RANDOX

EDUCATIONAL GUIDE

5th Generation Total Bile Acids & Importance of Intrahepatic Cholestasis in Pregnancy

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Introduction

The synthesis and metabolism of bile acids (BAs) is determined by the timing and content of food ingestion, where they, in turn, regulate the subsequent metabolism of available fuel. There is large diversity between the bile salt profiles produced by different species, thought to be a result of the parallel evolution of multiple biochemical pathways which are responsible for the conversion of cholesterol, a hydrophobic and crucial cellular component, into a water-soluble molecule. After eating, BA levels spike approximately 6-fold in the duodenum before they are returned to the hepatocytes through the portal circulation. This spike occurs around 30 minutes after a meal. Circulatory BAs are also elevated around 1-2 hours after a meal[1] which have a high variability in response to metabolic state making it difficult to perform comprehensive analysis.

This guide details the structural and functional features of bile acids and their importance in intrahepatic cholestasis of pregnancy.

Bile Acids

Structure

Bile acids are of a diverse family of bile salts, which also include bile alcohols. These molecules all share a central sterol ring, but this is as far as the similarities reach. These molecules are planar and amphipathic and display a carboxyl tail. BAs display a hydrophilic hydroxyl group on one side and a hydrophobic methyl group on the other, causing the characteristic amphipathic nature of these molecules. Polarity and solubility will be determined by the structure of the specific BA and the species in which it is found. When found in low concentrations, BAs will dissolve in water. However, at high concentrations, the hydrophobic regions orientate inwards to repel water, while the hydrophobic regions line up towards water causing the BAs self-associate to form aggregates known as micelles[1].

Biosynthesis

BAs can be classified as primary, secondary, or tertiary, with each form gaining additional modifications. Primary BA biosynthesis occurs through 1 of 2 mechanisms, namely the classic or neutral pathway or the alternate or acidic pathway.

The majority of BAs are formed through the classic pathway which occurs in the liver and consists of hydroxylation of the cholesterol steroid nucleus, catalysed by the enzyme cholesterol 7-α hydroxylase (CYP7A1). The alternate pathway synthesises BAs from oxysterols rather than cholesterol and occurs primarily outside the liver. In this process, the sterol side chain is oxidized by sterol hydroxylase before 7α-hydroxylation occurs, catalysed by 25-hydroxycholesterol 7α-hydroxylase (CYP7B1)[2].

Oxysterols intermediates, which are formed in both pathways, undergo hepatic modification to become the BA, chenodeoxycholic acid (CDCA) or, in the presence of sterol 12α-hydroxylase (CYP8B1), they become cholic acid (CA), another important BA[2].

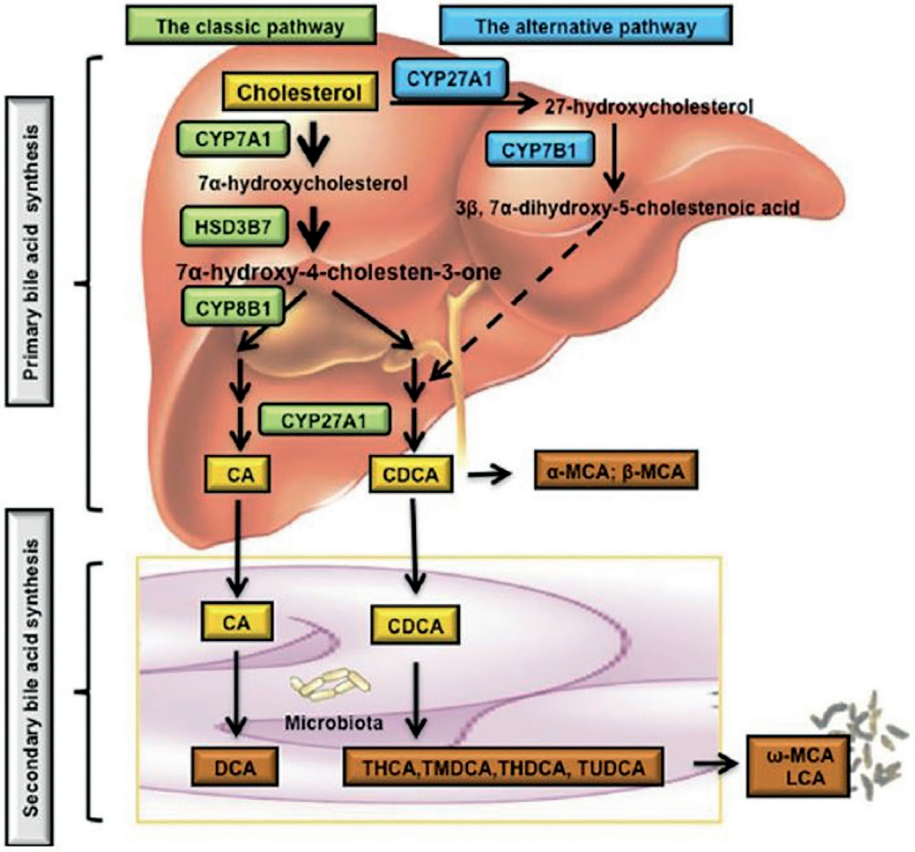

Figure 1. Bile acid synthesis showing both the classical pathway, yielding CA, and the alternative pathway, yielding CDCA and the modifications of these BAs into secondary BAs[7].

Hepatic conjugation of primary BAs takes place before they are excreted into the bile. There are 5 types of conjugation which occur at different locations on the BA structure[1]:

1. N-acyl amidation with glycine or taurine
2. Sulfation
3. Ester glucuronidation
4. Ethereal conjugation
5. N-aceytlglucosamination

The first of these conjugations is catalysed by bile acid CoA: amino acid N-acyltransferase (BAAT) and occurs on the terminal side of the carboxylic acid. This is the final stage in BA synthesis and functions to increase the solubility of BA. In addition, a negative charge is induced under the specific pH conditions of digestive fluids. This conjugation also reduces the permeability of BAs, inhibiting their diffusion through paracellular junctions. This causes the newly secreted, conjugated BAs to maintain high concentrations in the lumen of the small intestine, a feature which is essential for the digestion of lipids. Secondary BAs are formed through the removal, oxidation, or epimerisation of the nuclear hydroxyl groups. Further hepatic modifications result in tertiary BAs[2].

Transporter genes

BA transporter genes are self-regulated, allowing for the precise distribution of BAs in response to meal status. Some of these important transporters are mentioned in Table 1:

Table 1. Transporters of Bile Acids

TRANSPORTER GENE	DESCRIPTION
Apical sodium-dependent bile acid transporter	Responsible for the uptake of luminal BAs from the small bowel
Organic solute transporter (OSTα/β)	Secretes bile acids into the portal bloodstream
Sodium/taurocholate co-transporting polypeptide (NTCP)	Facilitates bile acid uptake in the liver

Function

Major functions of BAs include direct emulsification and solubilisation of luminal lipids. Additionally, their signalling activity has a variety of downstream effects, however, the function of a species of BA will be determined by its structural differences. The diversity seen in BAs, and the wide range of activity they display including synthesis, cycling and modification, enables stringent regulation of BA-stimulated responses[3].

The emulsification properties of BAs convert dietary fats into smaller fat droplets allowing them to be digested by pancreatic enzymes due to an increase in surface area. BAs are also essential for the absorption of several essential vitamins including A, D, E and K. These non-polar lipids are incorporated into micelles, facilitating their uptake.

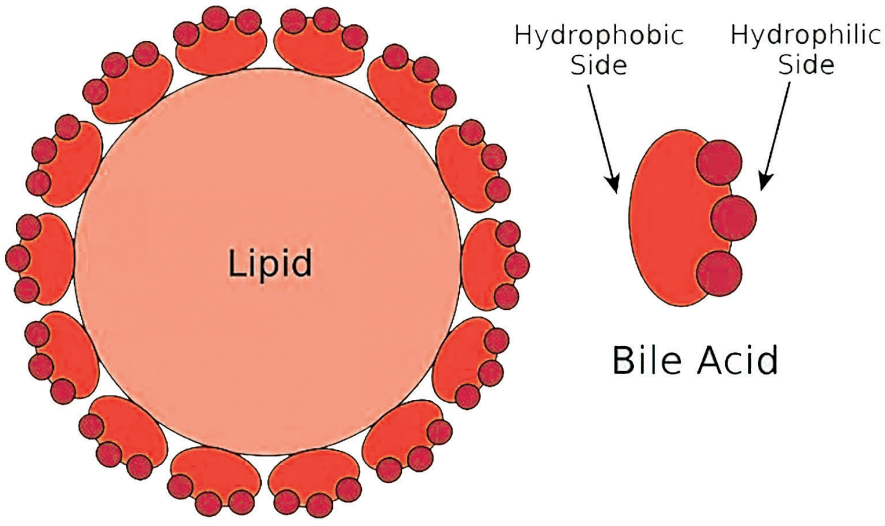

Figure 2. Illustration of the conformation of bile acids around a central lipid, forming a micelle[8].

As synthesis and degradation of BAs are regulated in response to levels of dietary cholesterol, changes in the normal processes are associated with the progression and severity of coronary artery disease[3].

Some of the major receptors associated with the metabolism of BAs are detailed below in Table 2[1]:

Table 2. Major Receptors Associated with Bile Acid Metabolism

RECEPTOR	ACTION
Farnesoid X receptor (FXR)	This receptor is essential to the normal function of the negative feedback cycle that regulates BA synthesis and delivery. The activation of FXR increases the expression of small heterodimer partner (SHP) which, in turn, inhibits the expression of CYP7A1, and therefore the classic pathway of BA synthesis. SHPs also block the expression of NTCP, decreasing portal circulation uptake of BAs. These actions are responsible for maintaining BA levels below a toxic concentration. FXR also increases the clearance of triglycerides from the liver through the modification of a variety of genes involved in lipid transport and metabolism. In addition, FXR, through SHP, modulates hepatic gluconeogenesis by suppressing enzymes crucial to this pathway. Finally, FXR is responsible for the hepatic uptake of LDL and VLDL through increased production of the transmembrane proteoglycan, syndecan-1, which is involved in LDL binding.
TGR5	Located on the cell membrane, this receptor is responsible for the activation of vascular endothelial cells, resulting in vasodilation and increased hepatic blood flow. This receptor is also responsible for the relaxation of the smooth muscle of the gallbladder wall, allowing it to fill with bile and the secretion of chloride into the bile from the gallbladder. Finally, TGR5 also actions anti-inflammatory effects in monocytes and macrophages, and inhibits the production of pro-inflammatory cytokines.

Intrahepatic Cholestasis of Pregnancy

During pregnancy, the liver adapts its metabolic processes to account for the ever-changing requirements of the growing foetus. A major alteration occurs in the metabolism of glucose. Increasing insulin resistance and changes in hormone secretion result in changes in lipid metabolism. BA transport is also increased resulting in increased concentration of BAs in the blood. This is usually a slight increase, presenting no risk to the mother or foetus. Under normal pregnancy conditions, BAs are transported from the foetus to the mother as the former does not have the mechanisms to degrade BAs yet[4].

Intrahepatic cholestasis of pregnancy (ICP) is a multifactorial disorder characterised by abnormal liver enzyme levels, pruritis with or without a skin rash, and elevated BA concentrations, namely CA and CDCA. Typically manifesting in the second or third trimester, ICP is the most common pregnancy-related liver disease, with a prevalence of up to 5.6% in the USA[5]. Other symptoms include abdominal pain, nausea and vomiting, jaundice, insomnia, irritability, and depression. In addition, the shortage of fat-soluble vitamins can result in elongated prothrombin times, perinatal haemorrhages, and bleeding into the central nervous system[4]. Symptoms normally resolve spontaneously upon the delivery of the foetus, however, ICP is between 45-90% likely to return in subsequent pregnancies.

There are several risk factors associated with ICP pathophysiology which remains enigmatic:

- Genetic
- Ethnical
- Hormonal
- Nutritional
- Environmental

The primary complication of ICP is stillbirth. The normal transport of BAs from the foetus to the mother is reversed in ICP resulting in the accumulation of BAs in the foetal compartment. While the mechanisms of ICP remain elusive, it is hypothesised that elevated BA levels cause abnormal cardiomyocyte contraction and rhythm transmission in the heart of the foetus or a dose-dependent vasoconstrictive effect on placental chorionic veins[5]. Other complications include up to 60% likelihood of preterm birth[4], meconium-stained amniotic fluid and neonatal respiratory distress syndrome[5].

A total bile acid (TBA) concentration of 100µmol/L is considered the cut-off for ICP. Studies show that TBA >100µmol/L is associated with a significantly higher incidence of perinatal death and adverse perinatal outcomes when compared with a TBA concentration of <100µmol/L[5].

The clinical utility of serum BA concentration is becoming increasingly clear, with UK guidelines including recommendations for the weekly monitoring of BAs after an ICP diagnosis has been made. Reservations about this testing are mainly based on the argument that the quantification of TBA does not accurately reflect the presence of the BAs associated with the progression of ICP. However, recent data displays a directly proportional relationship between increased TBA concentration and CA and CDCA concentration[6].

Randox 5th Generation Bile Acids

For the *in vitro* quantification of Total Bile Acids in serum and plasma, the Randox 5th generation Total Bile Acids assay is an enzymatic, colourimetric assay supplied in a 2-shot liquid format.

The 5th generation assay incorporates a more complex enzyme cycling method compared to previous generations to amplify the signal, ultimately improving sensitivity and precision while also reducing interference from haemolytic and lipemic samples. Haemolysis and lipaemia are common in neonatal and pregnant patients, making the 5th generation test ideal for testing on these patients.

Assay Principle

Two reactions are combined in this kinetic enzyme cycling method. In the first reaction, bile acids are oxidised by 3-α hydroxysteroid dehydrogenase with the subsequent reduction of Thio-NAD to Thio-NADH. In the second reaction, the oxidised bile acids are reduced by the same enzyme with the subsequent oxidation of NADH to NAD. The rate of formation of Thio-NADH is determined by measuring the specific absorbance change at 405nm. Enzyme cycling means multiple Thio-NADH molecules are generated from each bile acid molecule giving rise to a much larger absorbance change, increasing the sensitivity of the assay.

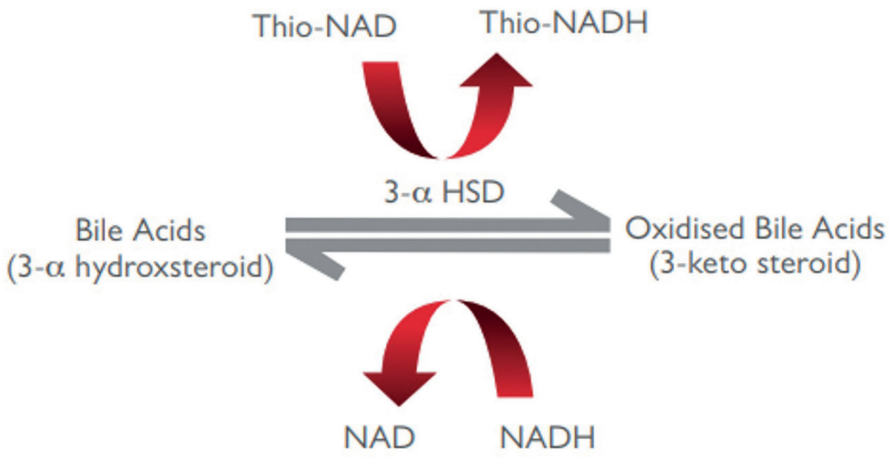

Figure 3. 5th generation TBA assay principle.

Features

- Superior methodology utilising an advanced enzyme cycling method, the test displays outstanding sensitivity and precision when compared to traditional enzymatic-based tests
- Excellent measuring range of 2.16 - 238µmol/l. The normal upper range for bile acids in a fasting serum sample is 10µmol/l
- Exceptional correlation of r=0.99 when compared against other commercially available methods
- Two shot liquid ready-to-use reagent for convenience and ease-of-use
- Stable to expiry when stored at +2 to +8°C
- Applications available detailing instrument-specific settings for a wide range of clinical chemistry analysers
- Complementary controls and calibrators available offering the complete testing package

Conclusions

Bile acid synthesis and metabolism are dictated by the amount and timing of food consumption. BAs play a major role in the removal of lipids and the uptake of a variety of essential vitamins. ICP is a common, multifactorial disorder of pregnancy associated with elevated BA concentrations. While symptoms of ICP often resolve spontaneously upon delivery of the foetus, this disease is associated with increased rates of stillbirth and pre-term delivery.

The 5th generation TBA assay utilises a more complex enzyme cycling method than previous generation tests, boasting improved sensitivity and precision. This assay displays excellent correlation with other available methods to aid in the detection and monitoring of ICP.

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