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Effect of Medium-chain Triacylglycerols on Anti-obesity Effect of Fucoxanthin

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Abstract: Dietary effects of medium-chain triacylglycerols (MCT) and fucoxanthin (Fc) on abdominal fat weight were determined using KK-Ay obese mouse. Experimental diet contained MCT(0.9%), Fc (0.1%), or MCT (0.9%)＋Fc (0.1%). The abdominal fat weight of mice fed with Fc was significantly lower than that of mice fed with MCT. Uncoupling protein 1 (UCP1), a key molecule for metabolic thermogenesis, was clearly expressed in the white adipose tissue (WAT) of mice fed Fc, but little expression in that of the mice fed MCT. The anti-obesity effect of Fc was increased by mixing Fc with MCT. This increase would be due to the increase in the absorption rate of Fc by MCT.

Key words: fucoxanthin, obesity, medium-chain triacylglycerol

1 INTRODUCTION

Fucoxanthin is a characteristic carotenoid of brown seaweeds, such as *Undaria pinnatifida*, *Hijikia fusiformis* and *Sargassum fulvellum*. It has a unique structure including an allenic bond and 5,6-monoepoxide in the molecule. Anticarcinogenic effects, apoptosis induction on cancer cells, anti-inflammatory effects and radical scavenging activity are known as biological activities of fucoxanthin[1–4]. Furthermore, we have found that fucoxanthin shows anti-obesity effect with an interesting molecular mechanism[5]. Feeding with fucoxanthin significantly reduces white adipose tissue (WAT) in rats and mice with a clear expression of UCP1 protein and mRNA in WAT, while there was little expression of UCP1 in WAT in mice fed control diet. UCP1 is normally expressed only in brown adipose tissue (BAT). It dissipates the pH-gradient generated by oxidative phosphorylation, releasing chemical energy as heat. UCP1 expression in WAT by fucoxanthin intake leads to oxidation of fatty acids and heat production in WAT. The substrate oxidation would directly reduce WAT in animals. Considered as break- through discoveries for an ideal therapy for obesity, regulation of uncoupling protein 1 (UCP1) expression in adipose tissue by food constituent needs to be further explored.

Medium-chain triacylglycerols (MCT) are also being promoted as potential agents in the prevention of obesity[6]. MCT refers to mixed triacylglycerols of saturated fatty acids with chain length of 6-10 carbon[7]. Compared to long-chain triacylglycerols (LCT), MCT are smaller molecules and have a lower melting point, thus being liquid at room temperature[8]. The physico-chemical property of MCT tends to be more rapid and complete hydrolysis upon digestion as compared with that of LCT. The absorption and metabolism of MCT are also different from those of LCT. The products of MCT hydrolysis, monoacylglycerol and medium chain fatty acids (MCF), are rapidly absorbed through the stomach mucosa into the hepatic portal vein after ingestion. The medium chain fatty acids are bound to serum albumin and transported in the soluble form of fatty acids and enter to systemic circulation through the portal vein directly to the liver, without being incorporated in the chylomicron. They do not accumulate in adipose tissue or muscle. Since MCF leave the intestinal mucosa by the portal vein system, they reach the liver more rapidly compared to the longer molecules[8]. In addition, transportation of the MCF into the mitochondria and its β-oxidation rate is more rapid than those of long chain fatty acids. These characteristic digestion, absorption, and metabolism of MCT are recognized to be due to the increased energy expenditure and thus reducing weight gain in the animals

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fed MCT[9]. Human studies have also shown an increase in energy expenditure with regard to consuming MCT containing meals[10,11].

In the present study, we investigated anti-obesity effects of mixtures of fucoxanthin and MCT using KK-Ay obese mouse. Fucoxanthin is soluble in MCT or in fish oil at room temperature, while solubility of fucoxanthin in vegetable oil is very low. Antioxidants such as vitamin E (Vit.E) are generally used in the products of unstable carotenoids such as astaxanthin and fucoxanthin to protect against oxidation. However, solubility of fucoxanthin into Vit.E is very low. In the present study, we could easily mix fucoxanthin with Vit.E using MCT as a medium.

2 MATERIALS AND METHOD

2.1 Fucoxanthin and MCT

Dried powder of wakame (*Undaria pinnatifida*) was obtained from Riken Vitamin (Tokyo, Japan). Wakame lipid obtained by extraction of powder with acetone was subjected to silicic acid column chromatography using n-hexane/acetone (7 : 3, v/v) as mobile phase to separate fucoxanthin (Fc). The separation was repeated three more times for further purification of Fc. Isolated Fc showed a purity of more than 78% on high performance liquid chromatography (HPLC). HPLC was carried out using Hitachi L-7000 system with the reversed - phase column (Develosil ODS-UG-5; 250 × 4.6 mm i.d., 5.0-μm particle size, Nomura Chem. Co.) fitted with a 10 × 4.0 mm i.d. guard column containing the same stationary phase. A mixture of methanol and acetonitrile (70 : 30, v/v) at a flow rate of 1.0 mL/min was used as mobile phase. Fc was monitored at 450 nm using UV-Vis detector. Peak identification was carried out by the comparing with standard. The standard fucoxanthin was prepared as previously described (1). MCT and Vit.E mixture were kindly donated by Riken Vitamin. Main fatty acids of MCT were caprylic acid (C8 : 0; 58.3%) and capric acid (C10 : 0; 41.5%).

2.2 Animals and diets

Female KK-Ay mouse (3weeks old) was obtained from Japan CREA Co. (Tokyo, Japan). They were housed at controlled temperature (23 ± 1℃) and humidity (50%) room under a 12 : 12-h light-dark cycle. After acclimation for 1 week by feeding control diets, mice were randomly divided into three groups of seven mice and given free access to water and the experimental diet. The diet was prepared according to the recommendation of the American Institute of Nutrition (AIN-93G)[12]. Composition of experiment diets are shown in Table 1. Fatty acid composition of dietary fat are shown in Table 2. After feeding of experimental diets for 4 weeks, mice were starved for 12 hours and anesthetized with diethyl ether. Mice were killed by exsanguination and their blood was withdrawn at the abdominal artery. Abdominal WAT, BAT, and liver were rapidly removed and weighed. Both adipose tissues and liver were frozen in liquid nitrogen for Western blot analy-

Table 1 Composition of Experimental Diets (in grams).

	Group		
ingredients	MCT	Fc	Fc + MCT
Soybean oil[1]	125.10	134.10	124.10
Medium-chain triglyceride[2]	9.00		9.00
Vitamin E[2]	1.00		1.00
Fucoxanthin (Fc)		1.00	1.00
Corn starch[3]	346.28	346.28	346.28
Casein[3]	216.00	216.00	216.00
Dextrinized cornstarch[3]	114.99	114.99	144.99
Sucrose[4]	87.12	87.12	87.12
AIN-93 mineral mixture[3]	35.00	35.00	35.00
AIN-93 vitamin mixture[3]	10.00	10.00	10.00
L-cystine[5]	3.00	3.00	3.00
Choline bitartrate[5]	2.50	2.50	2.50
Cellulose[3]	50.00	50.00	50.00
Tert-Butyl hydroquinone[5]	0.01	0.01	0.01

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[4] Kanto kagaku Reagent division, Tokyo, Japan
[5] SIGMA-ALDRICH Japan, Tokyo, Japan

Table 2 Fatty Acid Composition of Dietary Fat.

Fatty acid (wt %)	Dietary fat		
	MCT	Fc	Fc + MCT
8:0	1.7	N.D[a]	1.9
10:0	1.5	N.D[a]	1.8
16:0	10.0	10.7	10.1
18:0	3.6	3.7	3.6
18:1n-9	23.3	23.7	23.2
18:1n-7	1.4	1.6	1.7
18:2n-6	50.0	51.2	49.8
18:3n-3	5.5	5.8	5.4
18:4n-3	N.D[a]	N.D[a]	N.D[a]
20:5n-3	N.D[a]	N.D[a]	N.D[a]

N.D[a]: Not detected

sis and enzymatic activity measurement, respectively. The study protocol was approved by the committee of Hokkaido University.

2.3 Fatty acid composition of dietary fat

The fatty acid composition of the dietary fat was determined by gas chromatography (GC). After conversion of fatty acyl groups in the fat to their methyl esters as described previously by Prevot and Mordet[13], GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho Co, Ltd, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column [Omegawax 320 (30 m × 0.32 mm i.d.); Supelco, Inc., Bellefonte, PA]. The injection port and flame ionization detector were operated at 250 and 260℃, respectively. The column temperature was held at 200℃. Component peaks were identified by comparison with standard fatty acid methyl esters and quantified by a Shimadzu Chromatopac C-R6A integrator (Shimadzu Seisakusho Co., Ltd.).

2.4 Analysis of blood and liver

Adiponectin and Leptin level of the blood plasma were analyzed by commercial ELISA kit, namely, Mouse Adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd. Tokyo Japan) and Mouse Leptin Assay Kit (L) -IBL (IBL Co., Ltd. Gunma Japan), respectively.

Liver (50 mg) was homogenized by a Polytron in 1 mL Tris buffer saline (20 mM Trizma base, 137 mM NaCl, pH 7.6). Triacylglycerol concentration and protein concentration were analyzed by enzymatic kits. Triglyceride E-test Wako (Wako Pure Chemical Industries, Osaka, Japan) and DC protein assay kit (Bio-Rad Laboratories, California USA) were used for this purpose. For the analysis of lipid metabolic enzyme activity, liver (*ca*. 300 mg) was homogenized with 7 volume of 0.25 mol/L sucrose and centrifuged at 500 × g for 10 min[14]. The supernatant was used for analysis of Carnitine palmitoyl transferase activity according to the method described by Ide *et al*.[15]. The supernatant was further centrifuged at 9000 × g for 10 min to isolate mitochondria. Glucose-6-phosphate dehydrogenase activity of this fraction was measured as described by Keley and Kletzien[16].

2.5 Western blot analysis

Each tissue was homogenized in 5-10 volume of a solution containing 10 mM Tris-HCl, and 1 mM EDTA (pH 7.4) for 30s with a Polytron. After centrifugation at 1500 g for 5 min, the fat cake was discarded, and the infranatant (fat-free extract) was used for Western blot analysis of UCP1 as described previously[17]. Total protein content in BAT and WAT was measured with a DC protein assay kit (Bio-Rad Laboratories, California USA). Supernatants (30 μg protein/lane) were separated by 10% SDS-polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membrane. The membrane was incubated with UCP 1 antibody (Sigma, Saint Louis, USA) for one hour and then was incubated with a secondary antibody rabbit IgG-conjugated horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for one hour at room temperature. The membrane was treated with the reagents in the chemiluminescence detection kit (ECL system, Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. β-Actin was detected as a control with β-Actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.6 Statistical analysis

The level of significance for differences between the groups was tested using one-way ANOVA and the Student's *t*-test.

3 RESULTS

3.1 Body weight and adipose tissue weight

All animals remained healthy though the experimental period. There was no significant difference ($P > 0.05$) in body weight and in energy intake between the groups (Fig. 1). Uterinary and mesentery WAT significantly ($P < 0.05$) decreased by the supplementation of fucoxanthin (Fc) or Fc +MCT as compared with MCT group (Fig. 2). This effect was found more clearly in Fc+MCT than that in Fc alone. On the other hand, BAT content was significantly ($P < 0.01$) higher in the mice fed Fc or Fc+MCT than in the mice fed MCT diet (Fig. 2).

3.2 Adiponectin and leptin level of blood plasma, liver lipid content, and enzymatic activity

There was no significant difference ($P > 0.05$) in the adiponectin level of blood plasma (Fig. 3). Diet with Fc +

MCT significantly ($P<0.05$) reduced the leptin level of blood plasma as compared with MCT diet, while no significant difference ($P>0.05$) in the leptin level was found between Fc group and MCT group. Liver triacylglycerol concentration decreased in Fc group and Fc+MCT group as compared with that in MCT group, but significant difference ($P<0.05$) was only found in Fc (Table 3). Carnitine palmitoyltransferase activity increased by feeding Fc or Fc +MCT, but not significantly ($P>0.05$). Glucose-6-phosphate dehydrogenase activity did not change in each group.

3.3 UCP1 protein expression in BAT and WAT

There was no difference in the UCP1 protein expression in BAT among the three groups (Fig. 4). On the other hand, UCP1 expression in WAT increased by feeding Fc and significant increase ($P<0.05$) was found in Fc+MCT group (Fig. 5).

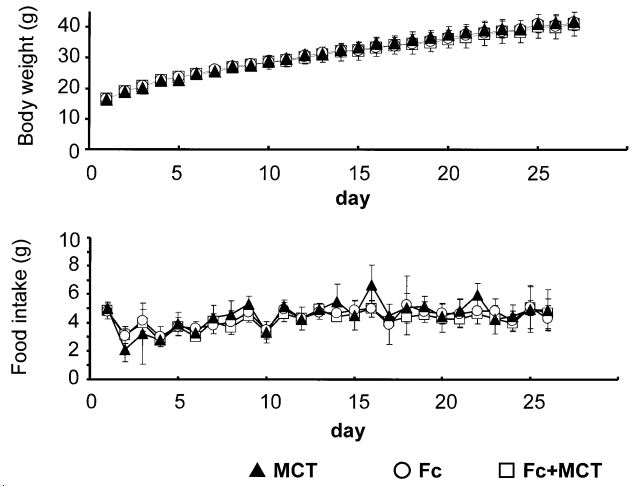

Fig. 1 Body Weight and Food Intake of Mouse.

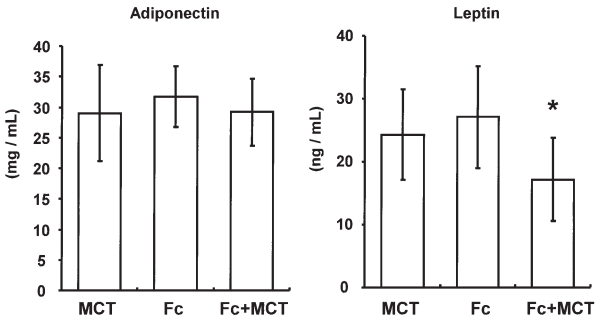

Fig. 3 Leptin and Adiponectin Concentration in the Blood Plasma. *$P<0.05$ vs MCT

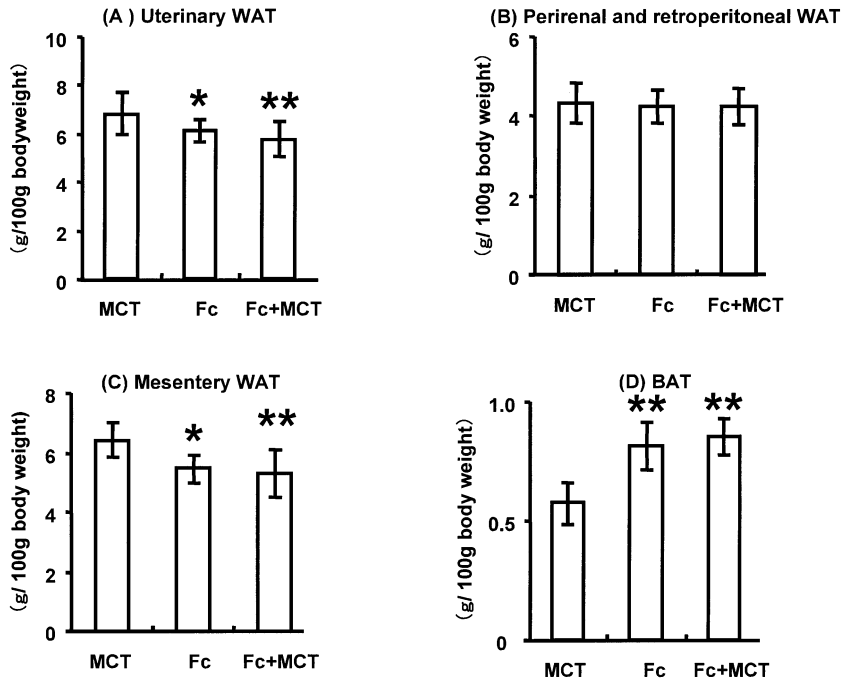

Fig. 2 Relative Adipose Tissue Weight of KK-Ay Mice Fed MCT Diet (MCT), 0.1% Fc diet (Fc), 0.1% Fc +MCT diet.
(A) Uterine WAT , (B) Perirenal and retroperitoneal WAT, (C) Mesentery WAT, (D) Brown adipose tissue *$P<0.05$ vs MCT **$P<0.01$ vs MCT

Table 3 Lipid Content and Enzyme Activities in the Liver.

	Group		
	MCT	Fc	Fc + MCT
Liver triacylglycerol (mg/g protein)	238 ± 64	167 ± 33*	205 ± 46
Carnitine palmitoyl transferase (μmol/min/mg protein)	2.1 ± 0.7	2.6 ± 0.6	2.5 ± 0.6
Glucose-6-phosphate dehydrogenase (μmol/min/mg protein)	2.6 ± 0.5	2.6 ± 0.3	2.9 ± 0.8

*P<0.05 vs MCT

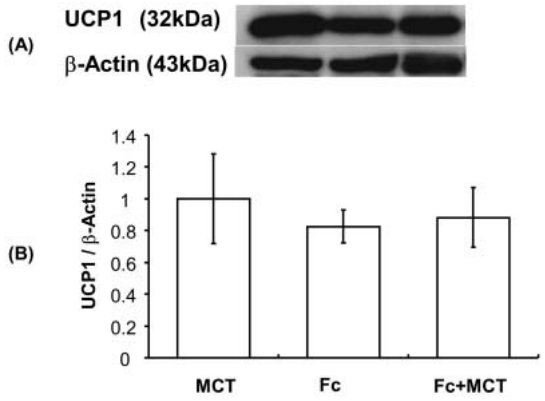

Fig. 4 (A) Western Blot Analysis of Uncoupling Protein1 (UCP1) in BAT. (B) Level of UCP1 Protein Expression Relative to β-Actin.

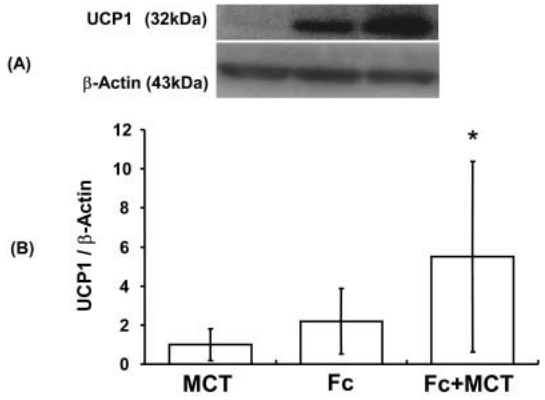

Fig. 5 (A) Western Blot Analysis of Uncoupling Protein1 (UCP1) in WAT. (B) Relative Expression Level of UCP1 Protein to β-Actin. *P<0.05 vs MCT

4 DISCUSSION

The increasing incidence of obesity is becoming a medical problem in the world. Weight loss can be achieved by different means but the maintenance after weight loss in the long term is rarely shown. Therefore, identification of substances that can decrease or prevent obesity remains a requirement. MCT has different physical properties as compared with long-chain triacylglycerols, with MCT showing a greater increase in energy expenditure in animal studies and human studies[18]. Thus, MCT can be useful agents in the prevention of obesity. On the other hand, a great deal of interest has been focused on adaptive thermogenesis by UCP1 as a physiological defense against obesity[19,20] UCP1 is a key molecule for anti-obesity. UCP1 expression is known to be a significant component of whole body energy expenditure, and its dysfunction contributes to the development of obesity. In a previous study on the anti-obesity effect of fucoxanthin, we have found that fucoxanthin reduced abdominal fat accumulation through UCP1 expression in WAT[5]. In the present study, we used three dietary fat containing MCT (0.9%), Fc (0.1%), MCT (0.9%)+Fc (0.1%). As shown in Fig. 2, the weights of uterinary and mesentery WAT were significantly lower in Fc-fed mice than in MCT-fed mice. This result indicates the stronger anti-obesity effect of Fc (0.1%) than MCT (0.9%). We have found the clear expression of UCP1 protein and mRNA in WAT of KK-Ay mice fed Fc (0.4%)[5]. The same result was also observed in WAT of mice fed Fc (0.1%) (Fig. 5), while little expression in that of MCT-fed mice. Furthermore, BAT weight was significantly higher in Fc-fed mice than in MCT-fed mice (Fig. 2). UCP1 is generally known to be expressed in BAT. Although there was no significant difference in BAT content, increase in BAT of mice fed Fc would affect the reduction of abdominal fat of the mice. Therefore, the energy dissipation via the generation of heat by UCP1 expression would be more effective on the reduction of abdominal fat than the enhancement of energy expenditure induced by MCT feeding.

In most studies on the anti-obesity effect of MCT, the concentration of MCT in the diet is around 5%. The lower effect of MCT in the present study would be due to the lower concentration of MCT (0.9%). On the other hand, the effect of Fc on the reduction of abdominal fat increased by mixing Fc with the low concentration of MCT (Fig. 2).

UCP1 expression of mice fed Fc+MCT was higher than those of mice fed Fc or MCT alone (Fig. 5). Leptin is secreted from adipocyte and adjusts the body weight or insulin sensitivity[21]. Leptin suppresses the appetite to control body weight. However, obesity patient has leptin resistance and leptin level in the blood keeps high in the patient. Therefore, plasma leptin level is used as an index of body fat accumulation. Leptin level of obese model mice (KK-Ay) fed Fc + MCT was significantly lower than those of other mice fed MCT and Fc alone.

Dietary fucoxanthin is converted to fucoxanthinol after absorption[22]. Absorption rate is generally affected by the composition of food matrix. We found that the solubility of Fc in soybean oil is very low, while Fc can easily dissolve in MCT or in fish oil. MCT diet also contained Vit.E (0.1%). Vit.E might be effective for the prevention of Fc against oxidation and/or decomposition. Thus, the higher anti-obesity effect of Fc with MCT than Fc alone would be due to the increase in the absorption rate of Fc and in the oxidative stability of Fc.

When the UCP1 expression in WAT was compared between mice fed with purified fucoxanthin and seaweed lipids containing fucoxanthin, the higher UCP1 level was found in the mice fed seaweed lipids than those fed purified fucoxanthin, although the fucoxanthin content was the same in both groups (unpublished data). This suggests that the absorption rate of fucoxanthin is strongly affected by the presence of other components, especially lipids. The synergistic relationship between Fc and other lipids need to be clarified by further study.

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