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ORIGINAL ARTICLE

Influence of ursodeoxycholic acid on hepatic lipid deposition, blood glucose, and insulin levels in high-fat -fed Sprague–Dawley rats

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Abstract

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is becoming a growing public health concern. MASLD often coexists with abnormal glucose metabolism. Currently, there is no standard treatment for MASLD. **Aim:** To elucidate the influence of ursodeoxycholic acid (UDCA) on hepatic lipid deposition, blood glucose, and insulin levels in high-fat diet-induced Sprague–Dawley (SD) rats. **Methods:** SD rats were randomly assigned to three groups: Group A, which comprised a normal-diet control group; Group B, which comprised a high-fat-fed group; and Group C, which comprised rats treated with UDCA combined with a high-fat diet. At the end of week 4, we performed oral glucose tolerance tests, measured liver enzymes, triglyceride (TG) and total cholesterol (TC) contents, identified morphological changes of the liver, and quantified the expression of major genes in fatty acid, glucose, and bile acid metabolic pathways using real-time polymerase chain reaction. **Results:** In comparison to Group A, Group B had higher postprandial glucose, insulin, C-peptide, and hepatic TG and TC concentrations (all $p < 0.05$); metabolism-related genes were differentially expressed in Group B. In comparison to Group B, the values of these indices were all decreased in Group C (all $p < 0.05$); UDCA reversed the mRNA expression of metabolism-related genes in Group C (all $p < 0.05$). **Conclusion:** UDCA administration decreased hepatic lipid content, blood glucose, and insulin levels in SD rats. **Relevance for patients:** UDCA may be helpful in the treatment of MASLD and its related comorbidities in clinical practice.

Keywords: Ursodeoxycholic acid; Metabolic dysfunction-associated steatotic liver disease; Glucose; Insulin; Gene expression

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1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease.[1] The total prevalence of MASLD worldwide was estimated to be 32.4% and is growing at an astonishing rate.[2] Therefore, there is an urgent need for effective management of this disease. However, the pathogenesis of MASLD remains unclear. There is increasing evidence of a strong correlation between obesity and MASLD.[3] MASLD typically begins as simple steatosis and can advance to metabolic

dysfunction-associated steatohepatitis (MASH). Its characteristics are steatosis, inflammation, and fibrosis. MASH can further progress toward cirrhosis and, in a subset of patients, eventually culminate in hepatocellular carcinoma. Steatosis results from triglyceride (TG) accumulation in hepatocytes due to *de novo* lipogenesis (DNL).[4] Intrahepatic lipid accumulation may be partially induced by elevated hepatic fatty acid (FA) influx, enhanced DNL, and/or reduced clearance of lipid through secretion or oxidation.[5] In the liver, the synthesis of new FAs starts with acetyl-CoA; acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, which is further utilized by FA synthase (FAS) to synthesize palmitate.[6] The last reaction in FA synthesis is mediated by FAS, and FAS is believed to determine the maximal capacity for liver DNL.[7] The enhancement of lipogenic gene transcription by glucose and insulin requires carbohydrate-response element-binding protein (ChREBP).[8] Carnitine palmitoyltransferase 1 (CPT-1) acts as the inhibitory enzyme in mitochondrial FA oxidation, responsible for the transport of long-chain FA into the mitochondria.[9]

Upregulation of hepatic fibrillar collagen genes has been shown to be associated with MASH.[10] Additionally, correlations have been observed between bile acid (BA) levels and MASH severity in obese individuals. BAs are generated from cholesterol through classic and alternative pathways in human hepatic tissue. Cholesterol 7α-hydroxylase (CYP7A1) initiates the classic pathway; this pathway produces over 75% of total BA, and further steps in the synthesis of BA require cytochrome P450 family 8 subfamily B member 1 (CYP8B1).[11,12] BAs function as metabolic integrators that coordinate glucose, lipid, and energy balance. BAs exert their regulatory effects via specific BA-activated receptors, including nuclear receptor superfamily members—such as farnesoid X receptor (FXR)—and G-protein-coupled receptor superfamily members—such as Takeda G-protein-coupled receptor-5 (TGR5).[13] In humans, steatosis can be detected noninvasively by imaging assessments, comprising ultrasonography and tomographic techniques. Although the diagnosis of MASLD is straightforward, the diagnosis of MASH requires liver biopsy.[4,14] Since MASLD is associated with a spectrum of metabolic dysfunctions, including insulin resistance, abnormal glucose levels, obesity, and dyslipidemia, its pathogenesis remains unclear.[15] Ursodeoxycholic acid (UDCA) is structurally derived as the 7β-hydroxy epimer of the primary BA chenodeoxycholic acid.[16] Numerous studies have established a relationship between BA levels and improved liver biochemical parameters.[17] UDCA protects against oxidative and inflammatory damage and mitochondrial injury during the progression of obesity-associated diseases. A previous study reported that UDCA could improve insulin resistance and liver function in cirrhotic rats.[18] In the current study, we aim to determine whether UDCA could improve the abnormal metabolism in patients with MASLD and to further explore the underlying mechanisms involved. We developed an obese rat model using high-fat diet (HFD) administration to study the effects of UDCA on the hepatic lipid content, glycemic parameters, and insulin secretory function, and quantified the gene transcripts governing lipid, fibrosis, BA, and glycemic homeostasis. This study provides a foundation for UDCA therapy in MASLD and its comorbidities for future clinical application.

2. Methods

2.1. Animals and diets

After one week of environmental adaptation, pathogen-free male Sprague–Dawley (SD) rats (n = 30; 4–6 weeks old; 100–150 g, supplied by the Laboratory Animal Center of ShanXi Medical University, China) were randomly assigned to three groups: Group A (the control group, n = 10) received standard chow containing 66.5% carbohydrate, 10.2% fat, and 23.3% protein; Group B (the HFD group, n = 10) fed on a HFD containing 38.4% carbohydrate, 54.2% fat, and 7.4% protein; and Group C (the UDCA-treated group, n = 10), comprised HFD-fed rats with concurrent UDCA administration. (0.25 $g \cdot kg^{-1}$, BC grade, purity ≥ 99.0%, Sangon Biological Engineering Technology and Services Co. Ltd., China; dissolved in normal saline) daily by gavage for four weeks[18], and the dose of UDCA was based on the previous study.[19] Lard was the major constituent of the HFD. All rats were kept in specific pathogen-free cages (n = 5 per cage) under a 12 h light/dark cycle at controlled temperature. Food and water were available freely. We determined daily food intake by subtracting uneaten food from the total amount provided over 24 hours. We euthanized all rats by intraperitoneal injection of sodium pentobarbital (45 $mg \cdot kg^{-1}$ body weight) four weeks after HFD. The Animal Ethics Committee of the Second Hospital of ShanXi Medical University of China approved all experimental protocols (No.20240729, TaiYuan, China).

2.2. Biochemical indices analysis

At four weeks after HFD, serum contents of TG, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) were determined using the Reitman–Frankel colorimetric assay according to the manufacturer's protocol (Jiancheng Corp, China). The procedure details were described in a previous study.[18]

2.3. Oral glucose tolerance test and insulin release test

After four weeks of HFD feeding, rats were fasted overnight and administered glucose (5 g D-glucose·kg^{-1} body weight) by oral gavage. We collected tail blood at 0, 60, and 120 min, and measured blood glucose using a glucometer (Accu-Chek Active, Roche Diagnostics, Switzerland). Insulin and C-peptide levels were measured using a rat enzyme-linked immunosorbent assay kit (RD system; Insulin: NBP3-00515; C-peptide: NBP3-18792, Bio-Techne Corporation, USA). The area under the curve (AUC) for the oral glucose tolerance test (OGTT), insulin, and C- peptide levels was estimated as previously reported.[20] The details of the procedure were described in a previous study.[18]

2.4. Measurement of triglyceride and total cholesterol contents in the liver

Liver tissues were harvested from rats following anesthesia (0.5% pentobarbital sodium [45 mg·kg^{-1} body weight] via intraperitoneal injection) and euthanasia. We extracted TG and TC from the liver and measured them according to the supplier's guidelines (Nanjing Jiancheng Corp, China). The procedure details were described in the previous study.[21]

2.5. Examination of liver histopathology

At the end of the experiment, all rats were euthanized as described in **Section 2.4**. Liver tissues ($n = 5$) were randomly collected by researchers blinded to the experimental groups. We fixed the specimens in 10% formalin, then processed them for paraffin embedding, sliced them into 4 μm serial sections, and stained them with hematoxylin and eosin (H&E) for histopathological assessment; Masson's trichrome was used to evaluate hepatic collagen deposition. We analyzed the images using Image-Pro Plus software (7.0, Media Cybernetics, USA). The details of the procedure were described in the previous study.[18]

2.6. Measurement of quantitative real-time polymerase chain reaction

Total RNA was isolated from hepatic tissue samples. cDNA was then reverse-transcribed using the PrimeScript RT Master Mix (RR036A, Takara Biomedical Technology Co., Ltd., Japan) according to the supplier's protocol. The resulting cDNA was subsequently subjected to quantitative real-time polymerase chain reaction in a 20 μL reaction volume, utilizing SYBR Premix Ex Taq™ II (RR820A, Takara Biomedical Technology Co., Ltd., Japan) and gene-specific primers (Table 1). Amplification was performed using a real-time PCR system (ABI 7300/7500, Applied Biosystems, USA). The procedure details were described previously.[18] Relative mRNA levels were quantified using the ΔΔCq method, with β-actin serving as the housekeeping gene for normalization. All primers were synthesized by Sangon Biological Engineering Technology and Services Co. Ltd. (China).

2.7. Statistical analyses

The results are shown as mean ± standard deviation. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS 25.0, IBM, United States). Intergroup comparisons were performed using analysis of variance. Post-hoc analyses were conducted using either least significant difference (when variance homogeneity was confirmed) or Tamhane's T2 test (when variance homogeneity was violated). A p-value < 0.05 was regarded as statistically significant.

3. Results

3.1. Physical conditions and serum biochemical indices analysis

At baseline, the three rat groups had similar body weight. After four weeks, relative to Group A, both Group B and Group C exhibited significantly elevated mean body weights ($p < 0.01$), and Group B also showed markedly increased serum concentrations of ALT, AST, GGT, TG, ALP (all $p < 0.01$), and LDL-C ($p < 0.05$). However, compared with Group B, body weight in Group C did not change significantly. Furthermore, Group C showed significantly decreased serum AST, GGT, TG, TC (all $p < 0.01$), and ALT ($p < 0.05$) levels compared to Group B (Table 2).

3.2. Oral glucose tolerance test and insulin release test

Following four weeks of HFD, no significant difference in fasting blood glucose was detected between Group A and Group B (fasting: 5.47 ± 0.59 vs. 5.82 ± 0.79 mmol/L, $p > 0.05$), Nevertheless, postprandial glucose concentrations at both the 1 h and 2 h time points were markedly elevated in Group B compared to Group A (1 h: 6.25 ± 0.50 vs. 7.68 ± 1.85 mmol/L; 2 h: 7.10 ± 0.43 vs. 8.20 ± 0.96 mmol/L, all $p < 0.05$).

In comparison to Group B, no significant difference in fasting and 1 h blood glucose after meal was detected in Group C (fasting: 5.82 ± 0.79 vs. 5.04 ± 0.53 mmol/L; 1 h: 7.68 ± 1.85 vs. 6.40 ± 1.87 mmol/L, all $p > 0.05$), while the glucose level of 2 h after meal was significantly lower in Group C (8.20 ± 0.96 vs. 6.24 ± 0.87 mmol/L, $p < 0.01$).

In comparison to Group A, fasting, 1 h and 2 h insulin levels after meal were significantly increased in Group B (fasting: 14.74 ± 0.86 vs. 16.88 ± 1.94 mIU/L [$p < 0.05$]; 1 h: 33.23 ± 4.80 vs. 37.44 ± 5.06 mIU/L [$p < 0.05$], 2 h:

Table 1. Primers used for quantitative polymerase chain reaction

Target gene	Protein encoded	Primer sequence (from 5' to 3')	Amplicon (bp)
Fasn	FAS	F: GGATGTCAACAAGCCCAAGTA R: TTACAGAGGAGAAGGCCACAA	101
Cpt1a	CPT-1	F: GGAGAGAATTTCATCCACTT R: ATGGCTTGTCTCAAGTGCTT	92
Mlxipl	ChREBP	F: AATCCCAGCCCCTACACC R: CTGGGAGGAGCCAATGTG	60
Cyp7a1	CYP7A1	F: TGGCTGTCATGTGCAGGCGCCAA R: ACGCACTGCCTCAGCAAACACACA	159
Cyp8b1	CYP8B1	F: ACCGCAGGCCCTGGGAGCCACC R: ACGTCCCCGTGCTTGGCCCGC	121
Col4a1	COL4A1	F:GCGATAGCACGATAGGACAAGAAG R: GTCCAGGAGGTCCAGGTTCAC	168
Nr1h4	FXR	F: CGGGAAACCCCACTGACACGCCC R: ACGAGGCGGTCCCTAGCTCAGT	85
Pck1	PEPCK	F: ACGGGCGGCTGCTGGCCCACAT R: AGCGGCCCAGCTGGCTCTGCCCAC	189
G6pc	G6PC	F: GGTGTGAGCGCCCCGAGTGGGT R: TGCAGCAGGCCCAAGGAGGCCACG	190
Gpbar1	TGR5	F: GCCCGCTGTGGGGGCCACTGCCCT R: GGGTGCATCACGGCACACCGCCCGC	109
Actb	β-actin	F: AAGTCCCTCACCCTCCCAAAAG R: AAGCAATGCTGTCACCTTCCC	96

Abbreviations: ChREBP: Carbohydrate-response element-binding protein ; COL4A1: Collagen type IV alpha 1 chain; CPT-1: Carnitine palmitoyltransferase 1; CYP7A1: Cholesterol 7α-hydroxylase; CYP8B1: Cytochrome P450 family 8 subfamily B member 1; FAS: Fatty acid synthase; FXR: Farnesoid X receptor; G6PC: Glucose-6-phosphatase catalytic subunit; PEPCK: Phosphoenolpyruvate carboxykinase; TGR5: Takeda G-protein-coupled receptor-5.

19.33 ± 3.41 vs. 24.80 ± 3.71 mIU/L [$p < 0.01$)]). Group C displayed a significant reduction in fasting insulin levels relative to Group B (16.88 ± 1.94 vs. 14.91 ± 0.60 mIU/L; $p < 0.05$), while 2 h insulin levels after meal were markedly lower in Group C (24.80 ± 3.50 vs. 20.51 ± 1.76 mIU/L, $p < 0.05$).

In comparison to Group A, Group B had higher fasting and 2 h C-peptide levels after meal (fasting: 129.21 ± 3.55 vs. 135.19 ± 3.87 pmol/L; 2 h: 122.57 ± 4.13 vs. 128.27 ± 4.17 pmol/L, all $p < 0.01$). Compared to Group B, fasting C-peptide levels were not significantly different (fasting: 135.19 ± 3.87 vs. 131.32 ± 2.46, $p > 0.05$) and 1 h and 2 h C-peptide levels after meal were significantly lower (1 h: 177.64 ± 7.03 vs. 171.30 ± 4.13 pmol/L, $p < 0.05$; 2 h: 128.27 ± 3.94 vs. 122.20 ± 3.56 pmol/L, $p < 0.01$) in Group C.

Compared to Group A, Group B exhibited a significantly greater AUC for OGTT relative to Group A (12.53 ± 0.94 vs. 15.78 ± 1.91 mmol/L·h, $p < 0.05$); and the AUC values for the concentrations of insulin increased (50.27 ± 6.80 vs. 58.28 ± 6.40 mIU/L·h, $p < 0.05$), the AUC values for the concentrations of C-peptide were not significantly different (302.77 ± 8.44 vs. 309.38 ± 5.35 pmol/L·h, $p > 0.05$). In comparison to Group B, the AUC values for the OGTT decreased after UDCA treatment in Group C (15.78 ± 1.91 vs. 11.53 ± 2.51 mmol/L·h, $p < 0.05$), and the AUC for insulin levels were not markedly different (58.29 ± 6.40 vs. 52.37 ± 3.61 mIU/L·h, $p > 0.05$), the AUC for C-peptide concentrations was significantly decreased in Group C (309.38 ± 5.35 vs. 298.06 ± 5.15 pmol/L·h, $p < 0.01$) (Figure 1).

Table 2. Biometric parameters and serum biochemical analysis in the three groups at the end of the study

Group	A (Normal, n = 10)	B (HFD, n = 10)	C (HFD + UDCA, n = 10)
Body weight (g)	237.58 ± 11.71	366.57 ± 46.67**	360.81 ± 58.59
Food intake (g/24 h)	30.45 ± 1.30	31.90 ± 3.70	31.13 ± 2.17
AST (U/L)	56.71 ± 22.98	98.42 ± 19.30**	49.64 ± 10.21##
ALT (U/L)	23.28 ± 7.85	44.57 ± 15.51**	25.27 ± 4.46#
ALP (U/L)	59.84 ± 10.40	118.56 ± 38.56**	97.81 ± 43.10
GGT (U/L)	23.08 ± 3.24	31.39 ± 2.10**	22.97 ± 2.35##
TG (mmol/L)	1.24 ± 0.47	2.66 ± 0.90**	1.38 ± 0.68##
TC (mmol/L)	1.60 ± 0.56	2.14 ± 0.70	1.17 ± 0.13##
LDL-C (mmol/L)	1.38 ± 0.64	1.98 ± 0.78*	1.39 ± 0.56
Liver TG (mmol/g)	29.42 ± 4.01	45.38 ± 2.61**	41.17 ± 2.41##
Liver TC (mmol/g)	22.42 ± 4.16	33.35 ± 3.98*	29.41 ± 1.51#

Notes: *p < 0.05 vs. Group A; **p < 0.01 vs. Group A; #p < 0.05 vs. Group B; ##p < 0.01 vs. Group B; Group A: Rats fed on a normal chow; Group B: Rats fed on an HFD; Group C: Rats fed on an HFD intervened with UDCA. Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; HFD: High-fat diet; LDL-C: Low-density lipoprotein cholesterol; TC: Total cholesterol; TG: Triglycerides; UDCA: Ursodeoxycholic acid.

3.3. Triglyceride and total cholesterol contents in the liver

Relative to Group A, Group B displayed a marked elevation in hepatic TG and TC concentrations, and Group C displayed a marked decrease in hepatic TG and TC concentrations relative to Group B (all p < 0.01, p < 0.05, respectively) (Table 2, Figure 2).

3.4. Morphological changes in the liver

In comparison to Group A, hepatic lipid droplet accumulation was substantially more pronounced in Group B than in Group A. In comparison with Group B, Hepatic lipid droplet accumulation in Group C was reduced. In comparison with Group A, collagen deposition (blue staining) in Group B was increased, and in comparison with Group B, collagen deposition was decreased in Group C (Figure 2).

3.5. The expression of genes related to lipid, glucose, and bile acid metabolism in the liver

In Group B, relative to Group A, the mRNA expression of *Mlxipl* (encoding ChREBP) was upregulated by approximately 1.1 fold (p < 0.01), *Fasn* (encoding FAS) mRNA level was upregulated by approximately 1.0 fold (p < 0.01), *Cpt1a* (encoding CPT-1) mRNA expression levels decreased by approximately 50% (p < 0.05), *Col4a1* (encoding collagen type IV alpha 1 chain [COL4A1]) mRNA expression levels increased by 7.0 fold (p < 0.01), *Cyp7a1* (encoding CYP7A1), *Cyp8b1* (encoding CYP8B1), *Nr1h4* (encoding FXR), *Gpbar1* (encoding TGR5), *Pck1* (encoding phosphoenolpyruvate carboxykinase [PEPCK]) and *G6pc* (encoding glucose-6-phosphatase catalytic subunit [G6PC]) mRNA levels in Group B were not markedly changed (all p > 0.05). In comparison to Group B, *Mlxipl* mRNA level decreased by approximately 1.8-fold (p < 0.05) in Group C; *Fasn* mRNA level decreased by approximately 1.7-fold (p < 0.05); *Cpt1a* mRNA level was reduced by approximately 50% (p < 0.05); *Col4a1* mRNA level was downregulated by roughly 50% (p < 0.05). Additionally, the mRNA levels of *Cyp7a1* and *Cyp8b1* decreased by approximately 72% and 75%, respectively (all p < 0.05); *Nr1h4* mRNA expression was downregulated by approximately 75% (p < 0.05); *Gpbar1* mRNA expression was upregulated by approximately 36%; *Pck1* and *G6pc* mRNA expression reduced by approximately 65% and 55%, respectively in Group C (all p < 0.05) (Figure 3).

4. Discussion

The prevalence of MASLD is increasing rapidly, and fibrosis is the major determinant in the progression of MASLD.[22] Currently, there are no approved therapies for MASLD.[3,4] UDCA has a hepatoprotective effect[23], and we studied its effects in the treatment of MASLD. After four weeks of HFD consumption, hepatic TG and TC contents were elevated, concomitant with increased serum AST, ALT, and GGT activities. Notably, Group C demonstrated reduced hepatic TG and TC levels and lower serum AST, ALT, and GGT levels when contrasted with Group B. These results indicate that UDCA administration is capable of attenuating hepatic lipid accumulation and ameliorating liver enzyme profiles. Our results are comparable to those

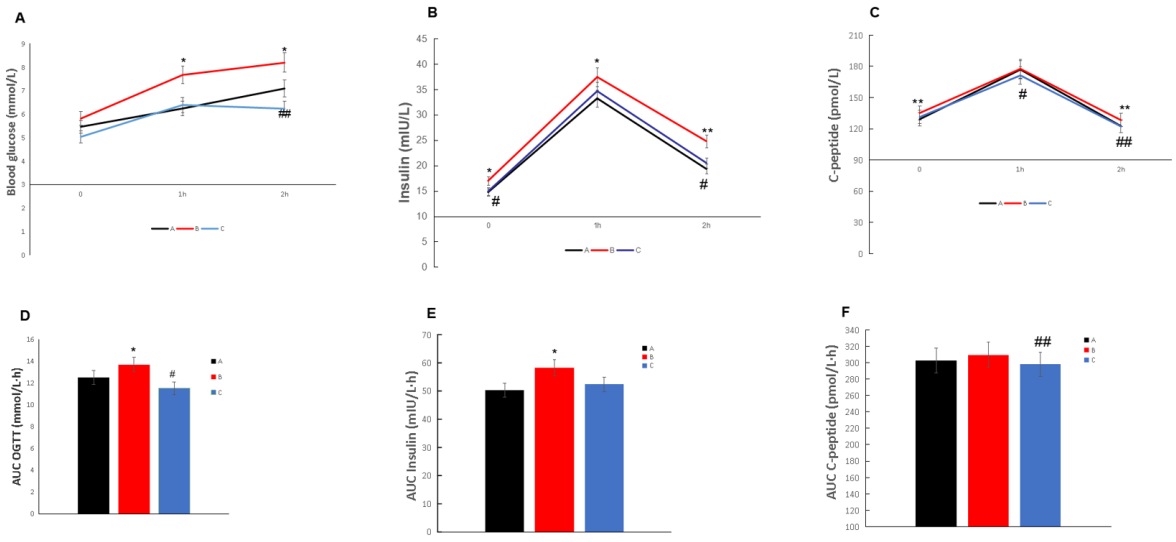

Figure 1. Oral glucose tolerance test (OGTT) and insulin release test. (A) OGTT across the three groups. (B) Insulin levels during OGTT in the three groups. (C) C-peptide levels during OGTT in the three groups. (D) Areas under the curve (AUCs) for the OGTT in the three groups. (E) AUCs for insulin levels during OGTT in the three groups. (F) AUCs for C-peptide levels during OGTT in the three groups.
Notes: $^{*}p < 0.05$ vs. Group A; $^{**}p < 0.01$ vs. Group A; $^{\#}p < 0.05$ vs. Group B; $^{\#\#}p < 0.01$ vs. Group B; $n = 10$ in each group; Group A: Normal control; Group B: High-fat diet; Group C: High-fat diet intervened with ursodeoxycholic acid.

of Nadinskaia *et al.*[24] in humans and Li *et al.*[25] in C57BL mice. However, Mueller *et al.*[26] reported that after UDCA treatment for three weeks, serum and liver TG increased in people with obesity, whereas liver TC remained unchanged. The different results may be affected by food ingredients, drug dosage, sample size, treatment duration, and the severity of fatty liver. Therefore, a rigorously designed clinical trial for further confirmation is required.

Hepatic steatosis arises from an imbalance where the synthesis of triglycerides surpasses their clearance and vice versa.[27] We measured the mRNA expression levels of *Fasn*, which is the key gene that encodes the enzyme involved in FA synthesis; *Mlxipl*, the gene that encodes ChREBP, the upstream regulator of FAS[8]; and *Cpt1a*, the gene that encodes CPT-1, the key regulatory enzyme in FA oxidation. Our analysis revealed that, compared with Group A, Group B exhibited elevated levels of both *Mlxipl* and *Fasn* gene expression, alongside diminished *Cpt1a* mRNA levels. In comparison to Group B, *Fasn* and *Cpt1a* mRNA expression levels decreased in Group C, indicating that UDCA inhibited the levels of genes associated with FA synthesis and oxidation. Our results are similar to those of Chen *et al.*[28]

Synthesis of BA is the most important cholesterol catabolism pathway. After BA synthesis, they are excreted into the gallbladder and secreted into the duodenum.[13] Mouzaki *et al.*[29] showed that synthesis of BA was elevated in patients with MASH. Excessive BA deposition in the liver causes inflammation.[30] The levels of BA are related to the degree of MASH, and the effect of UDCA on BA is controversial.[26] We measured the expression levels of genes related to BA metabolism in the liver and found that, in comparison to Group A, the gene expression of *Cyp7a1* and *Cyp8b1* was not significantly altered in Group B; in comparison to Group B, *Cyp7a1* and *Cyp8b1* mRNA expression decreased in Group C, indicating that UDCA suppressed the expression of genes associated with BA synthesis. Our findings are consistent with those of Li *et al.*[25] and Taylor *et al.*[31] Inhibition of BA synthesis is associated with decreased cholesterol excretion, and this may be its subsequent response to the decreased lipid synthesis. Therefore, in addition to inhibition of the expression of genes associated with FA synthesis and oxidation, UDCA also suppressed the mRNA levels of genes associated with BA synthesis, suggesting that the balance of lipids and BA metabolism is critical to improving MASLD.

Metabolic dysfunction-associated steatotic liver disease starts with triglyceride accumulation in hepatocytes and can progress to fibrosis or cirrhosis. *Col4a1* is a gene associated with liver fibrosis; it is also a pro-inflammatory cytokine, which can result in liver injury.[32,33] We subsequently measured the gene expression of *Col4a1*. The expression of *Col4a1* was elevated in Group B relative to Group A, yet was attenuated in Group C compared to Group B. Our findings are comparable to previous reports

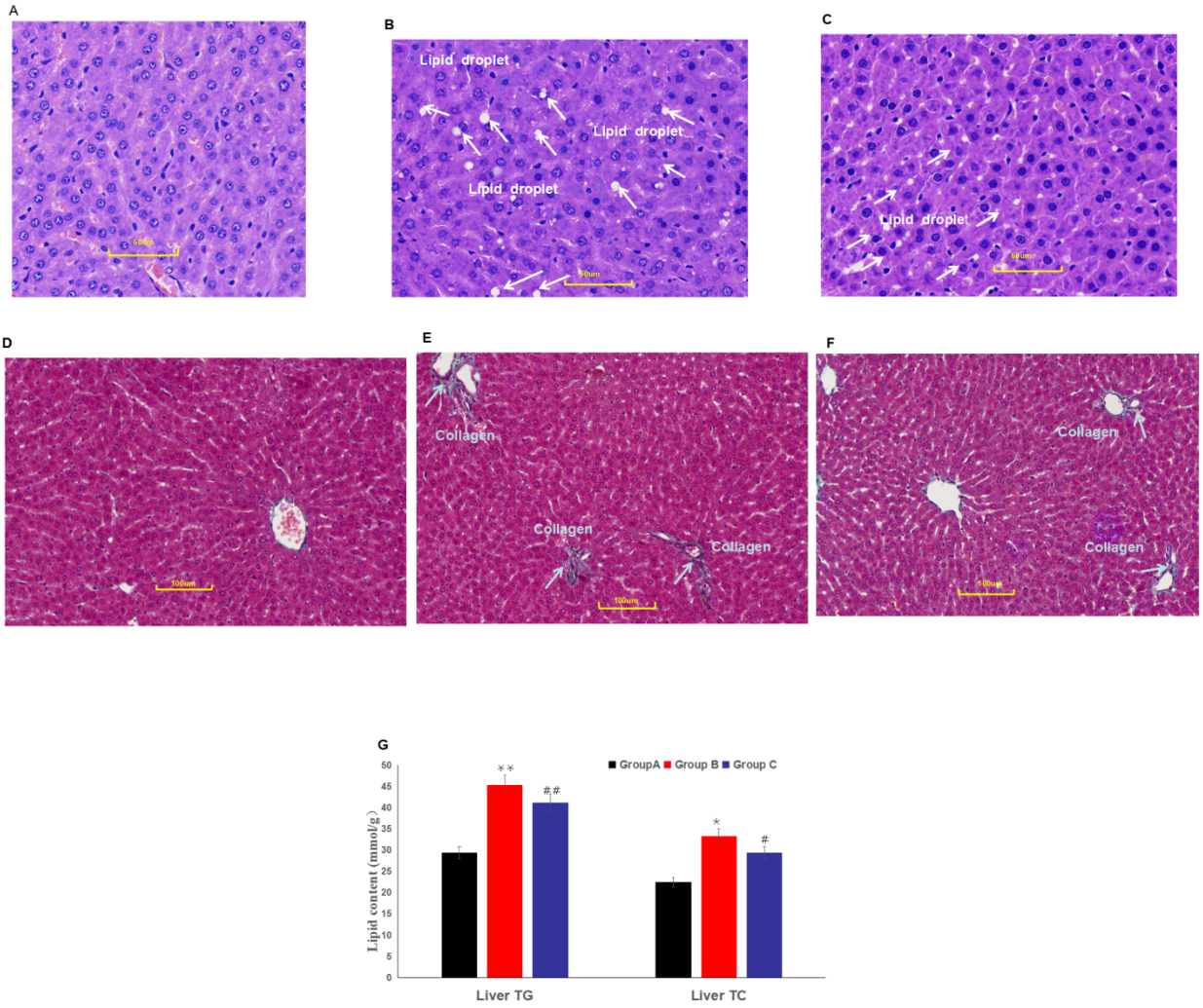

Figure 2. Histological examination of the liver using hematoxylin and eosin (H&E) and Masson's trichrome (MTC) staining. (A, B, C) Liver tissues stained with H&E in the three groups at week 4 (Groups A, B, C, respectively). Scale bar: 60 μm; magnification: 200×. (D, E, F) Liver tissues stained using MTC in the three groups at week 4 (Groups A, B, C, respectively). Scale bar: 100 μm; magnification: 100×. (G) Lipid content of the liver tissues.
Notes: $^{*}p < 0.05$ vs. Group A; $^{**}p < 0.01$ vs. Group A; $^{\#}p < 0.05$ vs. Group B; $^{\#\#}p < 0.01$ vs. Group B; $n = 10$ in each group; Group A: Normal control; Group B: High-fat diet; Group C: High-fat diet intervened with ursodeoxycholic acid; White arrow points to lipid droplet; blue arrow indicates collagen.
Abbreviations: TC: Total cholesterol; TG: Triglyceride.

of Elhini *et al.*[34] in humans, suggesting UDCA treatment can influence *Col4a1* gene expression.

Bile acids play a pivotal role in modulating glucose, lipid, and energy metabolism by activating *Nr1h4* and *Gpbar1*. We subsequently measured *Nr1h4* mRNA levels in the hepatic tissue and found that, in comparison to that in Group A, liver *Nr1h4* mRNA levels in Group B were not markedly changed; moreover, compared to that in Group B, it was lower in Group C. Our findings are similar to the previous reports by Chiang and Ferrell[35] and align with the study by Brevini *et al.*[36] that reported UDCA suppression on *Nr1h4* signaling.

In addition, MASLD is closely related to hyperglycemia. Hepatic gluconeogenesis is largely regulated at the transcriptional level by control of *Pck1* and *G6pc*, genes that encode the major rate-limiting enzymes for this process.[37] In previous studies, the mRNA levels of *Pck1* and *G6pc* have been shown to increase in ob/ob mice but decrease after UDCA treatment.[28] In our study, compared to Group A, after HFD consumption for four weeks, the AUC values of the OGTT increased, insulin levels increased, and *Pck1* and *G6pc* mRNA levels were not substantially altered. The results suggest that additional mechanisms may contribute to the regulation of glucose homeostasis, and further

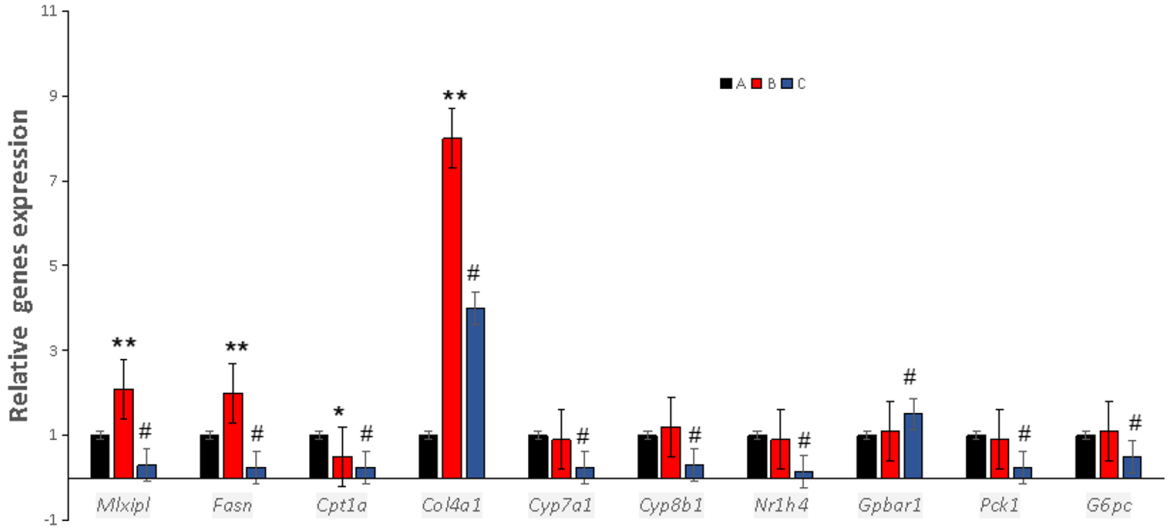

Figure 3. Comparison of mRNA expression in the liver across three groups
Notes: *$p < 0.05$ vs. Group A; **$p < 0.01$ vs. Group A; #$p < 0.05$ vs. Group B; $n = 5$ in each group; Group A: Normal control; Group B: High-fat diet; Group C: High-fat diet intervened with ursodeoxycholic acid.

validation is required. Compared to Group B, the AUC values of the OGTT decreased, insulin levels declined to varying degrees, and *Pck1* and *G6pc* mRNA levels also decreased in Group C. It has been shown that FXR activates gluconeogenic pathways in the liver[38], suggesting that inhibition of *Pck1* and *G6pc* gene expression by UDCA might be associated with the inhibition of *Nr1h4* gene expression by UDCA.

Takeda G-protein-coupled receptor-5 is present in the hepatic epithelial cells and Kupffer cells. It exerts anti-inflammatory activity through the blockade of the production of pro-inflammatory cytokines.[34] Compared to wild-type mice, TGR5-knockout mice exhibit exacerbated liver damage and increased pro-inflammatory factor levels.[39] We found that, compared to that in Group A, *Gpbar1* mRNA level was not significantly altered in Group B, and in comparison with Group B, *Gpbar1* mRNA level was markedly enhanced in Group C. Our findings align with previous research, suggesting that UDCA may increase *Gpbar1* gene expression in HFD-fed rats.[40,41]

Above all, we found that UDCA intervention decreased liver TG and TC contents and glucose levels, altered insulin secretion, and repressed the expression of genes associated with BA synthesis, inflammation, lipid metabolism, and hepatic gluconeogenesis in HFD-fed rats. Previous clinical studies have shown that UDCA can improve certain parameters in MASLD[24,42], and our study further indicates that UDCA may be effective for the management of MASLD from the perspective of basic research. Therefore, UDCA can be a potential treatment for MASLD and its related comorbidities in the clinic, and large-scale clinical trials for further validation are required.

This investigation is subject to several constraints. Firstly, the sample size of experimental animals was relatively small. Secondly, both the HFD intervention and UDCA administration periods were of limited duration. Thirdly, as the current data were derived exclusively from animal models, their translational relevance to human clinical practice remains to be validated through large-scale population-based studies.

5. Conclusion

In conclusion, UDCA intervention improved MASLD and its related metabolic disorders in SD rats. These effects may be related to its influence on genes related to lipid, BA, and glucose metabolic regulation.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

All animal care and experimental protocols were approved by the Animal Ethics Committee of the Second Hospital of Shanxi Medical University of China (No.20240729).

Consent for publication

Not applicable.

Availability of data

Original data are available from the corresponding author upon reasonable request.

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