Andrographolide Prevents High-Fat Diet–Induced Obesity in C57BL/6 Mice by Suppressing the Sterol Regulatory Element-Binding Protein Pathway

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ABSTRACT
Sterol regulatory element-binding proteins (SREBPs) are major transcription factors regulating the expression of genes involved in biosynthesis of cholesterol, fatty acids, and triglycerides. We investigated the effect of the specific SREBP suppressor andrographolide, a natural compound isolated from Andrographis paniculata, on the regulation of SREBP signaling by use of Western blot, reporter gene assay, and quantitative real-time polymerase chain reaction analysis. In addition, the antiobesity effects of andrographolide were evaluated in C57BL/6 mice with high-fat diet (HFD)–induced obesity. Our results showed that andrographolide downregulated the expressions of SREBPs target genes and decreased cellular lipid accumulation in vitro. Further, andrographolide (100 mg/kg per day) attenuated HFD-induced body weight gain and fat accumulation in liver or adipose tissues, and improved serum lipid levels and insulin or glucose sensitivity in HFD-induced obese mice. Andrographolide effectively suppressed the respiratory quotient, energy expenditure, and oxygen consumption, which may have contributed to the decreased body-weight gain of the obese mice fed with a HFD. Consistently, andrographolide regulated SREBP target genes and metabolism-associated genes in liver or brown adipose tissue, which may have directly contributed to the lower lipid levels and enhanced insulin sensitivity. Taken together, our results indicated that andrographolide ameliorated lipid metabolism and improved glucose use in mice with HFD-induced obesity. Andrographolide has potential as a leading compound in the prevention or treatment of obesity and insulin resistance.

Introduction
The prevalence of obesity and associated metabolic disorders is increasing rapidly (James et al., 2004). It is characterized by an excessive increase in adipose tissue mass and dysregulation of lipid metabolism (Després and Lemieux, 2006). Currently, obesity has become a major health problem worldwide, increasing the risk of diabetes (Hwang et al., 2009), cardiovascular disease, and chronic metabolic disease (Festi et al., 2004). The swelling figures of obesity, which are accompanied by rising healthcare costs for obesity-associated diseases, such as diabetes and cardiovascular disease, have put pressure on governments and industry to find an effective solution. In addition to managing obesity and associated metabolic disorders through diet manipulation and exercise, antiobesity agents to improve energy imbalance and lipid metabolism are urgently needed.

It is widely assumed that lipid metabolism is primarily governed by a number of proteins, including sterol regulatory element-binding protein (SREBP), liver X receptor (LXR), and 3-hydroxy-3-methylglutaryl-CoA (Kim and Spiegelman, 1996;...
Seo et al., 2004). In mammals, there are two SREBP genes, SREBP-1 and -2, which express three major SREBP proteins. Most data suggested that the two SREBP-1 isoforms 1a and 1c primarily regulated fatty acid metabolism and that SREBP-2 was the main regulator of cholesterol metabolism (Horton et al., 2002). Recent studies have implicated the SREBP pathway as important in the development of a range of pathologic conditions associated with obesity and the metabolic syndrome, such as liver steatosis and hyperlipidemia (Moon et al., 2012). These discoveries suggest that suppression of SREBP might be an effective strategy to treat obesity and obesity-related metabolic diseases (Xiao and Song, 2013).

A large quantity of natural products oppose SREBP actions and are thus potential therapeutic agents for obesity and associated metabolic disorders. Although the natural products betulin and prunetin, which suppress SREBP actions, have been reported to improve diet-induced obesity in animal models (Tang et al., 2011; Ahn et al., 2013), more antiobesity agents that are safe, effective, and well characterized are needed. Our study screened a library of natural products and identified andrographolide, an active and important labdane diterpenoid isolated from rhizomes of Andrographis panica, as significantly opposing SREBP's signaling. To determine the regulatory mechanism of SREBP signaling, we used andrographolide as an effective molecule for suppression of SREBP and evaluated its molecular mechanism via reporter gene assay, Western blot analysis, and quantitative real-time polymerase chain reaction (qRT-PCR) analysis. In addition, we used a mouse model of high-fat diet (HFD)–induced obesity to evaluate the preventive and therapeutic effects of andrographolide on metabolic disorders.

Materials and Methods

Andrographolide (C20H30O5, molecular weight 350.45, high-pressure liquid chromatography ≥98%) was obtained from the Shanghai R&D Center for Standardization of Traditional Chinese Medicine, Shanghai, People's Republic of China. Human hepatocyte carcinoma Huh-7 and HepG2 cells were purchased from the American Type Culture Collection (Manassas, VA). All other reagents used in this study were obtained commercially.

Generation of Huh-7/SRE-Luc and Reporter Gene Assays. The generation of Huh-7/SRE-Luc cells and the reporter gene assay were performed as previously described elsewhere (Tang et al., 2011). Huh-7/SRE-Luc cells were depleted of sterols by incubation for 16 hours in a medium containing lipoprotein-deficient serum. Then the cells were incubated individually with andrographolide at concentrations of 0.1, 0.3, 1.0, 3.0, and 10.0 μM in 96-well plates. The luciferase activity was measured after 16-hour treatment, and the intensity of luciferase was calculated as the percentage of cholecystokinin octapeptide absorbance as follows: (Absorbance of drug-treated sample/Absorbance of control sample) × 100%.

Determination of Intracellular Triglyceride and Cholesterol Levels. HepG2 cells were cultured (37°C; 5% CO2) in Dulbecco's modified Eagle's medium (5.5 mM d-glucose) supplemented with 10% fetal bovine serum. Cells were passaged and seeded on a 12-well plate; then they were quenched in serum-free Dulbecco's modified Eagle's medium overnight. After serum depletion, HepG2 cells were then incubated in high glucose (30 mM d-glucose) and treated with andrographolide (10 μM) for 24 hours. All cells were homogenized in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton), and protein concentrations in the cell lysates were measured using a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Cell lysates were then mixed with chloroform/methanol (2:1, vol/vol). An aliquot of the organic phase was collected, dried, and resuspended in isopropyl alcohol. The intracellular triglyceride (TG) and total cholesterol (TC) contents were measured by using commercially available test kits (Cayman Chemicals, Ann Arbor, MI). The intracellular lipid contents in HepG2 cells were expressed as micrograms of lipid per milligram of cellular protein.

Animals and Diets. All animals received care according to the institutional guidelines approved by Shanghai University of Traditional Chinese Medicine. Studies were performed on male C57BL/6 mice (4 weeks old) purchased from the SLAC Laboratory (Shanghai, People's Republic of China) and caged individually in a 12:12-hour light/dark cycle, temperature- and humidity-controlled environment. Forty male C57BL/6 mice were divided into five groups (n = 8) for the prevention strategy, and were given normal chow (10% calories from fat, 20% calories from protein, 70% calories from carbohydrate; D12450B, Research Diets, New Brunswick, NJ) or a HFD (60% calories from fat, 20% calories from protein, 20% calories from carbohydrate; Research Diets). The mice fed with HFD were administered andrographolide (100 and 50 mg/kg per day) or lovastatin (30 mg/kg per day) daily for 12 weeks by oral gavage. In the therapeutic strategy, the male C57BL/6 mice were fed the HFD for 16 weeks to induce obesity, and then the obese mice (n = 6 per group) were randomized to receive the HFD, or HFD plus andrographolide (100 and 50 mg/kg per day) or lovastatin (30 mg/kg per day) daily for another 4 weeks by oral gavage, with another eight normal mice fed normal chow as a control group. Body weight and daily food intake were measured every week.

Metabolic Measurements. The metabolic rate was assessed (MotoRater System; TSE Systems, Bad Homburg, Germany) after the mice had received different treatments for 12 weeks. Mice from each group were acclimated in a comprehensive laboratory animal monitoring system for 24 hours before testing. Measurements were taken over a 24-hour period during which the animals had ad libitum access to food and water. Data on O2 consumption (VO2; ml/h per kilogram) and CO2 production (VCO2; ml/h per kilogram) were collected every 1 hour for 24 hours. Respiratory quotient (RQ) was calculated as Volumes of CO2 released/Volumes of O2 consumed. Energy expenditure was calculated with the equation of Weir (1949): energy expenditure (EE) (kcal/kg per hour) = (3.818 × VO2) + (1.232 × VCO2).

Measurement of Fecal Cholesterol and Triglyceride Contents. Feces were collected over the final 3 days of andrographolide treatment. The feces were dried, weighed, and powdered, and the fecal cholesterol and triglycerides were extracted from 100 mg of the powdered feces with chloroform/methanol (2:1, v/v). An aliquot of the organic phase was collected and resuspended in isopropyl alcohol. The cholesterol and triglyceride were determined using commercially available test kits (Cayman Chemicals).

Serum and Hepatic Lipid Determination. Liver tissues were homogenized in chloroform/methanol (2:1, v/v). An aliquot of the organic phase was collected, dried, and resuspended in isopropyl alcohol. The serum and extracted hepatic triglycerides and cholesterol were determined according to the manufacturer's instructions (Cayman Chemical). Serum low-density and high-density lipoprotein cholesterol levels were measured with commercially available test kits (Jiancheng Bioengineering Institute, Nanjing, People's Republic of China).

Glucose Tolerance and Insulin Tolerance Tests. Mice were fasted for 24 hours and then injected intraperitoneally with 2 g/kg glucose or 0.75 U/kg insulin (Sigma-Aldrich, St. Louis, MO) on the third day before they were sacrificed. Glucose levels were measured from tail blood with the OneTouch Ultra blood glucose monitoring system.
Liver tissues were embedded in paraffin and sectioned at 10 µm thick sections in 2h at 80°C. These tissues were used to generate 10-µl of RIPA buffer (Pierce Biotechnology) containing Cocktail (Roche Diagnostics, Indianapolis, IN). Immunoblot analysis was performed as described elsewhere (Tang et al., 2011). The samples were blotted using SREBP1, SREBP2, Tomatoes, Beverly, MA) antibodies. A desaturase 1 (SCD-1), and actin or lamin B1 (Cell Signaling Technology). The primers used in the experiments are listed in Supplemental Table 1. β-Actin was used as an internal control to normalize all the mRNA levels.

**Immunoblot Analysis.** The nuclear extracts of mouse liver were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Waltham, MA). The concentrations of the extracted proteins were measured by BCA assay kit (Pierce Biotechnology). For whole-cell lysates, the harvested cells or the homogenized liver tissue were suspended in 120 µl of RIPA buffer (Pierce Biotechnology) containing Cocktail (Roche Diagnostics, Indianapolis, IN). Immunoblot analysis was performed as described elsewhere (Tang et al., 2011). The samples were blotted using SREBP1, SREBP2, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), low-density lipoprotein receptor (LDLR) (Abcam, Cambridge, MA), ATP-binding cassette subfamily G member 5 (ABCG5) (Novus Biologicals, Oakville, ON, Canada), fatty acid synthase (FAS), stearoyl-coenzyme A desaturase 1 (SCD-1), and actin or lamin B1 (Cell Signaling Technologies, Beverly, MA) antibodies.

**Histologic Analysis of Liver and Adipose.** Liver tissues were embedded in Tissue-Tek O.C.T. cryostat molds (Leica) and frozen at −80°C. These tissues were used to generate 10-µm thick sections in a cryostat. Tissue sections were stained in 0.5% oil red O and counterstained with Mayer’s hematoxylin for 1 minute. Adipose tissues and other liver tissues were embedded in paraffin and sectioned at 5 µm onto poly-l-lysine–coated slides. For histology, sections were stained with H&E.

**Statistical Analysis.** All experiments were repeated at least three times, and each experiment was performed with triplicate samples. Results are expressed as mean ± S.D. Statistical analyses were performed using SPSS 9.0 (SPSS/IBM, Chicago, IL). Group differences were assessed by Student’s t test or analysis of variance. *P < 0.05 and **P < 0.01 were considered statistically significant.

**Results**

**Andrographolide Downregulates the Expression of SREBP Target Genes and Decreases Cellular Lipid Levels.** We found by reporter gene assay that andrographolide inhibited human sterol regulatory element promoter activity in a dose-dependent manner (Fig. 1A). In addition, cell viability results showed no significant cytotoxicity of andrographolide on the growth of Huh-7/SRE-Luc cells (Fig. 1B). Andrographolide at 10 µM inhibited the transactivity of (ACL), fatty acid desaturase 1 (FADS1), FADS2, and glycerol-3-phosphate acyltransferase, were significantly reduced by andrographolide treatment (Fig. 1C). Similarly, all eleven genes involved in cholesterol synthesis—such as SREBP-2, HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, lanoster synthase (LS), sterol-C4-methyl oxidase-like, squalene epoxidase, 24-dehydrocholesterol reductase (DHCR24), 7-dehydrocholesterol reductase (DHCR7), farnesyl diphosphate synthetase (FDP), mevalonate kinase, and farnesyl diphosphate farnesyl transferase 1 (FDPT1)—were significantly downregulated by andrographolide (Fig. 1D). In contrast, LXR target genes, including ATP-binding cassette ABCG5 and ABCG8, were not affected by andrographolide (Fig. 1E).

Furthermore, andrographolide (10 µmol) notably lowered intracellular cholesterol and triglyceride levels (Fig. 1F, P < 0.01) compared with the vehicle control in HepG2 cells, suggesting that andrographolide may have an inhibitory effect on the intracellular cholesterol and triglyceride accumulation.

**Andrographolide Reduces Body Weight Gain and Increases Energy Expenditure in Mice with HFD-Induced Obesity.** In the prevention strategy, the total body weight of mice in the HFD group significantly increased compared with that of mice in the vehicle group (35.25%, P < 0.01). In contrast to the HFD group, andrographolide treatment suppressed body weight gain by 9.35% and 20.14% at the doses of 50 and 100 mg/kg, respectively (Fig. 2B). Lovastatin as an inhibitor of HMGCR also reduced body weight by 14.75% (Fig. 2B). During the experimental period, there were no significant differences in food intake in the HFD group compared with the other treatment groups (Fig. 2A). Similarly, andrographolide significantly reduced body weight but did not affect food intake when compared with the HFD group in mice in therapeutic treatment strategy (Supplemental Fig. 1, A and B).

We further analyzed the energy expenditure and lipid absorption. In andrographolide-treated mice (100 mg/kg), the oxygen consumption (VO2), EE, and RQ were all significantly increased (Fig. 2, C–E), which may have contributed to their decreased body-weight gain. Although the RQ in the mice that had received lovastatin was elevated, the VO2 and EE were still similar to the HFD-fed mice (Fig. 2, C–E). On the other hand, lovastatin treatment significantly increased the fecal TC and TG (Fig. 2, F and G), consistent with previous reports (Nielsen et al., 1993). These results indicate that lovastatin increases lipid excretion to antagonize HFD-induced obesity. However, the fecal TC and TG in the mice that had received andrographolide at the doses of 50 or 100 mg/kg were similar to the HFD-fed mice (Fig. 2, F and G), suggesting that andrographolide had no effect on lipid absorption/excretion.

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Andrographolide Improves the Lipid Parameters in Blood, Liver, and Adipose Tissues in Mice with HFD-Induced Obesity. The lipid levels in blood and other tissues were measured (Fig. 3; Supplemental Fig. 1). The HFD significantly elevated the fasting serum TC, TG, and low-density lipoprotein cholesterol (LDL-C) concentrations compared with the mice in the vehicle group (Fig. 3, A–C; Supplemental Fig. 1, C–E). Andrographolide and lovastatin significantly decreased serum TC, TG, and LDL-C and did not affect serum high-density lipoprotein cholesterol compared

Fig. 1. Andrographolide suppressed the expression of SREBP target genes and blocks high-glucose-induced lipid accumulation in HepG2 cells. (A) Transcription activity assay of SRE-containing promoter and (B) cell viability assay to determine the effect of andrographolide on the inhibition of SRE-containing promoter and cell viability, respectively. Statistical analyses were performed with Student’s t test. *P < 0.05; **P < 0.01 versus vehicle control group. (C) mRNA expression of fatty acid metabolism, (D) cholesterol metabolism, and (E) other genes assessed by qRT-PCR in huh-7 cells. Expression was normalized to β-actin, and each bar represents the mean ± S.D. of triplicates of three independent experiments. Statistical analyses were performed with Student’s t test. *P, 0.05; **P, 0.01 versus vehicle control group. (F) Intracellular cholesterol and triglyceride levels cells assayed in HepG2. The data are shown as mean ± S.D., n = 4 for all groups. Statistical analyses were performed with Student’s t test. **P < 0.01 versus vehicle control group.
with the HFD group (Fig. 3, A–D; Supplemental Fig. 1, C–F) in the prevention and therapeutic strategy. Moreover, we found that the TG and LDL-C levels in the andrographolide group were close to those in the chow group (Fig. 3, B and C). Interestingly, although both andrographolide and lovastatin decreased the hepatic TC level, only andrographolide significantly reduced the TG in the mice livers in the prevention and therapeutic strategy (Fig. 3, E and F; Supplemental Fig. 2, A and B).

Histologic analysis showed that andrographolide significantly reduced the cell size of both white adipocyte tissue and BAT. However, lovastatin only reduced the size of brown adipocytes (Fig. 3G; Supplemental Fig. 2C). Oil red O and H&E staining showed that the accumulation of lipid droplets in the livers of HFD-fed mice significantly increased as compared with the normal chow-fed mice, whereas there were fewer lipid droplets in the livers of lovastatin-treated mice and these were rare in the livers of andrographolide-fed mice (100 mg/kg) (Fig. 3G; Supplemental Fig. 2C). This is consistent with the previous data that lovastatin and andrographolide decreased the hepatic lipid contents (Fig. 3, E and F; Supplemental Fig. 2, A and B). Furthermore, andrographolide at the dose of 100 mg/kg was more effective than lovastatin at the dose of 60 mg/kg.

**Andrographolide Improves Glucose and Insulin Resistance in Mice with HFD-Induced Obesity.** The HFD significantly elevated fasting blood glucose and serum insulin levels compared with the normal chow. However, this increase in fasting blood glucose and serum insulin levels ($P < 0.05$) was significantly reduced by the addition of andrographolide compared with the HFD group, but not by lovastatin in the prevention and therapeutic strategy (Fig. 4, A and B; Supplemental Fig. 3, A and B).

Furthermore, the HFD-fed mice exhibited impaired glucose and insulin tolerance compared with mice on a chow diet (Fig. 4, C–F; Supplemental Fig. 3, C–F). Although both andrographolide- and lovastatin-treated mice had significantly improved glucose tolerance, only andrographolide treatment of HFD-fed mice significantly improved the insulin resistance (Fig. 4, C–F; Supplemental Fig. 3, C–F). Collectively, these results demonstrate that andrographolide improves glucose and insulin resistance in mice fed with HFD.

**Andrographolide Regulates the Expression of Metabolic Genes and Proteins In Vivo.** SREBPs are important regulators of lipid homeostasis through the modulation of the expression of important downstream target genes (Horton et al., 2003). We compared the expression levels of SREBP target genes and other related genes in the liver and BAT of mice undergoing various treatments (Fig. 5, A–F). This is consistent with the previous data that lovastatin and andrographolide decreased the hepatic lipid contents (Fig. 3, E and F; Supplemental Fig. 2, A and B). Furthermore, andrographolide at the dose of 100 mg/kg was more effective than lovastatin at the dose of 60 mg/kg.

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Fig. 3. Effect of andrographolide on lipid levels in blood and tissues. Male C57BL/6 mice at 4 weeks of age were randomly grouped (eight per group). Lovastatin vehicle (30 mg/kg per day) or andrographolide (50 or 100 mg/kg per day) was administrated to mice by gastric irrigation once daily. After 12 weeks of treatment, the mice were subjected to different tests as indicated. And, andrographolide (50 or 100 mg/kg per day); Lov, lovastatin (30 mg/kg per day). (A) Blood total cholesterol (TC) levels, (B) TG, (C) LDL-C levels, and (D) high-density lipoprotein cholesterol (HDL-C) levels after 12-week treatment. Error bars represent S.D. Statistical analyses were performed with one-way analysis of variance (ANOVA) (Dunnett’s post-test).

*P < 0.05; **P < 0.01 versus HFD. (E) TC and (F) TG levels in liver after 12-week treatment. Error bars represent S.D. Statistical analyses were performed with one-way ANOVA (Dunnett post test). *P < 0.05; **P < 0.01 versus HFD. (G) Histologic analysis of liver, white adipose tissue (WAT), and BAT after 12-week treatment. Liver tissues sections were stained with oil red O or H&E to visualize lipid contents. WAT and BAT were stained with H&E. Bars = 100 μm.
and LDLR were reduced by 29–59% in the liver of andrographolide-treated mice (Fig. 5, A and B). Similarly, for genes in fatty acid metabolism, andrographolide significantly decreased the mRNA of SREBP-1 (~29%) and its target genes by 28–57%, including ACC-1, FAS, SCD-1, ACL, acyl-CoA synthetase, GAPT, FADS-1, and FADS-2 (Fig. 5C). Andrographolide treatment did not affect the mRNA levels of the ATP-binding cassette transporter A1 (ABCA1), ABCG5, or ABCG8 (Fig. 5B), suggesting that andrographolide specifically inhibits the SREBP processing but does not activate ABCA1, ABCG5, or ABCG8, which mediate the efflux and clearance of cholesterol as the target genes of LXR (Peet et al., 1998; Venkateswaran et al., 2000; Repa et al., 2002). On the other hand, the mRNA levels of peroxisome proliferator–activated receptor α and apolipoprotein E were slightly increased by andrographolide (Fig. 5, C and D), suggesting that andrographolide may accelerate lipolysis (Duval et al., 2007).

For carbohydrate metabolism genes, andrographolide significantly increased mRNA levels of the glucose-6-phosphate dehydrogenase, but did not alter the expression of phosphoenolpyruvate carboxykinase 1, insulin receptor substrate 1, or insulin receptor substrate 2 (Fig. 5E). Consistently, Western blotting results revealed that the hepatic protein levels of SREBP1, SREBP2, FAS, SCD-1, HMGCR, and LDLR were significantly lower in andrographolide-treated versus HFD-treated mice (Fig. 5G). However, andrographolide treatment did not affect the expression of ABCG5 (Fig. 5G).

Lovastatin had no significant effect on the hepatic mRNA expression of SREBP1 or SREBP2, in turn stimulating FAS.

Fig. 4. Effect of andrographolide on diet-induced glucose intolerance and insulin resistance in mice. The mice in this analysis are the same as those used in Fig. 3. (A) Blood glucose and (B) blood insulin in HFD-fed mice improved by andrographolide treatment. Error bars represent S.D. Statistical analyses were performed with one-way analysis of variance (ANOVA) (Dunnett’s post-test). *P < 0.05 versus HFD. (C) Effect of betulin on glucose tolerance in HFD-fed mice as determined by glucose tolerance test (GTT). (D) Quantification of the area under the curve (AUC) from the GTT in (C). Error bars represent S.D. Statistical analyses were performed with one-way ANOVA (Dunnett’s post-test). *P < 0.05 versus HFD. (E) Effect of andrographolide on insulin resistance in HFD-fed mice as determined by insulin tolerance test (ITT). (F) Quantification of the AUC of the ITT in (E). Error bars represent S.D. Statistical analyses were performed with one-way ANOVA (Dunnett’s post-test). *P < 0.05 versus HFD. And, andrographolide; Lov, lovastatin.
Fig. 5. Alterations of mRNA or protein levels in liver or BAT of mice treated with andrographolide. The mice in this analysis are the same as those used in Fig. 3. (A and B) The relative expressions of SREBP2 target genes in liver. (C) The relative levels of SREBP1 target genes in liver. (D) The relative levels of lipoprotein metabolism genes in liver. (E) The relative levels of carbohydrate metabolism genes in liver. (F) The relative levels of metabolism genes in BAT. (G) The relative protein expressions in mouse liver tissues. For each group, equal amounts of total RNA or protein from the tissues of 3 to 5 mice were subjected to qRT-PCR quantification or Western blot analysis as described in Materials and Methods. Protein expression was normalized to actin (whole-cell lysate) or lamin B1 (nuclear extracts), and each bar represents the mean ± S.D. of three independent experiments. Statistical analyses were performed using one-way analysis of variance (Dunnett’s post-test). *P < 0.05; **P < 0.01 versus HFD. And, andrographolide; Lov, lovastatin.
and SCD-1 activation and thereby increasing fatty acid synthesis. In addition, lovastatin increased the hepatic mRNA of ABCG5 and ABCG8, suggesting that lovastatin may prevent diet-induced obesity at least partly by decreasing lipid absorption/increasing excretion, which is consistent with the previous report (Jeon et al., 2004).

BAT plays a very important role in energy metabolism, and andrographolide dramatically enhanced the expression of uncoupling protein 2 and decreased the expression of FAS, SCD-1, and HMGCGR (Fig. 5F), suggesting that lipid synthesis dropped and fatty acid oxidation was enhanced after andrographolide treatment. This is consistent with our observation that andrographolide reduced the cell size of BAT.

As for the therapeutic strategy, similar hepatic gene alterations of SREBP target genes and metabolism-related genes in the andrographolide-treated mice were observed (Supplemental Fig. 4, A–C). All together, these data indicate that andrographolide regulates SREBP target genes and metabolism-associated genes in liver and BAT, which may directly contribute to the lower lipid level and enhanced insulin sensitivity.

**Discussion**

Andrographolide isolated from *Andrographis paniculata* has been shown to possess antioxidant (Trivedi et al., 2007), anti-inflammatory (Bao et al., 2009), antiatherosclerosis (Thisoda et al., 2006), and anticancer properties (Chao et al., 2013). Here, we characterized the antiobesity effects of andrographolide by suppressing the SREBP pathway.

SREBP is a basic helix-loop-helix-leucine zipper transcription factor, which is synthesized as a precursor protein that is localized to the endoplasmic reticulum (Brown and Goldstein, 1997; Shimano 2001). It is activated in a cholesterol-dependent manner: when cellular sterol levels fall, the precursor is transported to the Golgi apparatus by SREBP cleavage-activating protein and is cleaved by two proteases, designated site-1 and site-2 proteases, to release the mature, transcriptionally active form (Brown and Goldstein, 1997). The active form enters the nucleus and binds to specific sterol regulatory elements on the promoter regions of cholesterogenic genes to positively regulate the transcription of target genes, resulting in the maintenance of cellular lipid levels (Horton, 2002). SREBP-2 controls the expression of numerous genes involved in cholesterol homeostasis, including HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, FAPS, DHCR7, DHCR24, LSS, FDFPT1, and LDLR (Field et al., 2001; Horton et al., 2003). SREBP1 preferentially regulates the lipogenic process by activating genes involved in fatty acid and triglyceride synthesis, including ACC, FAS, SCD-1, ACL, acyl-CoA synthetase, GAP, FADS-1, and FADS-2 (Shimano, 2001; Horton et al., 2003; Gosmain et al., 2005).

LXR is another sterol-regulated transcription factor. Activation of LXR stimulates the expression of a variety of genes, including ABCA1, ABCG5/8, and Cyp7a1, that mediate the efflux and clearance of cholesterol (Peet et al., 1998; Venkateswaran et al., 2000; Repa et al., 2002). However, LXR/RXR can bind the liver X receptor response element motif located in the SREBP-1c promoter and activate SREBP-1c transcription in the presence of LXR agonist (Repa et al., 2000; Yoshikawa et al., 2001). Therefore, although activation of LXR by pharmacologic ligands reduces serum cholesterol level and protects against atherosclerosis (Joseph et al., 2002; Terasaka et al., 2003), it also leads to hepatic steatosis and hypertriglyceridemia due to the induction of fatty acid synthesis through activation of SREBP-1c (Schultz et al., 2000). Our results showed that andrographolide down-regulated SREBP and its target genes but did not affect LXR target genes, including ABCA1, ABCG5, and ABCG8, suggesting that andrographolide might specifically suppress SREBP actions. The mechanism by which andrographolide opposes SREBP actions needs further investigation.

Statins, as an inhibitor of HMGR, which is the rate-limiting enzyme for de novo cholesterol synthesis, effectively reduces the serum cholesterol level through inhibition of cholesterol synthesis (Goldstein and Brown, 2009). They are the most widely prescribed drugs to treat hypercholesterolemia and associated metabolic disorder such as obesity. However, the inhibition of cholesterol synthesis by statins leads to the activation of the SREBP pathway and a rise of fatty acid biosynthetic enzymes in liver, which leads to other metabolic syndrome risk factors characterized by dyslipidemia and insulin resistance, also in part due to statin intolerance (Vladutiu, 2008; Vandenberg and Robinson, 2010; Mancini et al., 2011). The noncompliance of statin is often related to statin-induced myalgia (Harris et al., 2011). Furthermore, recent evidence suggests that high-dose statins may increase the risk of developing type 2 diabetes (Culver et al., 2012).

Andrographolide (100 mg/kg) decreased the lipid level to a greater extent than lovastatin (30 mg/kg) in the liver, white adipocyte tissue, and BAT (Fig. 3; Supplemental Fig. 2). Also, andrographolide was more effective than lovastatin in improving insulin sensitivity (Fig. 4; Supplemental Fig. 3). However, the effective dosage of andrographolide is much higher than lovastatin because of its poor solubility in water, which results in low bioavailability after oral administration (Bothiraja et al., 2009; Ren et al., 2009). At present, there are several methods that can be used to improve the solubility and oral absorption of andrographolide, including complexing with hydroxypropyl-β-cyclodextrin (Bothiraja et al., 2009) and solid dispersion with polyvinylpyrrolidone (Ren et al., 2009). The effect of the andrographolide complex on HFD-induced obesity needs to be further evaluated.

The increase in obesity around the world in the last decade has been overwhelming. Currently, orlistat is the only drug approved by the US Food and Drug Administration for long-term obesity management, but recently it has been reported that orlistat is implicated in severe liver and acute kidney injuries (Colon-Gonzalez et al., 2013). The history of weight-loss pharmacotherapy highlights the need for new, safer, and more effective approaches to treat and prevent obesity. Natural products from Chinese medicine are rich sources of weight-loss drug development. Many natural products exert antiobesity effects with few side effects. In our current study, we found that andrographolide suppressed SREBP actions and had a multitude of beneficial effects in vivo, indicating that it could be a potential therapeutic agent to treat obesity and metabolic diseases.

In conclusion, we have demonstrated that andrographolide opposes SREBP actions at the gene transcription level, regulates lipid metabolism, and enhances insulin sensitivity. Andrographolide may serve as a leading compound for pharmacologic control of obesity and metabolic diseases, especially type 2 diabetes.

**Authorship Contributions**

*Participated in research design:* L. Yang, Wang, Song.

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Contributed new reagents or analytic tools: Tang, Qi, Qim, Yang, Qia, Yang.

Performed data analysis: Ding, Li.

Wrote or contributed to the writing of the manuscript: Ding, Huang, L. Yang, Wang.

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