Petalonia binghamiae Extract and Its Constituent Fucoxanthin Ameliorate High-Fat Diet-Induced Obesity by Activating AMP-Activated Protein Kinase

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ABSTRACT: In this study, we investigated the antiobesity properties of *Petalonia binghamiae* extract (PBE) in mice in which obesity was induced with a high-fat diet (HFD). PBE administration (150 mg/kg/day) for 70 days decreased body weight gain, adipose tissue weight, and the serum triglyceride level in mice fed a HFD. PBE reduced serum levels of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase as well as the accumulation of lipid droplets in the liver. PBE restored the HFD-induced decrease in phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in epididymal adipose tissue. PBE increased the phosphorylation of AMPK and ACC and decreased the expression of SREBP1c in mature 3T3-L1 adipocytes. In addition, we further explored the active compound responsible for AMPK activation by PBE in 3T3-L1 adipocytes. Fucoxanthin isolated from PBE increased the phosphorylation of AMPK and ACC with increasing LKB1 phosphorylation in mature 3T3-L1 adipocytes. Taken together, these data suggest that PBE (or fucoxanthin) exert improving effects on HFD-induced obesity by promoting β-oxidation and reducing lipogenesis.

KEYWORDS: *Petalonia binghamiae*, fucoxanthin, 3T3-L1 adipocyte, high-fat diet-induced obesity, AMP-activated protein kinase

■ INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and increased health problems.[1] Obesity is a leading preventable cause of death worldwide, with increasing prevalence in adults and children, and it was considered as one of the most serious public health problems of the 21st century.[2] Excessive fat accumulation in the body, especially in white adipose tissue, causes obesity and results in an increased risk of many serious diseases, including diabetes, cardiovascular disease, nonalcholic fatty liver disease, hypertension, hyperlipidemia, and other health problems.[3−5] Therefore, antiobesity foods and food ingredients may avert obesity, possibly leading to the prevention of lifestyle-related diseases, if they can effectively reduce visceral fat mass.[6]

AMP-activated protein kinase (AMPK) is a metabolic master switch that is activated by LKB1 under conditions of intracellular stress, including glucose deficiency, hypoxia, and reactive oxygen species activity.[7,8] In the process of energy depletion, AMPK inhibits de novo fatty acid synthesis by inactivating acetyl-CoA carboxylase (ACC) and stimulates fatty acid oxidation by up-regulating the expression of carnitine palmitoyltransferase-1 (CPT-1), peroxisome proliferator-activated receptor α (PPARα), and uncoupling protein.[9] As a cellular energy regulator, AMPK plays a major role in glucose and lipid metabolism and in the control of metabolic disorders such as diabetes, obesity, and cancer.[10,11] Thus, AMPK has emerged as a therapeutic target for metabolic disorders.[12]

The edible brown alga *Petalonia binghamiae* (J. Agaradh) Vinogradova is a traditional food in fishery areas of northeast Asia. *P. binghamiae* has an aggregate of several leaves that are

15−50 mm in width and 100−250 mm in length. *P. binghamiae* extracts have been shown to possess antioxidant properties.[13] We previously reported that an ethanolic extract of *P. binghamiae* had an antidiabetic effect on streptozotocin-induced diabetic mice.[14] Moreover, we demonstrated that its water-soluble extract exerted antiobesity effects in rats with high-fat diet (HFD)-induced obesity.[15] These multiple physiological activities of *P. binghamiae* may thus offer many advantages for potential applications in the nutraceutical industry. However, it is needed to identify their active constituents and the real molecular events that underline their beneficial effects.

Fucoxanthin, which is a major marine carotenoid derived from brown seaweeds, has several physiological activities, including anticancer,[16] anticarcinogenic,[17] anti-inflammatory,[18] antioxidant,[19] and antiobesity[20] effects. We have recently reported that fucoxanthin isolated from *P. binghamiae* exerted antiobesity effects by inhibiting the differentiation of adipocytes at both intermediate and late stages as well as by inhibiting glucose uptake in mature adipocytes.[21] Yet, it is not addressed whether fucoxanthin activates AMPK, which plays an important role in fatty acid β-oxidation.

In this study, we investigated the effects of PBE administration on body weight gain, adipose tissue, liver weight, and the serum triglyceride level in mice fed a HFD by examining its effect on AMPK activation. In addition, to identify the possible
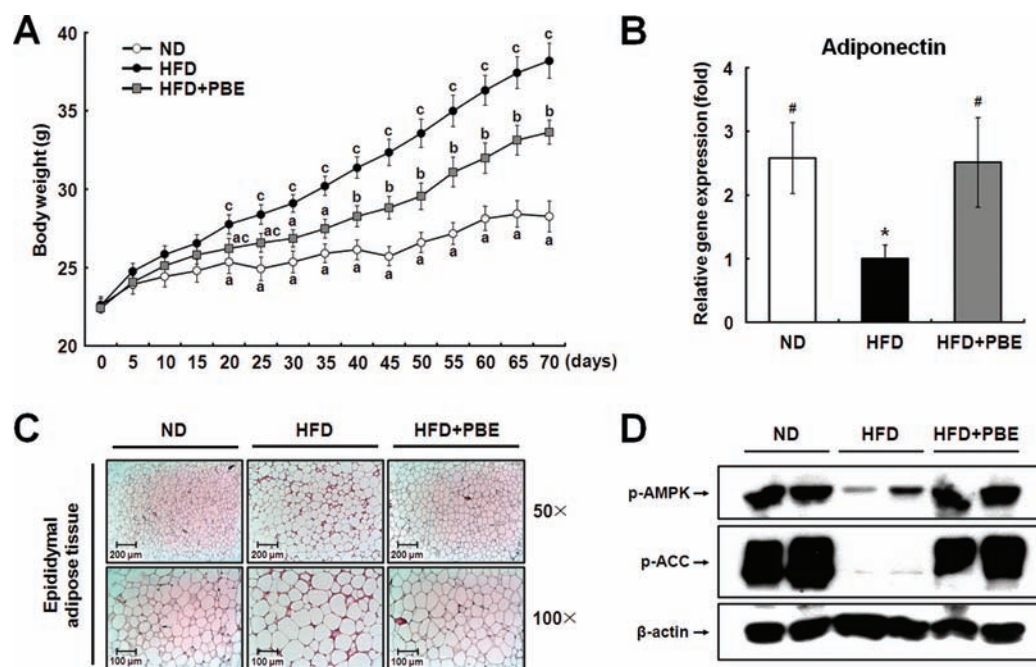
Figure 1. Effect of *Petalonia binghamiae* extract (PBE) on mice with high-fat diet (HFD)-induced obesity. Body weight changes in mice fed a normal diet (ND), HFD, or HFD+PBE (150 mg/kg/day). (A) Body weight was measured at 5-day intervals for 70 days. All results are shown as the mean ± SE (n = 10). Mean separation was performed using a Duncan's multiple range test. Different letters indicate significant differences ($p < 0.05$). (B) Real-time RT-PCR analysis of adiponectin mRNA expression in epididymal adipose tissue. All values are presented as the mean ± SD (n = 10; *$p < 0.05$ as compared to ND and #$p < 0.05$ as compared to HFD). The data shown are representative of three independent experiments. (C) Hematoxylin and eosin (H&E)-stained photomicrographs of epididymal adipose sections are shown at magnifications of 50× and 100×. (D) Expression of p-AMPK and p-ACC in epididymal adipose tissue as determined by Western blot analysis.
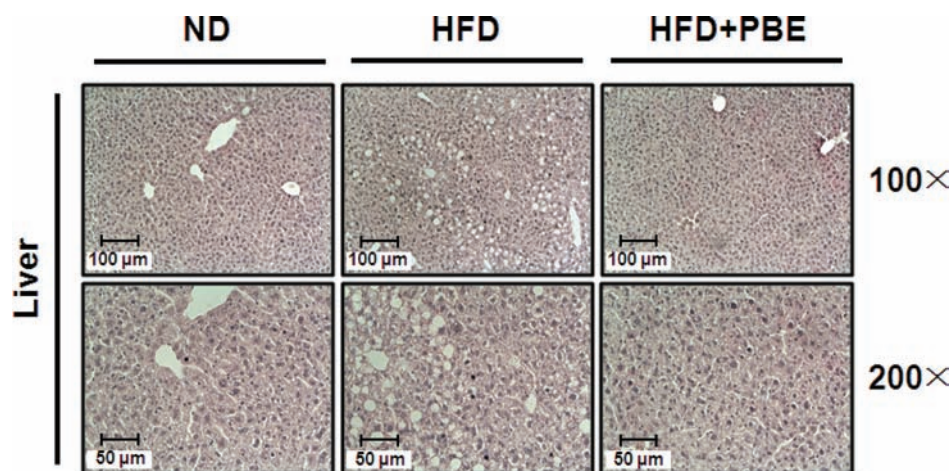
Figure 2. Effect of *Petalonia binghamiae* extract (PBE) on fatty droplets in the livers of mice fed a normal diet (ND), high-fat diet (HFD), or HFD +PBE (150 mg/kg/day). Hematoxylin and eosin (H&E)-stained photomicrographs of liver tissue sections are shown at magnifications of 100× and 200×.

active constituents contained in PBE, we investigated the effects of fucoxanthin, which was isolated from PBE, on the AMPK signaling pathway in mature 3T3-L1 adipocytes. Our finding is the first report to demonstrate that PBE and fucoxanthin enhance fatty acid β-oxidation via AMPK activation.

■ MATERIALS AND METHODS

Chemicals and Materials. Dulbecco's modified Eagle's medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS), and penicillin-streptomycin (PS) were obtained from Gibco (Grand Island, NY). Phosphate-buffered saline (PBS; pH 7.4), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dime-thylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO). The Lactate Dehydrogenase (LDH) Cytotoxicity Detection Kit was purchased from Takara Shuzo Co. (Otsu, Shiga, Japan). Antibodies against phospho-Ser431-LKB1 were purchased from Santa Cruz Biotechnol-ogy (Santa Cruz, CA). Antibodies against AMP-activated protein kinase (AMPK) α, phospho-Thr172-AMPK α (p-AMPK), acetyl-CoA carboxylase (ACC), and phospho-Ser79-ACC (p-ACC) were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against sterol regulatory element binding protein 1c (SREBP1c) were obtained from BD Biosciences (San Jose, CA). All other reagents were purchased from Sigma Chemical Co. unless otherwise noted.

Preparation of PBE and Fucoxanthin. Brown seaweed *P. binghamiae* was collected off the coast of Jeju Island, South Korea. PBE and fucoxanthin were prepared as described previously.[14,21] Briefly, lyophilized powder (1 kg) of *P. binghamiae* was extracted twice in 10 L of 80% ethanol at room temperature for 48 h. The procedure described above was repeated twice, designated PBE. The PBE solution was lyophilized and then stored at −20 °C until use. The yield of PBE from dried *P. binghamiae* was approximately 11.0%. The fucoxanthin of PBE was isolated by high-performance liquid chromatography (HPLC) using a Waters 2695 Alliance system (Waters Corp., Milford, MA) equipped with a system controller, autoinjector, column oven, and Waters 2998 photodiode array (PDA) detector. Fucoxanthin was isolated using a SymmetryPrep C18 column (300 × 7.8 mm ID; 7 μm) at 40 °C. PBE (40 μL, 100 mg/mL) was injected into the column and monitored by recording the UV spectra of the irradiated samples at wavelengths between 210 and 600 nm. The mobile phase for the prep-HPLC solvent system consisted of methanol (A) and water (B). The gradient mobile phase program was as follows: a 47 min gradient was started with 60% A, linearly increased to 100% A over 36 min, linearly reduced to 60% A over 2 min, and held for 7 min at a flow rate of 1.6 mL/min. The fucoxanthin peak was fractionated using a fraction collector and concentrated in a rotary evaporator at 40 °C. ^{13}C NMR spectra were collected using a JEOL FT/NMR 400 spectrometer (JEOL Ltd., Tokyo, Japan). ^{13}C NMR data were recorded at 400 and 100 MHz, respectively, with reference to the solvent signals. FAB-MS were obtained with *m*-nitrobenzyl alcohol matrix on JEOL, JMS-700 spectrometer. The spectral data for fucoxanthin were consistent with those reported previously.[22,23] The purity of fucoxanthin was >92% by HPLC analysis.

Quantitative Analysis. To quantify fucoxanthin contained in PBE, PBE was dissolved in MeOH and filtered through a 0.45 μm polytetrafluoroethylen (PTFE) syringe filter (Advantec Toyo Roshi Kaisha Ltd., Tokyo, Japan). High performance liquid chromatography (HPLC) was performed using a Waters 2695 Alliance system equipped with a system controller, autoinjector, column oven, and Waters 2998 photodiode array detector. PBE (10 μL) was injected onto a Sunfire RP 18 column (250 × 4.6 mm ID; 5 μm) at 30 °C. The mobile phase consisted of MeOH (A) and water (B), and the gradient elution programs was as follows: a 30 min gradient was started using 60% A, linearly increased to 100% over 20 min, held for 5 min, then finally returned to the initial conditions and for 4 min with a flow rate at 0.8 mL/min. Fucoxanthin was detected at 450 nm; the spectrum range was from 200 to 600 nm. The calibration curve for fucoxanthin analysis was calculated using Waters Empower 2 chromatography software. Fucoxanthin content in PBE was 3.57 ± 0.028 mg/g. Although PBE was the mixture of several constituents, fucoxanthin was detected as a main peak on HPLC chromatogram using UV detector.[24]

Animals. The animal study protocol was approved by the Institutional Animal Care and Use Committee of Jeju National University. After purchase, 30 male 4-week-old C57BL/6 mice (Nara Biotech Co., Ltd., Seoul, Korea) were adapted for 1 week to a specific temperature (22 ± 2 °C), humidity (50 ± 5%), and light schedule (light from 08:00 to 20:00). The animals were housed in plastic cages (two animals per cage) and given free access to drinking water and food. After adaptation, the mice (now 5 weeks old; 22.5 ± 1.1 g) were randomly divided into three groups of 10 mice each. One group (normal diet, ND) was fed a 10% kcal fat diet (D12450B, Research Diets, New Brunswick, NJ; protein 19.2%, carbohydrate 67.3%, fat 4.3%, and other 3.85 kcal/g), while the other two groups (HFD and HFD+PBE) were fed a 60% kcal fat diet (D12492, Research Diets; protein 26.2%, carbohydrate 26.3%, fat 34.9%, and other 5.24 kcal/g). PBE was dissolved in 0.1% carboxymethyl cellulose (CMC), and the mixture was administrated orally to the animals at a dosage of 150 mg/kg/day for 70 days. The oral administration volume was approximately 100 μL per 10 g of weight. The mice in the ND and HFD groups were administered 0.1% CMC.

Measurement of Body Weight, Food Intake, Epididymal Adipose Tissue Weight, Perirenal Adipose Tissue Weight, and Liver Weight. Body weight and food intake were measured once every 5 days for 70 days. At the end of the feeding period, mice were

anesthetized with diethyl ether after an overnight fast. The epididymal adipose tissue, perirenal adipose tissue, and liver tissue were rapidly removal from the sacrificed mice, and the weights of the tissues were determined.

Biochemical Analysis. After 70 days, the mice were sacrificed by an ether anesthesia overdose. Blood samples were drawn from the abdominal aorta into a vacuum tube and allowed to stand at room temperature for 30 min for clotting. Serum samples were then collected by centrifugation at 1000*g* for 15 min. The serum triglyceride (TG), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) concentrations were assayed using a commercial kit (Asan Pharm, Seoul, Korea) and an automatic blood analyzer (Kuadro, BPC Biosed, Rome, Italy).

Histology. After the blood had been drained from the livers, the livers and epididymal adipose tissues were fixed in a 10% neutral formalin solution for 48 h. The tissues were subsequently dehydrated in a graded ethanol series (75−100%) and embedded in paraffin wax. The embedded tissue was sectioned (8 μm-thick sections), stained with hematoxylin and eosin (H&E), and examined by light microscopy (Olympus BX51; Olympus Optical, Tokyo, Japan); photographs were taken at total magnifications of 50×, 100×, and 200×.

Cell Culture and Differentiation. 3T3-L1 preadipocytes obtained from the American Type Culture Collection (Rockville, MD) were cultured in DMEM containing 1% PS and 10% BCS at 37 °C under a 5% CO_2 atmosphere. To induce differentiation, 2-day postconfluent preadipocytes (designated day 0) were cultured in MDI differentiation medium (DMEM containing 1% PS, 10% FBS, 0.5 mM IBMX, 1 μM dexamethasone, and 5 μg/mL insulin) for 2 days. The cells were then cultured for another 2 days in DMEM containing 1% PS, 10% FBS, and 5 μg/mL insulin. Thereafter, the cells were maintained in postdifferentiation medium (DMEM containing 1% PS and 10% FBS), and the medium was replaced every 2 days. The effects of PBE on cell viability and cytotoxicity were determined by MTT and LDH assays, respectively. Mature 3T3-L1 adipocytes were cultured in DMEM containing 1% PS, 10% FBS, and PBE for 24 h. MTT (400 μg/mL) was added to each well, and the plate was incubated at 37 °C for 4 h. The liquid in the plate was then removed, and dimethyl sulfoxide was added to dissolve the MTT−formazan complex. The optical density was then measured at 540 nm. The effect of PBE on cell viability was evaluated by comparing the relative absorbance of treated cultures with that of control cultures. The cytotoxic effect of PBE was measured using an LDH Cytotoxicity Detection Kit. LDH activity in the medium and cell lysate was measured to evaluate cytotoxicity according to the manufacturer's instructions (LDH released into the medium/maximal LDH release × 100).

Western Blot Analysis. Adipose tissue was homogenized in ice-cold buffer containing lysis buffer [1× RIPA (Upstate Biotechnology, Temecula, CA), 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 1 mM NaF, and 1 μg/mL each of aprotinin, pepstatin, and leupeptin]. 3T3-L1 cells were washed with ice-cold PBS, collected, and centrifuged. The cell pellets were resuspended in lysis buffer and incubated on ice for 1 h. The adipose tissue and 3T3-L1 cell debris were then removed by centrifugation, and the protein concentrations in the lysates were determined using Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA). The lysates were then subjected to electrophoresis on 10% polyacrylamide gels containing sodium dodecyl sulfate and were transferred to polyvinylidene difluoride membranes. The membranes were blocked with a solution of 0.1% Tween-20 in Tris-buffered saline containing 5% bovine serum albumin (BSA) at room temperature for 1 h. After incubation overnight at 4 °C with primary antibody, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Immunodetection was performed using the ECL Western blotting detection reagent (Amersham Biosciences, Piscataway, NJ).

RNA Preparation and Quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction (Real-Time RT-PCR) Analysis. Total RNA was extracted from adipose tissue and 3T3-L1 adipocytes using the TRIzol reagent according to the manufacturer's instructions and then treated with DNase (Wako Pure Chemical

Industries, Ltd., Osaka, Japan). cDNA was synthesized from 1 μg of total RNA in a 20 μL reaction volume using a Maxime RT PreMix Kit (iNtRON Biotechnology, Seongnam, Kyunggi, Korea). The following primers were used for real-time RT-PCR: adiponectin, 5′-GAC CTG GCC ACT TTC TCC TC-3′, and 5′-GTC ATC TTC GGC ATG ACT GG-3′; carnitine palmitoyltransferase-1a (CPT-1a), 5′-ACC CTG AGG CAT CTA TTG ACA-3′, and 5′-TGA CAT ACT CCC ACA GAT GGC-3′; and β-actin, 5′-AGG CTG TGC TGT CCC TGT AT-3′, and 5′-ACC CAA GAA GGA AGG CTG GA-3′. The samples were prepared using the iQ SYBR Green Supermix (Bio-Rad Laboratories) according to the manufacturer's instructions. The mRNA expression levels of adiponectin, CPT-1a, and β-actin were determined by quantitative real-time RT-PCR performed using the Chromo4 Real-Time PCR System (Bio-Rad Laboratories). The formation of a single product was verified by melting curve analysis. The expression levels of adiponectin and CPT-1a were normalized to that of β-actin. The data were analyzed using Opticon Monitor software (ver. 3.1; Bio-Rad Laboratories).

Statistical Analysis. All values are expressed as the mean ± standard deviation (SD) or standard error (SE). One-way analysis of variance was used for multiple comparisons. Treatment effects were analyzed by a paired t-test or Duncan's multiple range test using SPSS software (ver. 12.0; SPSS Inc., Chicago, IL). Differences were considered statistically significant at $p < 0.05$.

■ RESULTS

PBE Ameliorated HFD-Induced Obesity. After 70 days on a HFD, the mean body weight and body weight gain in the HFD group were higher than the corresponding values in the ND group, indicating that the HFD induced obesity (Figure 1A and Table 1). PBE administration (150 mg/kg/day) signifi-

Table 1. Effects of *Petalonia binghamiae* Extract (PBE) Supplementation on Final Body Weight and Body Weight Gain in High-Fat Diet (HFD)-Induced Obese Experimental Group after 70 days[a]

	ND	HFD	HFD+PBE
initial body weight (g)	22.61 ± 0.53	22.60 ± 0.42	22.43 ± 0.26
final body weight (g)	28.26 ± 0.99 a	38.20 ± 1.12 c	33.64 ± 0.77 b
body weight gain (g)	5.65 ± 0.60 a	15.60 ± 0.78 c	11.21 ± 0.67 b
intake of PBE (mg/kg of body weight/day)			150

[a]All values are expressed as the means ± SE ($n = 10$). Mean separation was performed using Duncan's multiple range test. Different letters indicate significant differences ($p < 0.05$).

cantly decreased mean body weight and body weight gain in the HFD+PBE group relative to the non-PBE-treated HFD group (11.9% and 28.1% lower, respectively). Moreover, the mRNA expression of adiponectin was lower in the HFD group than in the ND group but was restored in the HFD+PBE group (Figure 1B).

Histological analysis of epididymal adipose tissue confirmed that after 70 days, the adipocyte size was markedly increased in the HFD group as compared to the ND group, whereas the adipocyte size was markedly decreased in the HFD+PBE group as compared to the HFD group (Figure 1C). The epididymal and perirenal adipose tissue weight values were also significantly higher in the HFD group than in the ND group. The epididymal and perirenal adipose tissue weight values were significantly lower in the HFD+PBE group (24.8% and 28.5%, respectively) than in the HFD group (Table 2). Food intake did not differ significantly between the HFD group and the HFD+PBE group, although there was the difference in food

Table 2. Effects of *Petalonia binghamiae* Extract (PBE) Supplementation on Food Intake, Epididymal Adipose Tissue Weight, Perirenal Adipose Tissue Weight, and Serum Levels of TG in High-Fat Diet (HFD)-Induced Obese Experimental Group after 70 days[a]

	ND	HFD	HFD+PBE
food intake (g/cage/5 day)	26.57 ± 0.40 a	21.19 ± 0.28 b	21.07 ± 0.23 b
epididymal adipose tissue (g)	0.86 ± 0.05 a	2.02 ± 0.12 c	1.52 ± 0.06 b
perirenal adipose tissue (g)	0.50 ± 0.06 a	1.23 ± 0.07 c	0.88 ± 0.06 b
TG (mg/dL)	92.29 ± 4.86 a	138.43 ± 9.15 b	100.00 ± 7.76 a

[a]All values are expressed as the means ± SE ($n = 10$). Mean separation was performed using Duncan's multiple range test. Different letters indicate significant differences ($p < 0.05$).

intake between normal diet and HFD groups. However, the serum TG level was significantly lower in the HFD+PBE group (27.8%) than in the HFD group (Table 2). Next, we investigated the proteins responsible for fatty acid β-oxidation in epididymal adipose tissue. As shown in Figure 1D, the expression of phosphorylated forms of AMPK and of its immediate substrate (phosphorylated forms of ACC) was higher in the HFD+PBE group than in the HFD group.

PBE Reduced Signs of Liver Pathology. We next examined the effects of PBE on the serum levels of GPT and GOT in HFD-induced mice. PBE administration significantly reduced the levels of these markers of cell damage. The serum levels of GPT and GOT were significantly lower in the HFD+PBE group (40.5% and 14.4%, respectively) than in the HFD group (Table 3). In addition, liver weight was significantly
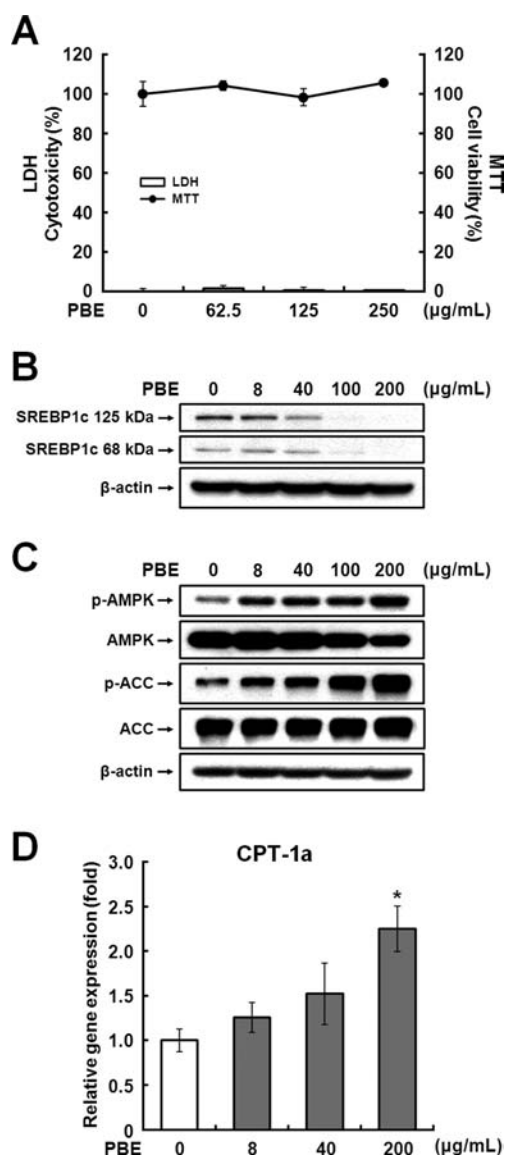
Table 3. Effects of *Petalonia binghamiae* Extract (PBE) Supplementation on Serum Levels of GPT and GOT and Liver Weight in High-Fat Diet (HFD)-Induced Obese Experimental Group after 70 days[a]

	ND	HFD	HFD+PBE
GPT (IU/L)	8.57 ± 0.85 a	17.29 ± 3.03 b	10.29 ± 0.68 a
GOT (IU/L)	42.29 ± 1.62 a	57.43 ± 3.14 b	49.14 ± 1.89 a
liver weight (g)	1.07 ± 0.06 a	1.30 ± 0.08 b	1.07 ± 0.04 a

[a]All values are expressed as the means ± SE ($n = 10$). Mean separation was performed using Duncan's multiple range test. Different letters indicate significant differences ($p < 0.05$).

lower in the HFD+PBE group than in the HFD group (Table 3). Figure 2 presents representative photomicrographs of liver tissue samples stained with H&E. H&E analysis of the liver revealed greater fatty accumulation in the HFD group compared with the ND group; however, no fatty accumulation was observed in the livers from the HFD+PBE group.

PBE Reduced the Expression of SREBP1c and Activated the AMPK Pathway in Mature 3T3-L1 Adipocytes. We first determined the maximal concentration of PBE to be added to mature 3T3-L1 adipocytes based on MTT and LDH assays (Figure 3A). We then investigated the effect of PBE on the expression of sterol regulatory element binding protein 1c (SREBP1c) in mature 3T3-L1 adipocytes. PBE reduced the expression of SREBP1c, a transcription factor that regulates lipogenesis, in a dose-dependent manner (Figure 3B).

Figure 3. Effect of *Petalonia binghamiae* extract on the expression of SREBP1c and phosphorylation of AMPK and ACC in mature 3T3-L1 adipocytes. 3T3-L1 preadipocytes were induced to differentiate as described in the Materials and Methods. (A) On day 8, viability and cytotoxicity were assessed by MTT and LDH assays, respectively. (B–D) On day 8, mature 3T3-L1 adipocytes were incubated for 16 h with serum-free DMEM containing 0.2% BSA (serum-free medium). The cells were then treated with postdifferentiation medium containing various concentrations of PBE for 24 h. (B) Western blot analysis of the dose-dependent expression of SREBP1c. (C) Western blot analysis of the dose-dependent expression of p-AMPK and p-ACC. (D) Real-time RT-PCR analysis of the dose-dependent expression of CPT-1a. All values are presented as the mean ± SD ($n = 3$; *$p < 0.05$ as compared to conditions without PBE). The data shown are representative of three independent experiments.

Consistent with our in vivo data, PBE also markedly induced the phosphorylation of AMPK and ACC in a dose-dependent manner (Figure 3C). Thus, we investigated the downstream effects of AMPK activation by treating mature 3T3-L1 adipocytes with PBE. PBE significantly increased the expression of *CPT-1a* mRNA (200 µg/mL: 2.25-fold increase), which is involved in fatty acid β-oxidation, as compared to the control (without PBE) (Figure 3D).

Fucoxanthin Activated the AMPK Pathway in Mature 3T3-L1 Adipocytes. To identify the possible active constituents contained in PBE, we isolated fucoxanthin from PBE (Figure 4A) and investigated its effect on AMPK signaling in mature 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were cultured in postdifferentiation medium and then exposed to 10 µM fucoxanthin for various time periods. Consistent with our PBE data, fucoxanthin induced AMPK and ACC phosphorylation in a dose-dependent manner (Figure 4B). Moreover, fucoxanthin increased the phosphorylated form of LKB1, which is responsible for the phosphorylation of AMPK (Figure 4B). Additionally, fucoxanthin significantly increased the level of *CPT-1a* mRNA (10 µM: 1.98-fold increase) as compared to the control (without PBE) (Figure 4C).

DISCUSSION

Obesity is a major public health problem in industrialized and developing countries. Currently available drugs for the treatment of obesity have undesirable side effects; therefore, there is great demand for a safe but therapeutically potent antiobesity drug. Thus, there has been increased interest in the search for antiobesity phytonutrients that effectively reduce visceral fat mass.

PBE is known to exert several pharmacological effects, including lowered blood glucose levels, improved glucose tolerance, increased transcriptional activity of PPARγ, and increased glucose uptake.[14] However, no fatty acid β-oxidation effect has as yet been reported. In the present study, we investigated the antiobesity potential of PBE by targeting fatty acid β-oxidation through AMPK signaling using mice with HFD-induced obese mice. In this animal model, body weight gain, adipose tissue weight, and the serum TG level were significantly lowered by PBE administration with no change in food intake.

Obesity is caused by an increase in the number and size of adipocytes derived from fibroblastic preadipocytes in adipose tissue.[25] The HFD group had a higher number of large adipocytes in the epididymal adipose tissue than the HFD+PBE group. The HFD+PBE group also exhibited fewer pathological symptoms. Adiponectin mRNA expression in the HFD group was about 2.5 times lower than that in the ND group. Interestingly, PBE increased the mRNA expression of adiponectin to approximately the level observed in the ND group. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism.[26] Obesity, diabetes, and atherosclerosis have been associated with reduced adiponectin levels.[27] Thus, our data suggest that PBE administration may be beneficial in the treatment of metabolic diseases.

In general, the development of fatty liver is strongly associated with obesity.[28] HFD feeding induced an accumulation of numerous fatty droplets, which is typical of fatty liver. PBE administration reduced the accumulation of lipid droplets and the signs of liver pathology and also protected against an increase in liver tissue weight. Additionally, PBE administration significantly reduced the levels of biochemical markers of liver function, including serum GPT and GOT. These results suggest that PBE protects against the development of HFD-induced fatty liver; however, the molecular mechanisms underlying these beneficial properties of PBE are unknown.

As a metabolic master switch, AMPK activation is associated with metabolic organs, including the liver, skeletal muscle, and pancreas, as well as adipose tissue.[29] At the molecular level, our
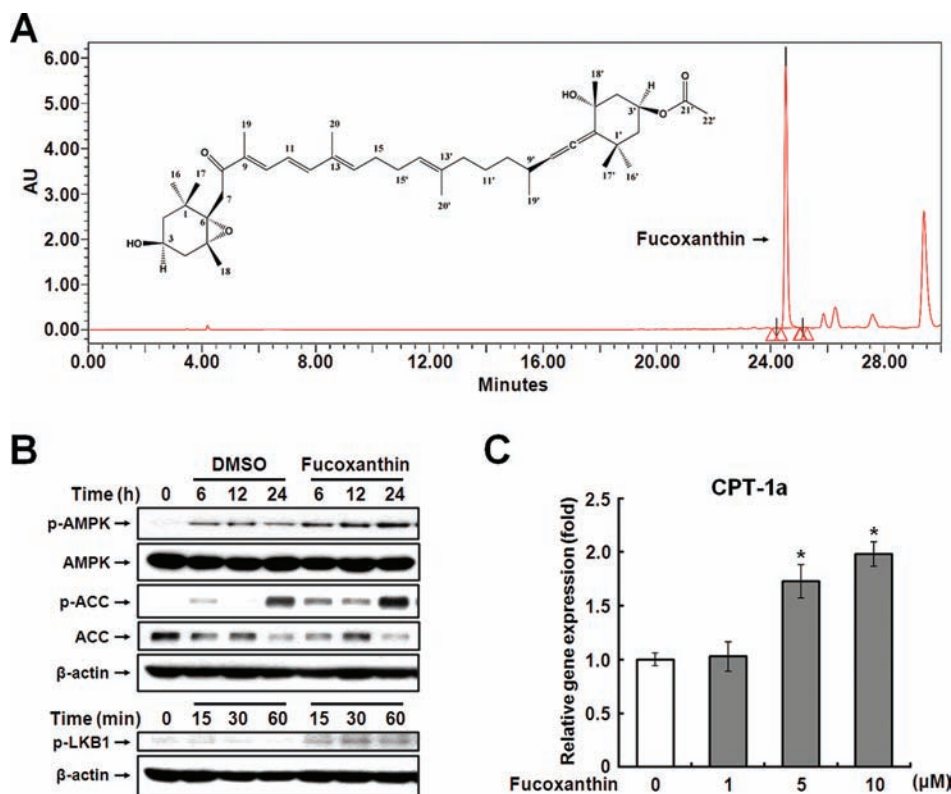
Figure 4. Effect of fucoxanthin isolated from *Petalonia binghamiae* extract on the phosphorylation of LKB1, AMPK, and ACC in mature 3T3-L1 adipocytes. (A) Structure of fucoxanthin and HPLC chromatograms of PBE obtained by the PDA detector system (wavelength: 450 nm). 3T3-L1 preadipocytes were induced to differentiate as described in the Materials and Methods. (B) Western blot analysis of the time course of p-LKB1, p-AMPK, and p-ACC. On day 8, mature 3T3-L1 adipocytes were incubated for 16 h with serum-free medium. The cells were then treated with postdifferentiation medium containing 10 μM fucoxanthin for various time periods. (C) Real-time RT-PCR analysis of the dose-dependent expression of CPT-1a. After incubation with serum-free medium, cells were treated with postdifferentiation medium containing various concentrations of fucoxanthin for 24 h. All values are presented as the mean $\pm$ SD ($n = 3$; *$p < 0.05$ as compared to conditions without fucoxanthin). The data shown are representative of three independent experiments.

data showed that HFD feeding significantly depressed the activation of AMPK in C57BL/6 mouse epididymal adipose tissue. However, the level of phosphorylated forms of AMPK returned to normal after PBE administration for 70 days, suggesting the beneficial role of PBE in activating the AMPK signaling pathway. Therefore, we tested whether PBE affected the AMPK signaling pathway in vitro using mature 3T3-L1 adipocytes. Consistent with in vivo data, our results showed that in mature 3T3-L1 adipocytes, PBE increased the phosphorylation of AMPK and ACC and the mRNA expression of CPT-1a.

Chronically high levels of fatty acids in the blood, typically observed in obesity, are correlated with many detrimental metabolic consequences such as insulin resistance.[30] AMPK activation increases fatty acid β-oxidation by reducing malonyl-CoA through the inhibition of ACC, and this process upregulates CPT-1a expression.[31] CPT-1a regulates long-chain fatty acid transport across the mitochondrial membrane.[32] Moreover, sterol regulatory element binding protein (SREBP) transcription factors regulate the expression of lipogenic enzymes, including ACC, fatty acid synthase, and 3-hydroxy-3-methylglutaryl CoA reductase.[33] PBE reduced the expression of SREBP1c in mature 3T3-L1 adipocytes. These results strongly suggest that the beneficial properties of PBE in mice with HFD-induced obesity were exerted by both increasing fatty acid β-oxidation through the AMPK signaling pathway and decreasing de novo lipogenesis in adipose tissue.

To putatively identify the active compounds, we purified fucoxanthin from PBE. We then investigated the effect of fucoxanthin on the AMPK signaling pathway in mature 3T3-L1 adipocytes. Fucoxanthin increased the phosphorylation of LKB1, AMPK, and ACC and the mRNA expression of CPT-1a. These results suggest that, similar to PBE, fucoxanthin both promotes fatty acid β-oxidation and inhibits de novo lipogenesis in adipose tissue, as our previous report showed that fucoxanthin inhibited the expression of SREBP1c in 3T3-L1 adipocytes.[21]

In conclusion, PBE administration in mice with HFD-induced obesity reduced body weight gain, adipose tissue weight, adipose tissue cell size, and fatty droplet accumulation in the liver. Additionally, PBE administration increased AMPK and ACC phosphorylation in animal adipose tissue. Moreover, PBE and its component fucoxanthin activated the AMPK signaling pathway and inhibited the expression of SREBP1c in mature 3T3-L1 adipocytes. Taken together, our findings demonstrate that PBE (or fucoxanthin) may improve HFD-induced obesity by increasing fatty acid β-oxidation and inhibiting lipogenesis in adipose tissue.

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Notes

The authors declare no competing financial interest.

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