Fucoxanthin and Fucoxanthinol Enhance the Amount of Docosahexaenoic Acid in the Liver of KKAy Obese/Diabetic Mice

Takayuki Tsukui,[†] Kensuke Konno,[†] Masashi Hosokawa,*,[†]
Hayato Maeda,[†] Tokutake Sashima,[§] and Kazuo Miyashita[†]

Faculty of Fisheries Sciences and Creative Research Institute, Hokkaido University, Hakodate,
Hokkaido 041-8611, Japan

This study examined the effect of dietary fucoxanthin or fucoxanthinol on the amount of docosahexaenoic acid (DHA) in the liver of KKAy mice, a model for obese/type II diabetes. In the first experiment, mice were fed diets containing crude fucoxanthin or glyceroglycolipid for 4 weeks. Results showed a significant increase in the level of DHA in mice fed 0.53% crude fucoxanthin, from 2.3% in control mice to 5.1% of fatty acid composition of total liver lipids. On the other hand, in mice fed crude glyceroglycolipid, the level of DHA as a proportion of total liver fatty acids remained unchanged. To clarify the enhancement of hepatic DHA, in the second experiment, KKAy mice were fed a diet containing purified fucoxanthin or its deacetylated derivative, fucoxanthinol. Results from a quantitative analysis using an internal standard showed that in mice fed 0.2% fucoxanthin, the amount of hepatic DHA was 2-fold higher than in control mice, whereas DHA levels in the small intestine remained unchanged. Furthermore, 0.2% fucoxanthinol led to 1.8- and 1.2-fold increases in the amount of hepatic DHA and arachidonic acid compared to control mice, respectively. These results indicate for the first time that dietary fucoxanthin and fucoxanthinol enhance the amount of DHA in the liver of KKAy mice.

KEYWORDS: Fucoxanthin; fucoxanthinol; docosahexaenoic acid; liver; KKAy mouse

INTRODUCTION

Fucoxanthin is a major marine carotenoid, found in edible seaweeds such as *Undaria pinnatifida* and *Sargassum fulvellum.* Its structure (**Figure 1**), which include an allenic bond and a 5,6-monoepoxide, differs from that of common carotenoids such as *β*-carotene and lycopene. Fucoxanthin has been reported to demonstrate anticaricinogenic (*1*) and anti-inflammatory effects (*2*) as well as apoptotic effects in cancer cells (*3−5*) and radical scavenging activity (*6*). We also recently reported that dietary fucoxanthin suppresses the weight gain of white adipose tissue (WAT) in a mouse model of obesity/diabetes, KKAy (*7*). Interestingly, fucoxanthin induces uncoupling protein 1, which is a key molecule involved in metabolic thermogenesis, in the WAT of KKAy mice. In addition, we have reported that fucoxanthin inhibits intercellular lipid accumulation during the differentiation of 3T3-L1 adipocyte cells (*8*). Orally administered fucoxanthin is known to be metabolized to fucoxanthinol (**Figure 1**) and amarouciaxanthin A, which are detected in the liver and serum in mice (*9*). However, to date there are no
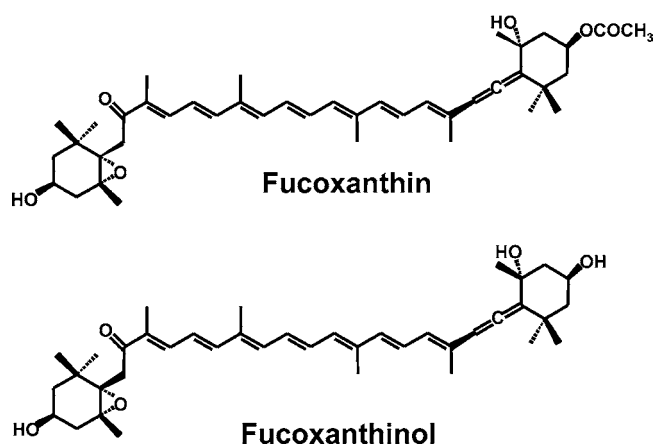

Figure 1. Structures of fucoxanthin and fucoxanthinol.

studies examining the effect of fucoxanthin and its metabolite fucoxanthinol on hepatic function including fatty acid biosynthesis.

Highly unsaturated n-3 fatty acids (HUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have attracted considerable interest in both nutraceutical and pharmaceutical fields (*10*). DHA and EPA have been reported to have pivotal roles in a number of physiological functions

* Author to whom correspondence should be addressed (telephone +81-138-40-5530; fax +81-138-40-5530; e-mail hoso@fish.hokudai.ac.jp).
† Faculty of Fisheries Sciences.
§ Creative Research Institute.

including cardioprotection (*11*), reduction of triacylglycerol and cholesterol (*12*), and anti-inflammatory (*13*, *14*) and anti-cancer effects (*15*, *16*). Furthermore, DHA is known to be an essential fatty acid for brain functions (*17*) and its development, particularly in premature infants (*18*, *19*). These n-3 HUFA are absorbed directly in the body following the intake of fish oil, and the fatty acid compositions in the liver and serum are subsequently altered. In addition, DHA and EPA could be biosynthesized through desaturation and elongation reaction steps beginning with α-linolenic acid (18:3n-3) in the liver (*20*, *21*). Arachidonic acid (20:4n-6), which is one of an n-6 HUFA, is synthesized from linoleic acid (18:2n-6) (*20*, *21*). However, because Δ-6 desaturase is the rate-limiting enzyme in HUFA biosynthetic pathways, mammals rely on a dietary supply of fish oil as source of DHA (*22*, *23*).

In a previous study of the fatty acid composition of liver lipids in obese Zucker rat (*24*) and normal Wistar rats (*25*), fenofibrate and clofibric acid increased the proportion of 20:4n-6, but not DHA. However, little is known about natural compounds that have the ability to increase hepatic DHA levels, except for direct consumption of fish oil containing DHA and EPA. Because the liver plays a central role in whole body lipid metabolism, natural compounds that enhance hepatic DHA are expected to exhibit beneficial effects in preventing lifestyle-related diseases and in the development of brain function.

In the current study, we focused on the effect of fucoxanthin and its metabolite fucoxanthinol on the biosynthetic pathway of DHA in the liver. The present results establish that dietary fucoxanthin and fucoxanthinol enhance the amount of DHA in the liver of obese and diabetic KKAy mice.

MATERIALS AND METHODS

Preparation of Samples. Crude fucoxanthin and glyceroglycolipid were prepared from *Undaria pinnatifida* according to the procedure described in our previous paper (*7*). Crude fucoxanthin used in experiment 1 contained 78% fucoxanthin and 22% glyceroglycolipid. Crude glyceroglycolipid contained 70% glyceroglycolipid, 0.2% fucoxanthin, and 29.8% other components such as chlorophyll and phospholipids. To obtain purified fucoxanthin for use in experiment 2, 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 was prepared from purified fucoxanthin by hydrolysis with porcine pancreas lipase, type II (Sigma, St. Louis, MO). One gram of fucoxanthin and 5 g of taurocholic acid sodium salt were once dissolved in methanol, and the solvent was dried under nitrogen. Then 20 g of lipase (Sigma) in 1 L of potassium phosphate buffer (0.1 M, pH 7.0) was added to the mixture of fucoxanthin and taurocholic acid and dispersed by sonication. After incubation at 37 °C for 2 h, the reaction mixture was extracted with methanol/diethyl ether (1:1, v/v), and the diethyl ether phase containing fucoxanthinol was separated. Fucoxanthinol was further purified by silica gel column chromatography using acetone/*n*-hexane (1:1, v/v). This reaction and purification procedure was performed multiple times to obtain sufficient fucoxanthinol for experiment 2. The purity of fucoxanthinol was 99% by HPLC analysis.

Animals and Diets. All procedures for the use and care of animals for this research were approved by the Ethical Committee of Experimental Animal Care at Hokkaido University. KKAy mice (3 weeks old, female) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The mice were housed at 23 ± 1 °C and 50% relative humidity under a 12 h light/dark cycle and had free access to food and drinking water. After a 1 week adaptation period, the mice were divided into groups of seven and were fed experimental diets for 4 weeks. The basal diet used in this study was AIN-93G. Crude fucoxanthin (0.26 and 0.53%) and glyceroglycolipid (1.24%) were added to the basal diet for experiment 1. Purified fucoxanthin and fucoxanthinol were added to

Table 1. Composition (Grams per 100 g of Diet) of the Diets Used in Experiment 1

	control	crude fucoxanthin (0.26%)	crude fucoxanthin (0.53%)	crude glycero-glycolipid
β-cornstach	37.1	37.1	37.1	37.1
casein	18.7	18.7	18.7	18.7
α-cornstarch	12.3	12.3	12.3	12.3
KC flock	4.7	4.7	4.7	4.7
AIN-93G mineral mix	3.3	3.3	3.3	3.3
AIN-93G vitamin mix	0.93	0.93	0.93	0.93
sucrose	9.3	9.3	9.3	9.3
L-cystine	0.28	0.28	0.28	0.28
choline bitartrate	0.23	0.23	0.23	0.23
TBQ	0.0013	0.0013	0.0013	0.0013
soybean oil	13.1	12.8	12.6	11.3
fucoxanthin	0	0.18	0.36	0.003
glycolipid	0	0.8	0.17	1.2

Table 2. Fatty Acid Composition of Dietary Lipids Used in Experiment 1

	control	crude fucoxanthin (0.26%)	crude fucoxanthin (0.53%)	crude glycero-glycolipid
16:0	10.6	10.8	11.1	11.0
18:0	4.1	4.0	4.0	3.8
18:1n-9	24.0	22.8	22.6	21.5
18:2n-6	50.8	51.6	51.4	49.3
18:3n-3	5.8	5.7	5.7	6.0
18:4n-3	ND[a]	ND[a]	ND[a]	2.4
20:5n-3 (EPA)	ND[a]	ND[a]	ND[a]	0.9

[a] Not detected.

diets at 0.1 and 0.2% in experiment 2. The concentrations of crude fucoxanthin used in experiment 1 were 0.26 and 0.53% to adjust the fucoxanthin concentration to the same as that of Wakame lipid used for observation of antiobesity effect on KKAy mice in our previous study (*7*). At the end of the 4 week period, animals were killed under ether anesthesia after a 12 h starvation. The liver and small intestine were removed rapidly in their entirety, weighed and frozen in liquid nitrogen.

Fatty Acid Composition Analysis. Liver lipids were extracted using the method of Folch et al. (*26*). The fatty acid composition of the lipid obtained was analyzed using gas–liquid chromatography after methylation according to the method of Christopher and Glass as described by Prevot and Mordret (*27*). In experiment 2, the amount of individual fatty acids was quantified using 17:0 as an internal standard.

Statistical Analysis. All values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA, and differences between the test groups and the control group were evaluated by Dunnett's *t* test ($p < 0.05$).

RESULTS AND DISCUSSION

Experiment 1: Effect of Crude Fucoxanthin and Glyceroglycolipid on the Proportion of DHA in Liver Fatty Acids in KKAy Mice. KKAy mice were fed crude fucoxanthin and glyceroglycolipid diets, as shown in **Table 1**. Fatty acid compositions of 0.26 and 0.53% crude fucoxanthin diets were approximately the same as a control diet with 13.1% soybean oil (**Table 2**). In contrast, the crude glyceroglycolipid diet contained 18:4n-3 and EPA at 2.4 and 0.9%, respectively.

The inclusion of crude fucoxanthin and glyceroglycolipid in the diet did not affect food intake during the 4 weeks. In addition, whole body and liver weights and the total amount of liver lipids did not differ significantly among diet groups (data not shown). Interestingly, crude fucoxanthin diets significantly

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Table 3. Fatty Acid Composition of Liver Lipid of KKAy Mice Fed Crude Fucoxanthin and Glyceroglycolipid Diets[a]

	control	crude fucoxanthin (0.26%)	crude fucoxanthin (0.53%)	crude glycero-glycolipid
16:0	22.1 ± 0.3	21.7 ± 0.4	22.8 ± 0.6	22.8 ± 0.3
18:0	7.4 ± 0.1	12.0 ± 0.5*[b]	11.3 ± 0.6*	10.2 ± 03
18:1n-9	31.5 ± 1.8	27.9 ± 1.3	23.2 ± 2.6	26.4 ± 1.7
18:2n-6	22.2 ± 1.3	16.6 ± 0.3*	19.5 ± 0.6	20.7 ± 0.9
18:3n-3	1.1 ± 0.1	0.5 ± 0.04*	0.8 ± 0.05	1.0 ± 0.04
18:4n-3	ND[c]	ND	ND	0.1 ± 0.01
20:4n-6	5.4 ± 0.2	9.1 ± 0.5*	8.0 ± 0.6	7.5 ± 0.4
20:5n-3(EPA)	0.2 ± 0.03	0.3 ± 0.01	0.3 ± 0.07	0.7 ± 0.4
22:5n-3	0.1 ± 0.04	0.2 ± 0.01	0.2 ± 0.04	0.3 ± 0.04
22:6n-3 (DHA)	2.3 ± 0.1	4.1 ± 0.1*	5.1 ± 0.7*	3.4 ± 0.1

[a] Values are means ± SEM of seven mice. [b] *, $p < 0.05$ versus control. [c] Not detected.

increased the proportion of DHA in the fatty acids of liver lipids (**Table 3**). The proportion of DHA in mice fed a diet containing 0.53% crude fucoxanthin was found to be more than twice that of the control group. In addition, increases in the proportion of stearic acid and arachidonic acid (20:4n-6) were also observed in the group receiving diets supplemented with crude fucoxanthin. In contrast, the proportions of oleic acid (18:1n-9) and α-linolenic acid (18:3n-3) in the fatty acid composition of liver lipids were reduced.

Because 18:4n-3 and EPA were not detected following 4 weeks on crude fucoxanthin diets, the increase in DHA is thought to be dependent on conversion from 18:3n-3 through activation of the n-3 HUFA biosynthetic pathway in the liver. However, diets containing crude glyceroglycolipid did not result in a significant increase in the proportion of DHA despite the fact that the precursors of DHA biosynthesis, 18:4n-3 and EPA, were contained in the diet. These results suggest that Δ-6 desaturase activity in liver is low and that the conversion from 18:4n-3 and EPA to DHA takes place very slowly in KKAy mice fed crude glyceroglycolipid.

Experiment 2: Effect of Fucoxanthin and Fucoxanthinol on the Amount of DHA in the Liver of KKAy Mice. Crude fucoxanthin used in experiment 1 contained 78% fucoxanthin and 22% glyceroglycolipid. To clarify the enhancement in total hepatic DHA by crude fucoxanthin observed in experiment 1, we used purified fucoxanthin and carried out a quantitative assessment of DHA by using an internal standard in experiment 2. As orally administered fucoxanthin is known to be metabolized to fucoxanthinol and then to amarouciaxanthin A in the mouse, we therefore also examined the effect of fucoxanthinol on hepatic DHA levels. The chemical composition of these diets and the fatty acid composition of dietary lipids are shown in **Table 4**. Fatty acid compositions were approximately the same in the control, fucoxanthin- and fucoxanthinol-containing diets.

Throughout the 4 week duration of the experiment, food intake did not differ among treatment groups. The body weight gain for mice fed 0.2% purified fucoxanthin was less than that recorded for other groups (data not shown). However, liver weights and the total amount of liver lipids did not differ among groups (**Figure 2**).

The consumption of diets supplemented with fucoxanthin led to an increase in the total amount of hepatic DHA in KKAy mice. In mice fed 0.1 and 0.2% purified fucoxanthin, the amount of hepatic DHA was 1.7 and 1.9 times higher than that of the control group (**Figure 3**). In addition, an increase in 22:5n-3 was observed in mice fed 0.2% fucoxanthin diet (data not shown). There was a trend for the amount of hepatic 20:4n-6

Table 4. Composition (Grams per 100 g of Diet) of the Diets Used in Experiment 2

	control	0.1% fuco-xanthin	0.2% fuco-xanthin	0.1% fuco-xanthinol	0.2% fuco-xanthinol
β-cornstarch	37.0	37.0	37.0	37.0	37.0
casein	18.6	18.6	18.6	18.6	18.6
α-cornstarch	12.3	12.3	12.3	12.3	12.3
KC flock	4.7	4.7	4.7	4.7	4.7
AIN-93G mineral mix	3.3	3.3	3.3	3.3	3.3
AIN9-3G vitamin mix	0.93	0.93	0.93	0.93	0.93
sucrose	9.3	9.3	9.3	9.3	9.3
L-cysteine	0.28	0.28	0.28	0.28	0.28
choline bitartrate	0.23	0.23	0.23	0.23	0.23
TBQ	0.0013	0.0013	0.0013	0.0013	0.0013
soybean oil	13.51	13.41	13.31	13.41	13.31
fucoxanthin	0	0.1	0.2	0	0
fucoxanthinol	0	0	0	0.1	0.2

Table 5. Fatty Acid Composition of Dietary Lipids Used in Experiment 2

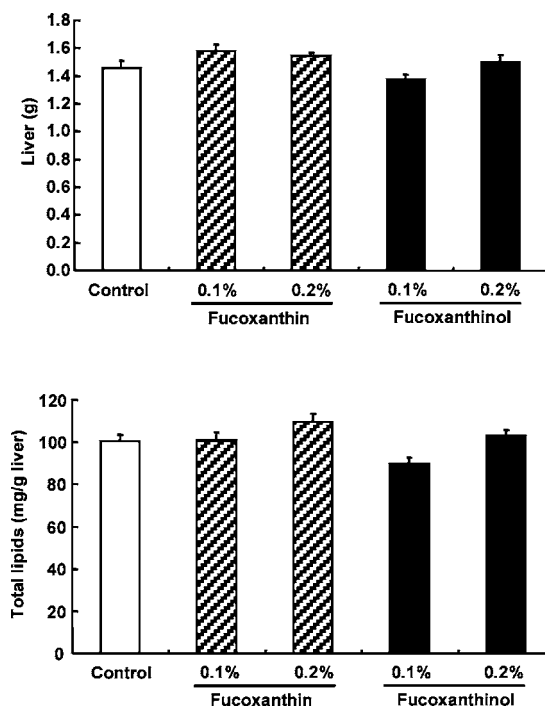|	control	0.1% fuco-xanthin	0.2% fuco-xanthin	0.1% fuco-xanthinol	0.2% fuco-xanthinol
16:0	10.3	10.3	10.6	10.5	10.3
18:0	3.1	3.1	3.0	3.1	3.0
18:1n-9	22.2	21.6	21.6	21.6	21.3
18:2n-6	53.7	54.9	54.7	54.1	55.2
18:3n-3	6.6	6.7	6.6	6.8	6.7

Figure 2. Weight of liver and total liver lipid in KKAy mice fed fucoxanthin- and fucoxanthinol-containing diets. Mice were fed diets containing purified fucoxanthin or fucoxanthinol at 0.1 and 0.2% for 4 weeks, respectively. Values are expressed as mean ±SEM ($n = 7$).

to increase with fucoxanthin diets, although this increase was not significant (**Figure 3**).

The amount of hepatic DHA was also significantly enhanced by feeding diets containing 0.2% purified fucoxanthinol with a 1.8-fold increase measured compared to the control group
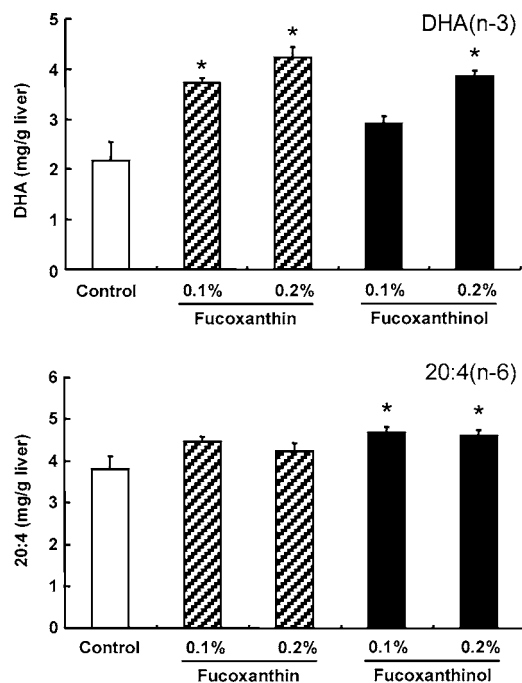
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Figure 3. Effect of fucoxanthin and fucoxanthinol on the amount of DHA and arachidonic acid in the liver of KKAy mice. Mice were fed diets containing purified fucoxanthin or fucoxanthinol at 0.1 and 0.2% for 4 weeks, respectively. Values are expressed as mean ±SEM ($n = 7$). An asterisk shows significant difference at $p < 0.05$ versus control mice.

(**Figure 3**). Furthermore, an increase in 20:4n-6 was observed in mice fed fucoxanthinol diets (**Figure 3**). These results show that dietary fucoxanthinol, which is a metabolite of fucoxanthin in the body, increases the amount of DHA and 20:4n-6 in the liver of KKAy mice. Fucoxanthin is metabolized to fucoxanthinol and then to amarouciaxanthin A in mice (9). 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. On the other hand, in the small intestine of mice fed 0.2% purified fucoxanthin or fucoxanthinol, an increase in DHA or 20:4n-6 was not observed (**Figure 4**). Because the liver has been considered to be the primary tissue for desaturation and elongation of unsaturated fatty acids, the increase in DHA and 20:4n-6 seen with fucoxanthin- and fucoxanthinol-supplemented diets was thought to result from a modification in the biosynthesis and degradation of HUFA in liver. However, an increase in hepatic 20:4n-6 was less compared to DHA, especially in mice fed fucoxanthin diets. It is therefore proposed that DHA (n-3) and 20:4n-6 may be synthesized by independent pathways involving n-3 and n-6 specific enzymes (28, 29) and that the degradation of 20:4n-6 is faster than that of DHA.

DHA is the end product in the n-3 HUFA biosynthesis pathway from 18:3n-3, through desaturation and elongation steps. However, Δ-6 desaturase is known to be the rate-limiting enzyme in such pathways in mammals (22, 23). In the current study, dietary glyceroglycolipid binding 18:4n-3 and EPA did not significantly enhance DHA levels in liver fatty acids, although both are precursor forms of DHA.

It has also been reported that synthetic peroxisome proliferators, fenofibrate (24) and clofibric acid (25), enhance the biosynthesis of 20:4n-6 from 18:2n-6 through activation of Δ-6 desaturase in normal and obese rats. Recently, a PPARα ligand, rosiglitazone, has been reported to increase DHA and EPA in adipose tissue but not in the liver, in a mouse model of type II diabetes (30). In addition, vitamin A deficiency has also been
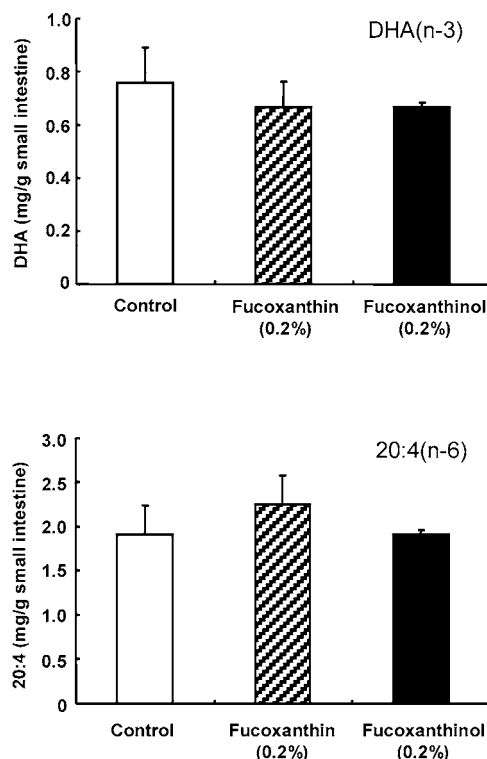

Figure 4. Effect of fucoxanthin and fucoxanthinol on the amount of DHA and arachidonic acid in the small intestine of KKAy mice. Mice were fed diets containing purified fucoxanthin or fucoxanthinol at 0.1 and 0.2% for 4 weeks, respectively. Values are expressed as mean ±SEM ($n = 7$).

reported to enhance the proportion of DHA in the liver of rats fed 18:3n-3 (31). Thus, DHA biosynthesis has been reported to be regulated by synthetic compounds and nutritional factors. However, there are only a few studies regarding the enhancement of DHA biosynthesis through the regulation of de novo synthesis. In the present study, we found for the first time that dietary fucoxanthin and fucoxanthinol enhance the amount of DHA in the liver of KKAy mice. Because purified fucoxanthin and fucoxanthinol diets contained only 18:3n-3 as precursor fatty acid of DHA, increased DHA is considered to be converted from 18:3n-3 through elongation and desaturation steps. Therefore, the results obtained in this study suggest that dietary fucoxanthin and fucoxanthinol may modify the biosynthesis and metabolic pathways of n-3 and n-6 HUFA.

DHA has many physiological effects related to health, growth, and development. Conversely, Wang et al. has reported that hepatic DHA levels in C57BL/6JLep[ob/ob] obese mice were lower than that in C57BL/6JLep[ob/+] lean mice, although the expression of elongase and desaturase was higher in obese than in lean mice (32). Administration of DHA to KKAy mice has been reported to reduce blood glucose and plasma free fatty acid levels (33). These results indicate that DHA is effective in preventing obesity and diabetes. Therefore, the novel effect of fucoxanthin and fucoxanthinol in increasing total DHA in the liver may contribute many beneficial effects.

In previous studies, we have reported that dietary fucoxanthin suppresses visceral fat accumulation in KKAy mice (7) and that fucoxanthin and fucoxanthinol inhibit 3T3-L1 preadipocyte differentiation (8). Thus, the multifunctional nature of fucoxanthin and its metabolite fucoxanthinol makes them highly suitable for the prevention of lifestyle-related diseases. However, further studies are required to evaluate the mechanisms responsible for the increase in hepatic DHA seen with these compounds.

In conclusion, our results show that dietary fucoxanthin and fucoxanthinol enhance the amount of DHA and 20:4n-6 in the liver of KKAy mice.

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