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Licochalcone A Prevents Adipocyte Differentiation and Lipogenesis via Suppression of Peroxisome Proliferator-Activated Receptor γ and Sterol Regulatory Element-Binding Protein Pathways

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ABSTRACT: Licochalcone A (LA) has been shown to exert multiple pharmacological effects, including anti-inflammatory, antiparasitic, antifungal, anticancer, and osteogenic activities. The present study investigated the ability of LA to suppress the differentiation of 3T3-L1 preadipocytes, and its antiobesity activity was explored using high fat diet (HFD)-fed ICR mice. During the terminal differentiation process, 3T3-L1 preadipocytes were treated with LA, and the lipid contents were quantified along with any changes in the expression of biomarkers associated with adipocyte differentiation and lipogenesis. The results show that LA significantly reduced lipid accumulation and down-regulated the expression of peroxisome proliferator-activated receptor γ, CCAAT/enhancer binding protein α, sterol regulatory element-binding protein 1c, and their target genes (fatty acid binding protein, fatty acid synthase, stearoyl-CoA desaturase 1, and glycerol-3-phosphate acyltransferase). In an animal study, body weight, triglyceride, cholesterol, and nonesterified fatty acid levels in the group given 10 mg/kg LA were significantly decreased by 14.0, 48.2, 58.9, and 73.5%, respectively. Transverse microcomputed tomography indicated that visceral fat depots in LA-treated mice were markedly reduced when compared with those of the HFD control group. In summary, these results suggest that LA exerts an antiobesity effect and that it is a candidate for future clinical trials.

KEYWORDS: *licochalcone A, 3T3-L1 preadipocytes, adipocyte differentiation, peroxisome proliferator-activated receptor γ, CCAAT/enhancer binding protein α*

INTRODUCTION

The International Obesity Task Force estimates that at least 1.1 billion adults worldwide are overweight, including 312 million who are obese.[1] By 2015, these numbers are projected to increase to 2.3 billion and over 700 million, respectively.[2] Additionally, 15% of children are currently estimated to be obese, and this percentage continues to grow. Obesity is a dreadful but preventable disease associated with a number of ailments, such as type 2 diabetes, hypertension, dyslipidemia, coronary heart disease, gallbladder disease, and some cancers.[3]

Current strategies for the management of obesity include diet, exercise, and behavior modification. Managing obesity with diet and exercise alone, while successful in the short term, is not successful over the long-term for the majority of patients, as any weight loss is difficult to maintain. Research has shown that only 20% of overweight individuals are successful at long-term weight loss when success is defined as losing at least 10% of the initial body weight and maintaining that loss for at least 1 year. Given the limitations of changing diet alone, medications and alternative treatment options have been sought. Currently, there are only two classes of drugs on the market. The first class consists of sympathomimetic drugs including phentermine, diethylproprion, benzphetamine, and phendimetrazine, which are approved only for short-term use. The second class is the pancreatic lipase inhibitor orlistat. In the past, there have been additional weight-loss drugs on the market, but these have been removed because of concerns about their side effects and the associated risks that outweigh their benefits. For example, sibutramine was withdrawn from the market in the European Union in January 2010 because of an increased risk of heart problems.[4] Therefore, the need for additional safe and effective drug therapies is urgent, and there is growing interest in herbal remedies because such remedies have minimal or no side effects.

Licochalcone A (LA) is a major phenolic constituent of the *Glycyrrhiza* plant, of which the root is commonly called licorice,[5] and is also found in *Brassica rapa* L., which is a widely cultivated plant that has a large edible white or yellow root, commonly known as a turnip. LA has been shown to have osteogenic,[6] anti-inflammatory,[7,8] antiparasitic,[9] antifungal,[10] anticancer,[11,12] and antihyperglycemic[13] activities. In this study, we investigated whether LA has an inhibitory effect on adipocyte differentiation and lipogenesis in 3T3-L1 preadipocytes. We also examined the antiobesity effect of LA using ICR mice fed a high-fat diet.

MATERIALS AND METHODS

Materials. Licochalcone A was isolated from *B. rapa* L. and kindly supplied by Prof. N. I. Baek of Kyung Hee University (Yongin, Korea). Antibodies against peroxisome proliferator-activated receptor γ (PPAR-γ), CCAAT/enhancer binding protein α (C/EBPα), and actin were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

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Table 1. Composition of the Experimental Diets

	RD 10% kcal		HFD 45% kcal	
	g %	kcal %	g %	kcal %
protein	19.2	20	24	20
carbohydrate	67.3	70	41	35
fat	4.3	10	24	45
total		100		100
kcal/g	3.85		4.73	
ingredient	**g**	**kcal**	**g**	**kcal**
casein, 80 mesh	200	800	200	800
L-cystine	3	12	3	12
corn starch	315	1260	72.8	291
maltodextrin 10	35	140	100	400
sucrose	350	1400	172.8	691
cellulose, BW 200	50	0	50	0
soybean oil	25	225	25	225
lard	20	180	177.5	1598
mineral mix S10026	10	0	10	0
dicalcium phosphate	13	0	13	0
calcium carbonate	5.5	0	5.5	0
potassium citrate	16.5	0	16.5	0
vitamin mix V10001	10	40	10	40
choline bitartrate	2	0	2	0
FD&C yellow dye #5 (RD) red dye #40 (HFD)	0.05	0	0.05	0
total	**1055.05**	**4057**	**858.15**	**4057**

Reverse transcriptase and Taq polymerase were supplied by Promega (Mannheim, Germany). Protein extraction, EASY BLUE total RNA extraction, and ECL reagent kits were from Intron Biotechnology Inc. (Beverly, MA, USA). Other reagents and chemicals were of the highest grade commercially available.

Cell Viability Assay and Adipocyte Differentiation. Mouse embryonic fibroblast 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). To determine the cytotoxicity of LA, preadipocytes (5×10^3 per 96 well) were incubated in the presence of LA. After 48 and 96 h, 20 μL of MTS solution was added to each well and incubated for 30 min, and then the absorbance at 450 nm was measured using a microplate reader. 3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum and 100 units/mL penicillin−streptomycin at 37 °C in an atmosphere of 5% CO_2. To induce differentiation, 2 days after confluence (designated day 0), preadipocytes were placed in differentiation medium (DM) consisting of 5% FBS, 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, and 10 μg/mL insulin for 4 days, switched to post-DM containing 5% FBS and 10 μg/mL insulin on day 6, and then changed to 5% FBS medium, which was then refreshed every other day. During differentiation, 3T3-L1 cells were treated with 5 or 10 μM of LA from day 0 to day 4.

Oil Red O Staining. After 8 days of adipocyte differentiation, the cells were washed with phosphate-buffered saline (PBS) twice, fixed with 10% formalin for 1 h, and then stained with Oil Red O (Sigma, St. Louis, MO, USA) for 2 h at room temperature. Cells were photographed using a phase-contrast Olympus CKX41 microscope (Tokyo, Japan) in combination with a digital camera at 100× magnification. The stained lipid droplets were dissolved in isopropanol and quantified at 540 nm.

Animal Treatment and Serum Chemistry Analysis. The animal experiment protocol was reviewed and approved by the Institutional Animal Ethics Committee of Kyung Hee University. Five-week-old ICR mice (Orient Bio Inc., Seongnam, Korea) were housed in a temperature- (22 ± 2 °C) and humidity-controlled (50 ± 5%) room with a cycle of 12 h light/12 h darkness and free access to food and water. Mice were randomly divided into the following four groups: the regular diet fed group (RD), the high fat diet fed group (HFD),

Table 2. RT-PCR and Real-Time PCR Primer Sequences and Annealing Temperatures

gene	forward primer	reverse primer	annealing temp (°C)
SREBP 1c	GCGCTACCGGTCTTCTATCA	TGCTGCCAAAAGACAAGGG	58
	GCAGTCTGCTTTGGAACCTC	CCTCCTGTGTACTTGCCCAT	60
SCD1	CGAGGGTTGGTTGTTGATCTG	ATAGCACTGTTGGCCCTGGA	56
	GCGATACACTCTGGTGCTCA	CTGGCAGAGTCGAAGGG	60
FAS	GATCCTGGAACGAGAACAC	AGACTGTGGAACACGGTGGT	50
	CCCTTGATGAAGAGGGATCA	ACTCCACAGGTGGGAACAAG	60
GPAT	GGTAGTGGATACTCTGTCGTCCA	CAGCAACATCATTCGGT	58
	CCAGCCTGTGCTACCTTCTC	GAAGCTTCTTGTCCCACTGC	60
aP2	TCTCACCTGGCCTCTTCCTTTGGCTC	TTCCATCCAGGCCTCTTCCTTTGGCTC	55
	TCACCTGGAAGACAGCTCCT	AAGCCCACTCCCACTTCTTT	60
PPAR-γ	AGGCCGAGAAGGAGAAGCTGTTC	TGGCCACCTCTTTGCTCTGCTC	55
	CTTGTGAAGGATGCAAGGGT	GGATCCGGCAGTTAAGATCA	60
C/EBPα	GGGTGAGTTCATGGAGAATGG	CAGTTTGGCAAGAATCAGAGCA	50
	ATCCCAGAGGGACTGGAGTT	AGCATAGACGTGCACACTGC	60
CPT1	CCTGGGCATGATTGCAAG	ACAGACTCCAGGTACCTGCTCAC	60
	CATGTCAAGCCAGACGAAGA	TGGTAGGAGAGCAGCACCTT	60
actin	GGACTCCTATGGTGGGTGACGAGG	GGGAGAGCATAGCCCTCGTAGAT	58
	TGACAGGATGCAGAAGGAGA	CGCTCAGGAGGAGCAATG	60

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and two treatment groups fed a high fat diet plus LA at 5 mg/kg (LA5) or 10 mg/kg (LA10) (Table 1). The body weight was measured twice per week. After 3 weeks of treatment with LA, mice were anesthetized with diethyl ether after an overnight fast. Blood was collected from the heart aorta, and the abdominal and epididymal fat pads were removed, weighed, and frozen with liquid nitrogen. The plasma concentrations of triglycerides (TG), cholesterol, nonesterified fatty acids (NEFA), glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined using commercial kits (Stanbio Laboratory, Boerne, TX, USA) and an automatic analyzer (SMAR-TLAB, Mannheim, Germany). The plasma insulin concentration was determined using a mouse insulin immunoassay kit (Shibayagi, Gunma, Japan).

Microcomputed Tomography (CT) and Histological Analysis. For in vivo scans, mice were anesthetized by 1% isoflurane inhalation and positioned with both legs fully extended.[14,15] Micro-CT was carried out with a high-resolution Skyscan1076 scanner (SKYSCAN, Belgium) using a resolution pixel size of 35 μm, an exposure time of 400 ms, an energy source of 40 kV, and a current of 250 μA. Approximately 180 projections were acquired over a rotation range of 360°, with a rotation step of 0.6°. Scans were reconstructed for the abdominal region (between the proximal end of L1 and the distal end of L5). Transverse micro-CT and 3D images were analyzed using the CTAn and CTVol programs (SKYSCAN). For the histomorphometric analysis, the epididymal fat pads were fixed in 10% phosphate-buffered formalin acetate at 4 °C overnight and embedded in paraffin wax. The paraffin-embedded sections were cut at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) for microscopic assessment (Olympus, Tokyo, Japan).

Western Blot Analysis. To detect proteins from whole cell lysates, cells were washed with ice-cold PBS and broken down using a protein extraction kit. Insoluble protein was removed by centrifugation at 13000 rpm for 20 min. The protein concentration of the cell lysates was measured using a Bio-Rad protein assay kit (Hercules, CA, USA). To determine the levels of PPAR-γ and CEBPα protein expression in epididymal fat, the epididymal fat pads were removed and sonicated for 30 s, and then the protein concentration was measured as described above. Equal amounts of protein (40 μg/lane) were resolved by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene fluoride membrane (Millipore, Beverly, MA, USA), and hybridized with primary antibodies (diluted 1:1000) overnight at 4 °C. After incubation with a horseradish peroxidase-conjugated secondary antibody (diluted 1:2000) for 2 h at room temperature, protein bands were detected using an enhanced chemiluminescence Western blot detection kit (Amersham, Uppsala, Sweden), exposed to X-ray film, and then quantified by densitometric analysis.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Real-Time PCR. To determine the levels of gene expression of PPAR-γ, C/EBPα, aP2, sterol regulatory element-binding protein 1c (SREBP1c), stearoyl-coenzyme desaturase 1 (SCD1), fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase (GPAT), and carnitine palmitoyltransferase 1 (CPT1) in adipocytes and adipose tissue, RT-PCR and real-time PCR techniques were utilized. The total RNA was isolated from differentiated 3T3-L1 cells and epididymal adipose tissue using the EASY-BLUE total RNA extraction kit according to the manufacturer's instructions. For RT-PCR, single-strand cDNA synthesis was performed using 5 μg of RNA, oligo (15) dT primers, and reverse transcriptase in a total reaction volume of 50 μL. PCR reactions were performed in a total volume of 20 μL consisting of 2 μL of the cDNA product, 0.2 mM of each dNTP, 20 pmol of each primer, and 0.8 unit of Taq polymerase. PCR was performed at 95 °C for 30 s, followed by annealing for 30 s, and 72 °C for 1 min. The last cycle was followed by a final extension step at 72 °C for 10 min. The RT-PCR products were electrophoresed in 1% agarose gels at 100 V and stained with 0.5 μg/mL ethidium bromide. Real-time PCR was performed using 1 μL of cDNA in a 20 μL reaction volume with the LightCycler real-time PCR System (Roche Applied Science, Indianapolis, IN, USA). The double-stranded DNA-specific dye SYBR Green I was incorporated into the PCR buffer provided in the SYBR Premix Ex Taq reagent. The temperature profile of the reaction was 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and an extension at 72 °C for 1 min. The actin gene was used to correct for differences in RNA isolation, RNA degradation, and the efficiency of the reverse transcription. The annealing temperatures for both procedures are shown in Table 2.

Statistical Analysis. All data are expressed as the mean ± standard error (SE). Comparisons between groups were made using an ANOVA, and the significance was determined by Tukey's test. Differences of $p < 0.05$ were considered to be statistically significant.

RESULTS

LA Inhibits Adipocyte Differentiation and Lipogenesis via Down-regulation of Transcription Factors. First, we investigated the effect of LA on the proliferation of 3T3-L1 cells using the MTS assay. The growth profiles observed on the second and fourth days of culture in the presence of LA were similar to that of the control, but treatment with more than 20 μM LA resulted in cytotoxicity (data not shown). Therefore, 5 and 10 μM LA were employed in this study. To examine the effect of LA on the differentiation of preadipocytes into adipocytes, confluent 3T3-L1 preadipocytes were treated with

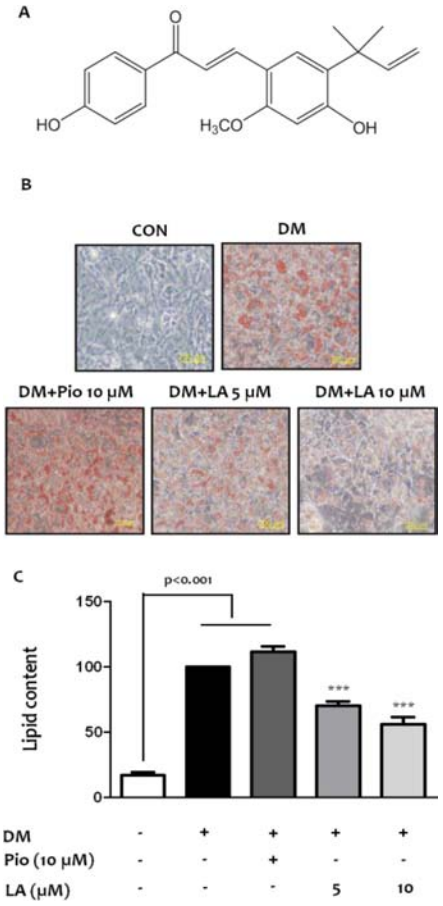

Figure 1. (A) Chemical structure of licochalcone A. (B) Effects of LA on 3T3-L1 preadipocyte differentiation. 3T3-L1 preadipocytes were treated with LA as indicated during differentiation. (C) On day 8, cells were stained with Oil Red O, and the lipid contents were quantified spectrophotometrically at 540 nm. Pio represents pioglitazone, a PPAR-γ agonist inducing adipocyte differentiation. Each bar represents the mean ± SE of three independent experiments. ***, $p < 0.001$, compared to cells in differentiation media (DM).

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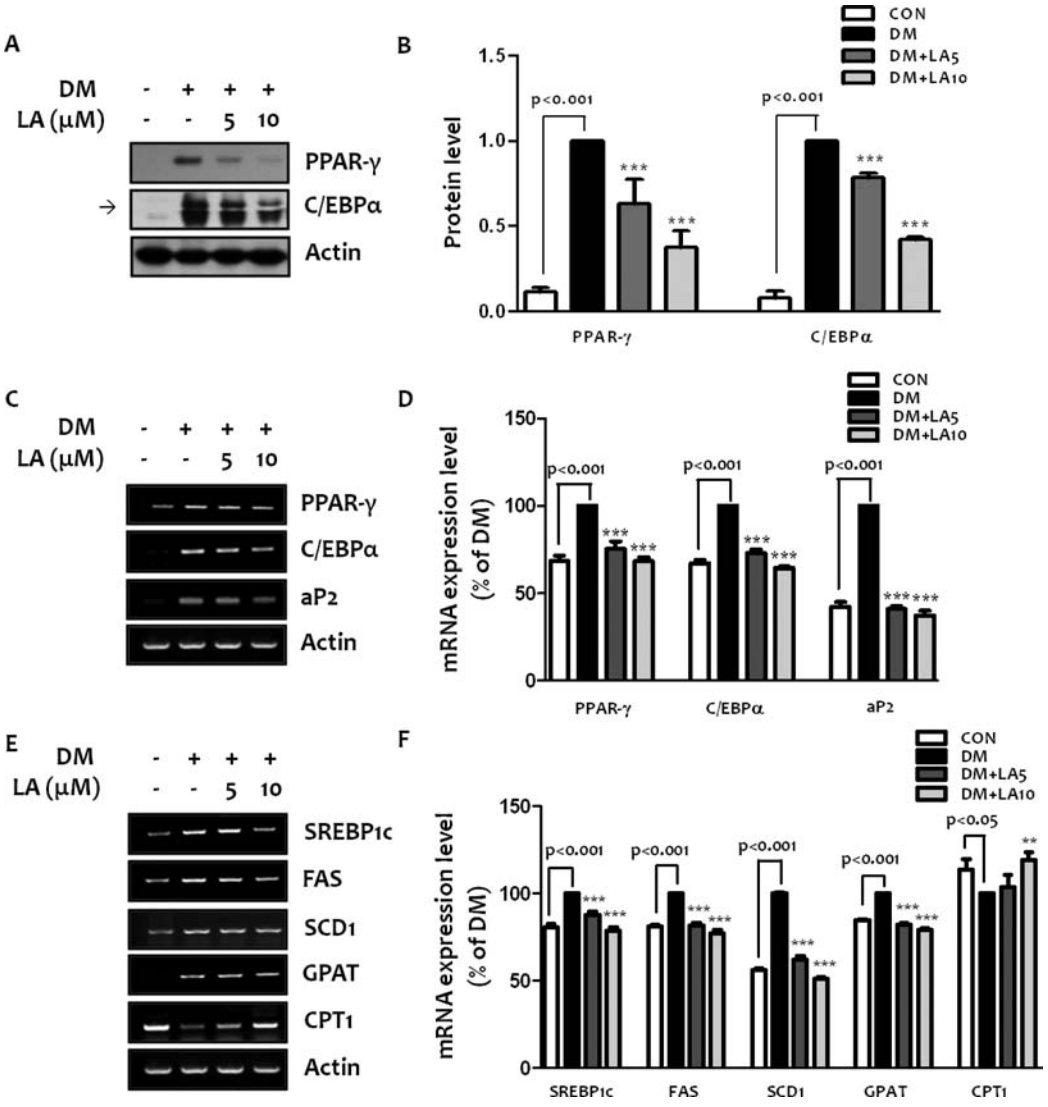

Figure 2. Effects of LA on the protein and gene expressions in 3T3-L1 preadipocytes. 3T3-L1 preadipocytes were differentiated in the presence of LA (0, 5, 10 μM) for 8 days. (A, B) Protein expressions of PPAR-γ and C/EBPα were determined by Western blot as described under Materials and Methods. (C, E) mRNA levels of PPAR-γ, C/EBPα, SREBP1c, and its target molecules (aP2, FAS, SCD1, GPAT, CPT1) were estimated by RT-PCR. (D, F) Transcripts of these genes were quantified by real-time PCR and normalized to actin. Data represent the mean ± SEM. **, $p < 0.01$, and ***, $p < 0.001$, compared to cells in differentiation media (DM).

the indicated concentrations of LA for 4 days. On the eighth day of culture, lipid accumulation was examined by Oil Red O staining as a marker of differentiation. The triglycerides presented in fully differentiated adipocytes should be highly stained with the Oil Red O solution, and the lipid contents were measured at 540 nm. We found that the level of triglycerides decreased in a dose-dependent manner (Figure 1B,C). To elucidate the mechanism of action of LA, the protein and gene expression levels of transcription factors for preadipocyte differentiation, such as PPAR-γ and C/EBPα, were examined using Western blot and PCR techniques, respectively. The protein and gene expression levels of both of these factors were significantly reduced in a dose-dependent manner (Figure 2A–D). The gene expression of aP2, a target molecule of PPAR-γ, was also markedly suppressed (Figure 2C,D). We also determined the gene expression levels of SREBP1c, a key transcription factor for TG biosynthesis, and its target enzymes (FAS, SCD1, and GPAT) using RT-PCR and real-time PCR techniques. As shown in Figure 2E, the gene expression levels of these proteins were significantly reduced in a dose-dependent manner, and these results were reaffirmed by our real-time PCR experiments (Figure 2F). In contrast, the gene expression level of CPT1, a rate-limiting enzyme for fatty acid β-oxidation, was markedly up-regulated and reached the level of vehicle-treated cells (CON) when compared with that of cells treated with 10 μM LA (Figure 2E,F).

Effects of LA on Body Weight and Metabolic Parameters. To investigate the antiobesity effect of LA, ICR mice were simultaneously fed HFD and LA for a 3 week period, and their body weight was measured twice a week. Compared with the RD group, the body weight of the HFD control group increased by 10.0%. However, the final body weights of LA5 and LA10 groups were significantly lowered by 12.7 and 14.0%, respectively, when compared with the HFD control group (Figure 3A). The abdominal and epididymal fat weights decreased in the LA-treated groups when compared with those in the HFD control group, although the difference was not statistically significant (Figure 3B). We also examined the

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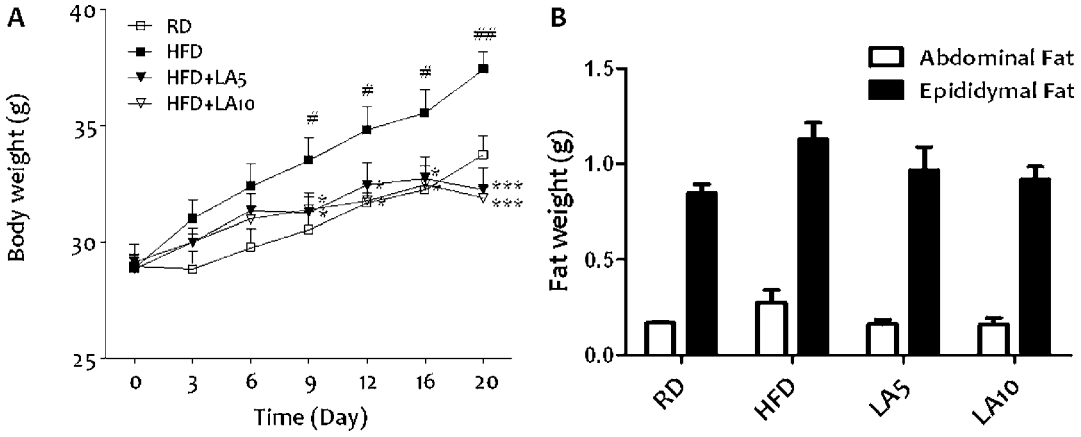

Figure 3. ICR mice were fed either a regular diet (RD) or high-fat diet (HFD) for 3 weeks with (5, 10 mg/kg/day) or without LA (n = 10): (A) effect of LA on body weight change during the 3 week period; (B) comparison of abdominal and epididymal fat weights between groups. Each bar represents the mean ± SEM from 10 mice. HFD+LA5, HFD fed 5 mg/kg of LA; HFD+LA10, HFD fed 10 mg/kg of LA. #, $p < 0.05$ vs RD, and ##, $p < 0.01$ vs RD. ∗, $p < 0.05$ vs HFD, and ∗∗∗, $p < 0.001$ vs HFD.

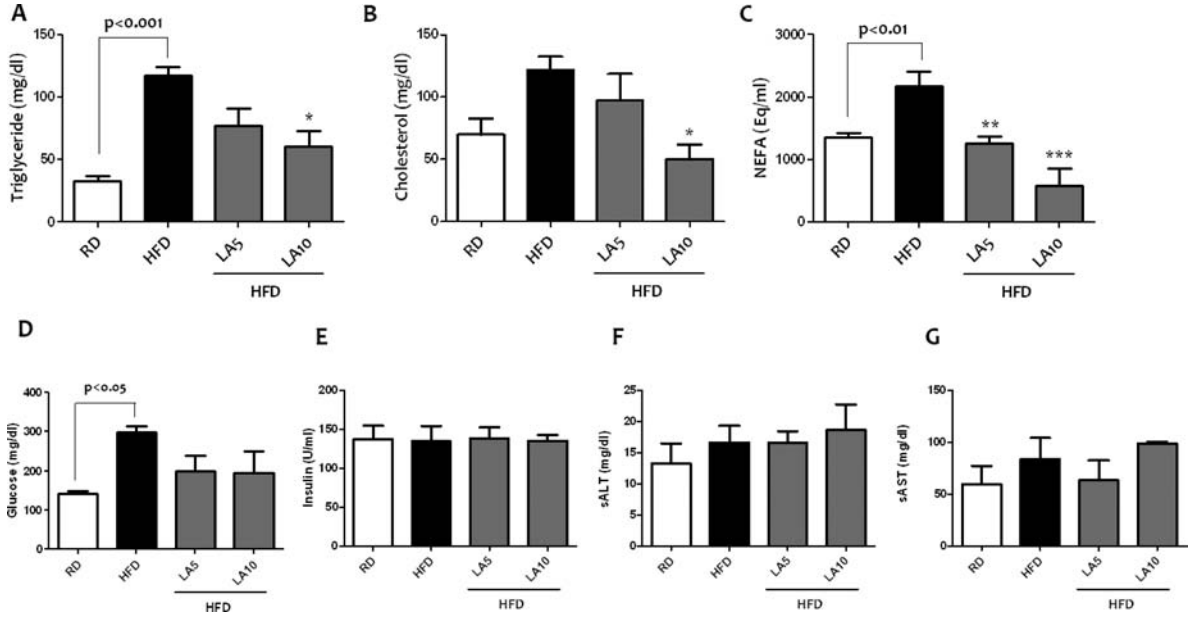

Figure 4. At the end of the treatment, plasma was obtained after an overnight fasting from RD or HFD mice treated with either vehicle or LA as described previously. The plasma concentrations of triglyceride (A), cholesterol (B), NEFA (C), glucose (D), insulin (E), ALT (F), and AST (G) were compared between groups. Each bar represents the mean ± SEM from 10 mice. ∗, $p < 0.05$ vs HFD, ∗∗, $p < 0.01$ vs HFD, and ∗∗∗, $p < 0.001$ vs HFD.

effects of LA on metabolic parameters in plasma at the end of the treatment. The triglyceride, cholesterol, and NEFA levels were increased by 3.6-, 1.7-, and 1.6-fold, respectively, in the HFD control group compared with those in the RD group. However, the LA5 and LA10 groups, respectively, showed 34.1 and 48.2% reductions in triglyceride, 20.1 and 58.9% reductions in cholesterol, and 42.1 and 73.5% reductions in NEFA (Figure 4A−C). The blood glucose level also increased by 2.11-fold in the HFD control group compared with that of the RD group, and the LA-treated groups showed some antihyperglycemic effects (Figure 4D). There were no significant differences in the plasma insulin, ALT, and AST levels between groups (Figure 4E−G).

Transverse Micro-CT of a Mouse Abdomen and Epididymal Fat Morphology. To evaluate the potential efficacy of LA as an antiobesity agent, the precise quantification of adipose tissue was determined by a high-resolution micro-CT scanner. The visceral (region 2 of Figure 5A) and subcutaneous (region 1 of Figure 5A) fat volumes of a representative HFD control mouse increased by 2.5- and 2.3-fold, respectively, compared with those of a mouse fed the regular diet. However, the visceral fat volumes of mice treated with 5 and 10 mg/kg LA were decreased by 62.3 and 81.5%, respectively, compared with that of the HFD control mouse. The subcutaneous fat volumes of LA-treated mice were also decreased by 50.1 and 72.8% when compared with that of HFD control mice. The visceral regions, shown in yellow in the 3D image of the micro-CT, largely disappeared in the LA-treated groups (lower panel of Figure 5A), and there were some differences in volume of the subcutaneous adipose tissue (blue region) between the HFD control group and the LA-treated groups. To validate the micro-CT image, a morphometric analysis of the epididymal fat pad was performed by H&E staining. As shown in Figure 5B, the number of enlarged

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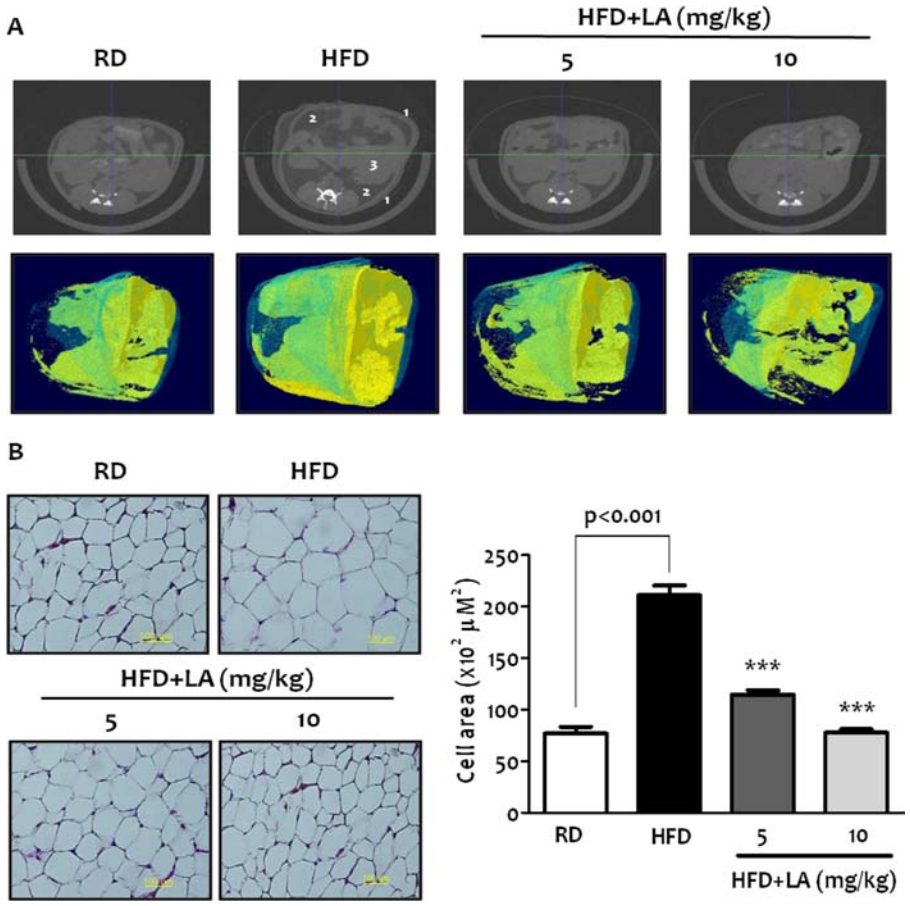

Figure 5. (A) Transverse micro-CT (upper panels) and 3D images (lower panels) of a mouse abdomen at the level of the L5-L1 intervertebral disk. Regions 1, 2, and 3 in the upper panel denote subcutaneous fat, visceral fat, and lean tissue, respectively. In the 3D picture, the visceral and subcutaneous fat depots are shown in yellow and blue, respectively. (B) The epididymal fat pad removed from each group was stained with hematoxylin and eosin. Magnification 200×.

adipocytes shown in the HFD control mouse significantly decreased in a dose-dependent manner.

Effects of LA on Transcription Factors for Lipogenesis in Epididymal Fat Tissue. Parallel to the in vitro assay, we examined the protein and gene expression of targets responsible for adipocyte differentiation and lipogenesis in the epididymal fat tissue. The protein and gene expression of transcription factors and their target molecules involved in adipocyte differentiation (PPAR-γ and C/EBPα) were all up-regulated in HFD control mice when compared with those in the RD group. However, the protein and gene expression levels of these molecules in the LA-treated mice were significantly suppressed, and the levels of the LA10 group resembled those of the RD group (Figure 6A–D). The expression of genes responsible for lipogenesis (SREBP1c and its target enzymes) was also markedly attenuated in the LA-treated groups (Figure 6E,F). CPT1 was the only gene to be up-regulated when the mice were treated with LA for a 3 weeks.

■ DISCUSSION

Obesity is defined as the phenotypic manifestation of abnormal or excessive fat accumulation that alters health and increases mortality. In terms of anatomy, we usually refer to the distribution of the body fat deposition. The ratio of waist-to-hip circumference measures the degree of central (visceral, abdominal) versus peripheral (subcutaneous) adiposity. Visceral fat is a major risk for metabolic disorders such as diabetes, hypertension, and cardiovascular diseases, whereas peripheral fat appears to be benign to metabolic complications.[16] Global strategies are focused on dietary and lifestyle modifications, that is, restricting caloric intake and increasing physical activity to slow the development of obesity.[17] A field of food research that has recently aroused considerable interest is the potential of natural products to counteract obesity.[18,19] The antiobesity effects of these compounds are mediated by affecting the regulation of various pathways, including lipid absorption, the intake and expenditure of energy, and the differentiation and proliferation of preadipocytes as well as increasing lipolysis and decreasing lipogenesis.[20]

Chalcones chemically consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system, and they have been described as powerful anti-inflammatory, antioxidant, and anticancer agents.[21] Chalcones have also been reported to have antiestrogenic, anti-infective, antiproliferative, antimicrobial, and antimitotic properties.[21] Dihydrochalcone phlorizin, which is currently used in the treatment of diabetes mellitus and obesity, belongs to this family of compounds.[22,23] Its principal pharmacological action is to produce renal glycosuria and to block intestinal glucose absorption through nonspecific inhibition of the sodium-glucose symporters located in the proximal renal tubule and mucosa of the small intestine.[22] Another member of the chalcone family, licochalcone A (LA; $C_{21}H_{22}O_4$, MW 338.4, Figure 1A), is the primary active

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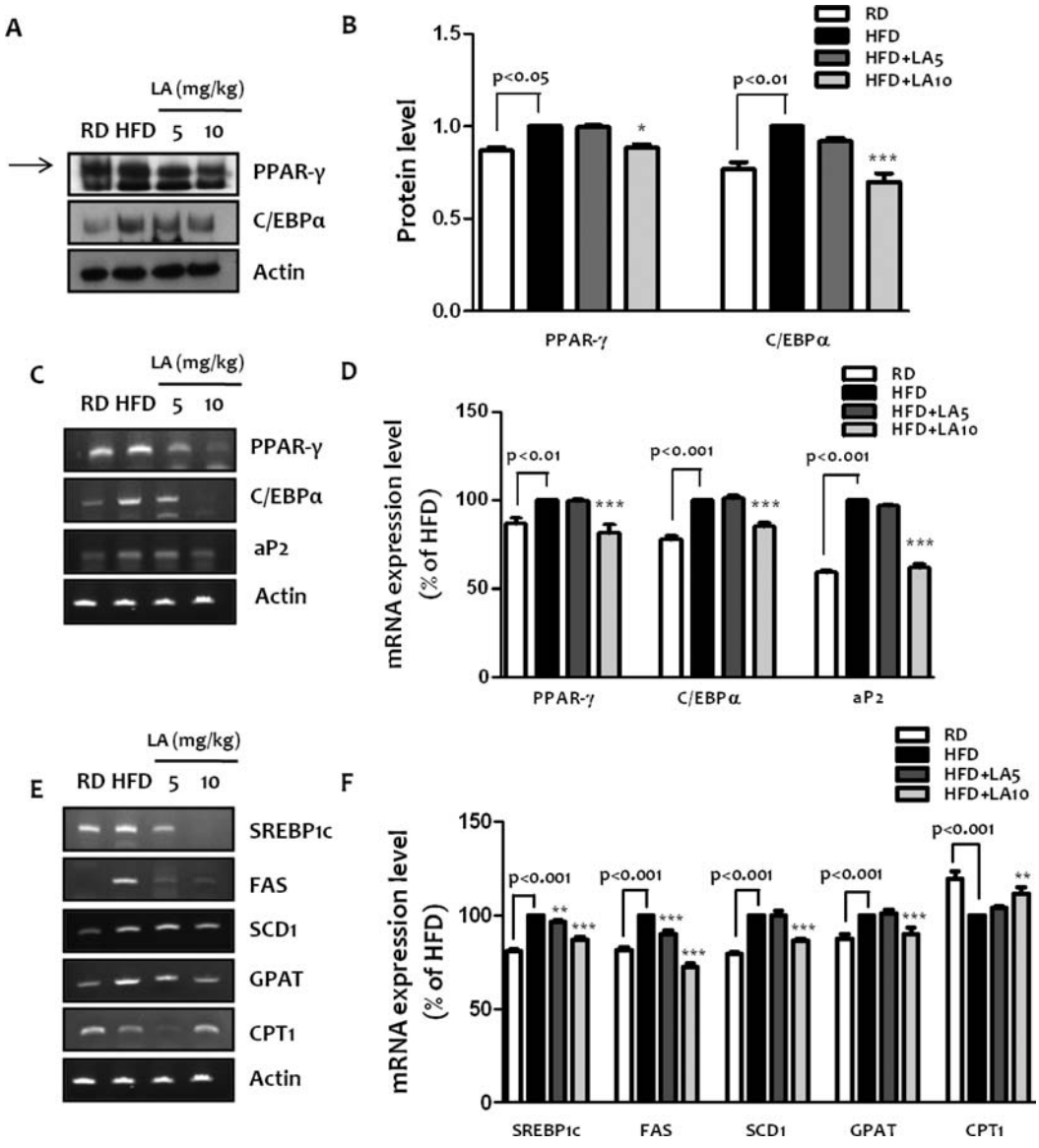

Figure 6. Effects of LA on the protein and gene expressions in the epididymal fat. (A, B) Protein expressions of PPAR-γ and C/EBPα were determined by Western blot as described under Materials and Methods. (C, E) mRNA levels of PPAR-γ, C/EBPα, SREBP1c, and their target molecules (aP2, FAS, SCD1, GPAT, CPT1) were estimated by RT-PCR. (D, F) Transcripts of these genes were quantified by real-time PCR and normalized to actin. Data represent the mean ± SEM. *, $p < 0.05$ vs HFD, **, $p < 0.01$ vs HFD, and ***, $p < 0.001$ vs HFD.

compound of *Glycyrrhiza inflata* and *Brassica rapa* L. Although LA has been shown to have multiple pharmacological effects such as anti-inflammatory, antitumor, antifungal, and antiparasitic properties,[7−12] its antiobesity effect has not been yet reported. Here, we investigated the ability of LA to suppress differentiation in 3T3-L1 preadipocytes, and the antiobesity activity of LA was further explored using high fat diet-induced obese ICR mice.

It is worth mentioning that obesity usually begins before the appearance of hyperglycemia, elevated blood pressure, and dyslipidemia. Therefore, prevention of obesity is an international public health priority given the significant impact of obesity on acute and chronic diseases, general health, development, and well-being.[24,25] In this context, LA might be a potential preventive agent for obesity, because LA prevented the weight gain of >10% when high fat diet-fed mice were administered LA for 3 weeks (Figure 3A). The abdominal and epididymal fat masses were also apparently reduced (Figure 3B), and these results were confirmed using transverse micro-CT imaging (Figure 5A). To evaluate the potential efficacy of LA, the precise quantification of adipose tissue is critical. The body composition and spatial stratification into visceral and subcutaneous adipose tissue (VAT, SAT) can be determined by noninvasive three-dimensional micro-CT. VAT is more closely correlated with obesity-associated pathologies and complications than either total adipose tissue or SAT.[26,27] Not surprisingly, removing SAT from the abdominal area through liposuction does not provide the health benefits that would be expected from the reduction in fat mass.[28] However, VAT is not the only factor contributing to the metabolic syndrome, and high levels of SAT have been associated with many metabolic risk factors, including aberrant levels of fasting plasma insulin, triglycerides, low-density lipoprotein, and cholesterol.[29,30] By using the CTAn and CTVol programs, we were able to analyze the geometry and volume of VAT and SAT (Figure 5A). The total fat volume

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(visceral and subcutaneous fat) of a representative HFD control mouse increased by 2.48-fold compared with that of mouse fed the regular diet. However, the total fat volume of mice treated with 5 and 10 mg/kg of LA decreased by 60 and 80%, respectively, compared with that of the HFD control mouse. Hypertrophy (large adipocytes) has been found to be strongly correlated with diet, whereas hyperplasia (many small adipocytes) is dependent on genetics.[31] In this study, a high-fat diet caused the hypertrophy of adipocytes, and treatment with LA reduced the hypertrophy in a dose-dependent manner (Figure 5B).

The differentiation process during which preadipocytes mature into adipocytes involves the following four stages: growth arrest, clonal expansion, early differentiation, and terminal differentiation. These stages are orchestrated by a transcriptional network involving the nuclear receptor PPAR-γ and members of the C/EBP family and a controversial proposed role for SREBP1c. SREBP1c is now believed to be an insulin-modulated transcription factor that is involved in the regulation of genes associated with triglyceride metabolism rather than adipogenesis. In contrast, PPAR-γ appears to be central to adipocyte differentiation and in the maintenance of the terminal differentiation state. During adipogenesis, the early expression of C/EBPβ promotes the expression of C/EBPα and PPAR-γ. C/EBPα acts together with PPAR-γ to maintain the differentiated phenotype.[32] In the present study, we found that LA significantly down-regulated transcription factors, including PPAR-γ and C/EBPα, and their downstream adipocyte-specific targets, such as aP2, in 3T3-L1 adipocytes and HFD-fed ICR mice (Figures 2A–D and 6A–D). We also found that LA suppressed the gene expression of SREBP1c, a transcription factor involved in lipogenesis, and its target enzymes, such as FAS, SCD1, and GPAT, in both cell and animal studies (Figures 2E,F and 6E,F). Another strategy to prevent or treat obesity and insulin resistance could be the alteration of the fatty acid oxidation pathway. Mitochondrial β-oxidation represents a crucial process in energy metabolism and is tightly regulated by interactions between the key enzymes CPT1 and acetyl-CoA carboxylase (ACC) via the intermediate malonyl-CoA.[33] LA stimulated the gene expression of CPT1 (Figures 2E,F and 6E,F), likely as the result of the indirect inhibition of ACC via AMP-activated protein kinase (AMPK) or the activation of nuclear receptor PPAR-α. LA did not activate AMPK in this study (data not shown), so the PPAR-α pathway is likely to be involved in the activation of CPT1 in the epididymal fat tissue. The stimulation of CPT1 activity may be an attractive means to accelerate peripheral fatty acid oxidation and hence ameliorate obesity and insulin resistance.

Taken together, our results suggest that licochalcone A prevents adipocyte differentiation and lipogenesis through the down-regulation of PPAR-γ, C/EBPα, and SREBP1c in 3T3-L1 preadipocytes. We have confirmed the antiobesity activity and mechanism of action of licochalcone A in an animal study. Although a clinical trial is required to demonstrate the therapeutic efficacy, licochalcone A holds promise as a therapeutic agent for the prevention and treatment of obesity.

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Notes

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