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Advancements in Laboratory Testing for Bile Acid Profiling and Clinical Implications

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Abstract

Bile acids are a diverse family of amphipathic sterols synthesized from cholesterol, primarily in the liver. They play crucial roles in digestion, lipid absorption, and various signaling pathways. Bile acid synthesis and metabolism involve complex processes, including the classical and alternative pathways, conjugation, and biotransformation by gut microbiota. Bile acids undergo enterohepatic circulation, and their homeostasis is maintained through a delicate balance of synthesis, reabsorption, and excretion. Receptors such as FXR and TGR5 are key regulators of bile acid signaling and metabolism. Profiling bile acids has become increasingly important for understanding their roles in health and disease. Liquid chromatography-mass spectrometry (LC-MS) is the most widely used technique for bile acid detection and quantification. However, challenges such as high costs and complex sample preparation have limited its clinical adoption. Bile acid profiles exhibit significant alterations in various diseases, including type 2 diabetes mellitus, non-alcoholic fatty liver disease, cholestatic liver disease, inflammatory bowel disease, Alzheimer's disease, and cancer. These changes often precede conventional diagnostic markers, highlighting the potential of bile acid profiling as a tool for early disease detection and monitoring. Despite the promise of bile acid profiling, translating research findings into clinical practice faces

several barriers, such as interspecies differences in bile acid metabolism and inconsistencies in study design and methodology. Overcoming these challenges and leveraging advances in technology and artificial intelligence could pave the way for personalized medicine approaches based on comprehensive bile acid profiling.

Keywords: Laboratory Testing, Bile acid profiling, Clinical implications Bile acid metabolism, Mass spectrometry

1. Introduction

Bile acids are amphoteric sterols synthesized from cholesterol in all vertebrates. The primary forms, cholic acid (CA) and chenodeoxycholic acid (CDCA), are predominantly produced through the action of 16 enzymes in the liver, catalyzing 17 distinct reactions within various intracellular compartments (di Gregorio et al., 2021). As a structurally diverse family of small molecules with interspecies variability, bile acids in mammals are primarily characterized by the C24 structure (Perino et al., 2021). These molecules fulfill multiple physiological roles, including aiding in the digestion and absorption of lipids and fat-soluble vitamins. Additionally, bile acids function as signaling molecules, exhibit antimicrobial properties, regulate cholesterol homeostasis, and help prevent the formation of gallstones and kidney stones. Bile acids are classified in several ways. Based on their origin, they are categorized into primary bile acids (PBA), secondary bile acids (SBA), and tertiary bile acids. Structurally, they can be divided into free bile acids (FBA) and conjugated bile acids (CBA). Furthermore, they may be classified as hydrophilic or hydrophobic, depending on their molecular characteristics.

Recent studies have increasingly highlighted the varied impacts of different bile acids on diseases. For instance, ursodeoxycholic acid (UDCA) has been shown to inhibit tumor progression, while deoxycholic acid (DCA) and lithocholic acid (LCA) are considered potential carcinogens (Lirong et al., 2022). Tauroursodeoxycholic acid (TUDCA) is recognized for its neuroprotective effects, including its ability to mitigate neuroinflammation and suppress pro-inflammatory factors. Both CA and DCA disrupt bacterial membranes to inhibit growth; however, DCA is more than tenfold more effective than CA in this function. Despite the diverse roles of bile acids, current clinical diagnostics rely solely on total bile acid (TBA) levels, which fail to provide comprehensive and accurate insights into disease status. Enhanced detection and analysis of bile acid profiles across various biological samples could lead to novel diagnostic and prognostic strategies. As the relationship between disease progression and bile acid profiles becomes clearer, profiling will likely play an increasingly vital role in clinical decision-making. This paper will explore the clinical significance of bile acid profiling from four perspectives: bile acid synthesis and metabolism, techniques for profiling assays, the role of bile acid profiling in disease, and the challenges and prospects for clinical applications.

2. Bile Acid Synthesis and Metabolism

2.1. Synthesis of Bile Acids

Bile acid synthesis and secretion exhibit considerable variation influenced by dietary intake, diurnal rhythms, sex, and age. Studies have shown that postprandial bile acid concentrations in the portal vein increase sixfold. Furthermore, synthesis peaks at 1:00 p.m. and 9:00 p.m., with a decline at midnight. Sex-related differences have also been observed, with females synthesizing more CDCA from a smaller bile acid pool compared to males. Interestingly, this contrasts with female mice, which possess a bile acid pool approximately 60% larger than that of males. Plasma concentrations of specific bile acids, such as CDCA, glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), and glycoursodeoxycholic acid (GUDCA), are influenced by age and exhibit sex-related differences. Young males aged 20–35 years demonstrate the highest concentrations, with levels decreasing with age. Differences between young males and females are particularly pronounced, with some bile acids significantly more abundant in males. Variations related to age, gender, and BMI have also

been identified. For instance, individuals with higher BMI exhibit elevated bile acid concentrations compared to those with lower BMI. During pregnancy, bile acid profiles undergo distinct alterations. Total bile acid (TBA) levels in pregnant women increase progressively throughout gestation. This increase correlates with a rise in conjugated bile acids and the CA-to-CDCA ratio, while unconjugated bile acid (UCBA) levels decrease. Gagnon et al. reported significant elevations in all maternal bile acid species during the second and third trimesters of pregnancy (Gagnon et al., 2021). These changes are likely due to the fetus synthesizing bile acids but being unable to eliminate them, necessitating maternal metabolism and excretion through the placenta.

The synthesis of bile acids in the liver occurs through two primary pathways: the classical pathway, also known as the neutral pathway, and the alternative pathway, referred to as the acidic pathway. The classical pathway, named for its intermediate product neutral cholesterol, involves cholesterol 7α-hydroxylase (CYP7A1), which catalyzes the hydroxylation of cholesterol at the 7α-position to form 7α-hydroxycholesterol (7α-OHC), the rate-limiting step in this pathway. Another key enzyme in the classical pathway is sterol 12α-hydroxylase (CYP8B1), which synthesizes CA and regulates the CA-to-CDCA ratio, thereby influencing the hydrophilicity of the bile acid pool. In contrast, the alternative pathway, predominant in the liver and extrahepatic tissues, begins with sterol 27-hydroxylase (CYP27A1), producing 27-hydroxycholesterol (27-OHC) and leading to CDCA synthesis. More than 90% of bile acids are synthesized via the classical pathway, with the remainder generated through the alternative pathway, which dominates before the neonatal expression of CYP7A1 (Nguyen et al., 2022).

The body exhibits adaptive mechanisms by enhancing the activity of enzymes in the alternative pathway during stress conditions such as cold exposure, poor diet, or liver disease. Cholesterol synthesis in the brain differs due to the blood–brain barrier, which prevents cholesterol transport. Instead, cholesterol is locally synthesized from 24S-hydroxycholesterol (24-OHC) through the action of the brain-specific enzyme cholesterol 24-hydroxylase (CYP46A1), resulting in intermediates that contribute to CDCA synthesis. This localized synthesis pathway is critical for maintaining brain cholesterol levels (Monteiro-Cardoso et al., 2021).

2.1. Conjugation and Biotransformation of Bile Acids

Bile acids undergo conjugation within hepatocytes prior to being secreted into the bile ducts. In addition to the classical conjugation with glycine or taurine through N-amidation, bile acids also form other types of conjugates, including sulfation, glucuronidation, and N-acetylglucosaminoglucosylation. The ratio of taurine-conjugated bile acids (TCBA) to glycine-conjugated bile acids (GCBA) is influenced by dietary patterns. A high-protein diet promotes the conjugation of bile acids with taurine, whereas a vegetarian diet favors conjugation with glycine. In humans, the GCBA-to-TCBA ratio is approximately 3:1, while in mice, more than 95% of bile acids are conjugated to taurine. This difference results in significantly higher passive absorption of bile acids from the intestinal tract in humans compared to mice (Durník et al., 2022).

The conjugation of bile acids with glycine and taurine is facilitated by bile acid–CoA synthetase (BACS) and bile acid–CoA: amino acid N-acetyltransferase (BAAT). This conjugation increases the ionization constant of bile acids, reducing their precipitation as bile salts and enhancing their hydrophilicity. Consequently, this reduces the cytotoxicity and membrane-solubility properties of bile acids, as well as their potential for crossing cell membranes.

In the colon, a portion of CA and CDCA conjugated with taurine or glycine is deconjugated by the bacterial enzyme bile salt hydrolase (BSH). Following this, they are dehydroxylated by bacterial 7α-dehydroxylase to form DCA and LCA, respectively. At this stage, the bile acid pool in the intestine primarily consists of approximately 30% CA, 40% CDCA, 20–30% DCA, and less than 5% LCA. Unlike BSH, which is widely distributed among gut microorganisms, 7α-dehydroxylation is carried out by a limited subset of anaerobic bacteria, predominantly

species of Clostridium within the phylum Firmicutes, which represent less than 0.025% of the gut microbiota and less than 0.0001% of the total microbiota (Winston & Theriot, 2020).
Interestingly, in addition to its well-known role in transforming conjugated bile acids into their unconjugated forms, BSH has been identified as an amine N-acyltransferase capable of attaching amines to bile acids. This results in the formation of bacterial bile acid amidates (BBAAs). Additionally, a small fraction of CDCA in the intestine is enzymatically converted by bacterial 7β-hydroxysteroid dehydrogenase (7β-HSDH) into the secondary bile acid UDCA.

2.2. Enterohepatic Circulation of Bile Acids

The primary bile acids in humans, along with their conjugated forms (CA:CDCA:DCA = 4:4:2), collectively comprise over 90% of the total bile acids (TBA). These bile acids undergo 4–12 enterohepatic circulation cycles daily, allowing a bile acid pool of approximately 3 g to function as effectively as one with 12–36 g of bile acids per day. Bile acid homeostasis is maintained through the synthesis of approximately 0.2–0.6 g of bile acids daily and the excretion of around 5% through the intestines and approximately 500 mg through urine. The bile acids excreted in feces are primarily free bile acids, such as DCA and LCA. In contrast, most bile acids excreted in urine are present in sulfated forms (Bathena et al., 2013).
After synthesis in the liver, bile acids conjugated with taurine and glycine are transported into capillary bile ducts via the bile salt export pump (BSEP) located on the hepatocyte's canalicular membrane (Fig. 2). Other conjugated bile acids combine with organic anions, organic cations, and reduced glutathione to form divalent anions, which are secreted into capillary bile ducts alongside cholesterol and phospholipids through multidrug resistance-associated protein 2 (MRP2) and multidrug resistance protein 1A (MDR1A). Bile salts, along with phospholipids, cholesterol, bile pigments, urea, and other substances, form bile. Among bile solutes, bile acid salts constitute the largest proportion, accounting for approximately 60–67% of the total solutes.
Typically, about 50% of the bile bypasses the gallbladder and enters the duodenum as part of the continuous enterohepatic circulation. The remaining 50% is stored in the gallbladder and released into the duodenum after meals in response to postprandial stimulation by enteroglucagon and cholecystokinin. Within the intestines, bile acids form mixed micelles with dietary lipids and their digestion products, facilitating the absorption of lipolysis products and fat-soluble vitamins.
Bile acids encounter difficulty in crossing the tight junctions of intestinal epithelial cells due to their large molecular size. Furthermore, the absence of specific bile acid transporters for active absorption in the upper and middle regions of the small intestine limits their uptake. Passive absorption of free bile acids (FBA) and a minor fraction of glycine-conjugated bile acids (GCBA) occurs along the entire length of the intestine. The majority of bile acids, however, are actively absorbed in the distal ileum by the apical sodium-dependent bile acid transporter protein (ASBT) located on the apical membrane of ileal epithelial cells. After absorption, they bind to the intestinal bile acid-binding protein (IBABP) at the basolateral membrane and are subsequently transported to the portal vein by organic solute transporter proteins α/β (OSTα/β) and multidrug resistance-associated protein 3 (MRP3).
Approximately 15% of conjugated bile acids fail to be absorbed in the terminal ileum and instead reach the colon, where intestinal microbiota convert them into secondary bile acids (SBA). Around 50% of deoxycholic acid (DCA) and smaller amounts of lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) are passively absorbed in the colon and then transported back to the liver via the portal vein. In total, approximately 95% of bile acids are reabsorbed in the intestine, with the majority being primary bile acids (PBA) and a smaller portion consisting of unconjugated bile acids.

Among the bile acids returning to the liver via the portal vein, about 75% of trihydroxy bile acids and 99% of dihydroxy bile acids are bound to albumin. These bile acids are reabsorbed into hepatocytes during the first hepatic circulation by the sodium taurocholate cotransporting polypeptide (NTCP) and the organic anion transporting polypeptide 1 (OATP1). Under normal physiological conditions, the ratio of absorbed bile acids—CA, CDCA, and DCA (including their conjugated and unconjugated forms)—in the liver is approximately 1:1.34:1. The hepatic reuptake rate of conjugated bile acids is three times higher than that of unconjugated bile acids (Trammell et al., 2020). A small fraction of bile acids bypasses hepatic reabsorption and enters the peripheral bloodstream, where the total bile acid concentration is only 10–16% of that in the portal vein.

2.3. Bile Acid Receptors

Bile acids function as signaling molecules by activating a variety of receptors, including the farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor (VDR), Takeda G-protein-coupled receptor 5 (TGR5), and constitutive androstane receptor (CAR). These receptors play essential roles in regulating bile acid synthesis and metabolism. Among them, FXR and TGR5 have been extensively studied.

FXR is crucial in maintaining bile acid homeostasis by regulating bile acid synthesis, metabolism, and transport within the enterohepatic circulation. It also significantly influences lipid, glucose, and energy metabolism. First identified by Forman et al. in 1995, FXR is a nuclear receptor that binds bile acids. In mammals, FXR exists in two major forms: FXRα and FXRβ. While FXRα is highly expressed in tissues such as the liver, ileum, kidney, adipose tissue, heart, and adrenal glands, FXRβ is a pseudogene in humans (Tian et al., 2022).

FXRα encodes four isoforms (FXRα1–FXRα4). Of these, FXRα1 and FXRα2 are predominantly expressed in the liver and exhibit moderate expression in the ileum and adrenal glands. In contrast, FXRα3 and FXRα4 are highly expressed in the ileum and moderately in the kidney. The potency of bile acids in activating FXR depends on their hydrophobicity, with CDCA being the most potent, followed by DCA, LCA, and CA.

Activation of intestinal epithelial FXR induces the synthesis of fibroblast growth factor 19 (FGF19; FGF15 in mice), which enters the portal vein and interacts with the FGFR4/Klothoβ receptor complex in hepatocyte membranes. This interaction inhibits CYP7A1 expression, either through the small heterodimer partner (SHP)-dependent pathway or non-SHP-dependent pathways, such as jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) pathways. FXR activation also regulates numerous transport proteins in the enterohepatic circulation, including upregulation of BSEP, MDR3, and MRP2 in hepatocytes. Additionally, it inhibits NTCP and OATP1 expression at the basolateral membrane while upregulating IBABP and OSTα/β in intestinal cells and downregulating ASBT in ileocytes.

TGR5, the first bile acid-specific G protein-coupled receptor identified, is expressed in almost all tissues except for high expression levels in gallbladder epithelial cells. In the liver, TGR5 is predominantly located in non-parenchymal cells, such as Kupffer cells, cholangiocytes, hepatic sinusoidal endothelial cells, and activated hepatic stellate cells. The potency of TGR5 activation by bile acids is ranked as LCA > DCA > CDCA > CA, with taurine-conjugated bile acids (TCBA) being more effective activators than glycine-conjugated or free bile acids (Sato et al., 2008).

Activation of TGR5 inhibits the expression of CYP8B1 in hepatic tissues and upregulates the alternative bile acid synthesis pathway, thereby reducing the production of 12α-hydroxylated bile acids (12HBAs, including CA, DCA, and their conjugates). Furthermore, TGR5 activation elevates cAMP levels in bile duct epithelial cells, promoting ASBT insertion into the parietal membrane for selective SBA reabsorption, which reduces the hydrophobicity of the bile acid pool. Additionally, TGR5 activation enhances chloride and bicarbonate secretion in bile duct epithelial cells, forming a protective bicarbonate barrier that shields these cells from bile acid

toxicity. Beyond bile acid homeostasis, TGR5 plays critical roles in energy metabolism, inflammatory responses, gastrointestinal function regulation, and other physiological processes.

3. Bile Acid Profile Detection Techniques

The human body comprises a diverse array of bile acids, characterized by similar chemical structures, numerous isomers, and varying physiological activities. The concentrations of these bile acids can differ by several orders of magnitude and are often found at trace levels in blood, feces, and urine. These properties create substantial challenges in bile acid profiling for research purposes. Traditional enzyme assays are restricted to measuring total bile acid (TBA) concentrations and cannot distinguish among different types of bile acids. Furthermore, enzyme assays that utilize 3α-hydroxysteroid dehydrogenase are only applicable to C24-steroids with a C3-OH group. Substitutions in this group result in detection failures, and the high detection limits of these assays make them unsuitable for specific samples, such as saliva. Current bile acid profiling methodologies include immunoassays (e.g., RIA and ELISA), spectrophotometry, nuclear magnetic resonance (NMR), and various chromatographic techniques. Chromatographic methods encompass thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and supercritical fluid chromatography-mass spectrometry (SFC-MS). Of these, HPLC or ultra-high-pressure liquid chromatography (UPLC) coupled with mass spectrometry (MS), particularly tandem mass spectrometry (MS/MS), is the most sensitive and widely used analytical tool for detecting and quantifying bile acids in both human and rodent specimens. The number of bile acids identified varies among LC-MS detection techniques, with current LC-MS methods capable of identifying approximately 8 to 145 different bile acids in plasma samples. Targeted metabolomics using HPLC-MS/MS has become the predominant technology for bile acid sample analysis, applied in approximately 70% of related studies. This method offers advantages such as minimal sample requirements, convenient pre-treatment, rapid analysis, high specificity, sensitivity, stability, separation capability, and a broad analytical range.

Despite these advantages, the methods have limitations. For instance, immunoassays such as ELISA and RIA may exhibit cross-reactivity, spectrophotometry suffers from low detection accuracy, NMR has limited sensitivity, TLC is only suitable for qualitative analysis, SFC has low reproducibility, and HPLC, LC-MS, GC-MS, and SFC-MS are associated with high costs and complex operations (Zhao et al., 2022). These constraints limit the broad adoption of clinical bile acid profiling. Recently, biosensor detection technology has been introduced into bile acid detection, presenting a promising future for clinical applications. Biosensors utilize bio-specific binding molecules as recognition elements, including enzymes, microorganisms, cells, tissues, nucleic acids, and other biologically active substances. They transform biological signals into electrical signals, which are then amplified for qualitative or quantitative analysis. For instance, Zheng et al. developed a novel biosensor employing a near-infrared polymethylene dye for the rapid detection of lithocholic acid (LCA). This biosensor facilitated the spontaneous formation of J-aggregates via the interaction between LCA and 3,3-Diethylthiatricarbocyanine iodide, resulting in significant redshifts in absorption and fluorescence spectra. Detection limits were as low as 70 μM in phosphate-buffered solutions and 60 μM in artificial urine. This biosensor effectively detects LCA without requiring specialized instruments, offering simplicity, quick response times, and low costs. However, research on biosensor technologies for bile acid detection is still limited, particularly for specific bile acids. Most existing studies rely on enzyme-catalyzed reactions to produce specific metabolites for measuring total bile acids in the body. Future advancements in science

and increased research investment are expected to enable biosensor technologies to achieve breakthroughs, such as multi-component bile acid detection, portable and timely analysis, and intelligent detection systems.

4. Bile Acid Profiles in Diseases

Early research has indicated a concentration gradient for total bile acids in circulating serum, portal vein serum, liver tissue, and bile at ratios of 1:3:80:2600. In human serum samples, TBA concentrations typically range from 2.1 to 8.0 μmol/L, with chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), cholic acid (CA), and ursodeoxycholic acid (UDCA) and their conjugated forms constituting 92.1% of the serum TBA. Plasma samples show TBA concentrations ranging from 1.4 to 6.5 μmol/L, with similar bile acid fractions as serum samples. Primary bile acids (PBA) comprise 65.8% of serum TBA, with glycochenodeoxycholic acid (GCDCA) and CDCA being the most abundant. In adult feces, TBA concentrations are generally low, with dry fecal content averaging 1–25 μmol/g. Notably, up to 90% of TBA in feces are secondary bile acids (SBA), primarily in free form. Additionally, urinary TBA concentrations average approximately 9 μmol/L, comparable to plasma or serum levels.

Clinical studies of bile acid profiles predominantly examine fifteen classical components: CA, taurocholic acid (TCA), glycocholic acid (GCA), CDCA, taurochenodeoxycholic acid (TCDCA), GCDCA, DCA, taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), LCA, taurolithocholic acid (TLCA), glycolithocholic acid (GLCA), UDCA, glycoursodeoxycholic acid (GUDCA), and tauroursodeoxycholic acid (TUDCA). Sample sources for bile acid profile analysis include peripheral blood, bile, intestinal contents, urine, and feces. Peripheral blood and feces are the most frequently studied specimens, though researchers have also explored bile acid profiles in non-traditional samples such as saliva, bronchoalveolar lavage fluid, and amniotic fluid. Bile acid concentrations in saliva are trace, being 3 to 6 orders of magnitude lower than those in blood, urine, or bile. Conversely, bile acids are present at relatively high concentrations in amniotic and follicular fluids, with follicular fluid TBA concentrations potentially being twice as high as serum levels. Caparrós-Martín et al. detected bile acids in the alveolar lavage fluid of 49 out of 121 patients (40%), with concentrations ranging from 0.003 to 1.095 μM. Studies in experimental mice and rats also highlight differences in bile acid pool composition and size, despite similarities in bile acid synthesis pathways to humans. Hamsters, which closely resemble humans in bile acid biosynthesis and metabolism, have been less frequently studied (de Aguiar Vallim et al., 2013).

In the early stages of disease, significant changes in bile acid profiles can occur even when peripheral blood TBA levels remain unchanged. For example, one study reported a 27-fold increase in liver tissue bile acid concentrations and a 1,400-fold increase in peripheral serum bile acids 12 hours after bile duct ligation in mice. A cohort study by Luo et al. (n = 645) found significant alterations in bile acid fractions, except for DCA, in patients with hepatic injury compared to healthy subjects. Bile acids also act as sensors of intestinal contents due to the critical role of microorganisms in their biotransformation and absorption. Thus, bile acid profile testing is essential for screening, diagnosing, and differentiating various diseases. This review subsequently explores the use and significance of bile acid profiling in various disease contexts.

4.1. Type 2 Diabetes Mellitus (T2DM)

It is widely recognized that serum total bile acid (TBA) concentrations in patients with T2DM either remain unchanged or exhibit slight elevations. Nevertheless, certain studies have documented a twofold increase in serum TBA levels among individuals with T2DM compared to healthy controls. Although discrepancies exist, the more consistent observation is an elevation in serum deoxycholic acid (DCA) levels and/or their conjugates in T2DM patients. Brufau et al. attributed the 40% increase in cholic acid (CA) synthesis in T2DM patients to a corresponding 33% expansion of their DCA pool. Furthermore, Cariou et al. identified a

positive correlation between DCA and fasting glucose levels, a finding supported by meta-analyses linking elevated DCA levels to the development of T2DM. Additionally, elevated proportions of taurine-conjugated bile acids (TCBA) were noted in fasting serum samples from individuals with T2DM and, to a lesser degree, in those with impaired glucose tolerance (IGT) (Wewalka et al., 2014).

A nested case-control study involving 1,707 matched pairs revealed that, after adjusting for multiple variables, serum concentrations of glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA) were positively associated with the risk of T2DM in patients with normal glucose regulation. Conversely, levels of CA, chenodeoxycholic acid (CDCA), and DCA were negatively associated. The prevailing hypothesis suggests that 12-hydroxy bile acids (12HBAs) contribute to worsening insulin resistance, manifesting externally as elevated fasting glucose or an increased diabetes risk. However, Lee et al. reported no significant differences in bile acid profiles among healthy controls, individuals with IGT, and those with T2DM. Nonetheless, various bile acids, along with TBA, were significantly linked to homeostasis model assessment of insulin resistance (HOMA-IR) levels. Wang et al. observed significantly reduced serum bile acid levels in response to oral glucose administration in early-stage T2DM patients compared to healthy controls (Wang et al., 2022).

Conversely, Chávez-Talavera et al. argued that peripheral blood bile acids, 12HBAs/non-12HBAs, and conjugated bile acids (CBA)/unconjugated bile acids (UCBA) ratios are not reliable predictors of T2DM onset in individuals with IGT. The discrepancy may be attributed to differences in the extent of insulin resistance among study participants. Notably, bile acid profile alterations induced by weight-loss diets were associated with improvements in insulin resistance in a cohort study. Additional research has revealed that postprandial plasma ursodeoxycholic acid (UDCA) levels were elevated in T2DM patients, with further increases observed following fat consumption. Elevated levels of unconjugated secondary bile acids (USBA), particularly DCA, in new-onset T2DM patients were associated with a heightened risk of cardiovascular disease. Conversely, higher plasma concentrations of CA, CDCA, GCA, and GCDCA were linked to a reduced likelihood of chronic kidney disease in the same population (Geng et al., 2024).

Farnesoid X receptor (FXR) treatment has been shown to regulate glucose homeostasis by modulating glycogen synthesis and hepatic gluconeogenesis via several pathways. For example, mebhydrolin, a selective FXR antagonist, inhibits gluconeogenesis and promotes glycogen synthesis through the FXR/miR-22-3p/PI3K/AKT/FoxO1 and FXR/miR-22-3p/PI3K/AKT/GSK-3β pathways, respectively. Activation of Takeda G protein-coupled receptor 5 (TGR5) enhances glucagon-like peptide-1 (GLP-1) secretion from intestinal L cells by stimulating cyclic AMP (cAMP) signaling, thereby boosting β-cell insulin synthesis and secretion. Conversely, FXR activation may inhibit GLP-1 secretion. Both TGR5 and FXR are extensively expressed in the pancreas, alongside their hepatic and intestinal distributions. Activation of FXR receptors in pancreatic β-cells promotes insulin release while inhibiting glucagon secretion and hepatic glucose output. Furthermore, TGR5 receptors in pancreatic α-cells induce GLP-1 secretion by upregulating proconvertase 1/3 (PC1/3) expression. Semova et al. proposed that insulin reduces 12HBAs levels by inhibiting Forkhead Box O1 (FoxO1), which in turn activates CYP8B1. Interestingly, reductions in CA and its derivatives increase the delivery of free fatty acids to ileal L cells, thereby enhancing GLP-1-mediated enteric insulin secretion.

Alterations in gut microbiota are also recognized as a pivotal factor in T2DM development. For instance, Gou et al. identified 11 microbial taxa, including Alphaproteobacteria, Deltaproteobacteria, Lactobacillales, Comamonadaceae, and Clostridiales, as potential

predictors of T2DM. Studies confirm that disruptions in gut flora reduce bile acid activation, decrease the production of free and secondary bile acids, and impair the activation of bile acid receptors such as FXR and TGR5 (Ma et al., 2019).

4.2. Non-Alcoholic Fatty Liver Disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological condition primarily characterized by excessive intracellular fat deposition in hepatocytes, excluding cases caused by alcohol or other clearly defined hepatotoxic factors. NAFLD includes non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), and related cirrhosis. In 2020, an international expert panel proposed replacing the term NAFLD with metabolic-associated fatty liver disease (MAFLD). At the 2023 European Association for the Study of the Liver (EASL) meeting, the term metabolic dysfunction-associated steatotic liver disease (MASLD) was recommended as a replacement for NAFLD. Given that most studies referenced here employed the term NAFLD, it will be retained in this discussion.

Research has demonstrated significantly elevated fasting serum TBA concentrations in NAFLD patients compared to healthy controls. The increase is relatively modest in NAFL patients, whereas it exceeds a twofold rise in NASH patients. Most studies attribute this elevation to primary bile acids (PBA), with a significant increase in DCA levels. Chen et al. identified significantly elevated serum GCA/TCA and GDCA/TDCA ratios in the early stages of chronic liver disease (NAFL and NASH). Leveraging these findings, they developed a diagnostic model incorporating GCA/TCA and GDCA/TDCA ratios alongside patient age and sex to distinguish between NAFL and NASH. Validation of this model yielded high accuracy, with area under the curve (AUC) values of 0.91 and 0.93 in the discovery and test cohorts, respectively.

With fibrosis progression, NAFLD patients exhibit significantly elevated serum TBA levels and a gradual reduction in the GCDCA/TCDCA ratio. Fecal TBA levels increase, accompanied by a decline in the conversion of PBA to secondary bile acids (SBA). Furthermore, the percentage of serum PBA increases, while SBA levels decrease steadily with advancing fibrosis. Kwan et al. observed that conjugated primary bile acids (CPBA), GCA, and GCDCA show the strongest correlation with fibrosis. An analysis by Liu et al. of serum bile acid profiles in 550 NAFLD patients revealed that combining serum bile acid profiles with clinical and biochemical biomarkers effectively identified mild fibrosis in males, females, obese, and non-obese subgroups (Liu et al., 2023). Although its performance in detecting significant fibrosis was less optimal, it significantly outperformed commonly used non-invasive fibrosis scores such as the fibrosis-4 index (FIB-4), NAFLD fibrosis score (NFS), and Hepamet fibrosis score (HFS).

Jung et al. observed that primary bile acids (PBAs) were significantly upregulated in non-obese non-alcoholic steatohepatitis (NASH) patients compared to those with non-alcoholic fatty liver (NAFL) within the discovery cohort, with no significant differences in the obese group. This aligns with Legry et al., who reported bile acid profile changes in NASH linked to metabolic characteristics, particularly insulin resistance, rather than hepatic necroinflammation. Additionally, a large cohort study noted higher plasma bile acid concentrations in NASH patients, independent of type 2 diabetes mellitus (T2DM) status. A significant negative correlation between total cholic acid (CA) and insulin sensitivity was found in NASH patients, suggesting elevated plasma bile acids as a unique feature of this group. Puri et al. highlighted an elevated total CA to total chenodeoxycholic acid (CDCA) ratio in NAFLD patients, irrespective of T2DM. Another study noted significantly higher 12-hydroxy bile acids (12HBAs), such as glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA), in NASH patients with higher BMI compared to controls. These findings underscore the need for further research, especially in lean NASH populations.

Nimer et al. found a significant association between conjugated primary bile acids (CPBAs) and lobular inflammation, where higher bile acid concentrations correlated with increased inflammation. Similarly, Puri et al. reported correlations between specific bile acids and histological features of NAFLD, such as steatosis and inflammation. Gender also influences bile acid profiles, with Fitzinger et al. reporting higher levels of total bile acid (TBA), total PBA, total CA, and total glycine-conjugated bile acids (GCBAs) in women compared to men. In alcoholic liver disease, changes in bile acid profiles were more pronounced, with significant elevations in glycochenodeoxycholic acid (GCDCA), glycoursodeoxycholic acid (GUDCA), TBA, PBA, and secondary bile acids (SBAs). Tauroconjugated bile acid (TCBA) levels were markedly higher in patients with alcoholic liver disease compared to heavy drinkers and those with alcoholic cirrhosis. Additionally, the TCBA/GCBA ratio served as a non-invasive marker for alcoholic cirrhosis severity. Notably, ursodeoxycholic acid (UDCA) and hyocholic acid have been linked to preserved ejection fraction status in metabolic-associated fatty liver disease (MAFLD) (Zhou et al., 2024).

A meta-analysis involving 43,229 MASLD patients demonstrated elevated circulating levels of TBA, UDCA, TCA, CDCA, taurochenodeoxycholic acid (TCDCA), GCA, GUDCA, GCDCA, tauroursodeoxycholic acid (TUDCA), and CA compared to healthy controls. Hepatic expression of bile acid-related enzymes, such as CYP7A1, was significantly upregulated in NAFLD patients, especially in NASH. However, bile salt export pump (BSEP) expression at the mRNA and protein levels remains debated. Recent findings suggest a compensatory increase in periportal BSEP membrane-localized abundance in NASH patients with high histologic scores, facilitating bile acid excretion.

Reduced farnesoid X receptor (FXR) expression in NAFLD patients disrupts critical metabolic pathways, increasing sterol regulatory element-binding protein 1c (SREBP-1c) expression and promoting adipogenesis. FXR activation reduces hepatic triglyceride levels and fibrosis by influencing genes involved in lipid metabolism and hepatic stellate cell activity. Additionally, FXR activation reduces monounsaturated and polyunsaturated fatty acids in the liver by inhibiting key enzymes and lipid uptake. Petersen et al. observed a strong correlation between hepatic diacylglycerol levels and insulin resistance, highlighting the metabolic significance of hepatic steatosis.

Alterations in gut microbiota contribute to NAFLD development. Reduced alpha diversity and an imbalance between anti-inflammatory and pro-inflammatory microorganisms characterize the gut microbiota of NAFLD patients. Decreased abundance of bile salt hydrolase (BSH)-active bacteria, such as Clostridium leptum and Eubacterium genera, affects secondary bile acid levels. These changes are more pronounced in obese and insulin-resistant individuals.

4.3. Cholestatic Liver Disease

Cholestatic liver diseases, including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), disrupt bile formation, secretion, or excretion. PBC involves granulomatous destruction of small intrahepatic bile ducts, while PSC features inflammation and fibrosis of bile ducts, leading to strictures. Impaired bile flow in cholestatic liver disease significantly elevates bile acid levels in blood and urine, with reductions in 7α-hydroxy-4-cholesten-3-one (C4) indicating inhibited bile acid synthesis in advanced disease (Braadland et al., 2022).

PBC patients exhibit significantly elevated serum TBA, PBA/SBA ratios, and conjugated/unconjugated bile acid ratios. Liu et al. identified eight bile acids, including DCA, GDCA, and UDCA, as markers distinguishing PBC patients from healthy controls. Pruritus, a common symptom in PBC, correlates with elevated serum TBA, GCA, and GCDCA levels. UDCA therapy increases the hydrophilicity of the bile acid pool, becoming the primary circulating bile acid post-treatment.

Similarly, PSC patients demonstrate elevated serum TBA, PBA, and CBA/UCBA ratios, with unchanged glycine/taurine-conjugated bile acid ratios. Mousa et al. reported elevated levels of CA, CDCA, and UDCA in PSC patients. Interestingly, studies have reported that patients with primary sclerosing cholangitis (PSC) exhibit significantly reduced glycine-to-taurine (G:T) conjugation ratios in chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA), while showing significantly increased ratios in ursodeoxycholic acid (UDCA). Moreover, patients with PSC combined with inflammatory bowel disease (IBD) displayed higher levels of primary bile acids (PBA) and lower levels of secondary bile acids (SBA) compared to those with IBD alone. UDCA treatment may elevate serum total bile acid (TBA) concentrations in PSC patients; however, this process does not appear to influence bile acid synthesis.

4.4. Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an idiopathic inflammatory condition involving the intestines, particularly affecting the ileum, rectum, and colon. The two primary forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). PSC is the most prevalent liver disease associated with IBD, as approximately 70% to 80% of PSC patients develop IBD. This significant association underscores the connection between IBD and the enterohepatic circulation. Patients with IBD demonstrate elevated proportions of PBA and conjugated bile acids (CBA) in their feces, alongside reduced proportions of SBA (especially DCA and lithocholic acid (LCA)). Specifically, fecal bile acid concentrations are elevated, while bile acid levels in the duodenum are decreased in CD patients (N. Li et al., 2021).

Most studies report elevated total fecal bile acids in UC patients, although a few have noted reductions. Crohn's disease patients exhibit higher fecal PBA levels and reduced SBA levels after bowel resection. It has been observed that serum PBA levels are elevated, serum SBA levels are reduced, and the PBA-to-SBA ratio is increased in IBD patients, with these trends being more pronounced during active disease phases. Additionally, Murakami et al. reported significantly lower serum DCA/(DCA + cholic acid (CA)) ratios in IBD patients compared to healthy controls, with no notable differences between UC and CD during remission or exacerbation phases.

A pediatric IBD study found that conjugated primary bile acids (CPBA) positively correlated with disease activity, while unconjugated secondary bile acids (USBA) were negatively correlated. Moreover, the PBA-to-SBA ratio is a promising indicator for stratifying disease activity in IBD patients. Lower concentrations of SBA, including DCA, have been reported in CD patients compared to controls, with levels being even lower in active disease states. Conversely, Feng et al. did not identify significant changes in serum TBA, PBA, SBA, CBA, and unconjugated bile acids (UCBA) in CD patients, but they noted reductions in DCA, glycodeoxycholic acid (GDCA), LCA, glyco-LCA (GLCA), and taurodeoxycholic acid (TDCA). UC patients showed decreased levels of CA, CDCA, UDCA, and glycoursodeoxycholic acid (GUDCA) but increased taurochenodeoxycholic acid (TCDCA) compared to CD patients.

Sun et al. reported significantly reduced serum SBA levels in UC patients and concluded that TDCA and DCA could serve as diagnostic markers for UC. Blood inflammatory markers in UC patients were positively correlated with fecal PBA and negatively correlated with SBA levels. However, another untargeted metabolomics study found elevated serum DCA levels in CD patients. This study achieved diagnostic concordance rates of 89.49% and 94.88% when using a combination of DCA and palmitoyl amide to differentiate healthy controls from patients in remission-phase and active-phase CD, respectively. Additionally, CD patients with disease durations exceeding 18 months exhibited elevated serum DCA, GDCA, and TDCA levels, while UC patients with disease durations exceeding 48 months demonstrated increased DCA and GDCA levels.

IBD patients exhibit reduced farnesoid X receptor (FXR) activation, leading to decreased inhibition of hepatic bile acid synthesis, increased bile acid production, intestinal bile acid malabsorption, diminished apical sodium-dependent bile acid transporter (ASBT) expression, and impaired gut microbiota. For example, serum and fecal DCA/(DCA + CA) ratios strongly correlate with the abundance of Clostridium intestinalis subgroup XIVa, a key group of bacteria involved in bile acid metabolism. Reduced microbial biodiversity, particularly in CD patients, is characterized by lower diversity, greater compositional variability, a decline in beneficial bacteria, and an increase in pathogenic species such as Escherichia coli. Intestinal resection in CD patients further decreases gut microbiota α-diversity, increasing Aspergillus species while reducing beneficial bacteria and Anaplasma phyla.

4.5. Alzheimer's Disease

Aging is associated with declining serum TBA levels, primarily due to reductions in unconjugated primary bile acids (UPBA) and USBA, alongside an age-related increase in the percentage of CPBA. In Alzheimer's disease (AD) patients, most studies report significantly lower serum CA and CA%, as well as elevated DCA and conjugated secondary bile acids (CSBA), with some studies noting minor elevations in serum CA 1. In males, serum CA and CA% levels decline progressively from cognitively normal mild cognitive impairment (MCI) to AD, while in females, this decline occurs only after the MCI stage (Chen et al., 2024).

Varma et al. observed that reduced serum levels of 7α-hydroxycholesterol (7α-OHC), CA, and CDCA correlated with increased cerebral amyloid deposition, accelerated white matter lesion accumulation, and faster cerebral atrophy, particularly in males. MahmoudianDehkordi et al. reported that elevated serum DCA/CA, GDCA/CA, and TDCA/CA ratios were significantly associated with cognitive decline. Additionally, these ratios negatively correlated with cerebrospinal fluid (CSF) Aβ1-42, with the GDCA/CA ratio positively correlating with amyloid deposition. Higher SBA-to-PBA ratios were also associated with increased CSF p-tau, reduced hippocampal volume, and impaired cerebral glucose metabolism.

AD patients exhibit alterations in gut microbiota, including a reduction in Firmicutes and an increase in Bacteroidetes, which is linked to elevated production of cytotoxic SBA. Increased intestinal permeability facilitates the reabsorption of bile acids into the bloodstream, allowing them to cross the blood–brain barrier and exacerbate brain damage. Approximately 80% of AD patients also present with diabetes or impaired glucose tolerance, which may contribute to elevated circulating DCA levels. Hydrophobic bile acids, particularly DCA, LCA, and glycochenodeoxycholic acid (GCDCA), may disrupt blood–brain barrier integrity and promote amyloid β plaque formation through the oxidation of docosahexaenoic acid.

4.6. Cancer

The profiles of bile acids have been extensively studied in the context of gastrointestinal-related cancers. It has been reported that serum concentrations of conjugated bile acids (CBAs) are notably elevated in individuals with hepatocellular carcinoma (HCC) up to a decade prior to disease onset. Furthermore, serum levels of taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), and glycocholic acid (GCA) were found to be higher in patients with pancreatic cancer, while serum taurolithocholic acid (TLCA) levels were significantly elevated in patients with gastric cancer. In patients with duodenal tumors, the levels of chenodeoxycholic acid (CDCA) and lithocholic acid (LCA) in duodenal fluid were increased and decreased, respectively. Among gastrointestinal-related cancers, HCC and colorectal cancer (CRC) have the most robust body of research regarding changes in bile acid profiles.

Compared to healthy controls, total bile acid (TBA) levels are elevated in HCC patients. Elevated levels of serum CBA (including GCDCA, GCA, and TCA) were positively associated with an increased risk of HCC, whereas unconjugated bile acids (UCBA), particularly reduced levels of CA and LCA, were negatively correlated with the risk of HCC. Increased CPBA levels

were linked to a higher risk of HCC in cases related to hepatitis B and C viruses. Dai et al. reported that TBA levels in HCC patients were lower than in those with chronic liver disease. Their study also revealed elevated levels of DCA, LCA, and GLCA, alongside decreased levels of TCDCA, GUDCA, and TUDCA in HCC patients. A close association was observed between tumor progression and the levels of TCDCA, TUDCA, GLCA, and GUDCA.

Serum DCA has been significantly elevated in patients with colorectal adenomas, underscoring its role as a precursor in colorectal carcinogenesis. Interestingly, no increase in DCA levels was observed in the ileal fluid of CRC patients; however, a decrease in UDCA percentage was noted. Recent studies suggest that serum bile acid profiles may aid in distinguishing between healthy individuals, patients with adenomas, and those with CRC. Xu et al. developed a diagnostic model for CRC incorporating LCA, Apolipoprotein A1, Apolipoprotein A2, and carcinoembryonic antigen, achieving an impressive area under the curve (AUC) of up to 0.995. Cross et al. demonstrated a positive association between serum GCDCA levels and CRC risk in women. In a nested case-control study, Kühn et al. found that serum levels of GCA, TCA, GCDCA, TCDCA, GHCA, GDCA, and TDCA were positively associated with a higher risk of colon cancer. Similarly, Loftfield et al.'s prospective study identified a correlation between serum levels of DCA, GDCA, TDCA, GLCA, TLCA, TCDCA, and GCA in women and CRC risk (Loftfield et al., 2022).

5. Challenges and Prospects

As highlighted, much of the research on bile acid profiles has relied on animal models. Fleishman identified several significant barriers to translating bile acid profiling assays into clinical practice, including differences in lipid metabolism between rodents and humans, species-specific variations in bile acid pools, receptor coding sequences, and signaling pathways. These discrepancies pose challenges for clinical applications. Additionally, the high cost of liquid chromatography-mass spectrometry (LC-MS), a common technique for bile acid analysis, limits its accessibility in clinical settings. Many clinical studies on bile acid profiling for disease diagnosis face limitations such as small sample sizes, inadequate differentiation between patient BMI, ethnicity, gender, and age, and a lack of focus on insulin resistance or medication effects on bile acid metabolism. Moreover, there is a scarcity of dynamic observational studies and variations in disease definitions and detection methodologies, leading to inconsistencies and even conflicting findings. Further, bile acid profiling in less commonly studied specimens, such as alveolar lavage fluid, amniotic fluid, and saliva, remains limited, further impeding its clinical adoption.

Advances in medical technology have created a growing need for personalized, efficient, and safe medical services. Solely relying on blood samples to measure TBA levels is insufficient to meet clinical demands. Since bile acids play pivotal roles in various physiological and pathological processes, and their composition varies widely across diseases, comprehensive profiling of bile acids, particularly peripheral blood bile acid profiles, could offer groundbreaking insights into systems biology. This approach, combined with artificial intelligence (AI) studies, holds potential for enhancing disease diagnosis and monitoring.

Available studies demonstrate that bile acid profile diversity provides a comprehensive reflection of early events in conditions such as type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), inflammatory bowel disease (IBD), Alzheimer's disease (AD), cholestatic liver disease, and other related disorders. Bile acid profiles also show diagnostic potential in pregnancy-related conditions. For instance, elevated levels of TBA, LCA, and DCA during early pregnancy, coupled with reduced CA and CDCA levels, are associated with an increased risk of gestational diabetes mellitus. In intrahepatic cholestasis of pregnancy, serum CA levels are elevated, with an amplified CA/CDCA ratio and increased CBA levels. Additionally, elevated GCDCA levels in esophageal saliva have been observed in patients with

Barrett's esophagus, and reduced fecal concentrations of LCA and DCA were noted in pediatric patients with recurrent C. difficile infections (X. Li et al., 2024).

Bile acid profiling plays a significant role in new drug development and patient care. Wu et al. demonstrated that Lactobacillus acidophilus alleviates cholestatic liver injury by activating the intestinal FXR receptor, enhancing bile acid excretion, and enriching bile salt hydrolase (BSH) enzymes, thereby increasing UCBA levels (Wu et al., 2024). Zhang et al. showed that LCA administration mitigated obesity induced by a high-fat diet and reduced levels of non-12-hydroxy bile acids. As research progresses, the relationship between diseases and bile acid metabolism is becoming increasingly clear, contributing to the precision and personalized diagnostic assessment needed in modern clinical practice.

Conclusion

Bile acid profiling has emerged as a crucial component in understanding the intricate roles of bile acids in both physiological and pathological states. Laboratory testing techniques, including advanced methodologies like LC-MS and biosensors, have significantly enhanced the capacity to detect and quantify bile acid profiles with high specificity and sensitivity. Despite these advancements, clinical translation is hindered by challenges such as high costs, interspecies variability, and inconsistencies in study methodologies.

The diversity of bile acid profiles offers a promising diagnostic tool across a spectrum of diseases, including type 2 diabetes, non-alcoholic fatty liver disease, inflammatory bowel disease, Alzheimer's disease, cholestatic liver diseases, and various cancers. Moreover, bile acid profiling holds potential in pregnancy-related conditions and personalized medicine. Continued research, coupled with the integration of artificial intelligence and advancements in biosensor technologies, will likely overcome existing barriers, paving the way for more accessible and precise diagnostic and therapeutic strategies.

Future efforts should focus on standardizing methodologies, increasing sample diversity, and exploring underutilized biological matrices to fully harness the diagnostic and therapeutic potential of bile acid profiling in laboratory medicine.

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