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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JUNE 2013

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Mulberry Anthocyanins Inhibit Oleic Acid Induced Lipid Accumulation by Reduction of Lipogenesis and Promotion of Hepatic Lipid Clearance

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ABSTRACT: Mulberry (*Morus alba* L.) has been considered to possess different benefits such as protecting liver; improving fever, urine excretion disorder, hypertension, and diabetic syndrome; and preventing cardiovascular diseases. Recently, mounting evidence has shown that mulberry anthocyanin extract (MAE) is beneficial to hyperlipidemia; however, the mechanisms remain unclear. The present study was aimed to investigate the protective effects of MAE on hepatocyte cultured with high fatty acid and the underlying mechanisms. By using human hepatoma cell HepG2 as cell model, the results showed that MAE suppressed fatty acid synthesis and enhanced fatty acid oxidation, contributing to amelioration of lipid accumulation induced by oleic acid (OA). Moreover, MAE also inhibited acetyl coenzyme A carboxylase (ACC) activities by stimulating adenosine monophosphate-activated protein kinase (AMPK). MAE attenuated the expression of sterol regulatory element-binding protein-1 (SREBP-1) and its target molecules, such as fatty acid synthase (FAS). Similar results were also found in the expressions of enzymes involved in triglyceride and cholesterol biosyntheses including glycerol-3-phosphate acyltransferase (GPAT), 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCoR), adipocyte-specific fatty acid binding protein (A-FABP), and SREBP-2. In contrast, the lipolytic enzyme expressions of peroxisome proliferator activated receptor α (PPARα) and carnitinepalmitol- transferase-1 (CPT1) were increased. This study suggests the hypolipidemic effects of MAE occur via phosphorylation of AMPK and inhibition of lipid biosynthesis and stimulation of lipolysis. Therefore, the mulberry anthocyanins may actively prevent nonalcoholic fatty liver disease.

KEYWORDS: *mulberry anthocyanins, hepatic lipid accumulation, lipogenesis, lipolysis, AMP-activated protein kinase*

INTRODUCTION

Obesity and body fat stacking are associated with several chronic diseases, including fatty liver, metabolic syndrome, and cardiovascular disease.[1] Fatty liver disease is related to expanded abdominal or visceral fat accumulation, high triacylglycerol (TG), low high-density lipoprotein cholesterol (HDL-C), high low-density lipoprotein cholesterol (LDL-C), and insulin resistance, which lead to impaired metabolic regulation in adipose tissue. The oral intake of fat and the subsequent lipolysis in visceral adipose tissue lead to increased levels of free fatty acid (FFA) and therefore result in TG accumulation and secretion of TG-rich lipoproteins, which in succession affect lipolipase activity and the distribution of lipoprotein subtypes.

The liver is where complicated biochemical reactions take place in response to metabolism of multiple nutrients including oxidation of triglycerides and synthesis of lipoproteins, cholesterol, and phospholipids. Abnormal lipid and lipoprotein metabolism are known to result in dyslipidemia such as elevation of plasma cholesterol and/or triglycerides and decreased levels of high-density lipoprotein (HDL) that contribute to increased prevalence of morbid obesity. Several proteins associated with dyslipidemia have been reported to play important roles in lipogenesis, including fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), sterol regulatory element binding proteins (SREBPs), adipose fatty acid-binding protein (A-FABP), and 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCoR), and expression levels of these enzymes are dynamic in response to cellular energy status. ACC catalyzes carboxylation of acetyl-CoA to produce malonyl-CoA in a biotin-dependent manner.[2] Malonyl-CoA is a substrate of FAS for fatty acid synthesis and also a potent inhibitor of carnitine palmitoyltransferase I (CPT1) for fatty acid β-oxidation. FAS is a multiple enzyme complex that catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA. Expression of ACC and FAS is regulated by multiple transcription factors. SREBPs, a transcription factor family, regulate the expression of enzymes for the synthesis of fatty acid, cholesterol, triacylglycerol, and phospholipid, suggesting that the protein family plays a central role in energy homeostasis via modulation of glycolysis, lipogenesis, and adipogenesis. In human beings, genes of the SREBP family mainly consist of SREBP-1 and SREBP-2, specifically responding to lipogenic and cholesterogenic metabolism, respectively.[3] Fatty acid binding proteins (FABPs), tightly binding to long-chain fatty acids, are members of the cytoplasmic protein family involved in intracellular free fatty

Received: March 19, 2013
Revised: May 30, 2013
Accepted: June 3, 2013
Published: June 3, 2013

acid trafficking and metabolism. Among the nine tissue-specific cytoplasmic FABPs, A-FABP has been widely studied in recent years for its association with atherosclerosis, type 2 diabetes, and insulin resistance.[4] Recent studies have shown that A-FABP mediates efflux of fatty acids in A-FABP-null mice.[5] In addition, this increase in lipid synthesis has been shown previously to be due to increased activities of HMGCoR, key enzymes of cholesterol.[6] HMGCoR is the precursor for cholesterol synthesis and catalyzes production of mevalonate from HMG-CoA.

In mammals, hepatic lipolysis is mainly controlled by several enzymes, including peroxisome proliferator-activated receptors (PPARs), CPT1, and adenosine monophosphate-activated protein kinase (AMPK), in response to nutritional and hormonal conditions. PPAR, a nuclear receptor family, consists of three subtypes PPARα, PPARβ/δ, and PPARγ. PPARα is highly expressed in the liver and promotes lipid uptake, β-oxidation of fatty acid, ketogenesis, and gluconeogenesis through regulating expression of genes involved in fatty acid catabolism.[7] As a result, it has been reported that PPARα is a potential target for improvement of lipid disorders, diabetes, and obesity.[8,9] CPT1 is localized in the mitochondrial outer membrane and synthesizes acylcarnitine for fatty acid transport across the inner mitochondrial membrane. AMPK is a serine threonine kinase comprising a heterotrimeric complex and regarded as an energy sensor in most tissues. AMPK regulates cellular lipid metabolism through increase of fatty acid oxidation and constitutive lipoprotein exocytosis.[10] In general, AMPK activation leads to inhibition of energy-consuming anabolism (lipogenesis, protein synthesis, and gluconeogenesis) and enhancement of energy-producing catabolism (glucose uptake and fatty acid oxidation). In the liver, activated AMPK phosphorylates and inactivates the rate-limiting enzymes of lipogenesis such as ACC,[11] suggesting that AMPK/ACC signaling plays a critical role in hepatic lipid homeostasis.[12]

Mulberry, the fruit of *Morus alba* L., is traditionally used in edible food. It has some pharmacological effects, such as fever reduction, antifever diuretics, liver protection, eyesight improvement, blood pressure reduction, and cardiovascular disease prevention. Mulberry extracts (MWE) contain high amounts of anthocyanins, a polyphenol exhibiting antioxidant and antitumor properties.[13−17] Many herbally derived polyphenolic compounds have been suggested to be capable of preventing obesity via inhibiting hypolipidemia and reducing adipose tissue mass, thus suppressing the occurrence of the metabolic, hepatic, and cardiovascular alterations associated with obesity.[18−22]

On the basis of previous reports that mulberry extract has an antiatherogenic effect, this study aimed to examine whether mulberry anthocyanin extract (MAE) could reduce hepatic lipid induced by oleic acid (OA). Hepatic regulation of lipid synthesis, oxidation, and clearance was also investigated. Taken together, MAE has not only antioxidant activity but also lipid-lowering capability, suggesting that MAE has the potential to enhance hepatic lipid clearance.

MATERIALS AND METHODS

Materials. Tris-base, sodium dodecyl sulfate (SDS), NaCl, bovine serum albumin (BSA), Nonidet P-40, gallic acid, and sodium deoxycholate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered solution (PBS), trypsin−EDTA, fetal bovine serum, and powdered Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco/BRL (Gaithersburg, MD, USA). Antibody against FAS proteins was purchased from BD Transduction Laboratories (San Diego, CA, USA). Antibody against HMGCoR proteins was purchased from Millipore Co. (Billerica, MA, USA). AMPK inhibitor was purchased from Merck Millipore Bioscience.

Preparation of MAE. Mulberry (fruit of *M. alba* L.) was obtained from the Taichung District Agricultural Research Station in Tai-Pin, Taichung, Taiwan. MAE was prepared from the lyophilized fruit of mulberry (100 g), with a 3-fold volume of methanol containing 1% HCl for 1 day at 4 °C. The extract was filtered and then concentrated under reduced pressure at 30 °C. The precipitate was collected and allowed to stand on an Amberlife Diaion HP-20 resin column for 24 h, then cleaned in distilled water (5 L) containing 0.1% HCl solution and eluted with methanol. The filtrate was collected and lyophilized to obtain 5 g of MAE and stored at 4 °C before use (Amberlife Diaion, USA).

HPLC Assay for MAE. Total anthocyanins were extracted using the Fuleki and Francis method (1968).[23] Separation of anthocyanins was conducted on a Luna C18 column (2.00 mm × 150 mm, 3.0 μm, Phenomenex, Inc., Torrance, CA, USA) using an HPLC system consisting of a Finnigan Surveyor module separation system and a photodiode array (PDA) detector (Thermo Electron Co., USA) A linear gradient from 98% A to 30% B in 45 min was used for HPLC analysis of anthocyanins. Solvent A was water containing 1% formic acid, and solvent B was acetonitrile containing 0.1% formic acid. The flow rate was 0.2 mL/min. Absorption spectra of anthocyanins were recorded from 240 to 600 nm with an in-line PDA detector.

Cell Line and Cell Culture. Human hepatoma HepG2 cells were obtained from American Type Culture Collection and grown in DMEM supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (HyClone R, Thermo Scientific, Logan, UT, USA). The cells were cultured at 37 °C in a humidified atmosphere of 95% air to 5% CO_2.

Cytotoxicity Assay. HepG2 cells were seeded at a density of 1×10^5 cells/well into a 24-well plate and treated with OA and MAE at various concentrations for 24 h. The 0.5 mg/mL MTT reagent was added to each well and incubated for 4 h. The medium was removed, and isopropanol was added to dissolve the MTT−formazan complex. The absorbance was measured at 563 nm with a spectrophotometer (Hatachi 3210, Hitachi, Tokyo, Japan).

Nile Red Stain. HepG2 cells were seeded in a 6-well plate (3×10^6 cells/well) and treated with 500 mM OA and indicated concentrations of MAE for 24 h. The cells were washed twice with PBS and fixed with 4% formaldehyde in PBS for 1 h and then stained with 1 μg/mL Nile red for 30 min at room temperature. After staining, the distribution of lipid in cells was immediately analyzed by a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA). Lipid-bound Nile red fluorescence was detected using inverted fluorescence microscopy.

Western Blot Analysis. HepG2 cells were seeded in a 10 cm dish (1×10^6 cells/well) and treated with 500 mM OA at indicated concentrations of MAE for 24 h. The proteins of the cells were harvested in a cold radio immunoprecipitation assay (RIPA) buffer (1% NP-40, 50 mM Tris-base, 0.1% SDS, 0.5% deoxycholic acid, 150 mM NaCl, pH 7.5) containing leupeptin (1.7 μg/mL) and sodium orthovanadate (10 μg/mL). The cell mixture was vortexed at 4 °C for 4 h. All mixtures were then centrifuged at 12000 rpm at 4 °C for 10 min, and the protein contents of the supernatants were determined with the Coomassie Brilliant Blue total protein reagent (Kenlor Industries, Costa Mesa, CA, USA) using bovine serum albumin as the standard. Equal amounts of protein samples were subjected to SDS−polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% nonfat milk powder with 0.1% Tween-20 in TBS and then incubated with the first antibody at 4 °C overnight. Thereafter, membranes were washed three times with 0.1% Tween-20 in PBS and incubated with the secondary antibody to anti-mouse horseradish peroxidase (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Antibodies against AMPK, phospho-AMPK, and ACC were purchased from Cell Signaling Technology (Beverly, MA, USA). FAS, SREBP-1c, SERPB-2, A-FABP, CPT1, and GPAT antibodies

were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody against HMGCoR was purchased from Upstate Biotechnology (Charlottesville, VA, USA). Band detection was revealed by enhanced chemiluminescence using ECL Western blotting detection reagents and exposed ECL hyper film in FUJIFILM LAS-3000 (Tokyo, Japan). Protein quantification was determined by densitometry using FUJIFILM-Multi Gauge V2.2 software (FUJIFILM, Stockholm, Sweden).

Statistical Analysis. Data were analyzed by an unpaired t test and represented as the mean ± SD. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Ingredients of Anthocyanin Compounds of MAE. To determine the compounds of MAE from mulberry, the anthocyanin contents were determined by HPLC and spectrophotometry. HPLC analysis of the anthocyanin compounds showed the retention times of cyanidin-3-glucoside and cyanidin-3-rutinoside were 29.9 and 31.5 min, respectively (data not shown). The MAE included 301.75 ± 7.96 mg cyanidin-3-glucside and 108.79 ± 3.35 mg cyanidin-3-rutinoside (Table 1).

Table 1. HPLC Analysis of the Identified Anthocyanic Compounds Showed Retention Times of Cyanidin-3-glucoside and Cyanidin-3-rutinoside of 29.9 and 31.5 min[a]

peak	anthocyanic composition	content (mg/g sample)
1	cyanidin-3-glucoside chloride	301.75 ± 7.96
2	cyanidin-3-rutinoside chloride	108.79 ± 3.35

[a]HPLC chromatogram of MAE. Chromatograms were monitored at 518 nm, which corresponded to anthocyanic compositions. Anthocyanic compositions corresponding to peaks are marked. Peaks: 1, cyanidin-3-glucoside; 2, cyanidin-3-rutinoside.

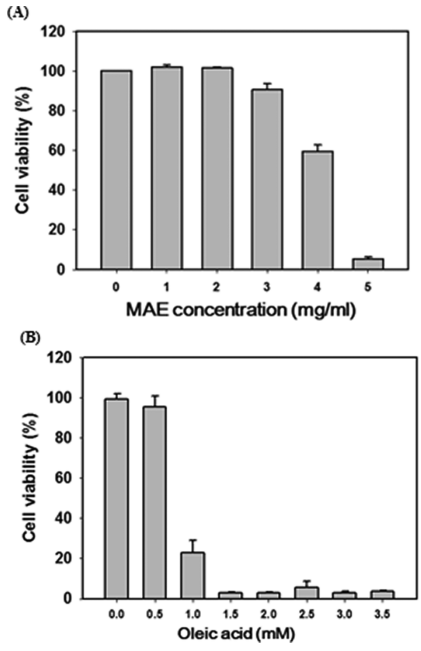

Figure 1. Cytotoxicity of HepG2 cells treated with MAE and OA: (A) cultured HepG2 cells were treated with various concentrations of MAE for 24 h and analyzed by MTT assay; (B) HepG2 cells were treated with OA under different concentrations (0.1−3.5 mM) for 16 h and analyzed by MTT assay. The data are the mean ± SD from four samples for each group.

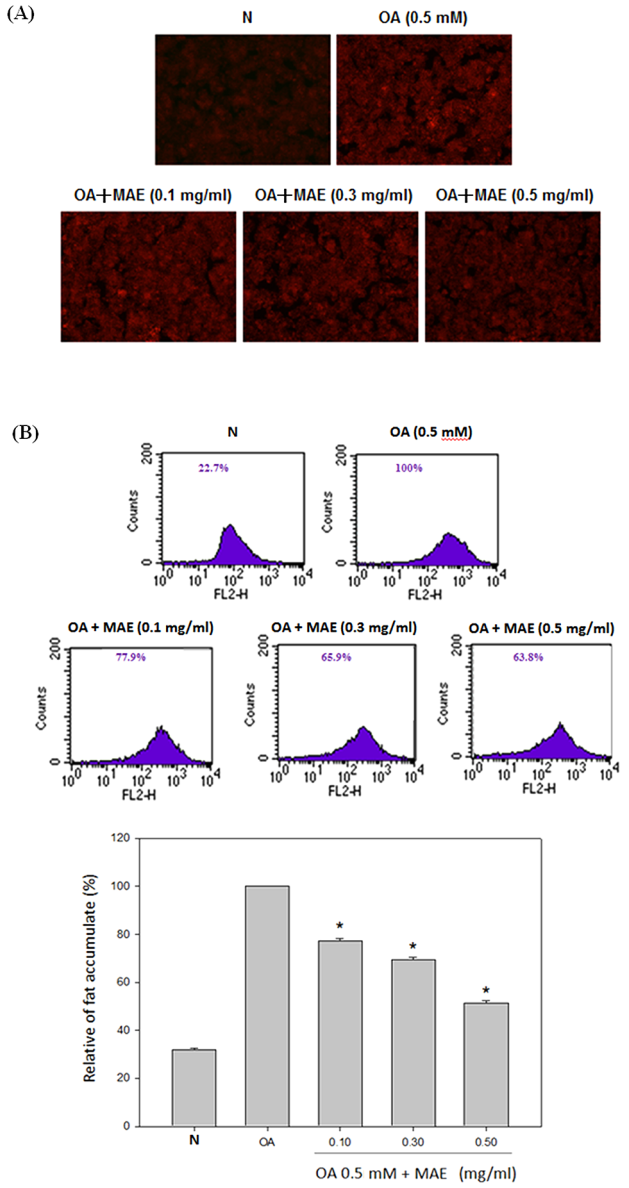

Figure 2. MAE attenuates OA induced lipid accumulation in HepG2 cells. HepG2 cells were treated with indicated concentrations of MAE (0.1, 0.3, 0.5 mg/mL) in the presence of 0.5 mM OA for 24 h. Cells were stained with Nile red (A) and analyzed by flow cytometry (B). The data are the mean ± SD from three samples for each group. (*) $p < 0.05$ in relation to the OA-induced group. N, normal; OA, oleic acid.

Cytotoxicity of MAE and OA in HepG2 Cells. To evaluate the effect of MAE and OA on the cell viability of HepG2 cells, various concentrations MAE and OA were treated with HepG2 cells for 24 and 16 h, respectively, and then assayed by MTT. Following 24 h of exposure of 4 mg/mL MAE, approximately 38−42% of cell numbers were decreased (Figure 1A). Figure 1B displays the cell viability of OA to HepG2 cells at 0−3.5 mM concentrations; 0.5 mM OA was not cytotoxic to HepG2 cells, whereas 1 mM OA inhibited 75−83% cell growth. Accordingly, 0.5 mM OA cotreated with MAE (0.1, 0.3, and 0.5 mg/mL) was selected for the following experiment.

Effect of MAE on OA Induced Lipid Accumulation. In this study, Nile red staining was used to examine the inhibitory effects of MAE on lipid accumulation in HepG2 cells. Lipid accumulation was induced by 0.5 mM OA in liver cells (Figure

dx.doi.org/10.1021/jf401171k | J. Agric. Food Chem. 2013, 61, 6069−6076

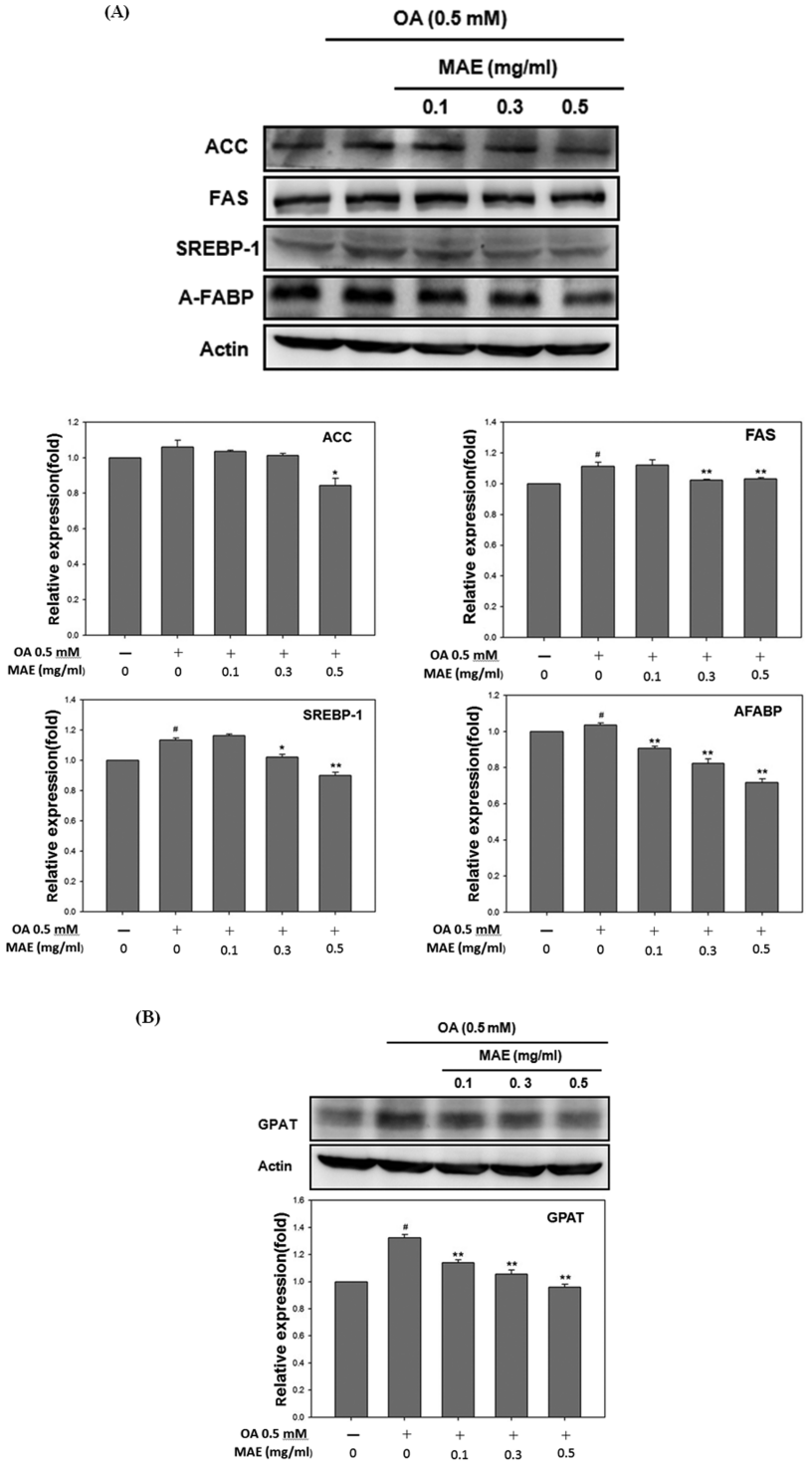

Figure 3. MAE treatment decreases FAS, ACC, SREBP-1, A-FABP, and GPAT protein expression. HepG2 cells were cotreated with OA at indicated concentrations of MAE (0.1, 0.3, 0.5 mg/mL) for 24 h. FAS, ACC, SREBP-1, A-FABP (A) and GPAT (B) protein were detected by Western blot analysis and quantified by densitometric analysis. The results from three independent experiments are expressed as the mean ± SD. (∗) $P < 0.5$, compared with OA-induced group; (∗∗) $P < 0.05$, compared with OA-induced group; (#) $P < 0.05$, compared with control group.

2A). However, the lipid content was remarkably decreased by cotreatment with 0.5 mg/mL MAE. Afterward, flow cytometry was used to quantitate the lipid accumulation state. As compared with the OA treated group, 0.1, 0.3, and 0.5 mg/mL MAE reduced levels of lipid accumulation to 22.1, 34.1, and 37.2%, respectively (Figure 2B), indicating the lipid-lowering potential of MAE.

Effect of MAE on Triglyceride Biosynthesis Expression. Various signaling pathways such as FAS, ACC, SREBP-1, and GPAT as well as A-FABP regulate the fatty acid synthesis and lead to triglyceride synthesis. Therefore, we investigated whether MAE decreased triglyceride synthesis-related proteins expression using Western blot analysis. As shown in Figure 3A, 0.5 mM OA treatment of HepG2 cells significantly increased

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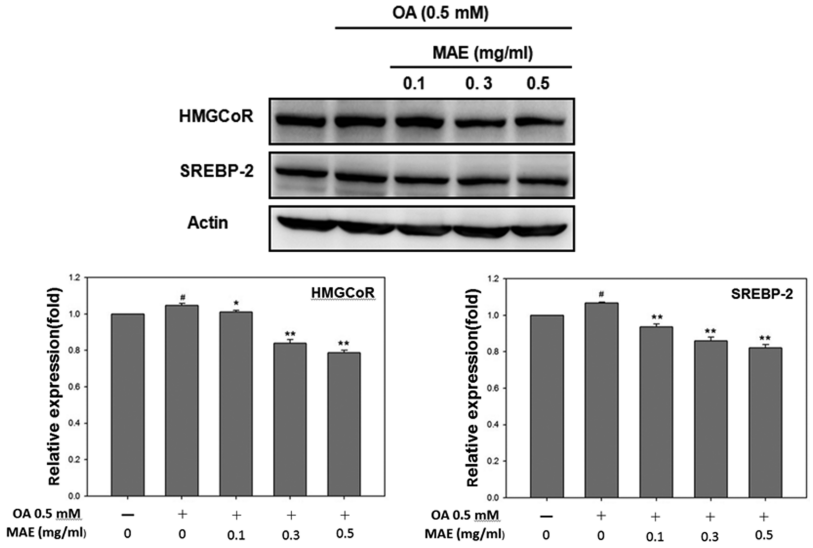

Figure 4. MAE treatment decreases HMGCoR and SREBP-2 protein expression. HepG2 cells were cotreated with OA at indicated concentrations of MAE (0.1, 0.3, 0.5 mg/mL) for 24 h. Protein was detected by Western blot analysis and quantified by densitometric analysis. The results from three independent experiments are expressed as the mean ± SD. (∗) $P < 0.05$, compared with OA-induced group; (∗∗) $P < 0.05$, compared with OA-induced group; (#) $P < 0.05$, compared with control group.

FAS, SREBP-1, and A-FABP expression but only slightly increased ACC. Data showed that MAE (0.5 mg/mL) decreased 18% ACC, 6% FAS, 23% SREBP-1, and 31% A-FABP expression ($P < 0.001$), as compared to OA treatment. GPAT catalysis is the committed step in the pathway of glycerolipid biosynthesis. We analyzed the level of MAE mediated GPAT expression following OA treatment for 24 h. The decreased GPAT expression was found by treating different concentrations of MAE compared with the OA-induced group ($P < 0.001$) (Figure 3B). These results hint that MAE attenuated hepatic lipid accumulation by regulating fatty acid and triglyceride synthesis.

Effect of MAE on Cholesterol Biosynthesis Related Proteins Expression. HMGCoR is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. Besides, SREBP-2 is known to up-regulate genes involved in cholesterol biosynthesis and uptake. We further investigated whether the MAE mediated HMGCoR and SREBP-2 expression. Cells were cotreated with 0.5 mM OA and different concentrations of MAE (0.1, 0.3, and 0.5 mg/mL) for 24 h. Figure 4 shows the MAE inhibited HMGCoR expression induced by OA in a dose-dependent manner (3, 18, and 24% by 0.1, 0.3, and 0.5 mg/mL MAE treatment, respectively). Similar results were also found for SREBP-2. These results suggested that MAE suppressed HMGCoR and SREBP-2 expression in HepG2 cells.

Effect of MAE on Free Fatty Acid Related Protein Expression. Long-chain acyl-CoA is transported into mitochondria via regulation of CPT1 and then is catabolized to acetyl-CoA via β-oxidation. Upon ACC inactivation by AMPK, the decreased level of malonyl-CoA elevates CPT1 activity, contributing to increase fatty acid oxidation.[23] In addition, nuclear translocation of PPARα induces transcription of genes for fatty acid utilization, for example, AMPK and CPT1.[24] To investigate the effects of MAE on the expression of PPARα, CPT1, AMPK, and phospho-AMPK, HepG2 cells were treated with OA (0.5 mM) or co-incubated with MAE (0.1, 0.3, and 0.5 mg/mL) for 24 h. As shown in Figure 5A, PPARα expressions were dose-dependently elevated in response to MAE treatments. In parallel to increased PPARα expression, CPT1 was increased to 104, 118, and 122% of control in the presence of 0.1, 0.3, and 0.5 mg/mL MAE, respectively ($P < 0.001$). In addition, our results showed that MAE (0.1−0.5 mg/mL) treatments significantly induced AMPK activation as compared to OA alone (Figure 5B). These findings indicated that MAE induced activation of AMPK and expression of PPARα and CPT1, which may increase fatty acid oxidation and attenuate hepatic lipid accumulation.

DISCUSSION

The hepatic hypolipidemic mechanism of MAE showed high relevant expression of lipogenic enzyme (SREPB-1, FAS, ACC, and A-FABP), cholesterol biosynthesis (SREBP-2 and HMGCoR), TG biosynthesis (GPAT), and fatty acid β-oxidation (PPARα and CPT1) in HepG2 cells. In this study, we also proved MAE inhibited cellular lipid accumulation through activating AMPK and suppressing the lipogenic enzyme. Activation of AMPK leads to regulation of a number of downstream targets involved in lipid metabolism.[25] Among the AMPK downstream targets, HMGCoR and ACC have been well identified.[26]

In a previous study, MWE and MAE possessed antioxidative and antiatherosclerogenesis abilities in vitro.[22] Both MWE and MAE showed great ability in scavenging free radicals, inhibiting LDL oxidation, and decreasing atherogenic stimuli in macrophages, whereas the efficacy of MAE was 10-fold greater than that of MWE. These results demonstrated anthocyanic components in mulberry extracts were regarded as indispensible in preventing atherosclerosis. Mulberry has been identified as being rich in anthocyanins, including cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside. In the previous paper, Prior et al. demonstrated anthocyanins from blueberries or strawberries reduced the body weight and body fat of rats fed a high-fat diet.[27] Anthocyanin-rich food reduced efficiently hyperglycemia in type 2 diabetic mice via inducing AMPK and PPARα expression in the liver and then resulted in significantly decreased liver and serum lipid contents.[28] Furthermore, MWE

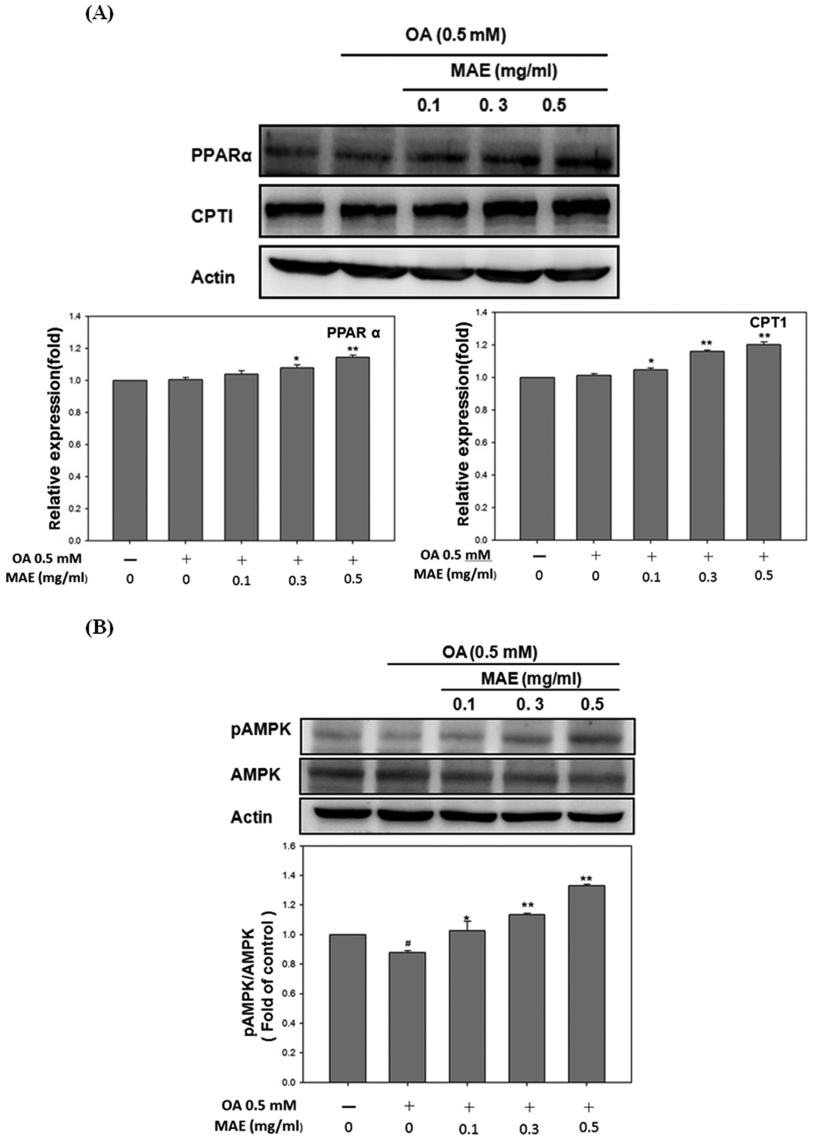

Figure 5. MAE treatment increases PPARα and CPT1 (A) and pAMPK/AMPK protein (B) expression. HepG2 cells were cotreated OA at indicated concentrations of MAE (0.1, 0.3, 0.5 mg/mL) for 24 h. Proteins were detected by Western blot analysis and quantified by densitometric analysis. The results from three independent experiments are expressed as the mean ± SD. (∗) $P < 0.5$, compared with OA-induced group; (∗∗) $P < 0.05$, compared with OA-induced group; (#) $P < 0.05$, compared with control group.

reduced HepG2 cellular lipid accumulation by increasing AMPK phosphorylation, thereby inhibiting the expression of SREBP-1, FAS, and ACC and further suppressing TG and cholesterol synthesis.[29] As well, we used lesser doses of MAE compared with MWE in this study.

One of the AMPK downstream targets, ACC, is an important enzyme for synthesis of malonyl-CoA, which inhibits CPT1, a transporter of long-chain fatty acyl groups into the mitochondria to undergo β-oxidation.[30] Other papers have also suggested that suppression of ACC by AMPK phosphorylation leads to a fall in malonyl-CoA content and a subsequent decrease in fatty acid synthesis and increase in fatty acid oxidation through the regulation of CPT1.[30] In our study, MAE treatments increased CPT1 expression and AMPK phosphorylation and decreased ACC protein expression (Figures 3 and 5). These data suggested the activation of AMPK by MAE inhibited the protein expression of ACC, increased CPT1 levels, and stimulated fatty acid oxidation.

Lipid accumulation in the liver may be caused by enhanced lipogenesis, activation of lipid uptake, and lowering of lipid catabolism. FAS and GPAT are key enzymes in de novo fatty acid and TG synthesis, which are known to be regulated by SREBP-1 in mammals.[31,32] Dietary studies with normal, transgenic, and knockout mice have established SREBP-1 as a dominant transcription factor regulating gene expression of lipogenic enzyme in the liver.[33] In this study, expressions of FAS, GPAT, and SREBP-1 were changed by cotreatment with OA and MAE (Figures 3 and 4). These results revealed that the effect of MAE on lipid reduction was associated with decreased expression of SREBP-1 and its downstream lipogenic genes. Activation of AMPK by metformin or an adenosine analogue suppresses expression of SREBP-1, a key lipogenic transcription factor in the liver.[34] Therefore, these data support the ability of MAE to suppress FAS expression through AMPK activation and suppression of SREBP-1 in HepG2 cells.

PPARα is expressed at high levels in the liver, kidney, and heart and has been implicated to play an important role in

dx.doi.org/10.1021/jf401171k | J. Agric. Food Chem. 2013, 61, 6069−6076

obesity-related metabolic diseases.[35,36] It is a nuclear fatty acid receptor and coordinates transcriptional activation of peroxisomal fatty CPT1.[37] Moreover, PPARα promotes mitochondrial fatty acid uptake and oxidation.[38] In this study, MAE significantly increased the expression of PPARα and CPT1, affecting fatty acid β-oxidation in HepG2 cells (Figure 5A). Several studies have indicated hypolipidemic effects also occur.[39,40] We presume MAE, containing anthocyanins, maybe enable lipid-lowering effects.[22,29,41]

Overall, we provide evidence MAE likely plays a significant role in reducing HepG2 cellular lipid accumulation by increasing AMPK phosphorylation, thereby inhibiting expression of SREBP-1, FAS, and ACC and further suppressing TG and cholesterol synthesis. Furthermore, MAE also stimulates lipolysis in HepG2 cells. Our results provide insight into how MAE affects lipid metabolism in vitro. These findings might be beneficial in informing various treatment strategies for nonalcoholic fatty liver disease in the future.

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Author Contributions

‖Y.-C.C. and C.-J.W. contributed equally to this study, thus sharing the corresponding authorship.

Funding

Funding for this research was provided by the National Science Council, Taiwan, under Grant NSC 101-2622-B-040-001-CC2.

Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

The protein expression pAMPK/AMPK in Figure 5b were reversed in the version of this paper published June 17, 2013. The correct version published June 26, 2013.