AN UPDATE ON SERUM BILE ACIDS
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Bile acids are synthesised by hepatocytes from cholesterol and are the major constituent of bile. The physiological pH within the biliary tree and small intestinal lumen dictates that most bile acids exist as negatively charged bile salts with ionised carboxylic acid side-chains. In this configuration, they act as powerful, water-soluble detergents that play a key role in intestinal digestion and absorption of lipids, cholesterol and fat-soluble vitamins. Within the small intestine, bile acids solubilise ingested lipids and their digestion products and promote their absorption by forming rapidly diffusible mixed micelles. Bile acids also optimise the activity of several digestive enzymes, facilitate absorption of calcium and iron, stimulate motilin release and mucus secretion, promote colonic motility and water and electrolyte absorption, have bacteriostatic effects and can bind intestinal enterotoxins. Independent of their detergent action, bile acids stimulate mucin secretion by gall bladder epithelium. Synthesis and secretion of bile acids represent the major pathway of cholesterol elimination from the body.

Cholic and chenodeoxycholic acids are primary bile acids that are formed directly from cholesterol. They are the major bile acids found in the bile of dogs; cholic acid predominates in the bile of cats. Secondary bile acids are largely formed by intestinal bacterial metabolism of primary bile acids; small quantities are produced by hepatocytes by modification of primary acids. All bile acids have a steroid nucleus that is hydroxylated at the C3 position. Additional hydroxylation may take place at the C7 and C12 positions. Greater hydroxylation renders a bile acid more hydrophilic.

Almost all bile acids are conjugated prior to secretion into bile canaliculi. Conjugation converts them from weak to strong acids. In dogs, conjugation is chiefly with taurine and minimally with glycine or glucuronate. Conjugation in cats is almost exclusively with taurine, even in taurine-depleted states. Conjugation is not essential for secretion but it increases the water solubility of bile acids at an acidic pH, decreases their passive mucosal absorption from the biliary and intestinal lumina, and ensures that intra-luminal concentrations are high to promote bile flow and intestinal fat digestion and absorption. Conjugation also prevents precipitation of bile acids by calcium and other divalent cations.

The rate-limiting step in the enterohepatic circulation of bile acids is active secretion into bile canaliculi. Within canaliculi, bile acids act osmotically to promote bile flow. During transit, the bile acids form mixed micelles with phospholipids solubilised from the luminal face of the canicular membranes; this prevents toxic injury to biliary epithelium. Secreted bile acids are stored and concentrated in the gall bladder. Gall bladder contraction to discharge bile into the duodenum is mediated by the autonomic nervous system and by cholecystokinin release from the duodenal mucosa following ingestion of a meal.

Bile acids are highly conserved. Approximately 90-95% are reabsorbed from the intestines and returned via the portal vein to the liver to be reutilised. Most absorption occurs actively via specific receptors in the distal ileum. Efficient extraction of returning bile acids by hepatocytes (chiefly periportal cells) is assisted by membrane transport molecules. Approximately 75-95% of the bile acids are extracted from the portal blood in its first pass through the liver. Because hepatocellular extraction of portal venous bile acids is less efficient than intestinal absorption, small concentrations of bile acids may be detectable in serum of healthy cats and dogs.
rate of intestinal absorption is the major factor determining the fasted and post-prandial total serum bile acid (TSBA) concentrations in health. A small increment in the concentration is expected post-prandially; in cats and dogs, the peak concentration occurs approximately two hours after ingestion of a meal.

Bile acids remaining in the intestines may be deconjugated and dehydroxylated by anaerobic bacteria. Deconjugated acids are passively absorbed by intestinal enterocytes and returned to the liver for recycling. Dehydroxylation leads to the formation of secondary bile acids (deoxycholic acid from cholic acid, and lithocholic and ursodeoxycholic acids from chenodeoxycholic acid). Secondary bile acids may be passively resorbed and recycled or excreted in the faeces. Hepatocellular recycling of lithocholic acid involves not only reconjugation but also sulphation, thereby increasing its likelihood of being subsequently excreted in faeces.

Faecal loss of bile acids is replenished by hepatic synthesis. Synthesis of both bile acids and cholesterol by hepatocytes is regulated by the volume of bile acids returning to the liver from the intestines. The rate-limiting step in synthesis is that catalysed by cholesterol 7α-hydroxylase. The activity of this enzyme increases in conditions of bile acid wastage (e.g. intestinal malabsorptive syndromes) and in cholestasis; it is also stimulated by thyroxine and glucocorticoids.

In health, there is minor excretion of serum bile acids in urine. In cholestatic liver disease, hepatocytes may sulphate bile acids to increase their water solubility and enhance renal clearance, particularly in cats. Sulphation and glucuronidation of bile acids may also occur to a limited extent in the kidneys. Measurement of the ratio of urine non-sulphated (or total urine sulphated and non-sulphated) bile acids to urine creatinine may in future be used to screen for hepatic dysfunction in small animals. However, preliminary data suggest that the diagnostic sensitivity of such assays in dogs is inferior to that of TSBA assays.

Measurement of the TSBA concentration is routinely used in cats and dogs to assess hepatic function and portal venous perfusion. Most diagnostic laboratories utilise a direct enzymatic method to measure all serum conjugated and unconjugated 3α-hydroxylated bile acids by spectrophotometry or radioimmunoassay. The test protocol involves collection of serum after a 12 hour fast and 2 hours post-prandially. In healthy animals, the TSBA concentration is expected to be low during the fast as almost all bile acids will be stored in the gall bladder. The post-prandial assay is the more sensitive screening test because it involves challenging the enterohepatic bile acid cycle. The disparity in sensitivity between the two assays is particularly pronounced in cats. In most dogs, the fasting assay is often sufficient to determine the presence or absence of functionally significant acquired hepatobiliary disease. Both assays should be performed if portosystemic shunting is suspected as it is not unusual for the latter to be associated with a normal fasting concentration but pronounced elevation of the post-prandial value. The fasted TSBA assay is less sensitive and considerably less specific than a fasted blood ammonia assay in detecting portosystemic shunting. The post-prandial TSBA assay yields similar information to the ammonia tolerance test but is far easier to perform and does not pose a hazard to patients with hepatic encephalopathy.

An increased TSBA concentration may reflect hepatic parenchymal disease, cholestasis or intra- or extra-hepatic portosystemic shunting. With shunting, the increase reflects direct diversion of bile acids resorbed from the intestines into the systemic circulation. In parenchymal disease, the increase may reflect diminished hepatocellular uptake of bile acids from portal circulation.
blood and/or impaired conjugation and secretion into canaliculi. In both parenchymal disease and cholestasis, bile acid transport within hepatocytes may be redirected away from canaliculi and into the space of Disse. High intraluminal pressures within the biliary tree in cholestasis may also promote this regurgitation of bile acids into blood via disrupted intercellular junctions or transcellularly across biliary epithelium. As the TSBA concentration is expected to be elevated in cholestasis, the assay is not advocated in hyperbilirubinaemic patients.

The TSBA assays have several limitations. They do not permit specific diagnoses to be made, although detection of high amplitude post-prandial spiking may strengthen suspicion of shunting and values approaching 100 μmol/L may support a diagnosis of hepatic encephalopathy in animals with consistent clinical signs. Post-prandial assays may not be feasible in anorexic patients. Sample haemolysis and lipaemia may cause inaccurate results in spectrophotometric TSBA assays. Drugs such as rifamycins, fusidic acid, cyclosporine and bumetanide can specifically inhibit hepatocytic uptake of bile acids to boost the serum concentration.

Intestinal hypomotility may reduce the sensitivity of the post-prandial assay. The post-prandial concentration may also be underestimated if the meal consumed has been inadequate to stimulate gastric emptying or gall bladder contraction, if there has been delayed gastric emptying or if there is intestinal hypermotility. The same holds true for animals with ileal disease or ileal resection, even though there may be a compensatory increase in passive absorption of bile acids from the colon. In cholecystectomy patients, enterohepatic cycling of bile acids is increased during fasts so that the fasted TSBA concentration tends to be slightly higher and the post-prandial concentration is slightly lower and peaks earlier than in healthy patients.

The fasted TSBA concentration may occasionally exceed the post-prandial value. This may reflect spontaneous gall bladder contraction during fasts (a common phenomenon in dogs) or individual variation in gastric emptying, intestinal transit time or cholecystokinin responses.

Unexpected increases in the TSBA concentration may be observed in animals with small intestinal bacterial overgrowth (SIBO). Bile acids deconjugated by intestinal bacteria such as clostridia and \textit{Bacteroides} species can be passively absorbed at all intestinal levels and may bind strongly to serum albumin so that the efficiency of their uptake from portal blood by hepatocytes is reduced. A gas chromatography-mass spectrometry assay of serum total unconjugated bile acids (TUBA) has been advocated in the diagnosis of SIBO in dogs. However, the assay reliability has been questioned. In one study, most dogs with SIBO or idiopathic antibiotic-responsive diarrhoea (ARD) did not have an increased serum TUBA concentration and the concentration did not correlate with bacterial counts obtained on quantitative duodenal bacterial culture. There was also no significant difference between the serum TUBA concentrations assayed before and during antibiotic therapy in dogs with ARD. False positives were observed in some healthy dogs and could theoretically occur in alimentary tract diseases other than SIBO (e.g. colitis). False negative results can also be anticipated in some dogs with SIBO as only certain bacterial species produce hydrolases capable of deconjugating bile acids. Measurement of the post-prandial rather than the fasted serum TUBA concentration (or measurement of both) may improve the sensitivity and specificity of the test in the diagnosis of SIBO.

At high intra- or extracellular concentrations, certain bile acids are cytotoxic and contribute to disease progression in chronic cholestatic hepatobiliary disorders. Periportal hepatocytes are
particularly vulnerable to such injury. Direct extracellular toxicity can occur via the detergent action of bile acids on phospholipid membranes, leading to modification of cell surface receptors and membrane-signalling systems and potentially hepatocellular apoptosis or necrosis. Apoptosis can be induced by plasma membrane lipid peroxidation, an increase in the intracellular ionised calcium concentration with activation of cathepsin B and protein kinases, or by direct mitochondrial injury. High intracellular concentrations of bile acids can disrupt mitochondrial electron transport, causing generation of reactive oxygen species that can trigger peroxidation of organelle membranes. Hydrophobic bile acids can also promote cholestasis. This effect may be mediated by inhibition of actin polymerisation, leading to disruption of the pericanalicular cytoskeleton of hepatocytes and dilation of the canaliculi. Lithocholic acid can form insoluble complexes with calcium to plug canaliculi. Certain bile acids are also pro-inflammatory, stimulating mast cell degranulation and activation of neutrophils and Kupffer cells. Some promote expression of major histocompatibility class I and II antigens by hepatocytes and biliary epithelium, increasing the risk of cytotoxic T-lymphocyte attack. Hydrophobic bile acids can also promote oxysterol-induced apoptosis of gall bladder epithelial cells and, in rats and humans, colorectal tumour development.

In general, the cytotoxicity of bile acids is correlated with the degree of molecular hydrophobicity which in turn is influenced by the degree of hydroxylation. Lithocholic acid is more toxic than deoxycholic and chenodeoxycholic acids which are in turn more toxic than cholic acid. Conjugation reduces hydrophobicity, with taurine conjugates being less hepatotoxic than glycine conjugates. Ursodeoxycholic acid (UDCA), the dihydroxy 7β-epimer of chenodeoxycholic acid, is hydrophilic and non-toxic in most mammalian species.

Use of synthetic UDCA has been advocated in cats and dogs with chronic cholestatic disorders but, to date, little has been published to confirm its efficacy or to document its effect on endogenous bile acid metabolism and progression of hepatobiliary lesions in these species. With therapeutic use in humans, UDCA becomes the dominant bile acid in serum and urine, substituting for hydrophobic bile acids in hepatic synthesis and in competition for ileal transport receptors. UDCA appears to protect hepatocyte mitochondrial membranes against hydrophobic bile acid injury, thereby preventing apoptosis. It also protects hepatocytes from oxidative injury by enhancing their synthesis of glutathione and metallothionein. UDCA also reduces cholesterol secretion into bile, has an anti-inflammatory action and may decrease hepatic collagen synthesis. Its immunomodulatory effects include a decrease in antibody production by plasma cells, interleukin synthesis by T-lymphocytes and expression of histocompatibility antigens by hepatobiliary cells. One of its major beneficial actions is to promote the flow of bicarbonate-rich bile. Because of this choleretic effect, use of UDCA is contraindicated in animals with extrahepatic bile duct obstruction.

A pilot study on the effect of daily oral administration of UDCA at 15 mg/kg to healthy dogs over a one week period revealed no significant effect on the fasted and post-prandial TSBA concentrations, provided that UDCA was not administered at the time of feeding. A second study using healthy dogs showed that administration of a comparable dose of UDCA with feed could significantly increase the TSBA concentration above 25 µmol/L at 2, 3 and 4 hours post-prandially. Lengthier studies are needed as data in humans and cats indicate that increases in the fasted and post-prandial TSBA concentrations should occur with long term UDCA therapy.
REFERENCES


