RESEARCH ARTICLE

Anti-obese property of fucoxanthin is partly mediated by altering lipid-regulating enzymes and uncoupling proteins of visceral adipose tissue in mice

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This study investigated the anti-obesity effects of fucoxanthin in diet-induced obesity mice fed a high-fat diet (20% fat, wt/wt). The mice were supplemented with two doses of fucoxanthin (0.05 and 0.2%, wt/wt) for 6 wk. Fucoxanthin significantly lowered body weight and visceral fat-pads weights compared with the control group without altering food intake. In epididymal adipose tissue of fucoxanthin-fed mice, adipocyte sizes and mRNA expression of lipogenic and fatty acid β-oxidation enzymes were significantly altered in a dose-dependent manner. Plasma leptin level was significantly lower in the fucoxanthin groups than in the control group, while the adiponectin level was elevated. Fucoxanthin significantly down-regulated various lipogenic enzyme activities in epididymal adipose tissue with a simultaneous decrease in fatty acid β-oxidation activity. The 0.2% fucoxanthin supplement led to increase mRNA expression of uncoupling protein-1 (UCP-1) and UCP-3 in brown adipose tissue and that of UCP-2 in the epididymal white adipose tissue. However, the 0.05% fucoxanthin only elevated UCP-1 mRNA expression in epididymal white adipose tissue. These results suggest that the anti-obesity effect of fucoxanthin could be mediated by altering lipid-regulating enzymes and UCPs in the visceral fat tissues and plasma adipokine levels.

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Adipose tissue / Anti-obesity / Fucoxanthin / Lipid metabolism / mRNA expression

1 Introduction

Many epidemiological studies have observed that consumption of high phenolic foods is associated with the prevention of some diseases [1, 2]. Fucoxanthin, an edible seaweed carotenoid, has a unique structure including an allenic bond and 5, 6-monoepoxide (Fig. 1), which differs from that of common carotenoids such as β-carotene and lycopene [3]. Fucoxanthin is mainly present in marine plants such as *Undaria pinnatifida*, *Sargassum fulvellum*, *Laminaria japonica* and *Hizikia fusiformis*. It has been reported that fucoxanthin exhibits beneficial biological effects on anti-carcinogenic [4], antihypertensive [5] and radical scavenging activity [6]. In recent studies, Maeda *et al.* [3, 7] showed the anti-obesity effect of crude fucoxanthin (0.4% fucoxanthin-rich fraction in diet, wt/wt) and pure fucoxanthin (Fx) (0.2% in diet, wt/wt) from *U. pinnatifida* in KK-A^y mice, type 2 diabetic animal models. Fucoxanthin has been reported to inhibit intercellular lipid accumulation during adpiocyte

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Abbreviations: BAT, brown adipose tissue; **β-oxidation,** fatty acid β-oxidation; **FAS,** fatty acid synthase; **Fx,** pure fucoxanthin; **G6PD,** glucose-6-phosphate dehydrogenase; **GAPDH,** glyceraldehyde-3-phosphate dehydrogenase; **ME,** malic enzyme; **UCP,** uncoupling protein; **WAT,** white adipose tissue

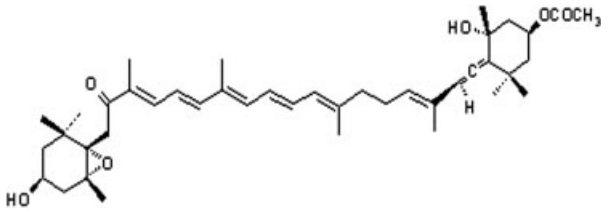

Figure 1. Structure of fucoxanthin.

differentiation of 3T3-L1 cells [8]. Although many researches have previously focused on the anti-obesity effect of fucoxanthin *in vitro* and *in vivo* [3, 7], there is still little known about the anti-obesity action of fucoxanthin on lipid-regulating metabolic enzymes in diet-induced obese mice.

Animal models for development of obesity have been important to understand the metabolic changes that occur with excessive accumulation of adipose tissue [9]. In general, diet-induced obese animal models have been very informative as they show the role of specific dietary components in the development of obesity [10, 11].

Therefore, this study investigated the anti-obesity efficacy of Fx isolated from *U. pinnatifida* that is one of the most popular edible seaweeds in Korea using diet-induced obese mice. In order to attempt to find a possible mechanism that is responsible for the anti-obesity effects of fucoxanthin, activities and gene expressions of lipid-regulating enzymes were measured with uncoupling protein (UCP) gene expression in visceral and brown adipose tissues (BAT).

2 Materials and methods

2.1 Preparation of fucoxanthin

Fucoxanthin was isolated from the dried *U. pinnatifida* brown algae that were harvested from a clean region of the South Sea in Wando county, Jeonnam, Korea. The isolated Fx was provided from Amicogen (Jinju, Korea). To obtain lipid extracts containing fucoxanthin, dried *U. pinnatifida* seaweed (50 kg) was extracted using a solution of H_2O: ethanol (1:6, v/v) in the dark for 6 h. The extractant was recovered by centrifugation and subsequently filtered through a filter pad (pore size 0.4 μm, 300 mm × 300 mm). The supernatants were condensed and then freeze-dried for 48 h. To obtain purified fucoxanthin, the crude lipid extracts containing fucoxanthin were dissolved in acetone and subjected to silica gel column chromatography (resin, Merck Kieselgel 66; 70–230 mesh, 7.5 cm × 60 cm), which was washed with *n*-hexane/acetone (8:2, v/v). Fucoxanthin was then eluted with *n*-hexane/acetone (6:4, v/v) to remove impurities. Part of the purified fucoxanthin was then used for the quantitative analysis by the HPLC. The HPLC system consisted of a 307 pump (Gilson, France), a UV-visible absorbance detector (Gilson). Fucoxanthin was separated on a symmetry C18 (4.6 × 250 mm, Waters, Ireland) column, attached to a precolumn of Pelliguard. The mobile phase used was acetone/hexane (90:10, v/v) with the flow rate of 0.5 mL/min. The fucoxanthin was detected at 450 nm and quantified from the peak area by using a standard curve with purified fucoxanthin (97.5%) as well as fucoxanthin (94%) from the Carotenature (Switzerland). The purity of fucoxanthin was 97.5% by HPLC analysis.

2.2 Animals and diets

Thirty male C57BL/6N mice aged 4 wk were purchased from Orient (Seoul, Korea). The mice were all individually housed in polycarbonate cages at 22 ± 2°C on a 12-h light–dark cycles. The mice were all treated in strict accordance with Kyungpook National University guidelines for the care and use of laboratory animals.

All mice were fed pellets of commercial chow for 1 wk after arrival. The mice were randomly divided into three groups ($n = 10$), fed a high-fat diet containing 20% fat (10% corn oil + 10% lard, wt/wt), a high-fat diet plus 0.05% fucoxanthin (wt/wt, 0.05Fx) or a high-fat diet plus 0.2% fucoxanthin (wt/wt, 0.2Fx) for 6 wk. The composition of the experimental diet was based on the AIN-76 semisynthetic diet (American Institute of Nutrition, 1977, 1980). The mice had free access to food and water *ad libitum*. Food consumption and body weight were measured daily and weekly, respectively.

At the end of the experimental period, the mice were anesthetized with Ketamine after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. After collecting the blood, white adipose tissues (WATs) from epididymal, perirenal and interscapular sites and interscapular BAT were removed, rinsed with a physiological saline solution and then weighed immediately. All samples were stored at −70°C until analyzed.

2.3 Plasma leptin and adiponectin determination

The plasma leptin and adiponectin levels were determined using a quantitative sandwich enzyme immunoassay kit (ELISA kit, Shibaygi, Japan).

2.4 Histological analysis of WAT

Epididymal WAT was removed from the mice and fixed in a buffer solution of 10% formalin. The fixed tissue was processed routinely for paraffin embedding, and 4-μm sections were prepared and dyed with hematoxylin–eosin. The stained areas were viewed using an optical microscope with a magnifying power of × 200.

2.5 Lipid metabolism-related enzyme activities

To measure the lipid-regulating enzymes activities in WAT, the epididymal WAT was prepared according to the method developed by Hulcher and Oleson [12] with a slight modification. Fatty acid synthase (FAS) activity was determined by a spectrophotometric assay based on measuring the malonyl-CoA-dependent oxidation of NADPH according to the methods by Nepokroeff *et al.* [13] with a slight modification. One unit of enzyme activity represented the oxidation of 1 nmol of NADPH *per* minute at 37°C. The malic enzyme (ME) activity was measured according to the method of Ochoa [14] by monitoring the production of NADPH at 340 nm. The enzyme activity represented by the formation of 1 nmol NADPH *per* minute at 37°C. The glucose-6-phosphate dehydrogenase (G6PD) activity was determined using the method of Pitkänen *et al.* [15], with a slight modification. The activity measured the reduction of 1 nmol NADP *per* minute at 340 nm using a spectrophotometer. Fatty acid β-oxidation (β-oxidation) activity was measured spectrophotometrically by monitoring the reduction of NAD to NADH in the presence of palmitoyl-CoA as described by Lazarow [16], with a slight modification. The protein content was determined by the method of Bradford [17] using bovine serum albumin as the standard.

2.6 RNA extraction and quantitative real-time RT-PCR analysis

Total RNA was extracted from epididymal WAT and BAT using TRIZOL reagent (Invitrogen, Carsbad, CA) according to the manufacture's instructions. The RNA samples were quantified spectrophotometrically. Complementary DNA was synthesized using Moloney murine leukemia virus RT (Fermentase, Burlington, ON, Canada), random hexamers, deoxyribonucleoside triphosphates and 5 μg of total RNA. After first-strand complementary DNA synthesis, the RNA expression was quantified by a quantitative real-time RT-PCR using SYBR green PCR reagents (Applied Biosystems, Foster City, CA) and the SDS7000 sequence-detection system (Applied Biosystems). Gene-specific mouse primers were used for FAS, 5′-ATTGCATCAAGCAAGTGCAG-3′ (forward), 5′-GAGCCGTCAAACAGGAAGA G-3′ (reverse); ME, 5′-AGGGCACA TTGCTTCAGTTC-3′ (forward), 5′-TGTACAGGGCCA GTTTACCC-3′ (reverse); G6PD, 5′-GGTACCTACAAGT GGGTGAA-3′ (forward), 5′-AGATGG TGAAAAGG GAAGAT-3′ (reverse); β-oxidation, 5′-ACCTTCACTTGGGCATGTTC-3′ (forward), 3′-TTCCAAGCCTCGAAGATGAG-3′ (reverse); UCP-1, 5′-TC AGGATTGGCCTCTACGAC-3′ (forward), 5′-TGCATTCTGACCTTCACGAC-3′ (reverse); UCP-2, 5′-TCAGAGCAGGAGGTTACAGT-3′ (forward), 5′-TCAACCCCTTCATTACAGAC-3′ (reverse); UCP-3, 5′-GC TGAGATGGTGACCTACG-3′ (forward), 5′-CGGGTCTTT ACCACATCCAC-3′ (reverse); glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-ACCACAGTCCATGCCATCAC-3' (forward), 5'-TCCACCACCCTGTTGCTGCTGTA-3' (reverse). The relative quantitation values were calculated by analyzing the changes in the SYBR green fluorescence during PCR, according to the manufacturer's instruction. The C_1 values obtained were the threshold cycles at which a statistically significant increase in SYBR green emission intensity occurred. Using the $2^{-\Delta\Delta Ct}$ method, the fold changes were calculated; transcripts of GAPDH were also amplified from the samples in order to assure normalized quantitative real-time RT-PCR detection [18].

2.7 Statistical analysis

All data are presented as the mean ± SE. The statistical analyses were performed using the statistical package for social science software program. Significant differences among the groups were determined by a one-way ANOVA. Duncan's multiple-range test was performed if differences were identified between the groups at $p < 0.05$. The correlation analysis was by the Pearson test.

3 Results

3.1 Body weight, food intake and food efficiency ratio

Supplementation of fucoxanthin seemed to suppress body weight gain compared with the control group from the third week during 6 wk (Fig. 2). Interestingly, 0.05% fucoxanthin only showed an early anti-obesity effect among three groups. The final body weights were significantly lower in the two fucoxanthin groups (0.05 and 0.2%) than in comparison to the

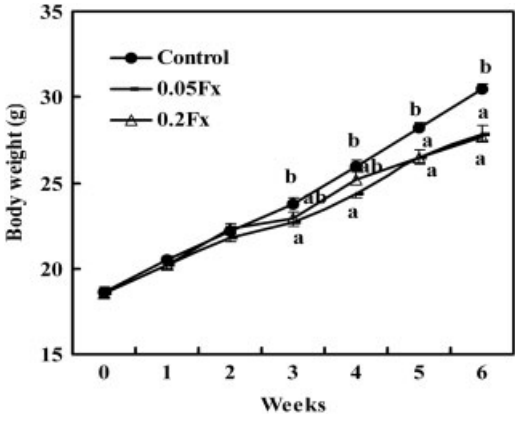

Figure 2. Effect of fucoxanthin supplementation on weekly changes of body weight in high-fat fed mice. Mean ± SE, $n = 10$, [ab]Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test. 0.05Fx, 0.05% fucoxanthin-supplemented group with a high-fat diet; 0.2Fx, 0.2% fucoxanthin-supplemented group with a high-fat diet.

control group (Table 1). After 6 wk, average body weight gain calculated was significantly lower in the 0.05 and 0.2% fucoxanthin groups than in the control group by 15 and 19%, respectively (Table 1). As such, the body weight-lowering effect of fucoxanthin was significant regardless of its dose. Although two doses of fucoxanthin did not affect food intake in high-fat fed mice, fucoxanthin lowered the food efficiency ratio compared with the control mice by 10% (Table 1).

3.2 Fat-pads weight and adipocyte sizes

Relative weights of adipose tissues were summarized in Table 2. Fucoxanthin significantly reduced the weight of epididymal and perirenal adipose tissues compared with the control group, but not that of interscapular adipose tissue. In this study, 0.05 and 0.2% fucoxanthin supplement lowered total WAT weight compared with the control group by 13.3 and 18.8%, respectively, although this effect was not dose dependent. However, epididymal adipocyte sizes of fucoxanthin groups were significantly smaller in a dose-dependent manner (Figs. 3A and B).

3.3 Plasma leptin and adiponectin levels

Plasma leptin level was significantly lower in the 0.05 and 0.2% fucoxanthin groups than in the control group, whereas adiponectin level was significantly elevated by fucoxanthin (Table 3). About 0.05% dietary fucoxanthin was more effective in elevating the plasma adiponectine concentration than 0.2% fucoxanthin. The leptin level exhibited a negative correlation with adiponectin level in the plasma ($r = -0.506$, $p < 0.01$) and a positive correlation with body weight ($r = 0.726$, $p < 0.01$) (data not shown).

3.4 Lipid-regulating enzyme activities in epididymal adipose tissue

As summarized in Table 4, two doses of fucoxanthin significantly lowered activities of lipogenic enzymes, FAS, ME and G6PD, in the epididymal fat tissue compared with the control group. Especially, the FAS activity exhibited a positive correlation with epididymal fat tissue weight ($r = 0.558$, $p < 0.01$), perirenal fat tissue weight ($r = 0.467$, $p < 0.05$) and body weight ($r = 0.463$, $p < 0.05$), respectively (data not shown). However, β-oxidation activity of epididymal fat tissue was higher in the 0.05 and 0.2% fucoxanthin groups than in the control group by 3.9-folds and 4.1-folds, respectively.

3.5 mRNA expression of lipid-regulating enzymes and UCPs in adipose tissues

Fucoxanthin significantly down-regulated mRNA expression of FAS, ME and G6PD in the epididymal fat tissue compared with the control group. In contrast to the lipogenic gene, mRNA expression of β-oxidation was up-regulated in fucoxanthin-supplemented groups. The effects of fucoxanthin on mRNA expression of FAS and β-oxidation were significant in a dose-dependent manner (Fig. 4). The 0.2% fucoxanthin supplement led to increased mRNA expression of UCP1 and UCP3 in interscapular BAT and that of UCP2 in the epididymal WAT, whereas the 0.05% fucoxanthin only elevated UCP1 mRNA expression in epididymal WAT (Fig. 5).

4 Discussion

Dietary fat is one of the most important environmental factors associated with the occurrence of obesity. Although diet-induced obese animals are considered a more comparable model for human obesity than genetically obese animals [19], the molecular mechanism of fucoxanthin for anti-obesity has not been elucidated in diet-induced obesity. High-fat diet (≥30% calories from fat) has been extensively used to induce obesity in rodents associated with insulin resistance and hyperglycemia [20, 21].

This study demonstrated that high-fat diet supplemented with 0.05 and 0.2% fucoxanthin for 6 wk resulted in a significant reduction in body weight gain without affecting

Table 1. Effects of fucoxanthin supplementation on body weight, food intake and food efficiency ratio in high-fat fed mice

	Control	0.05Fx[a)]	0.2Fx[b)]
Initial body weight (g)	18.65 ± 0.26	18.56 ± 0.25	18.58 ± 0.26
Final body weight (g)	30.46 ± 0.31[A)]	27.80 ± 0.36[B)]	27.69 ± 0.68[B)]
Body Weight Gain (g/day)	0.27 ± 0.00[A)]	0.23 ± 0.01[B)]	0.22 ± 0.01[B)]
Food Intake (g/day)	3.29 ± 0.03	3.24 ± 0.03	3.18 ± 0.05
FER[c)]	0.080 ± 0.002[A)]	0.072 ± 0.003[B)]	0.072 ± 0.004[B)]

Mean ± SE, $n = 10$, [AB]Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$ as determined by a one-way ANOVA test.

a) 0.05% fucoxanthin-supplemented group with a high-fat diet.

b) 0.2% fucoxanthin-supplemented group with a high-fat diet.

c) Food efficiency ratio (FER) = body weight gain/food intake.

Table 2. Effects of fucoxanthin supplementation on weights of white adipose tissue and brown adipose tissue in high-fat fed mice

	Control	0.05Fx[a)]	0.2Fx[b)]
Epididymal WAT (mg/g BW)	46.99 ± 1.75[A)]	38.35 ± 1.61[B)]	35.43 ± 2.22[B)]
Perirenal WAT (mg/g BW)	18.77 ± 0.69[A)]	14.93 ± 0.89[B)]	13.30 ± 0.80[B)]
Interscapular WAT (mg/g BW)	35.77 ± 1.10	34.72 ± 2.13	33.69 ± 1.30
Interscapular BAT (mg/g BW)	10.63 ± 0.75	11.65 ± 0.59	10.34 ± 0.47
Total WAT (mg/g BW)	101.5 ± 6.00[A)]	88.00 ± 5.25[B)]	82.42 ± 9.36[B)]

Mean ± SE, $n = 10$, [AB]Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$ as determined by a one-way ANOVA test.
a) 0.05% fucoxanthin-supplemented group with a high-fat diet.
b) 0.2% fucoxanthin-supplemented group with a high-fat diet.

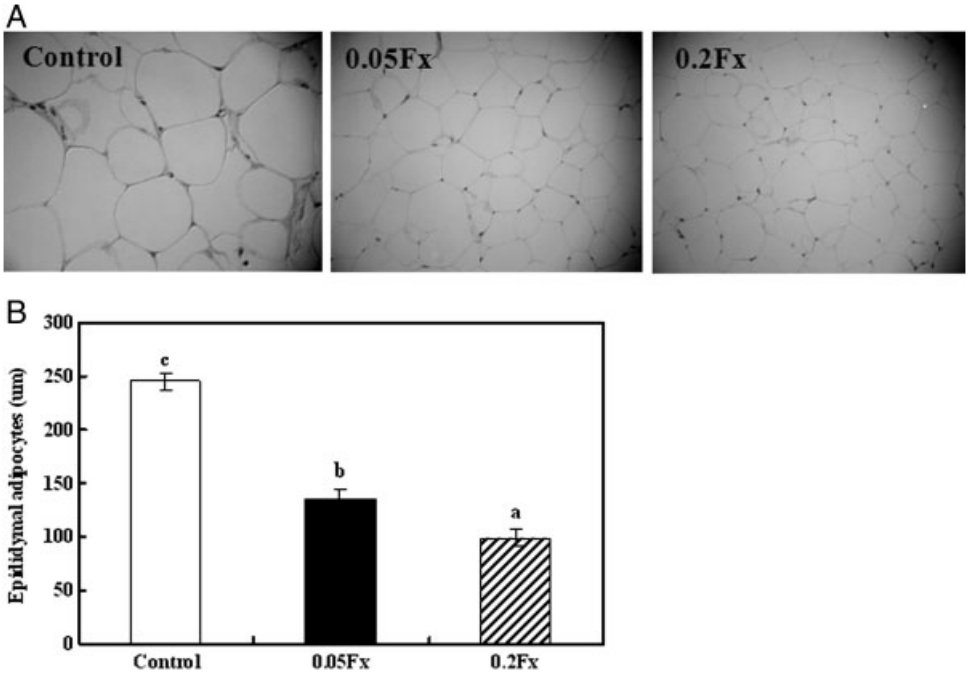

Figure 3. Effect of fucoxanthin supplementation on light micrography (A) and sizes (B) of epididymal adipocyte in high-fat fed mice (× 200). Mean ± SE, $n = 10$, [abc]Means not sharing a common letter are significantly different between groups at $p < 0.05$ as determined by a one-way ANOVA test. 0.05Fx, 0.05% fucoxanthin-supplemented group with a high-fat diet; 0.2Fx, 0.2% fucoxanthin-supplemented group with a high-fat diet.

Table 3. Effects of fucoxanthin supplementation on plasma leptin and adiponectin levels in high-fat fed mice

	Control	0.05Fx[a)]	0.2Fx[b)]
Leptin (ng/mL)	1.90 ± 0.02[A)]	1.81 ± 0.01[B)]	1.78 ± 0.01[B)]
Adiponectin (μg/mL)	2.39 ± 0.65[C)]	8.00 ± 0.62[A)]	4.89 ± 0.61[B)]

Mean ± SE, $n = 10$, [ABC]Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$ as determined by a one-way ANOVA test.
a) 0.05% fucoxanthin-supplemented group with a high-fat diet.
b) 0.2% fucoxanthin-supplemented group with a high-fat diet.

food intake, and hence decreasing FER compared with the control group. Recently, Maeda *et al.* [7] showed that 0.2% dietary fucoxanthin significantly lowered body weight gain in KK-A^y mice, an obese-diabetic model, but not 0.1% fucoxanthin. Interestingly, it was found that low dosage of fucoxanthin, 0.05%, significantly suppressed the increase in body weight gain as well as visceral fat-pads weights, epididymal and preirenal WAT, compared with the control group. Many studies have reported that phenolic compounds possessing antioxidant properties decrease body weight gain [22]. Obesity is characterized at the cell biological level by an increase in the number and size of

Table 4. Effects of fucoxanthin supplementation on lipid-regulating enzyme activities of epididymal WAT in high-fat fed mice

	Control	0.05Fx[a)]	0.2Fx[b)]
FAS (μmol/min/mg protein)	$0.137 \pm 0.01^{A)}$	$0.077 \pm 0.00^{B)}$	$0.076 \pm 0.00^{B)}$
ME (μmol/min/mg protein)	$1.68 \pm 0.20^{A)}$	$0.91 \pm 0.13^{B)}$	$0.93 \pm 0.10^{B)}$
G6PD (μmol/min/mg protein)	$2.80 \pm 0.32^{A)}$	$1.24 \pm 0.27^{B)}$	$1.27 \pm 0.15^{B)}$
β-oxidation (μmol/min/mg rotein)	$2.22 \pm 0.51^{B)}$	$8.75 \pm 2.14^{A)}$	$9.17 \pm 2.35^{A)}$

Mean ± SE, $n = 10$, [AB]Means in the row not sharing a common letter are significantly different between groups at $p < 0.05$ as determined by a one-way ANOVA test.
a) 0.05% fucoxanthin-supplemented group with a high-fat diet.
b) 0.2% fucoxanthin-supplemented group with a high-fat diet.

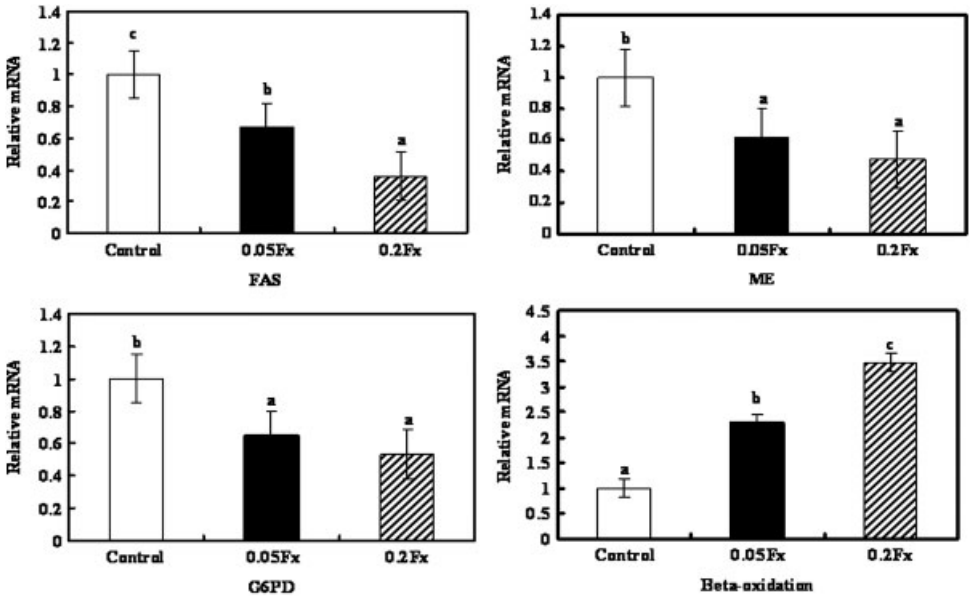

Figure 4. Gene expressions of lipid-regulating enzymes in epididymal WAT using quantitative RT-PCR analysis. Mean ± SE, $n = 8$, mRNA was normalized to GAPDH and expressed as a ratio to the corresponding amount in the control group. [abc]Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test. FAS, fatty acid synthase; ME, malic enzyme; G6PD, glucose-6-phosphate dehydrogenase; β-oxidation, fatty acid β-oxidation; 0.05Fx, 0.05% fucoxanthin-supplemented group with a high-fat diet; 0.2Fx, 0.2% fucoxanthin-supplemented group with a high-fat diet.

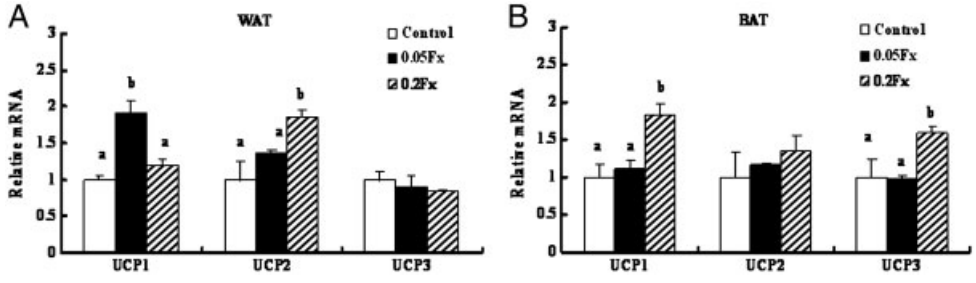

Figure 5. Gene expressions of UCPs in epididymal WAT (A) and BAT (B) using quantitative RT-PCR analysis. Mean ± SE, $n = 8$, mRNA was normalized to GAPDH and expressed as a ratio to the corresponding amount in the control group. [ab]Means not sharing a common letter are significantly different among groups at $p < 0.05$ as determined by a one-way ANOVA test. UCP-1,2,3, uncoupling protein-1,2,3; 0.05Fx, 0.05% fucoxanthin-supplemented group with a high-fat diet; 0.2Fx, 0.2% fucoxanthin-supplemented group with a high-fat diet.

adipocytes differentiated from fibroblastic pre-adipocytes in the adipose tissue [23]. Most of the anti-obesity effects of 0.05% fucoxanthin were similar to that of 0.2% focoxanthin in high-fat fed mice, while epididymal adipocyte sizes were dramatically diminished in a dose-dependent manner along with a decrease in its mRNA expression of lipid-regulating enzymes. Dietary manipulations by high-fat diet can effect increased changes in size of epididymal fat pads of the rat [24] and mice [25]. In general, visceral fat distribution in obesity is closely linked with metabolic complications and coronary heart disease [26]. The WAT is a primary site of energy storage in the form of triglycerides droplet, and it accumulates triglycerides during nutritional excess [27]. It was also confirmed that a positive correlation between body weight and visceral fat weights exists (epididymal: $r = 0.684$, $p < 0.01$ and perirenal: $r = 0.840$, $p < 0.01$). As such, the reduced body weights observed in fucoxanthin-supplemented groups were partly due to a decrease in fat-pad tissues. In accordance to another study [7], fucoxanthin supplementation prevented the diet-induced elevation of visceral fat and adipocyte sizes and revealed a marked anti-obesity effect.

It was also found that visceral (epididymal and perienal) fat weights showed a positive correlation with plasma leptin level, however, a negative correlation with plasma adiponectin level in diet-induced obese mice. In most obese subjects, plasma leptin concentration is high because of the increased leptin secretion from adipose tissues [28], and adipocyte size is correlated with a decrease in serum leptin concentration [29]. In this study, two doses of fucoxanthin, supplemented with 0.05 and 0.2% level in diet, significantly lowered the plasma leptin concentration compared with the control group, whereas it significantly elevated adiponectin concentration. In contrast to the findings in this study, 0.1% dietary fucoxanthin provided to KK-A^y mice did not alter plasma leptin or adiponectin concentration, but 0.2% fucoxanthin only significantly lowered plasma leptin levels [7, 30]. Adiponectin is very highly expressed in adipose tissues and it can increase β-oxidation in tissues and causes weight loss in mice [31]. Plasma adiponectin level in humans decreases with an increase in body weight or obese condition [32], which seems to be correlated with insulin resistance and hyperinsulinemia [33, 34]. As such, fucoxanthin exhibits a beneficial effect on improving the plasma adipokine level, thus enhancing β-oxidation.

Since metabolic response in adipose tissues is important in preventing and treating obesity and its related disorders [29, 35], the effect of fucoxanthin on lipid-regulating enzymes in the visceral adipose tissues was investigated in high-fat-fed mice. High-fat diet generally induces an increase in lipogenic enzyme activities as well as gene expression of these enzymes. Among the visceral fat tissues, epididymal fat tissue is the most sensitive to obesity [36]. Both 0.05 and 0.2% fucoxanthin supplement inhibited lipogenic enzymes activities, FAS, ME and G6PD, whereas fucoxanthin led to stimulate fatty acid β-oxidation activity in the epididymal WAT compared with the control group. Especially, the FAS activity in the epididymal WAT showed a positive correlation with its tissue weight ($r = 0.558$, $p < 0.01$). Newly synthesized fatty acids are used as substrates for triglyceride synthesis [37]. The present data also indicated a close correspondence between lipid metabolic enzyme activities and their mRNA expressions in epididymal fat tissue. Accordingly, fucoxanthin significantly down-regulated mRNA expression of FAS, ME and G6PD, whereas it up-regulated mRNA expression of β-oxidation in the epididymal fat tissue. Although the FAS and β-oxidation activities were not significantly different between two different doses of fucoxanthin in epididymal fat tissue, their mRNA expression levels were in a dose-dependent manner. The mechanisms of anti-obesity have been proposed as decreased energy intake and increased energy expenditure, decreased pre-adipocyte differentiation and proliferation, decreased lipogenesis and increased lipolysis and fat oxidation [38]. This study indicates that a significant reduction of the epididymal fat tissue weight in fucoxanthin-supplemented mice could be partly due to the decreased lipogenesis and increased fat oxidation.

This study showed that high-dose (0.2%) fucoxanthin up-regulated the gene expression of UCP1 and UCP3 in BAT and UCP2 in epididymal WAT; however, low-dose (0.05%) fucoxanthin only up-regulated that of UCP1 in epididymal fat tissue. It is known that BAT in rodents has an important role in the prevention and therapy of obesity [39]. The UCP1 in BAT is known as a significant component of whole body energy expenditure and its dysfunction contributes to the development of obesity [40]. UCP1 expression in WAT would also be an increasingly attractive target for the development of anti-obesity therapies [41]. UCP2 and UCP3 are expressed in various tissues, such as the BAT, skeletal muscle, WAT, liver, kidney, lung and the immune system. In particular, UCP2 and UCP3 in skeletal muscles regulate the thermogenesis in obese mice [42]. However, much evidence indicates that, in contrast to UCP1, UCP2 and UCP3 are not crucial for nonshivering thermogenesis, but a major function of UCP2 and UCP3 is to attenuate mitochondrial production of free radicals in mitochondria, in cells, and *in vivo* and to protect against oxidative damage [43]. For these reasons, UCP2 and UCP3 can be important targets for treatment of aging, degenerative diseases, diabetes and perhaps obesity [44]. Thus, high doses of fucoxanthin seemingly enhanced the thermogenic capacity of BAT compared with those of control mice and low doses of fucoxanthin resulted induction of the UCP1 gene expression in WAT. In Maeda *et al.*'s findings, 0.4 [3], 0.15 [7] and 0.2% [30] levels of dietary fucoxanthin, those are higher doses than 0.05% in which were used in this study, resulted to increase the UCP1 gene expression in WAT of KK-A^y mice.

In conclusion, this study provided direct evidence that dietary fucoxanthin, even with a 0.05% dose, can exhibit

potential anti-obesity effect in high-fat diet-induced mice, which may be mediated by altering plasma adipokine level, down-regulating fat production while up-regulating β-oxidation and UCP gene expressions in visceral adipose tissues. Fucoxanthin may act as a regulator of lipid metabolism in fat tissues and its health-promoting effect could be beneficial for preventing obesity.

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