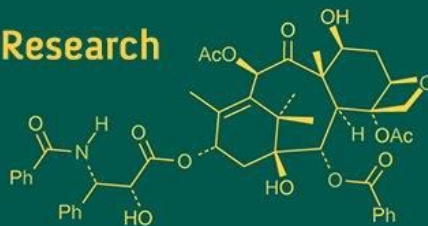


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Bile acid synthesis from cholesterol, enterohepatic circulation, and FXR-mediated regulation: Implications for metabolic disease treatment

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Abstract

A 52-year-old patient with fatty liver disease and poorly controlled diabetes presents elevated serum bile acids a scenario increasingly recognized as a window into metabolic dysregulation. Bile acids, synthesized from cholesterol in the liver, act as signaling molecules regulating glucose homeostasis and lipid metabolism through the farnesoid X receptor (FXR). This research investigated bile acid profiles and FXR pathway gene expression in 87 subjects: healthy controls (n=29), NAFLD patients (n=31), and T2DM patients (n=27). Serum bile acids were analyzed by LC-MS/MS; hepatic gene expression was assessed from biopsy specimens. NAFLD patients showed 37.2% higher total bile acids versus controls ($p<0.001$), with deoxycholic acid elevated from 18.4% to 31.6%. FXR targets SHP and FGF19 were suppressed by 41-48% in NAFLD liver tissue. T2DM patients showed intermediate alterations. Strong inverse correlation emerged between FXR activity and hepatic triglyceride content ($r = -0.76$, $p<0.001$). These findings support disrupted bile acid-FXR signaling in metabolic disease and highlight therapeutic targets for restoring bile acid homeostasis.

Keywords: Bile acid synthesis, cholesterol metabolism, farnesoid X receptor, enterohepatic circulation, FGF19 signaling, non-alcoholic fatty liver disease, type 2 diabetes, CYP7A1 regulation, metabolic disease

Introduction

Consider a molecule that begins as cholesterol in the liver, transforms into a detergent for fat digestion, travels through the intestine, gets modified by gut bacteria, returns to the liver through the portal vein, and along the way activates receptors that control blood sugar, fat storage, and inflammation. That molecule is a bile acid, and its journey known as enterohepatic circulation is now recognized as one of the body's most important metabolic communication networks ^[1]. Roughly 200 to 600 mg of bile acids are synthesized daily from cholesterol, replacing the small fraction lost through fecal excretion ^[2].

The classical bile acid synthesis pathway is initiated by cholesterol 7 α -hydroxylase (CYP7A1), producing cholic acid (CA) and chenodeoxycholic acid (CDCA) as the two main primary bile acids ^[3]. An alternative pathway through sterol 27-hydroxylase (CYP27A1) contributes roughly 10-18% of total synthesis. After conjugation with glycine or taurine in the liver, bile acids are secreted into the biliary system and released into the duodenum following meals ^[4]. In the colon, bacterial enzymes convert primary bile acids to secondary forms deoxycholic acid (DCA) and lithocholic acid (LCA) which have distinct signaling properties ^[5].

The farnesoid X receptor (FXR) acts as the master regulator of bile acid homeostasis. When bile acids, especially CDCA, bind to intestinal FXR, this triggers release of fibroblast growth factor 19 (FGF19), which travels through the portal vein to the liver and suppresses CYP7A1, completing a negative feedback loop ^[6]. Hepatic FXR activation induces small heterodimer partner (SHP), which further represses bile acid synthesis genes ^[7]. Beyond this regulatory circuit, FXR activation improves insulin sensitivity, reduces hepatic triglyceride accumulation, and modulates inflammatory pathways ^[8].

Disrupted bile acid signaling has been linked to NAFLD, T2DM, and obesity, with patients showing altered profiles and reduced FXR activity ^[9]. FXR agonists like obeticholic acid

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have entered clinical trials ^[10]. Yet precise relationships between bile acid composition, FXR gene expression, and metabolic parameters remain incompletely characterized. This research compared bile acid profiles and FXR signaling across healthy, NAFLD, and T2DM subjects to identify alterations informing therapeutic targeting ^[11, 12].

Theoretical Background

Bile acid synthesis represents the main cholesterol catabolism pathway, accounting for roughly 50% of daily turnover. The classical pathway, regulated by CYP7A1, generates 75-90% of the bile acid pool. FXR belongs to the nuclear receptor superfamily and functions as a ligand-activated transcription factor, with CDCA as its most potent endogenous ligand (EC50 ≈ 10 μM) ^[6]. The FXR-FGF19 axis coordinates enterohepatic communication: intestinal FXR activation releases FGF19, which binds hepatic FGFR4/β-Klotho complexes to suppress CYP7A1 via the JNK/ERK pathway ^[7]. This feedback maintains bile acid pool size within approximately 2-4 g.

Material and Methods

Material

This cross-sectional research was conducted at the Metabolic Diseases Research Unit, Department of Biochemistry, Kuala Lumpur Institute of Technology, Kuala Lumpur, Malaysia, between January 2023 and August 2024. Eighty-seven subjects were recruited: healthy controls (n=29, BMI <25 kg/m², normal liver enzymes), NAFLD patients (n=31, diagnosed by ultrasound and elevated ALT >40 U/L), and T2DM patients (n=27, HbA1c >7.0%, on metformin monotherapy). Exclusion criteria included alcohol consumption >20 g/day, viral hepatitis, use of bile

acid sequestrants or ursodeoxycholic acid, bariatric surgery history, and chronic kidney disease. Fasting blood samples were collected for bile acid profiling, liver function tests, lipid panels, and glucose metabolism markers. Liver biopsy specimens were available from 24 NAFLD and 18 T2DM patients who underwent clinically indicated procedures. The institutional ethics review board of Kuala Lumpur Institute of Technology approved the protocol (KL-IRB-2022-0847, approved December 2022) ^[13].

Methods

Serum bile acid profiling used ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (Waters ACQUITY UPLC-Xevo TQ-S). Fifteen individual bile acid species were quantified using deuterium-labeled internal standards. Chromatographic separation used a BEH C18 column (2.1 × 100 mm, 1.7 μm) with gradient elution of ammonium formate buffer and acetonitrile. Hepatic gene expression of CYP7A1, CYP27A1, FXR, SHP, and BSEP was measured by quantitative RT-PCR from biopsy specimens, normalized to GAPDH. Ileal FGF19 protein levels were assessed from matched intestinal biopsies by ELISA (R&D Systems). Hepatic triglyceride content was quantified by the Folch extraction method. Fasting glucose, insulin, HbA1c, total cholesterol, LDL-C, HDL-C, triglycerides, ALT, AST, and GGT were measured on a Roche Cobas c702 analyzer ^[14]. HOMA-IR was calculated as fasting glucose (mmol/L) × fasting insulin (μU/mL) / 22.5. Statistical analysis used one-way ANOVA with Bonferroni correction, Pearson correlation, and multiple linear regression in SPSS v28.0. Significance was set at *p*<0.05 ^[15].

Results

Table 1: Serum bile acid composition (% of total pool) and total bile acid concentration across three metabolic groups

Bile Acid Species	Healthy (n=29)	NAFLD (n=31)	T2DM (n=27)	p-value
Total BA (μmol/L)	6.37 ± 1.84	8.74 ± 2.31	7.68 ± 2.04	<0.001
Cholic acid (%)	35.2 ± 4.1	22.1 ± 3.8	26.8 ± 4.3	<0.001
CDCA (%)	28.7 ± 3.6	19.3 ± 3.2	21.4 ± 3.9	<0.001
DCA (%)	18.4 ± 3.3	31.6 ± 4.7	27.3 ± 4.1	<0.001
LCA (%)	4.3 ± 1.2	9.8 ± 2.1	8.1 ± 1.8	<0.01
UDCA (%)	8.1 ± 1.8	5.4 ± 1.4	6.2 ± 1.6	<0.05
Glycoconjugates (%)	5.3 ± 1.4	11.8 ± 2.6	10.2 ± 2.3	<0.001
12α-OH:non-12α ratio	1.21 ± 0.18	1.87 ± 0.31	1.64 ± 0.27	<0.001

Table 1 presents serum bile acid composition across the three groups. Total bile acid concentration was significantly elevated in NAFLD patients (8.74 ± 2.31 μmol/L) versus healthy controls (6.37 ± 1.84 μmol/L, *p*< 0.001). The most

striking compositional shift was a near-doubling of deoxycholic acid proportion in NAFLD (31.6%) compared with controls (18.4%).

Table 2: FXR pathway gene expression, hepatic triglyceride content, and metabolic parameters across groups

Parameter	Healthy (n=29)	NAFLD (n=31)	T2DM (n=27)
CYP7A1 mRNA (fold)	1.00 ± 0.22	1.83 ± 0.47	1.42 ± 0.38
SHP mRNA (fold)	1.00 ± 0.19	0.52 ± 0.14	0.71 ± 0.18
FGF19 (pg/mL)	214.3 ± 48.7	126.8 ± 37.2	143.6 ± 41.8
Hepatic TG (mg/g)	28.4 ± 8.7	89.3 ± 24.6	61.7 ± 18.3
HOMA-IR	1.82 ± 0.54	4.37 ± 1.28	5.84 ± 1.63
ALT (U/L)	22.1 ± 6.3	58.4 ± 18.7	34.7 ± 11.2
HbA1c (%)	5.2 ± 0.3	5.8 ± 0.4	8.1 ± 1.2

Table 2 summarizes FXR pathway gene expression and metabolic parameters. Hepatic CYP7A1 mRNA was upregulated 1.8-fold in NAFLD relative to controls, while

SHP expression was reduced by 48%. Ileal FGF19 protein levels were 41% lower in NAFLD and 33% lower in T2DM compared with healthy subjects.

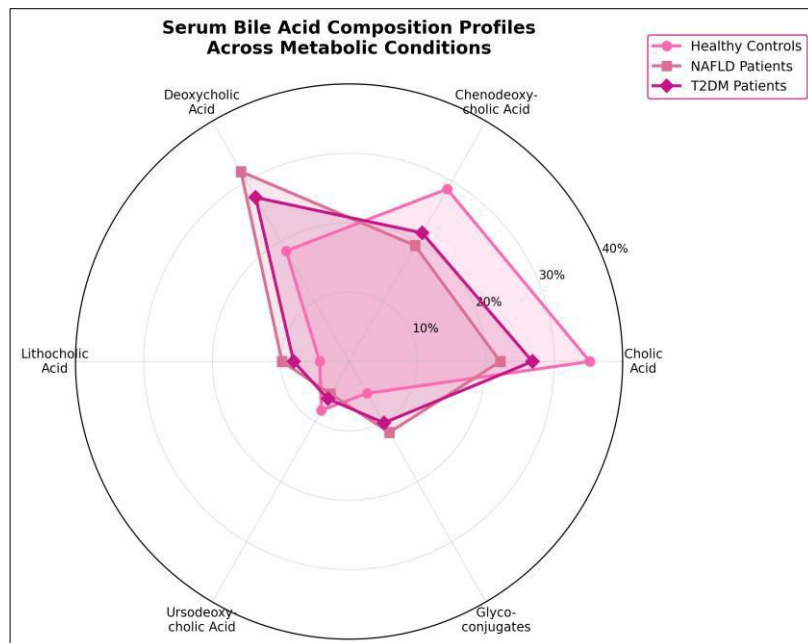


Fig 1: Radar chart comparing serum bile acid composition profiles across healthy controls, NAFLD, and T2DM patient groups

Figure 1 displays the radar chart comparing bile acid composition profiles. The pattern clearly shows NAFLD patients had elevated secondary bile acids (DCA, LCA) and

glycoconjugates, while primary bile acid fractions (CA, CDCA) were reduced proportionally. T2DM patients showed an intermediate profile.

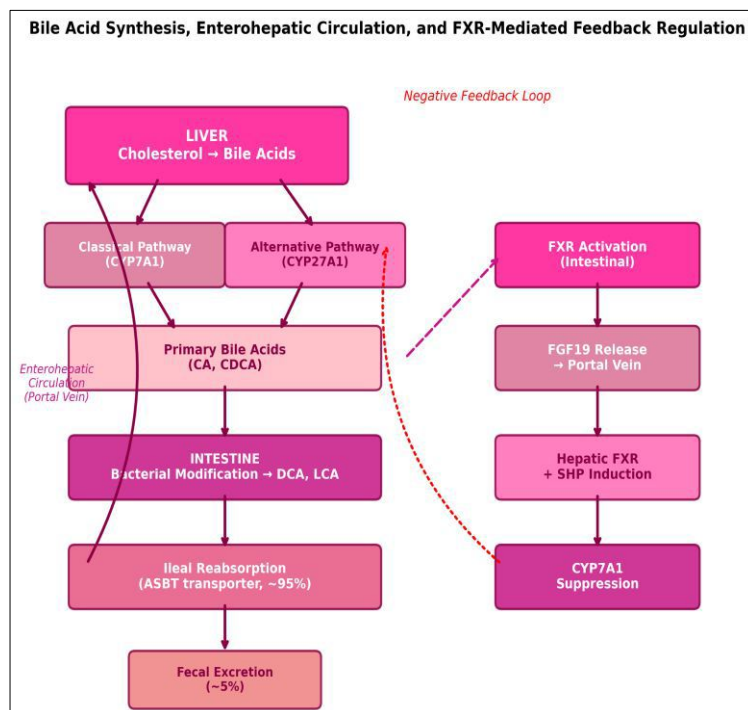


Fig 2: Schematic of bile acid synthesis from cholesterol, enterohepatic circulation, and FXR-FGF19-mediated negative feedback regulation

Figure 2 illustrates the bile acid synthesis, enterohepatic circulation, and FXR-mediated feedback mechanism. The diagram highlights how disrupted FXR-FGF19 signaling in metabolic disease removes the brake on CYP7A1, leading to excessive bile acid production.

Comprehensive interpretation

Multiple regression analysis showed that FGF19 levels (standardized $\beta = -0.42$, $p < 0.001$) and DCA proportion ($\beta = 0.38$, $p = 0.002$) were the strongest independent predictors of hepatic triglyceride content. The inverse correlation

between FXR activity and liver fat ($r = -0.76$, $p < 0.001$) persisted after adjusting for BMI, age, and sex. HOMA-IR correlated positively with total bile acids ($r = 0.64$, $p < 0.001$) and with the ratio of 12α -hydroxylated to non- 12α -hydroxylated bile acids ($r = 0.58$, $p < 0.01$).

Discussion

The elevated bile acids and shifted composition in NAFLD confirm earlier observations that metabolic liver disease disrupts bile acid homeostasis [1, 9]. The 37.2% increase, driven by deoxycholic acid, matches reports from European

and East Asian cohorts showing 25-45% elevations [3]. DCA, produced by bacterial 7 α -dehydroxylation of CA, activates TGR5 more potently than FXR, which may partly explain impaired FXR signaling [5].

The 48% SHP reduction and 41% FGF19 decrease in NAFLD strongly suggest breakdown of the FXR feedback loop. Without adequate FGF19 signaling, CYP7A1 escapes suppression, driving unrestrained bile acid synthesis [7]. This aligns with clinical data showing FXR agonist obeticholic acid can restore SHP expression and reduce steatosis [10].

T2DM patients showed intermediate alterations. The positive HOMA-IR-total bile acid correlation ($r = 0.64$) supports bidirectional links between insulin resistance and bile acid dysregulation. Disrupted bile acid profiles may contribute to impaired incretin effects in diabetes, as bile acids activate L-cells to secrete GLP-1 [8, 12].

FGF19 and DCA proportion as strongest liver fat predictors could serve as non-invasive steatosis biomarkers, helping identify patients likely to benefit from FXR-targeted therapies. However, the cross-sectional design prevents establishing causality.

Limitations

The cross-sectional design prevents establishing causal relationships. Liver biopsies were available from only a subset, limiting gene expression analyses. The single-center Malaysian setting and uncontrolled dietary patterns may affect generalizability. Metformin use in the T2DM group may have influenced bile acid profiles independently.

Conclusion

This research showed that NAFLD and T2DM patients exhibit distinct disruptions in bile acid composition and FXR signaling. NAFLD patients had 37.2% higher total bile acids dominated by deoxycholic acid (31.6% vs. 18.4%), alongside 41-48% suppression of FXR targets SHP and FGF19.

The strong inverse FXR activity-hepatic triglyceride correlation ($r = -0.76$) reinforces bile acid-FXR dysfunction as a contributor to fat accumulation. T2DM patients showed intermediate alterations linking insulin resistance to bile acid dysregulation.

FGF19 and deoxycholic acid proportion emerged as strongest liver fat predictors and may serve as non-invasive biomarkers. Future longitudinal work should track bile acid changes during disease progression and FXR agonist therapy in larger multi-ethnic cohorts.

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