

## Scientific Background Information

# Bile Acids: Diseases and Biological Functions

**Over the last decade, researchers have discovered many important functions of bile acids. Biochemically, bile acids are the end-product of the cholesterol metabolism and play an important role in the regulation of lipid digestion.**

The bile acid pool consists of approximately 3 g of conjugated and unconjugated bile acids. Food intake stimulates the release of bile acids from the gallbladder into the small intestine. On average, an adult human secretes approximately 0.5g of bile acids daily. These bile acids are synthesized in the liver, secreted and subsequently efficiently reabsorbed in the ileum from where it is transported back to the liver via portal blood, for re-secretion. This process is referred to as enterohepatic circulation of bile acids (Li & Chiang, 2014)<sup>1</sup>.

Recent publications have shown that bile acids have a hormone-like function (Chiang et al., 2002)<sup>2</sup>. As signaling, molecules bile acids interact with membrane bound G-protein coupled receptors and nuclear receptors of various tissues.

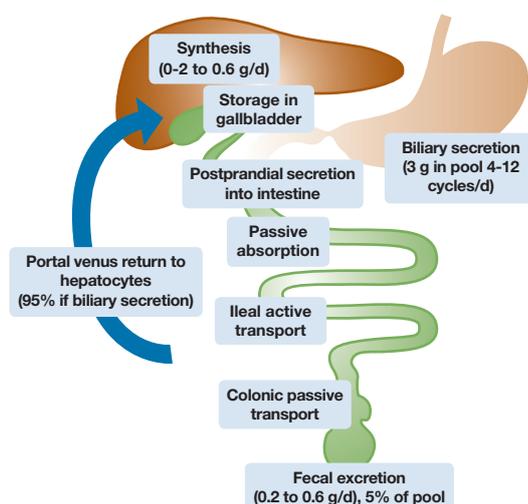


Figure 1: Based on Li and Chiang, Pharmacol Rev. 66:948-983, October 2014

## Bile Acids & Toxicology & Drug Metabolism

Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, with drugs accounting for 20-40% of all instances of fulminant hepatic failure (<http://emedicine.medscape.com/article/169814>)<sup>3</sup>. Since the 1970s, it has been known that liver injury is accompanied by increased bile acid levels, comprised largely of conjugated bile acids (Neale et al., 1971)<sup>4</sup>. Bile acids are also involved in regulating drug efficacy and toxicity via Cytochrome P450 mediated drug metabolism.

Cytochromes of the cytochrome P450 superfamily (CYP450) play a central role in the metabolism of pharmaceutical drugs. Approximately 50% of pharmaceutical drugs which are metabolized by CYPs, are processed by CYP3A4 (<http://www.pharmawiki.ch/wiki/index.php?wiki=CYP>)<sup>5</sup>.

CYPs do not only metabolize drugs, they are also involved in the regulation of bile acids and are regulated by bile acids. It has been shown that precursors of bile acids and secondary bile acids induce CYP3A4 via FXR and PXR, whereas the primary bile acid Chenodeoxycholic acid regulates CYP3A4 via the bile acid receptor FXR (Gnerre et al., 2004)<sup>6</sup>.

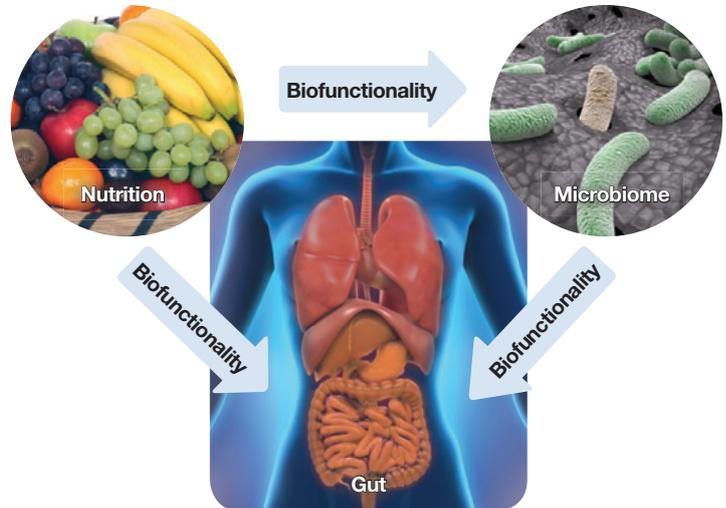
The measurement of aminotransferase is still the gold standard diagnostic test for liver function/injury. As many drugs cause symptomatic elevations in aminotransferase, the Biocrates Bile Acids Kit can be a great tool to improve established toxicological tests and give new and in-depth insights in drug metabolism.



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### Bile Acids & the Microbiome

Bile acids and gut microbiota are closely linked. Gut microbiota are involved in the biotransformation of bile acids through deconjugation, dehydroxylation, and reconjugation of bile acids, which alter bile acid composition and modulate FXR and TGR5 signaling (Li & Chiang, 2014)<sup>7</sup>. These gut microbiota-induced changes to bile acid composition in turn could influence bile acid receptor-mediated effects on glucose and lipid metabolism. A disrupted gut microbiome, including a reduction of bile salt hydrolase (BSH)-active bacteria, can significantly impair the metabolism of bile acids and may result in an inability to maintain glucose homeostasis, as well as normal cholesterol breakdown and excretion (Jones et al., 2014)<sup>7</sup>. In this context, the microbiome mediated composition of bile acids also potentially impacts the cytochrome P450 (CYP450) metabolized drugs.

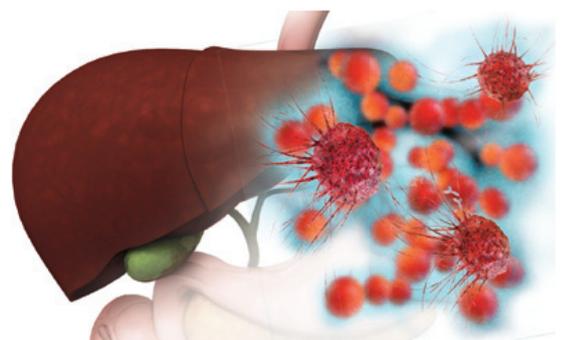


Disruption of the gut microbiome, followed by a change to the original bile acid composition could happen after intense medication with antibiotics or after bowel surgery.

The Biocrates® Bile Acids Kit\* measures a wide range of bile acids, including secondary bile acids. As microbiome-synthesized secondary bile acids play important roles as signaling molecules in cellular signaling processes (incl. drug metabolism), the Biocrates® Bile Acids Kit is a valuable tool to address questions concerning bile acid mediated signaling in drug metabolism, cardiovascular diseases, and diabetes.

### Bile Acids & Cancer

Bile acids have been implicated in cancer since the 1940s. In this context the role of some bile acids came to be regarded as cancer promoters rather than carcinogens. Here the composition of the bile acids might play an important role. Different bile acids show concentration dependent effects on gastrointestinal cells, promoting cancer or acting as carcinogens. Several studies could show that exposure to high concentrations of bile acid could induce formation of reactive oxygen species or DNA damage (Tocchi et al., 1996; Bernstein et al., 2005; Bernstein et al., 2011)<sup>8,9,10</sup>. Aside from the cancer-promoting effect of some bile acids, bile acids are natural ligands to the Farnesoid X receptor (FXR), which has been considered a multi-functional cell protector and a tumor suppressor in liver cancer (Huang et al., 2015)<sup>11</sup>.



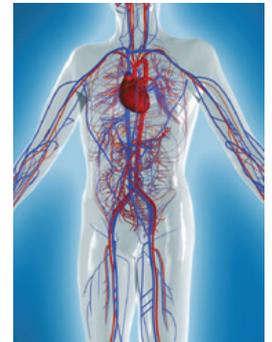
The Biocrates Bile Acids Kit provides a reproducible test which covers a broad spectrum of bile acids including primary, secondary, conjugated and unconjugated human bile acids as well as murine bile acids. This broad spectrum of bile acids, which is analyzed in a single mass spectrometric run, could enable researchers to establish individual profiles for cancer diagnosis and risk prevention.

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### Bile Acids & Cardiovascular Function

Bile acids regulate cardiovascular function by receptor-dependent and independent mechanisms. They can modify vascular tone by interacting with muscarinic receptors and transcription factors such as FXR and PXR. The ability of bile acids to interact with transcription factors, a variety of GPCRs, and potassium channels indicates that bile acids are promiscuous signaling molecules (Khurana et al., 2001)<sup>12</sup>. Notably, endogenous primary and secondary bile acids activate receptors with different efficacy (Porez et al., 2012)<sup>13</sup>.



The impact of bile acids on cardiovascular function is demonstrated by the fact that a number of pharma companies work on compounds which modulate bile acid receptors (FXR and/or TGR5) or bile acid sequestrants (Khurana et al., 2001)<sup>13</sup> to bind bile acids in the gastrointestinal tract.

BA/Ligand	Tissue/Model	Effect	Signaling/Mediator
CA	Rat heart	Bradycardia	Cholinergic stimulation
UDCA	Mouse heart	↓ allograft rejection	Immune-mediated
UDCA	Rat cardiac ischemia-reperfusion model	↓ myocyte apoptosis	Activation of PI3K-AKT
DCA, DCT CDCA	Endothelial cells	NO and K <sup>+</sup> current generation	↑ cytoplasmic [Ca <sup>2+</sup> ]
LCA	Cerebral arteries	Vasodilation	BKCa activation
CDCA	Endothelial cells	NOS upregulation	FXR activation
CDCA	Rat pulmonary artery endothelial cells	↓ ET-1 expression	FXR activation
CDCA GW4064	Rat aortic smooth muscle cells	↑ AT2R expression	FXR activation
CDCA	Endothelial cells; human esophageal cancer xenograft	↑ angiogenesis	FXR activation; COX-2-dependent VEGF production

Table1: ↓, decreased; ↑, increased; INT-747 is a synthetic CDCA derivative. GW4064 and 6 $\alpha$ -ethyl-chenodeoxycholic acid (6ECDCA) are synthetic FXR agonists; CAM, chorioallantoic membrane assay; CNV, choroidal neovascularization; VDCC, Voltage-dependent calcium channels ; Khurana et al., Clin Transl Sci., 2011

By measuring a broad spectrum of conjugated and unconjugated as well as primary and secondary bile acids, the Biocrates® Bile Acids Kit\* enables the analysis of the composition of bile acids and the total concentration of bile acids in the individual.

### Bile Acids & Diabetes

It has been suggested that cardiovascular diseases and type 2 diabetes may have a related origin (Anstee et al., 2013)<sup>14</sup>.

In addition to their traditionally-recognized role in cholesterol elimination and emulsification of dietary fat, bile acids exert regulatory effects on glucose and lipid metabolism via activation of FXR and TGR5 mediated signaling. The nuclear receptor FXR and membrane bound receptor TGR5 are the best studied bile acid receptors and targets for drug development concerning diabetes and cardiovascular diseases.



FXR regulates gluconeogenesis, glycogen synthesis, and insulin sensitivity and is activated in a bile acid species-dependent manner (Khurana et al., 2001)<sup>13</sup>. Haeusler et al. (2013)<sup>15</sup> showed that insulin resistance was associated with increased concentrations of cholic acid, deoxycholic acid, and their conjugated forms. They suggested the possibility of developing insulin-sensitizing therapeutics based on manipulation of the bile acid composition (Haeusler et al., 2013)<sup>15</sup>.

Thus, in diabetes research, the precise measurement of a broad spectrum of conjugated and unconjugated as well as primary and secondary bile acids, could bring new insights to a chronic disease with a permanent progressive number of cases.

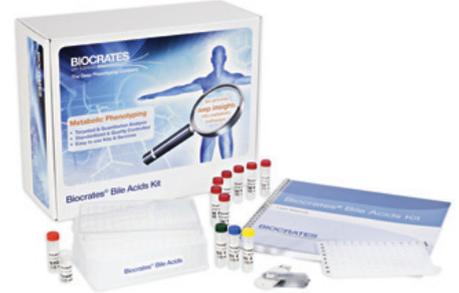
The Biocrates Bile Acids Kit enables not only the determination of the total concentration of bile acids, but also the quantification of specific bile acids which are related to insulin resistance.

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### Summary

The ready-to-use Biocrates® Bile Acids Kit\* measures a wide range of bile acids, including secondary bile acids. As microbiome-synthesized secondary bile play important roles as signaling molecules in cellular signaling processes (incl. drug metabolism), the Biocrates® Bile Acids Kit\* is a valuable tool to address questions concerning bile acid mediated signaling in drug metabolism, cardiovascular diseases, and diabetes.



### References

- 1 Li & Chiang, Bile Acid Signaling in Metabolic Disease and Drug Therapy., *Pharmacol Rev* 66:948-983, October 2014
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