

Dietary Fish Oil and *Undaria pinnatifida* (Wakame) Synergistically Decrease Rat Serum and Liver Triacylglycerol¹

Masakazu Murata,² Yoko Sano, Kenji Ishihara and Motoharu Uchida

Laboratory of Applied Microorganisms, Marine Biochemistry Division, National Research Institute of Fisheries Science, Fisheries Research Agency Kanazawa-ku, Yokohama 236-8648, Japan

ABSTRACT Japanese eating habits are characterized by the consumption of various food materials such as cereals, vegetables, fish, shellfish, marine algae and meat. Therefore, properties of functional substances in food materials may be enhanced or lessened by the combination of various food materials. In the present study, we examined how the combination of wakame and fish containing polyunsaturated fatty acids, which are typical Japanese food materials, affected rat lipid metabolism. Rats were fed one of four diets [control diet (C), AIN-76 diet with 5 g/100 g rapeseed oil; wakame diet (W) containing 19.1 g/100 g *Undaria pinnatifida* (wakame) dried powder in the C diet; fish oil diet (FO), AIN-76 diet with 4.1 g/100 g fish oil; wakame-fish oil diet (W + FO), the FO diet containing 19.1 g/100 g dried wakame powder] for 4 wk. We measured the concentration of lipids in serum and liver and hepatic activities of enzymes involved in fatty acid metabolism. The W diet, FO diet and W + FO diet significantly reduced the concentration of triacylglycerols in the serum and liver compared with the C diet. This decrease in the concentration of hepatic triacylglycerol was greatest in rats fed the W + FO diet. The activity of glucose-6-phosphate dehydrogenase, which is involved in fatty acid synthesis in the liver, of rats fed the W, FO and W + FO diets was lower than that in rats fed the C diet. However, the activities of malic enzyme and fatty acid synthetase did not differ among the four groups. In contrast, the W diet and W + FO diet increased the serum concentration of β -hydroxybutyrate. Further, the activity of 3-hydroxyacyl-CoA dehydrogenase, which is involved in fatty acid β -oxidation in the liver, was greater in rats fed the W diet (42%), the FO diet (154%) and the W + FO diet (381%) than in those fed the C diet. Because the decrease in the concentration of triacylglycerol in the liver was greatest when rats were fed wakame and fish oil at the same time (W + FO diet), we conclude that there was a synergistic process affecting fatty acid β -oxidation in the liver. These results suggest that the simultaneous consumption of fish (fish oil) and wakame decreases the concentration of triacylglycerol in the serum and liver. J. Nutr. 132: 742-747, 2002.

KEY WORDS: • *Undaria pinnatifida* (wakame) • fish oil • 3-hydroxyacyl-CoA dehydrogenase • fatty acid • β -oxidation • rats

Undaria pinnatifida (wakame), a brown seaweed that contains several minerals, vitamins and dietary fiber, is a constituent of traditional Japanese cuisine. Recently, it has been reported that wakame contains several elements with biological activities, such as coagulation protection in human blood and antitumor and antimutagenic activities (1-3). Further, we reported that dietary wakame decreased the concentration of triacylglycerol in rat serum and liver as a result of increased activities of hepatic enzymes involved in fatty acid oxidation. We concluded that wakame is a useful food material for the prevention and treatment of hypertriacylglycerolemia (4).

It is possible that the changes in the food habits of Japanese people, which had been characterized by the consumption of rice, vegetables and marine products, to a more European and American type of diet, characterized by the consumption of

meat and dairy products, have led to an increase in the incidence rates of various diet-related adult diseases. Traditional Japanese eating habits are characterized by the consumption of various food materials such as cereals, vegetables, fish, shellfish and marine algae, which are cooked by various techniques such as steaming, roasting, boiling and frying. Therefore, properties of the functional substances in food materials may be enhanced or lessened by the combination of various food materials, and the chemical changes that occur during the processing and the cooking steps. Therefore, it is necessary to analyze scientifically the influence of recent Japanese eating habits on health.

Many studies have indicated that fish oil rich in eicosapentaenoic acid (EPA)³ and docosahexaenoic acid (DHA) decreases the triacylglycerol concentrations in the serum and

¹ Supported in part by the Ministry of Agriculture, Forestry and Fisheries, Japan.

² To whom correspondence should be addressed. E-mail: murama@affrc.go.jp.

³ Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 16:0-CoA, palmitoyl-CoA; 18:1-CoA, oleoyl-CoA; 20:4-CoA, arachidonoyl-CoA; C diet, control diet; W diet, wakame diet; FO diet, fish oil diet; W + FO diet, wakame and fish oil diet.

liver as a result of the promotion of hepatic fatty acid β -oxidation (5–8).

The typical Japanese breakfast is often composed of rice, baked fish and miso soup with wakame. It is not known whether the triacylglycerol metabolism is changed by consumption of a diet containing both wakame and fish oil at the same time. To address this, we fed rats diets containing wakame and fish oil and examined in rats the influence of these diets on the concentration of lipids in the serum and liver, and the activities of fatty acid metabolizing enzymes in liver.

MATERIALS AND METHODS

Materials. The dried wakame powder was obtained from Riken Vitamin (Tokyo, Japan), and fish oil was obtained from Nippon Chemical Feed (Hokkaido, Japan). The constituents (g/100 g) of dried wakame powder were 17.2 g protein, 3.7 g lipid, 40.6 g carbohydrate, 3.1 g fiber and 5.0 g minerals (9). The fatty acid composition (g/100 g total fatty acid) of fish oil determined by gas-liquid chromatography (10) was as follows: 16:0, 10.3; 16:1, 8.1; 18:0, 2.6; 18:1, 11.4; 18:2, 1.3; 18:3(n-3), 1.0; 18:4(n-3), 5.1; 20:4, 1.8; 20:5(n-3), 34.2; and 22:6(n-3), 17.3. Palmitoyl-CoA (16:0-CoA), oleoyl-CoA (18:1-CoA) and arachidonoyl-CoA (20:4-CoA) were prepared according to the method of Kawaguchi et al. (11). Acetyl-CoA, acetoacetyl-CoA and malonyl-CoA were purchased from the Sigma Chemical (St. Louis, MO).

Animal and diets. Male Sprague-Dawley rats ($n = 28$) obtained from the Charles River (Kanagawa, Japan) were kept in an air-conditioned room (temperature, 20–22°C; humidity, 55–65%; lights on, 0700–1900 h), and fed a commercial nonpurified diet (Type NMF; Oriental Yeast, Tokyo, Japan) for 1 wk. After acclimation to the housing conditions, rats were divided into the following four dietary groups: control (C) diet; wakame (W) diet; fish oil (FO) diet; wakame and fish oil (W + FO) diet. The experimental diets were prepared according to the recommendations of the American Institute of Nutrition (AIN-76) [Table 1 (12)]. All of the diet ingredients were products of Oriental Yeast. Nutrient contents were standardized by subtracting the amounts of nutrients included in dried wakame powder from the basic AIN-76 diet. To standardize nutrient contents among the diets, we replaced 5 g/100 g diet of protein in the AIN-76 diet with protein of dried wakame powder in the W and W + FO diets. Therefore, dried wakame powder was added to these diets at a level of 19.1 g/100 g diet. In the W diet and W + FO diet, 19.1 g/100 g diet of the dried wakame powder in the diet contained 0.9 g of lipids. Therefore, we adjusted the concentration of rapeseed oil and fish oil to 4.1 g/100 g diet in the W diet and W + FO diet. Further, to adjust the fish oil concentration in the W + FO diet and FO diet, fish oil at 4.1 g/100 g diet and rapeseed oil at 0.9 g/100 g diet were added to the FO diet. However, mineral contents likely differed among the diets because wakame contains high levels of various minerals. In the present study, we did not adjust the mineral contents because it was impossible to completely standardize the mineral concentrations of all diets.

The care and treatment of the experimental animals conformed to the National Research Institute of Fisheries Science guidelines for the ethical treatment of laboratory animals. Rats were fed these experimental diets for 4 wk.

Enzyme assays. At the end of the experiment, rats were lightly anesthetized with diethyl ether, bled from the abdominal aorta and livers were quickly excised. Liver (~3 g) from each rat fed the experimental diets was homogenized with 7 volumes of 0.25 mol/L sucrose and centrifuged at 500 \times g for 10 min. The supernatant was re-centrifuged at 9000 \times g for 10 min to isolate the mitochondria. The mitochondrial fraction was washed twice with 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris-HCl (pH7.0) and finally suspended in the same medium to give a protein concentration of 20–25 g/L. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (13), malic enzyme (EC 1.1.40) (14) and fatty acid synthetase (15) activities were measured in the 9000 \times g supernatant fraction of the liver homogenate (16).

TABLE 1

Composition of the experimental diets

Ingredient	Diet			
	C	W	FO	W + FO
	g/100 g			
Casein ²	20.0	15.0	20.0	15.0
Lipid ²				
Rapeseed oil ³	5.0	4.1	0.9	0
Fish oil ⁴	0	0	4.1	4.1
Cellulose ²	5.0	4.33	5.0	4.33
Potato starch ²	15.0	15.0	15.0	15.0
AIN-76 vitamin mixture ²	1.0	1.0	1.0	1.0
AIN-76 mineral mixture ²	3.5	3.5	3.5	3.5
DL-Methionine ⁵	0.3	0.3	0.3	0.3
Dried wakame powder ⁶	0	19.1	0	19.1
Sucrose ²	50.2	37.66	50.2	37.66

¹ Abbreviations used: C, AIN-76 diet; W, AIN-76 diet with dried wakame powder; FO, AIN-76 diet with fish oil; W + FO, AIN-76 diet with dried wakame powder and fish oil.

² Oriental Yeast (Tokyo, Japan).

³ Aiku (Tokyo, Japan) provided the following fatty acids (g/100 g of total fatty acids): 16:0, 4.0; 18:0, 2.0; 18:1, 58.6; 18:2, 22.0; 18:3(n-3), 11.0.

⁴ Nippon Chemical Feed (Hokkaido, Japan) providing the following fatty acids (g/100 g of total fatty acids): 16:0, 10.3; 16:1, 8.1; 18:0, 2.6; 18:1, 11.4; 18:2, 1.3; 18:3(n-3), 1.0; 18:4(n-3), 5.1; 20:4, 1.8; 20:5(n-3), 34.2; 22:6(n-3), 17.3.

⁵ Wako Pure Chemical Industries (Osaka, Japan).

⁶ Riken vitamin (Tokyo, Japan). 19.1 g of dried wakame powder contained 0.9 g of lipid and their fatty acid composition (g/100 g of total fatty acids) was 16:0, 12.8; 18:0, 0.4; 18:1, 4.5; 18:2, 5.8; 18:3(n-3), 12.4; 18:4(n-3), 33.9; 20:4, 11.3; 20:5(n-3), 16.1; 22:6(n-3), trace.

The supernatant fraction obtained after centrifugation of the liver homogenate at 500 \times g for 10 min was used for measurements of the activities of the fatty acid oxidation enzymes except for carnitine palmitoyltransferase (EC 2.3.1.21) and acyl-CoA dehydrogenase (EC 1.3.99.3) (17). Carnitine palmitoyltransferase activity was measured in the isolated mitochondria solubilized with Triton X-100 according to the method described in Murata et al. (4). Acyl-CoA dehydrogenase activity was measured in isolated mitochondria according to the method described by Dommes and Kunau (18) and Dommes et al. (19) except that phenazine methosulfate was used as the primary electron acceptor (4). Acyl-CoA oxidase (EC 1.3.3.6) activity was measured in the 500 \times g supernatant fraction of liver homogenates as described elsewhere (20,21). Palmitoyl-CoA was used as a substrate for the carnitine palmitoyltransferase assay and palmitoyl-CoA, oleoyl-CoA, and arachidonoyl-CoA were used as substrates for the acyl-CoA dehydrogenase and acyl-CoA oxidase assays. Acetoacetyl-CoA was used as a substrate for 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (22) in the 500 \times g supernatant fraction of the liver homogenate. The activities of marker enzymes for cell organelles including succinate dehydrogenase (EC 1.3.99.1) (mitochondria) (23) and catalase (EC 1.11.1.6) (peroxisomes) (24) were determined in the 500 \times g supernatant fraction of liver homogenates.

Liver carnitine was determined in the perchloric acid extracts from the liver homogenate as described by Deufel and Wieland (25). Protein in fractions of the liver homogenate were determined by the methods of Lowry et al. (26)

Lipid analyses. The lipids in the serum and liver were extracted and purified (27). The concentrations of triacylglycerol, phospholipids and cholesterol in the extracts were determined as described by Hara et al. (28). The fatty acid composition of the fish oil was determined using gas-liquid chromatography (10). β -Hydroxybutyrate in the serum was measured enzymatically in a deproteinized sample as described elsewhere (29).

TABLE 2

Body weight, relative tissue weight and food intake of rats fed a control diet (C) or diets containing wakame (W), fish oil (FO) or both (W + FO) for 4 wk^{1,2}

	Diet				ANOVA (P-value) ³		
	C	W	FO	W + FO	Wakame	Fish oil	Wakame × Fish oil
Body, g	372 ± 9	360 ± 9	390 ± 12	363 ± 13	NS	NS	NS
Liver, g/100 g body	4.77 ± 0.19 ^a	4.02 ± 0.03 ^{bc}	4.56 ± 0.17 ^{ab}	3.89 ± 0.10 ^c	<0.001	NS	NS
Adipose tissue, g/100 g body	1.32 ± 0.06 ^a	0.83 ± 0.02 ^b	1.12 ± 0.10 ^a	0.73 ± 0.06 ^b	<0.001	NS	NS
Food intake, g/28 d	607.5 ± 17.1	625.5 ± 16.9	619.7 ± 15.4	625.0 ± 15.3	NS	NS	NS

¹ Values are the means ± SEM, n = 7.

² Different superscripts in a row indicate significant differences (P ≤ 0.05) among groups.

³ NS, not significant, (P > 0.05).

Statistical analyses. All values are expressed as the mean ± SEM. Data were analyzed by two-way ANOVA and post-hoc Duncan's multiple range test (30,31). Differences of P < 0.05 were considered significant. The analyses were performed by the macro statistics programs using Microsoft Excel (Microsoft, Redmond, WA).

RESULTS

The body weight of rats was 141.7 ± 8.9 g for the 28 rats at the beginning of this experiment. Throughout the experiment, food intake did not differ among groups (Table 2). Body weight gain tended to be less (P = 0.067) in rats fed the wakame-supplemented diets compared with rats fed diets without wakame. Moreover, rats fed the wakame-supplemented diets had lower relative liver and adipose tissue around the testis weights compared with rats fed the C diet (Table 2).

The serum triacylglycerol concentration was lower in rats fed the W, FO and W + FO diets than in rats fed the C diet (Table 3). Moreover, the FO and W + FO diets decreased the concentration of triacylglycerol compared with the W diet. The W, FO and W + FO diets decreased the concentration of cholesterol in the serum compared with the C diet, but there was no difference among rats fed W, FO and W + FO diets. The serum phospholipid concentration was lower in rats fed the W, FO and W + FO diets than in rats fed the C diet, and

that of rats fed W + FO diet was lowest among these dietary groups (Table 3).

The hepatic triacylglycerol concentrations of rats fed the W, FO and W + FO diets were significantly lower than in rats fed the C diet, with the decrease greatest in rats fed the W + FO diet (Table 3). The hepatic cholesterol concentrations of rats fed the W and W + FO diets also were significantly lower than that in rats fed the C diet. Liver phospholipid levels did not differ among the four dietary groups.

The serum β-hydroxybutyrate concentrations of rats fed the W, FO and W + FO diets were greater than that in rats fed the C diet, with the greatest increase in rats fed the W + FO diet (Table 4).

There were no differences in the hepatic activities of malic enzyme and fatty acid synthetase among the four groups. The hepatic activities of glucose-6-phosphate dehydrogenase in rats fed the W, FO and W + FO diets were lower than in rats fed the C diet, and the activities in rats fed the FO diet and W + FO diet were lower than in rats fed the W diet (Table 4).

The activity of acyl-CoA oxidase, which is the rate-limiting enzyme for fatty acid β-oxidation in liver peroxisomes, was significantly greater in rats fed the W, FO and W + FO diets compared with rats fed the C diet using both 16:0-CoA (saturated fatty acid-CoA) and 18:1-CoA (monosaturated fatty acid-CoA) as substrates (Table 4). There was no differ-

TABLE 3

Concentrations of liver and serum lipids in rats fed a control diet (C) or diets containing wakame (W), fish oil (FO) or both (W + FO) for 4 wk^{1,2}

	Diet				ANOVA (P-value) ³		
	C	W	FO	W + FO	Wakame	Fish oil	Wakame × Fish oil
Serum, mmol/L							
Triacylglycerol	2.20 ± 0.41 ^a	1.24 ± 0.07 ^b	0.90 ± 0.12 ^c	0.75 ± 0.06 ^c	<0.001	<0.001	NS
Cholesterol	4.03 ± 0.23 ^a	2.54 ± 0.16 ^b	2.74 ± 0.26 ^b	1.83 ± 0.28 ^b	<0.001	<0.001	NS
Phospholipids	2.63 ± 0.13 ^a	1.90 ± 0.09 ^b	1.61 ± 0.17 ^b	1.16 ± 0.09 ^c	<0.001	<0.001	<0.005
Liver, μmol/g							
Triacylglycerol	67.5 ± 14.0 ^a	14.5 ± 1.6 ^c	24.5 ± 3.9 ^b	8.7 ± 0.8 ^d	<0.001	<0.001	<0.001
Cholesterol	10.4 ± 0.7 ^a	5.7 ± 0.2 ^b	8.8 ± 0.9 ^a	5.3 ± 0.2 ^b	<0.001	NS	NS
Phospholipids	16.6 ± 0.5	18.6 ± 0.7	16.6 ± 0.6	18.3 ± 0.6	NS	NS	NS

¹ Values are means ± SEM, n = 7.

² Different superscripts in a row indicate significant differences (P ≤ 0.05) among groups.

³ NS, not significant (P > 0.05).

TABLE 4

β-Hydroxybutyrate concentration in the serum and enzyme activities involved in fatty acid synthesis and *β*-oxidation in the liver of rats fed a control diet (C) or diets containing wakame (W), fish oil (FO) and both (W + FO) for 4 wk^{1,2}

	Diet				ANOVA (P-value) ³		
	C	W	FO	W + FO	Wakame	Fish oil	Wakame × Fish oil
<i>β</i> -Hydroxybutyrate, $\mu\text{mol/L}$ serum	51.6 ± 3.8 ^c	90.5 ± 8.9 ^{ab}	77.5 ± 7.8 ^{bc}	104.4 ± 6.0 ^a	<0.001	<0.05	<0.05
Enzyme activities, nmol/(min · mg protein)							
Fatty acid synthetase	2.51 ± 0.43	1.76 ± 0.45	1.63 ± 0.28	1.65 ± 0.47	NS	NS	NS
Malic enzyme	7.10 ± 0.97	5.87 ± 0.87	5.58 ± 1.48	4.09 ± 0.94	NS	NS	NS
Glucose-6-phosphate dehydrogenase	104.2 ± 8.0 ^a	55.4 ± 3.9 ^b	21.0 ± 2.7 ^c	16.2 ± 1.3 ^c	<0.001	<0.001	<0.001
Acyl-CoA oxidase							
16:0-CoA	0.97 ± 0.05 ^b	1.32 ± 0.06 ^a	1.30 ± 0.03 ^a	1.44 ± 0.09 ^a	<0.001	<0.001	NS
18:1-CoA	1.92 ± 0.15 ^b	2.65 ± 0.14 ^a	2.71 ± 0.27 ^a	2.80 ± 0.52 ^a	<0.05	<0.01	NS
20:4-CoA	0.40 ± 0.07	0.50 ± 0.05	0.40 ± 0.04	0.51 ± 0.04	NS	NS	NS
Acyl-CoA dehydrogenase							
16:0-CoA	37.0 ± 2.0	36.6 ± 3.6	32.9 ± 4.6	37.0 ± 3.3	NS	NS	NS
18:1-CoA	24.8 ± 1.5	27.5 ± 1.1	23.5 ± 3.0	27.2 ± 1.2	NS	NS	NS
20:4-CoA	15.0 ± 4.0	20.4 ± 5.2	18.6 ± 4.6	17.7 ± 3.3	NS	NS	NS
Carnitine palmitoyl transferase	17.9 ± 1.0 ^b	17.1 ± 0.9 ^b	19.7 ± 0.9 ^a	21.7 ± 0.7 ^a	NS	<0.001	NS
3-Hydroxyacyl-CoA dehydrogenase	45.9 ± 1.4 ^d	65.1 ± 3.1 ^c	116.4 ± 7.2 ^b	220.9 ± 25.4 ^a	<0.001	<0.001	<0.05

¹ Values are the means ± SEM, *n* = 7.

² Different superscripts in a row indicate significant differences (*P* ≤ 0.05) among groups.

³ NS, not significant (*P* > 0.05).

ence in the activity of acyl-CoA dehydrogenase, which is the rate-limiting enzyme for fatty acid *β*-oxidation in liver mitochondria, among the rats fed each of the experimental diets using 16:0-CoA, 18:1-CoA and 20:4-CoA as substrates.

Another enzyme involved in fatty acid *β*-oxidation in liver mitochondria, carnitine palmitoyltransferase, which regulates the rate of transport of fatty acids across the mitochondrial membrane, had a greater activity in rats fed the FO and the W + FO diets than in rats fed the W and the C diets (Table 4). The activity of 3-hydroxyacyl-CoA dehydrogenase was greater in rats fed the W diet (42%), the FO diet (154%) and the W + FO diet (381%) than in rats fed the C diet (Table 4).

The hepatic carnitine concentration, which is the substrate for carnitine acyltransferase, was greater in rats fed the W and W + FO diets than in controls, with the increase greater in rats fed the W + FO diet (Table 5).

The concentration of protein in the liver 500 × g super-

natant fraction and mitochondrial fractions did not differ among the four dietary groups. The specific activities of succinate dehydrogenase in the liver 500 × g supernatant fraction and catalase in the liver mitochondrial fraction also did not differ (data not shown).

DISCUSSION

Until fairly recently, fish, shellfish, algae and vegetables were the main materials for food used in Japanese cuisine. However, the intakes of animal foods such as dairy products and meats have increased recently in Japan. Along with this change in eating habits, the incidence rates of diseases such as diabetes, arteriosclerosis, coronary arteries heart disease, thrombosis and allergy have increased. It is possible that fish, shellfish, algae and vegetables, which are the main food materials of Japanese cuisine, have preventative and therapeutic

TABLE 5

Liver carnitine concentration in rats fed a control diet (C) or diets containing wakame (W), fish oil (FO) or both (W + FO) for 4 wk^{1,2}

	Diet				ANOVA (P-value)		
	C	W	FO	W + FO	Wakame	Fish oil	Wakame × Fish oil
	<i>nmol/g Liver</i>						
Carnitine	13.4 ± 2.0 ^c	29.6 ± 3.4 ^b	22.5 ± 3.1 ^{bc}	53.1 ± 4.8 ^a	<0.001	<0.001	<0.01

¹ Values are the means ± SEM, *n* = 7.

² Different superscripts in row indicate significant differences (*P* ≤ 0.05) among groups.

effects against such diseases. To clarify how traditional Japanese eating habits contribute to the health of the Japanese, we examined the influence of diets composed of marine food materials, wakame and fish oil, on rat lipid metabolism.

In our previous study (4), a diet that was supplemented with dried wakame powder at 10 g/100 g diet significantly decreased the concentration of triacylglycerol in the serum and liver of rats compared with an unsupplemented diet. In this study, the W diet containing 19.1 g/100 g dried wakame powder also significantly decreased the concentration of triacylglycerol in the serum and liver compared with the C diet. The FO diet containing EPA and DHA significantly decreased the concentration of triacylglycerol in the serum and liver, and these data were comparable to other reports (32–34).

When wakame and fish oil were fed at the same time (W + FO diet), the serum concentration of triacylglycerol was 34% of that in rats fed the C diet, whereas the serum concentrations of triacylglycerol in rats fed the W and FO diets were 56 and 41% of that in rats fed the C diet, respectively. In addition, the hepatic triacylglycerol concentration in rats fed the W + FO diet also decreased to 13% of that in rats fed the C diet, whereas the W and FO diets decreased the concentration of triacylglycerol in the liver to 22 and 36% of that in rats fed the C diet, respectively. Thus, wakame and fish oil (W + FO diet) synergistically affected the decrease of triacylglycerol in the liver.

Because the absorption of lipids from the small intestine and/or the metabolism of lipids and fatty acids in the liver control the concentration of triacylglycerol in the serum and liver, it is possible that the W + FO diet modifies the rates of synthesis and degradation of fatty acids in the liver. Therefore, to clarify the mechanism(s) for the synergistically effect on the decrease of triacylglycerol in the liver of rats fed the W + FO diet, we compared the influences of the W, FO and W + FO diets on the activities of various enzymes involved in fatty acid synthesis in the liver.

Although the activity of glucose-6-phosphate dehydrogenase in rats fed the W, FO and W + FO diets decreased to ~ 50, 20 and 17% of that in rats fed the C diet, respectively, there was no difference in the activity of this enzyme between the FO diet group and the W + FO diet group. Further, the activities of fatty acid synthetase and malic enzyme of rats fed the W, FO and W + FO diets were almost the same as those of rats fed the C diet. Therefore, the difference in the concentration of liver triacylglycerols among the four diets was not due to differences in hepatic fatty acid synthesis. In contrast, the W, FO and W + FO diets significantly increased the concentration of β -hydroxybutyrate in the serum by ~80, 50 and 100% relative to that in rats fed the C diet. Therefore, we hypothesize that hepatic fatty acid β -oxidation in rats fed the W + FO diet tended to be increased; thus, we measured the activity of enzymes involved in hepatic fatty acid β -oxidation in rats fed the four diets.

There were no differences in the activities of acyl-CoA dehydrogenase and acyl-CoA oxidase among the four diet groups, and the W + FO diet had no effect on the specificity of fatty acyl-CoA for acyl-CoA oxidase and acyl-CoA dehydrogenase. However, the activity of 3-hydroxyacyl-CoA dehydrogenase in rats fed the W + FO diet was significantly increased to 381% of that in rats fed the C diet compared with 42% in rats fed the W diet and 154% in rats fed the FO diet. 3-Hydroxyacyl-CoA dehydrogenase catalyzes the third step in the mitochondrial fatty acid β -oxidation cycle, and inhibition of the activity of this enzyme would lead to the inhibition of activity of 2-enoyl-CoA accompanied by accumulation of 3-oxoacyl-CoA esters in the mitochondrion. The 3-oxoacyl-

CoA would inhibit fatty acid β -oxidation (35). Further, a deficiency in the activity of 3-hydroxyacyl-CoA dehydrogenase in pregnant woman leads to high levels of fat incorporated into the liver (36). Thus, 3-hydroxyacyl-CoA dehydrogenase has an important role in hepatic fatty acid β -oxidation and it is likely that the activation of this enzyme by the W + FO diet would increase fatty acid β -oxidation in the liver.

The liver carnitine concentration in rats fed the W, FO and W + FO diets was increased to ~121 and 68 and 296% of that in rats fed the C diet, respectively. Carnitine also plays an important role in fatty acid β -oxidation in the liver, and Hoppel (37) reported that an increase in the concentration of carnitine in the liver correlated with the rate of hepatic ketoacid production. Moreover, McGarry et al. (38) reported that carnitine stimulated ketogenesis from oleic acid. Therefore, the increase in the concentration of carnitine in the liver due to the W + FO diet would lead to an increase in hepatic fatty acid β -oxidation. In addition, McGarry et al. (38) also reported that increased fatty acid flux through the carnitine acyltransferase reaction was mediated by an elevation in liver carnitine concentration. However, in the present study, the increase in hepatic carnitine concentration in rats fed the W + FO diet did not reflect the activity of carnitine acyltransferase. Because the activity of carnitine acyltransferase is controlled by the levels of circulating glucose and free fatty acids in the serum, and the hepatic content of malonyl-CoA (35, 39), measurement of the concentration of glucose and free fatty acids in the serum, and the malonyl-CoA content in the liver in rats fed the W + FO diet would be required to clarify this point.

Thus, the W + FO diet synergistically increased the activity of 3-hydroxyacyl-CoA dehydrogenase and the concentration of carnitine in the liver, and decreased the concentration of triacylglycerol in the liver and serum. However, we cannot explain the mechanism(s) of the increase in the concentration of carnitine in the liver and in the activity of 3-hydroxyacyl-CoA dehydrogenase by the administration of the W + FO diet. Therefore, examination of the influences of the W + FO diet on hepatic fatty acid β -oxidation in more detail is necessary to clarify the mechanism of the synergistic liver triacylglycerol-lowering effect of the W + FO diet.

Although there was no difference in the food intake among the dietary groups, the relative liver and adipose tissue around the testis weight in rats fed the W + FO diet was significantly lower than those in rats fed the C diet in the present study. We conclude that this decrease was due to the promotion of the fatty acid β -oxidation in the liver by the W + FO diet.

In our previous study, the 10 g/100 g diet wakame diet did not decrease either the serum or liver cholesterol levels but in the present study, the W diet containing 19.1 g/100 g of dried wakame powder significantly decreased the concentration of serum and liver cholesterol compared with the C diet. It has been reported that wakame contains the polysaccharide, alginate, at ~30 g/100 g, and dietary alginate has been shown to decrease the concentration of cholesterol in the serum and liver (40,41). Therefore, it is probable that an increase in the alginate in the W diet decreased the concentration of cholesterol in the serum and liver.

The Wakame diet containing 10 g/100 g dried wakame powder increased the activity of acyl-CoA dehydrogenase, and the extent of the increase using 16:0-CoA as a substrate was higher than that using EPA-CoA as substrate in our previous study (4). However, although the activity of acyl-CoA oxidase in rats fed the W diet containing 19.1 g/100 g dried wakame powder was higher than that in rats fed the C diet using 16:0-CoA and 18:1-CoA as substrates, there was no difference

in the activity of acyl-CoA dehydrogenase between rats fed the W and the C diet in the present study. It is necessary to confirm whether the difference between these responses depends on the amount of dried wakame powder added to the diet.

The dried wakame powder used in this experiment contained 3.7 g lipid/100 g of dried wakame powder and the fatty acid composition (g/100 g total fatty acid) was 16:0; 12.8, 18:0; 0.4, 18:1; 4.5, 18:2; 5.8, 18:3(n-3); 12.4, 18:4(n-3); 33.9, 20:4; 11.3, and EPA; 16.1. Therefore, the EPA content of the W + FO diet was greater than that of the FO diet. However, the amount of EPA from dried wakame powder in the W + FO diet was only 0.14 g/100 g diet. On the other hand, the FO diet contained 1.71 g/100 g EPA and 0.87 g of DHA. It is unlikely that the fatty acids from the dried wakame powder altered hepatic fatty acid β -oxidation.

Many researchers are trying to identify the functional elements of food materials. However, because our diet is composed of various food materials, it is necessary to consider that the properties of functional substances in food materials may be enhanced or lessened by the combination of various food materials. When rats were fed wakame and fish oil at the same time, the concentration of triacylglycerol was lower than with consumption of either of the components alone, and this decrease was due to an increased rate of fatty acid oxidation in the liver. These results suggest that a diet composed of fish (fish oil) and wakame may be useful in the prevention of hyperlipidemia.

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