that of the control group respectively. In addition, 22:5n–3 increased in mice fed the 0.2% fucoxanthin diet. Hepatic 20:4n–6 also increased with the fucoxanthin diets, although this increase was not significant. A significant increase in hepatic DHA was found in mice fed diets containing 0.2% purified fucoxanthinol.[5] Furthermore, an increase in 20:4n–6 was observed in mice fed the fucoxanthinol diets. Fucoxanthin is metabolized to fucoxanthinol and then to amarouciaxanthin A in mice.[11] Therefore, fucoxanthinol and amarouciaxanthin A, but not fucoxanthin, are suggested to be key substances to enhance the amount of DHA in the liver of KKAy mice. DHA can be biosynthesized through desaturation and elongation reaction steps beginning with α-linolenic acid in the liver.[12,13] The increase in hepatic DHA found in the animals fed fucoxanthin might be due to the up-regulation of enzymatic activities related to the bioconversion of α-linolenic acid to DHA.

Mice in this study not only developed obesity but also hyperleptinemia and hyperinsulinemia along with insulin resistance. Therefore, glucose levels of mice fed the control diet reached levels higher than 400 mg/dl. On the other hand, mice fed the 0.1% and 0.2% purified fucoxanthin diets had significantly lower blood glucose concentrations of around 220 mg/dl and 170 mg/dl, respectively.[7] Furthermore, plasma insulin levels decreased in a dose-dependent manner after purified fucoxanthin intake.[7]

DISCUSSION

The rise in the prevalence of obesity is now recognized as a worldwide problem, with ominous implications for public health and health-related costs. It may be a second-most important preventable cause of death, exceeded only by cigarette smoking. Obesity is a potent risk factor for type-2 diabetes, hypertension, and dyslipidemia, comorbidities that markedly increase the risk of cardiovascular disease. Safe and effective anti-obesity component may be available in the form of nutrients found in seaweed.[14]

UCP1 is a dimeric protein present in the inner mitochondrial membrane of brown adipose tissue (BAT), and it dissipates the pH-gradient generated by oxidative phosphorylation, releasing chemical energy as heat. Thus, UCP1 expression is known as a significant component of whole body energy expenditure and its dysfunction contributes to the development of obesity. However, adult humans have very little BAT and most of fat is stored in WAT. Therefore, the induction of UCP1 in WAT by food

constituent would be important and could become an ideal therapy of obesity. From this viewpoint, the anti-obesity effect of edible seaweed carotenoid, fucoxanthin, is very interesting, as its activity depends on the protein and gene expressions of UCP1 in WAT.

n-3 Polyunsaturated fatty acids such as EPA and DHA have been reported to have pivotal roles in a number of physiological functions including cardio-protection activities, the reduction of triacylglycerol and cholesterol, as well as anti-inflammatory and anti-cancer effects.[2] The effects of EPA and DHA on lipid metabolism could be strongly correlated to the anti-obesity effect of marine lipids.[2] Therefore, the novel effects of fucoxanthin in increasing total DHA in the liver of rodents may be indirectly related to the health beneficial effects of fucoxanthin.

In conclusion, our results show that dietary fucoxanthin reduces the risk of many diseases by affecting two or more molecular targets.

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AUTHOR DISCLOSURES

Hayato Maeda, Takayuki Tsukui, Tokutake Sashima, Masashi Hosokawa, Kazuo Miyashita, no conflicts of interest.

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