Bile Acids and the Sterolbiome

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Disclosures

- Disclosure of Financial Relationships
  - None

- Off-Label Usage
  - None

from Ridlon JM, 2015

- **The human body is a complex ecosystem that on a cellular and gene level is predominantly bacterial.**

- **The liver synthesizes and secretes water soluble primary bile acids, which are converted by intestinal flora into numerous fat soluble compounds**

- **These bile acids are absorbed into the blood, returned to the liver, and accumulate in the biliary pool.**

from Ridlon JM, 2015

- **The various surfaces of body are an interconnected network of ecosystems made up of human cells, bacteria and archaea.**
  - **Skin**
  - **Oral**
  - **Gastrointestinal**
  - **Respiratory**
  - **Reproductive tract**

from Ridlon JM, 2015

“The gut microbial community through their capacity to produce bile acid metabolites distinct from the liver can be thought of as an *endocrine organ* with potential to alter host physiology, perhaps to their own favor. “

Bile basics

Primary and secondary bile acids

- **The two primary bile acids are synthesized from cholesterol in the liver:**
  - **Cholic acid**
  - **Chenodeoxycholic acid**

  About 16 enzymes are needed to convert cholesterol to bile acids.

- **The two secondary bile acids are made when intestinal flora act on the primary bile acids:**
  - **Deoxycholic acid**
  - **Lithocholic acid**

Bile acids and bilirubin are also conjugated

- **Liver cells also attach bile salts to glycine, taurine, sulfate or glucuronide (conjugation).**
- **Flora in the ileum and colon may undo this process. (deconjugation).**
- **Bilirubin pigments are also conjugated with glucuronic acid. If this does not happen as well as it should due to a genetic enzyme deficiency, Gilbert's syndrome occurs.**

Ridlon JM, 2015

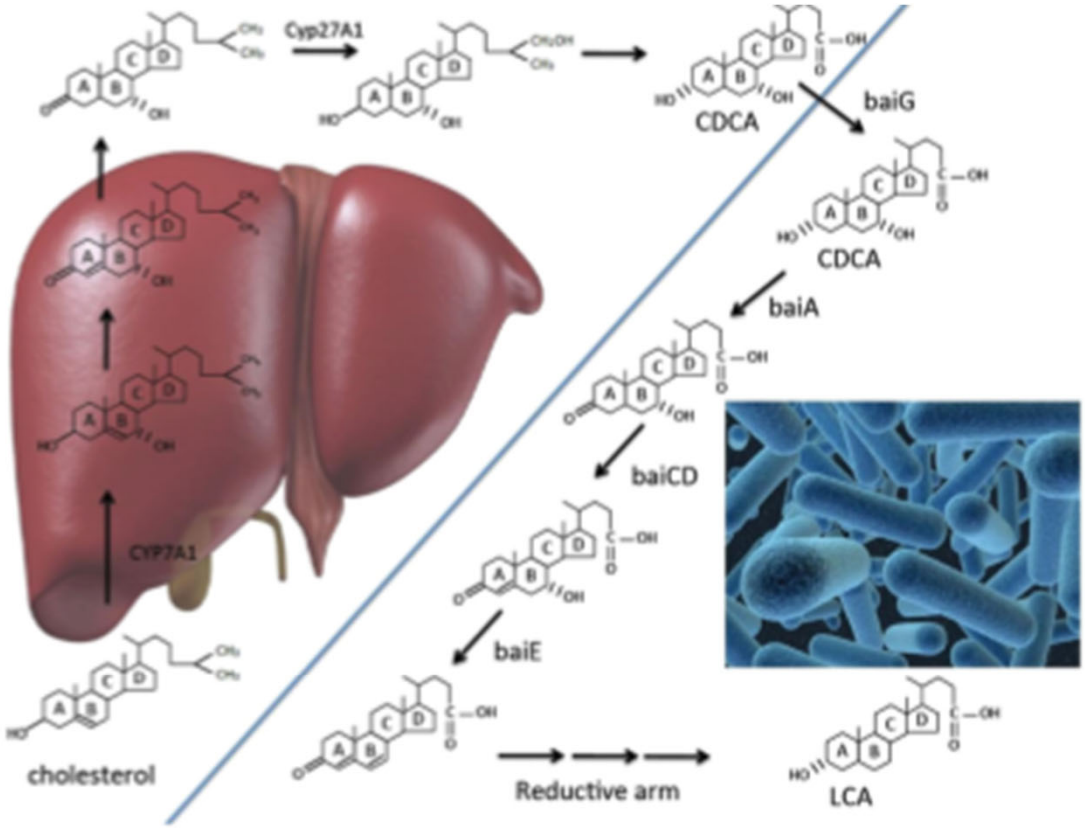
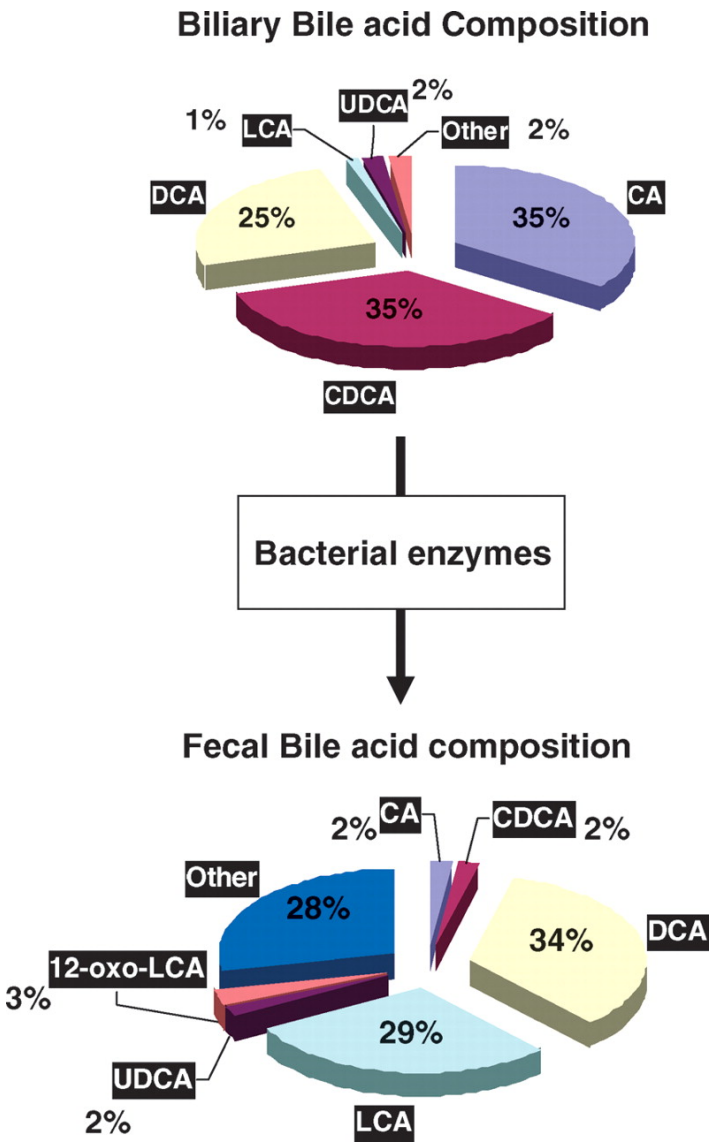


Figure 1 Pathway from cholesterol to lithocholic acid in the human ecosystem. CDCA is synthesized in the liver *via* the neutral pathway through a series of oxidative steps. LCA is produced by members of the gut microbiome through a multi-step biochemical pathway, the first half of which is oxidative followed by a net 2 electron reduction.

Composition of bile acids in the gallbladder and feces of healthy individuals. “Other” bile acids refer to oxo- and 3β-hydroxy derivatives of secondary bile acids.



Jason M. Ridlon et al. J. Lipid Res. 2006;47:241-259

Some bile salts are laxatives (Hofmann AF, 2008)

- **The primary bile acid chenodeoxycholic acid (CDCA) and the secondary bile acid deoxycholic acid (DCA) function as laxatives. They do this by increasing water secretion from the blood into the bowel.**
- **With increasing levels, these bile acids cause diarrhea. It is hypothesized that if these bile acids are deficient, functional constipation may occur.**

Cholesterol and bile

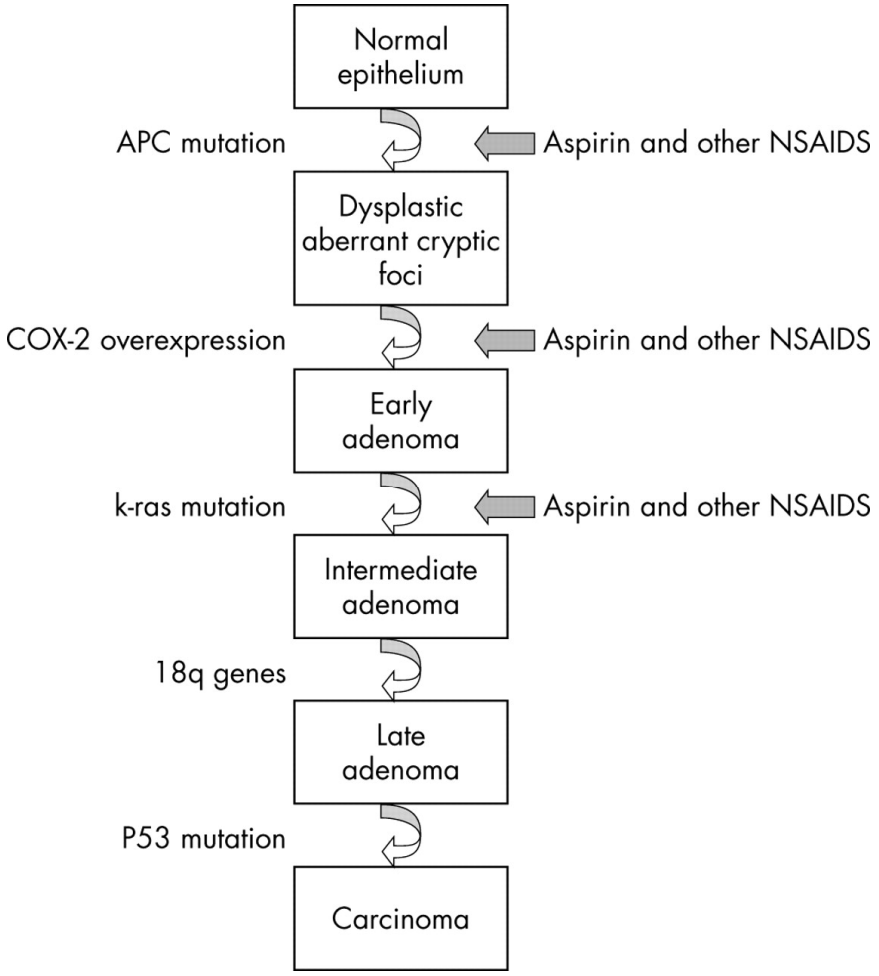
- **The major mechanism for removal of cholesterol from the blood is excretion into the bile. About 5% of the bile gets into the large intestine and is eliminated in the stool.**

Other bile facts

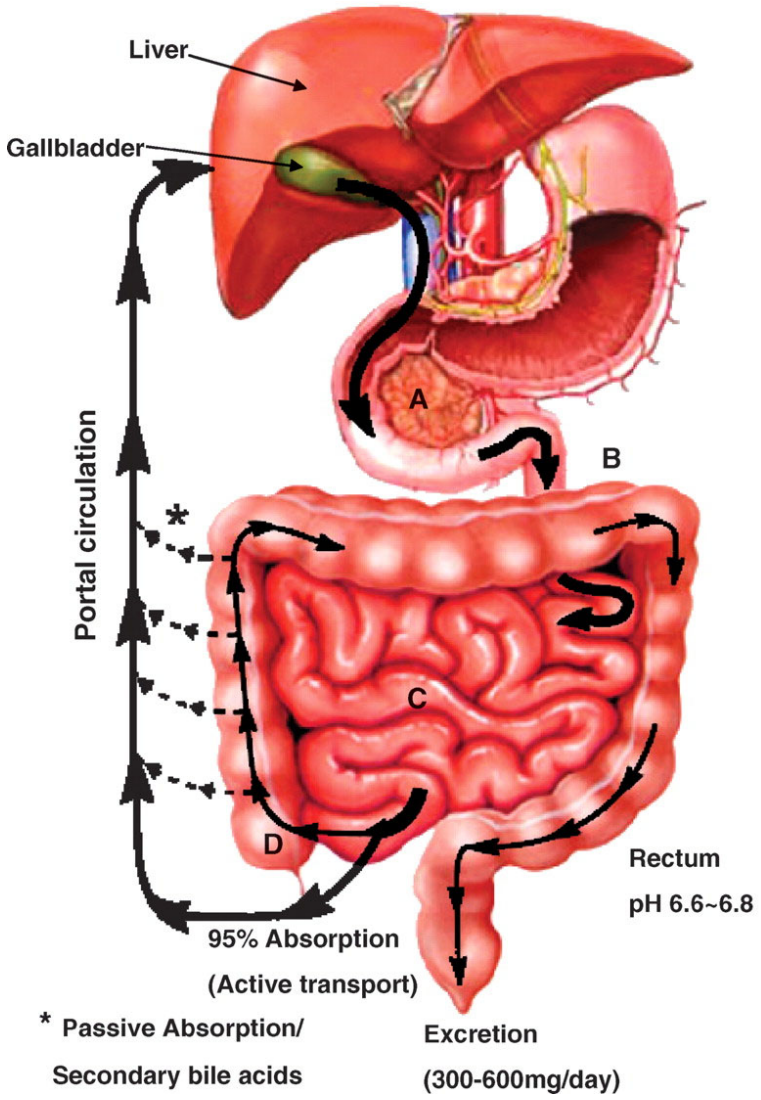
- **Primary bile acids form micelles with lecithin and cholesterol to mix fat and water, allowing absorption of fats and fat soluble vitamins.**
- **Secondary bile acid metabolites influence nuclear receptors**
- **Secondary bile acids may be carcinogens**
- **Bile acids can be therapeutic agents**
- **The biliary system is a route of detoxification**

The secondary bile acids (DCA and LCA) are carcinogens

Deoxycholic acid (DCA) stimulates the formation of the enzyme COX-2 which increases the risk of colonic polyps and colorectal cancer.



Anatomy, physiology, and microbiology of the gastrointestinal tract.



Small Intestine

A: Duodenum (25cm)
pH 5.7~6.4
~10^3 bacteria/ml
Lactobacillus
Streptococcus

B : Jejunum (1.0m)
pH 5.9~6.8
~10^4 bacteria/ml
Lactobacillus
Streptococcus Staphylococcus
Veillonella

C : Ileum (2.0m)
pH 7.3~7.7
10^6~10^8 bacteria/ml
Enterobacteria
Enterococcus
Bacteroides
Clostridium
Lactobacillus
Veillonella

Large Intestine

D. Cecum/Colon (150cm)
pH 5.7-6.8
~10^{11} bacteria/g
Bacteroides, Eubacterium, Bifidobacterium, Ruminococcus, Peptostreptococcus, Propionibacterium, Clostridium, Lactobacillus, Escherichia, Streptococcus, Methanobrevibacter

Jason M. Ridlon et al. J. Lipid Res. 2006;47:241-259

Hofmann AF, Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. Cell Mol Life Sci. 2008 Aug;65(16):2461-83.

During overnight fasting, about half of the secreted bile acids pass into the gallbladder; the remainder enter the small intestine.

Those bile acids entering the small intestine are absorbed into the blood and return to the liver where they are once again secreted into bile.

With time, most of the circulating bile acids become stored in the gallbladder.

When a meal is ingested, gradual gallbladder emptying occurs, delivering bile acids to the small intestinal lumen.

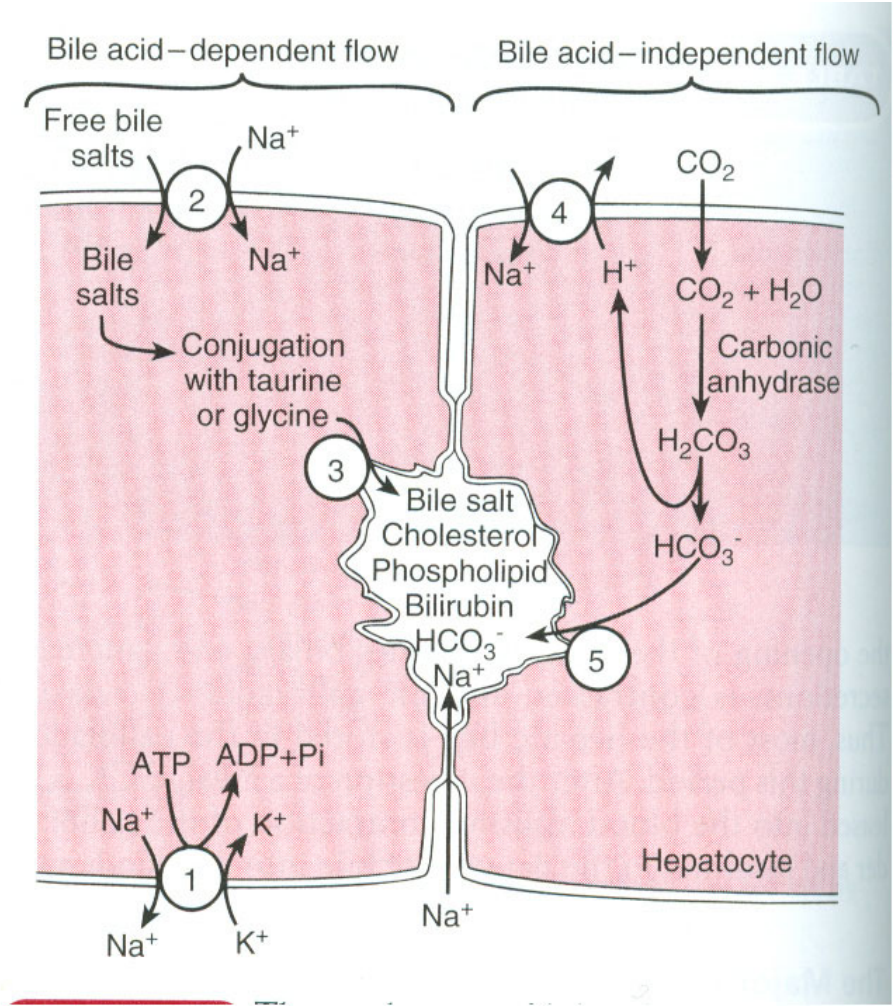
These are efficiently absorbed, returned to the liver, and the process starts over.

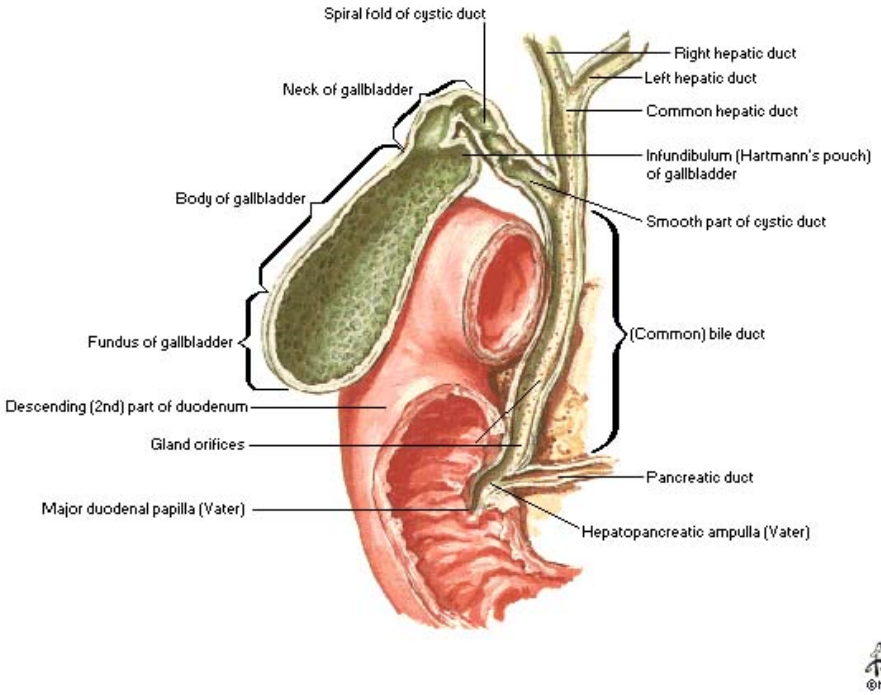
Constituents of bile

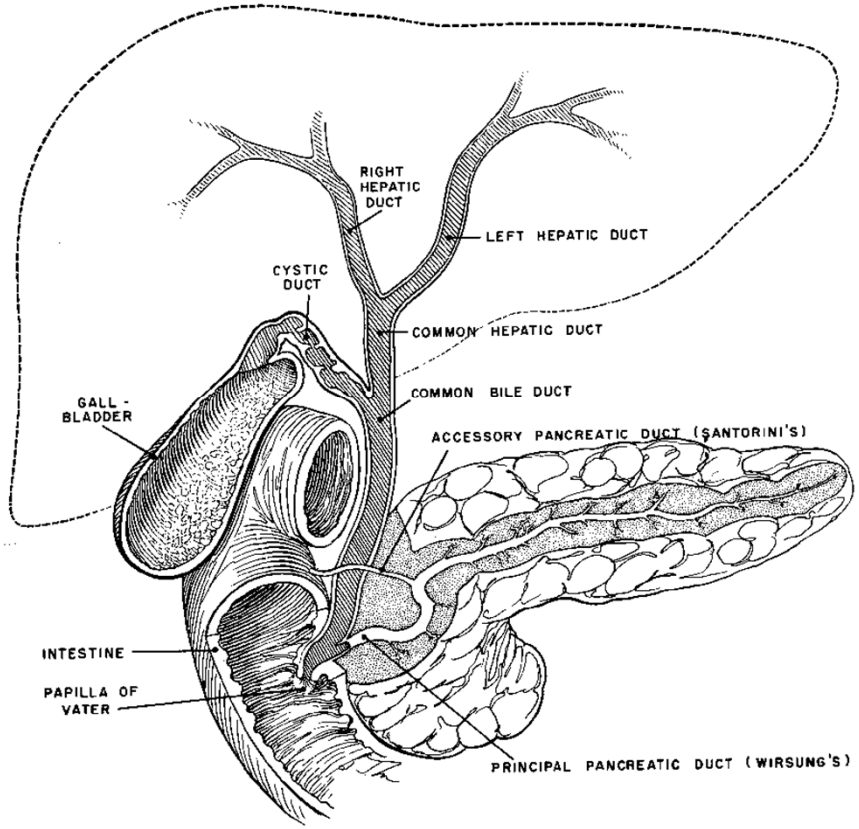
- **Bile acids**
- **Phospholipids (lecithin)**
- **Cholesterol**
- **Bicarbonate is secreted from bile ducts and liver cells to neutralize acids in the small intestine.**
- **Bile pigments (bilirubin)**

- **Sodium**

- **Bile also contains melatonin. Its function is as an antioxidant to protect the GI mucosa against the damaging effect of bile acids.**







BILIARY DUCTS

- **Right & Left Hepatic Ducts** — bile outflow from liver
- **Common Hepatic Duct** — junction of R and L hepatic ducts
- **Cystic Duct** — outflow from gall bladder
- **Common Bile Duct** — outflow of bile from gall bladder and liver
- **Hepatopancreatic Ampulla** — junction of bile and pancreatic ducts
- **Main Pancreatic Duct** — outflow from pancreas
- **Major Duodenal Papilla** — bile and pancreatic secretion into duodenum

GALL BLADDER body, neck and fundus

Cholecystokinin (CCK) promotes

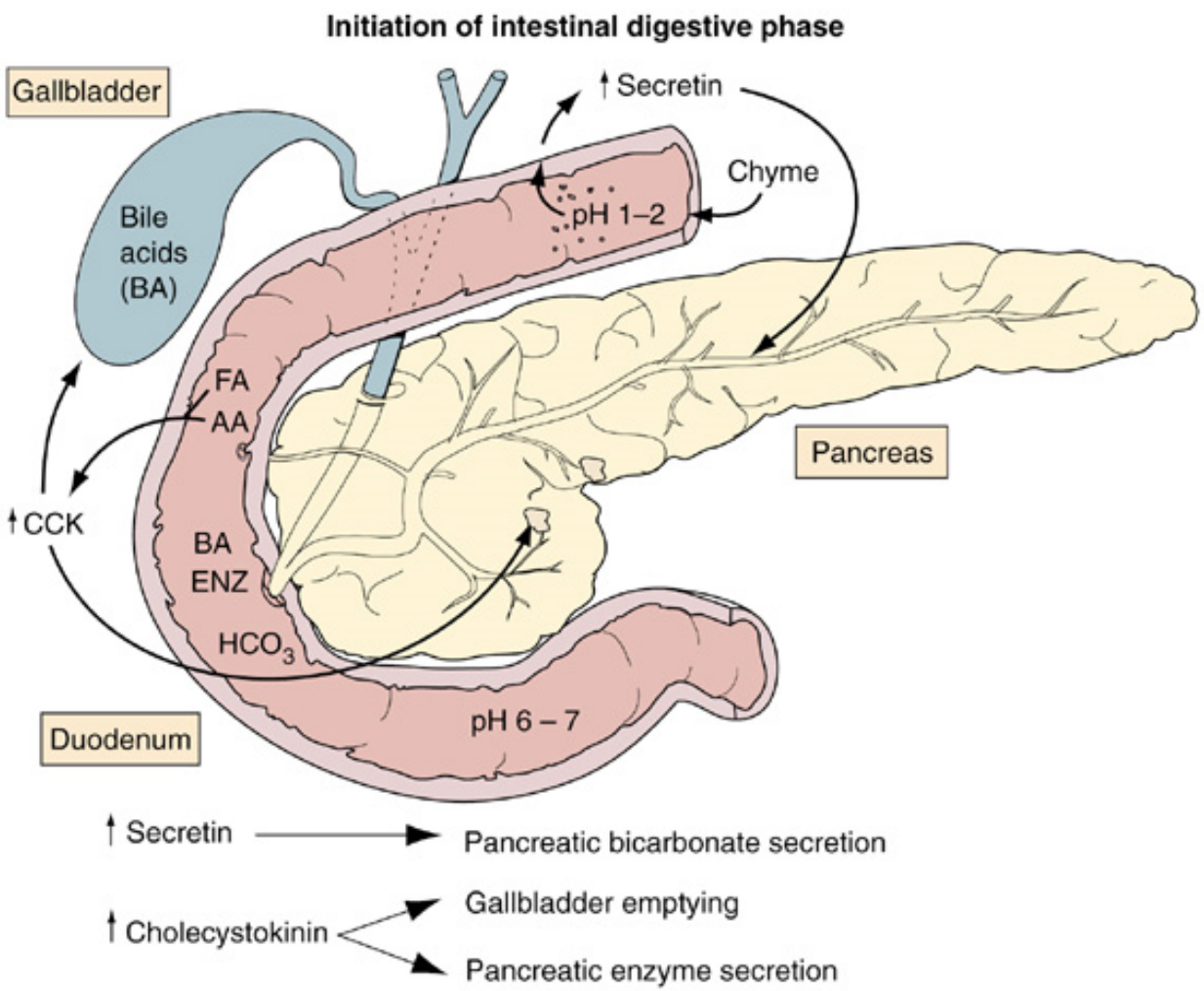
- **Gall bladder emptying**
- **Sphincter of Oddi relaxation**
- **Pancreatic secretion**
- **Gastric slowing**
- **Intestinal motility**

Secretin promotes pancreatic exocrine enzyme secretion from:

- **acini**

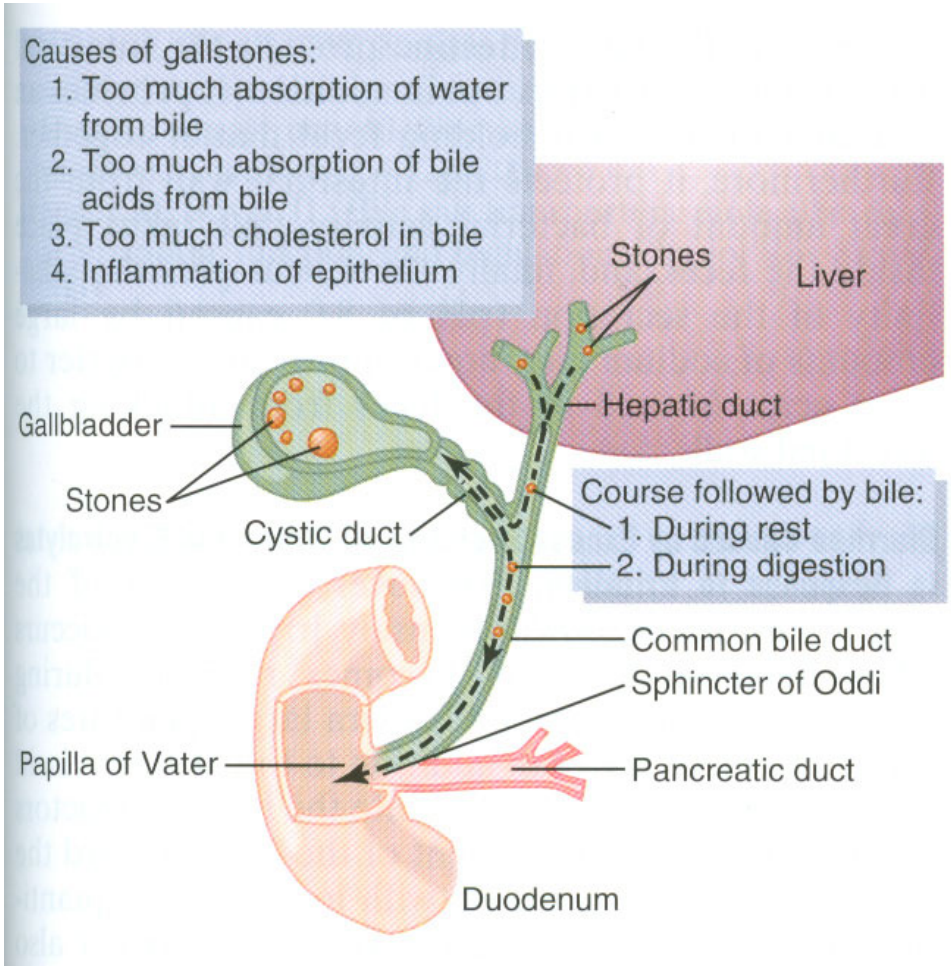
and bicarbonate secretion from:

- **Bile ducts**
- **Pancreatic ducts**
- **Brunner's glands**

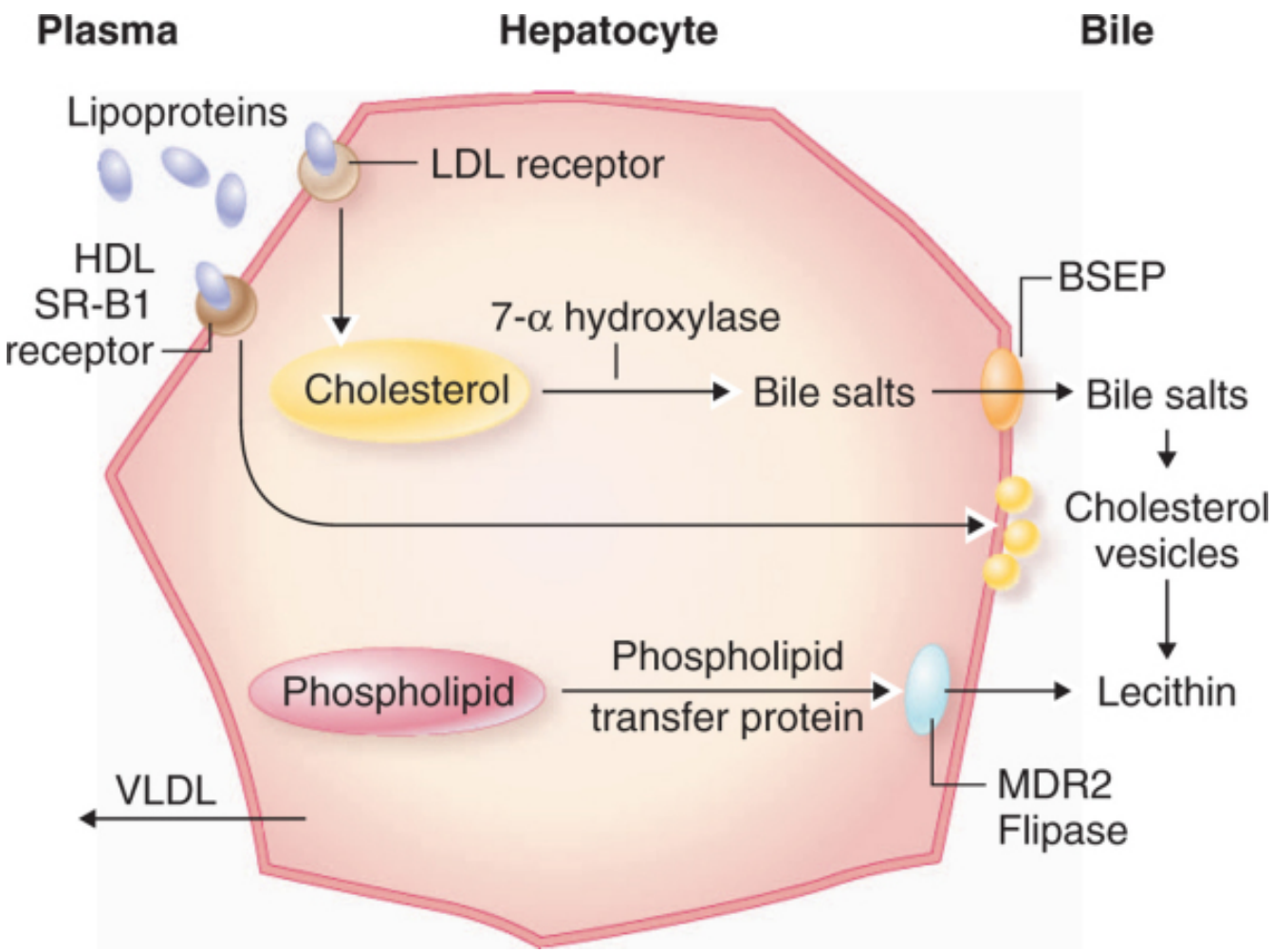


Gall stones are precipitates of cholesterol due to any of the following:

- Excess absorption of water
  - Over-concentrates bile
- Excess absorption of bile salts & lecithin
  - Cholesterol % of bile is too high
- **Reduction in terminal ileum reabsorption of bile salts**
- Excess cholesterol
  - Is a type of hyperlipidemia
- Inflammation of epithelium
  - Increases absorption of water, bile acids, etc
- Stasis of the gall bladder smooth muscle layer
  - Increases crystalization of cholesterol



Hepatocyte production of bile constituents



http://www.uptomed.ir/Digimed.ir/cecil/Cecil/HTML/605.htm

Obesity and rapid weight loss > the risk of gallstones

A study of over 90,000 morbidly obese patients reported a sevenfold risk of gallstone formation compared with normal weight population. (Stampfer MJ, 1992)

The risk is higher during a period of rapid acute weight loss (Worobetz LJ,, 1993)

Sludge, gravel and stones

- Between 10-38% of patients undergoing bariatric surgery develop gallstones (Magouliotis DE, 2017)

- UDCA administration in these patients significantly reduces the risk of stones (Magouliotis DE, 2017 – A metanalysis of 8 studies)

Dosages used:

- 500–600 mg UDCA
- 1000–1200 mg UDCA

Gallsludge

- **Biliary sludge** (microlithiasis) is a reversible suspension of precipitated particulate matter in bile in a viscous mucous liquid phase. The most common precipitates are cholesterol monohydrate crystals and various calcium-based crystals, granules, and salts. (Medscape)

Gallsludge

- The classic patient for oral bile-acid therapy is a mildly symptomatic individual with small radiolucent stones, who cannot or does not want to undergo surgery.

- Patients with biliary sludge or microlithiasis are believed to be gallstone patients at an early stage of their disease.

- Indeed, patients with biliary sludge-associated pancreatitis have been shown to benefit from UDCA treatment.(Testoni PA, 2000)

Hormones and gallbladder physiology

- Estrogen increases cholesterol and its saturation in bile and promotes gallbladder hypomotility (Mills JC, 2010)

- Diminished gallbladder motility is commonly seen during pregnancy (Cuevas A, 2004)

Hormones and gallbladder physiology

- Progesterone administered as subcutaneous implants alters bile flow from the liver to the gallbladder, decreasing filling.
- Progesterone also significantly impairs gallbladder emptying in response to cholecystokinin.
- These effects may contribute to the greater prevalence of gallstones and biliary motility disorders among women.

(Tierney S, 1999)

Hormones and gallbladder physiology

- Leptin, produced in adipocytes, enhances biliary lipid secretion and adiponectin could decrease it. (Ogiyama H, 2010)

- Increasing homocysteine may also increase lithogenic risk (Tazuma S, 2005)

Mucin and gallsludge

- Mucin glycoprotein is secreted by biliary epithelial cells and is a pronucleating protein.
- Decreased degradation of mucin by lysosomal enzymes may promote the formation of cholesterol crystals.
- For this reason NAC has been studied for gallsludge treatment.

Gallsludge treatments

- Choleretics
  - Chelidonium majus
  - Chionanthus virginicus
  - Cynara scolymus
  - Mentha piperitia
- Mucolytics
  - N-acetylcysteine
- Emulsifiers
  - phoshatidylcholine

Gallstone treatments

- Ox bile, chenodeoxycholic acid or Ursodiol

- Rowachol (a combination of menthol and other terpenes) was successful in completely resolving common bile duct stone dissolution as a single therapy in 42% of patients (treatment for up to 48 months). Menthol is choleretic, antibacterial and spasmolytic

(Sommerville KW, 1985)

- Combination therapy with Rowachol plus bile salts (chenodeoxycholic acid 250-750 mg/day) led to complete resolution of CBD stones in 73% of pts.

Rowachol ingredients

Supplement Facts

Serving Size: 1 capsule

Servings: 100

Amount per Serving	% Daily Value
Proprietary blend 67mg	*
Menthol	
Menthone	
Pinene	
Borneol	
Cineol	
Camphene	

* Daily Value not established

Other ingredients: Olive oil, gelatin, glycerin

Suggested use: Adults take 1 capsule 3-4 times a day.

Keep out of the reach of children

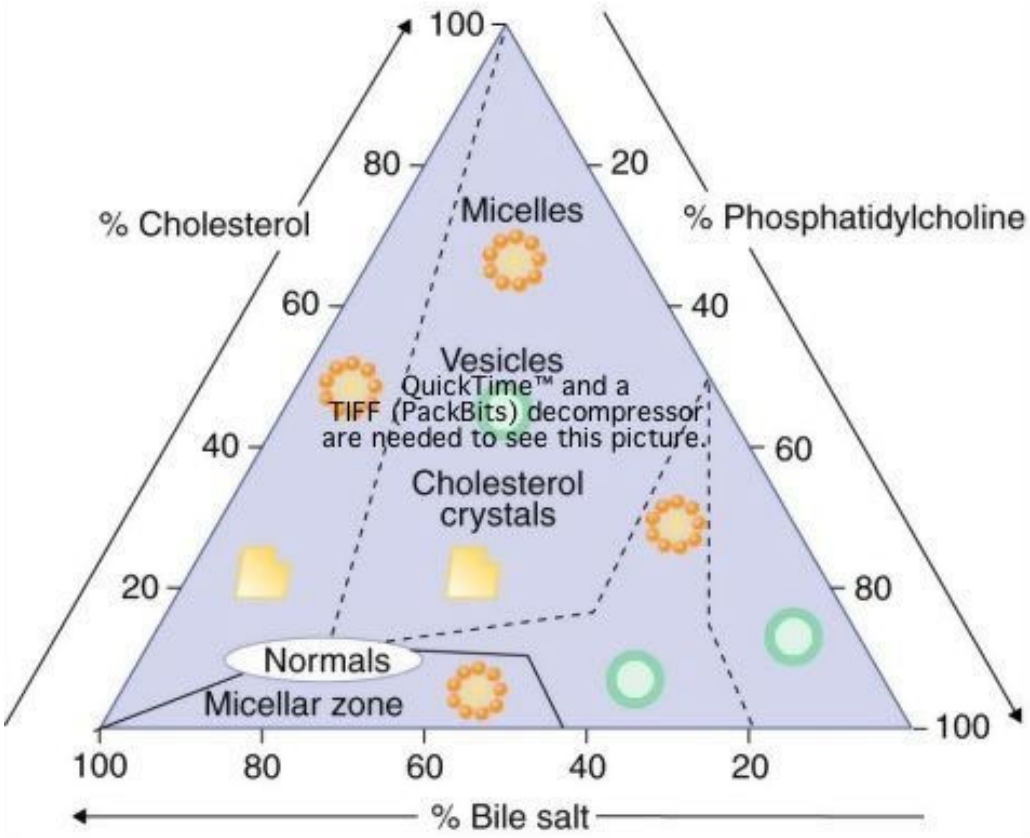
Dietary factors and the gallbladder

- The following dietary factors can lower triglycerides, lessen bile cholesterol saturation, and increase HDL.

(Marschall HU, 2007 and Cuevas A, 2004)

  - polyunsaturated fat
  - monounsaturated fat
  - fiber
  - caffeine.[15]
  - fish oil
  - moderate alcohol consumption

Triad of biliary lithogenicity



Bile acids, antibiotics and *Clostridium difficile.*

- **The secondary bile acids deoxycholic (DCA) and lithocholic acid (LCA) are produced by certain species of Clostridium bacteria (e.g. Clostridium scindens)**
- **In the colon, primary bile acids regulate growth of *Clostridium difficile.***

- **Secondary bile acids suppress growth of *C. diff.***

- **Antibiotics inhibit production of secondary bile acids by killing bacteria in the gut**

Happy balance of Clostridia

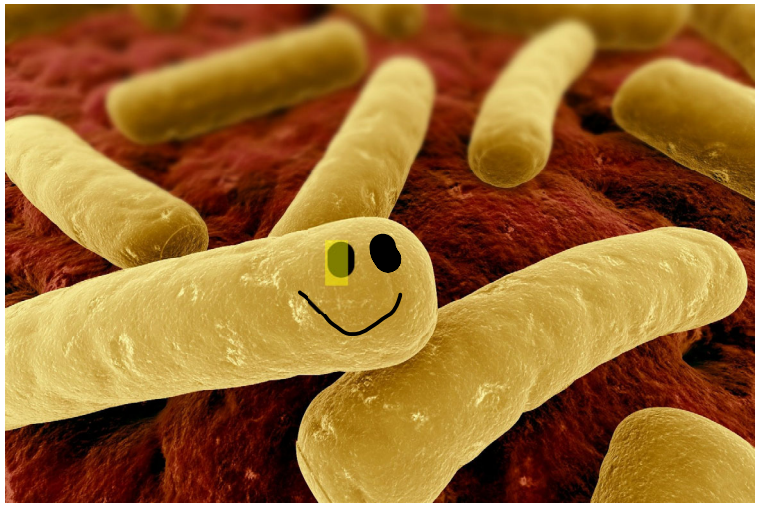
Clostridia species

↙

Secondary bile acids

↙

Suppression of C. diff growth



How antibiotics lead to Clostridium difficile enterocolitis

Antibiotics

↓

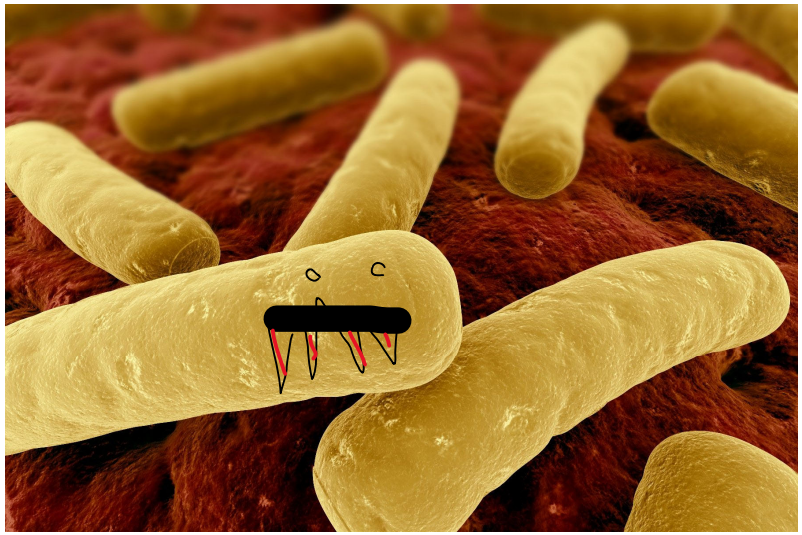
Reduction of Clostridia species

↓

Reduction of secondary bile acids

↓

Enhanced C. diff growth



Bile acids…
Are they hormones?

from Ridlon JM, 2015

- **Bile acids are hormones that regulate**
  - **their own synthesis and transport**
  - **glucose and lipid homeostasis**
  - **energy balance**
  - **inflammation**
  - **microbial growth**

Sterolbiome

- **The gut microbiome (sterolbiome) produces endocrine molecules from cholesterol-based molecules in the gut**

- **Bile acids are also known to fundamentally shape the gut microbiome and vice versa.**

Bile acids (BA) activate nuclear receptors

- **Farnesoid X receptor (FXR)**
- **Pregnane X receptor (PXR)**
- **G-protein coupled receptors (GPCR)**
- **Vitamin D receptor (VDR)**

FXR (farnesoid X receptor)

"FXR is the bridge between the liver and the small intestine to control BA levels and regulate BA synthesis." (Matsubara T, 2012)

By doing so it regulates:

- **Glucose**
- **Lipoproteins**
- **Lipid metabolism**
- **Inflammation**
- **Tumor suppression**
- **Drug metabolism**
- **Hepatic regeneration**
- **Fibrosis**
- **Cell differentiation**
- **Tumor formation**

Mechanism by which BA promote antimicrobial proteins

- **In the human liver, bile epithelial cells show intense immune responses to cathelicidin and for the vitamin D receptor.**
- **In cultured bile epithelial cells, chenodeoxycholic acid *(CDCA) and ursodeoxycholic acid (UDCA) stimulate cathelicidin production through 2 different nuclear receptors: the farnesoid X receptor and the vitamin D receptor, respectively.***
- **Vitamin D further increases the production of cathelicidin by both bile salts.**

(D'Aldebert, 2009)

PXR (pregnane X receptor)

- **PXR turns on phase 2 detoxification**
- **Therefore it regulates processing of xenobiotic (environmental) and endogenous (internally produced) compounds**
- **It is especially important in the detoxification of lithocholic acid (LCA)**
  - **LCA can induce mutations (it is a carcinogen)**

The poorly absorbed antibiotic, rifaximin, also activates the PXR gene

- **Rifaximin causes PXR-mediated inhibition of angiogenic factors in colorectal cells and may be a promising anticancer tool. Treatment with rifaximin also caused a significant reduction of cell growth and spread, blood vessel growth, and enzymes that allow metastasis. (Esposito G, 2016)**

- **This activation of PXR (by rifaximin) can enhance intestinal epithelial repair and may help to normalize intestinal barrier dysfunction observed in patients with inflammatory bowel disease. (Terc J, 2014) and (Wan YC, 2015)**

- **Rifaximin is bile soluble**

GPCR (G-Protein Coupled Receptor)

- **Short chain fatty acids also activate GPCR**

- **GPCR are found in the gallbladder, spleen, certain intestinal cells, some white blood cells, cells lining the bile ducts and in fat cells**

- **Bile acids robustly stimulate GPCR, to increase levels of the following:**
  - **incretin hormones (Kuhre RE, 2018)**
  - **glucose-dependent insulinotropic peptide (GIP)**
  - **glucagon-like peptide-1 (GLP-1)**
  - **glucagon**
  - **insulin**

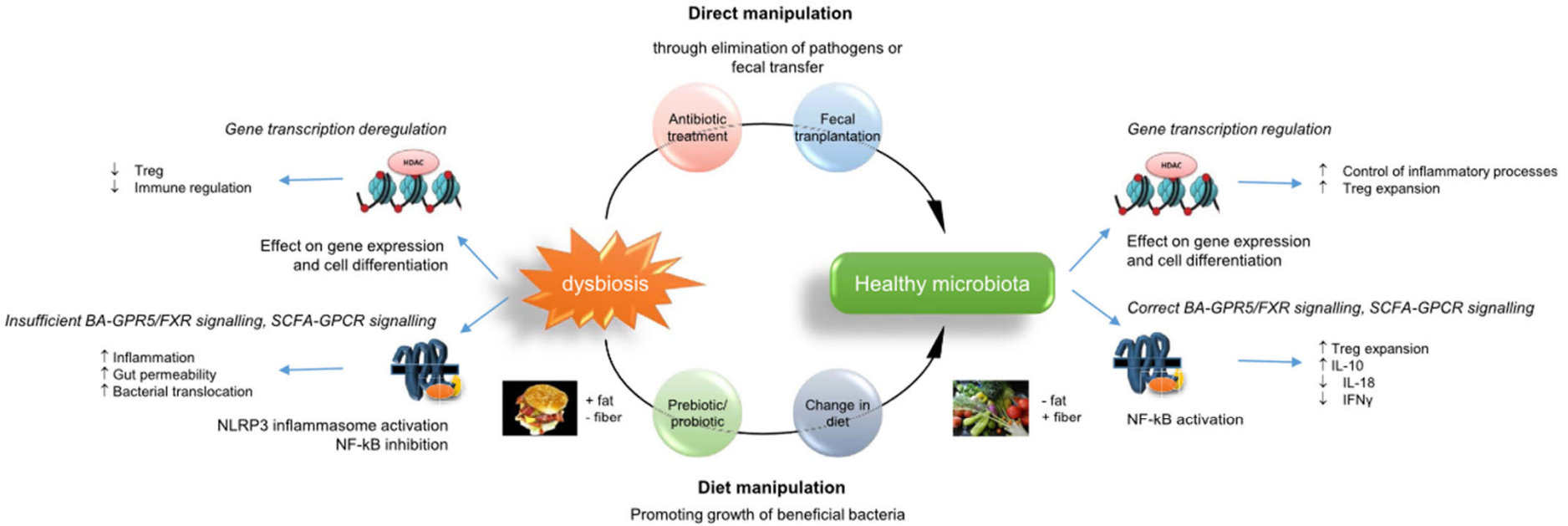


Fig. 2. Proposed intervention for gut dysbiosis recovery and schematic model on how diet could contribute to chronic inflammation. Diet induces changes to gut microbiota, and reduces the production of BAs and SCFAs, leading to impaired signaling activation, epigenetic transcriptional changes and resulting in alteration of gut homeostasis, Treg activity and immune homeostasis. Therapeutic targeting gut dysbiosis can be approached by a direct manipulation of the microbiota through antibiotic administration or faecal transplantation or by promoting growth of beneficial bacteria by dietary administration of prebiotic or probiotic or by consuming high fiber diet.

Clostrium scindens increases blood estrogen levels and converts cortisol to male sex hormones

- **Conjugated estrogen travels from the liver and bile to the small intestine and is on its way out of the body (in stool).**

- **Clostridium scindens can deconjugate the estrogen, allowing the hormone to be absorbed from the gut increasing a more active serum estrogen.**

- **Clostridium scindens can also convert glucocorticoids to male sex hormones. (Ridlon JM, 2013)**

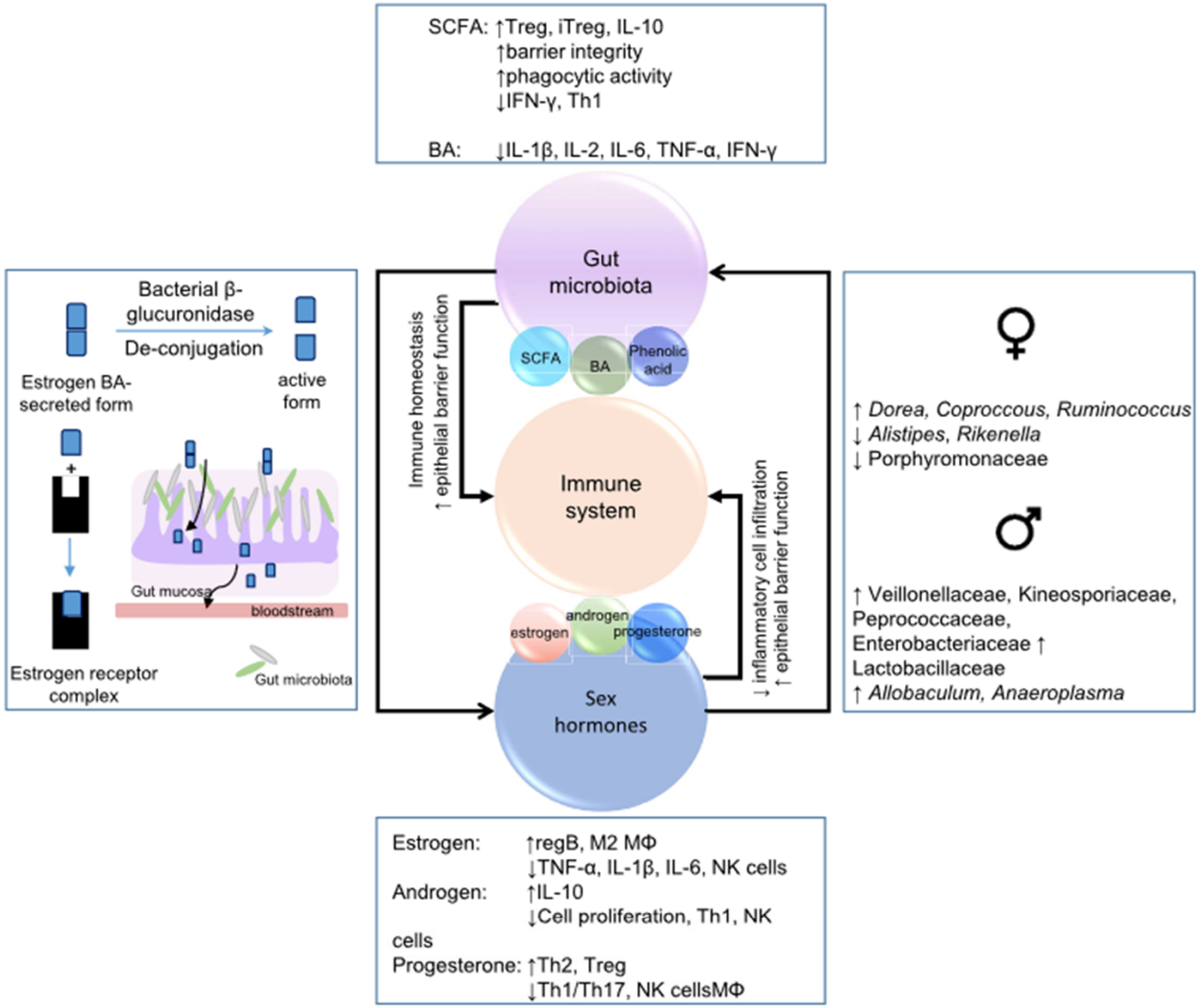


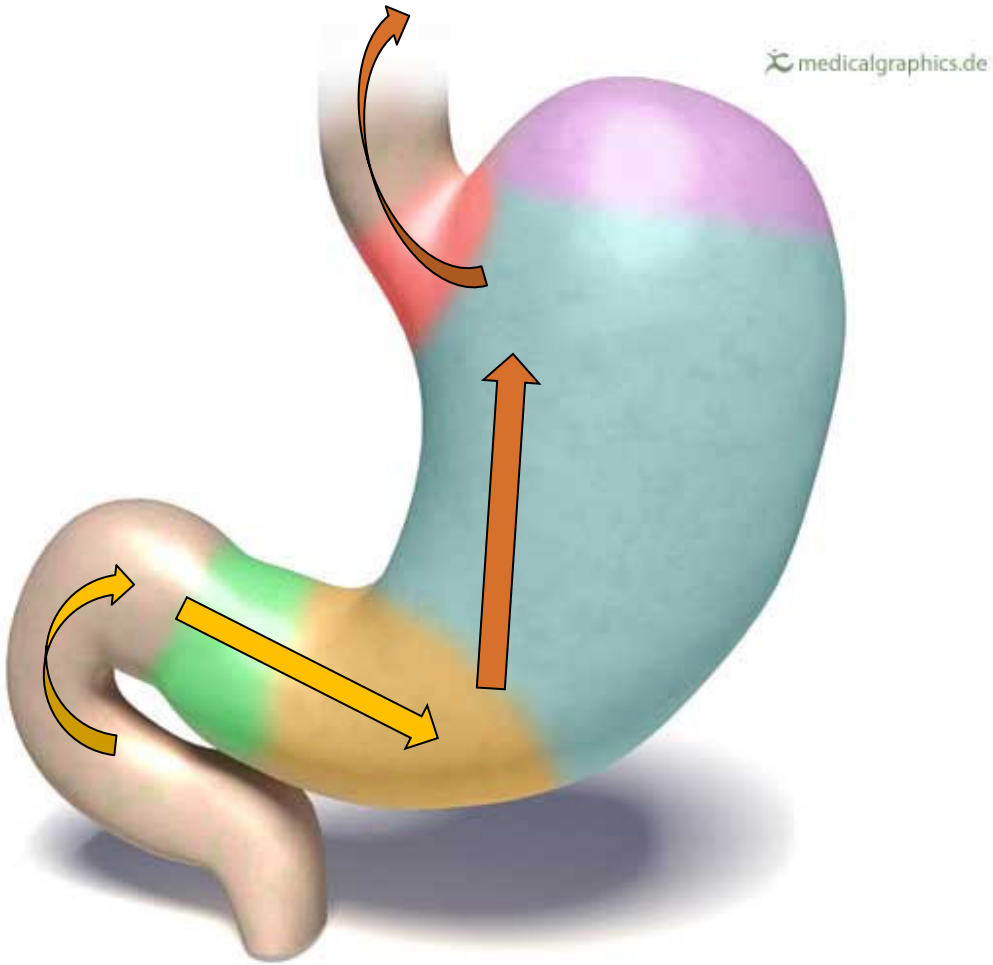
Fig. 1. Schematic representation of the interconnections occurring among immune system, gut microbiota and sex hormones. Even more evidence are indicating the establishment of a tight reciprocal regulation between immune functions, sexual hormones and gut microbiota. Gut microbiota composition contribute to the immune homeostasis by regulating through its composition and its metabolites immune system functions and are concomitantly shaped by the immune system. Studies in mammals have shown that sex hormones can also modulate microbiota composition and indeed could potentially contribute to the sex bias observed in autoimmunity. Interestingly, gut microbiota metabolizes estrogens by secreted β-glucuronidase leading to estrogen activation and their translocation into the bloodstream for reaching distal sites. The alteration of such interconnected regulation contributes to chronic inflammation and the onset of autoimmunity. Data reported on microbial abundance are taken from mice data [22,43–47,49]. BA, bile acid; IL, interleukin; iTreg, inducible regulatory T cells; MΦ, macrophages, NK, natural killer; regB, regulatory B cells; SCFA, short chain fatty acid; Th, T helper cells; Treg, regulatory T cells.

More on the Sterolbiome

Clostridia create more secondary BA

- **Higher levels of secondary BA increase the risk of gallstones**
- **Higher levels of secondary BA increase the risk of colorectal cancer**
- **High fat diet may further drive intestinal bacterial conversion to secondary BA**
- **UDCA may protect.**

Bile reflux and DGERD



DGERD (Hofmann AF, 2008)

- **Bile acids will be exclusively in conjugated form unless there is abnormal bacterial deconjugation in the duodenum. There is ongoing controversy as to the role of bile acids as a cause of esophageal inflammation or Barrett's esophagus, a condition in which the esophageal lining cells become transformed (pre-cancerous).**

- **One study found that refluxing duodenal content in patients with esophagitis contained unconjugated DCA, which we know is a carcinogen.**

DGERD (Hofmann AF, 2008)

- **In the healthy person, there is little reflux of duodenal fluid into the stomach or esophagus, because intragastric pressure is higher than intraduodenal pressure.**
- **In patients with disordered gastroduodenal motility or pyloric sphincter dysfunction, there may be duodenal reflux into the stomach that in turn leads to reflux from the stomach to the esophagus.**
- **Duodenal content is rich in bile acids, but also contains active pancreatic enzymes, as well as irritant digestive products generated by pancreatic enzymes.**

Bile reflux

- Reflux of duodenal contents through the pyloric valve is purported to be more common after cholecystectomy, but is also seen in patients with an intact gall bladder.

- The only research published about treatment of this condition are on sucralfate or ursodiol.

Treatments for bile reflux

- Sucralfate is an oral liquid sulfated disaccharide.

- Sucralfate's antiulcer activity is the result of formation of an ulcer- adherent complex that covers the ulcer site and protects it against further attack by acid, pepsin, and bile salts. (Medscape).

Treatments for bile reflux

- Ursodiol

SIBO and bile flow

- Methane excess slows intestinal motility
- Gases produced by small intestinal overgrowth of bacteria or archea increase intra-abdominal pressure
- Along with delayed gastric emptying (post-viral, diabetic, idiopathic), the factors above may promote bile reflux or dyskinesia

Botanical and other interventions for biliary disorders

- Berberine containing herbs
- Oregano, allicin, etc.
- Chelidonium, Chionanthus, Fumaria, Cynara, etc.

He K, Rhizoma Coptidis alkaloids alleviate hyperlipidemia in B6 mice by modulating gut microbiota and bile acid pathways. Biochim Biophys Acta. 2016 Sep;1862(9):1696-709

- **This study of Coptis (a berberine containing herb) found that Coptis treatment reduced**
  - **body weight gain**
  - **blood cholesterol level**
  - **blood triglyceride level**
  - **low-density lipoprotein cholesterol (LDL-C) level**
  - **blood lipopolysaccharide level**
  - **liver fat deposition**
  - **size of fat cells**

He K, Rhizoma Coptidis alkaloids alleviate hyperlipidemia in B6 mice by modulating gut microbiota and bile acid pathways. Biochim Biophys Acta. 2016 Sep;1862(9):1696-709

- **Coptis increased Akkermansia muciniphila in the gut of mice, whereas, the abundance of Escherichia coli and Desulfovibrio was suppressed.**

- **The observed effects be attributed to Coptis' effects on FXR**

Dose-response effect of berberine on bile acid profile and gut microbiota in mice. Ying Guo, 2016

Berberine has a strong direct effect on bile acids in the serum of mice.

There was an increase in primary and a decrease in secondary bile acids.

The yellow dots in the diagram on the left represent berberine.

The blue area represents the protective primary bile acids.

The red area represents the carcinogenic secondary bile acids,

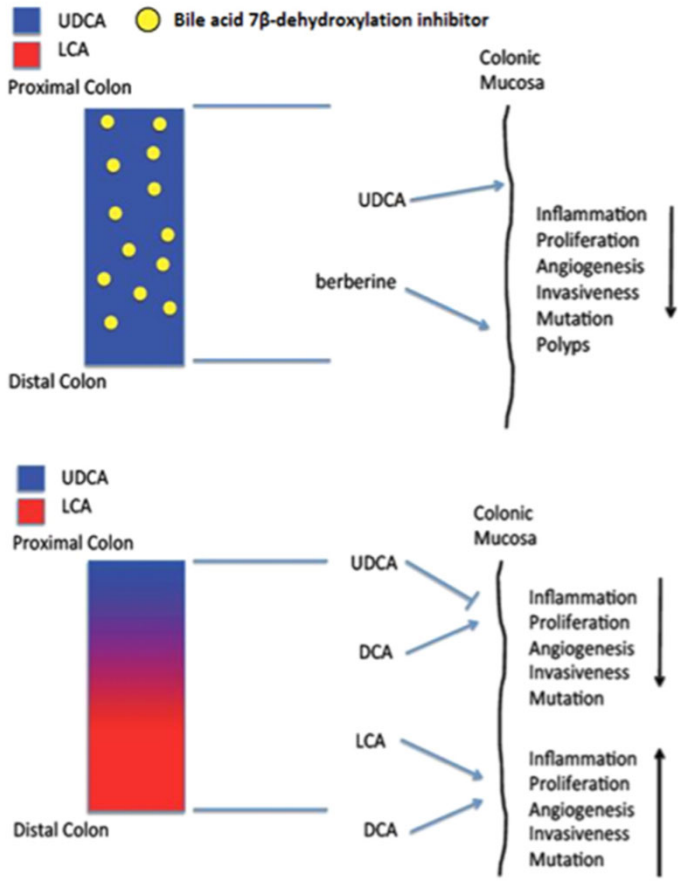


Figure 3 Targeted inhibition of bile acid 7β-dehydroxylating pathway hypothesized to improve therapeutic potential of UDCA in prevention of colon cancer development.