RESEARCH ARTICLE

Beneficial effects of mangiferin on hyperlipidemia in high-fat-fed hamsters

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Scope: Mangiferin, a natural polyphenol, has been shown to have hypolipidemic effect in rat and mouse. However, the mechanism of action is not well understood. This study was conducted to determine the effect and mechanism of action of mangiferin on hyperlipidemia induced in hamsters by a high-fat diet.

Methods and results: Forty male hamsters were randomly assigned to normal control, high-fat control, and high fat with mangiferin (50 and 150 mg/kg BW) groups. Mangiferin treatment significantly decreased final body weight, liver weight and visceral fat-pad weight, serum triglyceride (TG) and total free fatty acid (FFA) concentrations, hepatic TG levels and hepatic and muscle total FFA contents. Mangiferin upregulated mRNA expression of peroxisome proliferator-activated receptor-α (PPAR-α), fatty acid translocase (CD36) and carnitine palmitoyltransferase 1 (CPT-1), but downregulated mRNA expression of sterol regulatory element-binding protein 1c (SREBP-1c), acetyl CoA carboxylase (ACC), acyl-CoA:diacylglycerol acyltransferase 2 (DGAT-2) and microsomal triglyceride transfer protein (MTP) in liver. Mangiferin also stimulated mRNA expression of PPAR-α, CD36, CPT-1 and lipoprotein lipase (LPL) in muscle.

Conclusions: The results suggest that mangiferin may ameliorate hypertriglyceridemia partly by modulating the expression levels of genes involved in lipid oxidation and lipogenesis.

Keywords:
Acyl-CoA:diacylglycerol acyltransferase 2 / Hamster / Hyperlipidemia / Mangiferin / Peroxisome proliferator-activated receptor-α

1 Introduction

Hyperlipidemia is a well-known risk factor for several diseases, such as cardiovascular disease [1, 2], hepatic steatosis [3] and diabetes mellitus [4]. Thus, lowering blood lipids, especially triglyceride (TG) and total cholesterol (TC), is an important strategy in preventing the occurrence and progression of these diseases. Phytochemicals are attracting increasing attention because of their health benefits and their relatively low toxicity [5] and might be suitable for long-term supplementation.

Mangifera indica, commonly known as mango, is consumed worldwide as a fruit and culinary and flavoring agent. Mangiferin, a *C*-glucosylxanthone (1,3,6,7-tetrahydroxyxanthone-*C*2-β-D-glucoside), was first isolated from *M. indica* leaves and is also present in the pulp, peel, seed kernels and stem bark of mango. Mangiferin is also considered to be an active component of *Anemarrhena asphodeloides* [6]. In China, it has been used as a traditional medicine to

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Abbreviations: ACC, acetyl CoA carboxylase; **CD36,** fatty acid translocase; **CPT-1,** carnitine palmitoyltransferase 1; **DGAT-2,** acyl-CoA: diacylglycerol acyltransferase 2; **FFA,** free fatty acids; **HDL-C,** high-density lipoprotein cholesterol; **LDL-C,** low-density lipoprotein cholesterol; **LPL,** lipoprotein lipase; **MTP,** microsomal triglyceride transfer protein; **PPAR-α,** peroxisome proliferator-activated receptor-α; **SREBP-1c,** sterol regulatory element-binding protein 1c; **TC,** total cholesterol; **TG,** triglyceride

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treat diabetes for thousands of years and a raw material to produce functional food. Mangiferin has many important pharmacological properties, including antioxidant [7], anticancer [8], antiviral [9], immunomodulation [10] and hypoglycemic activities [11, 12]. It has been reported that mangiferin with exercise could significantly decrease blood TG and TC in KK/Ay mice [6]. Mangiferin also had a hypolipidemic effect in a hypoglycemic study in streptozotocin (STZ) diabetic rats [13]. However, the exact mechanism underlying its hypolipidemic effect is not well understood.

To further investigate the hypolipidemic effect of mangiferin and its possible mechanism, we first systematically studied the effect of mangiferin on lipid levels in blood, liver, and muscle using an animal model of hyperlipidemia in hamsters fed a high-fat diet. To more specifically identify the mechanisms of the hypolipidemic effect at the molecular level, we determined mRNA expression of genes involved in lipogenesis and lipid oxidation in liver and muscle.

2 Materials and methods

2.1 Animals and diets

Forty 7-wk-old male golden Syrian hamsters (*Mesocricetus auratus*, 80–90 g) were purchased from the Vital River Laboratory Animal Technology (Beijing, China). The animals were individually housed in stainless steel cages in a room at $22 \pm 2^{\circ}C$ on a 12-h light–dark cycle with free access to regular rodent chow and water. After a week of acclimatization, animals were randomly divided into four groups: a normal control group (NC, $n = 10$), a high-fat control group (HF, $n = 10$), a low-dose mangiferin group (high-fat diet + 50 mg/kg BW, $n = 10$) and a high-dose mangiferin group (high-fat diet + 150 mg/kg BW, $n = 10$). Hamsters from the NC group were fed a normal diet, while hamsters in the other groups were fed a high-fat diet. The composition of the experimental diets was based on the AIN-93G growth diet with some modifications. The normal diet contained 13.9% (cal) fat and the high-fat diet contained 33.0% (cal) fat (Table 1). The mineral and vitamin mixtures were AIN-93G formulas. Diets were freshly mixed in small amounts every week and stored at 0–4°C to avoid rancidity. Mangiferin (>90%, HPLC; Zhongxin Innova Laboratories, Tianjin, China) was given by gavage in 0.5% carboxymethyl cellulose (CMC) buffer solution. Hamsters in the NC and HF groups were given 0.5% CMC buffer only. Food consumption and weight gain were measured daily and weekly, respectively.

2.2 Sample collection

After 8 wk of mangiferin supplementation, the hamsters were anesthetized with pentobarbital after withholding food for 12 h, and blood samples were taken from the inferior vena cava. Serum was obtained by centrifuging the blood at 1500 rpm for 15 min at 4°C. Tissues collected were liver, epididymal adipose tissue, perirenal adipose tissue, soleus muscle, spleen, testicle and kidney. All excised tissues were rinsed with physiological saline and weighed. All tissue samples were flash frozen in liquid nitrogen and stored at −80°C until analyses. The study was approved by the Harbin Medical University Institutional Animal Care Committee and was conducted in accordance with Harbin Medical University guidelines for the care and use of laboratory animals (SYXK (Hei) 2006-010).

Table 1. Composition of the experimental diet (g/kg diet)

Ingredien	Normal diet	High-fat diet
Casein	200	200
Corn starch	635	330
Sucrose	0	200
Soybean oil	60	60
Lard	0	100
Cellulose	50	50
L-cystine	3	3
AIN-93G Mineral mixture	40	40
AIN-93G Vitamin mixture	10	10
Cholic acid	0	2
Choline bitartrate	2	2
Cholesterol	0	3
Total	1000	1000
kcal/g diet	3.88	4.36
Calorie from protein (%)	20.6	18.4
Calorie from fat (%)	13.9	33.0
Calorie from carbohydrate (%)	65.5	48.6

2.3 Measurement of serum parameters

Serum TG, TC, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and glucose levels were determined using enzymatic kits from Zhongsheng Beikong Biological Technique Company (Beijing, China).

2.4 Hepatic lipids and histology

After the animals were sacrificed, part of the liver was snap-frozen in liquid nitrogen, cut with a cryostat (Leica CM 1100) and stained with Oil Red O. Hepatic lipids were extracted using the method described by Folch et al. [14] with some modifications. In brief, 100 mg of liver tissue was homogenized in 1 mL of ice-cold phosphate-buffered saline using an IKA T-10 basic Ultra-Turrax (IKA, Germany) for 5 min. A 2.0-mL aliquot of chloroform/methanol (2:1, v/v) was added to the homogenate and vortexed for 60 s. After 12 hours of standing, the homogenate was centrifuged at 3500 rpm for 15 min. The bottom organic phase was dried under nitrogen gas. The residue was redissolved in 3% Triton X-100 and an aliquot was used for measurement of TG and TC using commercial kits as used for serum lipids.

2.5 Measurement of serum and tissue free fatty acid (FFA)

Serum FFA was measured by GC-MS (Polaris Q, Thermo Electron, USA) using the method described by Liu et al. [15]. Concentrations of individual fatty acids were calculated using peak area against an internal standard (Sigma). Total lipids were extracted from tissues (liver and muscle) as described in Section 2.4. After centrifugation, the bottom organic phase was dried under nitrogen and then directly esterified with sulfuric acid in MeOH according to Liu et al. [15]. Finally, the FFA content of tissues was assayed by GC-MS as for serum samples.

2.6 Gene expression analysis

Total RNA was extracted from stored frozen tissues (liver and muscle) from the different hamster groups using TRIzol reagent (Invitrogen, CA, USA). cDNA synthesis was performed with an oligo Dt-Adaptor primer and AMV Reverse Transcriptase XL as recommended by the supplier (DaLian Bao). The cDNA fragment was amplified by PCR using specific primers. The primer sequences and resulting RT-PCR products are shown in Table 2. Preliminary experiments were carried out with various cycles to determine the nonsaturating conditions for PCR amplification of all the genes studied. The final products were subjected to electrophoresis on 1.5% agarose gels and detected by ethidium bromide staining via UV light. All PCR products measured were normalized to the amount of β-actin cDNA in each sample. The mRNA levels are expressed as a ratio relative to β-actin mRNA.

2.7 Statistical analysis

All data are presented as mean ± SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS, Release 13.01S China; Beijing Stats Data Mining Co. permanent license). Significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by *post-hoc* multiple comparison tests. p Values < 0.05 were considered statistically significant.

3 Results

3.1 Effect of mangiferin on animal growth, tissue weight, and food and energy intake

There were no differences in body weight among the groups at the beginning of the experiment. However, after 8 wk, the body weight of hamsters in the HF group was significantly higher than that in the NC group. Mangiferin (50 and 150 mg/kg BW) treatment for 8 wk significantly reduced body weight. There was no significant difference in food intake among the groups, whereas energy intake was significantly lower in the NC group compared with the HF group. The relative weights of liver, epididymal adipose tissue and perirenal adipose tissue (g/100 g BW) were significantly lower in the mangiferin treatment groups than in the HF group, whereas spleen weight was not significantly different among the groups. Kidney and testicle weights were significantly higher in the mangiferin treatment groups than in the HF group (Table 3).

3.2 Effect of mangiferin on serum metabolites

Concentrations of serum TG, TC, HDL-C, and LDL-C were significantly higher in the HF group compared to the NC group. However, the HDL-C/TC ratio was significantly lower in the HF group. Mangiferin administration significantly reduced serum TG concentrations, but had no effect on serum TC, HDL-C/TC and LDL-C levels compared to the HF group (Table 4). Serum glucose concentrations did not significantly differ among the groups (data not shown).

Table 2. Primers used for the PCR reaction

Gene	Accession number	Sense and antisense	PCR product (bp)	Annealing temperature (°C)
β-Actin	AJ312092	5′GCTGTCCCTGTATGCCTCT3′ 5′CTCGTTGCCAATGGTGAT3′	343	56.7
LPL	AB194713	5′CAGCTGGGCCTAACTTTGAG3′ 5′CCTCTCTGCAATCACACGAA3′	215	54.6
PPAR-α	AJ555631	5′GAAACTGCCGACCTCAAAT3′ 5′CAGCATTCCGTCTTTGTTC3′	399	54.4
CD36	U42430	5′AGCCTCACCAGACTATTT3′ 5′ACTGTTCACTGCCACTTC3′	382	50.1
CPT-1	AY762566	5′ATCTTCCAGTTGGGCTACG3′ 5′GCAGGTCCACATCATTCG3′	163	54.1
SREBP-1c	U09103	5′AGACAAACTGCCCATCCATC3′ 5′CACCCTCCATAGCCACATCT3′	165	57.2
ACC	AF356089	5′TGTGAGCCTGAGGAATAGCA3′ 5′GAGCAATCCACCATCACTCA3′	223	52.8
DGAT-2	NM026384	5′TCTCAGCCCTCCAAGACATC3′ 5′ATGCCAGCCAGGTGAAGTAG3′	195	54.7
MTP	U14995	5′TGCAGAGACCCGTTCTTCTT3′ 5′CATGTGTCCAGGGCCTTAGT3′	226	54.0

Table 3. Effect of mangiferin on body weight, food intake, organs, and adipose tissues weights

	NC	HF	ML	MH
Body weight				
Initial, g	106.8±6.3	107.0±5.9	106.5±3.5	106.5±6.7
Final, g	133.5±4.2a	152.5±6.6b	141.0±7.9c	136.8±5.4ac
Organs				
Liver, g/100g BW	3.33±0.23a	4.25±0.27b	3.92±0.40c	3.61±0.25d
Kidney, g/100g BW	0.79±0.03b	0.74±0.06a	0.81±0.05b	0.79±0.04b
Spleen, g/100g BW	0.08±0.01	0.08±0.01	0.09±0.01	0.08±0.01
Testicle, g/100g BW	2.81±0.19a	2.41±0.12b	2.59±0.29b	2.79±0.17a
Adipose tissue				
Epididymal WAT, g/100g BW	1.78±0.41b	2.73±0.29a	2.26±0.30c	2.08±0.22bc
Perirenal WAT, g/100g BW	1.43±0.31c	1.92±0.29a	1.71±0.28ab	1.57±0.12bc
Food intake, g/day	8.10±0.21	7.93±0.27	8.04±0.40	8.11±0.25
Energy intake, kcal/day	31.44±0.81a	34.57±1.16b	35.06±1.76b	35.35±1.10b

Values are mean±SD, $n = 10$ hamsters in each group. NC: normal control group, HF: high-fat control group, ML: low-dosage mangiferin group (high-fat diet+50 mg/kg BW), MH: high-dosage mangiferin group (high-fat diet+150 mg/kg BW). a, b, c, d: Means in the same row with different online letters differ significantly, $p < 0.05$.

Table 4. Effect of mangiferin on serum lipid profile

	NC	HF	ML	MH
Triglyceride (mmol/L)	1.34±0.31a	3.00±0.58c	2.34±0.42b	2.02±0.65b
TC (mmol/L)	4.38±0.62a	6.68±0.58b	6.56±1.22b	6.34±0.42b
HDL-C (mmol/L)	0.63±0.08a	0.81±0.11b	0.81±0.23b	0.84±0.11b
HDL-C/TC (%)	0.15±0.02a	0.12±0.02b	0.12±0.02b	0.13±0.02ab
LDL-C (mmol/L)	3.48±0.59a	4.86±0.67b	4.54±0.88b	4.34±0.49b

Values are mean±SD, $n = 10$ hamsters in each group. NC: normal control group, HF: high-fat control group, ML: low-dosage mangiferin group (high-fat diet+50 mg/kg BW), MH: high-dosage mangiferin group (high-fat diet+150 mg/kg BW). a, b, c: Means in the same row with different online letters differ significantly, $p < 0.05$.

3.3 Effect of mangiferin on liver lipids and histology

Hamsters in the HF group had significantly higher hepatic TG and TC levels than animals in the NC group. Mangiferin treatment significantly decreased hepatic TG levels and the size of hepatic fatty droplets in hamsters fed a high-fat diet, but had no effect on hepatic TC (Fig. 1).

3.4 Quantitative changes in serum, liver and muscle FFAs in hamsters treated with mangiferin

Quantitative changes in serum, liver and muscle FFAs in hamsters in the different experimental groups are shown in Tables 5 and 6. Fifteen FFAs were detected in serum ($n = 8$) and fourteen FFAs in liver ($n = 8$) and muscle ($n = 8$). Total serum FFA, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 fatty acids and n-6 fatty acids significantly increased in the HF group. Mangiferin treatment significantly decreased serum total FFA, SFA, MUFA, PUFA, $n-3$ FFA and n-6 FFA. However, serum concentrations of γ-C18:3, C22:5 and C22:6 did not differ among the groups. Levels of most fatty acids in liver and muscle were also significantly increased in the HF group, but levels of C22:0 in liver and of γ-C18:3 and C22:5 in muscle were not significantly different. Conversely, muscle C20:5 was lower in the HF compared with the NC group. Liver and muscle levels of most fatty acids were significantly decreased in the mangiferin treatment groups. Mangiferin had no effect on liver C14:0, γ-C18:3 and C20:2 levels or muscle C14:0, γ-C18:3 and C20:5 levels.

3.5 Effect of mangiferin on mRNA expression in liver and muscle

To investigate the molecular mechanism of the hypolipidemic effect of mangiferin, expression levels of genes related to lipid metabolism were determined by RT-PCR. We measured gene expression for peroxisome proliferator-activated receptor-α (PPAR-α), lipoprotein lipase (LPL), fatty acid translocase (CD36), carnitine palmitoyltransferase 1 (CPT-1), sterol regulatory element-binding protein 1c (SREBP-1c), acetyl CoA carboxylase (ACC), acyl-CoA:diacylglycerol acyltransferase 2 (DGAT-2) and microsomal triglyceride transfer protein (MTP) in liver tissue (Fig. 2).

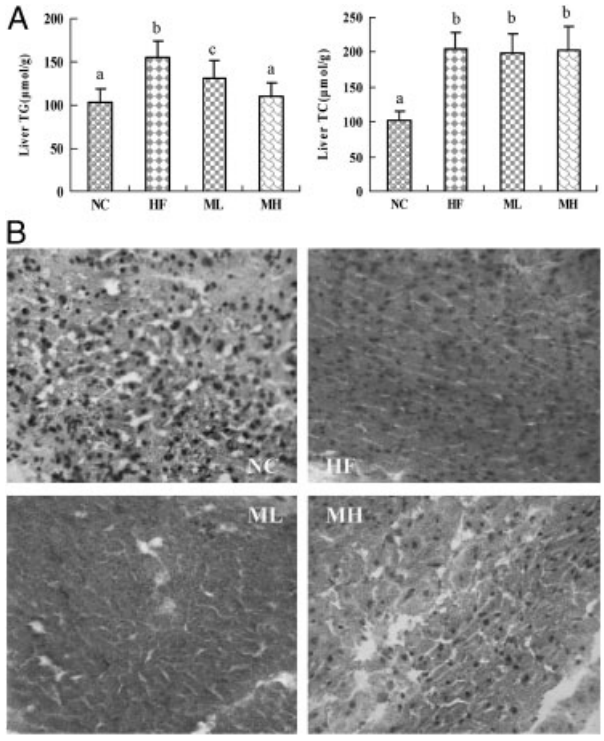

Figure 1. Hepatic TG and TC. (A) After 8 wk of mangiferin treatment, hepatic TG (left) and TC (right) levels were quantitatively measured. Values are mean ± SD ($n = 10$/group). (B) Oil Red O staining of liver tissue sections was used to assess lipid accumulation (200 ×). NC, normal control group; HF, high-fat control group; ML, low-dose mangiferin group (high-fat diet + 50 mg/kg BW); MH, high-dose mangiferin group (high-fat diet + 150 mg/kg BW). a, b, c: Means with different superscript letters differ significantly, $p < 0.05$.

Expression of PPAR-α and CPT-1 significantly decreased in the HF compared with the NC group. However, expression of SREBP-1c, CD36, ACC, DGAT-2, and MTP significantly increased. Treatment with mangiferin significantly enhanced mRNA expression of PPAR-α, CD36, and CPT-1. In contrast, mRNA expression of ACC, MTP, DGAT-2 and SREBP-1c was significantly decreased by mangiferin treatment. LPL mRNA expression did not differ among the groups (data not shown). Gene expression for PPAR-α, LPL, CD36 and CPT-1 was also determined in muscle tissue (Fig. 3). Expression of PPAR-α, LPL, and CPT-1 significantly decreased in the HF group and increased in the mangiferin treatment groups. Expression of CD36 also significantly increased in the HF compared with the NC group. Mangiferin treatment significantly increased CD36 mRNA expression compared with the HF group.

4 Discussion

Our results reveal that mangiferin administration significantly decreased serum TG and total FFA in hamsters fed a high-fat diet, but had no significant effect on serum TC, LDL-C, and HDL-C. We also observed that mangiferin treatment significantly reduced liver TG and FFA and muscle FFA in hamsters fed a high-fat diet. Furthermore, mangiferin treatment had no effect on fecal lipid excretion compared with the HF group (data not shown). The above results demonstrate that mangiferin can specifically affect triglyceride and fatty acid metabolism in hamsters. To explore the mechanisms of action, mRNA expression of genes involved in TG and fatty acid metabolism were determined by RT-PCR.

Miura et al. [6] showed that oral administration of mangiferin (30 mg/kg) with exercise for 2 wk significantly reduced blood TG and TC levels in KK-Ay mice. Muruganandan et al. [13] also reported that mangiferin (10 and 20 mg/kg, i.p.) treatment significantly decreased plasma TC, TG, LDL-C and increased HDL-C in STZ diabetic rats. KK-Ay mice, a widely used animal genetic model of type 2 diabetes mellitus, are characterized by severe obesity, hyperinsulinemia, insulin resistance [16], and hyperlipidemia [17]. STZ is a naturally occurring, broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to pancreatic insulin-producing β-cells in mammals. Diabetes and dyslipidemia can be induced by selective destruction of β-cells with a single rapid injection of STZ [18, 19]. However, the above two animal models may not be suitable for studying hyperlipidemia, since this rapidly increasing incidence of hyperlipidemia is attributed mainly to lifestyle rather than genetic or chemical factors. Therefore, animal models of diet-induced hyperlipidemia could mimic the pathophysiology of human metabolic hyperlipidemia better and provide more translatable knowledge [20]. Rats, mice and hamsters are rodents commonly used to test the hypolipidemic effect of plant extracts or phytochemicals. However, serum TG levels in mice and rats did not increase on a high-fat diet in a preliminary experiment we carried out (data not shown), in accordance with some previous studies [19–21]. Furthermore, it has been shown that cholesterol metabolism in hamsters closely resembles that in humans, in contrast to other rodent species [21, 22]. Therefore, hamsters were considered to be the best animal model for studying lipid metabolism in rodents, and the lack of effect of mangiferin on hypercholesterolemia in hamsters might be partly due to differences in cholesterol metabolism in hamsters compared with other rodent species.

Dietary TGs are digested by pancreatic lipase primarily in the lumen of the small intestine to fatty acids and monoglycerols that enter the cytosol of intestinal cells. These moieties are then reassembled into chylomicrons that enter the lymphatic system and eventually the systemic circulation. Chylomicrons are hydrolyzed to FFA by various lipases located on the endothelial surface of capillaries [23]. In liver, the major organ for lipid metabolism, FFAs enter hepatic cells, where they can either be oxidized in mitochondria to form ATP or esterified to produce TG for storage or incorporation into VLDL particles [24]. Considering the important

Table 5. Quantitative changes in serum FFAs in hamsters treated with mangiferin

Fatty acids (μg/mL)	NC	HF	ML	MH
C14:0	5.98±1.67a	14.23±3.36b	10.99±2.11c	9.70±1.89c
C16:0	629.08±48.12a	1003.60±77.89b	936.08±98.80b	736.60±79.68c
C16:1*n*−7	52.40±9.94a	80.70±14.35b	71.55±9.63bc	63.77±6.93c
C18:0	251.30±31.20a	381.53±35.92b	333.56±52.13c	281.69±34.75a
C18:1*n*−9	408.57±84.28a	844.91±144.13b	642.67±86.93c	498.50±57.41a
C18:2*n*−6	955.74±87.39a	1481.49±138.48b	1304.44±101.87c	1112.39±93.60d
γ-C18:3*n*−6	15.77±2.34	16.08±4.88	16.24±3.85	15.62±3.48
C18:3*n*−3	34.75±9.06a	64.75±12.38b	55.05±10.07bc	47.67±8.85c
C20:2*n*−6	9.18±2.01a	16.05±3.45b	14.79±2.43bc	13.02±1.78c
C20:4*n*−6	199.12±22.66a	289.70±25.07b	263.43±17.34c	214.49±26.30a
C20:5*n*−3	4.55±0.68b	6.19±1.22a	4.92±0.87b	4.28±1.59b
C22:4*n*−6	41.96±5.99a	53.42±8.55b	48.00±9.61ab	44.16±6.79a
C22:5*n*−3	21.38±4.99	22.43±6.30	22.58±6.09	22.26±4.02
C22:6*n*−3	27.82±6.86	27.83±4.71	27.87±4.14	25.67±6.61
C24:0	184.33±29.32a	246.79±48.06b	213.29±47.32ab	196.30±42.17a
Total SFA	1070.70±66.92a	1646.16±104.79b	1493.92±84.50c	1224.29±106.49d
Total MUFA	460.96±88.92a	925.61±154.22b	714.22±90.89c	562.27±59.30a
Total PUFA	1310.27±124.01a	1977.95±145.46b	1757.32±123.75c	1499.56±120.53d
Total *n*−3 FA	88.50±19.60a	121.20±13.96b	110.42±13.83bc	99.89±10.97ac
Total *n*−6 FA	1221.77±110.26a	1856.74±140.83b	1646.90±118.32c	1399.68±116.32d
Total	2841.93±242.91a	4549.71±250.68b	3965.45±232.12c	3286.12±208.07d

Values are mean±SD, $n = 8$ hamsters in each group. NC: normal control group, HF: high-fat control group, ML: low-dosage mangiferin group (high-fat diet+50mg/kg BW), MH: high-dosage mangiferin group (high-fat diet+150 mg/kg BW). a, b, c, d: Means in the same row with different online letters differ significantly, $p < 0.05$.

Table 6. Quantitative changes in liver and muscle FFAs in hamsters treated with mangiferin

	Liver				Muscle			
Fatty acids (mg/g)	NC	HF	ML	MH	NC	HF	ML	MH
C14:0	0.12±0.05a	0.20±0.03b	0.18±0.04b	0.017±0.04b	0.02±0.01a	0.04±0.03b	0.04±0.01ab	0.03±0.02ab
C16:0	11.62±1.65a	20.95±2.60b	18.35±3.60bc	16.58±3.76c	1.96±0.25a	2.60±0.59b	2.08±0.19a	1.85±0.18a
C16:1*n*−7	1.09±0.29a	2.99±0.71b	2.78±0.59b	1.46±0.33a	0.15±0.05a	0.22±0.18b	0.19±0.13b	0.15±0.06a
C18:0	8.67±1.86a	18.12±3.86b	13.52±2.28c	8.84±3.38a	1.16±0.17a	1.90±0.33b	1.48±0.09c	1.39±0.15c
C18:1*n*−9	19.17±4.93a	49.88±11.81b	39.40±5.90b	14.88±2.38a	0.98±0.26a	1.60±0.95b	1.25±0.38ab	1.15±0.22a
C18:2*n*−6	12.63±2.92a	25.82±2.61b	20.62±4.01c	21.07±3.74c	1.68±0.34a	2.67±0.76b	2.20±0.31c	1.89±0.25ac
γ-C18:3*n*−6	0.16±0.05a	0.38±0.13b	0.38±0.17b	0.48±0.15b	0.01±0.00	0.02±0.01	0.01±0.00	0.01±0.00
C18:3*n*−3	0.58±0.22a	1.15±0.52b	1.08±0.29bc	0.70±0.42ac	0.04±0.02a	0.09±0.05b	0.06±0.03a	0.06±0.01a
C20:2*n*−6	0.42±0.18	0.53±0.08	0.46±0.18	0.51±0.42	0.03±0.01a	0.07±0.02b	0.05±0.03bc	0.04±0.01ac
C20:4*n*−6	3.38±0.54a	8.28±1.52b	5.55±1.32c	6.28±0.77c	0.55±0.08a	0.80±0.12b	0.61±0.11a	0.55±0.05a
C20:5*n*−3	–	–	–	–	0.02±0.00a	0.01±0.00b	0.01±0.00b	0.01±0.00b
C22:4*n*−6	0.46±0.17a	1.31±0.27b	0.75±0.18c	0.69±0.18c	0.37±0.09a	0.66±0.19c	0.49±0.05b	0.43±0.05ab
C22:5*n*−3	0.19±0.08a	0.46±0.10b	0.35±0.07c	0.33±0.10c	0.29±0.06a	0.32±0.05a	0.19±0.05b	0.21±0.06b
C22:6*n*−3	7.19±1.49a	16.00±2.36b	10.10±1.50c	9.30±1.91c	3.04±0.42a	3.86±0.75b	2.81±0.43a	2.90±0.33a
Total SFA	20.41±3.34a	39.26±5.99b	32.05±5.67c	25.59±5.94a	3.14±0.38a	4.54±0.93b	3.60±0.25a	3.28±0.19a
Total MUFA	20.26±5.16a	52.88±12.42b	42.18±6.35b	16.35±2.60a	1.12±0.30a	1.82±1.12b	1.44±0.49ab	1.31±0.25ab
Total PUFA	25.01±4.70a	53.93±4.98b	39.29±6.39c	39.35±4.38c	6.04±0.97a	8.50±1.86b	6.43±0.59a	6.11±0.65a
Total *n*−3 FA	7.97±1.68a	17.61±2.63b	11.53±1.73c	10.33±1.72c	3.39±0.48a	4.05±0.57b	3.07±0.48a	3.19±0.37a
Total *n*−6 FA	17.04±3.33a	36.32±3.22b	27.75±4.99c	29.02±4.01c	2.65±0.51a	3.87±0.44c	3.36±0.34b	2.92±0.32ab
Total	65.68±12.41a	146.07±19.77b	113.52±16.97c	81.29±6.80d	10.30±1.56a	14.86±3.84b	11.47±1.18a	10.69±0.93a

Values are mean±SD, $n = 8$ hamsters in each group. NC: normal control group, HF: high-fat control group, ML: low-dosage mangiferin group (high-fat diet+50 mg/kg BW), MH: high-dosage mangiferin group (high-fat diet+150 mg/kg BW). a, b, c, d: Means in the same row with different online letters differ significantly, $p < 0.05$. – values cannot be detected.

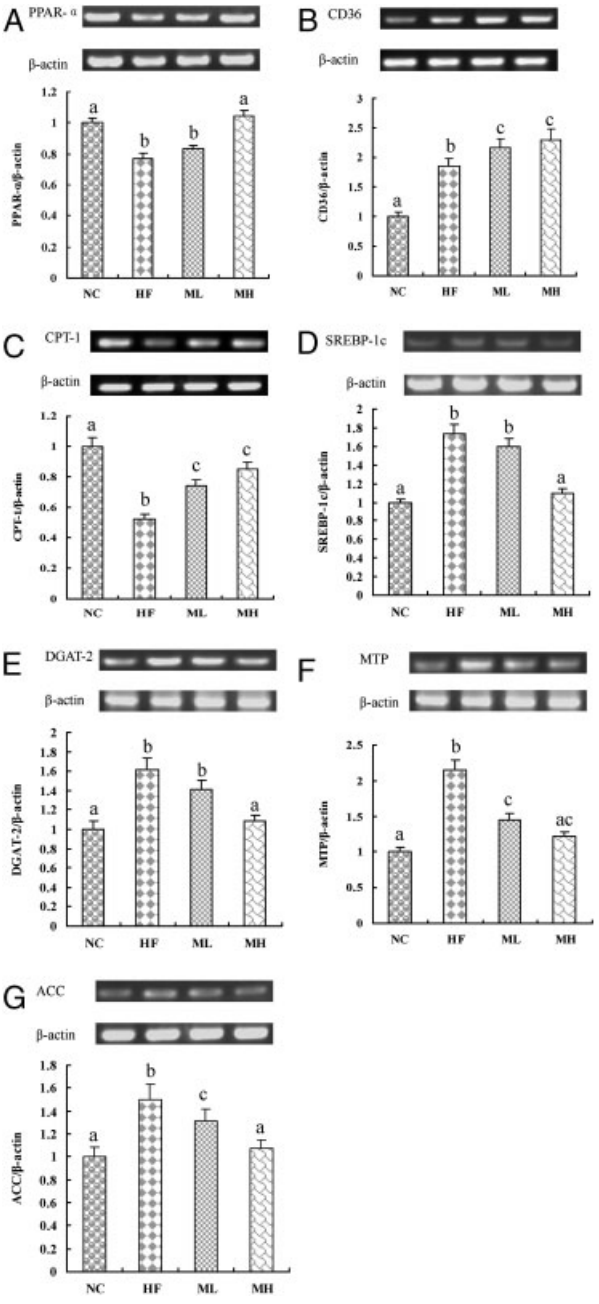

Figure 2. Hepatic expression of (A) PPAR-α, (B) CD36, (C) CPT-1, (D) SREBP-1c, (E) DGAT-2, (F) MTP and (G) ACC mRNA in hamsters treated with mangiferin for 8 wk. Total mRNA was extracted from liver using TRIzol. Relative mRNA levels were assessed by RT-PCR and results were normalized to β-actin. All values are mean ± SD ($n = 5$/group). a, b, c: Means with different superscript letters differ significantly, $p < 0.05$.

role of the liver in lipid metabolism, we investigated mRNA expression of genes involved in lipogenesis and lipid oxidation in this organ.

The decrease in TG and FFA levels in serum and liver observed in the mangiferin treatment groups could be

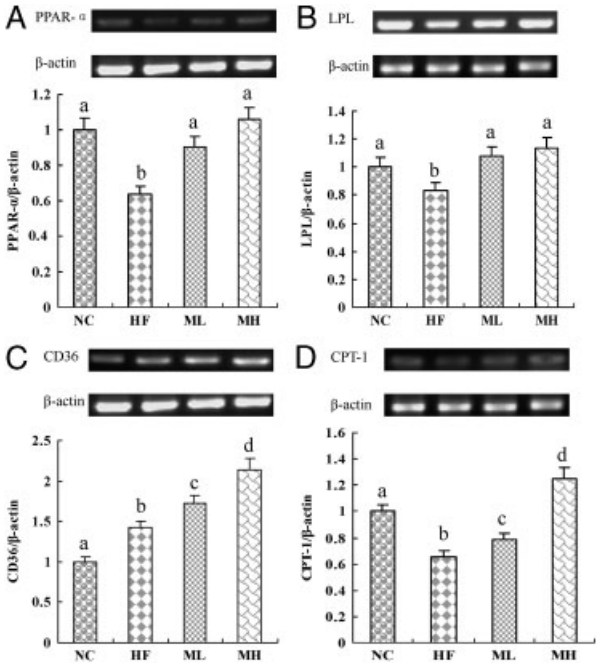

Figure 3. Muscle expression of (A) PPAR-α, (B) LPL, (C) CD36 and (D) CPT-1 mRNA in hamsters treated with mangiferin for 8 wk. Total mRNA was extracted from muscle using TRIzol. Relative mRNA levels were assessed by RT-PCR and results were normalized to β-actin. All values are mean ± SD ($n = 5$/group). a, b, c, d: Means with different superscript letters differ significantly, $p < 0.05$.

mediated by reductions in the expression levels of some hepatic lipogenic genes. DGAT is a microsomal enzyme that join acyl CoA to 1,2-diacylglycerol and thus constitutes the final step in TG biosynthesis. In mammals, DGAT occurs in two isoforms, DGAT-1 and DGAT-2, encoded by distinct gene families [25]. Although both isoforms are widely expressed and present at high levels in white adipose tissue, DGAT-1 is most highly expressed in the small intestine, whereas DGAT-2 is primarily expressed in the liver [26]. A previous study demonstrated that pharmacological reduction of DGAT-2 expression in liver using antisense oligonucleotides (ASOs) could markedly reduced hepatic lipids (diacylglycerols and TG) [25]. A significant decrease in DGAT-2 mRNA expression in liver after mangiferin supplementation was observed in our study. This indicates that mangiferin may reduce liver TG levels by inhibiting DGAT-2 gene expression.

MTP is a heterodimeric lipid transfer protein present in the endoplasmic reticulum of hepatocytes and intestinal cells. MTP plays an important role in VLDL assembly by mediating the transfer of hepatic lipids to nascent apolipoprotein B [27]. It was reported that MTP gene expression is enhanced in the liver of both fructose-fed hamsters and obese diabetic mice [28]. In the current study, MTP mRNA expression was significantly increased in hamsters fed a high-fat diet, and this overexpression was significantly

inhibited by mangiferin. Therefore, it is possible that the reduction in serum TG levels induced by mangiferin was the result of decreased MTP gene expression.

SREBP-1c is one of three SREBP isoforms that belong to the basic helix-loop-helix-leucine zipper (Bhlh-Zip) family of transcription factors [29]. SREBP-1c plays a crucial role in the dietary regulation of most hepatic lipogenic genes such as ACC [30], DGAT-2 [25] and MTP [28, 31]. We found that mangiferin treatment induced a significant decrease in SREBP-1c expression levels. The results indicate that the reductions in SREBP-1c, DGAT-2 and MTP levels induced by mangiferin treatment partly account for the TG-lowering effect of mangiferin in hyperlipidemic hamsters.

PPARs are ligand-activated transcription factors that belong to the nuclear receptor superfamily. Three PPAR receptor subtypes termed α, β and γ have been identified [32]. PPAR-α is highly expressed in liver, skeletal muscle and brown adipose tissue [32, 33], where it upregulates genes involved in fatty acid oxidation. In the present study, liver mRNA expression of PPAR-α in the HF group was significantly reduced compared with the NC group, in accordance with a previous study [34]. PPAR-α downregulation was accompanied by increases in serum, liver and muscle TG and FFA, which indicates that reduced lipid breakdown occurred in hamsters fed a high-fat diet. FAT/CD36 is involved in fatty acid uptake [35] and CPT-1 catalyzes fatty acid β-oxidation [36]. Both CD36 and CPT-1 are responsive to PPAR-α activation [37]. We found that liver CPT-1 expression was markedly decreased and liver CD36 expression was markedly increased in hamsters fed a high-fat diet. The opposite expression trends for CD36 and CPT-1 in the HF group are somewhat contrary to our expectations, but are in agreement with studies by Koonen et al. [38] and Lelliott et al. [39]. These results indicate that the increases in liver TG and FFA might be the result of increased FFA infusion and decreased lipid oxidation. In our study, mangiferin treatment significantly increased liver mRNA expression of PPAR-α, CD36 and CPT-1 and decreased mRNA expression of ACC. CPT-1 activity is inhibited by malonyl-CoA. Inhibition of ACC activity leads to a decrease in malonyl-CoA production, which results in suppression of fatty acid synthesis and, contrarily, enhancement of fatty acid β-oxidation [40]. These results demonstrate that mangiferin could markedly enhance FFA oxidation by activating PPAR-α and its target genes in liver.

As skeletal muscle is another major organ for lipid catabolism, the expression of genes involved in TG hydrolysis, fatty acid uptake and oxidation was measured. LPL is an enzyme that hydrolyzes circulating TG and delivers FFA to peripheral tissues for utilization and storage. It is highly expressed in skeletal muscle, which is a major TG clearance site [41]. We found that TG and FFA levels were reduced and LPL, CD36, CPT-1 and PPAR-α gene expression was significantly enhanced by mangiferin in skeletal muscle. These results suggest that the beneficial effect of mangiferin in the treatment of hyperlipidemia may be partly due to enhanced TG and FFA catabolism in skeletal muscle. Regrettably, owing to a lack of suitable antibodies, expression levels of proteins involved in lipogenesis and lipid oxidation were not investigated.

In summary, our results suggest that mangiferin has a specific effect on TG and FFA metabolism in hamsters fed a high-fat diet. Mangiferin supplementation probably ameliorates hypertriglyceridemia in these hamsters by downregulating genes involved in lipogenesis in liver and upregulating genes involved in fatty acid β-oxidation in both liver and skeletal muscle.

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