Section 8

LECTURE

Lipid Digestion, Absorption, and Malabsorption

Lipid Digestion, Absorption, and Malabsorption

I. Classic Fat Digestion/Absorption Experiment of M. Claude Bernard

A. Published first as "Memoire sur le Pancréas..." in Supplement aux Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences (Paris), Mallet-Bachelier, Tome I, pp 379-563 with 9 color plates, 1856. (Also published as a monograph, same year, by Ballière, Paris.)

1. Color plate No. 7-8 (shown in Slide 1 of lecture):
This illustrates the duodenal loop and mesentery of a rabbit following ingestion of dietary fat (mainly triglyceride). In it, Bernard (together with his illustrator, Borromée) elegantly separates the effects of bile and pancreatic juice on the absorption of fat and provides incontrovertible evidence for the importance of pancreatic juice in fat absorption (lacteals visibly white with chylomicra (emulsion particles that carry absorbed dietary lipid) distal to the separate entrance of the main pancreatic duct). This stands both as a classic of physiological illustration and the beginning of scientific investigation of intestinal absorption. However, Bernard omitted the appropriate control experiment which his pupil, A. Dastre, did 34 years later showing the essential functionality of bile in promoting intestinal absorption (Rechêrches sur la bile, Arch Physiol 2:315-330, 1890).

II. Lipid Digestion and Absorption

A. Composition of Dietary Fat

1. Long-chain triglycerides (TG), 95%

   a. Chemical energy - 9 Kcal/gram and essential fatty acids - precursors of prostanoids, leucotrienes, and lipoxins.

   b. Only the lipids of tissues (e.g., biomembranes, lipoproteins, etc.) and obviously not proteins or carbohydrates can be altered by the composition of the diet; "Tissue lipids reflect the environment".

2. Other lipids, 5%

   a. Complex membrane lipids: phospholipids (PL), sphingolipids, glycolipids, glycosphingolipids, etc.

   b. Cholesterol (Ch) (animal origin only) and other sterols (usually of plants and shellfish)

   c. Cholesteryl and other steryl esters (cholesteryl esters only from blood products, liver, etc.)

d. Waxes (esters of long-chain fatty acids and long-chain normal alcohols), usually of plant origin

e. Lipovitamins A, D, E, K, and their esters (fat soluble vitamins)

f. Natural hydrocarbons (carotene, squalene, etc.)

g. Environmental pollutants (in industrialized societies), aliphatic and aromatic hydrocarbons, pesticides, plasticizers, etc. Units of p.p.m., but are concentrated in "fat" stores.

B. 24 Hour Input-Output Fat Balance:

1. Input:

Exogenous: 1. Dietary Fats: → TG, 100 g; PL, 4-8 g; Ch, 0.5 g

Endogenous: 1. Biliary Lipids: → Bile Salts, ~ 30 g; PL*, 10-15 g; Ch, 1-2 g

2. Desquamated Cells: Mixed membrane lipids, 2-6 g

3. Dead Bacteria: Mixed membrane lipids, ~10 g

* Mostly (96%) "pure" lecithin (phosphatidylcholine)

2. Output: Fecal fat (~4 g) derived equally from dietary, biliary, cellular, and bacterial sources.

C. Physical State of Dietary Lipids in Water (Figure 1)

1. TG (100 g ≈ 120 mmoles). Insoluble nonswelling amphiphile (Class I polar lipid)
Forms crude emulsion particles when shaken with water. Orientation of surface monolayer of molecules: 3 fatty acid chains directed toward core, molecules in core oriented with sn-2 fatty acid directed in opposite direction to sn-1 and sn-3 fatty acids: 3 ester linkages on surface available for hydrolytic attack by digestive lipases which only function at and are in many cases activated by oil-water interfaces. Such crude emulsions are unstable in the absence of an emulsifier (see lecture notes by Dr. Carey on 'Physiological Chemistry of Gastrointestinal Lipids').

2. PL (mainly lecithins) (4-8 g ≈ 5-10 mmoles). Insoluble swelling amphiphiles (Class II polar lipids)
Form lamellar liquid crystals when allowed to swell spontaneously in water. When vigorously shaken in water, form multilamellar vesicles (liposomes). When sonicated in excess water, form small unilamellar vesicles - single phospholipid bilayer with polar head groups of lipids oriented toward core of particles and toward bulk water, respectively. Thermodynamically stable.

Figure removed due to copyright reasons. Please see:

Figure 1 Carey, M. C., D. M. Small, and C. M. Bliss. "Lipid Digestion and Absorption." *Annual Review of Physiology* 45 (1983): 651-677.

3. Ch (unesterified) (0.5 g ≈ 1 mmole). Insoluble nonswelling amphiphiles (Class I polar lipid) By itself, Ch crystallizes in water as monohydrate crystals. Ingested mainly as part of cellular membranes, i.e., mixed with swelling amphiphiles, phospho- and glyco-lipids. With the latter, Ch forms mixed liquid crystals (liposomes and vesicles) in water. Bulky steroid hydrocarbons moiety interdigitated between chains of PL and glycolipids, □OH group at lipid-aqueous interface.

4. Other dietary lipids: Those without polar groups (nonpolar lipids) or very weak polar groups are found principally dissolved in TG. Those with strong polar groups are generally oriented parallel to hydrocarbon chains of PL and glycolipids in bilayers or in emulsifying monolayers.

D. For effective hydrolysis, dietary fats must be dispersed as stable emulsion particles. (Figure 2)

1. Because dietary lipids are insoluble in water, the digestive lipases have been designed by evolutionary pressure to function at oil-water interfaces and have little or no activity on water-soluble substrates. True lipases display interfacial activation which has been shown to be due to the removal of a "lid" covering the active site (see below). Accordingly, the interfacial area/volume ratio ("interfacial concentration") of the lipid becomes a major determinant of hydrolytic rates. Emulsification increases the oil-water interfacial area (surface/volume ratio) by the dispersion of large oil masses into fine oil-in-water (O/W) emulsion particles.

2. Interfacial tension of pure TG in H_2O is ~15-20 mN/m. 20 kergs of energy required to emulsify a 1 cm drop of TG into 1 μm droplets (with a concomitant increase in surface area of 1000 times).

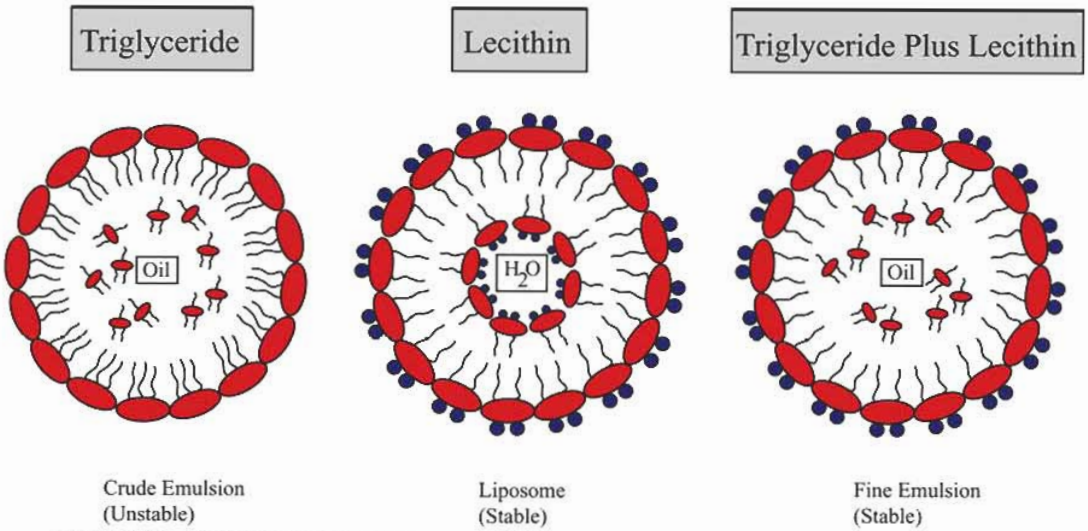

FIGURE 2. Dispersed states of major dietary lipids in water. In the triglyceride plus lecithin emulsion particles, some triglyceride is solubilized in the surface coat. (Figure by MIT OCW.)

3. 100 g of TG divided into 1 μm emulsion particles increases surface area to 600 m^2. Huge interface for lipase action but unstable owing to high surface-free energy.

4. Phospholipids are excellent emulsifiers, because

   a. PL-water interfacial tension is ~1-5 mN/m; much less energy required for emulsification

   b. 100 g TG in 1 μm particles (600 m^2) can be covered by 1 mmol (~1g) of PL as a monomolecular layer - not too different from dietary proportions

   c. PL monomers have negligible solubility in oil or in water - most molecules oriented at oil-water interfaces

   d. PL stabilizes emulsified droplets because head group charges prevent breaking and coalescence of the emulsion particles

   e. 3% solubility of TG in PL monolayer - This is the fraction available for hydrolysis by TG lipases

5. Many foods, e.g., milk, ice cream, salad dressings, and sauces are already prepared in emulsified form.

6. TG and PL interact easily to form emulsions with energy provided during grinding, cooking, marinating, blending, chewing, deglutition, and mixing in stomach. Also, PL as the emulsifying monolayer is in a suitable physical state for the hydrolysis of its sn-2 fatty acid ester bonds by pancreatic phospholipase A_2.

E. The Lipases Involved in Fat Digestion

Enzymatic hydrolysis of fatty acid ester linkage at the emulsion-water interface is essential for the absorption of dietary fats, be they TG, PL, cholesteryl or lipovitamin esters, etc. Result is smaller, more polar molecules that become dispersed in water of upper small intestinal contents as very small, "soluble" and "stable" particles - mixed micelles and unilamellar vesicles.

F. Topology of Lipases

1. *Human milk lipase.* Found in human, gorilla, ferret, mouse and cat milk (not to be confused with plasma lipoprotein lipases, traces of which are found in all milks including human and ruminants [cow]). M_r ~100,000. Essential cofactors for enzyme action are bile salts (a bile salt-activated lipase); hence, only function in duodenum/jejunum, pH optimum 6-8 (markedly stable at acid pH values, i.e., in stomach). Substrates are TG and other esters - can hydrolyze TG, DG, MG and lipovitamin esters dispersed as emulsions or micelles. Positional specificity with TG: sn-1 or sn-3 > sn-2 fatty acids. Reaction products: TG → G + 3 FA. Physiologic role: is capable by itself of hydrolyzing all the TG in a single infant feed (8 fl oz of milk) in 20 min at pH = 6.5 in duodenum/jejunum in presence of [BS] as low as 1.5 mM (< CMC). Principal reason for absence of steatorrhea in breast-fed newborns.

2. *"Gastric" lipase.* Found in essentially all animals, including humans, especially important in infants. In rodents produced in serous glands at base of circumvallate papillae (lingual lipase), produced in peripharyngeal serous glands in ruminanls (pharyngeal lipase), produced in gastric chief cells of all monogastric animals including humans (true gastric lipase). M_r ~50,000. No cofactor required. pH optimum 4.5 (activity range pH 2-7.5). Stable at pH 2. Major site of action is stomach. Substrate: dietary TG only. Effect of bile salts in duodenum is inhibition. Positional specificity: sn-3 >> sn-1 >> sn-2 fatty acids. Physiological reaction products: TG → DG + 1 FA (30% of dietary TG is therefore hydrolyzed in stomach during 1-4 hours of gastric lipolysis). Primary physiological roles for pre-duodenal release of FA (note: no FA in diet, induces rancidity):

   a. "Emulsogenic" when FA partially ionised in duodenum/jejunum ("acid soaps")

   b. FA on emulsion surface binds colipase and lipases, phospholipase A2, cholesteryl esterase

   c. FA (not TG) releases cholecystokinin

3. *Pancreatic lipase.* Responsible for quantitatively complete TG hydrolysis. M_r ~48,000. Essential cofactor is colipase since pancreatic lipase itself is inactivated by bile salts. pH optimum 8-9 (6.5 with colipase). Inactivated irreversibly at pH < 4.0. Site of activity: duodenum/jejunum. Substrate: TG only. Positional specificity: sn-1 and sn-3 fatty acids of TG; no activity on sn-2 linkage. Physiological reaction products: TG → MG + 2 FA. N.B.= x-ray structure in "resting" and working states solved - see below (Figure5).

4. *Pancreatic-lipase associated protein 1 and 2*: $PLAP_1$ has no known substrate; $PLAP_2$ is a TG lipase. Particularly important is suckling animals when pancreatic lipase (3.) is not developed. The audlt guinea-pig has $PLAP_2$ instead of the classic pancreatic lipase. The properties of $PLAP_2$ are, a) no interfacial activation, c) minimal stimulation by colipase, d) lower specific activity against TG, and e) will hydrolyse phospholipids and galactolipids but not sphingomyelin. Maximal activity in late fetal and early neonatal life.

5. *Pancreatic bile salt-activated lipase.* Constitutes about 5% of pancreatic lipase activity. M_r ~100,000. Primary bile salts induce dimer formation and thereby activation. Optimal pH range 6.5-9.0. Site of action: duodenum/jejunum. Substrates: TG and other esters, as well as ceramide (therefore also a ceramidase) in emulsions, liquid crystals, and also micellar substrates. Activity on micellar substrates > emulsions. Positional specificity with TG: sn-1 & sn-3 > sn-2 FA. Reaction products:
TG → G + 3 FA. This enzyme is the same enzyme as cholesteryl esterase and human milk lipase, the latter post-translationally modified. Major pancreatic lipase in animals whose dietary fat staples are wax esters, e.g., elasmobrancs (Leopard shark, dog fish). Physiological role: hydrolysis of MG, lipovitamins, steryl esters and ceramides.

6. *Pancreatic phospholipase A_2.* Intestinal luminal source of phospholipase activity. Only "lipase" secreted in proenzyme form. Activation requires enzymatic cleavage of an Arg-Ala bond by trypsin in the N- terminal chain. M_r ~14,000. Catalyzes the specific hydrolysis of sn-2 fatty acid ester linkages in a variety of phosphoglycerides (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, cardiolipin). It is without effect on sphingolipids (sphingomyelin, cerebrosides and gangliosides, etc.). These appear to be hydrolyzed by an alkaline active enzyme (pH optimum=9) on the brush border of absorptive cells and in bile -- possibly, further hydrolysis is by a lactase-ceramidase complex also on the "brush border, as well as No 5 above." Pancreatic phospholipase A_2 has an absolute requirement for Ca^{2+} ions which bind in a 1:1 stoichiometry to substrate and enzyme. Hydrolyzes PL in micelles, liquid crystals and on emulsion surfaces. Densely packed PL head groups such as in vesicles are hydrolyzed the slowest. Reaction products e.g. phosphatidylcholine (lecithin) ⟶ 1 lyso-PC (1-lysolecithin) + 1 FA. The saturated chain in PL is usually in the 1 position, therefore palmitoyl and stearoyl lysolecithins formed.

7. *Paneth cell lipases.* Poorly defined phospholipases of A2 class particularly active against phosphatidylglycerol (PG) and phosphatidylinositol (PI), also cholesteryl esterase: Physiological role unclear but may have a 'housekeeping' function in inhibiting small intestinal bacterial growth. Trivially labeled the "diffuse pancreas of the GI tract".

8. *Sphingomyelinase* secreted in human bile and as a "stalked" enzyme on intestinal (small and large) mucosa only. Active in cleaving the phosphocholine head-group of sphingomyelin (in milk, meat, liver, eggs, fish). pH optimum is 9.0 and is inhibited by phosphatidylcholine and bile salts. Is trypsin resistant. Biliary enzyme probably active in canalicular space and ileum where pH is highest-to date only found free in human bile.

9. *Microbial lipases.* Not involved in normal fat digestion in health. These lipases of the human colonic microflora are heterogeneous and poorly characterized. Can cleave all 3 fatty acid ester linkages of TG and both fatty and ester linkages of PL. They only become important in malabsorption syndromes when they hydrolyze fatty acid ester bonds, releasing long-chain fatty acids which can induce diarrhea (in addition to steatorrhea). Long-chain fatty acids are not absorbed from the colon to any major extent but short-chain

fatty acids (acetic, butyric, propionic, valeric) are. These are produced in abundance from microbial fermentation of the monosaccharide units of microbial digested dietary fiber, especially cellulose, hemicellulose, pectins, and GI mucins, but not lignins. (See section on fecal fat.)

G. Topographical Events in Fat Digestion and Absorption in Humans

1. Mouth - No Lingual Lipase in humans - mastication - swallowing
2. Stomach - fat digestion by gastric lipase (chief cells) released by gastrin, ? vagal, ? distension – interestingly lipase (not pepsin) is quantitatively the major protein in human gastric juice.
3. Physiological Function (Figure 3)
   a. TG → DG + FA; most active on sn-3 fatty acid ester bonds
   b. Short- and medium-chain FA (sn-3 FA from milk) absorbed passively by stomach mucosa, become bound to albumin and are transported by portal blood to liver.
   c. In stomach, long-chain FA are non-ionized and dissolve in core and surface (1:6 ratio) of crude TG emulsions; however, they stabilize TG emulsions when partially ionized at duodenal pH (see next section).

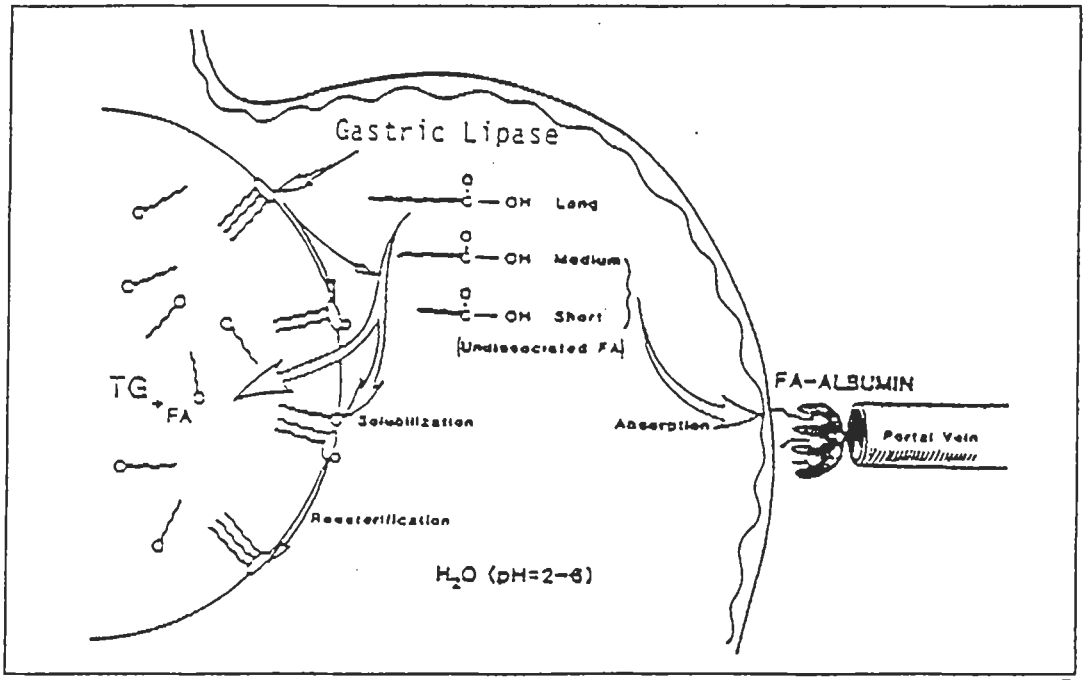

FIGURE 3: Physical state and fate of hydrolyzed cow's milk fatty acids during hydrolysis by gastric lipase in the stomach. Short- and medium-chain fatty acids are absorbed. Long-chain fatty acids are solubilized within and on the surface of the fat droplets.

d. FA in duodenum promote binding of the pancreatic lipase cofactor - colipase and other lipases to the emulsion surface

e. Duodenal FA are potent stimuli for CCK release (apart from being a gallbladder and pancreatic agonist, CCK is a short-duration meal-related satiety signal).

f. ~30% of TG is digested to DG and FA in the stomach after a normal adult meal.

4. Stomach-Pylorus (Figure 4)

a. Topographical site of action of gastric lipase (pH and time favorable)

b. Emulsification of dietary fat by
- Removal of protein envelopes by pepsin and HCl
- Retrograde pulsion of masses of fat by antral peristalsis against a closed pylorus
- Controlled release into duodenum via intermittent opening of pyloric canal
- Intermittent spurting of crude emulsion under high pressure from antrum through narrow pyloric canal ("colloid-mill" analog)
- Further fine emulsification in duodenal bulb when FA become partially ionized at pH 6.5 and migrate from TG interior to oil-water interface ("agent-in-oil" vs. "nascent soap" method of "spontaneous" emulsification)

c. A newly discovered gastric peptide ghrelin, an appetite-stimulating hormone which interacts with neuropeptide Y (NPY) and agouti-related peptide (AgRP) expressing neurons of the arcuate nucleus of brain, rises precipitously in blood when stomach is empty and falls after food is consumed. It stimulates not only feeding but also growth hormone secretion. Major pathway from stomach to brain is via gastric vagal afferents.

5. Duodenum-Jejunum

a. Hormonal coordination of the digestive sequence

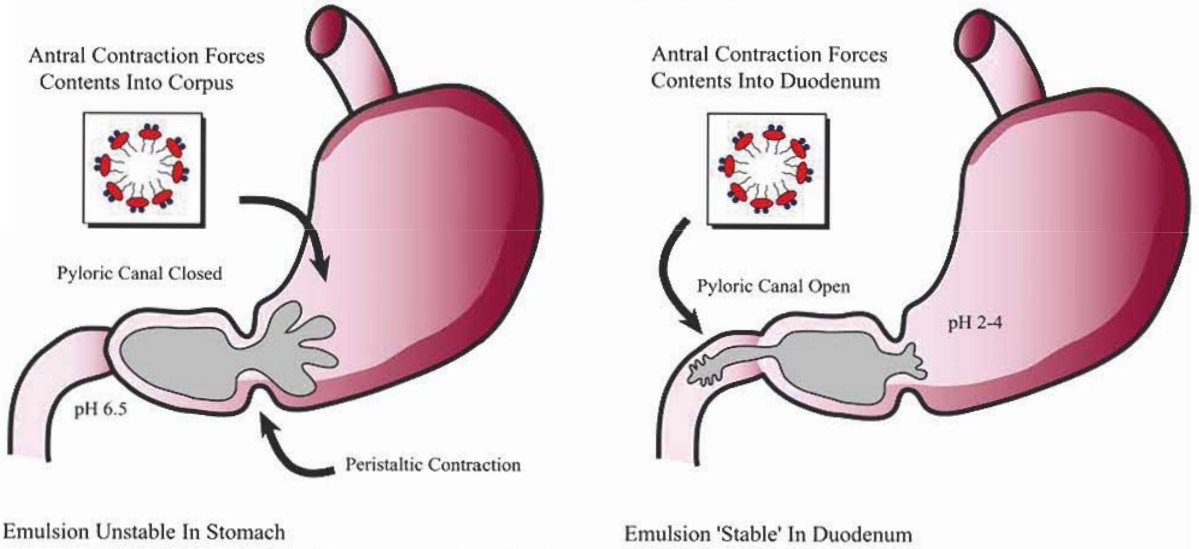

FIGURE 4: Role of the stomach and pyloric canal in the emulsification of dietary fat. (Figure by MIT OCW.)

1. FA (not TG) and acid (HCl) stimulate the release of CCK and secretin, respectively, from endocrine cells in duodenal-jejunum mucosa - possibility of a "CCK-releasing peptide" and a "secretin-releasing peptide"

b. Secretin:
   1. HCO_3^--rich fluid from the pancreas, biliary tree and Brunner's glands. HCO_3^- neutralizes gastric HCl
   2. Decreases gastric and duodenal motility, contracts pylorus
   3. Potentiates CCK

c. CCK:
   1. Strongest stimulant of enzyme secretion by the pancreas
   2. Contracts gallbladder and pylorus, stimulates intestinal motility, inhibits Oddi's sphincter
   3. Induces satiety of short duration

d. VIP increased 2° vagal activity - physiological role in fat digestion not clear.

e. GIP increased 2° vagal activity and dietary glucose (from starch and glycogen) - principal physiologic role as the gastrointestinal insulinotropic polypeptide.

f. Digestive milieu (pH, ionic strength, [lipid], [enzymes]) maintained relatively constant by these mechanisms.

6. Activation of Pro-enzymes and Cofactors -- Bile salts solubilize enterokinase (enteropeptides) from the duodenal brush border.

a. Trypsinogen —**Enterokinase**→ Trypsin

b. Pro-phospholipase A_2 —**Trypsin**→ Phospholipase A_2

c. Pro-colipase —**Trypsin**→ Colipase (N-terminal pentapeptide, sequence APGPR in humans may be satiety inducing, named "enterostatin")

d. Monomeric bile salt-dependent lipase (Ch esterase) —**Primary Bile Salts**→ Dimers of bile salt-dependent lipase (active)

H.Hydrolytic Sequences

1. Colipase binds TG substrate in the presence of bile salts (in the absence of colipase, bile salts displace pancreatic lipase from the interface into bulk water phase).

2. Lipase binds to colipase on the TG emulsion surface.

3. Lipase then binds substrate tightly and hydrolysis commences.

    a. TG → 2-MG + 2 FA
    b. 2-MG is not attacked by pancreatic lipase.

Figure removed due to copyright reasons. Please see:

van Tilbeurgh H., et al. "Structure of the pancreatic lipase-procolipase complex." *Nature* 359 (1992): 159-62.

van Tilbeurgh H., et al. "Interfacial activation of the lipase-procolipase complex by mixed micelles revealed by X-ray crystallography." *Nature* 362 (1993): 814-20.

I. Molecular Mechanisms (Figure 5)

1. At an interface lid and β5 loop spring open, revealing an oxyanion collar at the entrance to a hydrophobic canyon, the bottom of which contains the hydrolytic triad.
2. The open lid becomes H-bonded to one of the hydrophobic fingers of colipase.
3. The conformational changes unmask a continuum of hydrophobic amino acid residues that bind the lipase-colipase complex to the oil-water interface.

J. Physical-Chemical Phases Produced during TG and PL Hydrolysis (Figure 6)

1. Overall view and phase relations of physical phases during established fat digestion: Emulsion particles, multilamellar vesicles, unilamellar vesicles and mixed micelles. (Figure 6)

2. Packaging of FA (as "acid soaps") and MG and other lipids in micelles and vesicles - diffusion and passive penetration of apical membranes of enterocytes.

3. Major role of unilamellar vesicles (from emulsion interface) is to provide digested lipids for the micelles: micelles are kept saturated with cholesterol, FA and MG.

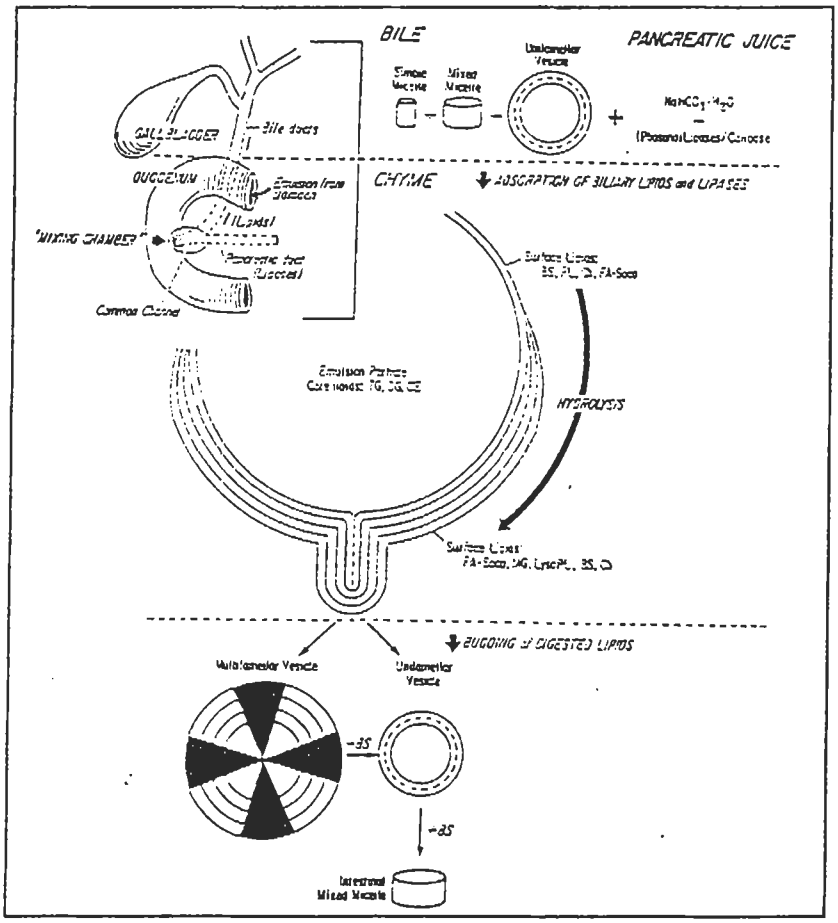

FIGURE 6: Physical-chemical sequences in lipid digestion and dispersion in the proximal small intestine.

4. Lecithin of emulsion surface or in mixed micelles:

$$\text{Lecithin} \xrightarrow[Ca^{2+}]{\text{Phospholipase } A_2} \text{1-Lysolecithin + FA}$$

K.Diffusion and Absorption

1. Micellar dispersion of hydrolytic products increases the rate of fat absorption by increasing their "concentrations" in the aqueous phase about 100,000 times compared with monomeric solubilities (see below).

2. Nernst diffusion layers of "unstirred water" which are contiguous with the absorptive membranes of the intestines are of physiological importance in that they influence the diffusivity of micellar/vesicle lipids from bulk water to the enterocytes.

3. The rate of flux (J) through the unstirred water layers is equal to the product of effective aqueous concentration (C) multiplied by the diffusion coefficient (D).
Example: (Approximate values)

Form	FA Conc. (mM)	D x 10^6 (cm^2/sec)	J x 10^6 (μmoles/cm·sec)
Molecules	10^{-5}	7	$7x10^{-5}$
Vesicles	1	0.1	1
Micelles	10	1	10

Hence, not only do micelles solubilize most fatty acids in the gut lumen (10x greater than vesicles), but the relative flux of micelles compared with molecules of fatty acid in monomeric solution is 142,000:1.

L. Uptake and Fate of Fatty Acids, Monoglycerides and Cholesterol by Absorptive Cells (Figure 7)

1. Uptake mechanism for fatty acids/MG's is unclear - passive diffusion or facilitated transport; a family of intramembrane fatty acid carriers have been described but properties not yet worked out.

2. Fatty acid binding protein – This FABP is a well-documented carrier at inner monolayer of apical membranes. One of the most abundant cytosolic proteins of enterocytes; most likely has a role in fatty acid trafficking to site of TG resynthesis.

3. Cholesterol is absorbed either passively or by facilitated diffusive transport and is then ejected back to the lumen, in part, by ABCG5 and ABCG8 forming a heterodimer on the apical enterocyte membrane — ABCG5 and ABCG8 are regulated by LXR/FXR, a nuclear transcription factor whose ligands are oxysterols.

4. Reesterification of FA and MG to reform TG and Ch with FA to form cholesteryl esters (CE) in endoplasmic reticulum.

5. Addition of several apolipoproteins (apo-B-48 especially) and emulsifying coat of resynthesized lecithin and CE to newly synthesized TG - chylomicron formation.

6. Exit of chylomicra across basolateral membrane to diffuse into villar lymphatics ⟶ thoracic duct.

7. Short/medium-chain fatty acids and MG - no reesterification, no chylomicron formation, diffuse to tributaries of portal vein, transported to liver, bound to albumin.

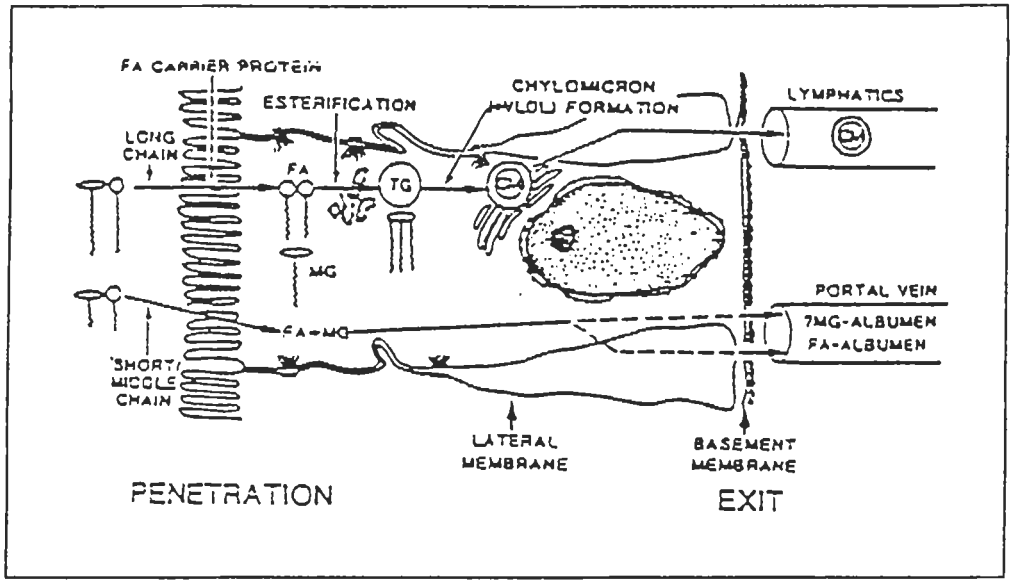

FIGURE 7. Fate of absorbed long- and short/medium-chain fatty acids (FA) and monoglycerides (MG) within intestinal absorptive cells. CM, chylomicron; VLDL, very low density lipoprotein

M. Ultimate Fate of Absorbed Dietary Fat

1. Catabolism of TG in core of chylomicra in vascular space by lipoprotein lipase (Remnant chylomicron with most of the CE taken up by liver)
2. Penetration of capillary and fat cell membranes by FA and MG
3. Resynthesis of TG by tissue lipase
4. Hydrolysis by other tissue lipases of stored TG in fat droplets to supply free fatty acid for metabolic demands

N. Fate of Fat in the Colon

1. Total hydrolysis of glycerides (TG) and other complex lipids such as PL and Ch esters (CE) by microbial lipases. Hence,

   a. TG $\longrightarrow$ 3 FA + glycerol
   b. PL $\longrightarrow$ 2 FA + glycerol + phosphate + choline
   c. CE $\longrightarrow$ 1 FA + Ch

2. Reduction of FA double bonds to form saturated fatty acids
3. Oxidation of fatty acid double bonds to form hydroxy fatty acids (e.g., oleic acid $\rightarrow$ 9-hydroxy stearic acid): potent cathartics
4. Formation of Ca^{2+} and Mg^{2+} soaps (divalent soaps)

5. Formation of Na^+ and K^+ soaps (monovalent soaps)

O. Physical-Chemical and Chemical State of Normal Fecal Fat (4-5 g/24 hrs)

Upon addition of an equal volume of H_2O, homogenization, and centrifugation, feces separate into 3 phases: oil, aqueous, and solid. See table below for the composition of each phase.

Chemical	Phase	% of Total Fat
Fatty Acids (including OH-FA)	oil or solid	70
$Na^+ + K^+$ soaps	aqueous or solid	
$Ca^{2+} + Mg^{2+}$ soaps	crystalline solids	10
Glycerides (TG & DG)	oil	0
Sterols ➢ Neutral (Ch)	solid	15
➢ Acidic (Bile Acids)	aqueous	
Other, PL from bacteria	oil or solid	5

P. Physical-Chemical Basis of Sudan III Test for Fecal Fat

1. Acetic acid conversion of $Mg^{2+}/Ca^{2+}/Na^+/K^+$ soaps to form fatty acids: m.p. of soaps > 250°C, m.p. of FA with identical chains < 85°C.

2. Heat in presence of ethanolic Sudan III. Ethanol evaporates, FA melt to form oils, Sudan III partitions into the oil globules and stains them.

3. Upon cooling, Sudan III precipitates as insoluble powder. FA precipitate as crystals at T < 45°C.

III. Lipid Malabsorption

A. Classification of Malabsorption Syndromes

1. Failure of digestion (intraluminal)
2. Failure of dispersion (intraluminal)
3. Failure of absorption (mucosal)
4. Failure of transport (lamina propria, lymphatics) (rare)

B.Work-Up of Malabsorption

1. Suspect
2. Prove presence of malabsorption: Steatorrhea
3. Distinguish faulty digestion/dispersion from faulty absorption/transport
4. Confirm specific diagnosis
5. Initiate specific treatment

C.Diagnostic Profile:

	Faulty Digestion	Faulty Absorption
Fecal fat	↑	↑
[Chemistry of fecal fat	*TG, DG (only if massive)*	*FA soaps]*
d-xylose	normal	↓
Small bowel x-ray	±	abnormal
Jejunal biopsy	normal	abnormal

D.Specific Examples with Pathophysiology

1. Following "mild" gastric/pyloric surgery (faulty digestion)

   a. Post-vagotomy
   b. Post-pyloroplasty
   c. Post-antrectomy and Billroth I anastomosis:

      1) Pathophysiology:
         i. Rapid gastric emptying (sometimes slow in post-vagotomy)
         ii. Defective gastric lipase action
      2) Gastric pH too high for effective gastric lipase action in post-vagotomy state
      3) Poor emulsification
      4) Loss of controlled release
      5) Poor mixing, protein envelopes of dietary fat not split by pepsin

   d. Note: Mainly chemical steatorrhea (10-20%): Clinical steatorrhea < 5-10%: malnutritional problems common

2. Following major gastric/duodenal-jejunal surgery (faulty digestion)

   a. Post-gastrectomy (Billroth II) steatorrhea

      - Defective gastric lipase action
      - Poor emulsification
      - Loss of controlled release
      - Impaired and delayed CCK and secretin release
      - Poor mixing of bile, pancreatic juice and dietary fat

3. *Bile salt deficiency* (termed "faulty digestion"; misnomer, really "faulty dispersion") (Biliary fistula, obstruction, liver disease, ileal resection, disease or bypass, etc.)

   a. Hydrolysis intact but none or poor dispersion of lipolytic products
   b. Absorption occurs by diffusion of monomers of MG and FA from liquid crystalline dispersions - no micellar phase, and vesicles large.
   c. Formation of calcium soaps in jejunum
   d. No micelles → no absorption of lipovitamins, monoglycerides, or Ch but FA absorption does occur

4. *Pancreatic insufficiency* (chronic pancreatitis, pancreatic carcinoma, cystic fibrosis, etc.) - faulty digestion

    a. Low HCO_3^-: duodenal/jejunal pH = 3-5, normal bile acids (glycine conjugates) precipitate
    b. Low lipase concentrations and acid inactivation of pancreatic lipase at pH < 4.0
    c. Low colipase
    d. Low phospholipase A_2
    e. Low Ch esterase (bile salt-dependent lipase)
    f. With defective lipolysis - TG enters the colon (see notes on colon above)

5. *Stasis syndromes with bacterial overgrowth* ("blind loop" syndrome) (mixture of faulty dispersion and faulty absorption)

    a. Peptide bond of conjugated bile salts split by cholyl-glycine and cholyl-taurine hydrolases of anaerobic bacteria. Free (unconjugated) secondary bile acids released.
    b. 7α-dehydroxylation of bile acids

        1. Cholic $\rightarrow$ deoxycholic acid
        2. Chenodeoxycholic $\rightarrow$ lithocholic acid

    c. Due to their high pK'a values, free bile acids may precipitate with fatty acids (enteroliths) or are absorbed rapidly in the jejunum by passive nonionic diffusion, thereby short- circuiting the enterohepatic circulation. Result: bile salt deficiency in jejunum.

    d. Importance of toxic effects of free secondary bile acids and other bacterial metabolites on absorptive membranes.

    e. Anaerobes consume vitamin B_{12} and the sugars of membrane glycoproteins including brush border enzymes, defective B_{12} availability and malabsorption of sugar, peptides and amino acids (in addition to fat) so pan-malabsorption results.

6. *Neonatal (especially premature) malabsorption* (mixture of faulty digestion and faulty dispersion)

    a. Ingesting ruminant or formula milk - no bile salt-dependent lipase
    b. Naso-gastric tube fed - little gastric lipase in stomach
    c. Relative hypochlorhydria - pH of stomach not optimal for gastric lipase
    d. Imature BS synthesis and ileal recptors: Small bile salt pool, jejunal bile salt concentrations < 2 mM
    e. Physiological immaturity of pancreas - low lipase, colipase, phospholipase A_2 and Ch esterase concentrations

7. *Mucosal cell disorders* (faulty absorption)

    Poor penetration - celiac sprue, collagenous sprue, tropical sprue, Whipple's disease; all involve loss of villi, absorptive cells sick.

8. *Lamina propria disorders* (faulty transport)

   Diminished synthetic enzymes for chylomicron and VLDL formation and release: abetalipoproteinemia, celiac sprue, tropical sprue, lymphangiectasias, etc. Mechanical blockage: congenital lymphatic blockage, microfilariasis, etc.

9. Crucial to Remember: Many malabsorption syndromes have a mixed pathophysiological basis

   Examples:

   a. Celiac sprue, Whipple's disease (gut enteropathy plus endocrinopathy; endocrine cells do not release CCK or secretin).

Defects:	1. Intraluminal	→	low [BS] [lipases] - functional GB and pancreatic failure
	2. Penetration	→	membrane surface diminished
	3. Resynthesis	→	cells sick
	4. Transport	→	mononuclear/plasma cells pack the lamina propria

   b. "Blind loop" syndrome (bile salt deficiency and enteropathy secondary to bacterial and free bile acid damage)

d. Defects:	1. Intraluminal	→	low [BS]
	2. Brush border	→	diminished enzymes
	3. Penetration	→	impaired (injured apical membranes)
	4. Resynthesis	→	impaired from "toxic" bile acids and bacterial toxins

IV. Hormonal Control of Appetite and Weight

A. Incompletely worked out, although the global obesity epidemic is spurring much basic research on this subject.

B. Divided into 1) those that act rapidly (usually via vagal afferents to the brain to influence individual meals) and 2) long term regulators released into blood in proportion to body fat and exert sustained inhibitory effects on food intake while increasing energy expenditure.

1a) Ghrelin – from specific endocrine cells when stomach is empty, travels to brain in vagal afferents, stimulates appetite via receptors on specific neuronal cells in arcuate nucleus of hypothalamus.

1b) Cholecystokinin (CCK); from duodenal/jejunal mucosa during eating, promotes sense of fullness to induce termination of a meal, molecule travels on vagal afferents to specific CCK receptors on brain neuronal cells.

2a) Insulin; from pancreas, augmented via VIP (see above). Released into blood in proportion to dietary intake of both fat and also sugars and in proportion to total body fat. Sustained inhibitory effect on food intake and increases calorie expenditure with dwindling levels sensed by the brain to increase appetite and metabolic efficiency.

2b) Leptin; from engorged adipocytes when body stores of fat are full; signals brain via blood stream through leptin receptors on specific neuronal cells of hypothalmus to decrease food intake over long term.

c) PYY_{3-36}; this member of the neuropeptide Y (NPY) protein family is secreted by bowel endocrine cells lining the distal ileum and colon. Levels increase in response to food, several hours after ingestion of meal. Thereby, blood levels are highest between meals. Experimentally high levels inhibit eating for up to 12 hours in both humans and rodents.

All these hormones involve specific neuronal cells and specific receptors in the arcuate nucleus of the hypothalmus, principally for long acting hormones by the blood stream and short acting hormones via vagal afferents. (See reference 16 in citation list for the neuronal ensembles and receptors that act as "accelerators" and "breaks" in response to peripheral GI signaling hormones to control food intake).

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