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A herbal extract with acetyl–coenzyme A carboxylase inhibitory activity and its potential for treating metabolic syndrome

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Abstract

Acetyl–coenzyme A carboxylase (ACC) plays a crucial role in fatty acid metabolism, and its inhibition is an effective approach for treating metabolic syndrome. Partially purified ACC from rat liver was used to screen herbs commonly used in Taiwanese folk medicine for ACC inhibitory effects. An ethanol extract of *Polygonum hypoleucum Ohwi* (EP), the Taiwan tuber fleece flower, was found to have the highest inhibitory activity (half-maximal inhibitory concentration = 30 μg/mL). We then tested the physiologic effects of EP using high-fat (HF) diet–fed C57BL/6J mice. After 4 weeks, body weight and levels of blood glucose, insulin, triacylglycerol, total cholesterol, and leptin were significantly reduced ($P < .05$) in mice fed a 3% EP-containing HF diet. The EP also improved the glucose tolerance and insulin sensitivity of HF diet–fed mice. In addition, EP at concentrations of 0.0725 and 0.145 mg/mL (2.5- and 5-fold higher than the half-maximal inhibitory concentration) was also effective in decreasing ACC and fatty acid synthase activity and the triacylglycerol content of HepG2 cells incubated in high-glucose (30 mmol/L) medium. These results show that EP, acting by inhibiting ACC activity, is effective in alleviating the symptoms associated with metabolic disease.

1. Introduction

Metabolic syndrome has become a major global public health problem. It is indicated by the clustering of certain risk factors, including insulin resistance, central obesity, hypertension, and dyslipidemia, which dramatically increases the risk of developing cardiovascular disease and type 2 diabetes mellitus [1,2]. In combating this global epidemic, changes in lifestyle are of prime importance. In addition, there is an urgent need for the discovery and development of new agents that are beneficial in relieving the metabolic perturbations.

Abnormal fatty acid metabolism and tissue fat burden may lie at the core of metabolic syndrome [3,4]. Modulation of fatty acid metabolism is therefore considered a potential approach for the treatment of obesity and metabolic syndrome. Acetyl–coenzyme A carboxylase (ACC, EC 6.4.1.2), an enzyme playing a crucial role in fatty acid metabolism, is an attractive target for the development of drugs active against obesity, diabetes, and other symptoms associated with metabolic disease [5,6]. Acetyl–coenzyme A carboxylase exists as 2 isoforms, ACC1 and ACC2, encoded by separate genes. Acetyl–coenzyme A carboxylase 1 is a cytosolic enzyme expressed mainly in lipogenic tissues (liver, adipose tissue, and mammary gland) and catalyzes the rate-limiting step in the biosynthesis of long-chain fatty acids. In contrast, ACC2 is associated with the mitochondrial membrane and is mainly expressed in the heart and skeletal muscle. Its product, malonyl–coenzyme A (CoA), is a potent inhibitor of fatty acid oxidation. By reducing malonyl-CoA levels, inhibition of ACC should be effective in reducing fatty acid synthesis and increasing fatty acid oxidation by, respectively, removing substrate and relieving malonyl-CoA inhibition of carnitine palmitoyltransferase–I [7]. In animal studies, reducing ACC expression by genetic manipulation,

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for example, ACC2 gene knockout [8] and ACC1 and/or ACC2 antisense oligonucleotides [9], and reducing ACC activity pharmacologically by isozyme nonselective inhibition [10,11] have been shown to be effective ways of treating and ameliorating metabolic syndrome.

In this study, partially purified ACC from rat liver was used to screen 20 herbs commonly used in Taiwanese folk medicine to explore their potential in treating metabolic syndrome; and an ethanol extract of *Polygonum hypoleucum Ohwi* (EP), the Taiwan tuber fleece flower, was found to have the highest ACC inhibiting activity. *Polygonum hypoleucum Ohwi* is a Chinese herb that has been used for the treatment of arthritis, rheumatoid arthritis, cough, influenza, and nephritis [12]. The antitumor and anti-inflammatory effects of a methanolic extract of *P hypoleucum Ohwi* have been demonstrated in an in vitro model [13-15]. However, whether extracts of this plant are effective in modulating lipid metabolism or treating metabolic syndrome has never been reported. We therefore performed an animal study to examine whether EP favorably affected a multitude of risk factors associated with high-fat diet–induced metabolic disorders. The functional effect of EP was also tested in hepatocytes with high-glucose–stimulated lipogenesis.

2. Materials and methods

2.1. Experiment 1: screening of herbs for ACC inhibitory activity

2.1.1. Purification of ACC from rat liver

The ACC was purified from rat liver following Tanabe et al [16]. To enrich ACC in the liver, 3 Wistar rats (body weight, 350 g) were fasted for 48 hours, and then received a standard diet (AIN-93M) for 24 hours and were given 30% sucrose as drinking water. After CO_2 asphyxiation, the rats were killed and the livers were removed. All subsequent steps were at 4°C. The livers were homogenized in 2 vol of 10 mmol/L potassium phosphate (K-P) buffer containing 0.25 mol/L sucrose, 5 mmol/L 2-mercaptoethanol, 1 mmol/L EDTA, and 1% protease inhibitor cocktail (1 mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride [AEBSF], 0.8 μmol/L aprotinin, 20 μmol/L leupeptin, 40 μmol/L bestatin, 15 μmol/L pepstatin A, and 14 μmol/L E-64) (Sigma, St Louis, MO). After centrifugation of the homogenate at 13 000*g* for 45 minutes, the supernatant (crude extract) was collected and brought to 30% saturation with ammonium sulfate; the mixture was left for 30 minutes and then centrifuged at 16 000*g* for 20 minutes. The precipitate was dissolved and dialyzed against 10 mmol/L K-P buffer, and then batch adsorbed to calcium phosphate gel; and ACC-containing protein was eluted with 200 mmol/L K-P buffer. After a second identical precipitation with $(NH_4)_2SO_4$, the protein fraction in 10 mmol/L K-P buffer was applied to a diethylaminoethyl (DEAE)-cellulose column equilibrated with the same buffer and eluted with a linear gradient of 10 and 500 mmol/L K-P buffer. Fractions containing protein (measured by UV_{280}) and exhibiting strong ACC activity were collected and pooled. After precipitating with $(NH_4)_2SO_4$ and dialysis, the purified enzyme solution was aliquoted and frozen at −70°C. The specific ACC activity was measured at the end of each purification step.

2.1.2. Preparation of TOFyl-CoA

5-(Tetradecyloxy)-2-furoic acid (TOFA), a hypolipidemic agent that inhibits ACC activity [10], was used to test the purified ACC. However, TOFA has to be converted into its CoA ester form (TOFyl-CoA) to act as an ACC inhibitor [10]. TOFyl-CoA was prepared by incubating 1 mL of 100 μmol/L TOFA in reaction mixture (50 mmol/L K-P buffer [pH 7.4] containing 0.2% bovine serum albumin [BSA], 4 mmol/L $MgSO_4$, 4 mmol/L adenosine triphosphate [ATP], and 0.4 mmol/L CoA) with the microsomal fraction (0.16 mg of protein). The microsomal fraction was prepared from a rat liver homogenate in 10 mmol/L K-P buffer by centrifugation at 12 000*g* for 20 minutes, followed by centrifugation of the supernatant at 105 000*g* for 1 hour to pellet the microsomes. Formation of TOFyl-CoA was confirmed by the absorption at 320 nm [10]. The reaction was stopped by adding 10% $HClO_4$, and the TOFyl-CoA was extracted from the precipitate by vortexing with 0.5 mL of ethanol and centrifugation at 12 000*g* for 5 minutes; the supernatant was stored at −70°C.

2.1.3. Western blots

The ACC protein levels were determined by Western blotting of the liver crude extracts and the fractions obtained at each purification step. Rabbit anti-human ACC1 antibodies (ADI, San Antonio, TX) were used as the primary antibody; and horseradish peroxidase–coupled anti-rabbit IgG antibodies (Amersham International, Amersham, United Kingdom), as the secondary antibody. According to the data sheet from the manufacturer, the primary antibody reacts with ACC1, but not with ACC2. Bound antibodies were detected by ECL chemiluminescence (Amersham). Protein concentrations were determined using the Bio-Rad (Hercules, CA) protein assay and BSA as the standard.

2.1.4. Preparation of herbal extracts

Twenty indigenous herbs commonly used in Taiwanese traditional folk medicine were selected and extracted with ethanol. All extracts were washed with 5% $NaHCO_3$ to remove acidic substances, such as citrate and fatty acid, which interfere with the ACC activity assay. All crude extracts were concentrated by lyophilization and then dissolved in dimethyl sulfoxide to make a 50-mg/mL stock solution; the samples were stored at −70°C until use.

2.1.5. Determination of ACC activity

The ACC activity was measured using the $^{14}CO_2$ fixation method [16]. Briefly, 7 μL of enzyme solution (70 μU) and 10 μL of the test sample or vehicle (the final

concentrations used are indicated in the figures) were added to 150 μL of 50 mmol/L Tris-HCl buffer (pH 7.5) containing 10 mmol/L potassium citrate, 10 mmol/L $MgCl_2$, 3.75 mmol/L glutathione, and 0.75 mg/mL BSA; and the mixture was preincubated for 5 minutes at 37°C. Substrate solution (1.5 μL each of ATP, acetyl-CoA, and $NaH^{14}CO_3$) was then added to give final concentrations of 3.75 mmol/L ATP, 0.125 mmol/L acetyl-CoA, and 7.5 mmol/L $NaH^{14}CO_3$ (0.26 μCi/μmol). The reaction was allowed to proceed for 10 minutes at 37°C, and then 40 μL of 5 N HCl was added. After evaporation with a stream of nitrogen under ventilation, the residue was dissolved in distilled water and mixed with 5 mL of scintillant; and the radioactivity was measured on a liquid scintillation spectrometer (Beckman LS6500, Fullerton, CA). *One unit of ACC activity* is defined as the amount that catalyzes the formation of 1 μmol of malonyl-CoA per minute.

2.2. Experiment 2: testing the physiologic benefits of EP in an animal study

2.2.1. Identification of P hypoleucum Ohwi and preparation of the ethanol extract

Polygonum hypoleucum Ohwi was collected from a local herbal store; and its authenticity was confirmed by the Department of Life Sciences, National Chung-Hsing University. The voucher specimen (Hsu 3156) was deposited in the herbarium of the same university. The air-dried stem and roots (1 kg) of *P hypoleucum Ohwi* were chopped up and immersed in 8 L of 95% ethanol at room temperature. The filtrate evaporated under reduced pressure, and the residue was collected. The yield of EP was 9% (g/g).

2.2.2. Animals and feed

Male C57BL/6J mice purchased from the National Applied Research Laboratories (Taipei, Taiwan) at 7 weeks of age were fed a high-fat diet containing 30% (wt/wt) butter (induction group, n = 14) or a low-fat diet containing 4% soybean oil (LF group, n = 7). After 5 months, the induction group was split into 2 groups that received a high-fat diet alone (HF group, n = 7) or supplemented with 3% (g/g) EP (HF/EP group, n = 7). The animals were kept in a room maintained at 23 ± 2°C on a controlled 12-hour light-dark cycle with free access to food and drinking water. Body weight was recorded weekly. The protocols for animal care and handling were approved by the Institutional Animal Care and Use Committee of the China Medical University.

2.2.3. Assessment of insulin sensitivity

After EP treatment for 3 weeks, the insulin sensitivity of all mice was assessed by the oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). For the OGTT, animals were fasted overnight; and then tail blood was collected before (0 minute) and at 30, 60, 90, and 120 minutes after oral administration of a 2.5-mol/L glucose solution (1.5 g/kg body weight). For the ITT, the animals were fed for 3 hours after overnight fasting; and then tail blood was collected before (0 minute) and at 30, 60, 90, and 120 minutes after intraperitoneal injection of a 0.1-U/mL solution of insulin (0.75 U/kg body weight). Blood glucose levels were measured using a MediSense Optium glucometer (Abbott Laboratories, Worcester, MA). The area under the curve for blood glucose (AUC_{glu}) over the 2 hours was calculated in both cases.

2.2.4. Measurement of biomedical indices

After EP treatment for 4 weeks, all mice were killed by carbon dioxide asphyxiation after 10 hours of fasting. Blood was collected from the orbital capillary, and serum was immediately separated and stored at −20°C until analysis. Enzyme-linked immunosorbent assays were used to measure serum insulin (Linco, St Charles, MO) and leptin (R&D, Minneapolis, MN). Lipids in the liver were extracted using the method of Folch et al [17]. Triacylglycerol (TG) and total cholesterol (TC) in serum and tissue extracts were measured by enzymatic methods using commercial kits (Randox Laboratories, Crumlin, Northland, United Kingdom). For ACC activity assays, a portion of the liver was homogenized at 4°C in 10 mmol/L K-P buffer containing 250 mmol/L sucrose, 5 mmol/L 2-mercaptoethanol, 1 mmol/L EDTA, and 1% protease inhibitor cocktail (Sigma). After sequential centrifugation at 13 000*g* for 45 minutes and 100 000*g* for 60 minutes at 4°C, the final supernatant was collected for ACC activity assay.

2.3. Experiment 3: testing the effect of EP on hepatocytes in a high-glucose system

2.3.1. Cell culture and treatment

Considering that the effect of EP might be best seen under conditions with increased de novo lipogenesis, a model of high-glucose–induced ACC activation in hepatocytes was used [18]. Human hepatoma HepG2 cells (Bioresource Collection and Research Center no. 60025) obtained from the Bioresource Collection and Research Center (Taipei, Taiwan) were grown in DMEM containing 5.5 mmol/L D-glucose, 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL penicillin in a humidified atmosphere of 5% CO_2 at 37°C and passaged every 3 days by trypsinization. For the study, the HepG2 cells were incubated in low-glucose (5.5 mmol/L) medium in 100-mm–diameter dishes (for enzyme activity assay) or 12-well plates (for TG assay). When 70% confluence was reached, the cells were maintained overnight in serum-free low-glucose medium and then switched to serum-free high-glucose (30 mmol/L) medium for 24 hours. To test the effects of EP, the cells were preincubated with high glucose for 6 hours; and then EP was added at final concentrations of 0.0725 and 0.145 mg/mL (2.5- and 5-fold higher than the half-maximal inhibitory concentration [IC_{50}]) in the high-glucose medium. The EP was prepared as a 100-mg/mL stock solution in absolute ethanol and appropriately diluted with medium. The same amount of absolute ethanol was used in the vehicle control. Cells were collected at different time points as indicated in the figures.

2.3.2. Intracellular enzyme activity and TG content

The harvested cells were homogenized in 10 mmol/L K-P buffer, the homogenate was centrifuged at 13 000*g* for 45 minutes, and the supernatant was collected and used for assay of ACC activity (as above) or fatty acid synthase (FAS) activity. The FAS activity was measured using a spectrophotometric method [19]. The above supernatant containing 100 μg of protein was added to 1 mL of 0.5 mol/L K-P buffer (pH 7) containing 33 nmol acetyl-CoA, 100 nmol malonyl-CoA, 100 nmol nicotinamide adenine dinucleotide phosphate (NADPH), 1 μmol EDTA, and 1 μmol β-mercaptoethanol; and the oxidation of NADPH was followed at 340 nm. A correction was made for the rate of NADPH oxidation in the absence of malonyl-CoA. *One unit of FAS activity* is defined as the amount of enzyme protein required to synthesize 1 nmol of palmitic acid (equivalent to the oxidation of 14 nmol of NADPH) per minute. For intracellular TG measurement, the cells were broken by sonication and freezing/thawing in distilled water; and then TG was measured using an enzymatic assay kit from Randox Laboratories.

2.3.3. Statistical analysis

Data are expressed as the mean ± SD. The significance of differences between groups was analyzed statistically by 1-way analysis of variance (ANOVA) and Duncan multiple range test. In the cell culture study, the significance of differences between the experimental group and the corresponding vehicle control group was analyzed using Student *t* test. The data were transformed to log values for the statistical analysis if the variances were not homogeneous. The General Linear Model of the SAS package (SAS institute, Cary, NC) was used for both statistical analyses, and differences were considered significant at *P* less than .05.

3. Results

3.1. Experiment 1: screening of herbs for ACC inhibitory activity

A crude fraction of rat liver ACC was first prepared, Western blotting using anti-ACC1 antibody being used to follow the purification procedure (Supporting Figure 1).

Table 1
Purification of ACC from rat liver

Fraction	Protein (mg)	Total activity (U)	Specific activity (mU/mg)	Yield (%)
Crude extract	3825	2547	666	100
1st $(NH_4)_2SO_4$ precipitate	434	1373	3164	54
Ca-P eluate	52	243	4666	9
2nd $(NH_4)_2SO_4$ precipitate	28	165	5893	6
DEAE-chromatography and 3rd $(NH_4)_2SO_4$ precipitate	3.3	89	26928	3

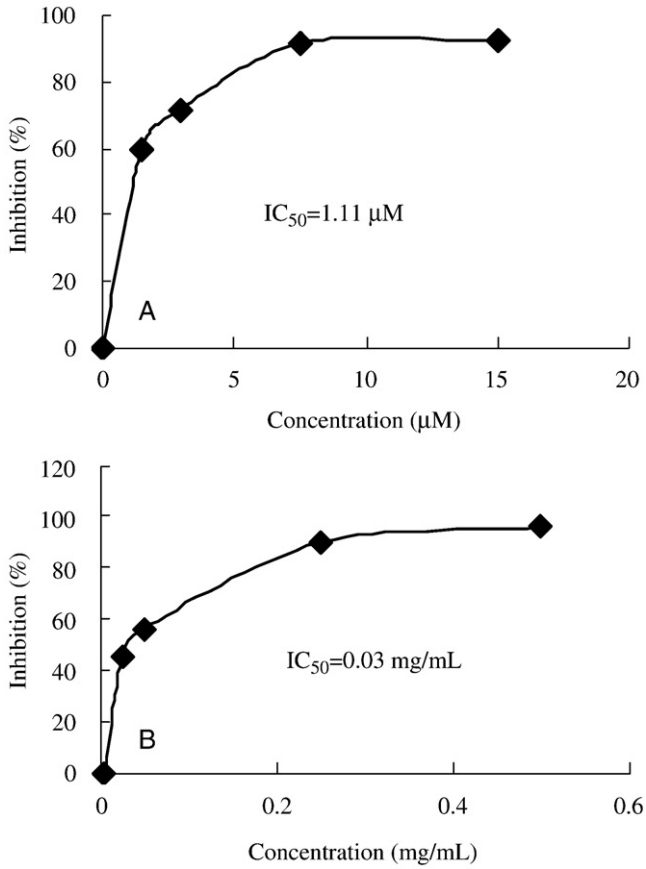

Fig. 1. Inhibition of ACC activity by TOFyl-CoA (A) or EP (B). Partially purified ACC was incubated with different concentrations of TOFyl-CoA or EP, and the inhibition of ACC activity was measured. The IC_{50} for TOFyl-CoA or EP is shown.

Table 1 shows the protein content and specific activity of ACC in the fractions obtained from each of the purification steps described in "Materials and methods." The specific activity of ACC increased during purification; and the overall purification was 40-fold, with a yield of 3%.

TOFyl-CoA, a potent ACC inhibitor, was used to verify the purified enzyme. When incubated with the purified protein, TOFyl-CoA inhibited the ACC activity in a dose-dependent manner (Fig. 1A). Inhibition was more than 90% at 7.5 μmol/L TOFyl-CoA, and the IC_{50} was 1.1 μmol/L. The observed inhibition is close to that reported by McCune and Harris [10], that is, 23% and 89% ACC inhibition for 0.25 and 2.5 μmol/L TOFyl-CoA, respectively. In contrast to TOFyl-CoA, citrate is an activator of ACC [7] and was supplied in the reaction mixture for the enzyme activity assay. When the potassium citrate was removed from the reaction mixture, the ACC activity was significantly reduced by 60% (data not shown). Thus, the purified protein was verified to have ACC activity and was used to screen the herbal extracts.

Among the 20 herbal extracts tested, EP was most effective in inhibiting ACC activity. Fig. 1B shows that ACC

Table 2
Body weight and adipose tissue weight of mice fed the experimental diets for 4 weeks

	LF	HF	HF/EP
Body weight, g	26 ± 1.2 c	37 ± 1.8 a	35 ± 3.2 b
Subcutaneous fat weight, g	0.35 ± 0.14 b	1.92 ± 0.44 a	1.61 ± 0.47 a
Retroperitoneal fat weight, g	0.13 ± 0.04 b	0.66 ± 0.12 a	0.61 ± 0.17 a
Epididymal fat weight, g	0.47 ± 0.19 b	1.89 ± 0.31 a	1.72 ± 0.49 a

The values are the mean ± SD (n = 7). The significance of differences among groups LF, HF, and HF/EP was analyzed by 1-way ANOVA and Duncan multiple range test. Values for which the groups do not share a letter are significantly different ($P < .05$).

activity was inhibited by EP in a dose-dependent manner. Inhibition was more than 90% at 250 μg/mL, and the IC_{50} was 30 μg/mL.

3.2. Experiment 2: testing the physiologic benefits of EP in an animal study

A high-fat diet containing 30% butter was used to induce metabolic syndrome in C57BL/6J mice. The HF group showed a significantly higher body weight and adipose tissue weight (subcutaneous, retroperitoneal, and epididymal fat pads) than the normal control group (LF) ($P < .05$, Table 2). Table 3 shows that blood glucose; serum levels of insulin, TG, TC, and leptin; and liver TC levels in the HF group were all significantly higher than those in the LF group. Serum nonesterified fatty acids and liver TG in the HF group were slightly higher than those in the LF group, but the differences did not reach statistical significance. Glucose intolerance and insulin resistance were also seen in the HF group, as shown by the significantly higher blood glucose levels (Fig. 2A, B) and the AUC_{glu} (Fig. 2C) over the 2-hour period of the OGTT and ITT in the HF group compared with the LF group. These results indicate that the symptoms of obesity, hyperglycemia, hyperlipidemia, and insulin resistance were successfully induced in the HF mice.

When 3% EP was incorporated in the high-fat diet and the animals were treated for 4 weeks (HF/EP group), the body weight, blood glucose, and serum levels of insulin, TG, TC, and leptin were all significantly lowered compared with those in the untreated HF animals ($P < .05$), whereas adipose tissue weight and liver TG and TC did not differ significantly from those in the HF group (Tables 2 and 3). In addition, EP improved the glucose intolerance and insulin resistance induced by the HF diet, as shown by the blood glucose levels and AUC_{glu} over the 2-hour period of the OGTT and ITT (Fig. 2A-C). The ACC activity in the liver was also measured

Table 3
Serum parameters, liver lipids, and liver ACC activity of mice fed the experimental diets for 4 weeks

	LF	HF	HF/EP
Blood glucose, mmol/L	6.17 ± 1.33 b	8.83 ± 2.50 a	7.67 ± 1.11 b
Serum insulin, ng/mL	0.39 ± 0.11 b	0.60 ± 0.28 a	0.46 ± 0.28 b
Serum TG, mmol/L	0.99 ± 0.23 b	1.38 ± 0.18 a	1.14 ± 0.32 b
Serum TC, mmol/L	2.62 ± 0.4 c	4.68 ± 0.21 a	3.69 ± 1.08 b
Serum NEFA, mmol/L	1.27 ± 0.16 a	1.63 ± 0.45 a	1.28 ± 0.25 a
Serum leptin, ng/mL	467 ± 155 c	5910 ± 1623 a	4204 ± 2209 b
Liver TG, mmol/g	14.0 ± 2.2 a	16.9 ± 2.7 a	15.7 ± 2.9 a
Liver TC, mmol/g	17.5 ± 2.9 b	30.7 ± 10.4 a	32.9 ± 11.8 a
Liver ACC, μU/mg	50.4 ± 14.1 b	61.9 ± 19.9 a	60.2 ± 21.6 a

The values are the mean ± SD (n = 7). The significance of differences among groups LF, HF, and HF/EP was analyzed by 1-way ANOVA and Duncan multiple range test. Values for which the groups do not share a letter are significantly different ($P < .05$). NEFA indicates nonesterified fatty acids.

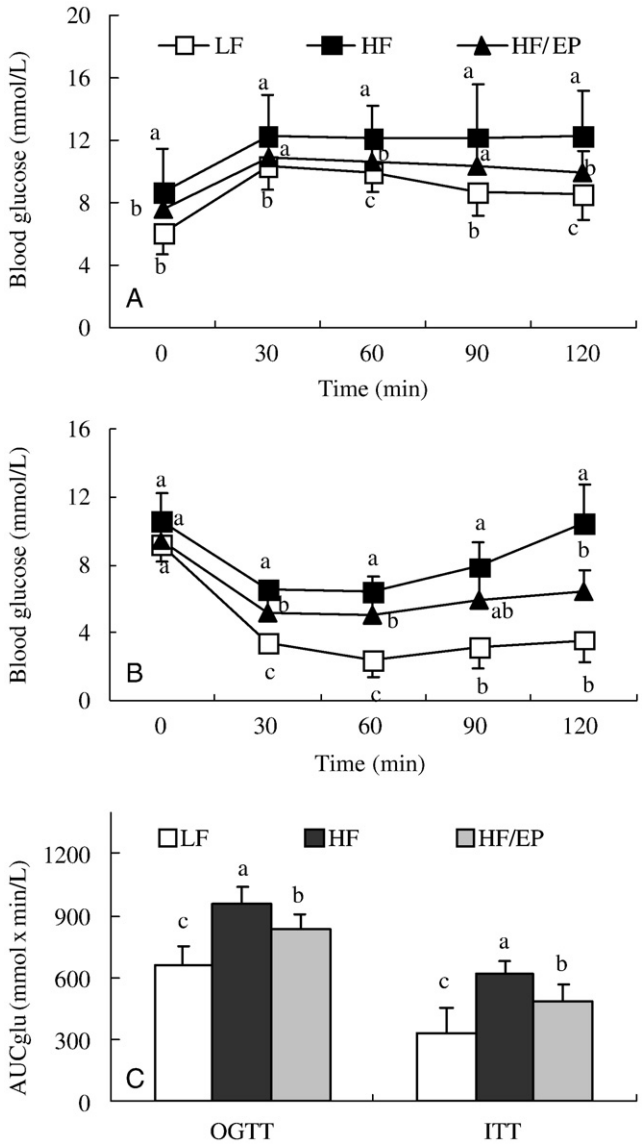

Fig. 2. Oral glucose tolerance tests (A) and ITTs (B) performed on mice fed the experimental diets (LF, HF, and HF/EP) for 4 weeks. The AUC_{glu} over 2 hours is shown (C). A glucose load (1.5 g/kg body weight) or an insulin load (0.75 U/kg body weight) was given; then, at the indicated time points, tail-blood was collected and blood glucose levels were measured. The values are the mean ± SD (n = 7). The significance of differences among groups LF, HF, and HF/EP was analyzed by 1-way ANOVA and Duncan multiple range test. Values for which groups do not share a letter are significantly different ($P < .05$).

in the 3 groups of mice, but no significant difference was found between the HF and HF/EP groups (Table 3).

3.3. Experiment 3: testing the effect of EP on hepatocytes in a high-glucose system

Although the animal study showed the physical benefits of EP, it failed to provide evidence that the benefits were mediated through ACC inhibition, as shown in the cell-free system in the first experiment. In addition, the effects of EP might be best seen under conditions with increased de novo lipogenesis. A model of high-glucose–induced ACC activation in hepatocytes [18] was therefore used to characterize the functional effects of EP in a cell-based system. Induction of ACC activity in HepG2 cells was successfully achieved by incubating the cells with 30 mmol/L glucose for 12 hours ($P < .05$, high-glucose cells vs low-glucose control; Fig. 3A). At the same time, the FAS activity and TG content of the high-glucose–treated cells were significantly increased compared with the low-glucose control (data not shown). To test if EP had an inhibitory effect, cells were stimulated with high glucose for 14 hours; and 2 doses (0.0725 and 0.145 mg/mL) of EP were added at 6 hours of high-glucose stimulation. The ACC and FAS activity and the TG content were then measured every 2 hours for 8 hours. Significant differences in enzyme activities and TG content between EP-treated cells and their corresponding vehicle control were seen at 4 hours of EP treatment (Fig. 3B-D), that is, at 10 hours of high-glucose stimulation, close to a time point for the highest ACC induction (Fig. 3A). Thus, EP inhibited the increase in ACC and FAS activities and TG content in HepG2 cells caused by high glucose; and the inhibition was greater at the higher concentration (0.145 mg/mL) of EP.

4. Discussion

Many reports have shown that ACC inhibition can favorably affect the etiology of metabolic syndrome. 5-(Tetradecyloxy)-2-furoic acid, a long-chain fatty acid analog and an ACC inhibitor, has been shown to reduce fatty acid synthesis in cultured hepatocytes [10] and to reduce plasma lipids and body weight in experimental animals [20,21]. CP-640186, which inhibits both ACC1 and ACC2, reduces fatty acid synthesis and increases fatty acid oxidation in hepatocytes and skeletal muscle cells and also

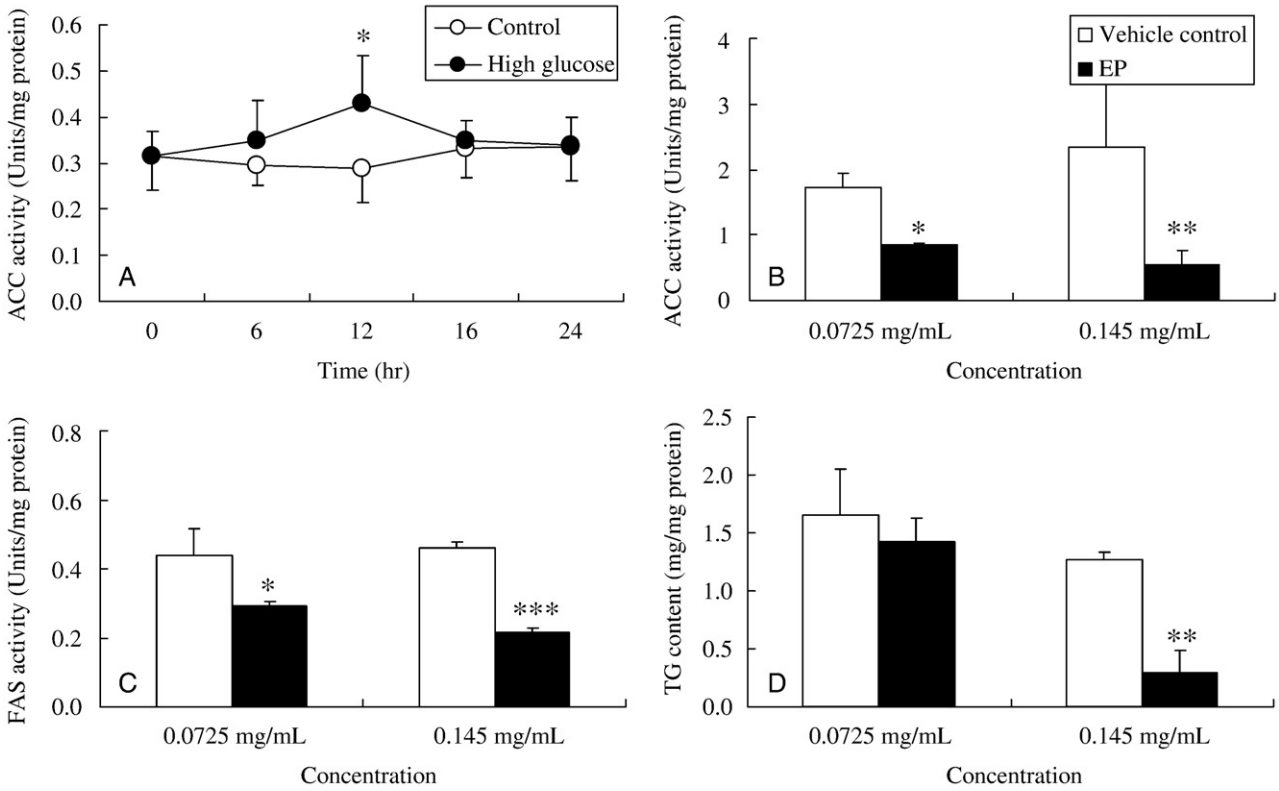

Fig. 3. Induction of ACC activity in HepG2 cells by high glucose (A) and ACC activity (B), FAS activity (C), and intracellular TG content (D) of high-glucose–stimulated HepG2 cells treated with vehicle or EP for 4 hours (see below). The ACC activity was measured in cells incubated in high-glucose (30 mmol/L) or low-glucose (5 mmol/L, control) medium over 24 hours (A). Cells were stimulated with high glucose for 14 hours, and 2 concentrations of EP (0.0725 and 0.145 mg/mL) were added at 6 hours of high-glucose stimulation; then ACC and FAS activity and the TG content were measured every 2 hours for 8 hours. The results at 4 hours of EP treatment are shown (B-D). The values are the mean ± SD (n = 3). The significance of differences between groups (ie, high-glucose cells vs low-glucose control or EP vs the corresponding vehicle control) was analyzed using Student *t* test. $^{*}P$ less than .05, $^{**}P$ less than .01, and $^{***}P$ less than .0001.

alleviates the symptoms of metabolic syndrome, including obesity, liver steatosis, and insulin resistance, in animals fed a high-sucrose diet [5]. Although ACC1 gene knockout is lethal [22], ACC2 gene knockout mice live normally, are leaner than the wild-type mice, and are protected from metabolic syndrome induced by a high-carbohydrate/high-fat diet [8,23,24]. A liver-specific ACC1 knockout mouse has been bred and shown to have less accumulation of hepatic TG when fed a lipogenic fat-free diet, although there is no impact on glucose homeostasis and body fat accumulation [25]. Using ACC1 and/or ACC2 antisense oligonucleotides, Savage et al [9] showed that inhibition of ACC1 and ACC2 expression prevents high-fat diet–induced nonalcoholic fatty liver and hepatic insulin resistance.

To avoid a compensatory response, the simultaneous inhibition of ACC1 and ACC2, thus reducing de novo fatty acid synthesis in lipogenic tissues and increasing fatty acid oxidation in skeletal muscle, is thought to be the most efficient way of treating metabolic syndrome. We believe our partially purified ACC was mainly ACC1 because (1) ACC2 is expressed to a lesser extent than ACC1 in the liver [6] and (2) the ACC2-containing mitochondrial fraction was removed from our preparation by centrifugation. Thus, it is clear that EP can inhibit ACC1, although the possibility that it also acts on ACC2 is not excluded.

Because the ACC inhibitory effect of EP was initially detected in a cell-free system, an animal study was needed to test the physiologic benefits of EP. In this study, a high-fat diet was used to induce metabolic syndrome in C57BL/6J mice. Although EP administration successfully ameliorated the hyperlipidemia, hyperglycemia, and insulin resistance induced by the high-fat diet, it did not reduce TG and TC accumulation in the liver or adipose fat mass, or the ACC activity in the liver. Given that ACC plays a central role in de novo lipogenesis [26], we speculate that the suppressive effect of EP on tissue lipid burden might be more marked in metabolic disturbances induced by a lipogenic diet (eg, fat-free diet or high-sucrose diet), rather than by a high-fat diet. Furthermore, the possibility of ACC inhibitors contributing to these metabolic benefits by acting through gastrointestinal tract (eg, inhibiting fat digestion and absorption in intestine) has to be considered.

As we speculated that a lipogenic condition might be required to show the effects of EP, a model of hepatocytes with high-glucose–stimulated lipogenesis was used. In this model, the ACC activity of the HepG2 cells was manipulated by modulating the glucose concentration in the medium. As shown in Fig. 3B, inhibition of ACC activity was observed in the EP-treated HepG2 hepatoma cell line preincubated with high glucose. Based on the synthesis of malonyl-CoA being the committed step toward the synthesis of fatty acids [26,27], EP treatment resulted in suppressions in FAS activity and cellular TG was expectable (Fig. 3C, D).

The ACC activity platform had been used pharmacologically for high-throughput screening for compounds with potential for treating metabolic syndrome. It has also been used to search for ACC inhibitors in foodstuffs; and (−)-epigallocatechin gallate; 9-oxooctadeca-10,12-dienoic acid; and 2-hydroxy-4-oxoheneicosa-5, 12, 15-trienyl/2-hydroxy-4-oxoheneicosa-12, 15-dienyl/2,4-dihydroxyheptadec-16-enyl/2,4-dihydroxyheptadec-16-ynyl acetate from green tea [28], red pepper [29], and avocado [30], respectively, have been identified as the active principles. However, effects of these compounds on metabolic syndrome were not tested in those studies. Using this screening tool, an ethanol extract from *P hypoleucum Ohwi* was found to have high potential for treating metabolic syndrome, which was shown in high-fat diet–fed animals and high-glucose–stimulated HepG2 cells in this study. Although long-chain fatty acyl-CoA is known to inhibit ACC [31], the inhibitory activity of EP is unlikely to be due to interference from free fatty acids in the herbal extract because no microsomal protein (source of the enzyme producing CoA esters) was included in the screening system. However, the possibility of the active component in the herbal extract being a CoA thioester of a fatty acid cannot be excluded.

Polygonum hypoleucum Ohwi is a Chinese herb that has been used for the treatment of arthritis, rheumatoid arthritis, cough, influenza, and nephritis [12]. Emodin, a compound isolated from *P hypoleucum Ohwi*, has been reported to have antitumor [13] and immunomodulatory [12,14,15] activities. However, an effect of the herb in reducing blood lipid levels or increasing insulin sensitivity has never been reported. Other than emodin, flavonoids including catechin, epicatechin, epicatechin-3-*O*-gallate, and procyanidin B2 were found in EP used in this study (study in progress). Epicatechin-3-*O*-gallate, rather than catechin and epicatechin, had been documented to inhibit ACC activity as effectively as (−)-epigallocatechin gallate [28]. Procyanidin B2, as a dimer of catechin and/or epicatechin, had never been reported to be an inhibitor of ACC, although its effect on ameliorating metabolic syndrome had been noticed recently [32].

Some adverse effects of ACC inhibitors must be considered. Malonyl-CoA plays an important role in controlling insulin secretion by the pancreas [33,34]. Inhibition of ACC could raise concern about impairing insulin secretion by β-cells, resulting in hyperglycemia, although simultaneously improving whole-body insulin sensitivity. However, in our study, this problem was not seen, as effective blood glucose lowering was seen during the OGTT in EP-supplemented mice. Another possible concern is compensatory appetite enhancement, as malonyl-CoA in the hypothalamus acts as a negative regulator of food intake [35,36]. In this study, hyperphagia was not observed in the EP-supplemented mice. In contrast to the decrease in malonyl-CoA levels in the hypothalamus caused by central administration (eg, intracerebroventricular injection) of ACC inhibitors [35,36], we believe that orally administered EP will have little or no effect on hypothalamic malonyl-CoA concentrations because of the blood-brain barrier.

The ACC activity can be controlled at the transcriptional level, as well as allosterically by small molecule modulators and by covalent modification, such as phosphorylation by cyclic adenosine monophosphate–activated protein kinase [6]. The screening platform used in this study is focused on allosteric effectors. Using this platform, the components of EP that act as allosteric regulators of ACC will be identified in future studies. Our results in high-fat diet–fed animals and high-glucose–stimulated HepG2 cells show that *P hypoleucum Ohwi,* a Chinese herb, is effective in alleviating the symptoms associated with metabolic disease and acts, at least in part, by inhibiting ACC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.04.012.

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