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Effect of Brown Seaweed Lipids on Fatty Acid Composition and Lipid Hydroperoxide Levels of Mouse Liver

M. K. Widjaja-Adhi Airanthi,*[,†] Naoya Sasaki,[†] Sayaka Iwasaki,[†] Nobuko Baba,[†] Masayuki Abe,[†,‡] Masashi Hosokawa,[†] and Kazuo Miyashita[†]

[†]Laboratory of Biofunctional Material Chemistry, Faculty of Fisheries Sciences, Hokkaido University, Hakodate, 041-8611, Japan

[‡]Kaneka Co., Takasago, Hyogo, Japan

ABSTRACT: Brown seaweed lipids from *Undaria pinnatifida* (Wakame), *Sargassum horneri* (Akamoku), and *Cystoseira hakodatensis* (Uganomoku) contained several bioactive compounds, namely, fucoxanthin, polyphenols, and omega-3 polyunsaturated fatty acids (PUFA). Fucoxanthin and polyphenol contents of Akamoku and Uganomoku lipids were higher than those of Wakame lipids, while Wakame lipids showed higher total omega-3 PUFA content than Akamoku and Uganomoku lipids. The levels of docosahexaenoic acid (DHA) and arachidonic acid (AA) in liver lipids of KK-A^y mouse significantly increased by Akamoku and Uganomoku lipid feeding as compared with the control, but not by Wakame lipid feeding. Fucoxanthin has been reported to accelerate the bioconversion of omega-3 PUFA and omega-6 PUFA to DHA and AA, respectively. The higher hepatic DHA and AA level of mice fed Akamoku and Uganomoku lipids would be attributed to the higher content of fucoxanthin of Akamoku and Uganomoku lipids. The lipid hydroperoxide levels of the liver of mice fed brown seaweed lipids were significantly lower than those of control mice, even though total PUFA content was higher in the liver of mice fed brown seaweed lipids. This would be, at least in part, due to the antioxidant activity of fucoxanthin metabolites in the liver.

KEYWORDS: brown seaweed lipids, fucoxanthin, omega-3 PUFA, DHA, antioxidant

INTRODUCTION

Seaweeds are used as foods worldwide. They are potentially good sources of nonstarch polysaccharides, minerals, trace elements, and certain vitamins.[1] Seaweed lipids have been reported to show high functionalities, although the lipids are found in relatively small quantities as compared with other major nutrients, carbohydrates and proteins.[2−4]

Average total lipid content of brown seaweed has been reported to be 1−6% per dry weight.[5] The lipids contained high level of omega-3 polyunsaturated fatty acids (PUFA) such as 18:4n-3 (stearidonic acid, SDA) and 20:5n-3 (eicosapentaenoic acid, EPA) together with omega-6 PUFA, 20:4n-6 (arachidonic acid, AA). Seaweed lipids also contain fucoxanthin, polyphenols, and fucosterol as bioactive phytochemicals. Among bioactive components of brown seaweed lipids, fucoxanthin has been known as a unique compound. Fucoxanthin, a characteristic carotenoid found in brown seaweeds, shows antiobesity effects based on specific molecular mechanism.[6,7] Fucoxanthin improves insulin resistance and decreases blood glucose level, at least in part, through the downregulation of several adipocytokines in WAT of animals.[7,8]

Tsukui et al.[9,10] also reported the increase in the proportion of docosahexaenoic acid (DHA) and AA in liver lipids of mice fed fucoxanthin. The feeding of fucoxanthinol, a main metabolite of fucoxanthin, also enhanced DHA and AA content in the liver lipids. DHA and AA can be biosynthesized through desaturation and elongation reaction steps beginning from α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LNA, 18:2n-6) in the liver, respectively. The increase in hepatic DHA and AA found in the animals fed fucoxanthin might be due to the upregulation of enzymatic activities related to the bioconversion of ALA to DHA and LNA to AA.[9,10]

The promotion effect of fucoxanthin on DHA and AA synthesis in the liver and the high content of PUFA in brown seaweed lipids suggest the increase in both PUFA in liver lipids by brown seaweed lipid feeding. To confirm this, we evaluated the fatty acid composition of liver lipids of mouse given the three kinds of brown seaweed lipids. On the other hand, PUFA such as DHA and AA are very easily oxidized in model systems as the oxidative stability of each PUFA is inversely proportional to its degree of unsaturation.[11,12] This suggests the induction of lipid oxidation by the increase in PUFA induced by brown seaweed lipid intake. In the present study, oxidation levels of liver lipids of mouse with or without seaweed lipid feeding were compared.

MATERIALS AND METHODS

Seaweed Lipid Extraction. Brown seaweeds used in this study were *Undaria pinnatifida* (Wakame), *Sargassum horneri* (Akamoku), and *Cystoseira hakodatensis* (Uganomoku). Akamoku and Uganomoku were collected freshly from Kamaishi, Iwate, Japan, in May and Hakodate, Hokkaido, Japan, in June, respectively. Samples collected were transported to the laboratory immediately in an iced condition and placed in a freezer (−18 °C) before further experiments. After more than 10 months storage, the frozen brown seaweeds, Akamoku and Uganomoku, were washed, cut, and dried with paper towels. Each seaweed (*ca.* 2 kg) was extracted with ethanol (*ca.* 4 L) overnight at room temperature under the dark. Wakame was obtained as dried powder from the market in Hakodate, Japan. The powder (*ca.* 2 kg) obtained was soaked in ethanol (*ca.* 4 L) overnight at room temperature under the dark. The

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Table 1. Composition (g/kg) of Experimental Diets Used in the Present Study

		Wakame lipids		Akamoku lipids		Uganomoku lipids	
diet ingredients	control	0.5%	2.0%	0.5%	2.0%	0.5%	2.0%
corn starch	346.3	346.3	346.3	346.3	346.3	346.3	346.3
dextrinized corn starch	115.0	115.0	115.0	115.0	115.0	115.0	115.0
casein	216.0	216.0	216.0	216.0	216.0	216.0	216.0
sucrose	87.1	87.1	87.1	87.1	87.1	87.1	87.1
cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0
oil							
soybean oil	135.1	130.1	115.1	130.1	115.1	130.1	115.1
seaweed lipids	0	5.0	20.0	5.0	20.0	5.0	20.0
L-cystine	3.0	3.0	3.0	3.0	3.0	3.0	3.0
AIN93G mineral	35.0	35.0	35.0	35.0	35.0	35.0	35.0
AIN93G vitamin	10.0	10.0	10.0	10.0	10.0	10.0	10.0
choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
tert-butylhydroquinone	0.014	0.014	0.014	0.014	0.014	0.014	0.014

solution was collected by filtration and concentrated in a rotary evaporator to obtain the extract. Chloroform−methanol−water (10:5:3, v/v/v) was added to the three different extracts in a separatory funnel. After shaking the funnel vigorously, the lower layer was evaporated under reduced pressure in a rotary evaporator. The last traces of the solvents and water remaining were removed under high vacuum to obtain seaweed lipids, namely, lipids from Wakame, Akamoku, and Uganomoku.

Total Phenolics of Extracted Lipids. Total phenolics was measured according to the method of Kuda et al.[13] An aliquot of seaweed lipid was weighed and dissolved in ethanol to adjust to a particular test concentration with a final volume of 100 μL. Each 100 μL of sample solution was added to 750 μL of 10% Folin−Ciocalteu solution and incubated at 37 °C. After 5 min, 750 μL of 6% Na_2CO_3 was added and the mixture allowed to stand at 37 °C for 30 min in the dark. The absorbance was measured at 750 nm. Total phenolics for each seaweed lipid was then calculated on the basis of a phloroglucinol standard curve and expressed as mg of phloroglucinol equivalent (PGE)/g lipid.

Fucoxanthin Content of Extracted Lipids. Fucoxanthin content was analyzed by high performance liquid chromatography (HPLC), as described previously.[5] The HPLC analysis was carried out with a Hitachi L-7000 HPLC system (Hitachi, Tokyo, Japan) equipped with a pump (L-7100), an autosampler (L-7200), a photodiode array detector (L-7455), and online analysis software (model D-7000). The analyses were carried out at room temperature using a reversed-phase column (Develosil-ODS UG-5, 250 × 4.6 mm i.d., 5.0 μm particle size; Nomura Chem. Co., Seto, Aichi, Japan) protected with a guard column having the same stationary phase. The mobile phase was methanol−acetonitrile (70:30, v/v), and the flow rate was 1.0 mL/min. Fucoxanthin was detected at 450 nm, and its content was estimated by the standard calibration curve using purified fucoxanthin (purity >98%). Purified fucoxanthin was isolated from the brown seaweed, Wakame, as described previously.[5]

α-Tocopherol Content of Extracted Lipids. HPLC was also used for the analysis of α-tocopherol (α-toc). The HPLC was carried out with the same system as described above except that fluoresence detector (Hitachi L-7485) was used for the analysis. The column used was Develosil 30-3 silica column (250 × 4.6 mm i.d). The mobile phase was hexane−2-propanol (99.2:0.8, v/v). The flow rate was 1.0 mL/min. The fluoresence detector was set at Ex. 298 nm and Em. 325 nm. The quantitative analysis was done with the calibration curve made using standard *dl*-α-tocopherol. *dl*-α-Tocopherol was obtained from Kanto Chemical Co. Inc., Tokyo, Japan.

Fatty Acid Analysis of Extracted Lipids. The fatty acid composition of sample lipid was determined by gas chromatography (GC) after conversion of fatty acyl groups in the lipid to their methyl esters. The fatty acid methyl esters were prepared according to the method by Prevot and Mordret.[14] Briefly, to an aliquot of total lipid (*ca.* 20 mg) 1 mL of *n*-hexane and 0.2 mL of 2 N NaOH in methanol were added, vortexed and incubated at 50 °C for 30 min. After the incubation, 0.2 mL of 2 N HCl in methanol solution was added to the solution and vortexed. The mixture was separated by centrifugation at 3000 rpm for 5 min. The upper hexane layer containing fatty acid methyl esters was recovered and subjected to GC analysis. The GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector (FID) and a capillary column (Omegawax-320; 30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA). The detector, injector, and column temperatures were 260, 250, and 200 °C, respectively. The carrier gas was helium at a flow rate of 50 kPa. Fatty acid content was expressed as weight percentage of total fatty acids.

Animal Experiments. All procedures for the use and care of animals were approved by the Ethical Committee of Experimental Animal Care at Hokkaido University, Japan. Animal experiments were carried out by using 4 weeks of age female KK-A^y mice that were obtained from Japan CREA Co., Osaka, Japan. The mice were housed at 23 ± 1 °C and at 50% humidity with a 12 h light/12 h dark cycle throughout the experiment. The mice had free access to drinking water and were fed a diet prepared according to the recommendations of American Institute of Nutrition (AIN-93G).[15] After acclimation for one week, mice were randomly divided into groups of seven and provided with the experimental diets. The body weight of each mouse was recorded daily, as well as diet and water intake.

The composition of the diets is shown in Table 1. The oil portion of the control diet contained all soybean oil (13.51% of total diet), while the experimental diets contained 13.01% or 11.51% soybean oil and 0.5% or 2.0% seaweed lipids, respectively. Three different forms of seaweed lipids, namely, Wakame lipids, Akamoku lipids, and Uganomoku lipids, were evaluated in comparison with the control in the experimental diets. All diets were vacuum-packed immediately after preparation and stored at −20 °C. To confirm the presence of fatty acid from each seaweed lipid of the diet, the lipid was extracted from the diet with chloroform/methanol (2:1, v/v). Fatty acid composition of the lipid was analyzed by GC after conversion of fatty acyl groups in the lipid to their methyl esters as described above.

Additional Animal Experiments. To confirm the accumulation of fucoxanthin metabolites in each tissue of mouse fed fucoxanthin,

additional animal experiments were carried out. Three weeks of age female or male KK-A^y were given the experimental diet containing 13.41% soybean oil and 0.1% purified fucoxanthin, or the diet contained 12.51% soybean oil and 1.0% Wakame lipids, respectively. Other dietary composition was the same as shown in Table 1. After acclimation for one week, mice were randomly divided into each group and provided with the experimental diets. Fucoxanthin was purified from Wakame lipids using silica gel column chromatography according to the procedure previously reported.[7]

Sample Collections. At the end of the experimental period (4 weeks), the mice were fasted for 12 h, and then the blood samples were taken from the mouse tail vein. Glucose levels in the blood were determined using a blood glucose level monitor (Glutest Neo; Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan). After 12 h of blood collection from the tail vein, mice were killed by exsanguination, and their blood was withdrawn at the abdominal artery. Liver, abdominal white adipose tissue (WAT), interscapular brown adipose tissue (BAT), and other tissue or organs were rapidly removed in their entirety and weighed. Blood plasma analysis was carried out by Analytical Center of Hakodate Medical Association (Hakodate, Japan). The analysis included measurement of the following parameters: total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerols.

Liver Lipid Analysis. Liver was perfused with 0.9% NaCl, and the lipids were extracted with chloroform/methanol (2:1, v/v) as described previously by Folch et al.[16] Liver samples from each mouse were analyzed separately. The fatty acid methyl esters from liver lipids were procured using the method of Prevot and Mordret[14] and analyzed by GC as described above. The lipids extracted were fractionated into neutral lipids and polar lipids using a cartridge of Sep-pak Silica (Nihon Waters, Tokyo, Japan). Total cholesterol and triacylglycerol levels of the liver were also determined by standard enzymatic assay methods, using Cholesterol E-Test Wako and Triglyceride E-Test Wako (Wako Pure Chemical Industries Ltd.).

Fucoxanthin Metabolites Analysis. After weighing each tissue or organ, total lipids were extracted according to the Folch method.[16] The protein content in the tissue was measured by the Lowry method.[17] An aliquot of the extracted lipids was dissolved in acetone and filtered with a 0.22 μm of membrane filter (PTFE Acrodisc; Wako, Osaka, Japan). The filtered sample was analyzed by HPLC equipped with two serially connected ODS columns (Develosil ODS-UG5) using methanol: hexane:dichloromethane: acetonitrile (10:2.5:2.5:85, v/v/v/v) as the mobile phase. Quantitative estimation of fucoxanthin metabolites was done using standard curve. The detection wavelength was set at 450 nm. Purified fucoxanthinol, a main metabolite of fucoxanthin, was used as authentic standard for the quantification of fucoxanthin metabolites. Fucoxanthinol (purity >99%) was prepared from purified fucoxanthin by hydrolysis with pancreas lipase according to a previous study.[9]

Hydroperoxide Analysis of Liver Lipids. Lipid hydroperoxide is known to react stoichiometrically with nonfluorescent diphenyl-1-pyrenylphosphine (DPPP) to give fluorescent DPPP oxide.[18] The hydroperoxide content of liver lipids was determined using this quantitative conversion of DPPP to DPPP oxide. Briefly, 10 mg of lipid extract was weighed and dissolved in 5 mL of chloroform (containing 10 mg/mL butylhydroxytoluene, BHT)/methanol (2:1, v/v). To a test tube with a screw cap, 100 μL of the sample solution and 50 μL of DPPP solution (1 mg/10 mL chloroform) were added and left for 60 min at 60 °C in a water bath. Then the solution was cooled in an ice bath and 3 mL of 2-propanol was added. The reaction mixture was diluted by 1:10 (v/v) before measurement by reversed phase HPLC. The HPLC analysis was carried out with a Hitachi L-2350 HPLC system (Hitachi, Tokyo, Japan) equipped with a pump (L-2130), an autosampler (L-2200) and a fluoresence detector (L-2485). All the HPLC analyses were done at 40 °C using a reversed-phase column (Develosil-ODS, UG-5), protected with a guard column (10 × 4.0 mm i.d.) having the same stationary phase. The mobile phase was butanol−methanol (10:90, v/v), and the flow rate was 1.0 mL/min. The fluoresence detector was set at Ex. 352 nm and Em. 380 nm. The hydroperoxide concentration in the sample solution was calculated from the DPPP oxide detected using a DPPP oxide standard curve. The hydroperoxides in the liver lipids were expressed as nmol/g liver.

Table 2. Total Phenolics, Fucoxanthin Content, and Fatty Acid Profile of Seaweed Lipids

	Seaweed lipids		
	Wakame lipids	Akamoku lipids	Uganomoku lipids
total phenolics[a]	26.96 ± 10.90	69.79 ± 4.79	79.00 ± 10.01
fucoxanthin[b]	16.16	21.19	20.10
fatty acid[c]			
14:0	1.47	3.75	3.46
16:0	5.87	18.33	17.45
18:1n-9	2.21	5.28	9.36
18:2n-6	4.11	5.64	9.12
18:3n-3	7.73	6.59	7.36
18:4n-3	41.99	13.57	16.42
20:4n-6	6.83	12.33	14.58
20:5n-3	17.33	14.36	13.32
total omega-3 PUFA	67.05	34.52	37.10

[a] Expressed as mg phloroglucinol equivalent per g lipid. [b] mg/g lipid. [c] Weight %.

Statistical Analysis. Results are shown as mean ± SD for seven mice. Differences between groups were examined for statistical significance using Dunnett's *t* test, except for analysis of hepatic hydroperoxide content and the levels of fucoxanthin metabolites in tissues, where Scheffe's test was used.

RESULTS

Composition of Brown Seaweed Lipids. Akamoku and Uganomoku lipids showed higher total phenolics than Wakame lipids (Table 2). Fucoxanthin contents of Akamoku and Uganomoku lipids were also higher than those of Wakame lipids, but the difference was small compared with that found in phenolics. The fucoxanthin levels of Akamoku and Uganomoku lipids were much lower than those found in the former study, where fucoxanthin content was 2−3 times higher than that found in the present study.[5]

Three kinds of brown seaweed lipids were rich in PUFA such as LNA (18:2n-6), ALA (18:3n-3), SDA (18:4n-3), AA (20:4n-6), and EPA (20:5n-3) (Table 2), their contents being in agreement with a former report.[5] SDA and EPA were not found in control soybean oil but in brown seaweed lipids at high levels, resulting in the increase in total omega-3 PUFA of dietary lipids by addition of these seaweed lipids from 6.31% (control) to 10.91% (Wakame lipids), 7.68% (Akamoku lipids), and 7.37% (Uganomoku lipids), respectively. AA was also detected in the experimental dietary lipids at levels of 0.54% (Wakame lipids), 1.13% (Akamoku lipids), and 1.26% (Uganomoku lipids), respectively, while no AA was found in the control dietary lipids.

Food and Water Intake, Body and Tissue Weight, and Lipid Levels of Plasma and Liver. Throughout the experimental period of 4 weeks, no significant difference was observed among the control groups and experimental groups in food intake (Table 3). Total water intake was significantly lower in 2% Wakame and Akamoku lipid fed mice than in control group. Body, liver, WAT,

Table 3. Final Body Weight, Total Food Intake, Total Water Intake, Liver Weight, WAT Weight, BAT Weight, and Muscle Weight of Mice Fed Control and Experimental Diets[a]

		Wakame lipids		Akamoku lipids		Uganomoku lipids	
	control	0.5%	2.0%	0.5%	2.0%	0.5%	2.0%
total food intake[b]	80.7 ± 20.3	70.7 ± 13.0	99.6 ± 8.2	68.8 ± 13.6	105.1 ± 8.7	77.8 ± 7.2	75.2 ± 6.6
total water intake[b]	332.5 ± 28.8	342.2 ± 22.5	243.1 ± 18.5*	446.7 ± 20.9	250.5 ± 14.7*	404.1 ± 27.3	424.5 ± 15.3
final body wt[c]	45.81 ± 1.22	44.69 ± 1.21	44.16 ± 0.71	45.22 ± 1.03	44.78 ± 1.02	46.06 ± 0.60	43.41 ± 1.07
final liver wt[c]	3.55 ± 0.10	3.62 ± 0.14	4.62 ± 0.26*	3.57 ± 0.16	4.00 ± 0.10	3.63 ± 0.15	3.86 ± 0.12
final WAT wt[c,d]	21.81 ± 0.77	23.36 ± 0.37*	21.25 ± 0.52	22.53 ± 0.39	21.43 ± 0.44	22.58 ± 0.43	21.08 ± 0.46
final BAT wt[c]	0.47 ± 0.02	0.57 ± 0.03	0.60 ± 0.03*	0.52 ± 0.02	0.55 ± 0.03	0.51 ± 0.04	0.53 ± 0.02
final muscle wt[c]	0.52 ± 0.01	0.52 ± 0.01	0.55 ± 0.01	0.50 ± 0.02	0.57 ± 0.01*	0.52 ± 0.01	0.56 ± 0.01
final spleen wt[c]	0.27 ± 0.02	0.24 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0.02	0.32 ± 0.02

[a] The values are mean ± SE for seven mice. *,**Significantly different from control (*$P < 0.05$, **$P < 0.01$). [b] Food intake and water intake are expressed as total g during experimental period. [c] Body weight and tissue weights are expressed as g/100 g body weight. [d] White adipose tissue (WAT) are made up of uterine, mesenteric, perirenal and retroperitoneal, and inguinal adipose tissues.

Table 4. Plasma Lipid Levels of Mice Fed Control and Experimental Diets[a]

		Wakame lipids		Akamoku lipids		Uganomoku lipids	
	control	0.5%	2.0%	0.5%	2.0%	0.5%	2.0%
total cholesterol (mg/dL)	155.9 ± 8.6	251.0 ± 4.5**	238.8 ± 6.8**	233.0 ± 11.7**	276.4 ± 5.4**	191.6 ± 11.7	257.4 ± 10.3**
HDL cholesterol (mg/dL)	75.3 ± 3.8	98.4 ± 2.3**	90.3 ± 2.3**	96.9 ± 2.7**	91.0 ± 1.7**	81.0 ± 4.5	88.9 ± 1.6**
triacylglycerol (mg/dL)	81.7 ± 7.0	78.6 ± 7.8	77.2 ± 9.8	112.7 ± 18.8	75.1 ± 8.3	157.6 ± 31.9	115.1 ± 19.8
phospholipids (mg/dL)	259.7 ± 11.9	330.9 ± 7.4**	306.3 ± 11.3**	336.7 ± 14.9**	358.7 ± 6.0**	305.3 ± 9.8**	349.9 ± 9.3**
free fatty acids (mg/dL)	1471.7 ± 137.8	1662.0 ± 121.1	1446.5 ± 125.4	1874.6 ± 225.7	1562.7 ± 112.1	1897.0 ± 206.8	1789.1 ± 180.9
glucose (mg/dL)[b]	260.3 ± 22.5	289.7 ± 33.2	252.2 ± 28.2	369.9 ± 51.1	256.9 ± 16.3	313.4 ± 39.2	308.1 ± 22.3

[a] The values are mean ± SE for seven mice. **Significantly different from control ($P < 0.01$). [b] Blood glucose levels were measured without fasting after 28 days of feeding.

Table 5. Liver Lipid Levels of Mice Fed Control and Experimental Diets[a]

		Wakame lipids		Akamoku lipids		Uganomoku lipids	
	control	0.5%	2.0%	0.5%	2.0%	0.5%	2.0%
total lipids (mg/g liver)	107.2 ± 20.5	83.6 ± 28.7	124.3 ± 29.7	91.2 ± 21.7	78.0 ± 14.4	98.3 ± 18.8	80.1 ± 11.3
neutral lipids (mg/g liver)	83.7 ± 19.4	46.6 ± 7.8**	54.5 ± 10.5	64.4 ± 18.3	27.7 ± 7.5**	51.5 ± 4.8**	31.9 ± 7.7**
polar lipids (μg/g liver)	20 ± 20	45 ± 22	61 ± 18**	34 ± 26	50 ± 11*	47 ± 18	48 ± 11*
total cholesterol (μmol/g liver)	8.07 ± 2.73	3.12 ± 1.21**	3.98 ± 1.03*	3.09 ± 1.80**	1.92 ± 1.06**	7.27 ± 1.50	4.12 ± 1.14**
triacylglycerol (μmol/g liver)	46.5 ± 9.7	17.2 ± 6.4**	13.3 ± 6.4**	26.8 ± 4.8**	19.4 ± 6.7**	27.1 ± 4.7**	16.7 ± 4.5**

[a] The values are mean ± SE for seven mice. *,**Significantly different from control (*$P < 0.05$, **$P < 0.01$).

BAT, muscle, and spleen weights were almost the same in these groups except for liver and BAT weights of 2.0% Wakame lipids, WAT weight of 0.5% Wakame lipids, and muscle weight of 2.0% Akamoku lipids.

Plasma total cholesterol, HDL cholesterol, and phospholipid levels of mice fed seaweed lipids increased significantly as compared with control group, while little difference was observed in triacylglycerol and glucose levels (Table 4). On the contrary, hepatic cholesterol levels decreased significantly by seaweed lipid feedings (Table 5). Neutral lipid contents and triacylglycerol levels in the liver lipids were also reduced by the seaweed lipid intakes (Table 5).

Fatty Acid Composition and Hydroperoxide Levels of Liver Lipids. Although the main source of lipid in all test diets was soybean oil (Table 1), an unexpected increase in highly unsaturated fatty acids such as AA and DHA was found in the liver lipids of mice fed Akamoku and Uganomoku lipids (Table 6). On the other hand, Wakame lipid feeding had no significant increase in AA and DHA as compared with control.

Table 6. PUFA Contents (Weight %/Total Fatty Acids) of Liver Lipids of Mouse Fed Control and Experimental Diets

fatty acid	control	Seaweed lipids (2%)		
		Wakame lipids	Akamoku lipids	Uganomoku lipids
18:2n-6	19.65 ± 0.49	18.44 ± 1.88	18.37 ± 1.02	20.57 ± 0.66
18:3n-3	0.18 ± 0.01	0.11 ± 0.01	0.12 ± 0.00	0.18 ± 0.01
18:4n-3	ND[a]	ND[a]	ND[a]	ND[a]
20:4n-6	6.12 ± 0.64	6.27 ± 1.04	10.79 ± 0.53**	10.92 ± 0.65**
20:5n-3	0.14 ± 0.01	0.71 ± 0.14**	0.53 ± 0.10**	0.48 ± 0.05**
22:6n-3	3.36 ± 0.22	4.45 ± 0.77	6.53 ± 0.79**	5.73 ± 0.41**
highly unsaturated fatty acids[b]	9.62	11.43	17.85	17.13

[a] Not detected. [b] Total content of 20:4n-6 + 20:5n-3 + 22:6n-3. **Significantly different from control ($P < 0.01$).

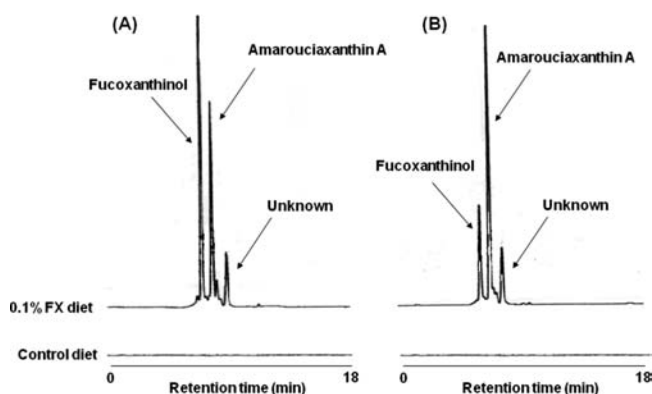

Figure 1. HPLC chromatogram of carotenoids found in the total lipids from liver (A) and abdominal WAT (B) of mouse fed control and 0.1% fucoxanthin (FX) containing diet.

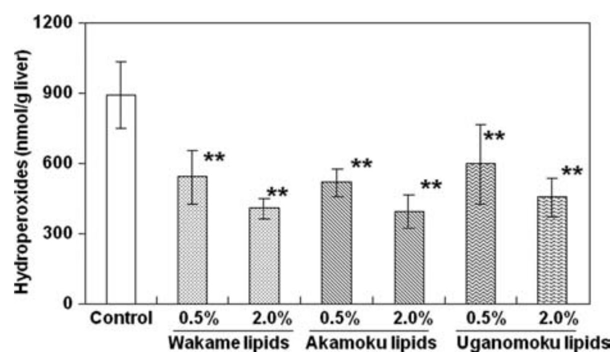

Figure 2. Lipid hydroperoxide contents (nmol/g liver) of liver lipids of mouse fed control and experimental diets.

Hydroperoxides were detected in the liver lipids (Figure 2). The hydroperoxide levels of the mice fed brown seaweed lipids were significantly lower than that of control mice.

Fucoxanthin Metabolite Contents. Figure 1 shows the common HPLC chromatogram of carotenoids found in the total lipids extracted from liver (A) and abdominal WAT (B) of female KK-A^y mice given 0.1% purified fucoxanthin containing diet. Three peaks appeared in the chromatogram. Two of them were identified as fucoxanthinol and amarouciaxanthin A by nuclear magnetic resonance and mass spectral analyses.[10] The other peak could not be identified because of its low concentration. Judging from the retention time and the UV absorption curve, it was considered to be a *cis*-isomer of fucoxanthinol or amarouciaxanthin A.

Total fucoxanthin metabolites detected in three kinds of WAT lipids was calculated to be 11.486 μg/mg protein (Table 7). This was more than 80% of those detected (13.613 μg/mg protein) in all kinds of tissue lipids. The main metabolite of WAT was amarouciaxanthin A, while that of other tissues was fucoxanthinol (Table 7 and Figure 1). The same result was obtained from the male KK-A^y mice given 1.0% Wakame lipids containing 17.6 mg fucoxanthin/g lipids (Table 8). More fucoxanthin metabolites were accumulated in WAT than in liver, and the main fucoxanthin metabolite was amarouciaxanthin A for WAT and fucoxanthinol for liver.

DISCUSSION

Fucoxanthin contents of Wakame, Akamoku and Uganomoku lipids (Table 2) were much lower than those reported previously.[5,6] Wakame lipids used in this study were extracted from dried powder obtained from the market in Hakodate. Fucoxanthin might be decomposed during the production and storage of the powder. Akamoku and Uganomoku were collected in May and June, respectively. Fucoxanthin content has been reported to vary with several biological and environmental factors.[5] It is generally increased during the growth of the seaweed, but decreased after that. Akamoku and Uganomoku stop their growth before May and June in each location, respectively. Furthermore, the extraction of lipids has been carried out after more than 10 months of storage in a freezer. During the storage, a part of fucoxanthin would be oxidized and decomposed. Gouveia and Empis[19] also have reported the stability of biomass of algae predominantly affected by light and oxygen, as well as storage temperature to a lesser extent. It is apparent that both factors, collecting season and long storage, would reduce the fucoxanthin contents in Akamoku and Uganomoku used in the present study.

Fucoxanthin shows an antiobesity effect through the uncoupling protein 1 (UCP1) induction of WAT mitochondria.[2−4] However, there was little change in body weight and abdominal WAT weights of mice fed brown seaweed lipids (Table 3). This would be due to the lower fucoxanthin level of these brown seaweed lipids used in the present study. Previous studies using Wakame lipids or purified fucoxanthin[6−8] have demonstrated that significant reduction of body and abdominal WAT weights was obtained by giving more than 100 mg fucoxanthin/100 g diet to KK-A^y mice. However, fucoxanthin levels of 0.5% and 2.0% Wakame lipids diets were calculated to be 8.08 and 32.32 mg/100 g diet, respectively. Those of 0.5% and 2.0% Akamoku and Uganomoku lipids diets were also calculated to be 10.6 and 42.38 mg/100 g diet, and 10.05 and 40.2 mg/100 g diet, respectively.

When fucoxanthin intake of mouse in the present study is roughly calculated from the fucoxanthin content of 2.0%

Table 7. Contents (mg/mg protein) of Fucoxanthin Metabolites in Total Lipids from Each Tissue and Organ of Mice Fed Purified Fucoxanthin (0.1%)

fucoxanthin metabolites	liver	uterine WAT	intestinal WAT	perirenal WAT	kidney	spleen	small intestine	large intestine
fucoxanthinol	0.308 ± 0.082	0.849 ± 0.316	0.671 ± 0.288	0.741 ± 0.288	0.402 ± 0.082	0.142 ± 0.048	0.293 ± 0.119	0.185 ± 0.113
amarouciaxanthin A	0.191 ± 0.037	2.672 ± 0.952	2.177 ± 0.669	2.336 ± 0.772	0.174 ± 0.031	0.080 ± 0.015	0.075 ± 0.017	0.063 ± 0.033
unknown	0.068 ± 0.016	0.715 ± 0.233	0.654 ± 0.180	0.672 ± 0.160	0.059 ± 0.010	0.023 ± 0.006	0.037 ± 0.013	0.027 ± 0.013

Table 8. Contents (mg/mg protein) of Fucoxanthin Metabolites in Total Lipids from Liver and WAT of Mice Fed Wakame Lipids (1.0%)

fucoxanthin metabolites	liver	epididymal WAT	mesenteric WAT	perirenal WAT
fucoxanthinol	0.197 ± 0.045	0.671 ± 0.284	0.400 ± 0.220	0.306 ± 0.154
amarouciaxanthin A	0.111 ± 0.043	2.961 ± 0.900	2.057 ± 1.273	1.100 ± 0.592
unknown	0.013 ± 0.005	0.933 ± 0.279	0.622 ± 0.436	0.371 ± 0.229

Akamoku lipids diet, average feed intake (>3 g diet per day), and average mouse body weight, it was 26.8 mg fucoxanthin intake/kg mouse/day. This fucoxanthin level was not enough to show an antiobesity effect in the mouse (Table 3). On the other hand, a recent study[20] demonstrated the significant reduction of abdominal WAT of obese female volunteers by intake of fucoxanthin less than 0.024 mg/kg/day (2.4 mg intake/day for volunteers with 100 kg average weight). This difference in the effectiveness between rodents and humans may be due to the different absorption rate and/or to different sensitivity to fucoxanthin.

Table 4 shows the effect of seaweed lipids on plasma lipid parameters and glucose level. Plasma total cholesterol, HDL cholesterol, and phospholipid levels of mouse fed seaweed lipids increased significantly as compared with control group. We have already reported that fucoxanthin intake significantly increased total cholesterol level as well as HDL cholesterol level.[21] Increased effect of xanthophylls such as canthaxanthin and astaxanthin on blood cholesterol levels has been also reported in rodents, although the mechanism has been still unclear.[22,23] Further study will be needed to clarify why xanthophyll intake increases circulating cholesterol. On the contrary, hepatic cholesterol levels decreased significantly by seaweed lipid feedings (Table 5). Neutral lipid contents and triacylglycerol levels in the liver lipids were also reduced by seaweed lipid intake (Table 5).

AA (20:4n-6) and DHA (22:6n-3) are the end products in the omega-3 and omega-6 PUFA metabolic pathway from LNA (18:2n-6) and ALA (18:3n-3), respectively. Key enzymes related to the bioconversion of omega-3 and omega-6 PUFA to AA and DHA are D5- and D6-desaturases, which can be modulated by various factors.[24,25] We have reported that fucoxanthin intake significantly increased AA and DHA contents probably due to upregulation of D6-desaturase expression.[9,10] Fucoxanthin content of both Akamoku and Uganomoku lipids was higher than that of Wakame lipids as shown in Table 2. This higher level of fucoxanthin would be due to the significant increase in AA and DHA found in the liver lipids of mice given Akamoku and Uganomoku lipids (Table 6).

AA and DHA are known to be very easily oxidized in model systems as the oxidative stability of each PUFA is inversely proportional on its degree of unsaturation.[11,12] Thus, the higher levels of these PUFA in the liver of mouse fed Akamoku and Uganomoku lipids might induce the peroxidation of liver lipids. However, lipid peroxidation level was significantly reduced by Akamoku and Uganomoku lipid feeding (Figure 2). The result in Figure 2 suggests the increase in hepatic antioxidant capacity of mice fed brown seaweed lipids.

Vitamin E (α-tocopherol) is one of the most well-known antioxidants. α-Tocopherol content of Akamoku and Uganomoku lipids was 1156 μg/g lipid and 1654 μg/g lipid, respectively. They were higher than those of Wakame lipids (91 μg α-tocopherol/g lipid) and soybean oil (80 μg α-tocoperol/g lipid). However, AIN 93G diet contained 150 mg α-tocopherol/kg diet and brown seaweed lipid content was 0.5 or 2.0%, suggesting little effect of higher amount of α-tocopherol in Akamoku and Uganomoku lipids on the lower hepatic hydroperoxide levels of mice fed these brown seaweed lipids.

Phenolic compounds of brown seaweeds are another candidate to explain the *in vivo* antioxidant activity of Akamoku and Uganomoku lipids found in the present study (Figure 2). Nakai et al.[26] screened the antioxidant activities of 50% ethanol extracts of 25 common Japanese seaweeds. They found that phlorotannins, major phenolics of brown seaweeds, showed strong scavenging potencies on superoxide anion radicals, which were around 5 times higher than that of catechin. Shibata et al.[27] also reported that several phlorotannins from the Japanese Laminariaceous brown seaweeds exhibited extraordinary superoxide anion radical scavenging abilities, which was around 2−10 times more effective than ascorbic acid and α-tocopherol.

It is clear that brown seaweed polyphenols have antioxidant activity. However, the evaluation of the effects exerted by seaweed polyphenols gives several problems when moving from simple experimental systems to the complexity of an animal body. The major problem is their bioavailability and the difficulties in unraveling the complex mechanisms of absorption and metabolism. To date most of the studies on the biological activities of seaweed polyphenols have been done in *in vitro* systems using cultivated cell lines. To ensure the involvement of brown seaweed polyphenols in the antioxidant effect on the liver lipid oxidation, basic research will be needed to define the absorption rate of seaweed polyphenols and to identify their metabolites.

In the present study, we could not find any compounds related to brown seaweed phenolic compounds or their metabolites in HPLC analysis of the extracted liver lipids (data not shown). Moreover, three kinds of brown seaweed lipids used in this study showed similar *in vivo* antioxidant activity (Figure 2), though Akamoku and Uganomoku lipids contained two times higher total phenolics than Wakame lipids (Table 2). These results suggest the

contribution of other antioxidants to *in vivo* antioxidant activity of brown seaweed lipids.

Contrary to seaweed polyphenols, the metabolic pathway of fucoxanthin is well documented. Fucoxanthinol and amarouciaxanthin A are known as major fucoxanthin metabolites.[28] Dietary fucoxanthin is hydrolyzed to fucoxanthinol in the gastrointestinal tract by digestive enzymes such as lipase and cholesterol esterase,[29] and then, some of the fucoxanthinol converts to amarouciaxanthin A in the liver.[28] Fucoxanthinol and amarouciaxanthin A have been detected in plasma and tissues of mouse with different accumulation ratio.[10,30,31]

Fucoxanthin metabolites were not analyzed in the first experiment using three kinds of brown seaweed lipids. Thus, additional study was conducted to clarify the fucoxanthin metabolites accumulating in KK-A^y mice by giving 0.1% purified fucoxanthin containing diet (100 mg fucoxanthin/100 g diet) or 1.0% Wakame lipid containing diet to the mouse. As shown in Tables 7 and 8, the main fucoxanthin metabolite was amarouciaxanthin A for WAT and fucoxanthinol for liver. This result is consistent with a former study, which showed that dietary fucoxanthin preferentially accumulates as amarouciaxanthin A in the adipose tissue and as fucoxanthinol in the other tissues such as liver.[29] We have reported the strong antioxidant activity of fucoxanthinol.[32] Thus, fucoxanthinol accumulation in the mouse liver would be one of the main reasons for the *in vivo* antioxidant activity of brown seaweed lipids.

AUTHOR INFORMATION

Corresponding Author

*E-mail: airanthi@fish.hokudai.ac.jp; airanthi@yahoo.com. Phone: +81 (138) 408892. Fax: +81 (138) 408892.

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ABBREVIATIONS USED

PUFA, polyunsaturated fatty acids; AA, arachidonic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid; LNA, linoleic acid; SDA, stearidonic acid; EPA, eicosapentaenoic acid; UCP1, uncoupling protein 1; WAT, white adipose tissue; BAT, brown adipose tissue; HDL, high density lipoprotein; *ca.*, circa; PGE, phloroglucinol equivalent; HPLC, high performance liquid chromatography; GC, gas chromatography; α-toc, α-tocopherol; AIN-93G, American Institute of Nutrition-93 Growth; PTFE, polytetrafluoroethylene; DPPP, diphenyl-1-pyrenylphosphine; ODS, octadecylsilane.

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