White Paper

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The Value of Expertise

Bile Acids:
Beyond Cholesterol Metabolism & Fat Absorption

Background

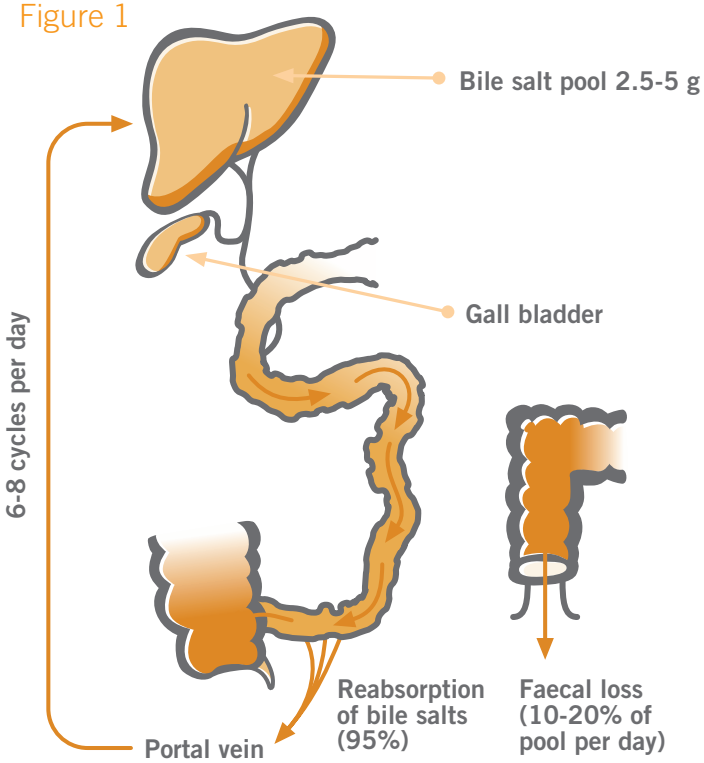
Bile acids are well recognized as essential for regulating cholesterol homeostasis and the digestion and absorption of fat through the intestine. In recent years, however, bile acids have emerged as signaling molecules with endocrine functions, acting as ligands for the G-protein coupled receptor TGR5 and the nuclear receptor farnesoid X receptor (FXR). This has elevated the importance of bile acids beyond fat digestion and into triglyceride, cholesterol, and glucose homeostasis. There is growing interest in bile acids as therapeutic targets for the treatment of obesity, type 2 diabetes, and hyperlipidemia.

Biochemistry and Physiology

Bile acids are synthesized in the hepatocyte and secreted through the bile canaliculi to the gall bladder for storage until food passes the intestine stimulating the release of the hormone cholecystokinin contracting the gall bladder to release bile to the intestine for further digestion of the fat (Figure 1).

The production of bile acids is accomplished by the cytochrome P450-mediated oxidation of cholesterol. The majority of these compounds are first hydroxylated at position seven by the enzyme cholesterol-7-alpha hydroxylase, thus producing chenodeoxycholic acid (Figure 2). Subsequently, beta oxidation and chain cleavage in the alkyl side chain at position 17 in ring D leads to formation of the carboxyl group at position 24. Chenodeoxycholic acid and cholic acid, which is also hydroxylated at the 12 position, are the two primary bile acids (Figure 2). The 7-hydroxyl group can be removed by intestinal bacteria producing deoxycholic acid from cholic acid, and lithocholic acid from chenodexoycholic acid. The latter are termed secondary bile acids. Lithocholic acid is rather insoluble and toxic to most cells at high levels.

Figure 1



Modified from Figure 7.5 in Burroughs AK, Westaby D: Liver, biliary tract and pancreatic disease in Clinical Medicine, Eds. Kumar P, Clark M. Elsevier 2002

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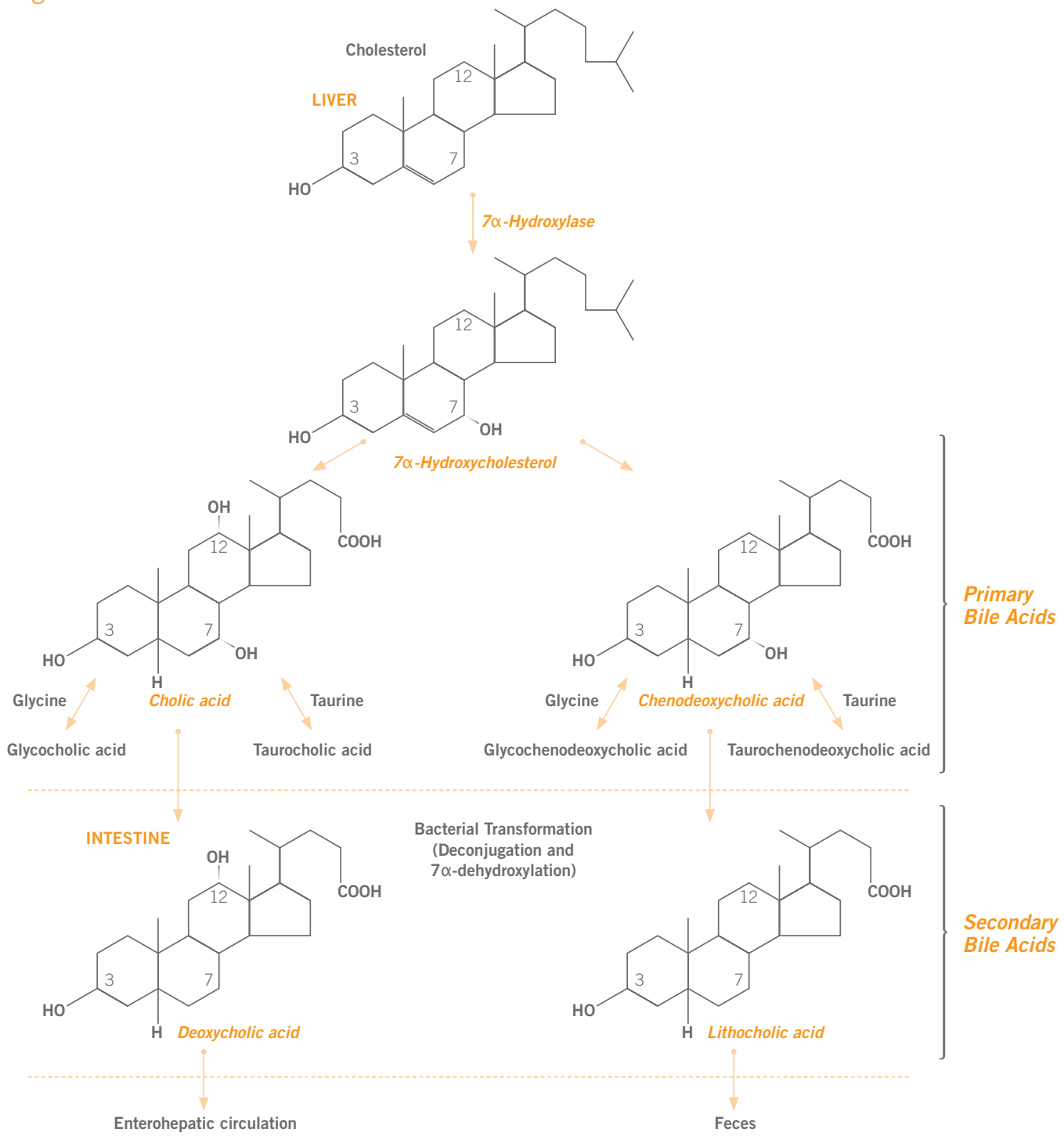
Figure 2



Modified from Figure 23-6 in Rafai, N, Warnick, GR, Remaley, AT: Lipids, Lipoproteins, Apolipoproteins, and other Cardiovascular Risk Factors in Tietz Fundamentals of Clinical Chemistry, Eds. Burtis CA, Ashwood ER, Bruns DE. Elsevier 2008

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Prior to secretion from the human liver the primary bile acids may be conjugated at position 24 by glycine or taurine. The human bile is conjugated to either glycine (75%) or taurine (25%). Conjugation increases water solubility and the capacity to solubilize lipids in the small intestine.

Approximately 800 mg of cholesterol is metabolized each day with about 50% used for bile acid synthesis.

Most of the bile acids (~95%) are absorbed by a combination of passive diffusion and active transport in the terminal ileum transporting the bile acids back to the liver through the portal vein (Figure 1). This process is called enterohepatic circulation. Besides the enterohepatic circulation, bile acids can also enter the systemic circulation through an alternative pathway when there is an overload of bile acid accumulation like in cholestatic liver diseases.

The overall bile acid pool is maintained by several important bile acid transporters discovered in recent years (see review papers in reference list for details). These bile acid transporters located at the basolateral membrane of the hepatocytes and in the proximal convoluted tubule cells in the kidney adapt to certain disease states keeping the bile acid homeostasis in equilibrium reducing the overall risk of toxic bile acid accumulation. Less than 10 percent of the bile acids are filtrated through the kidney. About 10-20% of the total pool per day is lost through feces.

Bile acids as signaling molecules

Besides their role in fat digestion and absorption, as suggested by a growing body of evidence now available, bile acids are crucial signaling molecules acting as ligands for the G-protein coupled receptor (GPCR) TGR5 and the nuclear receptor farnesoid X receptor (FXR) resulting in the secretion of GLP-1, PYY and oxyntomodulin from enteroendocrine L-cells. Hence bile acids decrease blood glucose levels and are anorexigenic through PYY and oxyntomodulin, causing weight loss. Specific bile acids may exert specific effects. For example, it is known that after bariatric surgery, the level of taurocholic acid increases dramatically in parallel with the improvements in weight and glucose control (insulin resistance). Neither glycholic acid nor cholate itself appear to increase.

Farnesoid X Receptor

The FXR-alpha is the main FXR involved in bile acid metabolism with chenodeoxycholate being the most potent natural agonists for FXR-alpha. The FXR-alpha is expressed in the liver, kidney, and the gut. Other bile acids such as lithocholic acid have been shown to be a potent agonist for the vitamin D receptor as well. The level of bile acid pool in the liver and the intestine is regulated by the FXR-alpha through the expression of the small heterodimer partner (SHP), a nuclear receptor, which through activation of multiple transporters and proteins located in the membranes of hepatocytes and enterocytes can either up or down regulate the efflux of bile-acids (see review papers in reference list for details).

The FXR-alpha regulates cholesterol metabolism through the repression of CYP7A1 (cholesterol 7-alpha-hydroxylase; see above). Increased bile-acids pool would stimulate the FXR-alpha, which in turn represses the CYP7A1 resulting in less cholesterol catabolism reducing the LDL-R expression and hence increase LDL-C levels. The opposite would

happen where low bile acids pool would lead towards reduced LDL-C levels. Other lipids and lipoproteins like HDL-C and triglycerides, and very-low density lipoproteins (VLDL) are all negatively associated with bile-acid flux, i.e. low bile-acids pool is associated with elevated lipoprotein levels and vice versa. There is evidence to suggest that this effect is mediated by FXR-alpha activation through SHP induction of the transcription factor sterol regulatory element-binding protein 1c (SREBP-1c) (see reference 1). Overall these data suggest that FXR-alpha can regulate cholesterol homeostasis including fatty-acid and triglyceride biosynthesis, through the bile acids pool.

TGR5

The TGR5 is expressed in the enteroendocrine L cells, which secrete the glucose lowering incretin hormone glucagon-like peptide-1 (GLP-1). An increasing body of evidence has suggested that TGR5 activated by bile-acids result in intestinal secretion of GLP-1 (reference 3). In obese type II diabetic subjects intrarectal admission of a bile acid (taurocholate) produced a dose-dependent increment in GLP-1, PYY, and insulin with reduction in circulating plasma glucose concentrations (reference 4).

In addition to this, the TGR5 receptor has also been associated with cell proliferation and apoptosis through its association with epidermal growth factor receptor (EGFR) and c-Jun N-terminal kinase (JNK) signaling pathways (reference 5 and 6).

Current drug development targeting bile acids metabolism

Specific TGR5 agonists are in development targeting the GLP-1 release. Obese animal models have showed improved liver and pancreatic function with enhanced glucose tolerance following the release of GLP-1 after administering a modified cholic acid, a potent TGR5 agonist, developed by Intercept Pharmaceuticals (INT-777) (reference 7). Similarly, a modified endogenous FXR ligand, chenodeoxycholic acid (INT-747), has now entered clinical trials for primary biliary cirrhosis and diabetes (ClinicalTrials.gov). In general FXR-alpha agonists could be used for treating hypertriglyceridemia (FXR-450; Wyeth), metabolic syndrome, type-2 diabetes, and obesity.

Another antidiabetic and antiobesity therapeutic approach is to decrease bile acids reuptake in the gut by inhibiting apical sodium-dependent bile transporters (ASBT) (reference 8). Encouraging Phase I results have been reported by Satiogen Pharmaceuticals.

Conclusions

In addition to their role in cholesterol metabolism and fat absorption, bile acids are important signaling molecules with endocrine functions. Bile acids regulate important pathophysiologic mechanisms leading to type 2 diabetes, obesity, and hyperlipidemia, hence making them attractive targets for novel therapeutics. Recognizing the importance of bile acids, PBI has developed a LC-MS/MS method to quantify multiple bile acids in serum and urine to support drug development in the above mentioned fields (see Table).

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Table. Bile acids test menu at Pacific Biomarkers Inc.

Full Name	Abbreviation	Status	
		Urine	Serum
Cholic Acid	CA	Available	Available
Deoxycholic Acid	DCA	Available	Available
Chenodeoxycholic Acid	CDCA	VUR	Available
Glycodeoxycholic Acid	GDCA	Available	VUR
Ursodeoxycholic Acid	UDCA	VUR	Available
Taurodeoxycholic Acid	TDCA	Available	VUR
Taurocholic Acid	TCA	Available	Available
Glycocholic Acid	GCA	Available	Available
Glycochenodeoxycholic Acid	GCDCA	VUR	Available
Taurochenodeoxycholic Acid	TCDCA	VUR	VUR
Tauroursodeoxycholic Acid	TUDCA	UD	UD
Glycoursodeoxycholic Acid	GUDCA	UD	UD
Lithocholic Acid	LCA	UD	UD
Taurolithocholic Acid	TLCA	VUR	VUR

VUR: validation under review. UD: under development.

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