CHAPTER 1

An Overview of Bile-Acid Synthesis, Chemistry and Function

DENNIS STAMP [1] AND GARETH JENKINS [2*]

[1] Retired Researcher (Dept of Nutrition, University of Toronto), Current address: 23 Fairmar Ave, Toronto, ON, M8Y2C7, Canada; [2] School of Medicine, Swansea University, Swansea SA28PP, UK

1.1 The Bile Acids

Bile acids (BAs) are a group of water-soluble steroids formed during the catabolism of cholesterol, and synthesised in the hepatocytes of the liver. The products, cholic acid (CA), and chenodeoxycholic acid (CDCA), are called primary bile acids. Figure 1.1 shows an overview of the pathways involved in these reactions. These primary BAs are then conjugated, mainly to either glycine or taurine. The conjugated BAs play a pivotal role in fat (and fat-soluble vitamin) digestion and absorption, reaching the colon via the gallbladder, bile duct, and duodenum. BAs are strongly cytotoxic, and are able to act as nuclear sensors, detecting and controlling their own concentrations within the body. Bile acids also play a major role in carcinogenesis of some tissues (liver, gallbladder, upper and lower GI tract). These roles will be described in the following pages and following chapters. BAs are stored in the gallbladder under extremely high concentration (>300 mM), achieved by a constant removal of water and electrolytes. About 5% of these bile acids go to the colon for excretion in the faeces,

* corresponding author

Issues in Toxicology
Bile Acids: Toxicology and Bioactivity
Edited by Gareth Jenkins and Laura J. Hardie

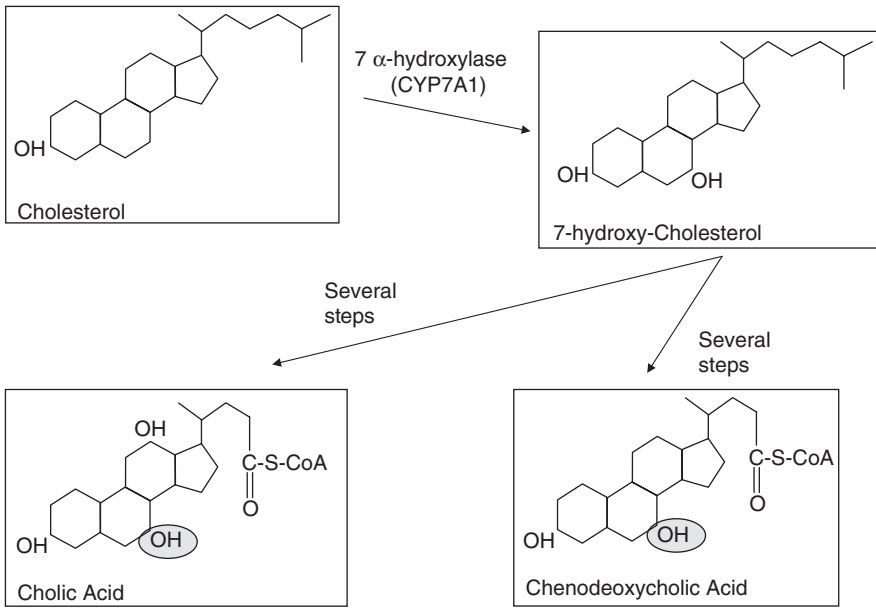

Figure 1.1 The classic pathway for the conversion of cholesterol into the primary bile acids CA and CDCA, involving the 7 α-hydroxylase enzyme (also known as CYP7A1). Simplified from Dr John Chiang.[1] The 7 OH group is highlighted with the shaded circle. This group is cleaved to produce the secondary BAs DCA and LCA.

and since cholesterol is a precursor of BA, this is the only time cholesterol is excreted from the body (as bile). Also present in bile are:

(1) Bilirubin and other pigments resulting from haem catabolism,
(2) Heavy metals such as copper or iron, in excess of bodily needs, and
(3) Lipophilic steroids and drug metabolites that would be insoluble in the urine.

In the colon, deconjugation of the conjugated primary bile acids occurs *via* the action of bacterial enzymes, producing free bile acids. Furthermore, the enzymatic action of the bacterial flora converts the bile acids into secondary BAs, by removing the hydroxyl group from the 7th carbon atom on the molecule. The specific enzyme responsible is 7 alpha-dehydroxylase, which forms deoxycholic acid (DCA) from cholic acid, and lithocholic acid (LCA) from chenodeoxycholic acid. These secondary bile acids then pass into the portal vein and reach the liver, where they join new primary BAs, they are then reconjugated to glycine or taurine in the canaliculi of the liver, and are then stored in the gallbladder. This recycling of bile acids is known as the enterohepatic circulation and can occur 10 times every day. Transport across the canalicular membrane of the liver, is an

ATP-dependent process, aided by the bile-salt excretion pump (BSEP) expression in the canalicular membrane. Conjugation increases the aqueous solubility of the bile acids, and renders these bile acids largely impermeable to the cell membranes of the intestine and duodenum; hence, they are unable to leave the intestinal lumen. This allows bile-acid levels to rise in the lumen, ultimately reaching sufficient concentrations to form micelles, which allow lipid emulsification and subsequent absorption.

Many other BAs are formed at lower levels both in the colon and liver by the bacterial flora and the conjugation with other biomolecules, but this chapter will focus on the more common bile acids; cholic and chenodeoxycholic acids (primary BAs), deoxycholic acid and lithocholic acids (secondary BAs), and their glycine and taurine conjugates. These are the main sub-types of bile acids, as seen in Table 1.1. There are some "minor" BAs that have significant importance. One is ursodeoxycholic acid (UDCA), which, as its name suggests, is abundant in bears, and much prized in Eastern medicine. Human bacterial flora can produce it as well, along with dozens of other BAs and their many isomers. Ursodeoxycholic acid plays a role in human cholesterol regulation, and its medical applications include dissolving gallstones and protecting cells from the harmful effects of other BAs like DCA in cholestatic diseases. When used medically, UDCA is not obtained from bears, but is synthesised from cholic acid, a byproduct obtained from the abattoir.

Table 1.1 Some of the biochemical properties of bile acids.

Bile acid	*Water solubility*	*CMC (mM)*	*CMpH*	*pKa*	*% in bile*
Free bile acids					
CA	273 um	13	6.65	5.2	Trace
DCA	28 "	10	7.08	6.2	Trace
CDCA	27 "	9	7.2	6.2	Trace
Glycine conjugates					
GCA	32 "	12	–	3.8	30
GDCA	6 "	6	–	4.8	15
GCDCA	7 "	6	–	4.3	30
Taurine conjugates					
TCA	Very sol	10	–	<2	10
TDCA	Very sol	6	–	<2	10
TCDCA	Very sol	7	–	<2	5
References	(12)	(12,13)	(12)	(14)	(15)

NB: In this table, BAs are divided into 3 groups: Free BAs, Glycine conjugates, and Taurine conjugates. CA = cholic acid, DCA = deoxycholic acid, and CDCA = chenodeoxycholic acid. The values quoted above represent human bile, and were taken from multiple sources. The amount of conjugated and free bile acids in bile is quite variable. Values in this table were determined for single BAs. In actuality, BAs exist as mixtures, and since they are detergents, they will influence each other's solubility characteristics. For example, taurine conjugates are strong sulfonic acids, capable of protonating other bile acids, and thus allowing them to enter the epithelium without any regards for established solubilities.

1.2 Conjugated Bile-Acid Biosynthesis

Figure 1.1 illustrates a condensed version of the classical pathway of bile-acid synthesis, a series of 12 enzymatic reactions that convert cholesterol, which is insoluble, into BAs, which are water soluble. The cholesterol is first converted to 7 alpha-hydroxy cholesterol, followed by the series of enzymatic transformations, eventually producing cholic and chenodeoxycholic acids (not all steps shown). The rate-limiting enzyme in this pathway is cholesterol 7 alpha-hydroxylase (CYP 7A1), which originates from microsomal cytochrome P-450 enzymes, expressed only in the liver hepatocytes.

Another indirect pathway (not shown in Figure 1.1) involves cholesterol reacting enzymatically with CYP 27A1, producing both 27-hydroxycholesterol and 3 beta-hydroxy-5-cholestanoic acid (omitted from the diagram for simplification). This is followed by a series of reactions, ending in the production of chenodeoxycholic acid. The inner mitochondrial membranes are the main reaction site for this pathway. In the adrenal glands, steroid acute response protein (StAR) delivers cholesterol to the mitochondrial membrane. StAR is necessary for steroidogenesis, and thus may provide a reliable source of cholesterol for these reactions.

Another pathway of some importance occurs in the brain; this is the cholesterol 24-hydroxylase pathway. About 25% of the body's cholesterol exists in the plasma membranes of myelin sheaths. Here, the blood–brain barrier prevents cholesterol exchanges with the circulating lipoproteins, which makes it difficult for cholesterol to leave the brain. The cytochrome P-450 enzymes (CYP 46), expressed almost exclusively in the endoplasmic reticula of the brain, allows formation of 24-hydroxycholesterol.

It is impossible to determine the relative contributions of each of these pathways to total bile-acid biosynthesis, due to the nature of the data. Some values were obtained from patients whose gallbladders had been surgically removed; other patients would be atypical due to illness, and many data were obtained from experimental animals, which may metabolise these compounds differently from humans. Also, the exact order of many of the reactions is not known, since the intermediates may act as substrates for more than one enzyme. Further details for these reactions can be found in reviews by Chiang,[1] Moore *et al.*,[2] and Fuchs *et al.*[3]

1.3 Bile-Acid Regulation

1.3.1 Bile-Acid Receptors (FXR)

The following is a brief overview of events in the area of BA synthesis, transport and regulation. More detailed descriptions are given in Chapter 2 and can be found in the review by Redinger.[4] Bile acids from the enterohepatic circulation, upon returning to the liver, inhibit further BA synthesis by suppressing the rate-limiting enzyme CYP7A1 (cholesterol 7 alpha-hydroxylase) in the hepatocytes. They do this by binding to, and activating, FXR, the Farnesoid X

receptor (NR1H4), a bile-acid receptor expressed in the liver, gut, kidneys and adrenal glands. In the liver, when bound to BA, FXR acts as a transcription factor to cause a feedback repression of BA synthesis.

FXR is one of 48 nuclear transcription factors so far identified, all of which reside inside the nucleus. They are involved in many biological processes, including cell growth and differentiation, embryonic development, and metabolism. They bind to ligands like bile acids, steroids and retinoids. BAs enter the cell and bind to FXR, resulting in conformational changes in structure, which allow them to react with, and influence, specific target genes. These reactions lead to the synthesis of inhibitory proteins, which repress the activity of the gene CYP7A1 (cholesterol 7 alpha-hydroxylase), the rate-limiting enzyme in BA biosynthesis. Another nuclear transcription factor to be discussed here is liver X receptor, (LXR), (NR1H3), which binds to cholesterol or its metabolic products. Other nuclear receptors like SHPs (small heterodimer partners), (NROB2), and SREBP, (sterol response element-binding proteins), have no identified ligands and are called orphan receptors.

FXR exists in alpha and beta forms and, when BA levels are high, the enzyme CYP7A1 is strongly repressed by a nuclear receptor cascade, in which activated FXR-alpha-BA ligand induces expression of the orphan receptor SHP. This shuts down the activity of another orphan receptor, liver receptor homologue-1, which is needed for CYP7A1 promoter activity.[5] Other indirect pathways also exist to repress CYP7A1 expression.[6] Hence, bile-acid levels in the liver control, through FXR, further bile-acid synthesis from cholesterol. All this ensures a constant, level of bile-acid production. Failure to achieve this regulated condition can lead to life-threatening conditions, such as liver, coronary, and cerebrovascular diseases.

Both FXR-alpha and -beta have been cloned. FXR has also been crystallised and its structure determined by X-ray crystallography. The shape of the cavity that holds a conjugated BA was determined.[7] FXR-alpha functions as a receptor for a wide range of bile acids, including cholic and deoxycholic acids, and their glycine and taurine conjugates. To keep BA levels constant in the liver, FXR can also induce the bile-salt export pump (BSEP) in the canalicular membrane. This is an ATP-dependent system and the main exporter of BA in the liver. In short, FXR suppresses the synthesis of new BAs and stimulates their billiary excretion, thus regulating BA levels and preventing excessive BA induced toxicity.

1.3.2 Cholesterol Receptors (LXR) (NR1H3) and (NR1H2)

Another receptor, LXR (Liver X receptor), also exists in alpha and beta forms, and acts as a receptor for cholesterol and its degradation products, which accumulate when cholesterol levels are high. LXRs are expressed in the liver and lower digestive tract, where they regulate cholesterol and bile-acid homeostasis. LXR-beta activates reverse cholesterol transport from the periphery to the liver.[8] LXR-alpha, which is found in the liver, promotes catabolism in the liver and drives catabolism of cholesterol to BAs. Its activation in the liver increases

cholesterol efflux and triglyceride production by inducing the expression of SREBP-1c, as well as its target genes in the liver. LXRs also regulates fatty-acid metabolism and exert anti-atherogenic effects by stimulating reverse cholesterol transport and cholesterol excretion (*via* BAs).

LXR-alpha and -beta form heterodimers with the retinoid X receptor (RXR), and are activated by cis-retinoic acid. The resulting compounds, RXR-LXR-alpha and -beta heterodimers, interact with DNA response elements. They bind to a D-4 element consisting of 2 hexanucleotides, direct repeat motifs separated by 4 nucleotides (DR-4). These heterodimers are permissive, and can be activated by ligands for both LXR and RXR.[9] The mechanisms whereby these receptors interface with DNA are still being deciphered, but they appear to be able to switch on CYP7A1 and drive BA synthesis. FXR and LXR, working together, coordinately regulate BA synthesis and oxysterol homeostasis, as well as fatty-acid and triglyceride control. These factors are targets for the development of therapeutic agents.

Entry of bile acids into the enterohepatic circulation from the gut is also controlled by bile itself. BA absorption into the ileal epithelium depends on a plasma membrane protein called the ileal bile-acid transporter (IBAT) gene (SLC10A2). The promoter for this gene also binds the FXR-bile-acid complex that starts the transcription that leads to synthesis of more transporters. As FXR activity is stimulated by BA, there is a positive feedback from BAs to IBAT, leading to up-regulated BA absorption and transport. Thus, we see bile acids have the potential to control their own reabsorption *via* a protein feedback mechanism (FXR-IBAT) as well as controlling their own catabolism in both a positive manner (LXR) and a negative manner (FXR-CYP7A1 and FXR-BSEP). The processes involved are obviously far more complicated than described here and are further explored in Chapter 2.

1.4 Chemistry of Bile Acids and Their Effects on Digestion

BA molecules are wedge-shaped, amphipathic structures, with a hydrophobic side (represented by the steroid side of the molecule), and a hydrophilic side (represented by the hydroxyl group, the amide carbonyl, and the ionised acidic groups of either glycine or taurine).[10] The hydrophobicity of the bile acids may well be linked to their intrinsic toxicity, with the more hydrophobic BAs being more toxic. The hydrophobicity is inversely related to the number of OH groups. Therefore LCA with only one OH group is highly hydrophobic and highly toxic, whereas, DCA and CDCA with 2 OH groups and CA with 3 OH groups are decreasingly hydrophobic and decreasingly toxic. The relative toxicity and bioreactivity of the different BAs are discussed in detail in later chapters.

Four to eight hundred mls of bile are secreted daily in humans and the contained BAs are strong detergents. They can be cytotoxic to the mucosal cell membranes, and can adversely affect many tissues, both intra- and extracellularly. Therefore, many strategies have evolved to control their distribution,

and maintain their concentration within narrow limits, to avoid cellular injury. For instance, bile is released into the small intestine only when there is food present (*via* cholecystokinin stimulated gallbladder contraction). The conjugated BAs are secreted into the duodenum as bile-acid anions, which mix with ingested food as it passes by. These BAs are conjugated and are thus largely impermeable to the cell membranes; hence, conjugated bile acids cannot leave the lumen of the upper GI tract. (The colon may be excluded from this since its bacterial flora can efficiently deconjugate them.) BAs are also signalling molecules, as described earlier,[1] that activate several nuclear receptors, and regulate many physiological pathways and processes to attain BA synthesis and cholesterol homeostasis. BAs also can induce signalling effects indirectly via their biological effects within the cell (*e.g.* the generation of ROS). These mechanisms, important in cancer development, are discussed in great detail in later chapters.

Bile acids help in the digestion of lipids and the products of digestion include dietary cholesterol, phospholipids, bilayers and fatty acids coming from the enzymatic breakdown of triglycerides. Association of these lipid derivatives with BAs forms mixed micelles, involving up to 40 BA molecules. The micellar mixture continues down the GI tract to the jejunum, where the contained lipids may diffuse into the epithelium to the portal veins. The micelles continue down to the distal ileum, where about 95% of the BAs are reabsorbed, and sent to the liver via the portal vein. This occurs several times during a typical high-fat meal, and forms the enterohepatic circulation (from the gallbladder, to the ileum, to the portal vein, and back to the liver). This cycling conserves BAs, thus avoiding the need to synthesise new BAs for each meal. The remaining $\sim$5% of bile/micelles enter the colon, where the colonic bacteria break them down to lithocholic acid and deoxycholic acids, which are excreted in faeces. In humans, the faecal BAs are all deconjugated, due to an efficient bacterial enzyme system that deconjugates them and removes the 7-hydroxyls from the molecule. This represents the only time cholesterol (in the form of deconjugated faecal bile acids) is excreted from the body.

Deconjugation and dehydroxylation reactions occur in the colon, leading to the formation of dozens of new distinct BAs, by the action of the colonic bacteria. The final products enter the enterohepatic circulation and reach the liver where they are reconjugated mostly to either glycine or taurine. Some lithocholic acid, the most toxic substance produced in the body and a known carcinogen, enters the liver where it is sulfated or esterified to glucuronic acid and excreted.

1.5 Micelles

BAs in aqueous solution spontaneously aggregate to form micelles, these also mix with lipid products during digestion to form mixed micelles and enhance absorption. Their general shape is cylindrical, and can become worm-like, depending on the lipid-to-bile ratio. A micelle is pictured in Figure 1.2. Hjelm *et al.*[11] describe micelles as; "having the polar lipids arranged radially, with their hydrophilic heads facing outwards into the aqueous phase. The BA molecules are

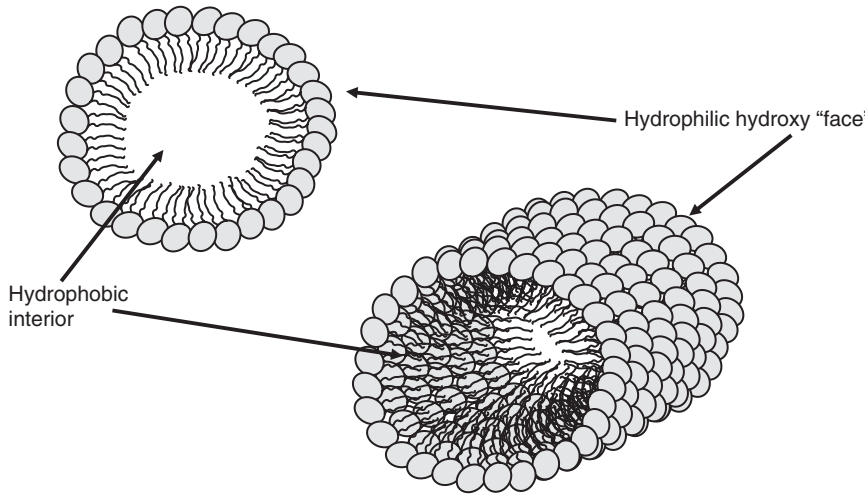

Figure 1.2 Structure of a mixed bile-acid/fatty-acid micelle, whereby the hydrophilic (OH groups of BA) are radially arranged on the outside of the micelle and the hydrophobic moieties are arranged on the interior. As well as a classic micelle, a cylindrical mixed micelle structure is also shown.

arranged perpendicularly between the polar heads. The hydrophobic faces of the BA molecules rest like a wedge between the heads of the alkyl chains of the lipid molecules; the hydrophilic face of the BA molecule faces the aqueous environment." This structure is the same for all micelles and they all have a negative surface charge. Micelles also serve to transport lipids and vitamins in the GI tract.

Micelles tend to aggregate, and there are many ways to measure their concentration, including surface tension measurements.[12] The midpoint of the concentration range over which micellar aggregation occurs is called the critical micellar concentration (CMC). Below the CMC, added bile-salt molecules dissolve in the form of monomers; above the CMC, added bile-salt molecules form micelles, leaving the monomeric concentration essentially constant. The pH at which CMC formation occurs is called the critical micellar pH, (CMpH). Table 1.1 lists values for some of the bile acids mentioned in this review.

Another term frequently used in this discussion is the pKa. Its relationship to pH is described in the Henderson–Hasselbalch equation. Some modifications to this equation have been made[12] to allow the calculation of many other physical/chemical values, including the CMpH (Table 1.1).

1.6 Biochemical Properties of Bile Acids and Their Effects on the GI Tract

Table 1.1 lists some of the characteristics of the more common bile acids, which are divided into 3 main classes: free bile acids, glycine and taurine conjugates.

1.6.1 Free Bile Acids

These include cholic and chenodeoxycholic (primary), and deoxycholic and lithocholic acids (secondary). Their pKa values range from 5.2–6.2 (Table 1.1) and they account for $\sim$2% of bile. They are present in the enterohepatic circulation and are precipitated at low pH. Free BAs (like DCA) are formed in the lower GI tract and are usually absent from the upper GI tract, but in patients with less acidic stomach environments (through acid-suppression medication or through loss of acid-secreting glands), the stomach pH can reach almost neutral pH values, allowing gastric bacteria to proliferate, and they can deconjugate any conjugated BA reaching the stomach from the duodenum.[16] Hence, free bile acids can be present in both the upper and lower GI tract, but the efficient liver-based conjugation process is constantly converting them to their conjugated counterparts.

1.6.2 Glycine-Conjugated BAs

With pKas of 3.8 to 4.8, these are the most abundant conjugated bile acids, (representing $>$70% of bile). It should be noted that conjugation restricts their entry into the epithelial cells, ensuring that they remain within the intestinal lumen and do not leak into the intra-cellular spaces to damage other organs. However, at pH values approaching their pKa values, they become un-ionised and can cross intestinal membranes to a certain extent.

1.6.3 Taurine Conjugated dBAs

Representing $>$20% of bile, are strong sulphonic acids with detergent properties. They are soluble in the normal acidic stomach, with pKa values of $<$2.[12] Thus they can partially enter the gastric epithelium. It is also known that epithelial diffusion barriers can be broken by BAs,[17] which further allow them entry into the epithelium.

1.7 The Effect of pH on Bile-Acid Solubility

Free and glycine-conjugated BAs are only slightly soluble in acid solutions. As the pH is increased, the solubility will increase.[12] This is a very important characteristic since it describes the solubility characteristics of the major BAs, and, it also explains their potential to enter the epithelium at physiological pH ranges.

Occasionally, free BAs and glycine-conjugated BAs are found in the stomachs of normal volunteers.[16] Normal stomach acidity will precipitate them, whereupon they will leave the stomach along with the rest of the partially digested food. If not precipitated by stomach acidity, these BAs can also enter the oesophagus of patients who suffer from the reflux disease GORD (gastro-oesophageal reflux disease). Approximately 87% of these GORD patients were

found to have BAs in their oesophagus,[18] mostly glycine conjugates.[19] Acid-suppressant therapy, the only medication used by these patients, will keep the pH of the refluxate at pH > 5 for over 20 h[20] and this may exacerbate GORD by preventing bile-acid precipitation in the stomach. The role of BAs in oesophageal cancer is described in some detail in Chapter 6.

From the above, it can be seen that different types of bile acids (free bile acids, taurine and glycine conjugates) will have access to the epithelial cells along the GI tract at normal physiological pH. For example, in the acidic stomach taurine conjugates would be soluble and potentially membrane permeable. In the more neutral pH small and large bowel, the free bile acids and the glycine conjugates would be soluble and permeable. Therefore, these different bile acids have the potential to start carcinogenesis across the whole GI tract, the bile type responsible being determined in each case by their solubility characteristic, their conjugation status and by their bioreactivity.

1.8 Potential Therapies for the Deleterious Effects of Bile Acids

Makeshima and his associates[21] postulated that LCA, a known colon carcinogen, is structurally similar to vitamin D, and like Vitamin D, it can activate the Vitamin D receptor, VDR. This would activate the gene CYP3A to make Cytochrome 450 enzymes to detoxify the LCA. Thus, adequate amounts of Vitamin D in the diet would protect against LCA-induced cancer, with the caveat that too much Vitamin D would have a potentially toxic hyper-calcemic effect. Experiments to find a drug to detoxify LCA, without at the same time affecting the calcium response are underway. This highlights the need for physiological balance. Bile acids play a fundamental role in normal human metabolism, excess BAs can be harmful, but so can the reduction of BA concentration, as is evidenced by the range of malabsorption diseases induced by lack of bile-acid absorption in the ileum.

The effect of antibiotic treatments on BA levels and the downstream effects of BAs are unknown. As bacterial de-conjugation and dehydroxylation is central to establishing and maintaining the normal bile pool, their decimation after antibiotic treatment can cause severe disruption. On the one hand, the reduction in free bile-acid production in the germ-free colon of patients on antibiotics could reduce the risks of carcinogenesis of the GI tract, through the action of free bile acids like DCA. It has certainly been shown in animal models that antibiotics increase the levels of conjugated bile acids in the lower GI tract.[22] However, the GI tract is not used to dealing with conjugated bile acids either and an increase in the level of conjugated bile in the lower GI tract could promote carcinogenesis at this site and indeed increased levels of conjugated BAs in the serum may drive carcinogenesis at other sites.[23]

Excessive amounts of BAs can accumulate in the GI tract (*e.g.* as a result of gallbladder surgery or cholecystectomy). These can be treated by the use of

polymeric compounds which serve as ion exchangers, exchanging anions (such as chloride) for BAs. These compounds are known as BA sequestrants, and they absorb BAs from the enterohepatic circulation, whereupon they can be excreted with the faeces. Problems associated with these medications include diarrhoea, flatulence, cramps, *etc*. Again highlighting the balance needed in maintaining physiological levels of BAs. Further dietary modulation of BA levels is being investigated. Fibre content of diets may potentially reduce overall BA levels by binding to and promoting the excretion of BA. However, there is controversy in this area and further research is needed. The role of probiotics in altering the bile-acid pool and specifically the DCA to CA ratio is another area of research that may yield interesting results in the near future.

Statins are compounds that inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, and they are the world's best-selling drugs and are used for lowering cholesterol. Statins are well studied and are believed to be quite safe. Because they reduce the levels of cholesterol, the precursor of the bile acids, statins may be the ideal drugs to use for BA-lowering in these GI tract diseases.

1.9 Summary

Bile helps in the digestion and absorption of fats. Its constituent bile acids (BAs) have detergent properties, and some can be carcinogenic. BAs can act as signalling molecules, entering the nuclei and reacting with the nuclear receptors and this could enhance or reduce BA synthesis. In this way, they control their own levels as well as those of their precursor, cholesterol. This controls cholesterol homeostasis and BA and lipid synthesis.

Taurine-conjugated BAs ($>20\%$ of bile), are strong sulfonic acids, and are completely soluble in the normal stomach, while glycine-conjugated BAs ($>70\%$ of bile), are only slightly soluble at acid pH. As the pH increases, glycine-conjugated BA solubility increases, as does that of the free bile acids. Thus, when in high-pH solutions, these BAs are able to enter the epithelial cells of the GI tract and promote carcinogenesis.

Experimental evidence has highlighted a role of BAs in the induction and proliferation of Barrett's oesophagus, the induction of gastric epithelial damage, potentially inducing colorectal carcinogenesis and in numerous diseases of the gallbladder and liver. Keeping bile acids at physiological levels and preventing their build-up would overcome many of these problems. However, radical efforts to remove BAs, or completely alter the natural balance of subtypes present, may have serious side effects.

References

1. J. Chiang, Bile acid regulation of gene expression, Roles of nuclear hormone receptors, *Endocrine Reviews*, 2002, **23**(4), 443–463.
2. D. Moore, S. Shigeaki and D. Wen Xie, The NR1H and NRR1I Receptors: Constitutive Androstane Receptor, Pregnene X Receptor, Farnesoid

X Receptor alpha and beta, Liver X Receptor alpha and beta, and Vitamin D Receptor, International Union of Pharmacology, LXII.
3. M. Fuchs, Bile Regulation of Hepatic Physiology 3, Regulation of Bile-acid synthesis: Past Progress and Future Challenges, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2003, **284**, 551–557.
4. R. N. Redinger, The coming of age of our understanding of the enterohepatic circulation of bile salts, *The Am. J. Surg.*, 2003, **185**, 168–172.
5. A. Shulman and D. Mangelsdorf, Retinoid X Receptor Heterodymers in the Metabolic Syndrome, *N. Engl. J. Med.*, 2005, **353**, 605–615.
6. E. Scotti, F. Gilardo, C. Godio, E. Gers, J. Krneta, N. Mitro, E. De Fabiano, D. Caruso and M. Crestani, Bile acids and their signalling pathways: eclectic regulators of diverse cellular functions, *Cell Mol. Life Sci.*, 2007, **64**, 2477–2491.
7. L.-Z. Mi, S. Devarakionda and J. M. Harp, Structural basis for bile-acid binding and activation of the nuclear receptor FXR, *Mol. Cell*, 2003, **11**, 1093–100.
8. P. Tontonoz and D. J. Mangelsdorf, Liver Receptor Pathway in Cardiovascular Disease, *Mol. Endocrinol.*, 2003, **17**, 985–993.
9. B. A. Janowsky, P. J. Wiley and T. R. Devi, An Oxystyerol-Signalling Pathway, Mediated by the Nuclear Factor LXR-alpha, *Nature*, 1996, **383**, 728–31.
10. A. Hofmann, Bile acids, The Good, the Bad, the Ugly, *News Phys. Sci.*, 1999, **14**(1), 24–29.
11. R. Hjelm, C. D. Schteingart and A. Hofmann, Form and Structure of Self-assembling Particles in Monoolein-bile salt Mixtures, *J. Phys. Chem.*, 1995, **99**, 16395–400.
12. A. Hofmann and K. J. Mysels, Review: Bile Acid Solubility and Precipitation in vitro and in vivo: The Role of Conjugation, pH, and Ca ions, *J. Lipid Res.*, 1992, **33**, 617–626.
13. A. Roda, A. F. Hofmann and K. J. Mycels, The influence of bile salts structure on self-association in aqueous solution, *J. Biol. Chem.*, 1983, **258**(10), 6370–83.
14. P. P. Nair and D. Kritchevski, *The Bile Acids' Chemistry, Physiology and Metabolism: Volume 1, Chemistry*, Plenum Press, NY, 1971, p. 289.
15. A. Hofmann and D. M. Small, Detergent properties of Bile Salts: Correlation with Physiological Functions, *Annu. Rev. Med.*, 1984, **18**, 333–376.
16. J. Theisen, D. Nehra and D. Citron, Suppression of Gastric Acid Secretions in Patients with Gastroesophageal Reflux Diseases Results in Gastric Bacterial Overgrowths and Deconjugation of Bile Acids, *J. Gasterointest. Surg.*, 2000, **4**(1), 50–54.
17. S. Batzri, J. Harmon, E. J. Schweitzer and R. Toles, Bile-acid accumulation in the Gastric Mucosal Cells, *Proc. Soc. Exp. Biol. Med.*, 1991, **197**(4), 393–9.
18. D. Gotley, A. P. Morgan and M. J. Cooper, Bile-acid concentration in the Refluxate of Patients with Reflux Esophagitis, *Br. J. Surg.*, 1988, **75**, 587–590.

19. W. K. Kauer, J. H. Peters, T. R. DeMeester and A. P. Ireland, Composition and Concentration of Bile Acid Reflux into the Esophagus of Patients with Gastroesophageal Reflux Diseases, *Surgery*, 1997, **122**(5), 874–81.
20. Jai Moo Shin and G. Sachs, Restoration of Acid Secretion Following Treatment with Proton Pump Inhibitors, *Gastroenterology*, 2002, **123**, 1588–97.
21. M. Makeshima, T. T. Lu and W. Xie, Vitamin D as an Intestinal Bile-acid sensor, *Science*, 2002, **296**(5571), 1313–6.
22. J. Guban, D. R. Korver, G. E. Allison and G. W. Tannock, Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of Lactobacillus salivarius and reduced bile salt deconjugation in the ileum of broiler chickens, *Poultry Science*, 2006, **85**, 2186–2194.
23. D. Stamp, Antibiotic therapy may induce cancers in the colon and breasts through a mechanism involving bile acids and colonic bacteria, *Medical Hypotheses*, 2004, **63**, 555–556.