Chapter

Dysregulation of Bile Acids in Patients with NAFLD

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Abstract

Bile acids are synthesized in the liver and tightly regulated through the enterohepatic circulation. Recent studies reveal that bile acids serve as hormone-like signaling molecules to activate nuclear receptors, notably farnesoid X receptor (FXR), regulating metabolic homeostasis of bile acids, cholesterol, lipids, and glucose. A connection between bile acids and nonalcoholic fatty liver disease (NAFLD) has long been recognized. Although inconsistent or even contradictory results are reported, a large body of evidence from clinical as well as preclinical studies demonstrates that bile acid homeostasis is disrupted in patients with NAFLD. The bile acid dysregulation gets worsening as NAFLD progresses from early stage simple steatosis to late stage nonalcoholic steatohepatitis (NASH) and NASH with fibrosis. As the risk factors for NAFLD, obesity and insulin resistance, which are often associated with NAFLD, contribute to the dysregulation of bile acids in patients with NAFLD. Total serum and fecal bile acid concentrations are mostly elevated in patients with NAFLD as a result of increased bile acid synthesis, elevated hepatic bile acids, and upregulation of bile acid transporters. The two negative feedback regulatory pathways for bile acid synthesis, FXR/SHP (small heterodimer partner) and fibroblast growth factor-19 (FGF19)/FGF receptor-4 (FGFR4), are impaired in patients with NAFLD.

Keywords: NAFLD, steatosis, fatty liver, NASH, bile acids, FXR, bile acid synthesis, enterohepatic circulation, bile acid transporters, FGF19

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease worldwide. It affects about 30% of the population in the United States [1, 2] and 10% of adolescents and children [3, 4]. NAFLD is a spectrum of metabolic disorders starting with simple steatosis characterized with excessive accumulation of triglycerides in the hepatocytes, progressing to nonalcoholic steatohepatitis (NASH) characterized with inflammation, to fibrosis and cirrhosis, and eventually to liver failure and hepatocellular carcinoma (HCC) [5–7]. Obesity and insulin resistance or diabetes are the most prevalent risk factors for development of NAFLD [8–13].

Bile acids are the metabolites of cholesterol and synthesized in the liver. It is well known that bile acids act as biological detergents to solubilize cholesterol and lipids in the bile and intestine, play important roles in cholesterol and lipid absorption and transport. Recent studies have revealed that bile acids can serve

as hormone-like signaling molecules to activate several nuclear receptors, notably the farnesoid X receptor (FXR) [14, 15]. The bile acids/FXR signaling plays critical roles in regulating a myriad of metabolic homeostasis including bile acids, cholesterol, lipids, and glucose [16–19], as well as inflammation/immunity [20–24] and liver regeneration [25–27].

Under physiological condition, bile acid homeostasis is maintained through multiple negative feedback loops for bile acid synthesis [18, 28–30] and a tightly regulated enterohepatic circulation of bile acids [31–34]. Since liver is the organ for bile acid synthesis and metabolism and biliary excretion of bile acids is the limiting step for the enterohepatic circulation [35, 36], impairment of liver function as a result of various liver disorders leads to dysregulation of bile acids. Indeed, the measurement of bile acids is considered a biomarker of liver function and serves as an indicator of hepatobiliary impairment or diseases [37–41]. On the other hand, excessive accumulation of bile acids in the liver causes liver damages by multiple mechanisms including disrupting the integrity of cell membranes through their detergent property [42–44], causing mitochondrial stress and promoting the generation of reactive oxygen species [45–48], and inducing endoplasmic reticulum stress [49–51] and inflammatory responses [52–54], resulting in cell death via apoptosis and/or necrosis [55–58].

Because of the reciprocal effects between liver damage and bile acid dysregulation, it is often difficult, if not impossible, to determine the cause-and-effect relation between liver damage and bile acid dysregulation for many liver disorders. In one hand, liver damage causes bile acid dysregulation. On the other hand, bile acid dysregulation potentially causes liver damage. The connection between NAFLD and bile acid dysregulation has long been recognized and reported [59–67]. It is well established that liver function is compromised in patients with NAFLD, especially advanced stages of NAFLD, such as NASH and NASH-associated fibrosis and cirrhosis, due to pathological and structural damages to the liver. Research interests and emphasis are recently condensed on investigating the contribution of bile acid dysregulation to the pathogenesis of NAFLD and developing therapeutic interventions for NAFLD by manipulating the bile acid signaling pathway [66–73]. However, the outcomes of clinical trials targeting bile acid signaling using ursodeoxycholic acid (UDCA) and obeticholic acid (OCA) to treat NASH patients are not very encouraging [74–79], indicating that our understanding on the relationship between bile acids and NAFLD is not complete or even may be misinterpreted.

Taken together, the link between bile acids and NAFLD has been firmly established. However, certain fundamental questions remain to be answered. How bile acid homeostasis is disrupted in patients with NAFLD? Whether dysregulation of bile acids is one of the manifestations of NAFLD or actually contributes to the development and/or progression of NAFLD? It only becomes possible to develop rationalized approaches to treat patients with NAFLD until those fundamental questions are fully addressed. In this chapter, the effects of NAFLD on bile acid homeostasis are reviewed and discussed.

2. Altered bile acid profiles in subjects with NAFLD

In human, cholic acids (CAs) and chenodeoxycholic acid (CDCA) are two primary bile acids synthesized in the liver and account for majority of bile acids in the bile acid pool. Upon excretion into intestine, primary bile acids can be converted into secondary bile acids by gut bacteria. Specifically, CA is converted into deoxycholic acid (DCA), while CDCA is converted into lithocholic acid (LCA) or UDCA in the intestine by dehydroxylation [80, 81] or 7β epimerization [82–84]. Majority

of primary and secondary bile acids are conjugated by either glycine or taurine in the liver, generating glycine- or taurine-conjugated bile acids [80, 81]. Under physiological conditions, total bile acid levels, as well as the composition of the bile acid pool, are regulated and maintained. However, under various pathological conditions, especially liver disorders, the bile acid pool size or total bile acids and bile acid pool compositions are altered. A large number of clinical and preclinical studies have revealed that bile acid profiles are altered in patients with NAFLD and rodent NAFLD models.

2.1 Altered bile acid profiles in patients with NAFLD

2.1.1 Serum bile acids

Under the physiological condition, serum bile acid concentrations are much lower than those in the enterohepatic system. However, when the enterohepatic cycling of bile acids is compromised due to hepatic injuries or intestine disorders, bile acids are spilled into the blood circulation system, altering serum bile acid concentrations, as well as compositions. Bile acid profiling in healthy populations has revealed that serum bile acid concentrations and compositions are age dependent [85]. Therefore, the serum bile acid profiles in adult and children with NAFLD are separately described in the following sections.

2.1.1.1 Serum bile acid profiling in adults with NAFLD

Currently, there are total nine clinical studies investigating serum bile acid levels and compositions in adults with NAFLD. In study 1 with 25 healthy subjects, 11 patients with steatosis, and 24 patients with NASH, it was found that serum bile acid profiles after overnight fasting were significantly altered in both steatotic and NASH patients, especially in patients with NASH [86]. The most prominent alteration is the markedly increased conjugated CA concentration. Taurine-conjugated CAs (TCAs) were elevated 4- and 2.2-fold, while glycine-conjugated CAs (GCAs) were increased 4.3- and 3.1-fold in patients with NASH and steatosis, respectively. Similarly, GCDCA levels were also elevated by 2- and 2.4-fold in patients with NASH and steatosis, respectively. Other bile acid species, including CA, GDCA, TDCA, and TCDCA, exhibited a trend of increase but their levels did not reach a statistical significance. It should be noted that patients with steatosis or NASH had significantly elevated insulin levels and exhibited insulin resistance, although the blood glucose levels were within the normal range. The patients, especially those with NASH, also had elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicating liver damage in those patients.

In study 2 with 15 healthy controls and 7 NASH patients, both fasting and postprandial bile acids were altered [87]. Total fasting serum bile acid levels were increase by more than twofold. Such increases in total bile acids are mainly due to significantly increased conjugated bile acids with both glycine and taurine, while unconjugated bile acids were not significantly altered. Both primary (CDCA and CA and their conjugated) and secondary (DCA and LCA and their conjugated) bile acids were markedly elevated. Similarly, postprandial serum bile acid levels were also markedly increased in patients with NASH, including total, conjugated and unconjugated, primary, and secondary bile acids. However, the relative ratios or the compositions of the serum bile acid pools were not significantly altered in both fasting and postprandial levels. Significant elevations in individual bile acids including DCA, GCA, GCDCA, and TCA were also noted. Other bile acid species including CA and CDCA were either not altered or slightly increased without reaching a

statistical significance. Patients with NASH had significantly elevated alkaline phosphatase (ALP), ALT, insulin, and homeostatic model assessment (HOMA) levels accompanied with significantly higher fast blood glucose levels when compared to the control subjects.

In study 3 with 24 healthy subjects, 25 steatotic, and 37 NASH patients, plasma bile acids after fasting were measured [88]. Total plasma primary bile acids (CA and CDCA) were gradually increased from controls to steatotic to NASH patients. On the contrary, total secondary bile acids (DCA and LCA) were gradually decreased from controls to steatotic to NASH patients. The increases in primary bile acids are mainly resulted from elevation of the conjugated bile acids, while the unconjugated primary bile acids (CA and CDCA) were comparable to those in the control subjects. Comparison between the two NAFLD groups, total conjugated CA and conjugated primary bile acids, was significantly higher in subjects with NASH compared to steatotic subjects. In addition, the compositions of the primary bile acid pools were also changed with significant increase in the ratios of total primary CA to CDCA, regardless of the status of diabetes. Although total secondary bile acids were lower in NASH patients, most of the individual secondary bile acids including GDAC, TDCA, TLCA, and GLCA were comparable among the three groups except for unconjugated DCA, which was significantly higher in NASH patients. Unconjugated UDCA levels were comparable among the three groups, while conjugated UDCA was significantly higher in NASH patients compared to steatotic and control subjects. It should be mentioned that AST and ALT levels were significantly elevated in both steatotic and NASH patients, indicating hepatic injury under the steatotic and NASH conditions. In addition, a large percentage of NASH patients (62.2%) were diabetic, while 20% of steatotic patients were diabetic with only one subject (4.2%) being diabetic in the control group.

In study 4 with 14 healthy controls and 7 patients with NASH, serum total bile acids were significantly elevated by 2.5-fold in patients with NASH compared to healthy control subjects [89]. Individual bile acids including GCA and TCA were markedly increased by 3.1- and 5.7-fold in patients with NASH, respectively. In addition, linear regression analysis revealed a significant association between NAFLD activity scores (NAS) and fasting total serum bile acid, GCA, and TCA concentrations. It should be mentioned that the fasting total bile acids, GCA and TCA serum, concentrations in healthy controls in the study were comparable to those reported previously for healthy adults [90].

In study 5 with 46 healthy control subjects and 13 patients with NAFLD, serum bile acids were dysregulated in patients with NAFLD [38]. Total serum bile acid levels were significantly increased by 4.7-fold from 2.8 μM in control subjects to 13.0 μM in NAFLD patients. Primary and secondary bile acids were elevated by 3.8- and 1.9-fold, respectively, in NAFLD patients. These increases in total, primary, and secondary bile acids are mainly due to much higher concentrations of conjugated bile acids in NAFLD patients (5.0 μM) than in control subjects (1.2 μM). Unconjugated bile acids were also slightly increased from 0.88 μM in control subjects to 1.30 μM in NAFLD patients without reaching a statistical significance.

In contrast to most of the previous studies, a recent study 6 with 32 patients with NASH and 26 non-NASH controls reported that plasma total, primary, secondary, unconjugated, and conjugated bile acids were not significantly different between the two groups [91]. The compositions of the plasma bile acid pools were also not altered either in patients with NASH when compared to non-NASH subjects. However, when the subjects were subcategorized into insulin resistance and insulin sensitive groups, significant changes in bile acid profiles were detected. Total serum CA (CA + GCA and TCA), unconjugated CA, total CDCA (CDCA + GCDCA + TCDCA), unconjugated CDCA, total primary and unconjugated primary, total

unconjugated, and non-12α bile acids were all significantly elevated in subjects with insulin resistance compared to insulin sensitive subjects. The authors therefore concluded that bile acid alterations were associated with insulin resistance but not NASH. The study also showed that body mass index (BMI), fasting plasma insulin concentrations, and HOMA values positively correlated with plasma CA and CDCA levels. It should be mentioned that the BMI and HOMA values were matched between the NASH patients and the non-NASH control subjects in this study. The average BMI was 40.2 for NASH and 39.4 for non-Nash subjects, indicating that both groups are severely obese. The average HOMA was 4.05 for NASH patients and 3.25 for non-NASH controls, indicating insulin resistance in both groups.

Consistent with the findings from the sixth study, another clinical study reported that patients with NAFLD exhibited comparable serum total bile acid concentrations to those in healthy control subjects [92]. The study included 16 healthy controls with an average BMI of 24.2, 14 overweight NAFLD patients with BMI of 28.3, and 12 obese NAFLD patients with an average BMI of 35.3. No significant alterations in fasting as well as postprandial serum total bile acid levels were detected between healthy control subjects and overweight or obese NAFLD patients.

In another study with 38 control subjects and 36 NASH patients, limited information about the characteristics of the studied subjects was provided and only data on three individual bile acid species were reported. The plasma concentrations of GCA, TCA, and TCDCA during fasting were significantly elevated in patients with NASH compared to control subjects [93]. Consistent with finding from most of the studies, another clinical study with 10 healthy controls, 39 steatotic, and 59 NASH patients reported that total serum bile acid levels were significantly elevated in patients with NAFLD [94].

The findings from six clinical studies, which provide detailed characteristics of the studied subjects as well as the corresponding bile acid profiles, are summarized in **Table 1**. The results from studies 1 to 5 are largely consistent. Serum total, primary, and conjugated bile acids were all significantly increased with limited changes for unconjugated bile acids. However, the secondary bile acids were significantly increased in studies 1, 2, and 5 but decreased in study 3. In contrast to the findings from studies 1 to 5, no significant alterations were detected in serum total, primary, secondary, conjugated, and unconjugated bile acids in the study 6.

Compared the characteristics of the control and NAFLD subjects, it is noticed that the control subjects in study 6 were severely obese with BMI 39.4 ± 5.9, while the control subjects in studies 1–5 have normal or close to normal body weights with BMI ranging from 24.5 ± 2.6 to 27.3 ± 5.8. The BMI values were matched between NAFLD patients and control subjects in study 6 but significantly different in studies 1–5. Compared with serum bile acid levels in a healthy population [85], the control subjects in study 6 had markedly increased total, primary, secondary, conjugated, and unconjugated bile acids. The results indicate that obesity or increased BMI is a contributing factor to the dysregulation of serum bile acids. Indeed, several studies have reported that subjects with overweight or obese had increased serum bile acid concentrations [85, 95, 96].

The second characteristic of the studied subjects that is different between study 6 and studies 1–5 is the status of insulin resistance in the control subjects. The serum insulin levels and HOMA values in study 6 are markedly higher than those in the other five studies, suggesting that insulin resistance is a contributing factor for the dysregulation of serum bile acids. Indeed, when all the subjects (NAFLD and control patients) in the study 6 were separated by insulin resistance status, primary bile acids, unconjugated bile acids, and non-12α bile acids, total CA and total CDCA were significantly increased in subjects with

Features	Subjects	Study 1 [87]	Study 2 [88]	Study 3 [89]	Study 4 [90]	Study 5 [92]	Study 6 [93]
Sample size	Control	25	15	24	14	46	26
	Steatosis	11		25			
	NASH	24	7	37	7	13	32
Age	Control	42.6 ± 9.2	43 ± 12	39.2 ± 12.4	42 ± 13	20–39	40.5 ± 11.7
	Steatosis	43.5 ± 10.7		54.6 ± 10.1			
	NASH	43.6 ± 12.6	48 ± 10	58.0 ± 8.8	48 ± 10	62.5 ± 16.5	41.3 ± 11.9
BMI	Control	24.5 ± 2.6	25 ± 2.7	27.3 ± 5.8	26 ± 2.7	<25	39.4 ± 5.9
	Steatosis	34.0 ± 4.0		32.6 ± 5.4			
	NASH	34.8 ± 4.7	32.0 ± 5.2	34.4 ± 4.2	32 ± 5.2	25.5 ± 2.8	40.2 ± 5.8
Insulin	Control	7.6 ± 3.1	7.6 ± 2.6	N/A	8.0 ± 3.0	N/A	15.7 ± 9.1
	Steatosis	20.6 ± 9.0		N/A			
	NASH	26.5 ± 14.9	40 ± 27	N/A	40 ± 27	N/A	18.9 ± 11.4
HOMA-IR	Control	0.9 ± 0.4	1.6 ± 0.6	1 (4.2%) diabetic	2.0 ± 1.0	0% DM	3.25 ± 2.05
	Steatosis	2.6 ± 1.1		5 (20%) diabetic			
	NASH	3.26 ± 1.6	13 ± 8.7	23 (62%) diabetic	12 ± 9.0	53.8% DM	4.05 ± 2.65
ALT	Control	17.6 ± 5.0	33 ± 11	22.7 ± 15.5	33 ± 11	Normal	27.3 ± 15.8
	Steatosis	44.4 ± 30.0		45.5 ± 24.0			
	NASH	84.6 ± 58.6	75 ± 36	57.1 ± 29.3	75 ± 36	64.1 (34.2–120)	36.5 ± 22.7
AST	Control	22.8 ± 5.7	N/A	22.3 ± 11.3	N/A	Normal	21.3 ± 11.9
	Steatosis	31.4 ± 15.4		45.6 ± 51.9			
	NASH	63.4 ± 46.7	N/A	42.4 ± 18.7	N/A	46.2 (26.8–79.8)	24.6 ± 17.5
Bile acids	Total	Increased	Increased	Increased	Increased	Increased	No changes
	Primary	Increased	Increased	Increased	Increased	Increased	No changes
	Secondary	Increased	Increased	Decreased	N/A	Increased	No changes
	Conjugated	Increased	Increased	Increased	Increased	Increased	No changes
	Unconjugated	N/A*	Increased but not significant	No changes	N/A	Slightly increased	No changes
	Compositions of bile acids	N/A	No significant changes	Significantly altered	N/A	N/A	No changes

**N/A, not available.*

Table 1.
Dysregulation of bile acids in adults with NAFLD with characteristics of the studied subjects.

insulin resistance compared to insulin sensitive subjects regardless of the status of NAFLD. These data strongly suggest that insulin resistance is a contributing factor to the dysregulation of bile acids, which is supported by the findings from previous studies [95–100].

The third different characteristic of the NASH patients in study 6 from studies 1 to 5 is the liver injury status. The NASH patients in study 6 exhibited much lower ALT and AST levels than those in the NASH patients in studies 1–5, indicating that the NASH patients in study 6 experienced minimal liver injury, while the NASH patients in other studies exhibited hepatic injury or damage with the ALT and AST levels above the physiological values. As discussed earlier, it is well established that liver injury potentially can cause dysregulation of bile acids [38–42]. The differing liver injury statuses may provide an explanation for the discrepancy in bile acid alterations between the study 6 and the other five studies. Taken together, the characteristic variations in BMI, insulin resistance, and hepatic injury of the studied subjects may all contribute to the inconsistency in serum bile acid levels reported in those studies.

2.1.1.2 Serum bile acid profiling in children with NAFLD

There are three studies conducted with children from ages 4 to 17 years old. In one study with 11 healthy controls (average age 12.8 years) and 16 patients with NASH (average age 13.7 years), total serum bile acid levels were significantly elevated by threefold in children with NASH compared to healthy controls [101]. More specifically, the absolute concentrations of CA, CDCA, DCA, and UDCA were all markedly increased. The percentages of CA and DCA in the total bile acid pools were significantly increased, while the percentages of CDCA in the pools were decreased with no changes in UDCA. It is noted that both ALT and AST levels in patients with NASH were increased, indicating hepatic injury in those patients. The children with NASH also exhibited insulin resistance with an average HOMA value of 4.3 ± 2.8 and overweight or obese with an average BMI of 33.8 ± 7.7.

In the second study with 105 healthy controls at ages 9.3 ± 2.5 and 92 children with NAFLD, which were further classified into two groups based on the stages of fibrosis: NAFLD-F0 group at ages 10.9 ± 3.7 and F ≥ 1 group at ages 11.5 ± 1.9, total serum bile acids were significantly decreased from 3.6 μM in control subjects to 1.73 μM in nonfibrotic (NAFLD-F0) patients accompanied by decreased glycine-conjugated bile acids and slightly increased taurine-conjugated and unconjugated bile acids [102]. The total serum bile acids in patients with more advanced NAFLD with fibrosis (NAFLD-F ≥ 1) were also decreased to 2.45 μM. Comparison between the two NAFLD groups, the serum bile acid levels increased by 41.9% in the NAFLD-F ≥ 1 group. These data indicate that serum bile acid levels decrease in the early stage of NAFLD, followed by an increase as NAFLD progresses to fibrosis. No significant differences were detected in the compositions of serum total bile acid pools among the groups. It should be mentioned that compared with control subjects (BMI 18.8 ± 4.2), children in the NAFLD groups were overweight (BMI > 26) with significantly elevated glucose and insulin levels. In addition, NAFLD patients had elevated AST and ALT levels, indicating hepatic injury in those patients.

In a most recent third study with 35 control children at ages 12.8 ± 4.2 and 41 NAFLD children at ages 13.7 ± 2.4, which were further divided into mild and moderate/severe NAFLD groups, no significant alternations in serum total, primary, and secondary bile acids were detected in children with NAFLD compared to control subjects [103]. Most of individual bile acid species (CA, CDCA, DCA, and LCA), conjugated and unconjugated bile acids, were comparable among the groups. Significant differences were only detected for unconjugated CDCA and unconjugated primary bile acids (CDCA + CA). Unconjugated CDCA and primary bile acids increased by 1.58-fold and 1.43-fold, respectively, in NAFLD children. After adjusted for age, sex, HOMA and BMI, unconjugated DCA, conjugated DCA

(GDCA and TDCA), and total DCA were significantly lower in NAFLD patients than those in the control group. Meanwhile, serum GLCA and total conjugated LCA (GCA + TLCA) were significantly decreased in NAFLD patients compared to control subjects.

The findings from the three clinical studies with detailed characteristics of the studied subjects are summarized in **Table 2**. The results from the three studies are largely inconsistent. In the second study with a larger size of samples, serum bile acid levels decrease in early stage of NAFLD and then increase during progression to fibrosis, but the levels were still below that in control subjects. In the third study with a medium size of samples, no significant alternations in serum total, primary, and secondary bile acids were detected in children with NAFLD. However, in the first study with smallest size of samples, total serum bile acid levels and compositions were significantly altered in patients with NASH compared to healthy controls.

Comparing the characteristics of studied subjects, the trend of bile acid alterations from decrease to no changes to increase correlates with the trend of gradually increased BMI from slightly overweight (26.5 ± 3.59) to severe overweight (29.6 ± 5.2) to obese (33.8 ± 7.7). Such correlation indicates that NAFLD-associated overweight or obesity may play important roles in influencing the bile acid homeostasis in children as well [85, 95, 96]. Another possible factor playing a role in bile acid dysregulation under the NAFLD conditions is the HOMA values, which were increased from 3.0 ± 3.0 in study 1 to 4.1 ± 3.2 in study 2 and 4.3 ± 2.8 in study 3. The differences in sample sizes of the studies certainly also contribute to the variations of bile acid levels among the studies. The sample sizes in the control groups ranged from 105 in study 2 to 35 in study 3 to 11 in study 1. Meanwhile, the sample sizes for NAFLD subjects were decreased from 92 in study 2 to 42 in study 3 to 16 in study 1. Taken together, serum bile acid levels were differentially altered in children with NAFLD. The differences in BMI, insulin resistance, and sample sizes may contribute to the variations of serum bile acids detected among the three studies.

2.1.2 Hepatic bile acids

There are three relevant studies investigating hepatic bile acids in patients with NAFLD. In the first study with 15 NASH patients and 8 control subjects, total hepatic bile acids were significantly increased in patients with NASH compared to control subjects [104]. The concentrations of individual bile acid species including CA, CDCA, and DCA were markedly higher in patients with NASH than those in the controls. It was also found that hepatic total bile acid levels were significantly correlated with hepatic inflammation status. Meanwhile, CDCA concentrations were positively correlated with fibrosis status in patients with NASH.

In a second study with liver tissues from 17 normal control subjects, 4 patients with simple steatosis, and 37 patients with NASH, significant alterations in hepatic bile acids were detected in NASH patients [105]. Hepatic CA and GDCA concentrations were markedly decreased by 69 and 91%, respectively, in patients with NASH compared to the control subjects. In contrast, hepatic TCA, TDCA, and GCDCA were significantly increased by approximately three, five, and twofold in NASH patients, respectively. Overall, hepatic total and conjugated bile acid concentrations were significantly higher in patients with NASH than those in controls. On the other hand, unconjugated bile acids were significantly decreased in patients with NASH. In patients with simple steatosis, total, conjugated, and unconjugated bile acids were all decreased without reaching a statistical significance mainly due to a small sample size of the group.

Features	Subjects	Study 1 [104]	Study 2 [105]	Study 3 [106]
Sample size	Control	11	105	35
	Steatosis		27	18
	NASH	16	65	23
Age	Control	12.8 ± 4.2	16 ± 3	9.3 ± 2.5
	Steatosis		10 ± 5	10.9 ± 3.7
	NASH	13.7 ± 2.4	12 ± 5	11.5 ± 1.9
BMI	Control	19.2 ± 3.4	20.5 ± 4.2	18.8 ± 4.2
	Steatosis		26.8 ± 3.9	26.0 ± 5.1
	NASH	33.8 ± 7.7	26.5 ± 3.5	29.6 ± 5.2
Insulin	Control	N/A*	3.2 ± 0.6	6.9 ± 4.9
	Steatosis		11.2 ± 4.8	11.0 ± 5.9
	NASH	N/A	12.4 ± 6.0	17.5 ± 11.4
HOMA-IR	Control	N/A	1.5 ± 0.3	1.3 ± 1.0
	Steatosis		2.6 ± 1.8	2.6 ± 1.5
	NASH	4.3 ± 2.8	3.0 ± 3.0	4.1 ± 3.2
ALT	Control	19.4 ± 4.4	24 ± 28	14.0 ± 8.5
	Steatosis		62 ± 20	25.5 ± 17
	NASH	54.1 ± 29.7	87 ± 59	68.5 ± 42.0
AST	Control	24.4 ± 11.5	25 ± 13	25 ± 8.5
	Steatosis		40 ± 8	23 ± 6.7
	NASH	33.9 ± 15.0	61 ± 29	40.9 ± 24.7
Bile acids	Total	Significantly increased	Significantly decreased	No significant changes
	Primary	Significantly increased	Significantly decreased	No significant changes
	Secondary	Significantly increased	Slightly decreased	No significant changes
	Conjugated	N/A	Significantly decreased	No significant changes
	Unconjugated	N/A	Slightly increased	No significant changes
	Compositions of bile acids	Significantly altered	No changes	No changes

**N/A, not available.*

Table 2.
Dysregulation of bile acids in children with NAFLD with characteristics of the studied subjects.

In a relevant third study with 20 control subjects and 22 diabetic patients, hepatic bile acid concentrations were significantly altered [106]. Among the 22 diabetic patients, 77.7% patients had NAFLD with NAS score of 2 or above. Consistently, majority of patients (68%) were overweight, obese, or severe obese, with hypercholesterolemia being detected in 86.4% of the patients. Total hepatic

bile acids were markedly reduced by 53% in diabetic patients compared to control subjects. The significant decrease in total bile acids is largely due to the marked reduction in conjugated bile acids. On the other hand, unconjugated bile acids were slightly increased by 33% without reaching a statistical significance. Among the conjugated bile acids, both glycine and taurine conjugated bile acids were significantly reduced in diabetic patients. However, the reductions were more severe in glycine conjugated than taurine-conjugated bile acids.

In summary, no clear consensus can be reached for hepatic bile acid profiles in patients with NAFLD. Both increases and decreases of hepatic bile acids were reported. Some specific bile acid species were markedly increased, while other species significantly decreased in the same patients. From the limited clinical studies, it can be concluded that hepatic bile acid homeostasis is dysregulated in patients with NAFLD. However, due to the complexity of bile acid regulation, variations in characteristics and stages of NAFLD patients, and lack of high quality clinical studies, it largely remains to be determined by the effects of NAFLD on hepatic bile acid homeostasis.

2.1.3 Fecal and urine bile acids

There are only one study investigating fecal bile acids in patients with NAFLD. The study has 25 healthy controls, 12 patients with steatosis, and 17 patients with NASH [107]. Total fecal bile acid levels were significantly higher in patients with NASH compared to healthy controls. Meanwhile, total fecal bile acids also showed an increased trend in steatotic patients without reaching a statistical significance. Primary, secondary, conjugated, and unconjugated bile acid concentrations all exhibited a gradual increase from healthy controls to steatotic to NASH patients. Unconjugated primary bile acids including CA and CDCA were significantly increased in NASH patients compared to healthy controls, while unconjugated secondary bile acids were not significantly different among the three groups. Patients with NASH had significantly higher concentrations of conjugated LCA compared to patients with steatosis. In addition, a higher ratio of primary to secondary bile acids in patients with NASH was also detected. However, the ratio of total conjugated over unconjugated bile acids was not significantly different among the groups. Correlation analysis revealed that fecal unconjugated primary bile acids positively correlated with steatosis, ballooning, fibrosis, NAS scores, and liver injury (AST and ALT levels). The results from the study demonstrated that fecal disposition of bile acids was enhanced in patients with NASH. However, it remains to be determined that such increase in fecal disposition of bile acids is resulted from impairment of intestine reabsorption of bile acids or enhanced biliary excretion of bile acids or both.

There is only one study with 15 healthy controls and 7 NASH patients to investigate urine bile acid profiles in patients with NAFLD. Urine total, primary, secondary, conjugated, and unconjugated bile acids all exhibited a trend of increase without reaching a statistical significance [87]. However, individual bile acid species including DCA, TCA, GCA, and GCDCA were significantly elevated in patients with NASH compared to control subjects. Consistently, total serum bile acid levels were also significantly increased by more than twofold in NASH patients compared to control subjects.

In summary, the findings from clinical studies to evaluate serum, hepatic, and urine bile acid profiles are inconsistent among the studies. The reasons for those inconsistent or even conflicting results are multiple folds. First, bile acid synthesis and serum concentrations fluctuate during the days and nights [108–112].

Although most of the samples were collected after fasting, there was no mentioning on exactly when the samples were collected in the studies. Second, NAFLD represents a spectrum of pathological liver conditions from simple steatosis to NASH with or without fibrosis. The severity of bile acid dysregulation appears NAFLD stage dependent. Bile acid alterations gets worsening in patients with advanced stages of NAFLD, such as NASH, compared to the patients with simple steatosis [86, 88, 101, 102, 107]. Some studies differentiate NAFLD patients into simple steatosis and NASH [86, 88, 101, 102, 107], while the others [38, 87, 89, 91, 103] do not, which certainly influences the outcomes of the studies. Third, NAFLD is often associated with various metabolic conditions, especially obesity and insulin resistance/diabetes. It has been reported that obesity and insulin resistance directly impacts bile acid homeostasis [85, 95–100]. Fourth, selection of the control groups varies from study to study [38, 86–89, 91], which certainly contributes to the discrepancy of the outcomes among the studies. Finally, the sizes of samples are relatively small in most of the studies with individual variations potentially masking the alterations.

2.2 Altered bile acid profiles in NAFLD animal models

Several rodent models for NAFLD have been developed [113–115], including high-fat cholesterol (HFC) and methionine- and choline-deficient (MCD) diet-induced or genetic deficient models, including leptin-deficient *ob/ob*, leptin receptor-deficient *db/db* mice, and *fa/fa* rats. Several preclinical studies were conducted to investigate the effects of NAFLD on bile acid homeostasis using NAFLD mouse or rat models. In one study, NAFLD was induced in rats with HFC diet [116]. Total hepatic bile acids were significantly increased in rats on HFC diet for 2 weeks. Primary, secondary, conjugated, and unconjugated bile acid concentrations were all increased after 2 weeks on HFC diet. Most bile acid species remained higher in rats on HFC diet for 8 and 14 weeks than those on regular diet. However, the levels of CA and DCA species declined from their peaks at 2 weeks, while CDCA species persistently increased for the entire treatment. In addition, CDCA species positively correlated with macrovesicular steatosis score, serum ALT levels, and quantified fibrotic area. Among the conjugated bile acids, glycine-conjugated bile acid species (GCA, GCDCA, GDCA, GLCA, and GUDCA) were predominate over taurine-conjugated bile acid species and positively correlated with macrovesicular steatosis score. The finding demonstrated that bile acid homeostasis is severely disrupted in HFC diet-induced NAFLD rats, especially the CDCA and glycine-conjugated bile acid species.

In another study with MCD-induced NASH mouse model, markedly increased serum concentrations of taurine-conjugated CA and β-muricholate (βMCA) were detected in mice on MCD diet for 2 or 8 weeks compared to mice on control diet, indicating dysregulation of serum bile acid in mice with NASH [117]. Similar findings were reported with *ob/ob* mouse model. Serum total bile acid concentrations were markedly elevated by sevenfold from 1.9 ± 1.0 μM in wt control mice to 14.9 ± 5.4 μM in *ob/ob* mice [118]. In contrast to the findings from the studies described above, a more recent study showed that total serum bile acid concentrations were not significantly different in HFD-induced NAFLD mice than mice on control diet [119].

Taken together, bile acid homeostasis is disrupted in NAFLD rodent models. Serum bile acid levels were markedly elevated in most of the studies. However, variations in serum bile acid concentrations exist in different NAFLD rodent models, may reflecting species difference between mouse and rat.

3. Alterations in bile acid synthesis in subjects with NAFLD

3.1 Alterations in bile acid synthesis in patients with NAFLD

Primary bile acids CA and CDCA are synthesized in the liver through either the classical or alternative synthesis pathways. In the intestine, CA can be converted into secondary bile acid DCA, while CDCA is converted into secondary bile acids LCA or UDCA (**Figure 1**). Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the classical pathway, while CYP8B1 is the rate-limiting enzyme for the production of CA. The two rate-limiting enzymes for the alternative pathway are CYP27A1 and CYP7B1 (**Figure 1**). Alterations in the expression levels of rate-limiting enzymes in the bile acid synthesis pathways result in dysregulation of bile acid homeostasis. A number of clinical studies have conducted to investigate the effects of NAFLD on bile acid synthesis.

3.1.1 CYP7A1

There are eight clinical studies investigating the expression of CYP7A1 in patients with NAFLD. Most of the studies revealed that CYP7A1 expression was dysregulated in patients with NAFLD. Among the eight studies, the results from five studies showed that the mRNA expression levels of CYP7A1 were significantly increased in patients with NAFLD [88, 91, 94, 101, 120], indicating that bile acid synthesis through the classical pathway is enhanced in patients with NAFLD. However, in a study with 17 normal control subjects, 4 patients with simple steatosis, and 37 patients with NASH, CYP7A1 expression was not altered in patients with steatosis or NASH [105]. In another study with 6 lean healthy controls, 20 obese normal controls, 20 patients with simple steatosis, and 20 patients with NASH [121], CYP7A1 mRNA expression significantly increased in obese normal control subjects, patients with steatosis, and NASH compared to healthy lean subjects. However, at the protein level, CYP7A1 expression was comparable in obese normal controls compared to healthy lean subjects. More

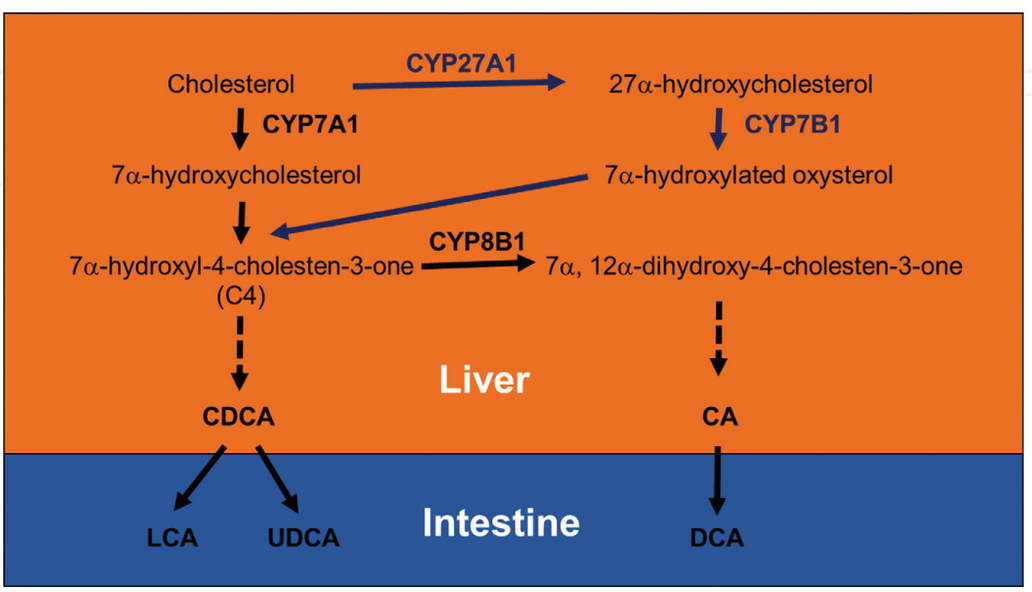

Figure 1.
Primary bile acids CDCA and CA are synthesized in the liver through classical (CYP7A1) and alternative (CYP27A1) bile acid synthesis pathways and converted into secondary bile acids LCA, UDCA, and DCA in the intestine.

strikingly, CYP7A1 protein expression was markedly decreased in patients with steatosis and especially with NASH, indicating that bile acid synthesis through the classical pathway is reduced in patients with NAFLD. In a study with 78 NAFLD patients, the subjects were divided into three groups based on the NAS scores, NAS 1–2, NAS 3–4, and NAS 5–8. The mRNA expression levels of CYP7A1 were comparable among the three groups, indicating that bile acid synthesis through the classical pathway remains unchanged during the progression of NAFLD [122].

Taken together, CYP7A1 expression was largely upregulated in patients with NAFLD, indicating that bile acid synthesis through the classical pathway is enhanced in patients with NAFLD. Discrepancy in CYP7A1 mRNA and protein levels was noted, indicating the importance of post-transcriptional regulation of CYP7A1 under the NAFLD condition.

3.1.2 CYP27A1

There are three clinical studies evaluating the effects of NAFLD on the expression of CYP27A1. The findings from the three studies are largely inconsistent. In one study, the expression levels of CYP27A1 were significantly induced in patients with NAFLD [101]. In contrast, a second study reported that CYP27A1 expression was significantly decreased in patients with NAFLD compared to control subjects [105]. A third study showed that CYP27A1 expression was not altered in NAFLD subjects [121]. Therefore, it can be concluded that the effects of NAFLD on CYP27A1 expression are inclusive.

3.1.3 Other enzymes

There are a couple of studies investigating other enzymes involved in bile acid synthesis, including CYP8B1 and CYP7B1. One study reported that the expression levels of CYP8B1 were decreased, while CYP7B1 levels were increased in patients with NAFLD [105]. The other study revealed that the expression levels of CYP8B1 were significantly increased in patients with NAFLD compared to control subjects [101]. Therefore, additional studies are required to determine the effects of NAFLD on CYP8B1 and CYP7B1 expression.

3.1.4 C4

7α-Hydroxy-4-cholesten-3-one (C4) is an intermediate of bile acid synthesis (**Figure 1**) and serves as an indicator for bile acid synthesis *in vivo* [123]. There are three studies investigating serum C4 levels in patients with NAFLD. In one study with 26 NAFLD patients and 16 healthy controls, the serum concentrations of C4 were not significantly different between the two groups, indicating that de novel bile acid synthesis was not changed in patients with NAFLD [38]. Consistent results were obtained with a second study which includes 26 healthy controls and 32 patients with NASH. The serum C4 concentrations were not significantly altered in patients with NASH compared to the control subjects [90]. However, in the third study with 25 healthy controls, 12 patients with steatosis, and 16 patients with NASH, serum C4 levels were significantly elevated in patients with steatosis and NASH compared to healthy control subjects, suggesting that bile acid synthesis is enhanced in patients with NAFLD. Correlation analysis revealed that the serum C4 concentrations were directly correlated with fecal total bile acid levels in the studied subjects [104]. Taken together, the serum C4 concentrations either increased or did not change in patients with NAFLD.

3.2 Alterations in bile acid synthesis in NAFLD animal models

There are five studies investigating the expression of enzymes involved in bile acid synthesis. In one study with high fat diet (HFD)–induced NAFLD mice, the mRNA expression levels of Cyp7a1 and Cyp8b1 were markedly decreased compared to control mice on regular diet [124], indicating that de novel bile acid synthesis through the classical pathway is reduced in NAFLD mice. Consistent with the finding, a study with *ob/ob* mice, the expression levels of Cyp7a1 were significantly decreased in *ob/ob* mice compared to lean wt mice [118]. However, in a study with HFD/streptozotocin (STZ)-induced NAFLD rats, the expression levels of Cyp7a1 were dramatically increased, while the expression levels of Cyp27a1 and Cyp7b1 were also significantly induced in NAFLD rats compared to control rats [125]. The findings indicate that bile acid synthesis through both classical and alternative pathway is increased in HFD/STZ-induced NAFLD rats. On the other hand, in one study with MCD-induced simple steatotic rats, the expression levels of Cyp7a1 were comparable between the steatotic rats and healthy control rats [126]. Consistently, a study with MCD-induced NASH in mice showed that the expression levels of Cyp7a1 were not altered in mice with NASH compared to control mice [117]. In addition, the expression levels of Cyp27a1 and Cyp8b1 were not significantly changed in steatotic mice compared to healthy control mice. The findings indicate that both classical and alternative bile acid synthesis pathways are not impaired in MCD-induced NASH mice. In summary, the effects of NAFLD on Cyp7a1, Cyp27a1, and Cyp8b1 expression are inconclusive in NAFLD rodent models, which are to a large extent different from the findings in patients with NAFLD, especially for CYP7A1.

4. Alterations in bile acid transporters in subjects with NAFLD

The enterohepatic circulation of bile acids is mediated by a series of bile acid transporters in the liver and intestine (**Figure 2**). After synthesis in the liver, bile acids are excreted into bile through the bile salt export pump (BSEP). Majority of bile acids are actively transported into enterocytes by the apical sodium-dependent bile acid transporter (ASBT). Bile acids exit the enterocytes on the basolateral side via the heterodimeric organic solute transporter α and β (OSTα/β) and then return to the liver through the Na^+/taurocholate cotransporting polypeptide (NTCP), completing the circulation. In the liver, other transporters are also capable of transport bile acids, including multidrug resistance associated protein 2 (MRP2) on the canalicular membrane and multidrug resistance-associated protein 3 (MRP3), MRP4, and organic anion-transporting polypeptides (OATP1B1 and OATP1B3) on the basolateral membrane. It should be emphasized that biliary excretion through BSEP is the rate-limiting step in the circulation and bile acid spilling into blood is mediated mainly by MRP3 and MRP4. Alteration in bile acid transporter expression has significant impact on bile acid compartmenting and homeostasis.

4.1 Alterations in bile acid transporters in patients with NAFLD

4.1.1 BSEP

As the canalicular bile acid transporter, BSEP expression was dysregulated in patients with NAFLD. Three clinical studies showed that BSEP mRNA expression

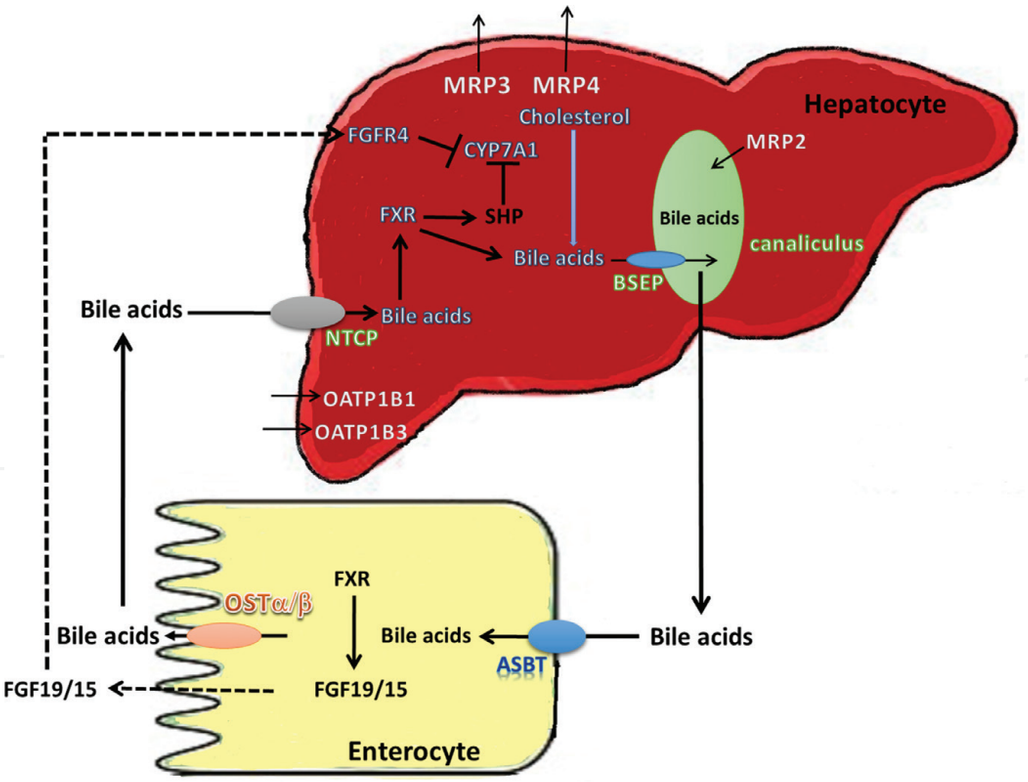

Figure 2.
Enterohepatic circulation of bile acids through a series of bile acid transporters, and regulation of bile acid synthesis by the FXR/SHP and FGF19/FGFR4 synthesis pathway.

was decreased in steatotic or NASH patients compared to control subjects [88, 91, 122]. A different study reported that BSEP mRNA expression levels were increased in patients with NASH compared to the patients with simple steatosis [118]. On the other hand, two studies revealed that BSEP mRNA expression was not altered in patients with NAFLD or diabetes compared to healthy control subjects [101, 106]. Finally, another study reported that BSEP mRNA levels were elevated in lean NAFLD patients but reduced in overweight or obese patients with steatosis or NASH [94], indicating that body weight of the patients influences the expression of BSEP under the NAFLD condition. Taken together, it can be cautiously concluded that BSEP expression was largely decreased in patients with NAFLD. The alterations BSEP expression may be influenced by the body weight of the patients.

4.1.2 NTCP

Three clinical studies showed that NTCP mRNA expression levels were significantly upregulated in patients with NAFLD compared to control subjects [88, 101, 94]. However, a different study reported that NTCP mRNA expression was significantly decreased as NAFLD progressed from earlier stage (steatosis) to late stage (NASH) [122]. On the other hand, one study reported that the mRNA levels of NTCP were significantly increased in patients with NASH compared to patients with simple steatosis. However, at the protein level, NTCP expression was significantly reduced in the patients with NASH compared to patients with simple steatosis [127], indicating the dominance of post-transcriptional regulation of NTCP under the NASH condition. Another study with diabetic patients reported that NTCP mRNA expression levels were comparable between diabetic patients

and control subjects [106]. In summary, NTCP expression was likely upregulated in NAFLD patients with certain inconsistency.

4.1.3 MRPs

In one study with NAFLD and one study with diabetic patients, MRP2 mRNA expression levels were not significantly altered in NAFLD and diabetic patients compared to control subjects [88, 106]. Another study reported that MRP2 mRNA expression levels were decreased as the NAFLD progressed from steatosis to NASH [122]. Supporting MRP2's role in NAFLD, it was found that a polymorphism in MRP2 was significantly associated with NAFLD [128]. Currently, there is only one study investigating the expression levels of MRP3 in patients with NAFLD. The MRP3 mRNA expression levels were significantly elevated in patients with NAFLD, especially with NASH, compared to the healthy control subjects [94]. In another study with diabetic patients, MRP3 and MRP4 expression levels were not significantly altered in diabetic patients compared to control subjects [106]. Taken together, the results from limited studies suggest that MRP3 and MRP4 were upregulated in patients with NAFLD, while the effects of NAFLD on MRP2 expression were minimal.

4.1.4 OATPs

There is currently only one study investigating the expression of OATPs in patients with NAFLD. Both OATP1B1 and OATP1B3 mRNA expression levels were significantly upregulated in patients with NAFLD compared to control subjects [101]. In a different study with diabetic patients, the expression levels of OATP1B1 were comparable in diabetic patients compared to control subjects [106]. Therefore, it can be cautiously concluded that OATP1B1 and OATP1b3 expression were largely induced in patients with NAFLD.

4.2 Alterations in bile acid transporters in NAFLD animal models

4.2.1 Bsep

Several studies have investigated the effects of NAFLD on Bsep expression in rodents. In one study with HFD/STZ-induced NAFLD rats, the Bsep mRNA levels were significantly downregulated in NAFLD rats compared to control rats [125], indicating reducing biliary excretion of bile acids in HFD/STZ-induced NAFLD rats. However, in two other studies with MCD-induced NAFLD rats and mice, the mRNA expression levels of Bsep were not altered in NAFLD rats or mice [126, 117]. Consistently, a study with obese zucker rats showed that Bsep expression was not significantly altered in obese ZR rats compared to control rats [129]. In another study with obese ZR rats, the expression levels of Bsep mRNA were significantly decreased in obese ZR rats, while Bsep protein levels detected by Western blot as well as immunohistochemistry were comparable between obese ZR rats and lean control rats [130]. In another study with *ob/ob* mice, the expression levels of Bsep mRNA were significantly increased in *ob/ob* mice compared to lean control mice. However, in contrast to the mRNA levels, Bsep protein levels were significantly decreased in *ob/ob* mice when compared to lean control mice [118]. Consistent with decreased Bsep expression in NAFLD mice, overexpression of Bsep increases hepatobiliary lipid secretion and reduces hepatic steatosis [131]. Taken together, Bsep expression was either not altered or decreased in NAFLD rodent models.

4.2.2 Ntcp

Currently, there are six studies evaluating the effects of NAFLD on Ntcp expression in rodents. In three studies, the expression levels of Ntcp were consistently decreased in animals with NAFLD compared to control animals [117, 125, 132]. Two studies were conducted in rats, while one study was carried out in mice. The common feature among the three studies is that NAFLD was induced by MCD for 8 weeks. In the same study [132], Ntcp expression levels were also significantly decreased when NAFLD was induced by HFD. On the other hand, another two studies with rats showed the Ntcp expression was not altered under the NAFLD condition [126, 130]. Different effects of NAFLD on Ntcp mRNA and protein expression were reported in a study with *ob/ob* mice [118]. The expression levels of Ntcp mRNA were not changed in *ob/ob* mice compared to the lean control mice. However, Ntcp protein levels were significantly lower in *ob/ob* mice than those in lean control mice. Taken together, the effects of NAFLD on Ntcp expression were largely consistent among the six studies, either no significant changes or decreased dependent on the species and methods by which NAFLD was induced.

4.2.3 Mrps

The effects of NAFLD on Mrp expression were extensively investigated mainly due to the fact that Mrps are important transporters for xenobiotics including drugs. Data from eight studies evaluating Mrp2 expression in NAFLD rodents are not consistent. Two studies with obese ZR rats reported consistent results that the expression levels of Mrp2 mRNA and protein were significantly downregulated in obese ZR rats compared to lean control rats [129, 130]. Consistent with downregulation of Mrp2 in obese zucker rats, Mrp2 expression levels were reduced in MCD-induced NAFLD rats compared to control rats on supplemented MCD [126]. On the other hand, other two studies showed that Mrp2 expression levels were not significantly altered in MCD-induced NAFLD rats or HFD/STZ-induced NAFLD mice compared to the control rats or mice [117, 125]. In a study with *ob/ob* mice, Mrp2 expression levels were significantly increased at the mRNA level but decreased at the protein level [118]. In contrast, the mRNA levels of Mrp2 were decreased but protein levels were increased in MCD-induced NAFLD rats [132]. In a comprehensive study to evaluate various NAFLD models with mice and rats, Mrp2 expression was significantly increased in athrogenic diet and MCD-induced NAFLD rats and all four types of NAFLD mouse models when compared to the corresponding control rats or mice. At the protein level, Mrp2 expression was only increased in MCD-induced NAFLD rats [133]. Compared with Mrp2, the data for the effects of NAFLD on the expression of Mrp3 and Mrp4 are more consistent among the studies. Mrp3 and/or Mrp4 expression were significantly upregulated in five studies with NAFLD rats or mice [117, 124, 126, 118, 133]. On the other hand, another three studies with HFD/STZ-induced NAFLD rats or obese ZR rats reported that the expression levels of Mrp3 and/or Mrp4 were not altered in NAFLD or obese ZR rats compared to the controls [125, 129, 130].

In summary, the effects of NAFLD on Mrp2 expression were inconsistent or even conflicting. The discrepancy between Mrp2 mRNA and protein levels was also noted in the studies, indicating that post-transcriptional regulation plays an important role in regulating Mrp2 expression under the NAFLD condition. On the other hand, the expression of Mrp3 and Mrp4 was largely upregulated in NAFLD rodent models.

4.2.4 Oatps

Currently, there are seven studies evaluating the effects of NAFLD on the expression of Oatps. The expression levels of Oatp1a1 mRNA and/or protein were consistently decreased in six studies [117, 118, 125, 129, 132, 133], while one study showed no changes [126]. There are three studies investigating Oatp1a4. In one study, the expression levels of Oatp1a4 were significantly reduced at both mRNA and protein levels in *ob/ob* mice compared to lean control mice [118]. In another study, the expression levels of Oatp1a4 mRNA were increased but its protein levels were decreased in various mouse and rat NAFLD models compared to the corresponding control mice or rats [133]. On the other hand, no alterations in Oatp1a4 expression were detected in MCD-induced NAFLD rats [126]. The effects of NAFLD on the expression of Oatp1b2 were very much consistent among the five studies. Oatp1b2 expression was significantly downregulated in four studies [117, 126, 132, 133], while no alterations in Oatp1b2 expression were detected in one study [125]. There are three studies investigating Oatp2b1. One study with *ob/ob* mice reported that Oatp2b1 mRNA levels were significantly upregulated in *ob/ob* mice compared to the lean control mice [118]. However, other two studies showed that Oatp2b1 was downregulated in obese ZR rats compared to lean control rats [129, 132]. Taken together, Oatp1a1 and Oatp1b2 were consistently downregulated, while the effects on Oatp1a4 and Oatp2b1 were inconsistent in NAFLD rodents.

5. Alterations in bile acid regulators in subjects with NAFLD

Bile acid synthesis is tightly regulated by multiple signaling pathways, mainly the FXR/SHP [134, 135] and FGF19/FGFR4 [136, 137] negative feedback loops (**Figure 2**). In the liver, activation of FXR by bile acids induces SHP expression, which in turn represses CYP7A1 expression, leading to reduced bile acid synthesis. In the intestine, activation of FXR by bile acids upregulates FGF19 (FGF15 in rodents). After entering the circulation, FGF19 binds to FGFR4 in the liver to activate the downstream signaling, which subsequently inhibits CYP7A1 expression, resulting in decreased bile acid synthesis. Those two negative feedback regulatory loops play critical roles in regulating bile acid synthesis and maintaining bile acid homeostasis. Impairment or dysregulation of the FXR/SHP and FGF19/FGFR4 signaling pathways interrupts bile acid balance.

5.1 FXR/SHP signaling pathway

5.1.1 In human

Most of the human clinical studies revealed that the FXR/SHP signaling pathway was dysregulated in patients with NAFLD. In one study with 10 healthy controls, 39 steatotic, and 59 NASH patients, both FXR and SHP mRNA levels were significantly downregulated [94]. In two studies with 20 or 11 normal control subjects and 20 NAFLD or 16 NASH patients, the expression levels of FXR were significantly deceased in NAFLD or NASH patients compared to control subjects [101, 138]. However, the expression levels of SHP remain comparable between the control subjects and NAFLD or NASH patients, indicating that the FXR signaling is impaired in NAFLD or NASH patients [101, 138]. In a study with 33 children (19 NAFLD patients and 14 control children), the FXR protein levels were gradually

decreased from control subjects to steatotic to NASH patients, indicating the worsening of FXR signaling as NAFLD progresses [139]. Consistently, in a study with 20 simple steatosis and 20 NASH patients, the FXR protein expression levels were significantly decreased in NASH patients compared to the patients with simple steatosis, although at the mRNA level, FXR expression was higher in patients with NASH than those in patients with simple steatosis [127]. On the other hand, in one study with 26 controls and 32 NASH patients, no differences were detected in the expression of both FXR and SHP between control and NASH subjects [91]. Finally, one study showed gender differences in FXR expression. A significant decrease in FXR expression was detected in female but not male NASH patients compared to control subjects, while SHP expression was significantly decreased in both male and female with NASH [122]. In summary, most of the studies revealed a decreased or impaired FXR signaling in patients with NAFLD, and such impairment gets worsening as NAFLD progresses from simple steatosis to NASH.

5.1.2 In rodent NAFLD models

Inconsistent results have been reported regarding the status of FXR signaling in NAFLD rodent models. In two studies with HFD or fructose-induced NAFLD mice, the FXR expression levels were significantly reduced in NAFLD mice compared to control mice [124, 140]. However, SHP expression remained unchanged in fructose-induced NAFLD mice while significantly increased in HFD-induced NAFLD mice. In another two studies with HFD/STZ or MCD-induced NAFLD rats, the FXR expression levels remained comparable between the NAFLD and control rats [125, 126]. Consistent with no changes in FXR expression, SHP expression was comparable between the two groups. In another study with *ob/ob* mice, FXR mRNA and protein were significantly increased in *ob/ob* mice compared to lean control mice, while no alterations in SHP expression was detected [118]. Finally, in a study with HFD-induced NAFLD mice, the FXR signaling status was investigated during the progression of NAFLD from simple steatosis to NASH, fibrosis, and hepatocellular carcinoma (HCC) on an HFD [141]. FXR signaling was strongly activated in the early stage of NAFLD (simple steatosis) evidenced by strong upregulation of FXR target genes including Bsep, Mrp2, and ATP-binding cassette subfamily G member 5 (Abcg5)/Abcg8. However, as NAFLD progressed, FXR signaling gradually decreased but was still higher than that in the control mice on regular diet. Taken together, the inconsistent results from the NAFLD rodent models indicate that the effects of NAFLD on the FXR signaling pathway are dependent on the methods by which NAFLD is induced as well as the species (mouse or rat).

5.2 FGF19/FGFR4 signaling pathway

A large number of clinical studies have demonstrated that the FGF19 signaling is dysregulated in patients with NAFLD. Serum FGF19 concentrations were significantly reduced in patients with simple steatosis or NASH compared to control subjects [88, 92, 101, 102, 139, 142–144]. The decreases in FGF19 concentrations were more severe in patients with NASH than the patients with steatosis, indicating the worsening of FGF19 signaling impairment as the NAFLD progresses from simple steatosis to NASH. On the other hand, there are two clinical studies showing that the fasting serum concentrations of FGF19 were not altered in patients with NAFLD compared to control subjects [107, 145]. Taken together, most of the clinical studies showed that the FGF19 signaling was reduced or impaired in patients with NAFLD.

6. Conclusions

A large body of evidence from clinical as well as preclinical studies has demonstrated that bile acid homeostasis is disrupted in subjects with NAFLD. The dysregulation of bile acids in patients with NAFLD gets worsening as the disease progresses from early stage simple steatosis to late stages NASH and NASH with fibrosis. Risk factors for NAFLD, especially obesity and insulin resistance, contribute to the dysregulation of bile acids in NAFLD patients.

Due to the complexity of bile acid regulation, small sample sizes in most of the clinical studies, variations in control subject selection, inherited differences in various rodent NAFLD models, and discrepancy in mRNA and protein levels of the target genes, inconsistent or even conflicting results, have been reported for serum and hepatic bile acid concentrations and compositions, as well as the expression levels of bile acid synthesis enzymes, transporters, and regulators. However, detailed examination and evaluation of the results from various studies, especially considering the characteristics of the studied subjects and the quality of each study, certain trends on alterations in serum and hepatic bile acid levels, bile acid synthesis, and regulation in patients with NAFLD are emerged.

As depicted in **Figure 3**, serum total bile acid concentrations are increased in patients with NAFLD, as a result of increased CYP7A1 expression and bile acid synthesis, elevated hepatic bile acids, and augment of MRP3 and MRP4 expression. Increased CYP7A1 expression and bile acid synthesis in patients with NAFLD are mainly due to the impairment of the FXR/SHP and FGF19/FGFR4 signaling pathways. Limited studies on investigating fecal and urine bile acids showed that both fecal and urine bile acid concentrations were elevated in patients with NAFLD, consistent with increased serum and hepatic bile acid levels in those patients.

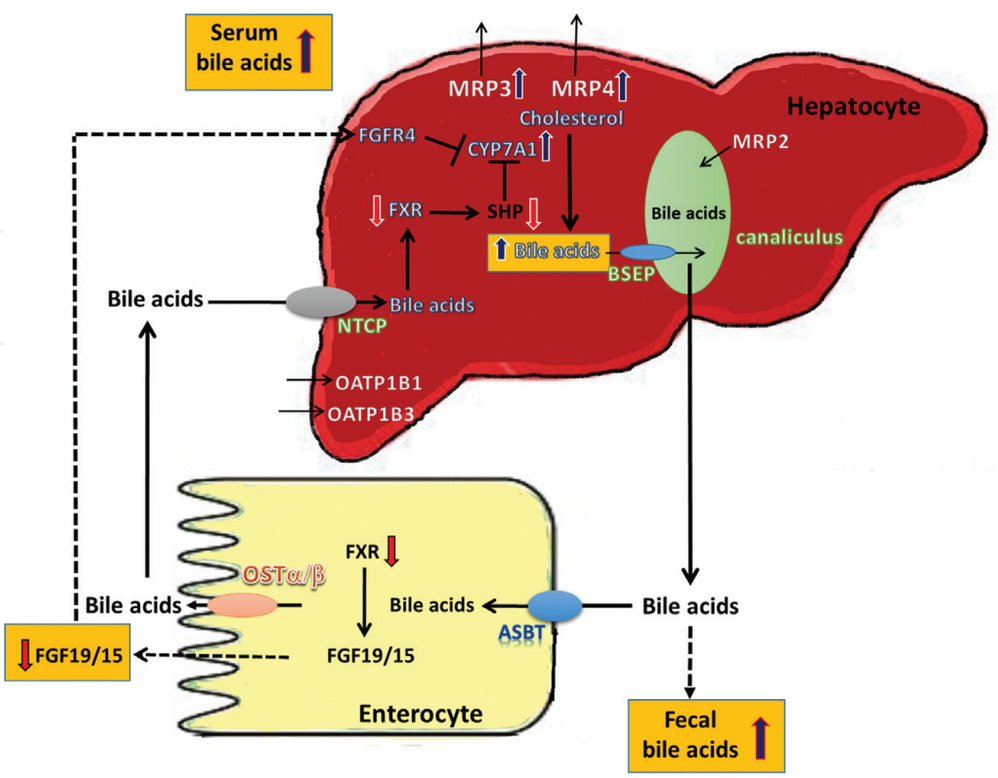

Figure 3.
Effects of NAFLD on serum, hepatic, and fecal bile acid concentrations as well as on bile acid synthesis (CYP7A1), transporters (MRP3 and MRP4), and regulators (FXR, SHP, FGF19/15).

7. Guidance for future studies

Future studies with high quality and large sample size are needed to solidify the trends depicted in **Figure 3**. The following points should be considered in the design of the future studies and interpretation of the findings. First, limited studies with children and adolescents revealed a different feature in bile acid dysregulation from adults with NAFLD. In contrast to the findings in adults, serum bile acid levels decrease in the early stage of NAFLD, followed by an increase as NAFLD progresses to fibrosis but the levels remain lower than those in the healthy control children. The effects of NAFLD on bile acid regulation appear different in children from adults. Second, the effects of NAFLD on bile acid homeostasis are stage dependent. No or mild effects of simple steatosis on bile acid regulation were detected, while significant alterations in bile acids are mostly detected in patients with NASH. A large percentage of previous studies did not separate the steatotic and NASH patients in the test groups, which certainly complicates the analysis and interpretation of data. Third, as risk factors for NAFLD, obesity and insulin resistance/diabetes are often associated with NAFLD. It is well documented that obesity and insulin resistance directly cause dysregulation of bile acids. Therefore, those risk factors should be adjusted or matched in the control group in order to reveal the exact effects of NAFLD on bile acid homeostasis. Among the clinical studies reported, only one study was conducted with a matched control group, in which a different conclusion was reached that NASH has no effects on bile acid regulation [93]. Fourth, in future studies using NAFLD rodent models, it should be emphasized that species differences between rodents and human and even between mouse and rat exist, especially in the effects of NAFLD on bile acid transporter expression. Finally, in the investigation of gene expression, both mRNA and protein levels should be detected and quantified for the target genes. Most of the previous studies only evaluated the mRNA levels. However, discrepancy between the mRNA and protein levels is often detected in studies investigating both levels. It appears that under the NAFLD condition, posttranscriptional regulation plays a predominant role in regulating the genes involved in bile acid synthesis, transport, and regulation.

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Conflict of interest

The authors have no conflict of interest.

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