Hypolipidemic Effects of Alkaloids from Rhizoma Coptidis in Diet-Induced Hyperlipidemic Hamsters

Abstract

This study was conducted to evaluate the antihyperlipidemic activity of five major alkaloids in Rhizoma Coptidis using high-fat- and high-cholesterol-induced hyperlipidemic hamsters. Hyperlipidemic hamsters were treated with coptisine, berberine, jatrorrhizine, palmatine, epiberberine, and total Rhizoma Coptidis alkaloids with a dose of 46.7 mg/kg × day for 140 days. Serum total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total bile acids were examined after alkaloid treatment. The results showed that all therapy agents prevented body weight gain, reduced the serum total cholesterol, and increased the high-density lipoprotein cholesterol of hamsters. Berberine, jatrorrhizine, and total Rhizoma Coptidis alkaloids decreased the triglyceride level in hyperlipidemic hamsters, while coptisine, jatrorrhizine, palmatine, and total Rhizoma Coptidis alkaloids significantly suppressed the elevation of the low-density lipoprotein cholesterol level. The fecal excretion of bile acids was significantly elevated by berberine, coptisine, jatrorrhizine, palmatine, total Rhizoma Coptidis alkaloids, and orlistat. Notably, total Rhizoma Coptidis alkaloids possess a much stronger lipid-lowering effect than the pure Rhizoma Coptidis alkaloids. Quantitative reverse transcription-polymerase chain reaction analyses revealed that Rhizoma Coptidis alkaloids could retard the synthesis of cholesterol by downregulating the mRNA expression of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and accelerate the clearance of lipids by upregulating the low-density lipoprotein receptor, cholesterol 7α-hydroxylase, and uncoupling protein-2 expression. These findings highlight the critical role of Rhizoma Coptidis alkaloids in hyperlipidemia treatment. Thus, they need to be considered in future therapeutic approaches.

Abbreviations

ASBT: apical sodium-dependent bile salt transporter
CYP7A1: cholesterol 7α-hydroxylase
DM: diabetes mellitus
FGF: fibroblast growth factor
HDL: high-density lipoprotein
HFHC: high fat and high cholesterol
HMGCR: 3-hydroxy-3-methyl glutaryl coenzyme A reductase
LDL: low-density lipoprotein
LDLR: low-density lipoprotein receptor
NC: normal control group
qRT-PCR: quantitative reverse transcription PCR
RC: Rhizoma Coptidis
TBA: total bile acids
TC: total cholesterol
TG: triglyceride
TRCA: total Rhizoma Coptidis alkaloids
UCP-2: uncoupling protein-2

Introduction

Over the last decades, there has been a significant increase in obesity in the general population of developed countries and a growing concern in the developing world as well. According to a survey published in 2010, there were 325 million obese individuals in China and this number will continue to increase [1]. Obesity is the main cause of dyslipidemia, which is associated with a higher risk of developing diabetes and cardiovascular disease [2]. Dyslipidemia is characterized by in-
creased total cholesterol, triglyceride, and LDL levels, and also by declined HDL levels [3, 4]. Controlling lipid levels can effectively interfere with the progression of atherosclerosis and reduce cardiovascular events [5]. Currently, amfepramome and simvastatin were widely used for the treatment of obesity. But these drugs have several adverse effects [6]. Therefore, the development of safe and efficient lipid-lowering drugs has attracted considerable research interest.

As early as Anno Domini 220, Rhizoma Coptidis (Coptis chinensis Franch, Ranunculaceae) was used in patients suffering from DM. Clinical studies have revealed that the plasma TG levels of type 2 diabetes mellitus patients were substantially increased, whereas HDL-C levels were markedly reduced in both men and women compared with the nondiabetic controls [7]. The lipid changes associated with DM were attributed to insulin resistance and were considered the major risk factors for cardiovascular disease in DM. Thus, the beneficial effects of RC on DM patients are probably related to the lipids regulation role of RC alkaloids [8]. In fact, berberine isolated from RC could upregulate LDLR expression via activating the extracellular signal-regulated kinase pathway and reducing serum cholesterol, TG, and LDL cholesterol in hypercholesterolemic patients [9]. Recent studies have suggested that berberine treatment significantly decreased blood lipid levels both in obese subjects and in Sprague Dawley rats [10–12]. Moreover, meta-analysis of clinical trials have revealed that the administration of berberine produced a significant reduction in total cholesterol, triglycerides, and LDL cholesterol levels, with a remarkable increase in HDL [13]. Nevertheless, little attention has been given to investigating the lipid-lowering effects of other RC alkaloids including coptisine, jatrorrhizine, palmatine, and epiberberine, and the underlying antihyperlipidemic mechanisms remain largely unknown.

The dynamic equilibrium of lipids in the body is subtly regulated by several key enzymes such as LDLR, HMGCR, CYP7A1, etc. The dietary modulation of cholesterol absorption of Syrian golden hamsters is similar to that of humans and it has been demonstrated that the lipoprotein profile of hamsters resembles that of humans fed an HFHC diet [14]. This study was therefore undertaken to investigate the antihyperlipidemic effects and mechanisms of pure RC alkaloids (coptisine, berberine, jatrorrhizine, palmatine, epiberberine; Fig. 1) and TRCA in hamsters. It is worth noting that TRCA exhibited the strongest weight-lowering effect in HFHC-induced hyperlipidemic hamsters compared with the pure RC alkaloid.

Results

The concentration of each RC alkaloid was calculated from the peak areas using the normalization method. As shown in Fig. 2, the alkaloids in the RC extract mainly include jatrorrhizine, epiberberine, coptisine, palmatine, and berberine, and the content of these alkaloids were 11.0%, 8.0%, 9.8%, 9.0%, and 47.0%, respectively.

The hyperlipidemic hamsters, weighing approximately 135 g, were obtained after 8 weeks of being fed an HFHC diet. As shown in Fig. 3, after 105 days of treatment, the body weight of each hamster reached its highest value, then gradually decreased, and finally returned to a stable value (see Supporting Information). Nevertheless, the body weight gain of the HFHC group was the largest, followed by the normal group, jatrorrhizine, coptisine, berberine, palmatine, epiberberine, orlistat, and TRCA (Table 1). After 120 days of treatment, animals supplemented with jatrorrhizine, coptisine, berberine, palmatine, epiberberine, orlistat, and TRCA weighed 6.3%, 8.2%, 9.1%, 10.5%, 11.2%, 12.2%, and 17.3% less than the HFHC group, respectively. These data suggested that both RC alkaloids and TRCA could slow down the weight gain of hamsters induced by an HFHC diet. Notably, TRCA exhibited the strongest weight-lowering effect compared with each pure RC alkaloid and orlistat.

The relative liver weight was not significantly different among groups, but the epididymal adipose weight of the drug-treated group was significantly different when compared to the HFHC.
In this study, an HFHC diet led to a significant increase in TC cholesterol, which will lead to impaired vascular reactivity [16]. Excessive triglycerides coincide with a high ratio of plasma LDL to HDL cholesterol level [15]. Besides, it has been demonstrated that a high triglyceride level in the plasma is a risk factor for cardiovascular disease independent of the high-density lipoprotein cholesterol level [15]. Therefore, the lipid-lowering effects of RC alkaloids were mainly achieved by mediating the lipid metabolism in hyperlipidemic hamsters rather than reducing body weight.

To determine the effects of RC alkaloids on the excretion and conversion of cholesterol, the feces of the hamsters were collected 3 days before the experiment and the 3 days prior to the termination of the study. As shown in Table 3, there was no obvious changes in the dry weight of hamster feces among all groups. The HFHC diet caused a significant increase in excreted cholesterol and TBA in the feces. RC alkaloid administration raised the level of cholesterol and TBA in the feces when compared to the HFHC group. It is well acknowledged that the fecal excretion of bile acids can be elevated by dietary cholesterol. In this study, all RC alkaloids and TRCA significantly enhanced the fecal excretion of bile acids. Meanwhile, the fecal cholesterol levels in the coptisine, jatrorrhizine, palmatine, berberine, TRCA, and orlistat groups were increased by 27.0%, 12.1%, 21.8%, 11.6%, 12.5% and 14.5%, respectively, compared to the HFHC group. These results suggested that both pure RC alkaloids and TRCA could promote the conversion of cholesterol into bile acids as well as accelerate the fecal excretion of bile acids.

To explore the molecular mechanisms for the antihyperlipidemic efficiency of RC alkaloids, the mRNA expression of lipid metabolism-related genes was investigated by qRT-PCR. As shown in Fig. 4, orlistat, berberine, and palmatine showed significant inhibition of HMGCR (p < 0.01). Oral administration of coptisine and TRCA also obviously suppressed the mRNA expression of HMGCR. Additionally, the CYP7A1 mRNA level was significantly increased by 38.4% and 53.8% with coptisine, and palmatine therapy, respectively, compared to the HFHC group (p < 0.01). The LDLR mRNA levels increased 3.1-, 3.0-, and 1.9-fold in berberine, jatrorrhizine, and palmatine supplemented groups, respectively, compared to the HFHC group (p < 0.01). Coptisine and TRCA also enhanced the LDLR mRNA expression in hamsters. HFHC administration significantly increased UCP-2 expression in comparison with the normal group. Among the tested treatments, berberine, palmatine, and TRCA administration elevated the UCP-2 mRNA expression by 20.1%, 35.4%, and 31.0%, respectively. These results suggested that pure RC alkaloids and TRCA could retard the synthesis of cholesterol by downregulating the mRNA expression of HMGCR and accelerate lipid metabolism by upregulating LDLR, CYP7A1, and UCP-2 mRNA expression in the liver of hyperlipidemic hamsters.

ELISAs are characterized by high selectivity as well as low limits of detection. In this study, ELISA kits were applied to the quantitative analysis of HMGCR, CYP7A1, and LDLR in the liver of hamsters. The HMGCR protein level in hamsters was found to decrease and this effect was promoted by RC alkald treatment. In accordance with the change in HMGCR mRNA expression, orlistat, coptisine, berberine, palmatine, and TRCA significantly reduced the protein levels of HMGCR by 12.00%, 8.24%, 6.70%, 9.61%, and 4.01%, respectively, compared with the HFHC group. CYP7A1 is the initial and rate-limiting enzyme involved in the conversion of cholesterol to bile acids. As can be seen from Fig. 5, the elevated level of CYP7A1 in the liver is possibly a reflection of increased circulation of bile acids in hamsters resulting from HFHC administration. In response to all pure RC alkaloids and TRCA, hamsters exhibited a higher level of CYP7A1, which promoted the conversion of cholesterol to bile acids and led to a depletion of the intrahepatic pool of cholesterol (Table 3). Among the tested samples, coptisine showed the highest CYP7A1 upregul-
ing activity, followed by palmatine, TRCA, jatrorrhizine, epiberberine, and berberine (increased by 17.01%, 15.10%, 11.32%, 9.43%, 7.55%, and 5.81%, respectively). LDLR was an important receptor involved in the endocytosis of cholesterol. As shown in Fig. 5, HFHC led to a lower content of LDLR in the hamsters. Except for epiberberine, the LDLR protein levels were greatly increased after coptisine, berberine, jatrorrhizine, palmatine, and TRCA treatment (increased by 11.49%, 22.09%, 30.08%, 22.14%, and 11.52%, respectively) compared to the HFHC group (p < 0.05).

**Discussion**

Currently, the rapid development of the world economy has resulted in a great variation in diet composition of most people in developing countries, that is, the shift from staple crops to more flesh (mainly pork in China). High-fat diets are associated with the development of chronic diseases such as Alzheimer's disease, diabetes and hyperlipidemia [17]. Therefore, harmonious regulation of the synthesis and metabolism of cholesterol is crucial to reduce the risk of these cardiovascular diseases.

The synthesis and metabolism of cholesterol of Syrian golden hamsters was similar to that of humans, therefore this animal model was used to evaluate the lipid-lowering effects of RC alkaloids. The body weight gain of all hamsters was consistent with the previous study [18]. Additionally, all therapy agents reduced the serum TC and increased HDL-C values. Berberine, jatrorrhizine, and TRCA decreased the TG level in hyperlipidemic hamsters, while coptisine, jatrorrhizine, palmatine, and TRCA significantly suppressed the elevation of the LDL-C level.

The cholesterol level in the body is subtly regulated by the excretion via conversion to bile acids in the liver as well as the synthesis of the bile acids in the gut. The bile acids are synthesized from cholesterol, which is mainly derived from dietary fat and cholesterol stored in the liver. The cholesterol level in the body is determined by the balance between the synthesis and excretion. Previous studies have shown that RC alkaloids can significantly increase the level of cholesterol and TBA in the feces when compared with the HFHC group [19].

### Table 1  The body weight and relative organ weight change of hamsters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dry weight (g/d)</th>
<th>Cholesterol (mg/dL)</th>
<th>Total bile acids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.17 ± 0.01</td>
<td>1.81 ± 0.04</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>HFHC</td>
<td>1.16 ± 0.00</td>
<td>2.45 ± 0.03</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Orlistat</td>
<td>1.17 ± 0.03</td>
<td>2.44 ± 0.02</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Berberine</td>
<td>1.17 ± 0.00</td>
<td>2.40 ± 0.07</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Coptisine</td>
<td>1.16 ± 0.01</td>
<td>2.43 ± 0.06</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Jatrorrhizine</td>
<td>1.16 ± 0.04</td>
<td>2.42 ± 0.05</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Palmatine</td>
<td>1.15 ± 0.02</td>
<td>2.41 ± 0.07</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>Epiberberine</td>
<td>1.16 ± 0.08</td>
<td>2.44 ± 0.01</td>
<td>1.50 ± 0.20 ±</td>
</tr>
<tr>
<td>TRCA</td>
<td>1.16 ± 0.05</td>
<td>2.44 ± 0.05</td>
<td>1.50 ± 0.20 ±</td>
</tr>
</tbody>
</table>

Values are means ± SD, n = 6 hamsters in each group. TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TBA: total bile acids; * p < 0.05, ** p < 0.01 vs. normal control; * p < 0.05, ** p < 0.01 vs. HFHC.
sis and metabolism of cholesterol. The regulation of HMGCR activity is the primary means for controlling the level of cholesterol biosynthesis, which is the target of statin drugs [19, 20]. Synthesized cholesterol and other dietary cholesterol is transported in the serum via LDLR-mediated uptake and HDL-mediated reverse transports [21], or catalyzed by CYP7A1 and then excreted in the bile [22, 23]. The present study demonstrated that the treatment of hyperlipidemic hamsters with berberine, palmatine, coptisine, and TRCA obviously suppressed HMGCR expression. Moreover, in response to berberine, coptisine, jatrohrrhizine, palmatine, epiberberine, and TRCA, hamsters exhibited a higher level of CYP7A1. Besides, berberine, jatrohrrhizine, coptisine, TRCA, and palmatine enhanced the LDLR expression. The combined effects of the increased LDLR and CYP7A1 levels may account for the declined TC level in the therapy groups. Meanwhile, the increased bile acids and cholesterol excretions in the feces of hyperlipidemic hamsters suggest a possibility of inhibiting cholesterol and bile acid absorption and reabsorption. ASBT is another well-known therapeutic target for antihyperlipidemic drugs. ASBT inhibitors would increase excretion of bile acids, causing increased catabolism of hepatic cholesterol to bile acids. It is recognized that decreased FGF15 and FGF19 expression in mice ileal will lead to increases in hepatic CYP7A1 expression and fecal bile acids excretion [24, 25]. Out et al. recently demonstrated that intestinal microbiota alters the Gata4-controlled expression of ASBT and affects the host bile acids reabsorption [26]. In light of these findings, it is important to investigate whether RC alkaloids regulate ASBT expression and determine the molecular mechanisms involved in the process. Kong et al. have recognized that berberine could activate the extracellular-regulated kinase pathway [9]. Since all RC alkaloids have the isoquinoline parent structure, it is rational for us to conclude that RC alkaloids also could activate the extracellular-regulated kinase pathway and increase LDLR expression. Mitochondrial uncoupling proteins are responsible for the generation of adenosine triphosphate (ATP) [27, 28]. Among them, UCP-2 was recognized as a critical regulator of cellular fuel utilization and whole body glucose and lipid metabolism. A study involving 603 subjects has revealed that uncoupling protein 2 is associated with obesity [29]. The increased transcriptional activation of hepatic UCP-2 can effectively prevent body weight gain, adiposity, and lipid disorders [30]. It has been demonstrated that UCP mediates lipid metabolism via mitochondrial fatty acid efflux to increase fatty acid oxidation [31]. The present study demonstrated that berberine, palmatine, and TRCA can elevate the UCP-2 mRNA expression in hamsters, indicating that these agents could increase energy expenditure [32]. These results suggested that RC alkaloids and TRCA could retard the synthesis of cholesterol by downregulating HMGCR expression and accelerate the metabolism of lipids by upregulating LDLR, CYP7A1, and UCP-2 expression in the liver of hyperlipidemic hamsters.

To demonstrate which mechanism is responsible for the observed lipid changes, a correlation coefficient analysis between the TC level and the expression of HMGCR, CYP7A1, and LDLR was conducted. A negative correlation was found between the TC level and the expression of HMGCR, CYP7A1, and LDLR content (r = 0.444). However, there was no significant correlation between the TC level and HMGCR and LDLR content (r = 0.06, p = 0.07, respectively). This indicates that among these three lipid metabolism-related proteins, CYP7A1 plays a vital role in mediating the TC level. It is not surprising that coptisine, berberine, jatrohrrhizine, palmatine, and epiberberine exhibited different antihyperlipidemic activities in the hamsters. Although these five alkaloids belong to the isoquinoline alkaloid family, they possess different substituent groups on the isoquinoline parent structure, and thus exhibit different pharmacological properties. Jung et al. have revealed that the presence of the methylenedioxy group in the D ring of epiberberine and greenlandine was responsible for butyrylcholinesterase inhibition activity [33]. Pharmacokinetic studies have revealed that TRCA, berberine, and coptisine were mainly distributed in the liver of rats [34]. Among them, berberine transported more easily across the intestinal barrier of diabetic rats [35]. These findings may explain the excellent anti-hyperlipidemic activities of TRCA, berberine, and coptisine in this study.
The use of a mono-compound purified from RC like berberine, palmatine, coptisine, jatrorrhizine, and epiberberine has proved to be a very powerful agent in modulating the numerous cellular signaling pathways including AMPK signaling [36], NF-kB signaling [37], PPAR signaling [38], etc. Therefore, the five alkaloids in TRCA may regulate either the same or different targets in various lipids metabolism pathways. Thus, it is not surprising that TRCA extracts exhibited greatly amplified antihyperlipidemic effects when compared with pure RC alkaloids. Nevertheless, it should be noted that other minor components in TRCA may also contribute to the lipid-lowering effect of TRCA. The precise and detailed mechanism of action for RC alkaloids on lipid metabolism remains to be elucidated.

Taken together, our results suggested that pure RC alkaloids and TRCA showed excellent lipid regulating activities and could reverse the body weight gain of hamsters induced by HFHC. These observed antihyperlipidemic effects can be attributed to the metabolism and excretion of cholesterol by RC alkaloids via downregulating the expression of HMGCR and upregulating the expression of LDLR and CYP7A1 as well as promoting the excretion of total bile acids in the feces. These findings highlight the critical role of RC alkaloids in hyperlipidemia treatment. Thus, they need to be considered in the future therapeutic approaches.

Materials and Methods

Plant materials and chemicals
RC was obtained from Good Agricultural Practices Demonstration Base in Shizhu City and authenticated by Prof. LuJang Yuan at Southwest University. Coptisine, berberine, jatrorrhizine, palmatine, and epiberberine with a purity of 99.0%, 99.3%, 98.4%, 98.9%, 98.8%, respectively (by HPLC analysis) were separated from RC according to previous methods of our lab [39]. Briefly, RC ethanol extracts were subjected to high-speed counter current chromatography using a solvent system consisting of CHCl3-MeOH-0.2 M HCl (4:2:2, v/v/v). Peak fractions were collected according to the elution profiles. Then, the main fractions were further purified by a Sephadex LH-20 column to obtain the five main alkaloids. The spectrum data of these compounds are shown in Figs. 1 S to 5 S, Supporting Information. Standard RC alkaloids were purchased from Chengdu Must Bio-technology Co., Ltd. and its purity was > 98% (batch number 14025939). Orlistat (99.9%) was purchased from Zein Pharmaceutical. TC, TG, LDL-C, and HDL-C in the serum were estimated using commercially available kits. Elisa Commercial kits were purchased from Beijing Bios Biotechnology Co., Ltd. All other chemicals used were of analytical grade.

Animals
Healthy 5-week-old male Syrian golden hamsters (Mesocricetus auratus) weighing 100 ± 10 g were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were raised in stainless steel wire-mesh cages individually in a room kept at 22 ± 2°C with 50–60% relative humidity and a 12-h light/dark cycle (light period: 8:00–20:00 h) and allowed free access to

Fig. 5 ELISA analysis of cholesterol metabolism-related protein in the livers of the hamsters. Orlistat, coptisine, berberine, palmatine, and TRCA significantly reduced the protein level of HMGCR. All pure RC alkaloids and TRCA significantly improved the expression of CYP7A1. Except for epiberberine, the LDLR protein levels were increased after RC alkaloid treatment. Values are means ± SD (n = 3). Δ p < 0.05, ΔΔ p < 0.01 vs. normal control; * p < 0.05, ** p < 0.01 vs. HFHC.
normal food and water. After acclimatization with the facility for 1 week, animals were randomly assigned to the normal or HFHC group. The HFHC diet was composed of 10% lard, 10% egg yolk powder, and 1% cholesterol. The normal diets were based on a modification of the recommendations of the American Institute of Nutrition. After 8 weeks, hyperlipidemic hamsters were determined by the lipid levels including serum TC, TG, LDL-C, and HDL-C and randomly divided into HFHC, coenzyme Q10, berberine, jatrorrhizine, palmatine, epiberberine, and TRCA (with a dose of 46.7 mg/kg×day), and TRCA groups (with a dose of 46.7 mg/kg×day containing 11.0% jatrorrhizine, 8.0% epiberberine, 9.8% coptisine, 9.0% palmatine, and 47.0% berberine) and 1.2 mg/kg×day of orlistat served as a positive control. Each group had six hamsters. In a subsequent experiment, except for the normal group, all hamsters were fed with HFHC diets. Hamsters were administered 0.7 mL of the corresponding drugs or saline (normal and HFHC groups) by gavage during the study period. Body weights were recorded every 7 days. Feces of each hamster were collected on the third day prior to termination of the study. After sacrifice of the hamsters, the relative organ weights (% ratio to final body weight) of selected vital organs were measured and subsequently kept in 10% neutral buffered formalin for pathological examination. This study was carried out in strict accordance with the recommendations in The Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental protocols were approved in May 2012 by the Institutional Animal Ethics Committee of Southwest University (Permit Number: 12–1036).

Observation for lipid metabolism in Syrian golden hamsters

TC, TG, LDL-C, and HDL-C were evaluated by commercially available kits (Huili Biotechnology) according to the manufacturer’s instructions. Briefly, the TC concentration was determined by the cholesterol oxidase method, TG was determined by the glycerol-3-phosphate oxidase-p-aminophenazone method, and LDL-C concentrations were quantified by precipitation with phosphotungstic acid and MgCl2. HDL-C concentrations were quantified by the same method used to determine total cholesterol after the removal of other lipoproteins. The concentrations of TBA were measured enzymatically using the 3-α-HSD method [40] by a commercially available kit (Shanghai Rongsheng Biotechnology Technology).

Bioassays of total cholesterol and bile acids in the feces

Feces of each hamster were collected 3 days prior to termination of the study for the analysis of cholesterol and bile acids. Feces powder (0.5 g) was extracted three times with 10 mL of 95% ethanol at 60 °C [41]. After being filtered, the supernatant was concentrated under reduced pressure, and the residue was dissolved in PBS to analyze the levels of TC and TBA.

Quantitation of mRNA expression using quantitative reverse transcription-PCR

Total RNA was prepared from livers using Trizol. cDNA was synthesized using SuperScript III First-Strand Synthesis System (Invitrogen). The qRT-PCR was performed on a Bio-Rad CFX connect real-time system. The sequences of the primers were as follows: HMGCR forward, 5′-TGG AGA TCA TGT GCT GCT TC-3′; reverse, 5′-GCC ACT ATG AGC GTG AAC AA-3′; LDLR forward, 5′-GAA AAG GCT ACT GCC TGT GC-3′; reverse, 5′-CCA GGA CCC GGT CAG TAG TA-3′; GAPDH forward, 5′-TCA TCC CTA AAG GA-3′; reverse, 5′-GCT CGG ATA TTC AAG GA-3′; UCP-2 forward, 5′-GGC TTC TGG GTA CCA TCC TA-3′; reverse, 5′-GCT CTC AGC CCT TGG TGT AG-3′; GAPDH forward, 5′-AAC TTT GGC ATT GTG GAA GG-3′; reverse, 5′-ACA CAT TGG GGG TAG GAA CA-3′. The following cycling conditions were used: one denaturing cycle at 95 °C for 3 min, and 35 cycles of amplification (95 °C for 10 s, 58 °C for 10 s, and 72°C for 30 s) were performed followed by 5 min of final extension at 72 °C. Relative RNA levels were determined by analyzing the changes in SYBR Green fluorescence during PCR by the 2-ΔΔCt method according to the manufacturer’s instructions. GAPDH was amplified in parallel for normalization [42]. The PCR products were confirmed by electrophoresis in 2% agarose gel stained with ethidium bromide. The purity of the amplified PCR products was determined by melting point analysis using ICycler software.

Quantitation of protein expression using ELISA

The livers of the hamsters were homogenized to detect the protein levels of HMGCR, LDLR, and CYP7A1 according to the manufacturers’ instructions of ELISA kits (Biovalue) using a BioTek Elx 808 absorbance microplate reader.

Statistical analysis

Statistical analysis was performed by SPSS software for Windows (version 15.0). Student’s t-test was calculated for comparison between normal and HFHC groups. Factorial significance of the interaction between lipid-lowering effects and body weights was assessed using an analysis of covariance (ANCOVA) with body weights as the covariate. Comparisons between treatment and control groups were performed by one-way or two-way analyses of variance (ANOVA) followed by post hoc Bonferroni’s test, when appropriate. P values < 0.05 were considered significant.

Supporting information

Details on the body weight of the hamsters and spectral data of the compounds are available as Supporting Information.

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Conflict of Interest

The authors declare no identifiable conflict of interest.

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