ACS FOOD SCIENCE & TECHNOLOGY

pubs.acs.org/acsfoodscitech

Article

Pyrroloquinoline Quinone Attenuates Fat Accumulation in Obese Mice Fed with a High-Fat Diet, *Daphnia magna* Supplied with a High Amount of Food, and 3T3-L1 Adipocytes

Nur Syafiqah Mohamad Ishak, Kazuto Ikemoto,* Midori Kikuchi, Mariko Ogawa, Kazeno Akutagawa, and Mitsugu Akagawa*

Cite This: https://doi.org/10.1021/acsfoodscitech.1c00301

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Obesity is a significant public health issue. Supplementation with pyrroloquinoline quinone (PQQ) may help prevent obesity by suppressing body fat accumulation. In the current study, we examined the effect of dietary PQQ on the inhibition of fat accumulation in obese mice, *Daphnia magna*, and adipocyte cells. PQQ significantly attenuated the total body fat and visceral fat volume in the abdominal region of mice. In *Daphnia*, body fat and body length increased with higher amounts of food; however, PQQ reduced body fat without affecting body growth. PQQ attenuated body fat accumulation under both high-fat and high-calorie consumption conditions. PQQ increased phosphorylation of LKB1 and AMPK, subsequently suppressing lipogenesis in adipocyte cell cultures. PQQ also promoted mitochondrial biogenesis by inducing the nuclear translocation of PGC1α in fat cells. Hence, dietary PQQ could prevent obesity by reducing body fat accumulation via suppressing lipogenesis and promoting mitochondrial biogenesis.

KEYWORDS: *obesity, PQQ, AMPK, body fat, lipogenesis, PGC1α, mitochondrial biogenesis*

INTRODUCTION

Obesity is a global health concern resulting from an increase in the consumption of high-energy diets that causes abnormal or excessive fat accumulation in the body. Obesity is often associated with various diseases, such as cardiovascular disease, type 2 diabetes, inflammation, and cancer.[1] Without efficient treatment, an estimated one-quarter of the global adult population will be overweight by 2030.[2] However, currently available fat-loss drugs elicit multiple toxic side effects.[3] Body fat accumulates when energy intake exceeds energy expenditure, particularly with an excess increase in dietary lipid intake.[3] Hence, increasing energy expenditure and reducing energy intake is the most efficacious strategy to treat obesity.[4] Consequently, to prevent diseases associated with obesity, implementing exercise routines and changes in diet are often suggested.[1,3] Many recent studies have focused on several specific food ingredients that are meant to enhance fat metabolism or energy expenditure to prevent high levels of body fat accumulation or reduce obesity.[5]

Pyrroloquinoline quinone (PQQ) is present at low concentrations in numerous dietary sources, including fermented soybeans (natto), tea, green peppers, parsley, kiwi fruit, green tea, and papaya.[6] Initially, PQQ was identified as a redox cofactor for bacterial dehydrogenases, including glucose dehydrogenase.[7,8] However, more recently, it has garnered significant interest owing to its impact on various physiological processes that could benefit human health. For instance, PQQ supplementation enhances cognitive function,[9] antioxidant potential, the immune system, and mitochondria-related functions in humans.[10] Moreover, PQQ is produced as a water-soluble PQQ disodium salt (BioPQQ) by fermentation, and its safety has been assessed by the EFSA panel on Dietetic Products, Nutrition, and Allergies (NDA).[11] In the US, BioPQQ has been filed as a new dietary ingredient (NDI) by the FDA, whereas in the EU, it has been registered as a novel food, enabling its use as a food ingredient.

Mitochondria are organelles that mediate fatty acid oxidation (FAO) for energy production. Transcriptional coactivator peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) is a key factor that promotes mitochondrial biogenesis and stimulates mitochondrial oxidative metabolism.[12,13] PGC-1α is highly expressed in brown adipocytes and skeletal muscles. Its expression can be upregulated by environmental stimuli and specific conditions, including cold exposure, exercise, and caloric restriction.[12,14] Furthermore, a study demonstrated a marked induction of PGC-1α expression in mice in response to PQQ exposure, resulting in stimulation of mitochondrial biogenesis.[15] Hence, PQQ may effectively reduce body fat accumulation by enhancing FAO via improved mitochondrial biogenesis.

PQQ reduces body fat accumulation and abates associated low-grade inflammation in a diet-induced obesity rat model.[16] However, the mechanism by which PQQ attenuates body fat

Received: August 12, 2021
Revised: October 19, 2021
Accepted: October 19, 2021

and affects fat metabolism remains unknown. In this study, we aimed to examine the dietary effects and mechanisms of PQQ in inhibiting body fat accumulation. First, we investigated the impact of PQQ on fat metabolism in mice with diet-induced obesity to observe changes in body fat and muscle composition. We also examined the levels of liver injury markers and cholesterol in serum analysis. Second, we questioned whether dietary supplementation with PQQ could help prevent body fat accumulation under conditions involving high food consumption or caloric intake. To test this query, we used water flea *Daphnia* as a model organism to study the relationship between food intake and PQQ supplementation on body fat content. Finally, we investigated the molecular mechanism underlying the attenuation of body fat accumulation mediated by PQQ using 3T3-L1 adipocyte cell cultures comprising fat cells derived from mice. We believe that our study makes a significant contribution toward the prevention and treatment of obesity as PQQ may effectively reduce body fat accumulation by suppressing lipogenesis and enhancing mitochondrial biogenesis.

MATERIALS AND METHODS

Experiment with Diet-Induced Obesity Mice. *Ethical Note.* All protocols were approved by the Animal Care and Experimentation Facility Committee of Mitsubishi Gas Chemical Company, Analysis Center (No. RP19-03). This study adheres to all legal requirements of the Japanese regulation of laboratory animal care and experiments with 3Rs and was performed in compliance with the ARRIVE guidelines.

Animals Treatment. Twenty-five male C57BL/6J mice (8 weeks old; Charles River Laboratories Japan, Yokohama, Japan) were used in this study. All animals were housed in an accredited animal facility that was maintained at a constant temperature (23 ± 3 °C), humidity (55 ± 15%), and a light/dark cycle (12/12 h). The mice were randomly divided into groups of five mice per cage [174 (W) × 278 (D) × 131 (H) mm; CLEA Japan, Tokyo, Japan] and provided with a normal diet (ND) (MF, Oriental Yeast, Tokyo, Japan; 13% fat, 61% carbohydrate, 26% protein; 3.57 kcal/g) and water *ad libitum*. Following a week of acclimation, the mice were divided into three groups: ND (n = 5), high-fat diet (HFD; n = 10), and HFD supplemented with BioPQQ (HFD+PQQ; n = 10). For the HFD and HFD+PQQ groups, the mice were fed an HFD-60 chow diet (Oriental Yeast, Tokyo, Japan; 60% fat, 22% carbohydrate, 18% protein; 4.93 kcal/g) with or without the addition of 0.02% w/w of PQQ (BioPQQ, Mitsubishi Gas Chemical, Tokyo, Japan). The acute oral LD_{50} value of BioPQQ was 1,000−2,000 mg/kg of body weight for male rats and 500−1,000 mg/kg of body weight for female rats.[17] The no-observed-adverse-effect level of 100 mg/kg of body weight/day for BioPQQ in rats has been determined in a previous subchronic toxicity study conducted for 13 weeks.[17] Thus, the BioPQQ dose used in the current study (ca. 18−26 mg/kg of body weight per day) was deemed nontoxic. The body weight of each mouse was measured twice per week, and chow supply was renewed every Monday and Friday throughout the experimental 6 week period. The weight of food was measured before and after feeding to evaluate the amount of food intake, and the average daily food intake per animal was calculated by dividing it by the number of mice (Table 1).

Fat Mass Measurement Using Bioimpedance Spectroscopy (BIS). During the final week of the experiment, the whole-body composition of each mouse was measured using an ImpediVet Bioimpedance Spectroscopy device (BIS; ImpediMed Limited, Brisbane, Australia) according to the manufacturer's instructions.[18] The weight and body length of each mouse were measured before BIS evaluations. Mice were anesthetized with 3% isoflurane during the measurement, and three consecutive measurements were performed at 10 s intervals. All measurements were performed by the same individual, under the same conditions, and on the same day.

Table 1. Effects of Dietary PQQ Supplementation on the Body Weights and Body Compositions of Mice under Conditions Involving Diet-Induced Obesity[a]

type of diet	HFD	HFD+PQQ	ND
number of mice	10	10	5
food intake per mouse per day (g)	3.4 ± 0.3	3.3 ± 0.5	3.2 ± 0.2
PQQ intake per mouse per day (mg)	0[a]	20.5 ± 0.7[b]	0[a]
initial body weight (g)	25.4 ± 0.6	25.2 ± 0.6	25.4 ± 1.7
final body weight (g)	38.8 ± 2.6[a]	37.4 ± 3.0[a]	29.3 ± 2.0[b]
body weight gain (%)	52.9 ± 7.6[a]	48.2 ± 10.8[a]	15.3 ± 5.0[b]
total body fat per body weight (%)	49.5 ± 1.4[a]	38.0 ± 2.7[b]	24.8 ± 1.9[c]
abdominal total fat (mm^3/%)	38.8 ± 1.4[a]	30.8 ± 1.4[b]	6.7 ± 0.9[c]
abdominal visceral fat (mm^3/%)	26.4 ± 0.9[a]	20.8 ± 1.3[b]	3.9 ± 0.5[b]
abdominal subcutaneous fat (mm^3/%)	12.4 ± 0.7[a]	10.0 ± 1.0[a]	2.8 ± 0.4[b]
lower body muscle (mm^3/%)	33.3 ± 1.0[a]	37.5 ± 1.4[b]	60.0 ± 0.9[c]

[a]The three diet groups were HFD, HFD+PQQ, and ND. Total body per body weight was measured using BIS, whereas abdominal fat and lower body muscle were measured using the μCT scan method. Total fat was obtained with the sum of the visceral fat and subcutaneous fat. Data indicate the mean ± SEM for the number of mice indicated in the table. The values with different superscript letters in a column are significantly different (one-way ANOVA with Tukey's post hoc test, $p < 0.05$).

Microcomputed Tomography (μCT) Imaging of Body Fat and Skeletal Muscle. After anesthetizing mice with 3% isoflurane, computed tomography (CT) images were acquired using 3D micro-CT (CosmoScanGXII; Rigaku, Tokyo, Japan) with a resolution of 148 × 148 × 148 mm^3, a tube voltage of 90 kV, and a tube current of 80 mA. A Cu 0.06 + Al 0.5 mm X-ray filter was used, and the FOV was set at 70 mm. The exposure time was set at 2 min, and the slice thickness (scanning width) was 20 mm for images captured in the standard mode. CT images were visualized and analyzed using the CT Atlas Metabolic Analysis Ver. 2.03 software (Rigaku) according to the protocol reported in a previous study.[19]

To measure the body fat volume, the Hounsfield unit (HU) value of the fat tissue was adjusted to between −350.0 and −140.0. The skeleton of the mice could be observed from a reconstructed μCT scan viewer. Here, the skeleton was readily identified as the defined region of interest. Body fat volume was measured at the fourth lumbar vertebrate. To analyze skeletal muscle volume, the HU value was set between 0 and 140 to quantify the skeletal muscle limited to both hind-limb regions.

Plasma Analysis. At the end of the experiments, blood was collected from the inferior vena cava under isoflurane anesthesia using a 25G needle syringe (Terumo, Tokyo, Japan). The collected blood was dispensed into a blood collection tube and allowed to stand at RT for 1 h. The blood samples were then centrifuged at 1,500 × g and 18 °C for 15 min; the supernatants were stored at −80 °C until plasma analyses were conducted. Plasma levels of aspartate aminotransferase (AST), aspartate aminotransferase (ALT), total cholesterol (T-CHO), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined using a Hitachi 7180 autoanalyzer (Hitachi, Tokyo, Japan).

Experiment Using *Daphnia magna*. *Daphnia Culture Conditions.* The experiments were performed using wild-type *Daphnia magna* purchased from Japan (Medaka Gakuen, Wakayama, Japan) and maintained in the laboratory by culturing 40 each of one-, two-, and three-week-old *Daphnia* fleas separately in 1 L of mineral water. The culture media was replaced every week, and the juveniles were removed before feeding. The *Daphnia* were fed with 6−8 × 10^7 cells

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

of *Chlorella vulgaris* (Nikkai Center, Tokyo, Japan) every Monday, Wednesday, and Friday each week. All cultures were stored at 22 ± 2 °C in an incubator with automatic controls under a light/dark cycle (16/8 h). The two- to three-week-old females with juveniles in the brood chamber of these cultures were cultured separately in a 500 mL beaker, and after 24 h, the newborn neonates were used for the experiments. To study the effect of PQQ supplementation on the *Daphnia* body, experiments were initiated with neonates (<24 h of age) obtained from females cultured with high food ration levels (8−9 × 10^5 cells/*Daphnia*/day of *Chlorella*). After 48 h, ten juveniles were transferred into a 300 mL beaker containing 15 mg/L PQQ supplemented in soft water for each treatment, and food (*Chlorella*) was added accordingly (low: 4 × 10^4 cells, medium: 2 × 10^5 cells, high: 8 × 10^5 cells per *Daphnia* per day). The culture was continued for at least 4 days. Body fat was quantified using Nile red staining according to a previously published protocol,[20] and body growth was measured via microscopic imaging. To study the effect of PQQ supplementation on overall *Daphnia* fitness, nine neonates (<24 h of age) were cultured individually in 50 mL test tubes with the following diet conditions: food only (*Chlorella*) or food supplemented with PQQ. PQQ was dissolved in the culture media and diluted to 1−2 mg/L. During the first 6 days of culture, *Daphnia* were fed with 1 × 10^5 *Chlorella* cells per day, and on days 7−14, the concentration was increased to 5 × 10^6 cells per day. The mineral water used for culturing *Daphnia* was replaced every Monday, Wednesday, and Friday, during which the survival and reproductive capacity (number of offspring produced) were recorded.

Nile Red Analysis and Microscopic Imaging. Nile red dye (FujiFilm Wako Pure Chemical; Osaka, Japan) stock solution was prepared in acetone and stored in the dark. The stock solution was diluted with soft water to prepare a 0.5 mg/L working solution. Live individuals were exposed to 2 mL of Nile red working solution in 24-well plates and incubated in the dark at 22 ± 2 °C for 1 h. The animals were then removed and washed in 100 mL of soft water for a few minutes before microscopic imaging and fluorescence quantification.

Daphnia were observed under a microscope (Nikon Eclipse TC2000-S) in a 35 mm culture dish containing 50% ethanol. Images were captured using a Nikon DS-Ri2 camera. The setting for the bright field was 3 ms of exposure time and 2.8 × gain, while a 500 ms exposure time and 7.6 × gain were set for fluorescence images captured under the RFP filter.

Fluorescence Quantification. For fat content quantification, Nile red fluorescence intensity was determined using a microplate fluorescence reader (Multimode Plate Reader ARVO X3, PerkinElmer, USA). The animals were placed individually in a 1.5 mL centrifuge tube, and the remaining water was removed. Each *Daphnia* was homogenized in 300 μL of isopropyl alcohol by sonication for at least 15 min. Subsequently, the cells were centrifuged at 10,000 × *g* for 5 min, and 200 μL of the supernatant was transferred to a 96-well plate to measure the fluorescence at excitation/emission wavelengths of 485 nm/535 mm for 0.1 s. An isopropyl alcohol solution without animal sample and dye was used as a blank. The fluorescence measurements were normalized by deducting the average result of the blank.

Body Length Measurement. From the bright-field images, the distance between the head and the base of the tail spine was measured to determine the body length of *Daphnia*. Measurements were performed using the ImageJ software for three fleas under each condition.

Experiments Performed Using the 3T3-L1 Cell Line. *Cell Culture Conditions.* The mouse 3T3 L1 cell line was obtained from the JCRB Cell Bank (JCRB9014, Osaka, Japan). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque; Kyoto, Japan) supplemented with 5% bovine calf serum (BCS) (Equitech-Bio, Inc.; Texas, USA) and penicillin-streptomycin mixed solution (× 100) (Nacalai Tesque) at 37 °C in an atmosphere containing 5% CO_2.

Differentiation of 3T3-L1 Cells. Adipocyte differentiation was induced by treating confluent 3T3-L1 preadipocytes with a hormone mixture containing 10% fetal bovine serum (FBS; Sigma-Aldrich), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Nacalai Tesque), 1 μM dexamethasone (DEX) (Nacalai Tesque), 2 μM rosiglitazone (Tokyo Chemical Industry, Tokyo, Japan), and 5 μg/mL insulin (Sigma-Aldrich). After 2 days of incubation at 37 °C in an incubator containing 5% CO_2, the medium was replaced with DMEM supplemented with FBS and insulin; thereafter, the medium was renewed every other day. Differentiated adipocytes were further cultured for 7−10 days. To test the inhibitory effect of PQQ on lipid accumulation, differentiated adipocytes were treated with up to 200 nM of PQQ in serum-free DMEM for 48 h. The fat content was determined using Oil Red O staining, whereas the lipid droplet size was measured using the BODIPY staining method.

Oil Red O Staining. Oil Red O dye (Sigma-Aldrich; Missouri, USA) stock solution was prepared in isopropyl alcohol, and staining was performed according to a previously published protocol.[20] Cellular neutral lipid content was normalized to the cellular protein concentration. The protein concentration of each lysate was determined using the BCA protein assay reagent kit (Nacalai Tesque) with bovine serum albumin (BSA) as a standard according to the manufacturer's instructions.

BODIPY Staining. Green-fluorescent BODIPY 493/503 (Nacalai Tesque) stock solution (5 mM) was prepared in dimethyl sulfoxide. The stock solution was diluted 2500-fold with PBS to prepare a working solution. Treated adipocytes on 8-well chamber slides were washed twice with PBS and then incubated in the BODIPY working solution to fluorescently label lipid droplets. Incubation was performed at 37 °C in the dark for 15 min. The cells were then washed with PBS, fixed with 4% paraformaldehyde in the dark for 30 min at RT, and washed with PBS thrice. After nuclear staining with DAPI (Nacalai Tesque), the cells were imaged using a fluorescence microscope BZ-9000 (Keyence; Osaka, Japan).

Triglyceride Quantification. 3T3-L1 adipocytes were cultured in 6- or 12-well plates. After treatment with PQQ, the adipocytes were washed twice with PBS, and total cellular lipids were extracted twice by incubating them with 500 μL of *n*-hexane/2-propanol (3:2, *v*/*v*) at 4 °C for 15 min. Triglyceride content in the extracts was determined using a Triglyceride E-Test Wako (FujiFilm Wako Pure Chemical), as previously reported.[21] The triglyceride content was normalized to the cellular protein concentration. Protein concentrations were measured using the BCA Protein Assay Kit (Nacalai Tesque) according to the manufacturer's protocol.

Protein Extraction and Western Blotting. After treatment with PQQ in serum-free DMEM, the 3T3-L1 adipocytes were washed twice with cold PBS. The cells were then lysed with radioimmunoprecipitation assay (RIPA) buffer containing 1% Triton X-100, a phosphatase inhibitor cocktail (Nacalai Tesque), and a protease inhibitor cocktail (Nacalai Tesque). The cell lysates were centrifuged for 10 min at 16,000 × *g* at 4 °C. SDS-PAGE and Western blotting were performed as previously reported.[21] Antibodies against p-AMPK*α* (p-Thr172), p-ACC (p-Ser79), p-SREBP-1c (p-Ser372), p-LKB1 (p-Ser428), AMPK*α*, ACC, LKB1, histone H3, goat anti-mouse IgG-HRP, and goat anti-rabbit IgG-HRP were obtained from Cell Signaling Technology (Beverly, MA, USA), those against PGC1*α* and SREBP-1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and those against fatty acid synthase (FAS) and ATGL were purchased from Abgent (San Diego, CA, USA). The anti-*β*-Actin monoclonal antibody was obtained from Proteintech (Rosemont, IL, USA), and MTCO1 was obtained from Boster Biological Technology (Pleasanton, CA, USA). Band intensities were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Immunofluorescence Staining. Immunostaining of the fixed 3T3-L1 adipocytes was performed according to a previously described method.[21] Primary antibodies against PGC-*α* and SREBP-1 were obtained from Santa Cruz Biotechnology. Cy2 conjugated anti-mouse IgG and Cy3 conjugated anti-rabbit IgG antibodies were purchased from GE Healthcare UK Ltd. (Buckinghamshire, UK) and were used as secondary antibodies. After nuclear staining with DAPI (Nacalai

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

Tesque), the cells were imaged using a fluorescence microscope BZ-9000 (Keyence; Osaka, Japan).

Preparation of Nuclear Extracts. Nuclear extracts for Western blotting were prepared as described previously.[20] Protein concentrations were determined using the BCA assay (Nacalai Tesque).

mRNA Isolation and Quantitative Reverse Transcription PCR (qRT-PCR). RNA isolation from 3T3-L1 adipocytes was performed using the SuperPrep Cell Lysis Kit for qPCR (Toyobo, Osaka, Japan) according to the manufacturer's guidelines. cDNA was synthesized using the SuperPrep RT Kit for qPCR (Toyobo) according to the manufacturer's instructions. qRT-PCR was performed with a Thermal Cycler Dice Real-Time System Single (Takara Bio, Shiga, Japan) using the THUNDERBIRD SYBR qPCR Mix (Toyobo) as described previously.[22] The following primer sequences were used: 5′-AGCACGGAGTGACCCAAAC and 5′-TGTACGTGGCTACATGGACCT for mouse *Nrf1*, 5′-ATTCCGAAGTGTTTTTCCAGCA and 5′-TCTGAAAGTTTTGCATCTGGGT for mouse *Tfam*, and 5′-CTACCACATCCAAGGAAGCA-3′ and 5′-TTTTTCGTCACTACCTCCCCG-3′ for mouse *18S rRNA* (as an internal control for all qRT-PCR). Primers were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Statistical Analysis. Statistical analyses were performed using the R software (version 3.4.3; R Core Team, 2017). For analysis of two groups, a two-tailed Student's *t*-test was used. Comparisons involving repeated measurements obtained with different statistical units were analyzed via one-way analysis of variance (ANOVA) using Dunnett's or Tukey's post hoc tests.

RESULTS

Dietary Effects of PQQ in Mice under Diet-Induced Obesity Conditions. Obesity was induced in ten male C57BL/6J mice by feeding them an HFD, which had a 60.7% fat calorie ratio feed (Supplementary Data Table S1). A control group of five male mice was fed ND with a low-fat calorie ratio of 12.6%. To test the dietary effect of PQQ against obesity, 0.02% of PQQ was added to the HFD chow fed to the male mice. After 6 weeks, compared to mice fed the ND, HFD-fed mice and HFD+PQQ-fed mice showed higher body weight (Table 1). Thus, dietary PQQ did not protect the mice against HFD-induced body weight gain. However, mice supplemented with PQQ appeared leaner than the HFD-fed mice (Figure 1a). Although no significant differences were observed with respect to food intake between the HFD and HFD+PQQ groups, PQQ treatment significantly reduced the HFD-induced increase in body fat content (Table1). Consumption of an HFD induced body fat accumulation, as it doubled the body fat content when compared to that observed in the mice of the ND group, whose total fat with respect to the body weight was set as the 100% marker (Figure 1b). The total fat content of the HFD+PQQ diet was 53.2% higher than the ND; hence, approximately half of the body fat accumulation was suppressed by PQQ intake during the consumption of the HFD (Figure 1b). Focusing on the main region of body fat accumulation (abdominal region), a μCT scan also showed a significant reduction in total fat with PQQ intake, with a more significant effect on the visceral fat compared to that on the subcutaneous fat (Figure 1c). These results indicate that PQQ can suppress the accumulation of body fat attributed to obesity, particularly that of visceral fat.

Muscle loss is another sign of obesity. Muscle volume quantification of the hind limbs performed using μCT scans showed that the mice in the HFD group had a significantly lower muscle volume when compared to the mice in the ND group (Table 1, Figure 1d). The muscle volume of mice in the HFD group was reduced to 55.5% due to obesity. Surprisingly,

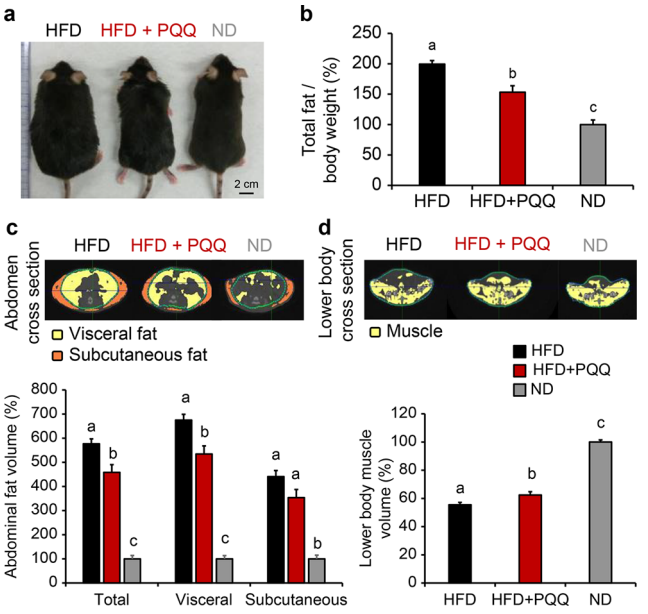

Figure 1. Effects of PQQ on the body composition of mice under conditions involving diet-induced obesity. PQQ reduced body fat accumulation, particularly that of visceral fat, and attenuated the loss of muscle in obese mice. (a) The appearance of representative mice from each dietary group after 6 weeks of feeding. Mice were divided into three groups (ND, $n = 5$; HFD, $n = 10$; HFD+PQQ, $n = 10$). (b) Total fat mass per body weight for mice fed with HFD, HFD+PQQ, and ND measured using bioimpedance spectroscopy. ND was set at 100%. (c) Fat volume in the abdominal region, as determined using μCT imaging. The top panel shows abdominal cross-section images for each group. The bottom panel shows the fat volume of mice fed with HFD, HFD+PQQ, and ND diets, where ND was set as 100%. Total fat is the sum of the visceral fat and subcutaneous fat volume. (d) Muscle volume in the hind-limb region as determined using μCT imaging. The top panel shows cross-sectional images of the lower body for each group. The bottom panel shows the lower body muscle volume of the mice fed the HFD and HFD+PQQ diets when compared to that observed in mice fed with ND, where ND was set as 100%. (b–d) Data of each graph indicate the mean ± SEM. Bars with different letters demonstrate significant differences ($p < 0.05$, one-way ANOVA, Tukey's post hoc test).

PQQ significantly attenuated muscle loss by retaining 62.5% of the muscle volume (Figure 1d). This may account for why only a small reduction in weight gain was observed in the HFD +PQQ group compared to the HFD group, even though the total body fat was reduced.

The effects of PQQ on the plasma biochemical parameters are shown in Table 2. The levels of ALT, T-CHO, LDL-C, and HDL-C were significantly lower in the ND group than the HFD group. Meanwhile, dietary PQQ significantly decreased the plasma levels of ALT and demonstrated a tendency to reduce plasma AST levels (Table 2), suggesting that PQQ can attenuate liver injury attributed to a high-fat intake. These results agreed with those of the autopsy in which we observed a reduction in abdominal fat accumulation and liver damage in mice supplemented with PQQ (Supplementary Figure S1). Although statistical significance was not observed, lipid analysis showed a reduction in cholesterol levels in the PQQ-supplemented group.

Dietary Effects of PQQ on *Daphnia* Fed with Different Amounts of Food. We investigated the influence of PQQ on body fat accumulation in *Daphnia* fed different amounts of

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

Table 2. Effects of PQQ on Plasma Biochemical Parameters in Mice under Conditions Involving HFD-Induced Obesity[a]

type of diet	HFD	HFD+PQQ	ND
number of mice	8	8	5
AST (IU/L)	50 ± 3	43 ± 1	40 ± 5
ALT (IU/L)	34 ± 3[a]	26 ± 2[b]	23 ± 2[b]
T-CHO (mg/dL)	189 ± 7[a]	171 ± 9[a]	88 ± 7[b]
LDL-C (mg/dL)	9 ± 1[a]	8 ± 1[a]	5 ± 1[b]
HDL-C (mg/dL)	96 ± 2[a]	88 ± 4[a]	61 ± 6[b]

[a]Plasma levels of AST, ALT, T-CHO, LDL-C, and HDL-C for three diet groups: HFD, HFD+PQQ, and ND. Data indicate the mean ± SEM for the number of mice specified in the table. The values with different superscript letters in a column are significantly different (one-way ANOVA with Tukey's post hoc test, $p < 0.05$).

food. *Chlorella* cells were used as the food, and low, medium, and high amounts corresponding to 4×10^4, 2×10^5, and 8×10^5 cells of *Chlorella*, respectively, were provided to each *Daphnia* flea per day. Body fat content was quantified using Nile red fluorescence staining. The results showed the *Daphnia* body fat and body length increased as the amount of food increased (Figure 2). The correlation (R) between body fat and food amount and body length versus food amount was over 0.9928 (Supplementary Figure S2), indicating a strong correlation. When PQQ was added to the water medium at a concentration of 20 mg/L with each food concentration, the body fat content was reduced by 14%−20%. Interestingly, with medium and high amounts of food, PQQ significantly lowered *Daphnia* body fat without affecting body length. The effect of PQQ intake on body fat reduction corresponded to a 31% reduction in food intake (Supplementary Figure S2).

We also examined the effect of PQQ on the *Daphnia*'s fitness based on its survival rate and reproductive ability. Exposure of *Daphnia* to PQQ did not affect its survival rate, as all fleas were healthy after a 14 day culture period (Supplementary Figure S3a). Regarding reproductive ability, the total number of offspring produced by *Daphnia* during the first and second brood was significantly increased with PQQ exposure (Supplementary Figure S2b).

PQQ-Mediated Inhibition of Lipid Accumulation in Adipocyte Cells. To elucidate the PQQ mechanism of action in adipocytes, mouse-derived 3T3-L1 preadipocytes were treated with IBMX, DEX, rosiglitazone, and insulin to induce adipocyte differentiation. Adipocyte cells were used for subsequent experiments. The 3T3-L1 adipocytes were then treated with PQQ (0−200 nM) for 48 h, and intracellular lipids were stained using Oil Red O dye. The lipid droplets became smaller as the concentration of PQQ increased (Figure 3a). The lipid droplets were also quantified; a significant reduction was observed in lipid content in PQQ-treated cells when compared to the control (Figure 3a). In addition, the lipid droplets were stained with a green-fluorescent BODIPY dye to measure the diameter of the lipid droplets. The PQQ treatment significantly decreased the diameter of the lipid droplets (Figure 3b). The triglycerides were also quantified in the treated adipocytes. The PQQ treatment significantly decreased the triglyceride content (Figure 3c).

We also evaluated the effect of PQQ on the phosphorylation levels of AMP-activated protein kinase (AMPK), which plays an essential role in the regulation of adipocyte lipid metabolism, along with those of the primary AMPK kinase, liver kinase B1 (LKB1). After 6 h of PQQ treatment, Western blotting results showed that the level of LKB1 and AMPK

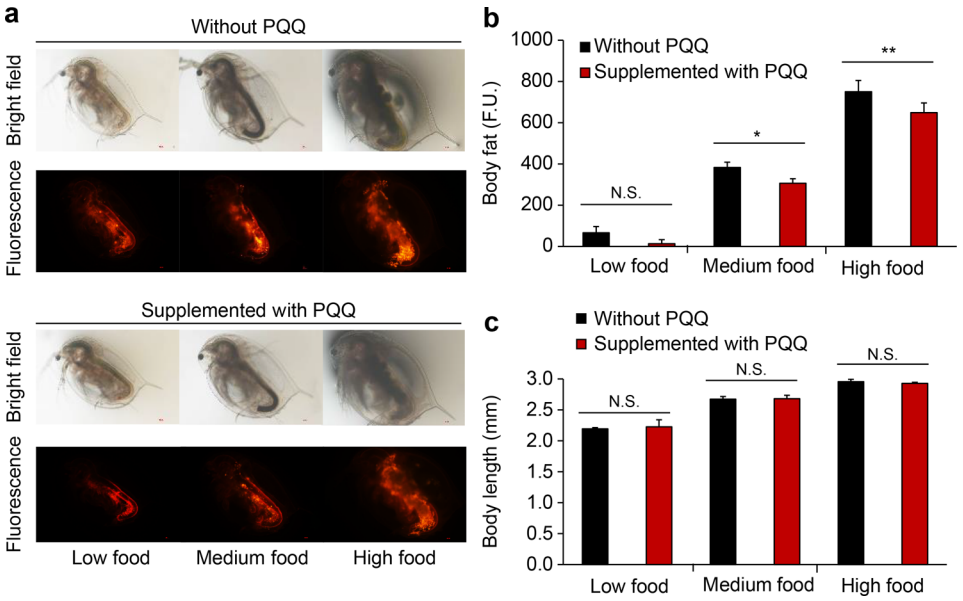

Figure 2. Effects of PQQ on the body fat and body length of *Daphnia* fed with different amounts of food. PQQ reduced body fat accumulation in *Daphnia*, when supplied with medium and high levels of food, without affecting the body length. (a) Images of *Daphnia* stained with the lipophilic dye Nile red to determine body fat. The images were captured under a bright field with a fluorescence filter for each designated group. Red fluorescence indicates the localization of body fat. Low food: low amount of food, *Chlorella* (4×10^4 cell/*Daphnia*/day); Medium food: medium amount of food, *Chlorella* (2×10^5 cell/*Daphnia*/day); High food: high amount of food, *Chlorella* (8×10^5 cell/*Daphnia*/day). (b) Body fat was quantified using the Nile red fluorescence intensity (FU) for each group. Data indicate mean ± SEM for five independent measurements of *Daphnia*. c. Body length for each group. Data indicate mean ± SEM of three independent measurements of *Daphnia*. Asterisks denote a statistically significant difference (*$p < 0.05$; **$p < 0.01$) between PQQ and non-PQQ treatments as determined using two-tailed Student's t-test; NS denotes not significant.

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

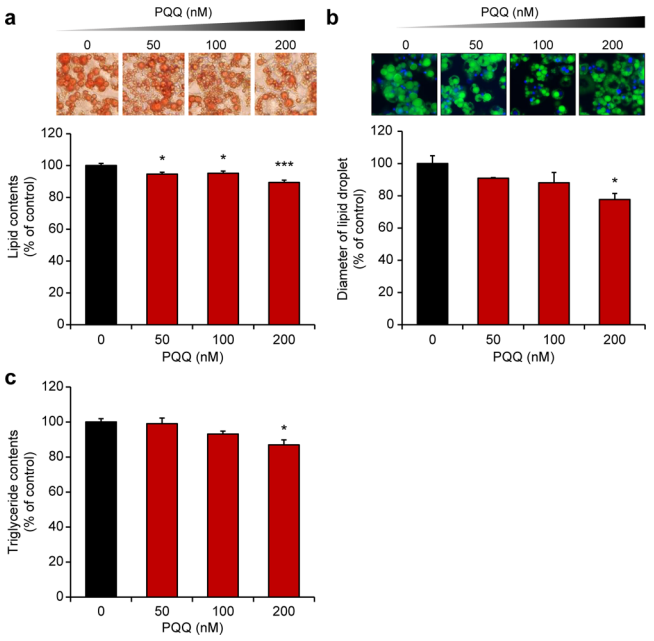

Figure 3. PQQ reduces the lipid content, size of the lipid droplets, and triglyceride contents in adipocyte cells. Differentiated 3T3-L1 adipocytes were cultured in a serum-free medium containing 0−200 nM of PQQ for 24 and 48 h. (a) Top panels show images of the intracellular lipid stained with Oil Red O dye after 48 h of PQQ treatment. The bottom graph shows the results of lipid content quantification. (b) Top panels show images of the lipid droplets stained with green-fluorescent BODIPY dye after 48 h of PQQ treatment. The bottom graph shows the results of the quantification of the diameters of lipid droplets. (c) The bar graph shows the result of triglyceride quantification in PQQ-treated adipocytes. Data demonstrate mean ± SEM derived from six measurements. Asterisks denote a statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) when compared with the vehicle-treated control as determined using one-way ANOVA followed by Dunnett's post hoc test.

phosphorylation in adipocytes increased in a PQQ concentration-dependent manner (Figures 4a and 4b). Therefore, we examined the phosphorylation levels of the ACC1 protein and found them to be increased after 15 h of PQQ treatment in a concentration-dependent manner (Figure 4c). In contrast, AMPK activation suppressed nuclear translocation of the transcription factor SREBP-1c via phosphorylation and reduced lipid biosynthesis. SREBP-1c regulates *Fas* gene expression, a fatty acid synthase that is important for lipid biosynthesis. In PQQ-treated cells, the phosphorylation level of SREBP-1c increased with increasing PQQ concentrations (Figure 4d), which could inhibit SREBP-1c localization to the nucleus. The expression level of *Fas*, a target gene of SREBP for fatty acid synthesis, was also suppressed with PQQ treatment (Figure 4e).

We also examined whether PQQ promotes nuclear translocation of PGC-1α, a transcription factor essential for regulating mitochondrial biogenesis. After culturing 3T3-L1 adipocytes in a serum-free medium containing 200 nM of PQQ for 15 h, immunofluorescence staining of PGC1-α was performed, and the cells were observed under a fluorescence microscope. The localization of PGC-1α into the nucleus was observed after PQQ treatment (Figure 5a). Furthermore, we performed cell fractionation to collect proteins from the nuclei and performed Western blotting to measure PGC-1α levels. The results showed that increasing the concentration of PQQ significantly increased PGC-1α levels in the nucleus (Figure 5b). We further confirmed that the protein levels of a mitochondrial marker, mtDNA-encoded cytochrome *c* oxidase subunit 1 (MTCO1), increased in differentiated adipocytes treated with PQQ for 24 h in a concentration-dependent manner (Figure 5c). Next, we examined the mRNA expression of PGC-1α target genes, nuclear respiratory factor 1 (*Nrf1*), and mitochondrial transcription factor A (*Tfam*). The qPCR results confirmed that the mRNA expression levels of *Nrf1* (Figure 5d) and *Tfam* (Figure 5e) were significantly increased after 20 h of PQQ treatment in the 3T3-L1 adipocyte cells. These results suggest that PQQ promotes mitochondrial biogenesis by inducing the nuclear translocation of PGC-1α.

DISCUSSION

PQQ is widely used as a food supplement, as it demonstrates a variety of functions related to health maintenance. Numerous *in vitro* and *in vivo* studies have revealed the potential health benefits of PQQ, including growth-promoting, antidiabetic, and antioxidant effects, as reviewed by Akagawa et al.[24] Furthermore, other studies have shown the positive effects of PQQ on mitochondrial metabolism and biogenesis in human subjects.[10,25] Here, we investigated whether PQQ can reduce body fat accumulation through mitochondrial biogenesis and fat metabolism and whether it is beneficial as an antiobesity supplement. We have demonstrated that PQQ supplementation can reduce body fat accumulation under conditions involving HFD-induced obesity in mice, in the model organism *Daphnia*, and in cultured adipocyte cells. Simpler organisms were used to study the effects of PQQ and its mechanism, as they are more convenient, cost-effective, and less time-consuming.

Obesity can occur with an increase in body fat content, body weight, muscle atrophy, and inflammation.[1,26,27] Previously, oral treatment with PQQ (10 and 20 mg/kg weight per day) significantly reduced food intake, body weight gain, and intra-abdominal fat content in rats fed a diet containing high fat (24.44%) and fructose (10%) content.[16] In addition, PQQ supplementation during gestation and lactation significantly reduced a Western-style diet (42% kcal from fat)-induced increase in fat mass in mouse offspring.[28] Our study revealed that oral PQQ supplementation significantly reduced the accumulation of body fat, particularly that of the visceral fat in the abdominal region in HFD-fed male mice (Figure 1); however, no significant differences were observed in food intake between the HFD and HFD+PQQ groups (Table 1). These results suggest that the effect of PQQ on the accumulation of body fat was not associated with changes in food consumption. In contrast, our data also showed that PQQ supplementation significantly reduced obesity-induced skeletal muscle loss in the lower body region (Figure 1), which agrees with the finding demonstrating no significant changes in HFD-induced body weight gain between the HFD and HFD+PQQ groups (Table 1). Hence, further studies are warranted to understand the mechanisms underlying the effect of PQQ on the skeletal muscles.

PQQ supplementation in obese female mice lowered the risk of their offspring developing nonalcoholic fatty liver disease (NAFLD).[28] Another study revealed that PQQ significantly reduced cholesterol levels in blood serum and obesity-associated low-grade inflammation.[16] Moreover, recently, dietary supplementation with PQQ has demonstrated

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

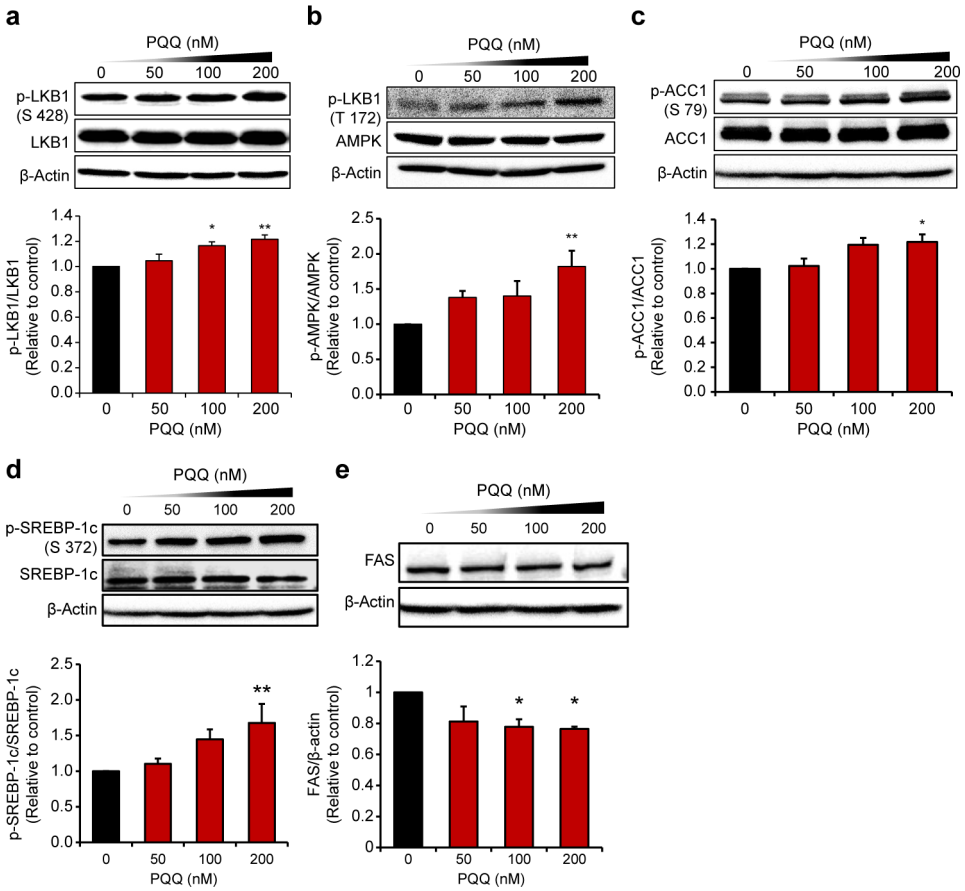

Figure 4. PQQ promotes the phosphorylation of LKB1 and AMPK and regulates downstream proteins that inhibit lipogenesis. (a) and (b) Differentiated 3T3-L1 adipocytes were cultured in a medium containing 0−200 nM PQQ for 6 h. (a) LBK1 phosphorylated at S428 (top blot), LBK1 (middle blot), and β-Actin (bottom blot) protein expressions were detected using Western blotting. The graph shows phosphorylated-LBK1 and LBK1 expression ratios relative to control. (b) AMPK phosphorylated at T172 (top blot), AMPK (middle blot), and β-Actin (bottom blot) protein expressions were detected using Western blotting. The graph shows phosphorylated-AMPK and AMPL expression ratios relative to control. (c−e) Differentiated 3T3-L1 adipocytes were cultured in a medium containing 0−200 nM PQQ for 15 h. (c) ACC1 phosphorylated at S79 (top blot), ACC1 (middle blot), and β-Actin (bottom blot) protein expressions were detected using Western blotting. The graph shows phosphorylated-ACC1 and ACC1 expression ratios relative to control. (d) SREBP-1c phosphorylated at S372 (top blot), SREBP-1c (middle blot), and β-Actin (bottom blot) protein expressions were detected using Western blotting. The graph shows phosphorylated-SREBP-1c and SREBP-1C expression ratios relative to control. (e) FAS (top blot) and β-Actin (bottom blot) protein expressions were detected using Western blotting. The graph shows FAS and β-Actin expression ratios relative to control. β-Actin protein was used as the control in all the Western blot experiments. All graphs show the band strength obtained from each Western blot as measured using ImageJ software. Data indicate mean ± SEM derived from 4 to 5 measurements. Asterisks denote a statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) when compared with the vehicle-treated control as determined using one-way ANOVA followed by Dunnett's post hoc test.

protective effects on the liver, mitigation of fat accumulation and oxidative injury via improved mitochondrial biogenesis, and maintenance of redox homeostasis in a high-fat-diet metabolic dysfunction-associated fatty liver disease chick model.[29] Our study also showed that PQQ reduced the levels of liver injury marker ALT and cholesterol levels. Although the cholesterol reduction results were not statistically significant, we hypothesize that significant results can be observed if the supplementation period is extended.

In addition, we investigated the effects of PQQ in *Daphnia* fed with different amounts of food. *Chlorella* was used as the food, which comprised 63% protein, 21% carbohydrate, and 16% fat (Supplementary Table S2). The three major types of macromolecules (carbohydrates, proteins, and fats) can be broken down into acetyl-CoA, an essential molecule used in the TCA cycle in mitochondria to provide energy for maintenance, growth, and the reproduction of biological systems.[30] Our results showed that PQQ reduced body fat accumulation during periods of high-calorie intake. Importantly, PQQ did not disturb normal body growth, whereas fitness based on reproduction ability was significantly improved, suggesting an improvement in health functions. Here, we examined two factors that led to high body fat content: HFD and high-calorie food intake. Dietary fat is broken down into fatty acids by lipase. Fatty acids are oxidized through the β-oxidation pathway that produces acetyl-CoA in the mitochondria, which is used as an energy source. HFD produces excessive fatty acids, which are stored in adipose tissues, leading to obesity. However, fatty acids are also synthesized in the body during periods of high-calorie food consumption. Excessive acetyl-CoA is the precursor of the fatty acid synthesis pathway, resulting in fatty acid accumulation in the adipose tissues. PQQ reduced body fat accumulation with both high-fat and high-calorie diet consumption.

To examine the mechanism underlying the effect of PQQ leading to a reduction in fat accumulation, we examined the

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

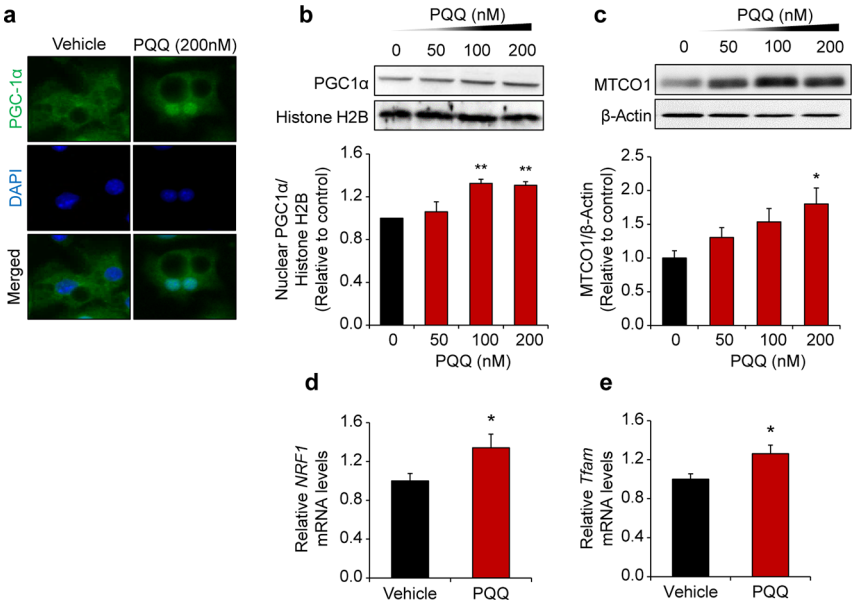

Figure 5. PQQ promotes mitochondrial biogenesis by enhancing PGC-1α levels in the nucleus, which promotes the expression of the downstream genes *Nrf1* and *Tfam*. Differentiated 3T3-L1 adipocytes were treated in a medium containing vehicle (control) or 200 nM PQQ for 15 h. (a) Fluorescence images captured after immunofluorescence staining targeting PGC-1α (top), nucleus region stained with DAPI (middle), and merged images (bottom). (b) PGC1α (top blot) and histone H2B (bottom blot) protein expressions in the nuclei fractions were detected using Western blotting. The graph shows the band strength obtained from each Western blot that was analyzed using ImageJ software. Data are shown as mean ± SEM (n = 3). (c) Differentiated 3T3-L1 adipocytes were cultured in a serum-free medium containing 0−200 nM of PQQ for 24 h. MTCO1 (top blot) and β-Actin (bottom blot) protein expressions were detected using Western blotting. Band intensity was measured using ImageJ software. Data are shown as mean ± SEM (n = 9). Asterisks denote a statistically significant difference (*p < 0.05; **p < 0.01) when compared with the vehicle-treated control as determined using one-way ANOVA followed by Dunnett's post hoc test. (d) and (e) Differentiated 3T3-L1 adipocytes were cultured in serum-free medium containing 200 nM of PQQ or vehicle (control) for 20 h. The mRNA expression level of *Nrf1* and *Tfam* was measured using real-time qPCR. *18S rRNA* was used as an internal standard. Data are shown as mean ± SEM (n = 8 or 12). (d) and (e) Asterisks denote a statistically significant difference (* p < 0.05) when compared with the control as determined using a two-tailed Student's t-test.

molecular level effects in the adipocytes. AMPK plays an essential role in regulating the energy balance in cells, making it a key focus in studies on obesity and metabolic syndrome.[31] AMPK activation (phosphorylation) is crucially involved in a broad range of systemic metabolic pathways that regulate energy homeostasis.[23] Thus, AMPK is a key target for the treatment of obesity and metabolic disorders.[23] Furthermore, as a downstream event of AMPK activation, phosphorylation of acetyl-CoA carboxylase 1 (ACC1) inactivates its functions to catalyze the conversion of acetyl-CoA to malonyl-CoA for lipid biosynthesis.

Sirtuin1 (SIRT1), an NAD^+-dependent deacetylase, activates LKB1 via deacetylation, which is followed by phosphorylation at Ser-428, and subsequently leads to AMPK phosphorylation at Thr 172.[32,33] Phosphorylated-AMPK (p-AMPK) phosphorylates ACC1 at Ser-79, which leads to the inhibition of ACC1 activity and the decreased synthesis of malonyl-CoA from acetyl-CoA, leading to the suppression of fatty acid biosynthesis.[34] In addition, p-AMPK regulates sterol regulatory element-binding protein-1c (SREBP-1c), a major regulator of lipogenic proteins, by phosphorylating Ser-372, leading to the inhibition of SREBP-1c nuclear activity. In turn, FAS expression is suppressed, which limits the lipogenesis pathway and lipid accumulation.[34,35] Our study showed that PQQ promoted the phosphorylation of LKB1 and AMPK and increased the inhibition of ACC1. PQQ also inhibited the nuclear translocation and activity of SREPB-1c via AMPK activation, which led to a reduction in FAS expression. This suggests that PQQ suppresses fatty acid biosynthesis through LKB1/AMPK signaling.

PGC-1α is essential for regulating mitochondrial biosynthesis.[12,13] The activation of PGC-1α by AMPK phosphorylation is required for regulating downstream gene expression and mitochondria.[36] A previous study showed that PQQ promotes mitochondrial biogenesis via AMPK activation, leading to the PGC-1 α/NRF1 complex that induces the induction of Tfam, which is involved in the expression of mitochondrial proteins.[37] Upon examination of adipocyte cells, we observed that PQQ promoted mitochondrial biogenesis by enhancing PGC1α activation in the nucleus along with the expression of *Nrf1* and *Tfam* genes. We confirmed that the number of mitochondria increased in both adipocyte cells and *Daphnia* (Supplementary Figure S4). The enhancement of mitochondrial biogenesis would lead to the improvement of β-oxidative metabolism in the mitochondria. The effect of PQQ on β-oxidation should be examined.

We also studied the effect of PQQ on lipase activity for fat (triglycerides) breakdown. No significant differences were observed between lipase activities in PQQ-treated and control *Daphnia* groups. PQQ also did not inhibit lipase activity *in vitro* (Supplementary Figure S5a,b). Our data suggest that the digestion and absorption of fat in the body remain unaltered with PQQ supplementation. These results were consistent with those observed with adipocytes, where PQQ treatment did not affect the protein expression of adipose triglyceride lipase and its activities (Supplementary Figure S5c,d). These findings suggest that fat breakdown is not involved in the inhibition of fat accumulation mediated by PQQ. Overall, our proposed

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

molecular mechanism for PQQ underlying reduction of fat accumulation via AMPK activation is summarized in Figure 6.

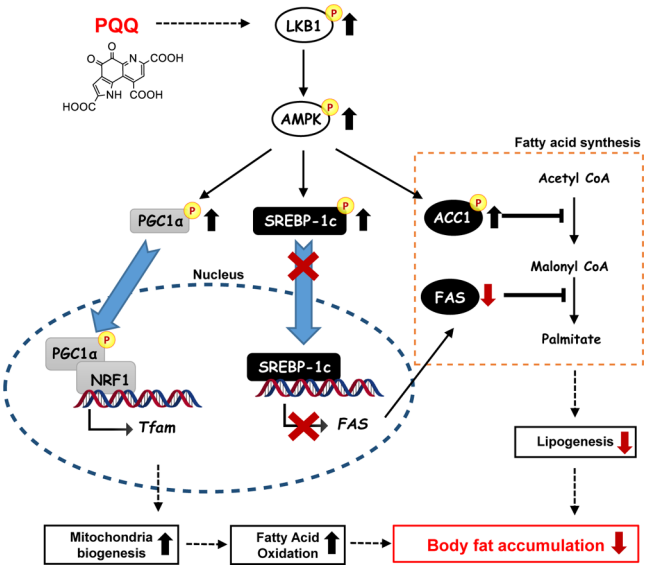

Figure 6. Proposed molecular mechanism of PQQ action in reducing fat accumulation. PQQ increases LBK1 and AMPK activities by promoting their phosphorylation. AMPK activation can mediate regulation of downstream factors, which 1) increases the phosphorylation of ACC1, 2) increases the phosphorylation of SREBP-1c, and 3) stimulates the nuclear translocation of PGC-1α. Phosphorylated ACC1 inhibits the conversion of acetyl-CoA to malonyl-CoA in the fatty acid synthesis pathway. However, phosphorylated-SREBP-1c prevents its translocation into the nucleus, which is required for FAS gene expression. This event helps to reduce FAS expression that suppresses the conversion of malonyl-CoA to palmitate. Subsequently, these events reduce lipogenesis for fat storage. In addition, the presence of PGC-1α in the nucleus eventually promotes the expression of mitochondrial genes, such *as Tfam,* which increases mitochondrial biogenesis and fatty acid oxidation. In summary, PQQ reduces body fat accumulation by 1) suppressing lipogenesis and 2) promoting fatty acid oxidation by enhancing mitochondrial biogenesis.

Although mechanistic studies were performed under *in vitro* conditions, further *in vivo* animal studies are required to confirm the molecular mechanisms underlying the effects of PQQ. Moreover, future studies must also assess the potency of PQQ treatment for phosphorylated LKB1 and AMPK levels and mitochondrial biogenesis in the adipose tissue under *in vivo* conditions.

Recent short-term human studies have reported improvements in cognitive function,[38] dry skin,[39] and lipid metabolism[40] following PQQ supplementation. Preclinical and human studies have also demonstrated that supplemental PQQ improves oxidative stress.[10,41] Additional clinical research on PQQ is needed to develop an efficacious therapeutic strategy for patients with obesity.

In conclusion, this study demonstrates that PQQ supplementation ameliorates body fat accumulation, leading to a reduction in total fat mass, muscle loss, liver injury, and cholesterol levels in HFD-fed mice. Additionally, PQQ significantly reduces body fat without affecting body growth in *Daphnia* supplied with a high amount of food. Moreover, reduction of fat accumulation by PQQ can be attributed to the activation of AMPK, which 1) reduces lipogenesis and 2) promotes mitochondrial biogenesis. Collectively, our findings suggest that PQQ exhibits therapeutic potential that can be exploited for the prevention and treatment of obesity. However, further human studies are warranted to elucidate and confirm the potential role of PQQ in obesity prevention and treatment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.1c00301.

Supplementary Table S1, nutrient composition of feed used in three diet groups; Supplementary Table S2, nutrient composition of *Chlorella*; Supplementary Figure S1, PQQ supplementation; Supplementary Figure S2, effects of PQQ on body fat and body length of *Daphnia* when given different amounts of food; Supplementary Figure S3, effect of PQQ on *Daphnia* survival and reproduction rates; Supplementary Figure S4, PQQ promoting mitochondria biogenesis in Daphnia and adipocyte cells; and Supplementary Figure S5, PQQ not affecting triglyceride breakdown when reducing body fat accumulation (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Kazuto Ikemoto − *Niigata Research Laboratory, Mitsubishi Gas Chemical Company, Inc., Niigata City, Niigata 950-3112, Japan;* orcid.org/0000-0002-5708-1636; Phone: +81-25-259-8226; Email: kazuto-ikemoto@mgc.co.jp; Fax: +81-25-258-4800

Mitsugu Akagawa − *Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai City, Osaka 599-8531, Japan;* orcid.org/0000-0002-8735-4738; Phone: +81-72-254-9460; Email: akagawa@biochem.osakafu-u.ac.jp; Fax: +81-72-254-9460

Authors

Nur Syafiqah Mohamad Ishak − *Niigata Research Laboratory, Mitsubishi Gas Chemical Company, Inc., Niigata City, Niigata 950-3112, Japan*

Midori Kikuchi − *Niigata Research Laboratory, Mitsubishi Gas Chemical Company, Inc., Niigata City, Niigata 950-3112, Japan*

Mariko Ogawa − *Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai City, Osaka 599-8531, Japan*

Kazeno Akutagawa − *Division of Applied Life Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai City, Osaka 599-8531, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsfoodscitech.1c00301

Author Contributions

N.S.M.I., K.I., and M.A. conceived and designed the study. N.S.M.I. and K.I. designed the mice experiment. N.S.M.I. and M.K. performed, analyzed, and interpreted the data. N.S.M.I. designed and performed the *Daphnia* experiment, and N.S.M.I. and K.I. analyzed and interpreted the data. M.O., K.A., and M.A. conducted the experiment using cultured adipocytes and analyzed the data. N.S.M.I. and M.A. wrote the manuscript. All

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

authors discussed the results, and K.I. and M.A. commented on and reviewed the manuscript.

Funding

The studies are sponsored only by Mitsubishi Gas Chemical Co., Inc. (Tokyo, Japan).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Summit Pharmaceutical International Corporation for allowing us to use their CosmoScanGXII small-animal 3D micro X-ray CT scanner (Rigaku) and for providing guidance on how to use it.

■ ABBREVIATIONS

μCT, microcomputed tomography; ACC1, acetyl-CoA carboxylase 1; ALT, aspartate aminotransferase; AST, aspartate aminotransferase; BCS, bovine calf serum; BioPQQ, PQQ disodium salt; BIS, bioimpedance spectroscopy; BSA, bovine serum albumin; DEX, dexamethasone; FAO, fatty acid oxidation; FAS, fatty acid synthase; FBS, fetal bovine serum; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; HU, Hounsfield unit; IBMX, 3-isobutyl-1-methylxanthine; LDL-C, low-density lipoprotein cholesterol; LKB1, liver kinase B1; MTCO1, mtDNA-encoded cytochrome *c* oxidase subunit 1; NAFLD, nonalcoholic fatty liver disease; ND, normal diet; NDI, new dietary ingredient; NRF1, nuclear respiratory factor 1; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; PQQ, pyrroloquinoline quinone; SIRT1, Sirtuin1; SREBP-1c, sterol regulatory element-binding protein-1c; T-CHO, total cholesterol; TFAM, transcription factor A

■ REFERENCES

(1) Tsatsoulis, A.; Paschou, S. A. Metabolically healthy obesity: Criteria, epidemiology, controversies, and consequences. *Curr. Obes. Rep.* **2020**, *9* (2), 109−120.

(2) Smith, K. B.; Smith, M. S. Obesity statistics. *Prim. Care* **2016**, *43* (1), 121−135.

(3) Thomas, E. L.; Frost, G.; Taylor-Robinson, S. D.; Bell, J. D. Excess body fat in obese and normal-weight subjects. *Nutr. Res. Rev.* **2012**, *25* (1s), 150−161.

(4) Chen, L. H.; Chen, Y. H.; Cheng, K. C.; Chien, T. Y.; Chan, C. H.; Tsao, S. P.; Huang, H. Y. Antiobesity effect of Lactobacillus reuteri 263 associated with energy metabolism remodeling of white adipose tissue in high-energy-diet-fed rats. *J. Nutr. Biochem.* **2018**, *54*, 87−94.

(5) Jeukendrup, A. E.; Randell, R. Fat burners: Nutrition supplements that increase fat metabolism. *Obes. Rev.* **2011**, *12* (10), 841−851.

(6) Kumazawa, T.; Sato, K.; Seno, H.; Ishii, A.; Suzuki, O. Levels of pyrroloquinoline quinone in various foods. *Biochem. J.* **1995**, *307* (2), 331−333.

(7) Goodwin, P. M.; Anthony, C. The biochemistry, physiology and genetics of PQQ and PQQ-containing enzymes. *Adv. Microb. Physiol.* **1998**, *40*, 1−80.

(8) Anthony, C. Pyrroloquinoline quinone (PQQ) and quinoprotein enzymes. *Antioxid. Redox Signaling* **2001**, *3* (5), 757−774.

(9) Itoh, Y.; Hine, K.; Miura, H.; Uetake, T.; Nakano, M.; Takemura, N.; Sakatani, K. Effect of the Antioxidant Supplement Pyrroloquinoline Quinone Disodium Salt (BioPQQTM) on Cognitive Functions. *Adv. Exp. Med. Biol.* **2016**, *876*, 319−325.

(10) Harris, C. B.; Chowanadisai, W.; Mishchuk, D. O.; Satre, M. A.; Slupsky, C. M.; Rucker, R. B. Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects. *J. Nutr. Biochem.* **2013**, *24* (12), 2076−2084.

(11) Turck, D.; Bresson, J. L.; Burlingame, B.; Dean, T.; Fairweather-Tait, S.; Heinonen, M.; Hirsch-Ernst, K. I.; Mangelsdorf, I.; McArdle, H. J.; Naska, A.; Neuhäuser-Berthold, M.; Nowicka, G.; Pentieva, K.; Sanz, Y.; Siani, A.; Sjödin, A.; Stern, M.; Tomé, D.; Vinceti, M.; Willatts, P.; Engel, K. H.; Marchelli, R.; Pöting, A.; Poulsen, M.; Schlatter, J. R.; de Sesmaisons, A.; Van Loveren, H. Safety of pyrroloquinoline quinone disodium salt as a novel food pursuant to Regulation (EC) No 258/97. *EFSA J.* **2017**, *15* (11), 5058.

(12) Wu, Z.; Puigserver, P.; Andersson, U.; Zhang, C.; Adelmant, G.; Mootha, V.; Troy, A.; Cinti, S.; Lowell, B.; Scarpulla, R. C.; Spiegelman, B. M. Mechanism controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **1999**, *98* (1), 115−124.

(13) Puigserver, P.; Spiegelman, B. M. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): Transcriptional coactivator and metabolic regulator. *Endocr. Rev.* **2003**, *24* (1), 78−90.

(14) Russell, A. P.; Feilchenfeldt, J.; Schreiber, S.; Praz, M.; Crettenand, A.; Gobelet, C.; Meier, C. A.; Bell, D. R.; Kralli, A.; Giacobino, J.; Dériaz, O. Endurance training in humans leads to fiber type − specific increases in levels of peroxisome. *Diabetes* **2003**, *52* (12), 2874−2881.

(15) Chowanadisai, W.; Bauerly, K. A.; Tchaparian, E.; Wong, A.; Cortopassi, G. A.; Rucker, R. B. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1α expression. *J. Biol. Chem.* **2010**, *285* (1), 142−152.

(16) Devasani, K.; Majumdar, A. Pyrroloquinoline quinone attenuates obesity associated low grade inflammation. *Obes. Med.* **2019**, *16*, 100134.

(17) Nakano, M.; Takahashi, H.; Koura, S.; Chung, C.; Tafazoli, S.; Roberts, A. Acute and subchronic toxicity studies of pyrroloquinoline quinone (PQQ) disodium salt (BioPQQ) in rats. *Regul. Toxicol. Pharmacol.* **2014**, *70* (1), 107−121.

(18) Aubertin, G.; Sayeh, A.; Dillenseger, J. P.; Ayme-Dietrich, E.; Choquet, P.; Niederhoffer, N. Comparison of bioimpedance spectroscopy and X-Ray micro-computed tomography for total fat volume measurement in mice. *PLoS One* **2017**, *12* (8), e0183523.

(19) Azegami, T.; Yuki, Y.; Sawada, S.; Mejima, M.; Ishige, K.; Akiyoshi, K.; Itoh, H.; Kiyono, H. Nanogel-based nasal ghrelin vaccine prevents obesity. *Mucosal Immunol.* **2017**, *10* (5), 1351−1360.

(20) Jordão, R.; Casas, J.; Fabrias, G.; Campos, B.; Piña, B.; Lemos, M. F.; Soares, A. M.; Tauler, R.; Barata, C. Obesogens beyond vertebrates: Lipid perturbation by tributyltin in the crustacean Daphnia magna. *Environ. Health Perspect.* **2015**, *123* (8), 813−819.

(21) Kusu, H.; Yoshida, H.; Kudo, M.; Okuyama, M.; Harada, N.; Tsuji-Naito, K.; Akagawa, M. Tomatidine reduces palmitate-induced lipid accumulation by activating AMPK via vitamin D receptor-mediated signaling in human HepG2 hepatocytes. *Mol. Nutr. Food Res.* **2019**, *63* (22), 1801377.

(22) Saihara, K.; Kamikubo, R.; Ikemoto, K.; Uchida, K.; Akagawa, M. Pyrroloquinoline quinone, a redox-active o-quinone, stimulates mitochondrial biogenesis by activating the SIRT1/PGC-1α signaling pathway. *Biochemistry* **2017**, *56* (50), 6615−6625.

(23) Zhang, B. B.; Zhou, G.; Li, C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab.* **2009**, *9* (5), 407−416.

(24) Akagawa, M.; Nakano, M.; Ikemoto, K. Recent progress in studies on the health benefits of pyrroloquinoline quinone. *Biosci., Biotechnol., Biochem.* **2016**, *80* (1), 13−22.

(25) Hwang, P. S.; Machek, S. B.; Cardaci, T. D.; Wilburn, D. T.; Kim, C. S.; Suezaki, E. S.; Willoughby, D. S. Effects of pyrroloquinoline quinone (PQQ) supplementation on aerobic exercise performance and indices of mitochondrial biogenesis in untrained men. *J. Am. Coll. Nutr.* **2020**, *39* (6), 547−556.

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

(26) Hariri, N.; Thibault, L. High-fat diet-induced obesity in animal models. *Nutr. Res. Rev.* **2010**, *23* (2), 270−299.
(27) Heo, J. W.; Yoo, S. Z.; No, M. H.; Park, D. H.; Kang, J. H.; Kim, T. W.; Kim, C. J.; Seo, D. Y.; Han, J.; Yoon, J. H.; Jung, S. J.; Kwak, H. B. Exercise training attenuates obesity-induced skeletal muscle remodeling and mitochondria-mediated apoptosis in the skeletal muscle. *Int. J. Environ. Res. Public Health* **2018**, *15* (10), 2301.
(28) Jonscher, K. R.; Stewart, M. S.; Alfonso-Garcia, A.; DeFelice, B. C.; Wang, X. X.; Luo, Y.; Levi, M.; Heerwagen, M. J.; Janssen, R. C.; de la Houssaye, B. A.; Wiitala, E.; Florey, G.; Jonscher, R. L.; Potma, E. O.; Fiehn, O.; Friedman, J. E. Early PQQ supplementation has persistent long-term protective effects on developmental programming of hepatic lipotoxicity and inflammation in obese mice. *FASEB J.* **2017**, *31* (4), 1434−1448.
(29) Qiu, K.; Zhao, Q.; Wang, J.; Qi, G. H.; Wu, S. G.; Zhang, H. J. Effects of pyrroloquinoline quinone on lipid metabolism and antioxidative capacity in a high-fat-diet metabolic dysfunction-associated fatty liver disease chick model. *Int. J. Mol. Sci.* **2021**, *22* (3), 1458.
(30) Turner, N.; Cooney, G. J.; Kraegen, E. W.; Bruce, C. R. Fatty acid metabolism, energy expenditure and insulin resistance in muscle. *J. Endocrinol.* **2014**, *220* (2), T61−79.
(31) Hardie, D. G. AMPK: A key regulator of energy balance in the single cell and the whole organism. *Int. J. Obes.* **2008**, *32* (S4), S7−S12.
(32) Lan, F.; Cacicedo, J. M.; Ruderman, N.; Ido, Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1: Possible role in AMP-activated protein kinase activation. *J. Biol. Chem.* **2008**, *283* (41), 27628−27635.
(33) Ruderman, N. B.; Xu, X. J.; Nelson, L.; Cacicedo, J. M.; Saha, A. K.; Lan, F.; Ido, Y. AMPK and SIRT1: A long-standing partnership? *Am. J. Physiol.-Endocrinol. Metab.* **2010**, *298* (4), E751−E760.
(34) Srivastava, R. A.; Pinkosky, S. L.; Filippov, S.; Hanselman, J. C.; Cramer, C. T.; Newton, R. S. AMP-activated protein kinase: An emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. *J. Lipid Res.* **2012**, *53* (12), 2490−2514.
(35) Li, Y.; Xu, S.; Mihaylova, M. M.; Zheng, B.; Hou, X.; Jiang, B.; Park, O.; Luo, Z.; Lefai, E.; Shyy, J. Y.; Gao, B.; Wierzbicki, M.; Verbeuren, T. J.; Shaw, R. J.; Cohen, R. A.; Zang, M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* **2011**, *13* (4), 376−388.
(36) Jäger, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B. M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (29), 12017−12022.
(37) Cheng, Q.; Chen, J.; Guo, H.; Lu, J. L.; Zhou, J.; Guo, X. Y.; Shi, Y.; Zhang, Y.; Yu, S.; Zhang, Q.; Ding, F. Pyrroloquinoline quinone promotes mitochondrial biogenesis in rotenone-induced Parkinson's disease model via AMPK activation. *Acta Pharmacol. Sin.* **2021**, *42* (5), 665−678.
(38) Itoh, Y.; Hine, K.; Miura, H.; Uetake, T.; Nakano, M.; Takemura, N.; Sakatani, K. Effect of the antioxidant supplement pyrroloquinoline quinone disodium salt (BioPQQTM) on cognitive functions. *Adv. Exp. Med. Biol.* **2016**, *876*, 319−325.
(39) Nakano, M.; Kamimura, A.; Watanabe, F.; Kamiya, T.; Watanabe, D.; Yamamoto, E.; Fukagawa, M.; Hasumi, K.; Suzuki, E. Effects of orally administered pyrroloquinoline quinone disodium salt on dry skin conditions in mice and healthy female subjects. *J. Nutr. Sci. Vitaminol.* **2015**, *61* (3), 241−246.
(40) Nakano, M.; Kawasaki, Y.; Suzuki, N.; Takara, T. Effects of pyrroloquinoline quinone disodium salt intake on the serum cholesterol levels of healthy Japanese adults. *J. Nutr. Sci. Vitaminol.* **2015**, *61* (3), 233−240.
(41) Zhu, B. Q.; Simonis, U.; Cecchini, G.; Zhou, H. Z.; Teerlink, Li. L.; Teerlink, J. R.; Karliner, J. S. Comparison of pyrroloquinoline quinone and/or metoprolol on myocardial infarct size and mitochondrial damage in a rat model of ischemia/reperfusion injury. *J. Cardiovasc. Pharmacol. Ther.* **2006**, *11* (2), 119−128.

https://doi.org/10.1021/acsfoodscitech.1c00301
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX