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ARTICLE

# Evaluation of the anti-obesity effect of the microalga *Phaeodactylum tricornutum*

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**Abstract** *Phaeodactylum tricornutum* (PT) is a classical model diatom widely used in ecology, physiology, biochemistry, and molecular biology studies. We evaluated the anti-obesity effects of PT powder based on a number of metabolic parameters in a model of diet-induced obesity. We fed C57BL/6 mice a high-fat diet supplemented with PT powder (15 or 30 % w/v) for 12 weeks, and determined energy intake, weight loss, and lipid profiles each week. PT powder significantly reduced body weight gain, and epididymal and perirenal adipose tissue weight via activation of AMPK and HMGCR pathways. Moreover, we found that fucoxanthin, the putative anti-obesity compound in PT, was effectively micellized and transferred to the soluble fraction at the ileum in an in vitro simulated digestion system. Our results indicate that PT powder has an anti-obesity effect and suggest that it is a candidate substance for the development of anti-obesity foods, supplements, and even drugs.

**Keywords** Fucoxanthin · Obesity · *Phaeodactylum tricornutum*

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## Introduction

Marine resources are diverse and abundant. Their importance has increased as terrestrial biological resources have decreased. Marine microalgae are considered important primary modulators of marine environments and play a critical role in supporting aquatic animals. As primary producers in the food chain, marine microalgae are of interest owing to their physiological and nutritional value (Mimouni et al. 2012). Over 15,000 compounds, including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, toxins, and pigments (lutein, β-carotene, etc.), have been isolated from cultured microalgae. The biomass of the diatom species *Phaeodactylum tricornutum* (PT) contains up to 35 % eicosapentaenoic acid (Peng et al. 2014). It contains 36.4 % crude protein, 26.1 % available carbohydrates, 18.0 % lipids, 15.9 % ash, and 0.25 % neutral detergent fiber on a dry weight basis (Ryckebosch et al. 2011). Fucoxanthin is its major pigment, and it plays a crucial role in the light-harvesting complexes of photosystems (Kawee-ai et al. 2013). Fucoxanthin is a carotenoid and is abundant in marine environments, especially in macroalgae and microalgae. It has received recent attention because it has an anti-obesity effect (Gammone and D'Orazio 2015). PT is a good source of fucoxanthin (Kim et al. 2012). Therefore, PT is expected to be beneficial for the management of obesity (Kawee-ai et al. 2013).

Obesity is a term used to describe the condition of being very overweight and is characterized by excessive body fat (Cottrell and Ozanne 2008). It is a risk factor for a number of chronic diseases, including diabetes, cardiovascular diseases, and cancer (Cottrell and Ozanne 2008). Therefore, the development of anti-obesity drugs has important economic and medical implications. However, the anti-

obesity effect of PT components has not been studied in vivo. In this study, we demonstrated the anti-obesity effects of PT in a dry powder state.

## Materials and methods

### Animals and experimental protocol

Four-week-old male C57BL/6 mice were purchased from Dooyeol Biotech (Korea). All mice (n = 84) were housed individually in cages under specific pathogen-free conditions at a temperature of 22–26 °C and maintained on a 12-h light/dark cycle with continuous access to food and water. After a 1-week adaptation period, mice were divided into four groups and fed specific diets for a period of 12 weeks. The normal diet (ND) group was fed a basal diet based on the standard AIN-76 diet containing 5 % corn oil ad libitum (Table 1). The high-fat diet group (HFD) was fed a diet consisting of 4.73 kcal/g with 45 % fat, 20 % proteins, and 35 % carbohydrates (Dooyeol Biotech) and permitted ad libitum consumption of water (HFD + Water). The HFD + PT powder group was fed a HFD containing 15 % (HFD + PT15 %) or 30 % (HFD + PT30 %) freeze-dried PT powder ad libitum. After 12 weeks, the mice were sacrificed by decapitation. Blood, liver, kidney, and epididymal white adipose tissue samples were collected, weighed, and stored at −80 °C. The food efficiency ratios (FER) were calculated as the ratio of body weight gain (g) to the total amount of food (g) ingested over the experimental period. The experimental protocol was approved by the Ethics Committee for Animal Experimentation of Chungbuk National University (Permit Number: CBNUA-633-13-01, Korea).

### Histological analysis

Mouse livers were fixed in 10 % neutral buffer formalin and embedded in paraffin. Sections (4 μm thick) were stained with hematoxylin and eosin (H&E) according to standard procedures. The livers were frozen in a deep freezer and embedded in optimal cutting temperature compound; 10 μm sections were stained with Oil Red O. Stained liver tissues were examined under a light microscope (Olympus, Japan).

### Western blot analysis

Western blot analysis was performed as described in a previous report (Hue et al. 2009). The livers were homogenized in a lysis buffer containing 20 mM HEPES (pH 7.5), 150 mM NaCl, 1 % Triton, 50 mM β-glycerophosphate (pH 7.2), 0.1 mM sodium vanadate, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl urea, 2 μg/mL leupeptin, and 4 μg/mL aprotinin. The soluble materials were separated by centrifugation. The lysates were resolved by 12 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred into nitrocellulose membranes. The membranes were blocked in Tris-buffered saline (10 mM Tris–Cl, pH 7.4) containing 0.5 % Tween 20 and 5 % nonfat dry milk, incubated with the first specific antibody in blocking solution for 5 h at room temperature, washed, and incubated with the secondary antibody for 1 h at room temperature. The protein bands were detected by chemiluminescence (Amersham Pharmacia Biotech, USA). The intensity of each band was quantified using the AlphaEase FC Program.

### Materials and reagents

Bile extract, α-amylase, pepsin, pancreatin, pancreatic lipase, and cholesterol esterase were purchased to simulate digestion from Sigma-Aldrich (USA). High-performance liquid chromatography (HPLC)-grade *tert*-butyl methyl ether (TBME), methanol, ethanol, water, and petroleum ether were supplied by Fisher Scientific (USA). Unless indicated, all other chemicals were purchased from Sigma-Aldrich. Dry PT powder was prepared by the freeze-drying method after harvesting from cultures as described in Kim et al. (Kim et al. 2012).

### Simulated digestion of PT powder

Simulated digestion was performed following previously described methods (Garrett et al. 1999a, b) with minor modifications. Briefly, the HFD (2 g) containing 15 and 30 % PT powder were each added to 10 mL of saline solution containing 120 mM NaCl, 5 mM KCl, and 6 mM $CaCl_2$. Then, 1000 units of α-amylase was added, and the pH was adjusted to 6.5. After adding saline solution to a volume of 12.5 mL, the samples were incubated at 37 °C for 5 min

**Table 1** Effect of PT powder on body weight and food intake in HFD-induced obese mice after 12 weeks

| Parameter | ND | HFD | HFD + PT15 % | HFD + PT30 % |
|---|---|---|---|---|
| Body weight gain (g) | 11.76 ± 2.05 | 20.33 ± 1.26 | 15.97 ± 1.43 | 10.45 ± 0.96 |
| Total food intake (g) | 254.28 ± 7.43 | 235 ± 3.75 | 241.66 ± 4.21 | 255.12 ± 14.84 |
| FER | 4.41 ± 0.65 | 8.66 ± 0.76 | 6.61 ± 0.69 | 4.1 ± 0.27 |

*FER* food efficiency ratios

in a shaking water bath (Lab Companion, Jeio Tech, Korea) at 95 rpm to simulate the oral phase of digestion. To mimic the gastric phase of human digestion, the pH of the sample was acidified to 2.2 with HCl, and 0.5 mL of porcine pepsin solution (0.075 g/mL in 0.1 N HCl) was added. The samples were filled with saline solution to a volume of 15 mL and incubated at 37 °C for 2 h in a shaking water bath at 95 rpm. To simulate the intestinal phase accurately and specifically, three intestinal regions were considered separately (the duodenum, jejunum, and ileum). For the duodenum, 250 mg of bile extract, 0.5 mL of pancreatic lipase (0.01 g/mL of 0.1 M sodium bicarbonate), and 0.5 mL of pancreatin (0.08 g/mL saline) were added, and the pH was increased to 4.7 by adding 1 M sodium bicarbonate. Samples were incubated for 30 min at 37 °C (final volume, 20 mL). For the jejunum, the pH was adjusted to 5.5, and samples were incubated for 1 h at 37 °C (final volume, 22.5 mL). Finally, the pH was adjusted to 6.5, and samples were incubated for 2 h at 37 °C (final volume, 25 mL) to mimic the ileum. At the end of each digestion, the bioaccessibility of fucoxanthin was examined by collecting digestates (1.4 mL) at each intestinal part, and the aqueous micelles were isolated by ultracentrifugation at 12,300×*g* for 30 min. The resulting micelles were filtered through a 0.45-μm GHP filter. To measure the digestive stability of fucoxanthin in PT powder, the entire digestive solution at each intestinal region was extracted using an equivalent volume of ethanol (20, 22.5, and 25 mL) by sonication for 1 h. The micelles and extracts were immediately used for the fucoxanthin analysis.

### Carotenoid extraction from PT powders, micelles, and digestates

One gram of each HFD containing PT powder (15 and 30 %) was mixed with 10 mL of ethanol and grinded thoroughly, followed by sonication for 1 h. The extraction process for PT powder was repeated in triplicate. After extraction, the supernatants were syringe filtered (PTFE filter) and analyzed by HPLC. Fucoxanthin contents were calculated based on diet weight and converted to an amount based on PT dry powder. To separate fucoxanthin in a simulated digestion experiment, each micelle and digestate (1 mL) was mixed with 3 mL of ethanol and extracted by vortexing for 30 s after stepwise addition of 1 mL of TBME, 1 mL of petroleum ether, and 5 mL of water. Tubes were centrifuged at 3500×*g* for 10 min at room temperature. The upper phase was collected, and the extraction with TBME and petroleum ether was repeated at least twice. Combined extracts were evaporated until dry using nitrogen purging. The residues were dissolved in an HPLC mobile phase solution. All procedures were carried out under subdued light, and 20 μL of *β*-apo-8′-carotenal was added as an internal standard to all samples.

### HPLC analysis

Fucoxanthin quantification was performed by HPLC analysis as described previously (Kim et al. 2012). In brief, a YMC carotenoid column (250 × 4.6 mm i.d. with a 3 μm particle size, Waters, Ireland) was used to analyze fucoxanthin from various sample solutions. Two mobile phases (methanol and water) were used with the following gradient program: the methanol/water ratio was increased from 90:10 to 100:0 over 20 min, and 100 % methanol was maintained for the next 5 min. After that, the ratio was decreased from 100:0 to 90:10 over 5 min, and this ratio was maintained for the final 5 min. The flow rate of the mobile phase was 0.7 mL/min with a column temperature of 35 °C. The chromatogram obtained at 450 nm was used for the quantitative analysis of fucoxanthin.

### Statistical analysis

Student's *t* tests were used to determine statistical significance. Data were expressed as mean ± SD.

## Results

### Body weight, food intake, and feed efficiency ratios

The average initial body weight of mice was 14.6 g and did not differ significantly among the four groups. We detected gradual increases in the body weights of mice in all groups during the experimental period. As shown in Table 1, the HFD induced a large increase in body weight. After 12 weeks, the final body weights were higher in the HFD group than in the ND group. Mice in the HFD + PT30 % group had similar body weights to those in the ND group. The mean body weight of mice in the HFD + PT30 % group was 52 % lower than that of mice in the HFD group. The mean body weight of mice in the HFD + PT15 % group was also lower than that of mice in the HFD group, but the magnitude of the difference was lower than what it was in the HFD + PT30 % group. Mice in the HFD + PT30 % and HFD + PT15 % groups exhibited higher consumption than mice in the HFD group. Although food intake did not differ considerably between the four groups, the FER was highest in the HFD group, lowest in the HFD + PT30 % group, and intermediate in the HFD + PT15 % and ND groups.

### Liver, epididymal adipose tissue, and perirenal adipose tissue weights

We weighed the livers and fat surrounding the organs. We did not observe significant differences in liver weight

between experimental groups (Fig. 1A). Epididymal adipose tissue weight (Fig. 1B) and perirenal adipose tissue weight (Fig. 1C) were significantly higher in the HFD group than in the ND group. However, PT powder reduced epididymal adipose tissue and perirenal adipose tissue weights. The epididymal and perirenal adipose tissue weights were 64 and 43 % higher in the HFD mice than those in the ND mice, respectively. The HFD + PT30 % and ND groups exhibited similar epididymal adipose tissue and perirenal adipose tissue weights. The epididymal adipose tissue and perirenal adipose tissue weights were lower in the HFD + PT15 % group than those in the HFD group.

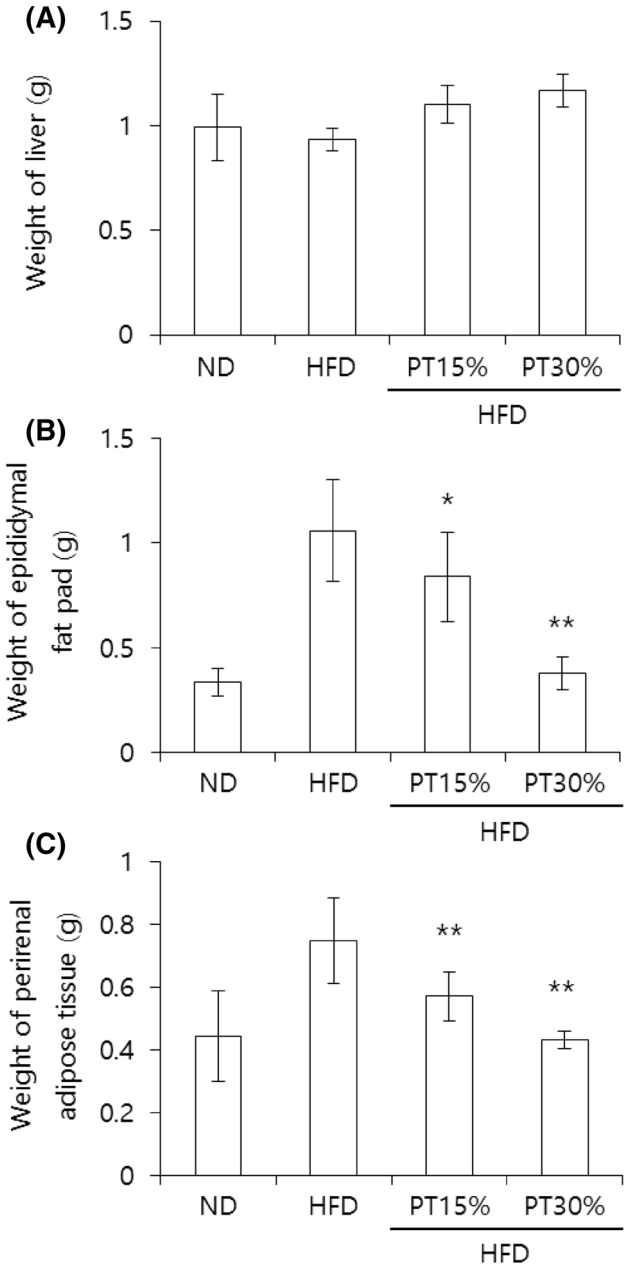


**Fig. 1** Effect of the experimental diets on liver weight (**A**), epididymal adipose tissue (**B**), and perirenal adipose tissue (**C**). The values shown are the mean ± SDs for three independent experiments. Statistical analysis was performed using *t* tests to compare the means of HFD groups with the ND group (*$p < 0.05$, **$p < 0.01$)

## Histological analysis of the liver

The livers of HFD mice had more lipid droplets than those of lean, ND mice. To determine the histological status, liver tissues were stained with Oil Red O and H&E, and examined under a light microscope. As shown in Fig. 2, the HFD-fed mice had larger fat droplets than the ND-fed mice. However, PT powder strongly decreased the size of lipid droplets. It also resulted in a decreased number of lipid droplets compared to the HFD-fed mice. Similarly, based on Oil Red O staining and H&E staining results, the accumulation of lipid droplets in the livers of HFD mice was remarkably reduced by PT powder intake.

## Effect of PT powder on the expression and phosphorylation of enzymes involved in fatty acid and cholesterol synthesis in the liver

We assessed the expression of p-AMPK, p-ACC, and HMG-CoA reductase (HMGCR), which are involved in lipid synthesis, in the livers of mice fed the HFD supplemented with PT powder. HFD suppressed the phosphorylation of AMPK and ACC, and PT powder restored it (Fig. 3). HMGCR expression was significantly lower in the livers of mice fed a HFD plus PT powder than in those of HFD-fed mice. The expression levels of p-AMPK in the livers of mice fed HFD plus PT powder were approximately 1.5-fold (PT15 %) and 1.46-fold (PT30 %) higher, and the expression level of p-ACC was approximately 1.5-fold (PT30 %) higher than those in the livers of mice fed a HFD. HMGCR expression levels in the livers of mice fed a HFD plus PT powder were approximately 0.8-fold (PT15 %) and 0.5-fold (PT30 %) lower than in those of HFD-fed mice.

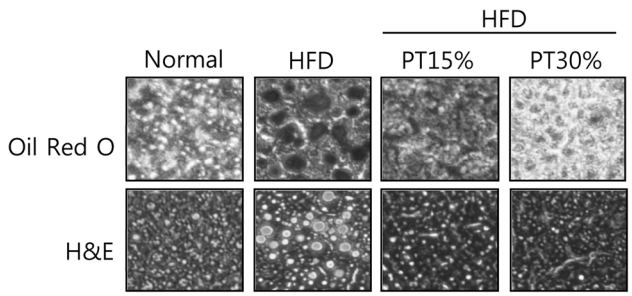


**Fig. 2** Histological analysis of liver tissues. Sections of liver tissue stained with Oil Red O and H&E are shown (magnification, ×200)

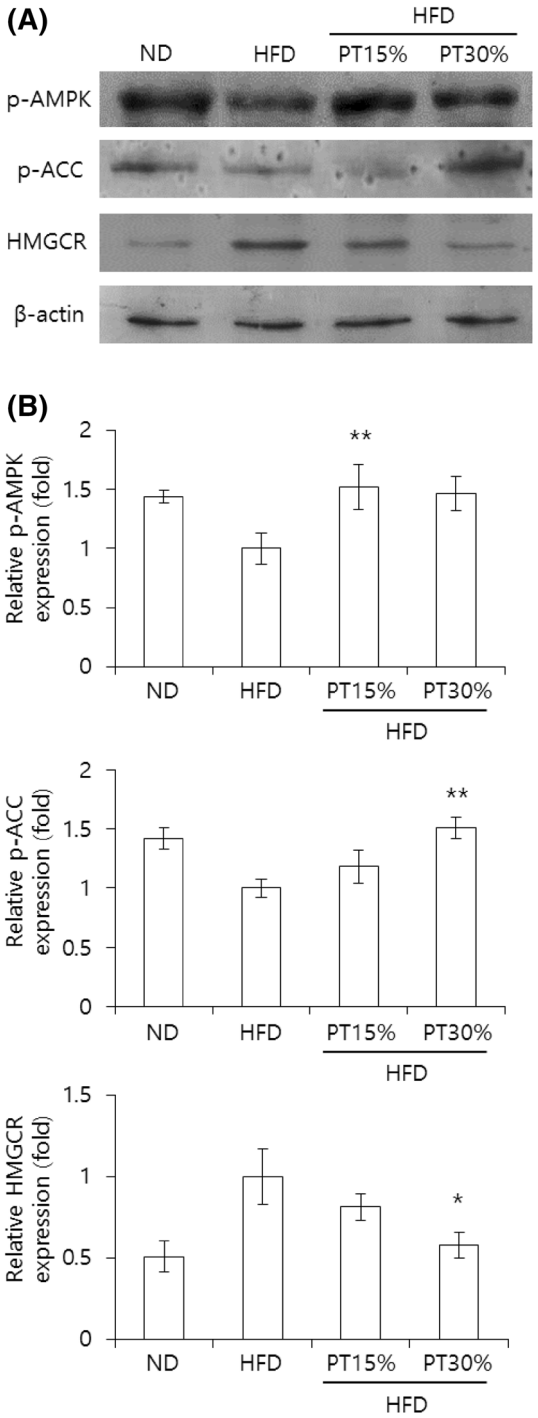


**Fig. 3** Effect of PT powder on the expression of lipid metabolism-related enzymes in the liver (**A**). The livers were homogenized, and the lysates were subjected to Western blot analysis for p-AMPK, p-ACC, and HMGCR (**B**). The intensity of each band was quantified using the AlphaEase FC Program. The values shown are the means ± SD for three independent experiments. Statistical analysis was performed using *t* tests to compare the means of the HFD groups with that of the ND group (*$p < 0.05$, **$p < 0.01$)

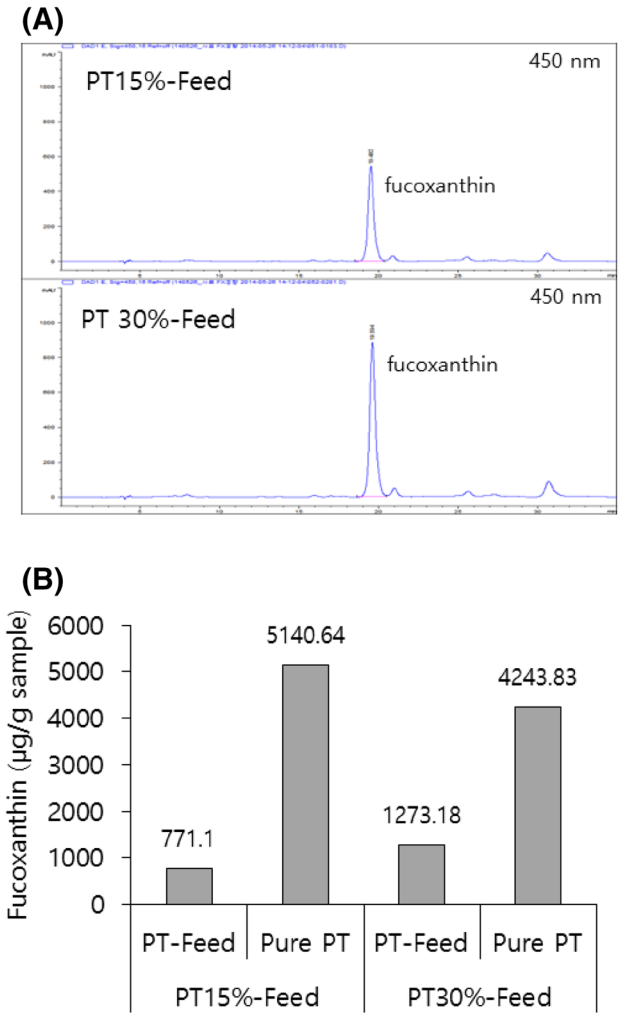


**Fig. 4** Comparison of fucoxanthin contents in mouse feed containing various amounts of PT powder (15 or 30 %). (**A**) HPLC chromatogram of an ethanol extract of mouse feed containing PT powder. HPLC eluent fractions were monitored at a wavelength of 450 nm for fucoxanthin, and the area under the fucoxanthin peak at 19.5 min was used for quantitative analysis of fucoxanthin in mouse feed. (**B**) Fucoxanthin contents of each diet are expressed as the amount of fucoxanthin in the whole diet and were then converted to amounts based on the amounts of added dry PT powder

## Fucoxanthin contents for each PT feed type

We examined the fucoxanthin content of each diet using HPLC methods. Based on previous results regarding extraction efficiency from PT powder (Kim et al. 2012), we used ethanol solvent to extract fucoxanthin from both diets. As shown in Fig. 4A, the HPLC chromatogram of PT30 %-Feed exhibited higher and wider peak of fucoxanthin than that of PT15 %-Feed. Diets containing differ-

ent amounts of PT powder had fucoxanthin contents that were within the expected ranges (771.1 and 1273.18 μg/g of diet for 15 and 30 % PT powder, respectively). By converting these values to fucoxanthin amount based on dry PT powder, we determined that fucoxanthin was stable in the PT powder during preparation, even though the exact amounts differed slightly between the diets.

### Bioaccessibility of fucoxanthin from PT powder during simulated digestion

To evaluate the bioaccessibility of fucoxanthin in PT powder, we simulated the digestion of each diet (i.e., those containing 15 and 30 % PT). Bioaccessibility was defined as the relative amount of fucoxanthin released from a complex food matrix during digestion; this released portion is potentially available for absorption. As shown in Fig. 5, the maturation of fucoxanthin micellization depended on the intestinal region. At the duodenum and jejunum, less than 1.2 % of fucoxanthin was micellized. However, fucoxanthin was effectively micellized and transferred to the soluble fraction at the ileum. We detected a similarly high bioaccessibility (39.97 and 44.10 %) for both diets. We observed significant chemical loss, especially at the jejunum and ileum, during simulated digestion. More than approximately 50 % of the fucoxanthin in PT powder was already degraded at the jejunum. As shown in Fig. 5, a substantial amount of fucoxanthin in PT powder can be released as absorbable micelles during digestion, and the specific conditions that characterize different intestinal regions, such as pH, digestive enzymes, and reaction time, strongly affect the micellization and digestive degradation of fucoxanthin.

## Discussion

The microalga PT is a potentially useful dietary supplement (Rebolloso-Fuentes et al. 2001) because microalgae-derived carotenoids are antioxidant and anti-inflammatory compounds. In this study, we demonstrated the anti-obesity effects of PT powder and analyzed the bioaccessibility of fucoxanthin, the putative anti-obesity compound. In a previous study, a fucoxanthin-rich fraction or fucoxanthin derived from *Undaria pinnatifida* was shown to exert anti-obesity and anti-fatty liver effects in HFD-induced and genetically modified obese animals (Jeon et al. 2010). *Undaria pinnatifida*-derived lipids, which contain 0.2 % fucoxanthin, decreased abdominal white adipose tissue weight in obese rats and mice. In addition, many studies demonstrated anti-obesity effects of fucoxanthin-rich lipid extracts or fucoxanthin obtained from microalgae in obese rodents (Maeda et al. 2007). However, until now, no study has demonstrated an anti-obesity effect of microalgae biomass containing fucoxanthin.

The mechanisms that mediate the anti-obesity effect of fucoxanthin are partially known. Fucoxanthin induces uncoupling protein 1 (UCP1) expression in white adipose tissue. UCP1 is mainly expressed in brown adipose tissue and acts in thermogenesis, energy expenditure regulation, and protection against oxidative stress (Miyashita et al. 2011). Fucoxanthin also prevents pancreatic lipase activity in vitro and triglyceride absorption in vivo (Matsumoto et al. 2010). A recent study showed that PT is a good source of fucoxanthin (Kim et al. 2012). Specifically, PT extract reduces weight gain and white adipose tissue accumulation by increasing UCP1 expression in white adipose tissue and by inhibiting the absorption of dietary fat via fucoxanthin (Kang et al. 2013). Our results are in

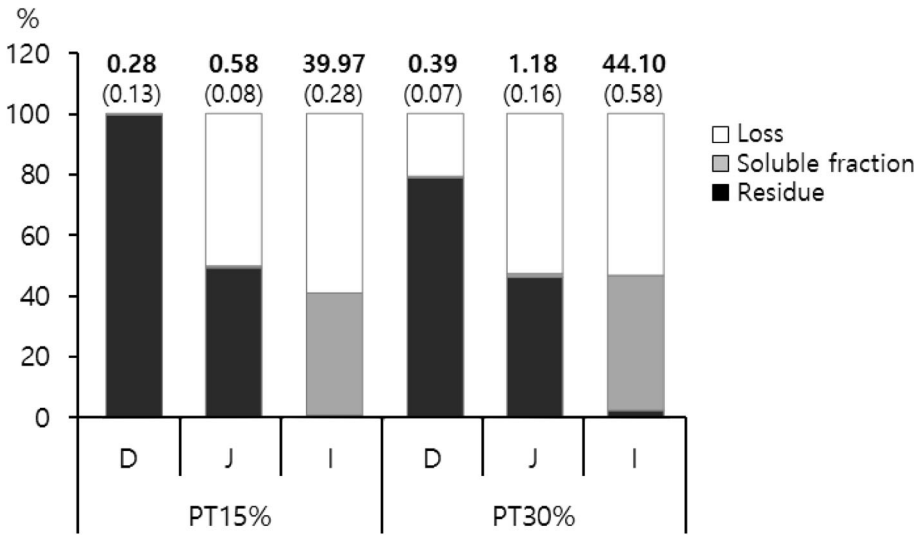


**Fig. 5** Influence of digestion on bioaccessibility and digestive stability of fucoxanthin in mouse diets containing different amounts of PT powder (PT15 and PT30 %) in three parts of intestine (*D* duodenum, *J* jejunum, and *I* ileum in *X* axis). *Bold* values above each bar denote fucoxanthin bioaccessibility (the % proportion of fucoxanthin present in the micelles compared with that contained in the nondigested PT powder (100 % in *Y* axis)). Values in parentheses are standard deviations ($n = 3$)

agreement with previous experimental data suggesting that PT powder administration in diet-induced models of obesity results in reduced body weight.

The biological activities of fucoxanthin are attributed to its distinct molecular structure, which includes an uncommon allenic bond, a 5,6-monoepoxide, and nine conjugated double bonds (Yan et al.1999; Hosokawa et al. 2009). Since such allenic bonds are highly susceptible to isomerization and oxidation, fucoxanthin is liable to degradation during processing and storage owing to exposure to heat, light, oxygen, and acidic or alkali solutions (Zhao et al. 2014). In fact, Hii et al. (2010) demonstrated that fucoxanthin is highly unstable when exposed to light and acidic pH.

Heating is a common and effective food processing technique. Moreover, temperature maintenance is an important factor influencing food quality. Therefore, the thermal stability of carotenoids has been widely investigated for various medium types in the presence or absence of air or light. Heating induces not only the degradation of all-*trans*, all-*cis*, and total carotenoids via oxidation, but also the formation of some *cis* isomers by isomerization, such as 9-*cis*, 13-*cis*, or 13, 15-di-*cis* forms, depending on treatment conditions (i.e., air and light exposure), medium, and carotenoid type (Pesek and Warthesen 1990; Manan et al. 1995; Sharma and Le Maguer 1996; Chen and Huang 1998; Henry et al. 1998; Lee and Chen 2002; Ax et al. 2003; Shi et al. 2003; Aman et al. 2005; Rios et al. 2005). Zhao et al. observed the degradation of total and all-*trans* fucoxanthin at all tested temperatures (25 and 100 °C) without air and light, and fucoxanthin degradation was promoted by exposure to both air and light (Zhao et al. 2014). In the present study, we demonstrated that fucoxanthin is stable in PT powder during preparation. The samples were not exposed to light or heat, and no acidic or basic solutions were added during feed production. Our results suggest that fucoxanthin is more highly stable in PT powder than are isolated compounds.

"Bioaccessibility" is a key indicator of the nutritional efficiency of compounds in food complexes. In this study, we considered the relative amount of fucoxanthin released from dried PT powder during digestion, which is potentially available for absorption. The released fucoxanthin was micellized and transferred to the soluble fraction at the ileum. Unexpectedly, we observed similarly high bioaccessibility (39.97 and 44.10 %) for the diets containing 15 and 30 % PT power. Based on these results, we suggest that the bioaccessibility of fucoxanthin is not dependent on the concentration of PT.

In this study, fucoxanthin restored AMPK phosphorylation and inhibited the activities of lipogenic enzymes such as ACC and HMGCR in the livers of HFD-fed mice. AMPK plays a key role as a regulator of body weight and the systemic glucose level (Kahn et al. 2005) because its activation positively regulates the signaling pathways of fatty acid oxidation and autophagy and negatively regulates gluconeogenesis and lipid synthesis (Kahn et al. 2005; Wu et al. 2010). Therefore, AMPK is a possible therapeutic target for the treatment of obesity and diabetes mellitus. After AMPK is phosphorylated, metabolic enzymes such as ACC and HMGCR are inactivated (Carling et al. 1987; Hardie 1992; Hardie et al. 1997). AMPK phosphorylation in C57BL/6 mice is inhibited by a HFD (Ejaz et al. 2009; Wu et al. 2010; Seo et al. 2011).

HMGCR inhibitors are used to lower serum cholesterol because the HMGCR enzyme is part of the mevalonate pathway, which is involved in cholesterol production (Farmer 1998). HMGCR reduces the risk of cardiovascular disease. ACC controls the metabolism of fatty acids (Tong 2005). It catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, which is involved in the production of new fatty acids. Moreover, ACC prevents the beta-oxidation of fatty acids in the mitochondria (Brownsey et al. 1997). When the energy status of a cell is low, ACC phosphorylation is increased, and phosphorylated ACC induces the activation of AMPK. Therefore, ACC has potential clinical applications for the production of an antagonist or the development of new therapies for obesity (Corbett and Harwood 2007). A recent study showed that fucoxanthin significantly reduces ACC gene expression (Gammone and D'Orazio 2015).

In conclusion, PT powder significantly reduced body weight gain and epididymal adipose tissue weight via activation of AMPK and HMGCR pathways. Moreover, we found that fucoxanthin, the putative anti-obesity compound in PT, was effectively micellized and transferred to the soluble fraction at the ileum in an in vitro simulated digestion system. Our results indicate that PT powder has an anti-obesity effect and suggest that it is a candidate substance for the development of anti-obesity foods, supplements, and even drugs.

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