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Hepatic cholesterol accumulation ascribed to the activation of ileum Fxr-Fgf15 pathway inhibiting hepatic Cyp7a1 in high-fat diet-induced obesity rats

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ABSTRACT

Aims: High-fat diet (HFD)-induced obesity resulting in cholesterol accumulation is one of the common pathogenic factors for lipids metabolic disorders. However, the potential mechanisms about cholesterol accumulation during obesity are still not clearly identified. Bile acids (BAs) as the natural ligands of farnesoid x receptor (Fxr) are demonstrated that can regulate the relevant enzymes and transporters at transcriptional level to determine the cholesterol homeostasis. Here, we explored the underlying mechanisms of hepatic cholesterol accumulation in HFD-induced obesity rats via the BAs-Fxr-enzymes/transporters signaling pathways.

Materials and methods: BAs and cholesterol levels as well as mRNA expressions of enzymes, transporters and nuclear receptors involving in cholesterol homeostasis in liver and ileum tissue were evaluated in 4-week HFD-induced obesity rats.

Key findings: HFD promoted BAs intestine passive absorption to increase the concentrations of BAs especially the chenodeoxycholic acids (CDCAs) in ileum of HFD-induced obesity rats. The increased CDCAs concentrations activated Fxr-Fgf15 pathway in ileum to result in the mRNA expression of Cyp7a1 in liver down-regulation, which inhibited cholesterol metabolizing into primary BAs to contribute to the cholesterol level increase in liver tissue in HFD-induced obesity rats.

Significance: The hepatic cholesterol accumulation should be ascribed to the activation of ileum Fxr-Fgf15 pathway by the increased BAs passive absorption into ileal enterocytes under the condition of rats fed with HFD, which inhibited hepatic Cyp7a1 gene transcription to reduce metabolic elimination of cholesterol. Moreover, these findings are expected to provide a cue for the treatment of cholesterol metabolism disorders in obesity patient.

1. Introduction

Obesity is becoming the leading cause of healthy problem globally [1]. According to the statistical data from World Health Organization in 2016, > 1.9 billion adults were overweight, of which over 650 million were obese. Furthermore, the latest study published in the UK's Lancet has been reported that the number of obese children and adolescents in the world is as high as 124 million [2,3]. Obesity, which always involves in dyslipidemia, is the high risk factor for lipid and glucose metabolism disorders including nonalcoholic fatty liver disease

Abbreviations: Abcg5/8, ATP-binding cassette sub-family G member 5/8; Asbt, apical sodium-dependent bile acids transporter; BAs, bile acids; Bsep, bile salt export pump; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; Cyp7a1, cholesterol 7α-hydroxylase cytochrome P450 7a1; DCA, deoxycholic acid; Fxr, farnesoid X receptor; Fgf15, fibroblast growth factor 15; G-, glycine conjugated bile acid; HDCA, hyodesoxycholic acid; HFD, high-fat diet; Hmgcr, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LCA, lithocholic acid; Ldlr, low density lipoprotein receptor; NAFLD, nonalcoholic fatty liver disease; NDCA, demethylation deoxycholic acid; Shp, small heterodimer partner; Srebps, sterol regulatory element-binding proteins; T-, taurine conjugated bile acid; TBAs, total bile acids; UDCA, ursodeoxycholic acid

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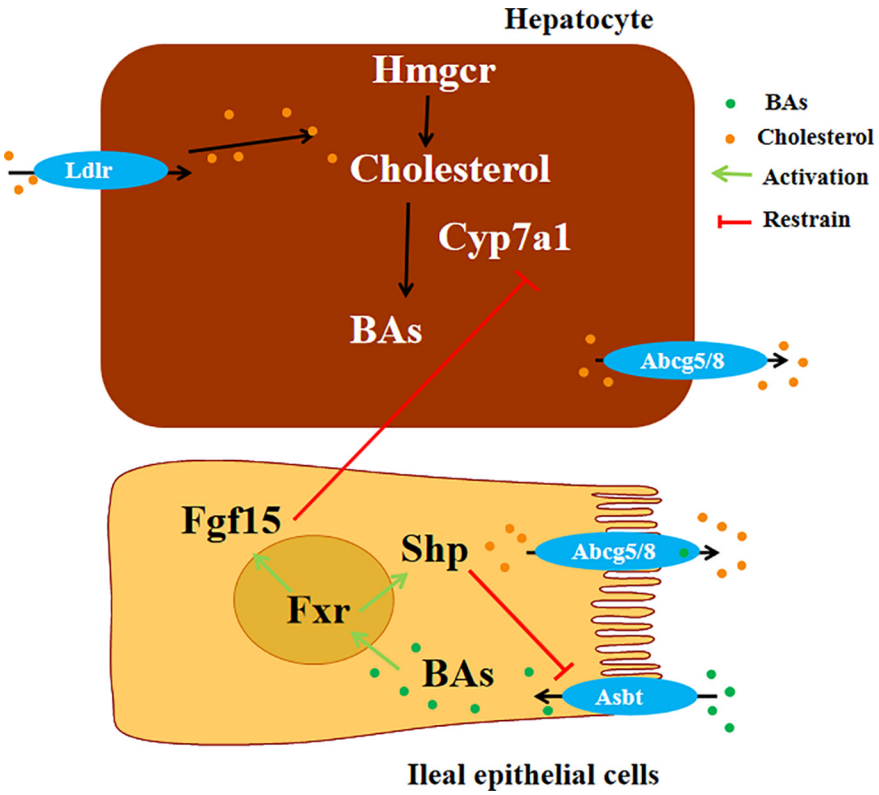

Fig. 1. Enzymes and transporters in liver and ileum are essential for mediation of various processes including absorption, synthesis, metabolism and excretion of cholesterol. (a) Hepatocyte: Hmgcr is rate-limiting enzyme of cholesterol synthesis; Cyp7a1 acts as the key metabolism enzyme for cholesterol metabolism and also the rate-limiting enzyme for BAs biosynthesis in liver; Ldlr mediate hepatic uptake of cholesterol; Abcg5/8 is pair of transporters mediate the cholesterol efflux from the hepatocyte to the bile flow; (b) Ileal epithelial cells: Apical Asbt is a key transporters in ileum to mediate BAs reabsorption from intestinal lumen; BAs-activated Fxr pathway down-regulates Asbt but increases Fgf15 release.

(NAFLD), cardiovascular diseases and diabetes et al. [4–6]. Importantly, cholesterol accumulation in vivo during the obesity is one of the most common pathogenic factors for NAFLD, atherosclerosis and hypertension [7–9]. However, the potential mechanisms about hepatic cholesterol accumulation under the condition of obesity are still not clear and need to be further identified.

Bile acids (BAs) play a pivotal role in determining the cholesterol homeostasis. Firstly, BAs as the amphiphile can increase the cholesterol solubility to promote intestinal passive absorption of cholesterol [10]. Furthermore, BAs are the major metabolites of cholesterol, excreted through the bile out of liver. Even more importantly, it has been demonstrated that BAs activating farnesoid x receptor (Fxr) in liver and ileum regulate the gene transcription of the relevant enzymes and transporters systems to mediate the homeostasis including absorption, distribution, metabolism and excretion of cholesterol in vivo [11–13] (the details were shown in Fig. 1). As the main absorption position of cholesterol and BAs, various transporters express at the membrane of the ileum epithelial cells. ATP-binding cassette sub-family G member 5/8 (ABCG5/8) is pair of transporters expressing at apical membrane of ileum epithelial cells mediate the cholesterol efflux from the enterocyte to the intestinal lumen [14,15]. Apical sodium-dependent bile acids transporter (Asbt) distributing in the apical membrane of ileum epithelial mediate about 95% of BAs absorption from the intestinal lumen into the enterocyte [16]. Additionally, enzymes and transporters in liver are essential for mediation of various processes including synthesis, metabolism, elimination and excretion of cholesterol and BAs [17,18]. In detail, rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmgcr) and transcription factors sterol regulatory element-binding proteins (Srebps) principally regulate the de novo synthesis of cholesterol; cholesterol 7α-hydroxylase (Cyp7a1) acts as the key metabolism enzyme for cholesterol metabolism and also the rate-limiting enzyme for BAs biosynthesis in liver [4,19]; the basolateral hepatic uptake of cholesterol is mainly mediated by the low density lipoprotein receptor (Ldlr) [20]; cholesterol secreting from hepatocyte into bile is mainly mediated by the Abcg5/8 that express at the canalicular membrane of hepatocyte. Moreover, the enzymes and transporters mentioned above are primarily regulated by BAs-Fxr signaling pathways in liver and ileum at transcriptional level. As such, we proposed that explore the mechanisms of cholesterol accumulation in liver during the obesity via the BAs-Fxr signaling pathways as well as their regulated enzymes and transporters systems mentioned above in rats.

Taken together, in the present study, we analyzed the BAs concentrations and compositions as well as the mRNA expressions of the relevant nuclear receptors, enzymes and transporters in liver and ileum to explore the mechanisms of the hepatic cholesterol accumulation under the condition of obesity in rats. We expected the findings from this study would provide a cue for identification of novel therapeutic targets for treatment of diseases associated with cholesterol disorders in obesity patients.

2. Materials and methods

2.1. Animals

Wistar male rats ageing 4 weeks with body weight around 60–100 g were obtained from the Experimental Animal Center of Lanzhou University (Lanzhou, China). All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Rats were randomly divided into two group, obesity group and control group. After one-week habituation period with standard diet, the diet of rats in obesity group was switched to the high-fat diet (Rodent diet D12492, Research Diets, USA) which derives 60% of calories from fat, and rats in the control group were fed with the commercial standard diet, and all the animals were consecutively fed for 4 weeks. During this period, the food intake of rats was measured daily to avoid differences in weight gain due to food intake, and the individual body weight was monitored weekly.

Table 1
The sequences for primers of related genes.

Protein code	Sequences for primes	
Fxr	Forward	GGAACTCCGGACATTCAAC
	Reverse	GTGTCCATCACTGCACATC
Shp	Forward	CCTGGAGCAGCCCTCGT
	Reverse	AACACTGTATGCAAACCGAGGA
Fgf15	Forward	AGGGCCAGAAACCTTCAAAC
	Reverse	GATCCATGCTGTCGCTCTC
Cyp7a1	Forward	CTGCAGCGAGCTTTATCCAC
	Reverse	CCTGGGTTGCTAAGGGACTC
Asbt	Forward	TGGGTTTCTTCCTGGCTAGACT
	Reverse	TGTTCTGCATTCCAGTTTCCAA
Hmgcr	Forward	CAAAGACAATCCTGGAGAAAATG
	Reverse	TGTTCCCTGCAGATCTTGTAAAT
Srebp2	Forward	CTGCAGCCTCAAGTGCAAAG
	Reverse	CAGTGTGCCATTGGCTGTCT
Ldlr	Forward	CTGTATTCACGGTAGCCGCC
	Reverse	TGGGTCACATTGATGCAGCC
Abcg5	Forward	CGCAGGAACCGCATTGTAA
	Reverse	TGTCGAAGTGGTGGAAGAGCT
Abcg8	Forward	GATGCTGGCTATCATAGGGAGC
	Reverse	TCTCTGCCTGTGATAACGTCGA
β-actin	Forward	CCGTGAAAAGATGACCCAGATC
	Reverse	GGTACGACCAGAGGCATACAGG

2.2. Sampling

After 4 weeks of dietary intervention, 5 rats were randomly selected from each group, and 1 h bile was collected by biliary cannulation with anesthetized rat. Meanwhile, after fasting for 12 h, blood sample was collected from abdominal aorta of the other 5 rats without bile collection under the condition of anesthesia, and liver, removed-contents ileum, abdominal adipose tissue samples were obtained from rats sacrificed by overdose anesthesia. The tissue samples were weighed after separation, and then a fraction of the liver and abdominal adipose tissue were immediately fixed in 10% formaldehyde, respectively. The rest of samples were frozen in −80 °C except that samples for mRNA expression analysis by real-time quantitative PCR (RT-qPCR) were stored in liquid nitrogen.

2.3. Evaluation of lipids levels in serum and tissues

The concentrations of cholesterol and triglycerides in serum, liver and ileum were determined by Microplate Reader (Multiskan FC, Thermo Scientific, USA) after processing according to the total cholesterol test kit (A110-1) and triglyceride test kit (A111-1) instructions (Jiancheng Bioengineering Institute, Nanjing, China), respectively. Briefly, the tissue was accurately weighed, using the weight (g): volume (ml) = 1:9 ratio, added 9 times the volume of acetone to homogenized in low temperature, after vortexed for a few minutes, centrifuged to take the supernatant to be tested.

2.4. Histopathology evaluation

The removed tissue samples of liver and adipose were fixed in 10% formaldehyde and then a portion was embedded in paraffin, which was cut to a thickness of 4 μm and stained with Hematoxylin and Eosin (H& E) to investigate the histopathology alterations. Additionally, Oil Red O staining was performed on frozen liver section to assess lipids content in liver.

2.5. Quantification analysis of the concentrations of total bile acids (TBAs) and individual BAs

The TBAs levels in liver and ileum were measured by TBA assay kit (Jiancheng Bioengineering Institute, Nanjing, China). Sample pretreatment was conducted following the manufacturer's instructions, and determined by the Microplate Reader. The concentrations of individual BAs including cholic acid (CA), chenodeoxycholic acid (CDCA) and β-muricholic acid (β-MCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyodesoxycholic acid (HDCA) and their glycine- (G) and taurine (T)-conjugated BAs in liver and ileum tissues were determined by HPLC/MS/MS (Agilent, Palo Alto, USA), and employed and demethylation deoxycholic acid (NDCA) as the internal standards. The individual BAs and internal standards were all purchased from Sigma Aldrich Company (St Louis, MO, USA). Briefly, liver and ileal tissue samples added methanol to homogenate, after centrifuge, took 50 μL of supernatant, added 150 μL of NDCA (4 μg/mL) internal standard methanol solution. Finally, with mixed and centrifuged, 20 μL supernatant was taken for HPLC/MS/MS analysis.

2.6. mRNA expressions determination by RT-qPCR

Total mRNA in liver and ileum were isolated from the frozen tissues using RNAprep Pure Tissue Kit (TianGen Biotech Corporation, LTD, Beijing, China) and according to manufacturer's instructions. Then, the complementary DNA was reversely transcribed from total mRNA by using the Fast Quant RT Kit (TianGen Biotech Corporation, LTD, Beijing, China). Finally, RT-qPCR was performed using Fast Start Essential DNA Green Master (Roche, Basel, Switzerland) and analyzed on LightCycler480 instrument (Roche, Basel, Switzerland). Relative mRNA expression levels were normalized to reference gene β-actin in the same sample and were calculated using the $2^{-\Delta\Delta Ct}$ method. Sequences for primers were listed in Table 1.

Fxr, farnesoid X receptor; Shp: small heterodimer partner; Fgf15, fibroblast growth factor 15; Cyp7a1, cholesterol 7α-hydroxylase cytochrome P450 7a1; Asbt, apical sodium-dependent bile acids transporter; Hmgcr, 3-hydroxy-3-methylglutaryl coenzyme A reductase; Srebp2, transcription factors sterol regulatory element-binding protein 2; Ldlr, low density lipoprotein receptor; Abcg5/8, ATP-binding cassette sub-family G member 5/8; β-actin as the reference gene was used to normalize expression.

2.7. Caco2 cell uptake experiment

The human colonic epithelial cell line Caco2 was seeded at a density of 2×10^4 cells/cm^2 in 6-well plates and maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Cells were routinely grown at 37 °C in a 5% CO_2 incubator. When the cell density exceeds 80%, a mixture of individual BAs containing 21 kinds of BAs was sterilized by filtration and added to the complete medium for investigation of the BAs intestinal absorption promoted by HFD. In detail, Caco2 cells were incubated with the individual BAs mixture mentioned above at a concentration of 100, 10, 1 μg/mL for 1 h in the presence or absence of high fat feed ethanol extract, respectively. After the intervention, cells were harvested for subsequent determination of TBAs concentration, using TBA assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Statistical analysis

All experimental data was expressed as the mean ± SD as indicated in the figure legends. Differences between two groups were tested by the Student's *t*-test. Differences were considered significant at a P value < 0.05 and 0.01 and have been indicated by asterisks in the graph accordingly.

3. Results

3.1. Lipids accumulated in liver of rats with obesity

A faster body weight gain trend was observed in the obesity group, which finally resulted in body weight significant increased by 32.9% as

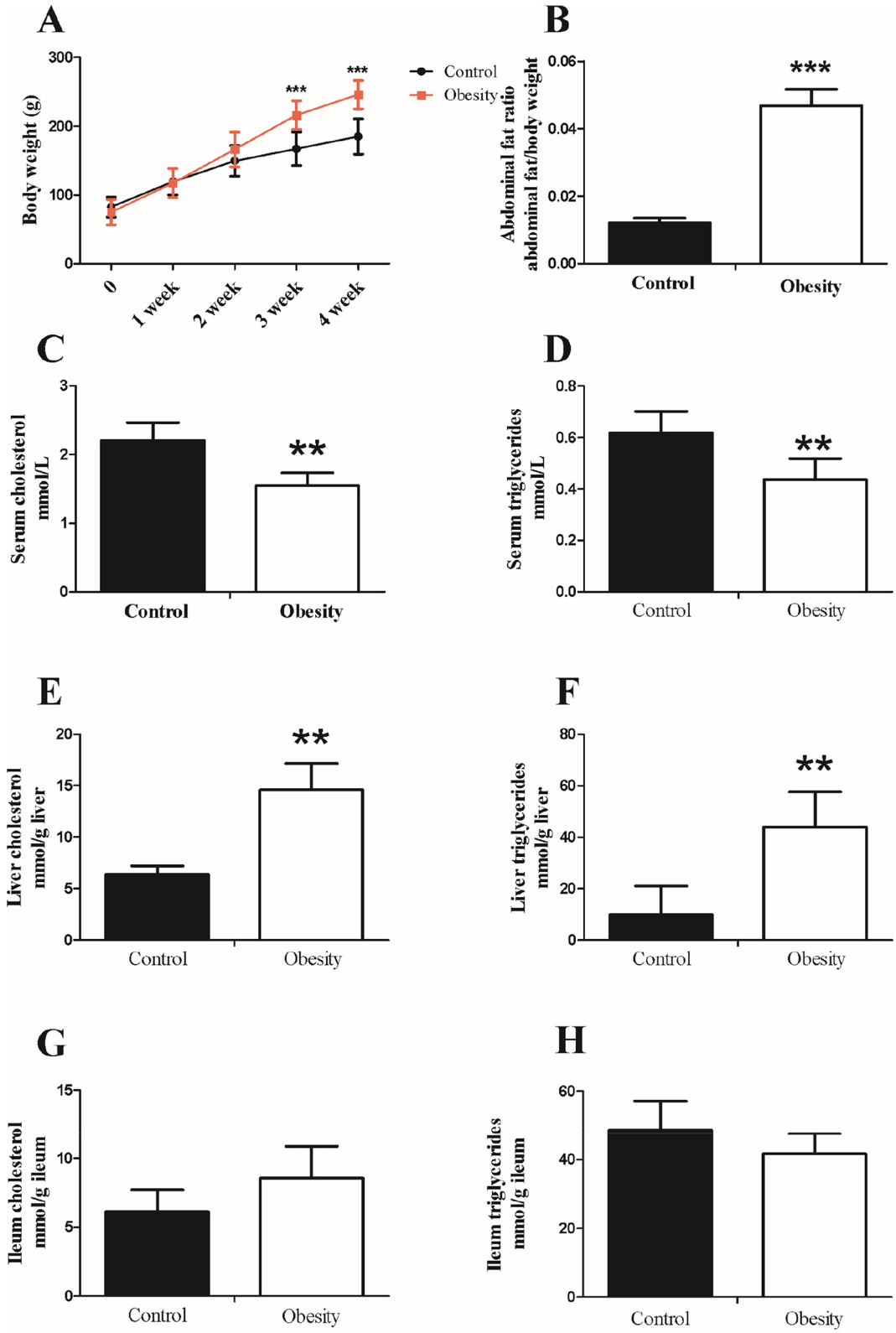

Fig. 2. High-fat diet induced significant weight gain and cholesterol accumulation. (A) Body weight, (B) abdominal fat ratio, (C–H) serum, liver and ileum cholesterol and triglycerides content. Results are shown as mean ± S.D. ($n = 5$ per group). $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group.

compared with the control group after 4 weeks HFD induction (Fig. 2A). Concomitantly, the ratio of abdominal fat to body weight in obesity group was 4 folds higher than that in the control group (Fig. 2B). Importantly, compared to the control group, it has been found that the concentrations of serum cholesterol and triglycerides decreased while hepatic cholesterol and triglycerides increased respectively by 1.29 and 3.47 folds in the obesity group (Fig. 2C–F). In addition, there came no difference between control group and obesity group in ileum cholesterol and triglycerides levels (Fig. 2E–F).

Four weeks of HFD induction altered the histological characteristic

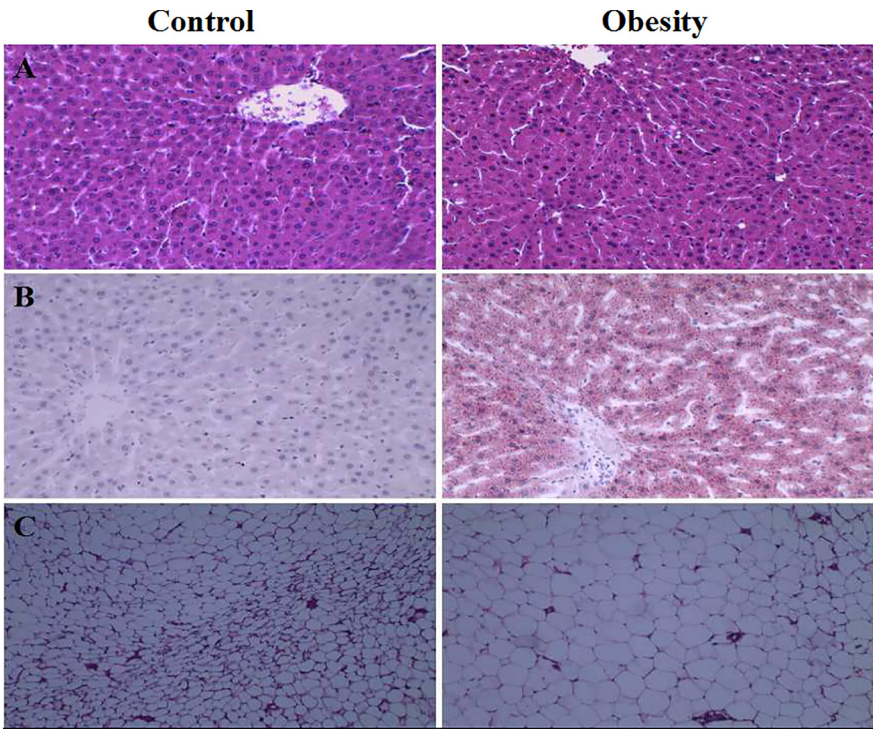

Fig. 3. High-fat diet causes changes in liver and abdominal fat cell volume. Hematoxylin and eosin-stained (A) and oil red O (B) stained liver sections, and hematoxylin and eosin-stained abdominal fat sections (C) from control and obesity group (20 × magnification). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in liver and adipose tissue. According to H&E and Oil Red O staining results described in Fig. 3, compared to the control group, apparent lipid deposition in liver was observed in the obesity group (Fig. 3 A and B), and the volume of abdominal adipose cells augmented (Fig. 3 C). Altogether, in the rats with HFD-induced obesity, lipids including cholesterol and triglycerides accumulated, especially in liver.

3.2. Hepatic BAs concentrations decreasing in obesity rats

As shown in Fig. 4A, in comparison with the control group, the hepatic TBAs concentration decreased obviously in obesity rats. Furthermore, the concentrations of GCA, CDCA, GCDCA, TCDCA, GDCA, β-MCA, GUDCA, HDCA LCA and GLCA in liver tissue revealed significant reduction in obesity group, especially the concentration of CDCAs including CDCA, TCDCA and GCDCA decreased by 75% (details shown in Fig. 4C). Exceptionally, compared with the control group, the concentrations of TCA and TDCA increased obviously in obesity group (Fig. 4C), and the concentrations of CA, DCA, TLCA, Gβ-MCA and Tβ-MCA did not show statistically different. As Fig. 4B described, the hepatic concentration of primary BAs was notably decreased under the condition of HFD-induced obesity as compared with the control group, but that of the secondary BAs did not show statistical difference.

3.3. BAs concentrations increasing in ileum tissue in obesity rats

As a result, the TBAs concentration in ileum tissue of the obesity rats profoundly increased by 5 folds as compared with the control (Fig. 5 A). For individual BAs concentrations in ileum tissue, the concentrations of CA, GCA, TCA, GCDCA, TCDCA, GDCA, TDCA, GUDCA, THDCA and Tβ-MCA obviously elevated in the obesity group, which exhibited the growth ratio of CDCAs was the most, when compared to the control group (Fig. 5 B). Moreover, as compared with control group, the concentrations of LCA decreased notably, and the concentrations of CDCA, DCA, TLCA, UDCA, HDCA, β-MCA and Gβ-MCA were slightly changed, but did not reach the statistical significant differences (Fig. 5B).

3.4. BAs concentrations in bile in obesity rats

As shown in Fig. 6A, in comparison with the control group, the TBAs concentration in bile of the obesity rats stayed constant. Furthermore, the concentrations of GCA, GCDCA, TCDCA, GDCA, TLCA, β-MCA, GUDCA and TUDCA in bile revealed significant reduction in obesity group. Exceptionally, compared with the control group, the concentrations of TCA increased obviously in obesity group (Fig. 6 B), and the concentrations of CA, TDCA, Tβ-MCA and THDCA did not show statistically different.

3.5. Alterations of mRNA expressions of the functional genes in liver and ileum of the obesity rats

In a further attempt to understand the mechanisms of cholesterol accumulation during the obesity in rats, we focused on the related enzymes and transporters involving in cholesterol homeostasis in liver and ileum. As a result (Fig. 7), in liver, as compared with the control group, it was found in obesity group that: the mRNA expressions of Cyp7a1, Hmgcr, Ldlr and Srebp2 respectively decreased by 61.6%; 38.7%, 72.4% and 66.7%. Otherwise, the mRNA expressions of Fxr, Abcg5 and Abcg8 exhibited no statistically significant difference between the control and the obesity groups.

In ileum, compared with the control group, Fxr and Npc1l1 mRNA expression unchanged, but its downstream target genes, Shp and Fgf15 mRNA expressions significantly up-regulated in rats with obesity (Fig. 8). Furthermore, the mRNA expressions of the key BAs reabsorption transporter Asbt down-regulated by 37.1% and Abcg5/8, the transporters are responsible for controlling cholesterol efflux into from enterocyte into the intestine lumen, up-regulated remarkably in the obesity group *v.s.* the control group (Fig. 8).

3.6. HFD promoted the passive intestinal absorption of BAs

To explore the mechanism resulting in the considerable increased BAs in ileum tissue under the condition of the mRNA expression of Asbt down-regulated, we proposed that the increased BAs absorption might be associated with the increased passive absorption that was promoted

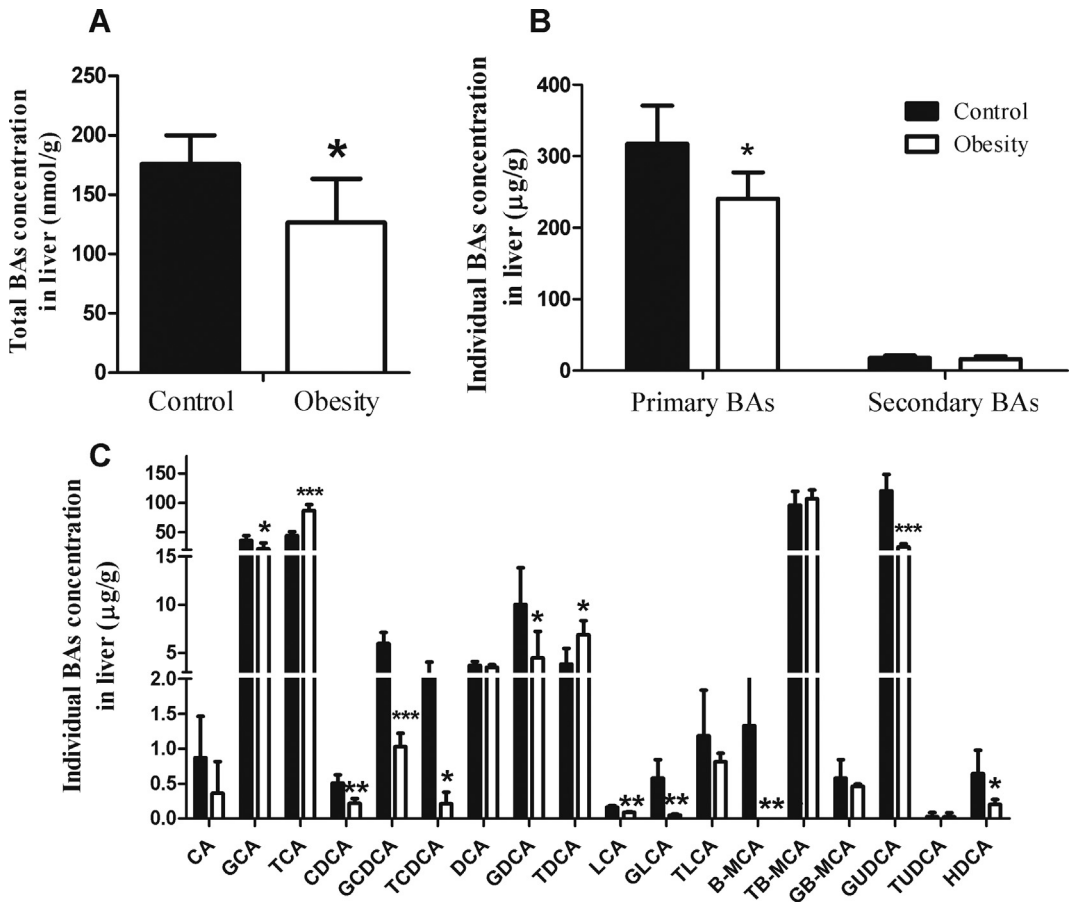

Fig. 4. Total BAs and individual BAs concentrations in liver. (A) total BAs concentration, (B) concentrations of primary and secondary BAs, (C) individual BAs concentrations. Results are shown as mean ± S.D. (n = 5 per group). *p < 0.05, **p < 0.01 versus control group.

by the HFD. As expected, the Caco2 cells BAs uptake experiment demonstrated that (Fig. 9), the TBAs concentration increased when the cells co-cultured with HFD especially increased significantly at the TBAs concentration of 10 μg/mL as compared with the cells without HFD incubation. This result suggested that HFD could promote the passive intestinal absorption of BAs to increase the BAs levels in ileum tissue. (See Fig. 8.)

4. Discussion

High-energy fast-food diet has filled the busy lives of people, which is the main cause of the spread of obesity into chronic epidemic diseases [21–23]. Obesity can induce a variety of metabolic disorders, such as lipid metabolism disorder and glucose metabolism disorder [24,25]. Importantly, cholesterol accumulated in the body under obesity

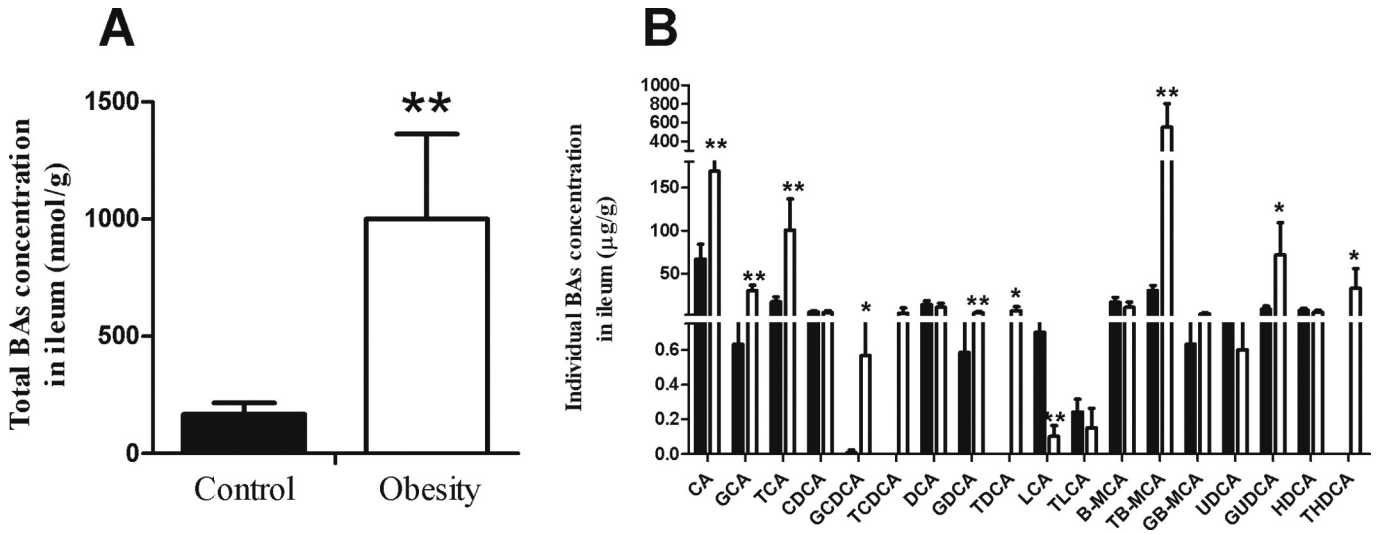

Fig. 5. Total and individual BAs concentration in ileum. (A) Total BAs concentration and (B) individual BAs concentrations. Results are shown as mean ± S.D. (n = 5 per group). *p < 0.05, **p < 0.01 versus control group.

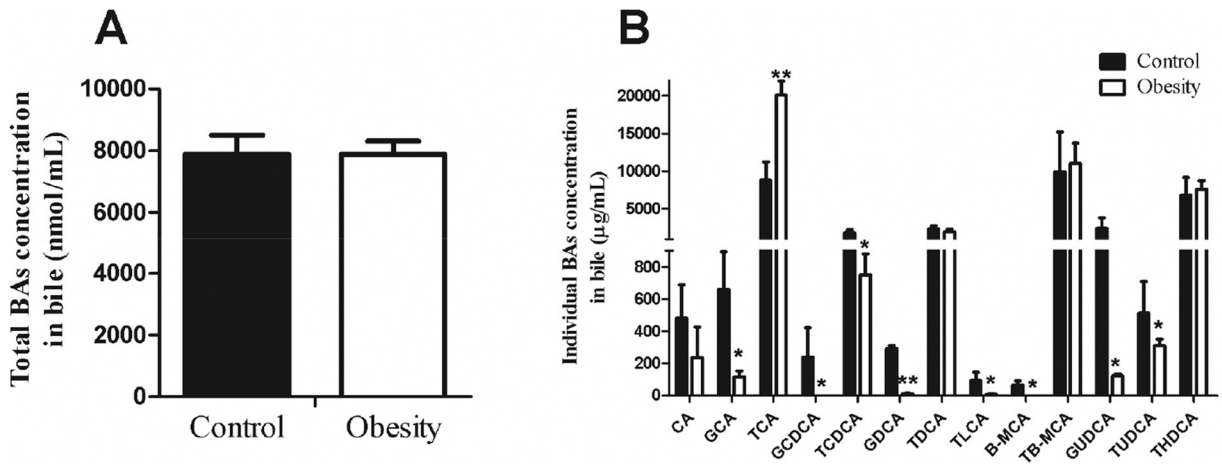

Fig. 6. Total and individual BAs concentration in bile. (A) Total BAs concentration, (B) individual BAs concentrations. Results are shown as mean ± S.D. ($n = 5$ per group). $^{*}p < 0.05$, $^{**}p < 0.01$ versus control group.

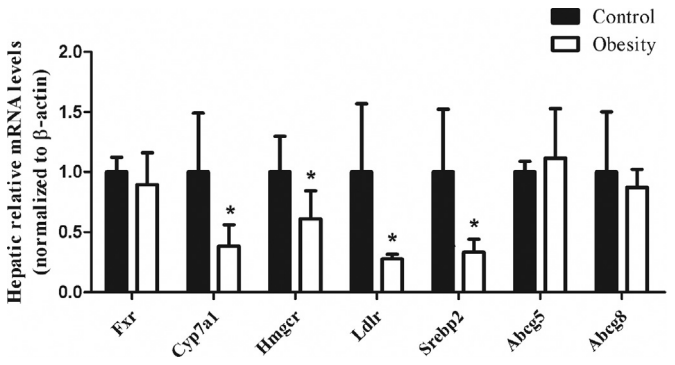

Fig. 7. mRNA expressions of hepatic related enzymes and transporters that involve in cholesterol homeostasis with HFD for 4 weeks. Results are shown as mean ± S.D. ($n = 5$ per group). $^{*}p < 0.05$, versus control group.

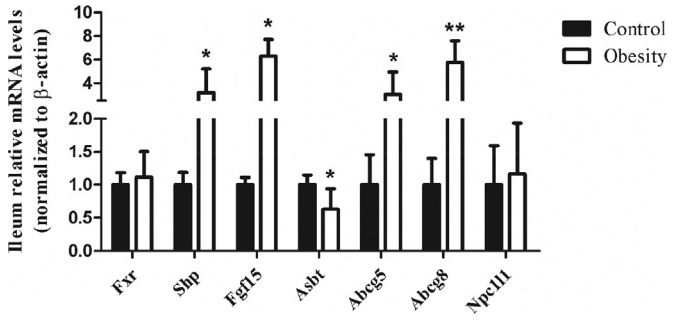

Fig. 8. mRNA expression of ileum related enzymes and transporters that involved in BAs and cholesterol homeostasis with HFD for 4 weeks. Results are shown as mean ± S.D. ($n = 5$ per group). $^{*}p < 0.05$, $^{**}p < 0.01$ versus control group.

condition is one of the common pathogenic factors leading to diseases such as NAFLD and atherosclerosis [26,27]. However, the mechanism of cholesterol accumulation under obesity conditions is still unclear and needs further exploration. In addition to their established role in cholesterol intestinal absorption, BAs as the signaling molecules activating the Fxr play a pivotal role in regulation the relevant enzymes and transporters systems to determine the cholesterol homeostasis in vivo, even more importantly, the BAs characteristic altered under the condition of obesity has been defined. Therefore, in order to explore the potential mechanisms of hepatic cholesterol accumulation on the context of obesity and expected to provide a basis for the clinical treatment of relevant diseases, the present study depicted the BAs alterations and changes in the mRNA expressions of cholesterol-related transporters and enzymes in HFD-induced obesity rats.

The results in the current study showed that HFD caused a rapid increase in body weight and fat accumulation in the body (Fig. 2), and lipid content determination and pathological section analysis further revealed that a significant increase in cholesterol levels in the liver tissue and lipid deposition were observed (Fig. 2-3). These results were consistent with the results of Yi-Syuan Lai et al. and Anuradha Rao et al. [28,29], indicating that there was significant liver cholesterol accumulation after 4 weeks of HFD induction. Which could be the underlying mechanisms of HFD-induced the hepatic cholesterol accumulation? The literature has shown that four main direct factors including uptake, synthesis, metabolism and excretion of cholesterol in hepatocyte affect liver cholesterol levels [30], additionally, the intestinal absorption of cholesterol from diet is another cholesterol source, which accounts for about 30% and is the indirect factor influencing cholesterol level in liver [31]. Meaningfully, the above-mentioned influent factors are mainly mediated by related transporters and enzymes. In detail, at the position of liver, the hepatic uptake of cholesterol is mediated by Ldlr; cholesterol biosynthesis, accounting for > 70% of cholesterol source, is restrictedly mediated by Hmgcr [32]; Cyp7a1 is the key metabolic enzyme determines metabolic elimination of cholesterol as well as it is also the limiting enzyme for BAs hepatic biosynthesis; Abcg5/8 at basolateral membrane of hepatocyte determines cholesterol excretion from hepatocyte into bile [33]. As the results described in the present study, it was found that only the down-regulated Cyp7a1 gene expression decreasing the cholesterol metabolizing into the primary BAs (the concentration of the primary BAs in liver decreased profoundly in obese rats) to reduce the metabolic elimination of cholesterol resulted in the cholesterol accumulation in liver after HFD-induced obesity for 4 weeks in rats (Fig. 6 and 4B), the down-regulated Ldlr and Hmgcr as well as the up-regulated Abcg5/8 could conversely improve the hepatic cholesterol accumulation. Furthermore, in ileum, the crucial position determining absorption of cholesterol in diet, except for the passive absorption, the Abcg5/8 expressing at the apical membrane of enterocytes play a role in efflux of cholesterol from enterocytes into intestinal lumen. However, as a result, the mRNA expression of Abcg5/8 up-regulated significantly and the cholesterol level in ileum tissue exhibited no great changes in rats with obesity (Fig. 7E–F, and Fig. 2G–H), which suggested at least that the increased cholesterol in liver could not ascribe to the intestinal absorption, moreover, significantly down-regulated Ldlr gene expression and reduced serum cholesterol levels also support this view. Inversely, the up-regulated Abcg5/8 genes expressions might be as a compensatory effect for alleviating of the cholesterol accumulation in liver. Collectively, the down-regulated gene transcription of Cyp7a1 mediating the remarkably

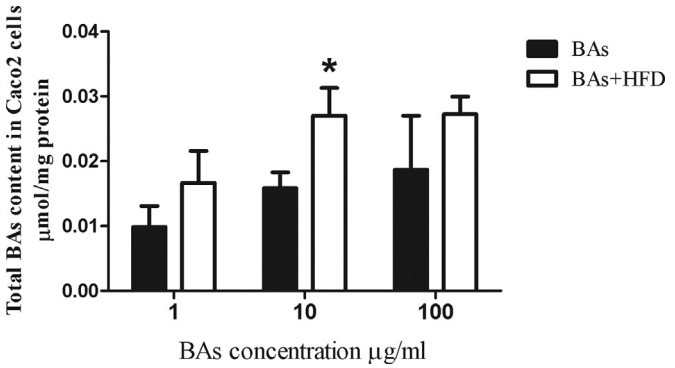

Fig. 9. Effect of HFD on the uptake of Caco-2 cells in different concentrations of BAs. Results are shown as mean ± S.D. $^{*}p < 0.05$ versus control group.

decreased metabolic elimination of cholesterol is the crucial factor to contribute to the hepatic cholesterol accumulation in rats with HFD-induced obesity for 4 weeks.

It has been demonstrated that the ileum Fxr-Fgf15 signaling pathway is the pivotal pathway that regulates the Cyp7a1 gene transcription via negative feedback regulation [20,34,35]. As described in Fig. 1, the hepatic Cyp7a1 gene transcription is inhibited by Fgf15 which is released from enterocyte to the surface of hepatocytes through the portal vein by activating Fxr in ileum [36]. In this experiment, the TBAs concentration especially the concentrations of the potent agonists of Fxr including TCDCA and GCDCA increased dramatically in ileum tissue (Fig. 5) and the ileal Fgf15 mRNA expression up-regulated significantly (Fig. 7C) in rats with obesity. Although there was no significant change in the mRNA expression of ileum Fxr in obese rats compared with the control group, a significant up-regulation of its downstream target gene Fgf15 mRNA expression indicated activation of this pathway [37]. Elevated concentration of TCDCA and GCDCA in ileal tissue was related to the high fat-solubility of CDCAs, which were more likely to enter the cell in a passive diffusion manner. Here, our findings indicated that the down-regulated expression of hepatic Cyp7a1 gene could be ascribed to the activation of ileum Fxr-Fgf15 pathway by increased BAs concentrations in ileum under the condition of HFD-induced obesity in rats.

> 95% BAs in vivo is reabsorbed from ileum, which is so-called BAs enterohepatic circulation [38–40]. During this process, Asbt expressing at the apical membrane of ileal epithelial cells mediates most of the BAs reabsorption, furthermore, a spot of BAs are reabsorbed via passive diffusion [16]. Therefore, we hypothesized that the increased BAs concentrations in ileum tissue of obese rats should be ascribed to the up-regulation of Asbt expression. Nevertheless, the mRNA expression of Asbt at ileum in the obesity rats decreased significantly (Fig. 7D), and for the reason, it could be associated with the up-regulated Shp mRNA expression (Fig. 7B). Shp is the downstream gene of Fxr, of which transcription is promoted by Fxr activation, and it is a repressive factor on Asbt gene transcription [16,41]. Consequently, the increased level of BAs as the Fxr potent agonists in ileum activate Fxr promoted Shp gene expression to result the Asbt mRNA expression down-regulated in rats with obesity. Apparently, the increase of BAs in ileum tissue was the contributor for Asbt down-regulation in obese rats, which suggested that it occurred before Asbt down-regulation. BAs as amphipathic molecules can combine with lipids to form micelles in intestine to facilitate lipids passive absorption [20,42], and it has been reported that the HFD indeed significantly increased intestinal permeability [43]. Here, we proposed that under the circumstance of rats fed with the HFD, the passive absorption of BAs into enterocytes elevated as well, which might be the key mechanism responsible for the BAs concentrations increasing in ileum in HFD-induced obesity rats. Indeed, in our experiment about the BAs uptake influenced by the HFD in Caco2 cells, it was found that the uptake of BAs into Caco2 cells could be promoted by HFD especially the BAs concentration in medium at 10 μg/mL (Fig. 8). Altogether, on the context of HFD-induced obesity for 4 weeks the elevated ileal BAs concentrations were associated with the increased passive absorption induced by HFD in rats.

In addition, with the level of cholesterol in the serum reduced and unchanged expression of Npc1l1, so we speculated that the cholesterol absorbed from the ileum may not increase. Meanwhile, we found in our study that the significant up-regulation of Abcg5/8 gene expression in the ileum can increase the reverse excretion of ileal cholesterol into the intestinal lumen, thereby balancing the cholesterol levels in intestinal cells.

Taken together, our findings in the current study indicated that, the increased BAs passive absorption promoted by HFD into ileal enterocytes activating Fxr-Fgf15 signaling pathway to inhibit Cyp7a1 gene transcription is the potential mechanism resulting in the hepatic cholesterol accumulation in rats with HFD-induced obesity for 4 weeks. Based on that finding, we inferred that the cholesterol-lowering effect of BAs sequestrants, one of the commonly used cholesterol lowering drugs in clinic, which can significantly reduce BAs ileum reabsorption via chelation with BAs, might be associated with the inhibition of the ileum Fxr-Fgf15-hepatic Cyp7a1 pathway [44,45]. It is, however, whether the cholesterol-lowering mechanism of BAs sequestrants has relationship with the Fxr-Fgf15-Cyp7a1 pathway needs to be further study. Moreover, there was a limitation in our study, although we had executed verification experiment in human Caco2 cells, the BAs pool in rat and human exhibits apparently distinction, there into, whether these findings are relevant for humans need to be further verified.

5. Conclusion

In conclusion, cholesterol accumulation is one of the primary symptoms during HFD-related obesity, and it is the high risk factor for NAFLD and cardiovascular diseases. However, the underlying mechanism about cholesterol accumulation under the condition of obesity is still not clearly identified. Therefore, the focus of this study is to explore the mechanism about the hepatic cholesterol accumulation in rats with HFD-induced obesity. As a result, the hepatic cholesterol accumulation should be ascribed to the activation of ileum Fxr-Fgf15 signaling pathway by the increased BAs passive absorption into ileal enterocytes under the condition of rats fed with HFD, which could inhibit hepatic Cyp7a1 gene transcription to reduce metabolic elimination of cholesterol. These findings are expected to provide clues to the mechanism of BAs sequestrants and offer a basis for the development of new drugs for the treatment of cholesterol metabolism disorders in obesity.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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