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Dietary Combination of Fucoxanthin and Fish Oil Attenuates the Weight Gain of White Adipose Tissue and Decreases Blood Glucose in Obese/Diabetic KK-A^y Mice

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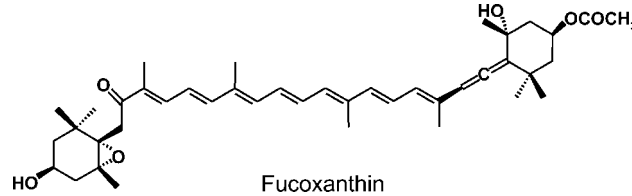
Fucoxanthin is a marine carotenoid found in edible brown seaweeds. We previously reported that dietary fucoxanthin attenuates the weight gain of white adipose tissue (WAT) of diabetic/obese KK-A^y mice. In this study, to evaluate the antiobesity and antidiabetic effects of fucoxanthin and fish oil, we investigated the effect on the WAT weight, blood glucose, and insulin levels of KK-A^y mice. Furthermore, the expression level of uncoupling protein 1 (UCP1) and adipokine mRNA in WAT were measured. After 4 weeks of feeding, 0.2% fucoxanthin in the diet markedly attenuated the gain of WAT weight in KK-A^y mice with increasing UCP1 expression compared with the control mice. The WAT weight of the mice fed 0.1% fucoxanthin and 6.9% fish oil was also significantly lower than that of the mice fed fucoxanthin alone. In addition, 0.2% fucoxanthin markedly decreased the blood glucose and plasma insulin concentrations in KK-A^y mice. The mice fed with the combination diet of 0.1% fucoxanthin and fish oil also showed improvements similar to that of 0.2% fucoxanthin. Leptin and tumor necrosis factor (TNFα) mRNA expression in WAT were significantly down-regulated by 0.2% fucoxanthin. These results suggest that dietary fucoxanthin decreases the blood glucose and plasma insulin concentration of KK-A^y along with down-regulating TNFα mRNA. In addition, the combination of fucoxanthin and fish oil is more effective for attenuating the weight gain of WAT than feeding with fucoxanthin alone.

KEYWORDS: Fucoxanthin; fish oil; white adipose tissue; blood glucose; insulin; TNFα; leptin; KK-A^y

INTRODUCTION

Adipose tissue is a major site of energy storage and plays an important role in fat and glucose metabolism. Furthermore, adipose tissue is now recognized as a major endocrine and secretary organ, releasing biologically active mediators termed adipokines (*1*, *2*). These adipokines have been shown to affect insulin sensitivity, glucose, and lipid metabolism in muscle, liver, and adipose tissue. In the current nutritionally rich environment, excessive white adipose tissue (WAT) leads to obesity and obesity-related disorders such as diabetes mellitus, hypertension, dyslipidemia, and cardiovascular disease through the disturbance of adipokine secretion from WAT (*3*). For example, the adipokine tumor necrosis factor-α (TNFα) is elevated in obesity and plays an important role in the development of insulin resistance and type 2 diabetes (*4*, *5*). In addition, resistin and leptin have been reported to affect insulin sensitivity (*6*) and are associated with the development of hypertension (*7*). On the other hand, adiponectin, which is known to play an important role in maintaining insulin sensitivity and glucose homeostasis, is reduced in obese, insulin-resistant rodent models (*8*).

Fucoxanthin (**Figure 1**) is a marine carotenoid found in edible brown seaweeds such as *Undaria pinnatifida* and *Hijikia fusiformis*. We previously reported that dietary fucoxanthin attenuates the development of WAT in the diabetes/obesity mouse KK-A^y (*9*). Interestingly, fucoxanthin up-regulated mitochondrial uncoupling protein 1 (UCP1), which plays an important role in energy expenditure, in WAT but not in brown



Fucoxanthin

Figure 1. Structure of fucoxanthin.

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Table 1. Composition of Experimental Diets

ingredient (g/1000 g diet)	control	0.1% FC[a]	0.2% FC	fish oil	0.1% FC + fish oil
soybean oil	135.10	134.10	133.10	65.10	65.10
fish oil				70.00	69.00
fucoxanthin		1.00	2.00		1.00
cornstarch	346.28	346.28	346.28	346.28	346.28
casein	216.00	216.00	216.00	216.00	216.00
dextrinized cornstarch	114.99	114.99	114.99	114.99	114.99
sucrose	87.12	87.12	87.12	87.12	87.12
AIN-93 mineral mixture	35.00	35.00	35.00	35.00	35.00
AIN-93 vitamin mixture	10.00	10.00	10.00	10.00	10.00
L-cystine	3.00	3.00	3.00	3.00	3.00
choline bitartrate	2.50	2.50	2.50	2.50	2.50
cellulose	50.00	50.00	50.00	50.00	50.00
tert-butylhydroquinone	0.01	0.01	0.01	0.01	0.01

[a] FC = fucoxanthin.

adipose tissue (BAT). These finding suggest that fucoxanthin is an effective compound for the prevention of obesity. However, it is not clear whether dietary fucoxanthin exerts a preventive effect on obesity-linked type 2 diabetes. In addition, there have been no reports on the combined effect of fucoxanthin and another compound.

Fish oil, which is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has beneficial effects on health (*10*). It is well known that dietary fish oil decreases serum triacylglycerol by stimulating lipid oxidation and inhibiting lipogenesis in the liver (*11*, *12*). Furthermore, fish oil has been reported to exhibit a synergistic effect with sesamin on hepatic fatty acid oxidation in rats (*13*) and attenuates fatty liver induced by conjugated linoleic acid in mice (*14*). Thus, the dietary combination of fucoxanthin with fish oil may be more effective for the prevention and improvement of obesity and diabetes.

In the present study, we estimated the WAT weight, blood glucose, and insulin concentration in KK-A^y mice fed fucoxanthin and fish oil. We also measured the expression level of UCP1 and adipokine mRNA in WAT of KK-A^y mice.

MATERIALS AND METHODS

Materials. Fish oil extracted from sardine was obtained from Nippon Suisan Kaisha Ltd. (Tokyo, Japan). *Undaria pinnatifida* dried seaweed was purchased from the marked in Hakodate, Japan.

Fucoxanthin Preparation. Lipid extracts containing fucoxanthin were obtained from commercial *Undaria pinnatifida* dried seaweed using acetone extraction. Fucoxanthin was purified from the lipid extracts by silica gel column chromatography with *n*-hexane/acetone (7:3, v/v). The purity of fucoxanthin (*all-trans*-fucoxanthin + *cis*-fucoxanthin) was >97% by HPLC analysis.

Animals Care. 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. In this study, we used female KK-A^y mice (3 weeks of age, CREA Japan (Tokyo, Japan)) because WAT deposition in female mice are higher than that of male mice. The mice were housed at 23 ± 1 °C and at 50% humidity with a 12 h light/12 h dark cycle. Animals had free access to drinking water and were fed a diet prepared according to the recommendations of American Institute of Nutrition (AIN-93G) (*15*). Fucoxanthin was at first added into soybean oil and then mixed to other component of AIN-93G diet. After acclimation for 1 week, mice were assigned five groups of seven mice and provided with the experimental diets. The composition of diets is shown in **Table 1**. Fatty acid composition of lipid added into each diet was analyzed by gas chromatography, as described in our previous report (**Table 2**) (*9*). After feeding with the experimental diets for 4 weeks, mice were starved for 12 h and anatomized under anesthesia by diethyl ether. Abdominal WAT and BAT were rapidly removed, weighed, and frozen in liquid nitrogen for Western blot analysis. Samples were also taken for mRNA expression analysis and stored in RNA later Storage Solution (Sigma Chemical Co., St. Louis, MO).

Table 2. Fatty Acid Composition of Dietary Lipid

fatty acid (wt %)	control	0.1% FC[a]	0.2% FC	fish oil	0.1% FC + fish oil
14:0	N.D.[b]	N.D.	N.D.	3.6	3.2
16:0	10.3	10.3	10.6	8.8	8.6
16:1n-7	N.D.	N.D.	N.D.	5.5	5.2
18:0	3.1	3.1	3.0	1.8	1.7
18:1n-9	22.2	21.6	21.6	12.8	13.3
18:1n-7	1.2	1.4	1.4	1.9	1.8
18:2n-6	53.7	54.9	54.7	23.6	25.1
18:3n-3	6.6	6.7	6.6	3.3	3.3
18:4n-3	N.D.	N.D.	N.D.	2.7	2.5
20:4n-6	N.D.	N.D.	N.D.	1.0	0.8
20:5n-3	N.D.	N.D.	N.D.	17.3	16.5
22:5n-3	N.D.	N.D.	N.D.	1.6	1.5
22:6n-3	N.D.	N.D.	N.D.	6.6	6.0
others	2.9	2.0	2.1	9.5	10.5

[a] FC = fucoxanthin. [b] N.D. = not detected.

Western Blot Analysis. Each tissue was homogenized in 5–10 volumes of a solution containing 10 mM Tris-HCl and 1 mM EDTA (pH7.4) for 30 s with a Polytron (PELLET PESTLES, KONTES, Vineland, NJ). After centrifugation at 1500*g* for 5 min, fat-free extract was used for Western blot analysis of UCP1, as described previously (*9*). Total protein content in WAT and BAT was measured with a DC protein assay Kit (Bio-Rad Laboratories, Hercules, CA). The supernatant was separated by 10% SDS–polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membrane. The membrane was incubated with an antibody against UCP1 (Sigma Chemical Co., St. Louis, MO) for 1 h and was then incubated with the secondary rabbit IgG-conjugated horseradish peroxidase antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. The membranes were treated with the reagents in the ECL chemiluminescence detection kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. β-Actin was detected as a control with β-Actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

mRNA Analysis. Total RNA was extracted from uterine WAT using the RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. Then, cDNA was synthesized from total RNA using the high-capacity cDNA archive kit (Applied Biosystems Japan Ltd., Tokyo, Japan). Real-time quantitative RT-PCR analysis was performed with an automated sequence detection system (ABI Prism 7000; Applied Biosystems Japan Ltd., Tokyo, Japan). PCR cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. TNFα, leptin, resistin, adiponectin, and GAPDH mRNA expression were measured by TaqMan Gene Expression Assays from Applied Biosystems (Japan Ltd., Tokyo, Japan). PCR primers (TNFα: Mm00445641_m1; leptin: Mm00434759_m1; resistin: Mm00445641_m1; adiponectin Mm00456425_m1; GAPDH: Mm99999915_g1) were purchased from Applied Biosystems (Japan Ltd., Tokyo, Japan).

Blood Glucose, Insulin, and Leptin Concentrations. Blood glucose levels were determined using a blood glucose monitor, Glutest Neo Sensor (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan), without fasting after 26 days of feeding. This sensor is a amperometric sensor with FAD-dependent glucose dehydrogenase and $Fe(CN_6)^{3-}$. The plasma insulin and leptin concentrations were analyzed using commercial ELISA kits, mouse insulin ELISA kit (U-type) (Shibayagi, Gunma, Japan), and mouse leptin assay kit (L)-IBL (IBL Co., Ltd., Gunma, Japan), respectively.

Statistical Analysis. Results are shown as means ± SD of seven mice. The data were analyzed with a one-way ANOVA, followed by a Bonferoni/Dunn analysis to detect significant differences in the means at the level of at $P < 0.01$ or $P < 0.05$.

RESULTS AND DISCUSSION

Adipose Tissue Weight of KK-A^y Mice Fed Fucoxanthin and Fish Oil. The body weight gain of KK-A^y mice during 4 weeks of feeding in the control group was 20.0 ± 2.0 g. Those

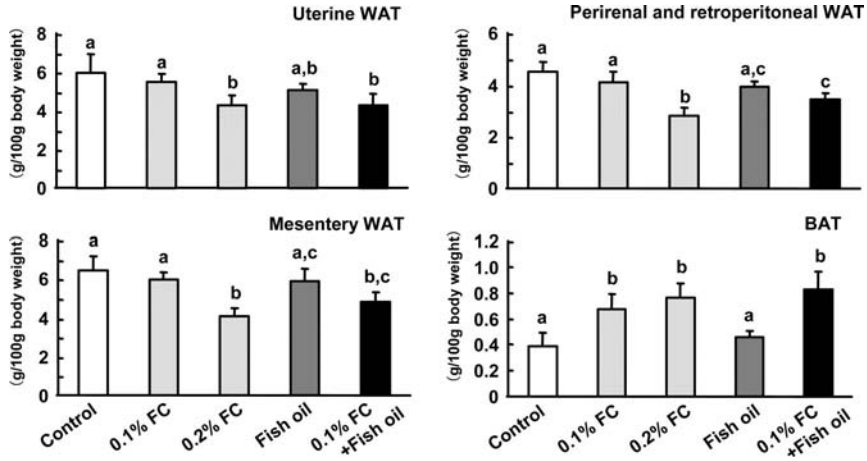

Figure 2. Adipose tissue weight of KK-A^y mice fed fucoxanthin and/or fish oil. KK-A^y mice were fed the experimental diets for 4 weeks. Values are expressed as means ± SD g WAT per 100 g body weight ($n = 7$). The different letters were significantly different from each other at $P < 0.01$. WAT= white adipose tissue, BAT = brown adipose tissue, and FC = fucoxanthin.

of the mice fed experimental diets were 19.2 ± 1.4 g with 0.1% fucoxanthin, 12.8 ± 1.4 g with 0.2% fucoxanthin, 17.2 ± 2.7 g with fish oil alone, and 18.0 ± 1.7 g with 0.1% fucoxanthin + fish oil. By feeding with 0.2% fucoxanthin, the body weight gain was significantly attenuated compared with that of the control mice ($P < 0.05$), although there was no significant difference in the amount of food intake.

The weight of uterine, mesentery, perirenal and retroperitoneal WAT normalized for body weight in the mice fed 0.2% fucoxanthin and 0.1% fucoxanthin + fish oil were significantly lower than in the control group (**Figure 2**). Furthermore, WAT weight of the mice fed 0.1% fucoxanthin + fish oil was significantly lower than that of the mice fed 0.1% fucoxanthin alone. These results indicate that the combination of fucoxanthin and fish oil exerts a beneficial effect on the attenuation of WAT weight gain of diabetic/obese KK-A^y mice by the additive effect of both compounds, but not by the synergistic effect. In contrast, the BAT weight normalized body weight, which is related to energy expenditure, was increased in the mice fed 0.1%, 0.2% fucoxanthin, and 0.1% fucoxanthin + fish oil compared with the control group, while fish oil alone had no affect. Other tissue weights were not affected by fucoxanthin and fish oil.

In a previous study, we reported that a diet containing 0.4% crude fucoxanthin (purity of 67.4%) suppresses the weight gain of WAT and enhances BAT weight in KK-A^y mice (*9*). In the current study, we observed that purified fucoxanthin in the diet is sufficient, even at a concentration of 0.2%, to suppress the weight gain of WAT and increase BAT weight in KK-A^y mice. On the other hand, there have been many reports about the antiobesity effects of fish oil (*16–18*). Hun et al. (*17*) reported a decrease in epididymal fat pad weight in KK-A^y mice by supplementing the diet with 3% fish oil for 12 weeks. However, we did not observe a significant suppressive effect on the development of WAT by fish oil, although there was a tendency for WAT weight to be lower in our study compared with the control diet. Four weeks feeding with 7% fish oil in the diet might be too short to elicit a significant effect. In addition, the antiobesity effect of fish oil might be affected by the kind of oils in the diet because lard was used in a previous report (*17*), whereas soybean oil was used in this study.

UCP1 Expression in WAT of KK-A^y Mice Fed Fucoxanthin and Fish Oil. UCP1 is expressed exclusively in BAT and plays a significant role in the control of energy expenditure and whole body energy balance (*19, 20*). We previously demonstrated that 0.4% crude fucoxanthin induces UCP1 expression in WAT,

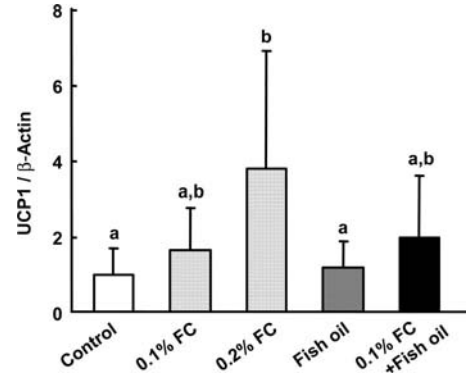

Figure 3. Expression of uncoupling protein 1 (UCP1) in white adipose tissue of KK-A^y mice fed fucoxanthin and/or fish oil. KK-A^y mice were fed the experimental diets for 4 weeks. UCP1 protein was detected by Western blot analysis and quantified by densitometry. The expression level of UCP1 was normalized to the β-actin level and expressed relative to control. Values are expressed as means ± SD ($n = 7$). The different letters were significantly different from each other at $P < 0.05$. FC = fucoxanthin.

but not in BAT, of KK-A^y mice (*9*). In this study, UCP1 in WAT was significantly up-regulated by 0.2% fucoxanthin, which is less than that in the previous study (**Figure 3**) (*9*). The expression level of UCP1 in uterine WAT by 0.2% fucoxanthin was approximately 4 times higher than that of the control. In addition, 0.1% fucoxanthin and 0.1% fucoxanthin + fish oil showed a tendency to increase UCP1 level compared to the control, although not significantly. However, there was no significant difference of UCP1 expression level between 0.1% fucoxanthin and 0.1% fucoxanthin + fish oil. Since WAT weight was lower in the mice fed the 0.1% fucoxanthin + fish oil diet than those fed 0.1% fucoxanthin alone, the suppressive effect of 0.1% fucoxanthin + fish oil on WAT weight gain seems to involve another mechanism by fish oil as well as the induction of UCP1 in WAT by fucoxanthin. Many studies demonstrate that fish oil, rich in EPA and DHA, decreases triglyceride in serum and liver through promotion of fatty acid β-oxidation and suppression of fatty acid synthesis in the liver (*11, 12*). There has been speculation that fish oil containing EPA and DHA attenuates triglyceride supply to adipose tissue through the regulation of fatty acid metabolism in the liver. Thus, the combined feeding of fucoxanthin and fish oil is suggested to suppress the weight gain of WAT through plural mechanisms.

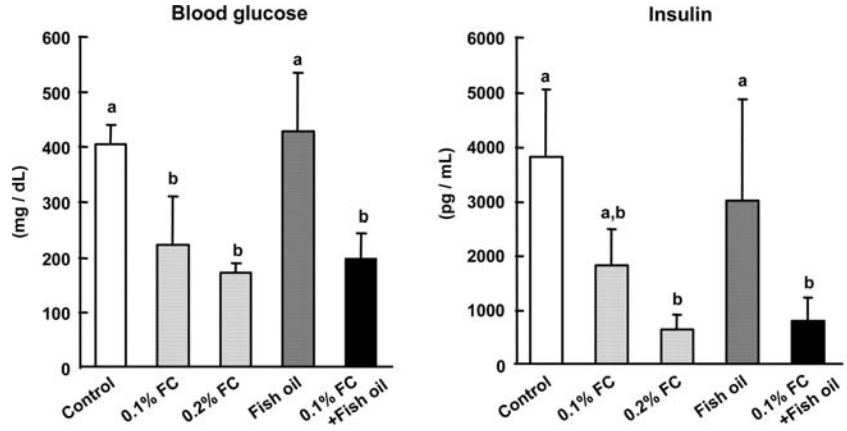

Figure 4. Blood glucose and insulin levels in KK-A^y mice fed fucoxanthin and/or fish oil. KK-A^y mice were fed the experimental diets for 4 weeks. Blood glucose levels were measured without fasting after 26 days of feeding. Plasma insulin levels were measured after 4 weeks feeding, followed by 12 h fasting. Values are expressed as means ± SD ($n = 7$). The different letters were significantly different from each other at $P < 0.01$. FC = fucoxanthin.

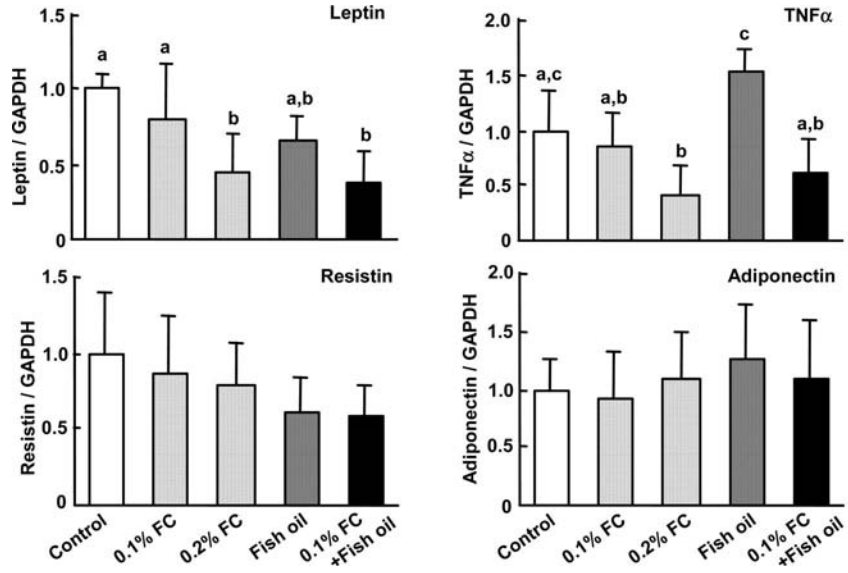

Figure 5. TNFα, leptin, resistin, and adiponectin mRNA expression in white adipose tissue of KK-A^y mice fed fucoxanthin and/or fish oil. KK-A^y mice were fed the experimental diets for 4 weeks. The expression levels of adipokine mRNA were measured by quantitative RT-PCR and expressed relative to control. Values are expressed as means ± SD ($n = 7$). The different letters were significantly different from each other at $P < 0.01$. FC = fucoxanthin.

Blood Glucose and Insulin Levels of KK-A^y Mice Fed Fucoxanthin and Fish Oil. KK-A^y mice develop obesity and show hyperleptinemia and hyperinsulinemia along with insulin resistance. Therefore, the KK-A^y mouse used in this study is a good model of obesity and type 2 diabetes (*21*). We measured blood glucose and insulin levels in KK-A^y mice fed to evaluate the antidiabetic effect of fucoxanthin. The mice fed 0.1% and 0.2% fucoxanthin had a significantly lower blood glucose concentration than the control mice (**Figure 4**). Furthermore, dietary fucoxanthin decreased plasma insulin level in a dose-dependent manner. In KK-A^y mice fed 0.2% fucoxanthin, there was a remarkable reduction in the plasma insulin level compared with the control mice. In addition, water intake, which is related to elevated blood glucose level in diabetic mice (*22*), was also decreased to ~40% of control by feeding of 0.2% fucoxanthin diet. Insulin resistance in peripheral tissue is one of the major pathogenic characteristics of type 2 diabetes. Therefore, our data indicate that fucoxanthin is an effective carotenoid for preventing and improving type 2 diabetes.

On the other hand, decreases in the blood glucose and insulin concentration were also observed in the mice fed the 0.1% fucoxanthin + fish oil diet. However, since their levels were not significantly different from 0.1% fucoxanthin alone, improvement of glucose and insulin level in KK-A^y is suggested to be dependent on fucoxanthin function. In this study, fish oil did not affect blood glucose and insulin level in KK-A^y, although Shimura et al. (*23*) reported that blood glucose was improved by DHA ethyl ester administration. Purified DHA ethyl ester may be more effective in decreasing blood glucose than fish oil.

Adipokine mRNA in WAT of KK-A^y Mice Fed Fucoxanthin and Fish Oil. Excessive visceral fat accumulation causes a disturbance of cytokine secretion from adipose tissue and is involved in the pathogenesis of type 2 diabetes, cardiovascular disease, and hypertension (*1, 2*). Many studies have reported that TNFα (*4, 5*) and leptin (*24*) secretions are elevated through the accumulation of fat in adipocytes and causes insulin resistance in obese animal models. Resistin has also been reported to regulate insulin sensitivity through the activation of TNFα in murine models (*6*) and to be regulated by 20:4*n*-6 (*25, 26*). In addition, adiponectin has been shown to contribute to the maintenance of insulin sensitivity (*27–29*).

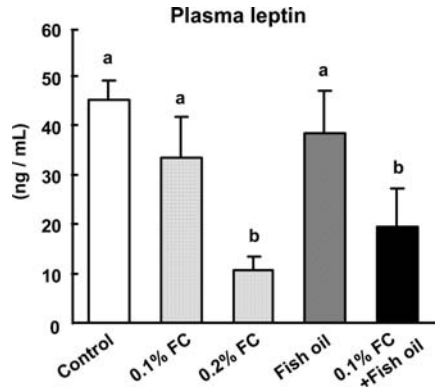

Figure 6. Plasma leptin concentration of KK-A^y mice fed fucoxanthin and/or fish oil. KK-A^y mice were fed the experimental diets for 4 weeks. The plasma leptin levels were measured by ELISA. Values are expressed as means ± SD ($n = 7$). The different letters were significantly different from each other at $P < 0.01$. FC = fucoxanthin.

Therefore, we measured the expression level of these adipokines mRNA in WAT of the mice fed fucoxanthin and fish oil using quantitative RT-PCR.

Leptin mRNA in WAT was remarkably reduced in the mice fed 0.2% fucoxanthin and 0.1% fucoxanthin + fish oil (**Figure 5**). Plasma leptin was also decreased by feeding 0.2% fucoxanthin and 0.1% fucoxanthin + fish oil diets (**Figure 6**). Furthermore, plasma leptin level was lower with 0.1% fucoxanthin + fish oil than with 0.1% fucoxanthin or fish oil alone. In general, leptin controls body weight and fat weight by regulating the food intake and energy expenditure (*30*, *31*). However, KK-A^y mice are known to have elevated plasma leptin levels and exhibit hyperleptinemia (*24*). Thus, the lower leptin level in the KK-A^y mice fed 0.2% fucoxanthin and 0.1% fucoxanthin and fish oil may reflect the size of WAT because leptin is mainly produced in adipocyte (*32*).

On the other hand, the TNFα mRNA level in WAT was remarkably lower in the mice fed 0.2% fucoxanthin diet than the control group, while resistin and adiponectin mRNA were not affected by fucoxanthin. This data implies that fucoxanthin improves insulin resistance and decreases blood glucose level, at least in part, through the down-regulation of TNFα mRNA in WAT of KK-A^y mice. However, the down-regulation of TNFα mRNA was not significant in WAT of the mice fed 0.1% fucoxanthin compared with the control mice, even though the blood glucose level was significantly decreased. Therefore, the reduction in blood glucose and insulin levels of KK-A^y mice by fucoxanthin seems to be induced by another signaling pathway as well as via TNFα-mediated signaling. Further studies are required to clarify the mechanisms by which fucoxanthin elicited improvements of blood glucose and insulin levels in diabetic/obese KK-A^y mice.

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LITERATURE CITED

(1) Matsuzawa, Y. The metabolic syndrome and adipocytokines. *FEBS Lett.* **2006**, *580*, 2917–2921.

(2) Kadowaki, T.; Yamauchi, T.; Kubota, N.; Hara, K.; Ueki, K.; Tobe, K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **2006**, *116*, 1784–1792.

(3) Walker, C. G.; Zariwala, M. G.; Holness, M. J.; Sugden, M. C. Diet, obesity and diabetes: a current update. *Clin. Sci.* **2007**, *112*, 93–111.

(4) Hotamisligil, G. S.; Shargill, N. S.; Spiegelman, B. M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **1993**, *259*, 87–91.

(5) Hotamisligil, G. S.; Arner, P.; Caro, J. F.; Atkinson, R. L.; Spiegelman, B. M. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* **1995**, *95*, 2409–2415.

(6) Kitagawa, Y.; Bujo, H.; Takahashi, K.; Shibasaki, M.; Ishikawa, K.; Yagui, K.; Hashimoto, N.; Noda, K.; Nakamura, T.; Yano, S.; Saito, Y. Impaired glucose tolerance is accompanied by decreases insulin sensitivity in tissues of mice implanted cells that overexpress resistin. *Diabetelogia* **2004**, *47*, 1847–1853.

(7) Aizawa-Abe, M.; Ogawa, Y.; Masuzaki, H.; Ebihara, K.; Satoh, N; Iwai, H.; Matsuoka, N.; Hayashi, T.; Hosoda, K.; Inoue, G.; Yoshimasa, Y.; Nakao, K. Pathophysiological role of leptin in obesity-related hypertension. *J. Clin. Invest.* **2000**, *105*, 1243–1252.

(8) Hu, E.; Liang, P.; Spiegelman, B. M. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J. Biol. Chem.* **1996**, *271*, 10697–10703.

(9) Maeda, H.; Hosokawa, M.; Sashima, T.; Funayama, K.; Miyashita, K. Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 392–397.

(10) Simopoulos, A. P. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* **1991**, *54*, 438–463.

(11) Gronn, M.; Christensen, E.; Hagve, T. A.; Christophersen, B. O. Effects of dietary purified eicosapentaenoic acid (20:5 (n-3)) and docosahexaenoic acid (22:6(n-3)) on fatty acid desaturation and oxidation in isolated rat liver cells. *Biochim. Biophys. Acta* **1992**, *1125*, 35–43.

(12) Berge, R. K.; Madsen, L.; Vaagenes, H.; Tronstad, K. J.; Gottlicher, M.; Rustan, A. C. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *Biochem. J.* **1999**, *343*. Pt. 1, 191–193.

(13) Ide, T.; Hong, D. D.; Ranasinghe, P.; Takahashi, Y.; Kushiro, M.; Sugano, M. Interaction of dietary fat types and sesamin on hepatic fatty acid oxidation in rats. *Biochim. Biophys. Acta* **2004**, *1682*, 80–91.

(14) Yanagita, T.; Wang, Y. M.; Nagao, K.; Ujino, Y.; Inoue, N. Conjugated linoleic acid-induced fatty liver can be attenuated by combination with docosahexaenoic acid in C57BL/6N mice. *J. Agric. Food Chem.* **2005**, *53*, 9629–9633.

(15) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(16) Tsuboyama-Kasaoka, N.; Takahashi, M.; Kim, H.; Ezaki, O. Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice. *Biochem. Biophys. Res. Commun.* **1999**, *257*, 879–885.

(17) Hun, C. S.; Hasegawa, K.; Kawabata, T.; Kato, M.; Shimokawa, T.; Kagawa, Y. Increased uncoupling protein2 mRNA in white adipose tissue, and decrease in leptin, visceral fat, blood glucose, and cholesterol in KK-Ay mice fed with eicosapentaenoic and docosahexaenoic acids in addition to linolenic acid. *Biochem. Biophys. Res. Commun.* **1999**, *259*, 85–90.

(18) Lombardo, Y. B.; Chicco, A. G. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. *J. Nutr. Biochem.* **2006**, *17*, 1–13.

(19) Crowley, V.; Vidal-Puig, A. J. Mitochondrial uncoupling proteins (UCPs) and obesity. *Nutr., Metab. Cardiovasc. Dis.* **2001**, *11*, 70–75.

(20) Porter, R. K. A new look at UCP1. *Biochim. Biophys. Acta* **2006**, *1757*, 446–448.

(21) Nishimura, M. Breeding of mice strains for diabetes mellitus. *Exp. Anim.* **1969**, *18*, 147–157.

(22) Lee, S. M.; Bressler, R. Prevention of diabetic nephropathy by diet control db/db mouse. *Diabetes* **1981**, *30*, 106–111.

(23) Shimura, T.; Miura, T.; Usami, M.; Ishihara, E.; Tanigawa, K.; Ishida, H.; Seino, Y. Docosahexanoic acid (DHA) improved glucose and lipid metabolism in KK-Ay mice with genetic non-insulin-dependent diabetes mellitus (NIDDM). *Biol. Pharm. Bull.* **1997**, *20*, 507–510.

(24) Masuzaki, H.; Ogawa, Y.; Aizawa-Abe, M.; Hosoda, K.; Suga, J.; Ebihara, K.; Satoh, N.; Iwai, H.; Inoue, G.; Nishimura, H.; Yoshimasa, Y.; Nakao, K. Glucose metabolism and insulin sensitivity in transgenic mice overexpressing leptin with lethal yellow agouti mutation: usefulness of leptin for the treatment of obesity-associated diabetes. *Diabetes* **1999**, *48*, 1615–1622.

(25) Drevon, C. A. Fatty acids and expression of adipokines. *Biochim. Biophys. Acta* **2005**, *1740*, 287–292.

(26) Haugen, F.; Zahid, N.; Dalen, K. T.; Hollung, K.; Nebb, H. I.; Drevon, C. A. Resistin expression in 3T3-L1 adipocytes is reduced by arachidonic acid. *J. Lipid Res.* **2005**, *46*, 143–153.

(27) Hotta, K.; Funahashi, T.; Bodkin, N. L.; Ortmeyer, H. K.; Arita, Y.; Hansen, B. C.; Matsuzawa, Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* **2001**, *50*, 1126–1133.

(28) Yamauchi, T.; Kamon, J.; Waki, H.; Terauchi, Y.; Kubota, N.; Hara, K.; Mori, Y.; Ide, T.; Murakami, K.; Tsuboyama-Kasaoka, N.; Ezaki, O.; Akanuma, Y.; Gavrilova, O.; Vinson, C.; Reitman, M. L.; Kagechika, H.; Shudo, K.; Yoda, M.; Nakano, Y.; Tobe, K.; Nagai, R.; Kimura, S.; Tomita, M.; Froguel, P.; Kadowaki, T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.* **2001**, *7*, 941–946.

(29) Berg, A. H.; Combs, T. P.; Du, X.; Brownlee, M.; Scherer, P. E. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat. Med.* **2001**, *7*, 947–953.

(30) Halaas, J. L.; Gajiwala, K. S.; Maffei, M.; Cohen, S. L.; Chait, B. T.; Rabinowitz, D.; Lallone, R. L.; Burley, S. K.; Friedman, J. M. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **1995**, *269*, 543–546.

(31) Pelleymounter, M. A.; Cullen, M. J.; Baker, M. B.; Hecht, R.; Winters, D.; Boone, T.; Collins, F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **1995**, *269*, 540–543.

(32) Frederich, R. C.; Hamann, A.; Anderson, S.; Lollmann, B.; Lowell, B. B.; Flier, J. S. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat. Med.* **1995**, *1*, 1311–1314.

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