

Section 11

LECTURE

Physiological Chemistry of Gastrointestinal Lipids

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Some Definitions

1. Lipids (a.k.a. "fats")
Biologically important lipids are molecules with aliphatic chains of at least 12 C atoms and/or aromatic/aliphatic structures with at least 3 fused rings. When water-binding functional groups (OH, COOH, NH, and C=O) are covalently attached (usually at one end) known as *polar lipids*.
2. Polar Lipids
Because of the presence of hydrophobic and hydrophilic properties in the same molecule, is termed an *amphiphile* (a.k.a. amphipath). Most biologically relevant lipids are polar lipids or amphiphiles.
3. Classification of Polar Lipids Based on Interactions with H₂O
 - Class I: Insoluble non-swelling amphiphiles (no bulk solubility, e.g., triglycerides, lipo-(fat-)soluble vitamins, cholesterol, and phytosterols, waxes)
 - Class II: Insoluble swelling amphiphiles (all membrane phospholipids, monoacylglycerols, and "acid soaps").
 - Class III: Soluble Amphiphiles
 - Type A. Form *micelles* with intermediate formation of *liquid crystals* (most have long aliphatic chains and strong polar functions).

(Important examples: straight-chain anionic, cationic, non-ionic and zwitterionic commercial detergents; biological detergents include lysophospholipids, long-chain thioesters of coenzyme A and carnitine, Na⁺ and K⁺ salts of long chain fatty acids)
 - Type B. Form *micelles* without intermediate formation of *liquid crystals*. Most have bulky aliphatic or aromatic ring structures with multiple polar functions.

(Important examples: All bile salts, sulfated bile alcohols, rosin soaps, fusidates, saponins; many drugs, including penicillins and phenothiazines)

Figure removed due to copyright reasons. Please see:

Small, D. M. "A Classification of Biological Lipids Based Upon Their Interactions in Aqueous Systems." *J Amer Oil Chem Soc* 45 (1968): 108-119.

Figure 1: Small's (1968) classification of lipids based on interactions with water

4. Self-Aggregated States

- (i). Liquid Crystals (L.C.): Intermediate physical states (mesophases) of many lipids with properties of both liquid and crystalline states (uncommon with carbohydrates and proteins). Crystalline order (long-range) in at least 1 dimension, liquid-like disorder in the other dimensions. When formed under the influence of heat – thermotropic LC; when formed under the influence of a solvent (usually H₂O) – lyotropic LC. In the latter, the molecules may pack into different physical states depending upon the quantity of H₂O.
- (ii) Micelles: Thermodynamically stable aggregates of amphiphilic molecules that form spontaneously above a critical micellar concentration (CMC) and critical micellar temperature (CMT) in aqueous (regular micelles) or organic solvent (reverse micelles) solutions. Number of molecules in the average micelle (aggregation number) can vary from 2 to 1000. Typical examples: soaps; detergents; bile salts.
- (iii) Emulsions: The most important biological dispersions, e.g., plasma lipoproteins, dietary fat emulsions. Dispersion of one liquid in a continuous phase of another liquid. The dispersed (discontinuous) phase consists of microscopic droplets (usually 0.1-100 μm in diameter).
- (iv) Solid crystals of lipids (found in gallstones, atherosclerotic plaques, etc.) always pathologic. Many lipid crystals when heated form oils often with intermediate thermotropic LC formation, e.g., cholesteryl esters in the cores of low density lipoprotein (LDL exhibit thermal transitions between liquid

crystalline and oily states).

5. Principal Mixed Lipid Systems in Living Organisms

- (i) **Stable Emulsions:** Emulsion droplets of lipid stabilized by another lipid (can also be a protein or carbohydrate) – called the emulsifier. Emulsifiers are amphiphiles that possess the proper balance of hydrophobic and hydrophilic properties to allow them to form a monomolecular film on the surface of emulsion droplets and thereby prevent coalescence and 'breaking' of the emulsion; however, creaming and flocculation can still take place. The most potent emulsifiers are molecules that are sparingly soluble in the dispersed (usually oil) phase as well as dispersion media (usually H₂O) but align at the oil-water interface. Major biological examples are serum lipoproteins, dietary fat in the upper small intestine, intracellular fat droplets.
- (ii). **Mixed Micelles:** Thermodynamically stable polymolecular aggregates formed spontaneously in solutions composed of more than 1 lipid-like chemical species. A key feature is that one of the chemical species must be capable of forming micelles alone in the solvent. When micelles incorporate within or upon themselves otherwise insoluble lipids (or other molecules) the process is called "solubilization". Examples: biliary and gut luminal mixed micelles; detergent extracts of cellular organelles, etc.
- (iii) **Mixed Liquid Crystals**

Liquid crystals (a.k.a. mesophases), forming usually in aqueous systems (lyotropic), containing more than one amphiphilic lipid or a mixture of lipids and proteins (rarely carbohydrates). In general, one of the participating molecules must be capable of forming liquid crystals on its own in the medium. Biological examples: all plasma and intracellular membranes; myelin sheets; mixed vesicles in bile and gut luminal contents; lipoproteinX in cholestatic serum, etc.

6. The 3 "P" Rules

(i) The Predictability Rule

By understanding the surface and bulk physical chemical interactions of pure lipids in aqueous systems, it is possible to predict the physical form of each biologically-relevant lipid in lipid-rich systems of the living organism: (a) Non-polar lipids: e.g. squalene, cholesteryl ester, long-chain fatty acids are found in oil droplets or oil-like phases; (b) Insoluble non-swelling amphiphiles: e.g. cholesterol - partition into membranes, emulsifiers, mixed micellar solutions; (c) insoluble swelling amphiphiles: e.g. phospholipids, glycolipids are found primarily in biomembranes, emulsifiers of plasma lipoproteins and dietary fat, etc.; (d) Soluble amphiphiles: e.g. bile salts, lysolecithin – enter membranes in small amounts, mostly found in micelles of bile and gut luminal contents.

(ii) The Predominance Rule

When a lipid species predominates in a biological aqueous system, the *in vitro* bulk and interfacial physical state of that lipid will govern the entire physical-chemical state of the system *in vivo*.

(iii) The Phase Rule

The fundamental thermodynamic reference condition of any physical-chemical system in the equilibrium state. The phase rule has been used successfully to build and analyze model systems of lipids representing bile, brain, atherosclerotic plaques, plasma and intracellular membranes, gut luminal contents during digestion, etc. Thereby, pathophysiological correlations can be made between analyzed native systems and model systems to decide the type and number of phases present in a biological or pathologic sample. Moreover, because phase diagrams are developed at equilibrium, such correlations allow one to say how much a biological system deviates from the equilibrium state, that is, how supersaturated it is. Such work has shown that many pathophysiological systems are indeed in the metastable supersaturated state in health.

7. How Lipids Traverse Biologic Membranes

A. As *single* molecules.

(i) Passive monomeric diffusion (down a concentration gradient). Nonionic much more than charged ionic ones because lipids are soluble in all "lipoid" biologic membranes.

(ii) Passive facilitated transport. Same as (i) above but employs a protein carrier.

(iii) Coupled Transport

a. Ion(s) plus substrate utilize a common carrier officially known as a symporter which employs an electrochemical gradient for the ion(s) generated by Na^+ , K^+ -ATPase.

b. Ion and substrate move in opposite directions utilizing an antiporter which exchanges an absorbed substrate with an ion, usually glutathione, HCO_3^- , etc.

(iv) Active Transport

Usually lipid substrate driven out of cell ("flipped") by energy derived from primary ATP hydrolysis. Protein is a member of ATP-binding-cassette, i.e., ABC transporter (approximately 50 predicted in human genome, also known as multi-drug resistance transporters (less useful name).

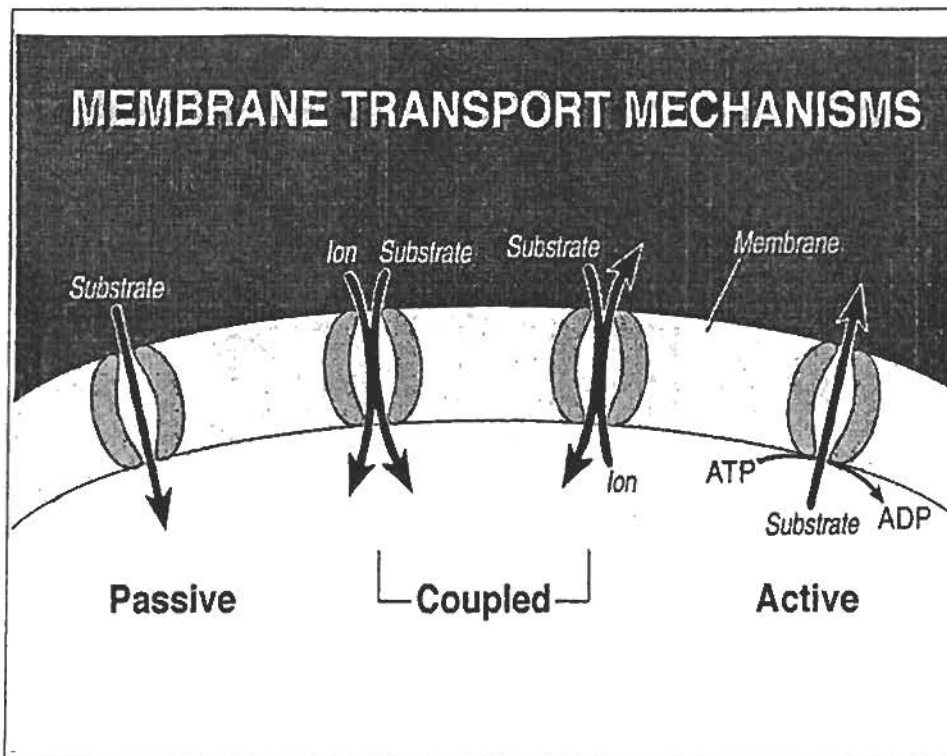


Figure 2: Membrane Transport Mechanisms

- B. As aggregated particles (usually specific emulsions, i.e., lipoproteins)
- (i) Endocytosis via clathrin-coated pits on plasma membrane, which recognizes a specific apolipoprotein on surface of particle, e.g., low density lipoprotein – LDL, chylomicron remnants, etc.
 - (ii) Selective or total lipid uptake into cell via scavenger receptors on plasma membrane, which recognize a specific apolipoprotein on surface of particles, e.g., selective lipid uptake of cholesteryl esters from high density lipoprotein – HDL by SRB1 on liver cells, adrenal cells, etc.
8. Control of Membrane Transporters
- A. Non-genomic – very fast. Activation, translocation and insertion as well as inhibition of degradation of cytoplasmic pool of transporters (various MAP, PKC kinases, etc.)
 - B. Genomic – slow via various nuclear transcription factors (FXR, PXR, LXR, PPAR (α , β , γ), RXR, etc.)
9. Examples
- A. Single molecules – bile salts and sterols (see Figure 3)

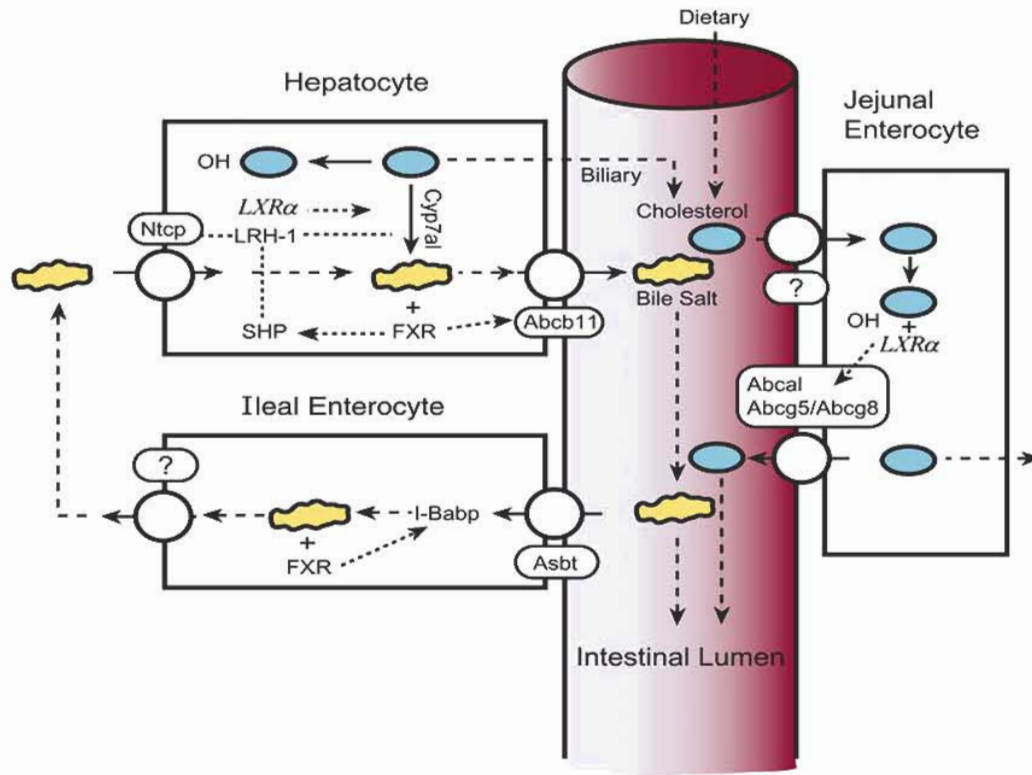


Figure 3: Nuclear Transcription Factor Control of Bile Salt and Sterol Metabolism
(Figure by MIT OCW.)

Bile Salts

(i) Uptake of bile salts from gut lumen.

- a. Distal Ileocytes: Apical Na^+ -coupled transporter (ASBT); cytosolic ileal lipid binding protein (ILBP); basolateral multi-drug resistance related protein isoform 3 (MRP3); approximately 90% of uptake. Maintains integrity of enterohepatic circulation of bile salts.
- b. Jejunal and ileal enterocytes; apical organic anion transport polypeptide isoform 3 (OATP3), (10%).
- c. Colonocytes: passive diffusion of deconjugated and undissociated secondary (bacterial modified) bile acids (10%).

(ii). Transport in Portal Blood

- a. Bound to serum albumin – 50% - most hydrophobic bile salts
- b. Bound to high density lipoprotein (HDL) – 50% - most hydrophilic bile salts

(iii) Basolateral, i.e., Sinusoidal Uptake by Hepatocytes

- a. Na -dependent taurocholate pump (NTCP) – 80%
- b. Several OATPs – 20%

- (iv) Secretion across canalicular membrane into bile
 - a. Bile salt export protein (BSEP) – monovalent bile salts – 98%
 - b. Multi-drug resistance related protein 2 (MRP2) – divalent bile salts – approximately 2-4% of enterohepatically cycling bile salts.

Sterols (Cholesterol and Phytosterols)

(i) GI Tract

- a. GI monomer uptake from the gut lumen is from micelles composed of bile salts and other digestive lipids
- b. Transport is believed to be either passive or facilitated diffusion or both, into the enterocyte.
- c. Absorption is highly efficient, but depending upon chemical structure, sterols are refluxed with different efficacy to intestinal lumen via the *ABCG5* and *ABCG8* half-transporters on the apical membrane. The nuclear transcription control is via *LXR α* with its obligate heterodimer partner RXR. Ligands are oxysterols and apparently not cholesterol itself.
- d. Approximately 50% of absorbed cholesterol is effluxed, but more than 98% of all phytosterols are effluxed.

(ii) Liver

- a. Most uptake from intestinal lipoproteins mainly chylomicron remnants and HDL (*ABCA1* is on the basolateral membrane of the enterocytes and is responsible in part for the synthesis of intestinal HDL).
- b. Cellular control involves *LXR α* /*RXR* via oxysterols.
- c. Transport into bile utilizes *ABCG5* and *ABCG8* on the canalicular membrane. Phytosterols have an absolute requirement for these transporters for secretion and are more efficiently transported into bile than is cholesterol.
- d. Dysfunctional mutations in either *ABCG5* or *ABCG8* give rise to sitosterolemia (or phytosterolemia) where absorption efficiency of plant sterols increases by one to two orders of magnitude and results amongst other features in premature atherosclerosis. When phytosterols are eliminated from bile, they can not be secreted otherwise and so accumulate in all tissues. Cholesterol secretion into bile is reduced only 50% in this autosomal recessive disorder.

- B. Emulsions: Plasma Lipoproteins – stable emulsion particles
Classes in rank order of increasing density are chylomicrons, VLDL, IDL, LDL, and HDL and the components are apoproteins, triglyceride (TG), cholesterol ester (CE), free cholesterol (Ch), and phospholipid (PL).

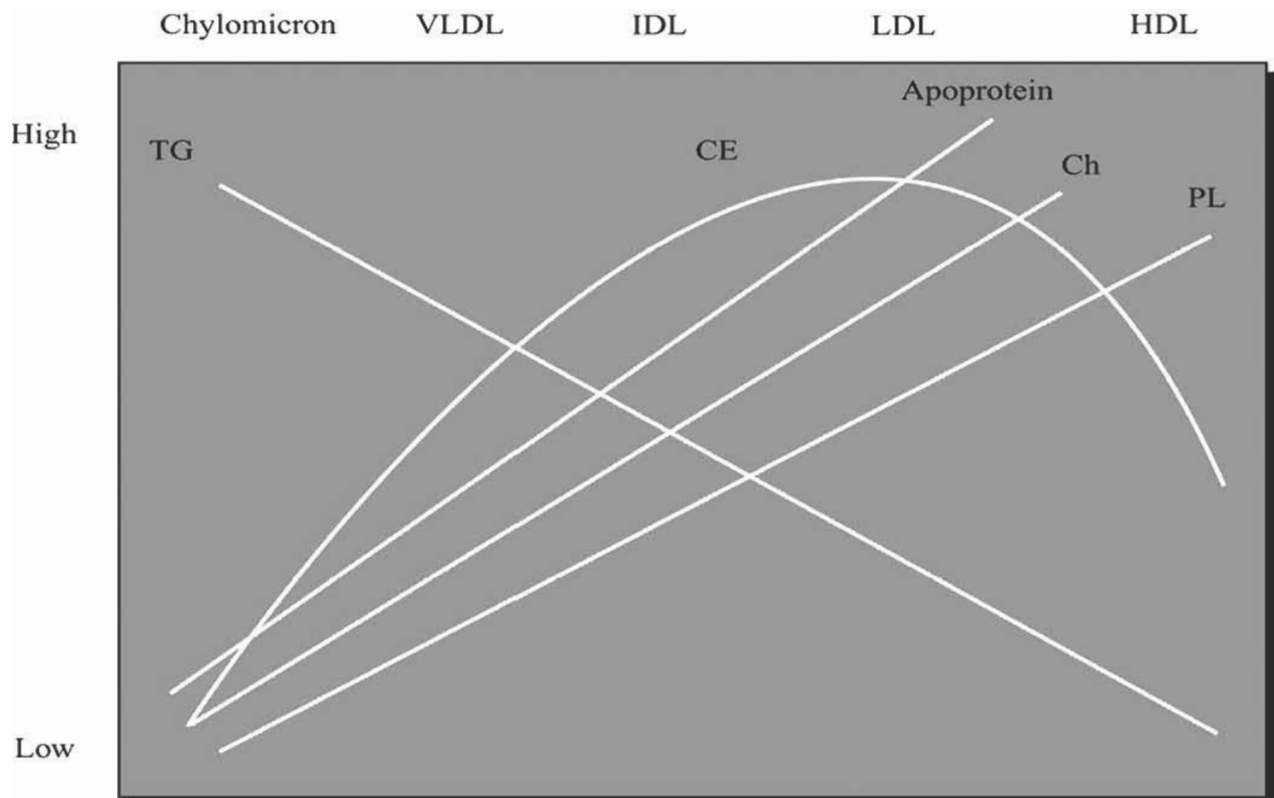


Figure 3. Chemical Composition Schema of Human Plasma Lipoproteins (Figure by MIT OCW.)

(i) Chylomicrons

- a. Biggest lipoproteins: principally TG and much less CE in core, synthesized in enterocytes, emulsified coat of PL and Ch (phospholipid has biliary origin resynthesized in enterocytes), ApoB-48 on surface needed to exit enterocyte; also ApoAI and AIV, passage of lymph to blood at thoracic duct level: in plasma acquire Apo CII.
- b. In peripheral tissues, lipoprotein lipase, a stalked enzyme on capillary bed activated by Apo CII – hydrolyzes most of the TG in the chylomicron core – FA and MG diffuse into tissues and adipocytes, etc.
- c. Particles shrink dramatically in size. Surface coat becomes redundant and forms nascent HDL – a bilayer of lipids stabilized by Apo AI and AII, which then binds LCAT (lecithin-cholesterol acyltransferase) of hepatic origin.
- d. Remnant particle binds ApoE in plasma. Sufficiently small to enter fenestrae of Disse's space and the sinusoids of the liver. Recognized by apolipoprotein E receptors and LDL-RP (related protein), also uses hepatic lipase and glycosaminoglycans as ligands. Internalized by clathrin-coated pits into hepatocytes. Major diseases, Type I and V hyperchylomicronemia.

- (ii) VLDL: Same structure as for chylomicrons but appreciably smaller –secreted by liver. Assembled in the SER/RER utilizing MTP (microsomal TG transfer protein). Exits cells by exocytosis – TG and CE in core with ApoB-100 on the

surface plus ApoE; emulsified coat, PL and Ch. Processed mainly by hepatic lipase to form IDL (intermediate density lipoprotein) and eventually LDL (low density lipoprotein) in rapid succession. VLDL has its own receptor and if it is defective on hepatocytes gives rise to mixed hypertriglyceridemia.

- (iii) LDL: Plasma product of VLDL. Delivers CE to peripheral tissues and liver. Endocytosis, secondary lysosomes; acidic dissociation of components. Acidic Ch esterase hydrolysis of CE to form Ch and FA. Hydrolysis of phospholipid to lysophospholipid and fatty acid. Apo B-100 to amino acids. Cholesterol via the SREBP transcription factor, activates ACAT, suppresses ApoE-100 receptor and suppresses HMGCoA reductase (rate-limiting enzyme in synthesis). For cholesterol to exit lysosomes, it needs a specific protein, Neimann-Pick type C protein with unknown function. In liver, most LDL cholesterol is utilized for *de novo* bile salt synthesis. Major disease of the ApoB-100 receptor: Familial hypercholesterolemia.
- (iv) HDL: "Mixed micelles" of the blood made from the redundant surface coats of VLDL and chylomicrons, as well as being made directly by liver and enterocytes. ApoAI, II, and E, LCAT on surface. HDL takes part crucially in "reverse cholesterol transport": Ch delivered from peripheral cells and macrophages via the apical ABCA1 transporter to ApoAI-lecithin particles. LCAT then uses one fatty acid from lecithin, forming lysolecithin (which returns to the liver on albumin) to esterify cholesterol on surface to form cholesteryl ester. Cholesteryl ester being membrane insoluble then moves into the core and swells the particle (mature quasi-sphered HDL). Can transfer its CE to TG-rich particles (VLDL) and LDL via the hepatic made CETP (cholesteryl ester transfer protein). On specific cells, recognized by SRB1, which takes up the cholesteryl ester only by docking the Apolipoprotein AI, principally on liver and steroidogenic tissues. The remaining particle regenerates nascent HDL and proceeds again to the periphery to repeat reverse cholesterol transport. Cholesteryl ester (after hydrolysis) and cholesterol of HDL go directly into bile to be eliminated from the organism as the free sterol in feces.

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