Chapter 7
Digestion and Absorption of Dietary Triglycerides

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1 Introduction

The mechanisms for digestion and absorption of dietary triglycerides have been extensively studied, reflecting the importance of triglycerides in normal human nutrition and in disease states. The Western diet contains a large amount of triglycerides, typically 60–120 g/day. Normally, 95 % or more of consumed triglycerides is digested and absorbed, providing 30–40 % of the total energy requirement. In addition, essential polyunsaturated fatty acids contained in dietary triglycerides are the precursors for important lipid-derived mediators such as prostaglandins and leukotrienes involved in regulation of diverse cellular functions. The processes for triglyceride digestion and absorption also significantly influence the efficiency of absorption of other important dietary lipids such as cholesterol and the fat-soluble vitamins.

The **stomach**, **liver**, **biliary tract**, **pancreas**, and **small intestine** all participate in normal triglyceride digestion and absorption. Inadequate triglyceride digestion and absorption result in excessive loss of fat in the stool (steatorrhea). The presence of *steatorrhea* is therefore the hallmark of a diverse group of digestive disorders that cause malabsorption. Dietary triglycerides also play important pathogenetic roles in some of the most prevalent diseases in Western society, including *obesity*, *atherosclerotic cardiovascular disease*, and several common types of *cancer*.

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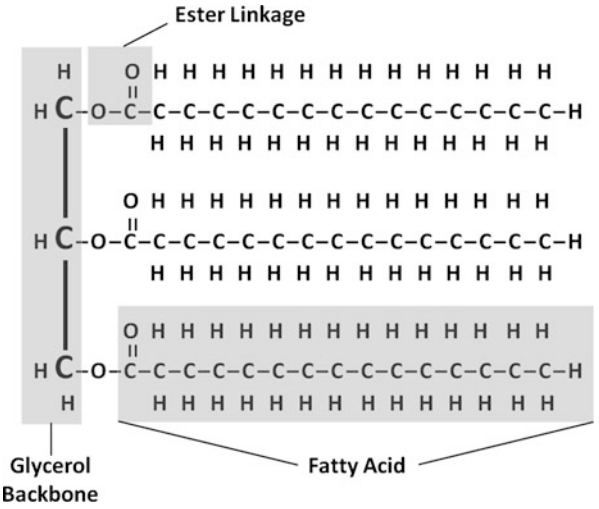

Fig. 7.1 Basic structure of triglycerides

2 Structure of Triglyceride

Dietary triglycerides consist of **three fatty acids** attached by ester linkage to a **glycerol backbone**, forming **triacylglycerol** (Fig. 7.1). Most dietary triglycerides are composed of various **long-chain** (C_{14}-C_{22}) saturated or unsaturated fatty acids, with only small amounts of triglycerides containing **medium-chain** (C_6-C_{12}) or **short chain** (less than C_6) fatty acids. Polyunsaturated fatty acids tend to be preferentially located in the 2 or β position of the glycerol backbone.

3 Triglyceride Digestion

3.1 Intragastric Events

Several important steps in triglyceride digestion occur within the stomach. **Lipase** activity is readily detected in the gastric fluid, and in normal adult humans appears to account for 10–30 % of triglyceride hydrolysis. Lipase is produced in **serous glands** at the ***base of the tongue*** or in **acinar cells** in ***pharyngeal tissue***, and the lipase is then swallowed along with the food bolus. Lingual lipase generating free fatty acids in the mouth and pharynx may play a role in **gustatory fatty acid signaling** (**fatty acid taste**). In humans, the lipase found in gastric fluid appears to originate mainly within **chief cells** of the ***gastric mucosa***. Secretion of gastric lipase can be stimulated by cholinergic agents, gastrin, and possibly other gastrointestinal hormones such as *cholecystokinin*. Gastric lipase has a **broad pH optimum** (**pH 2–6**) and is **resistant to pepsin** and therefore active within the environment of the stomach. Gastric lipase

emptied into the duodenum continues to be active within the environment of the small intestine, although pancreatic lipase is the enzyme responsible for most of the triglyceride hydrolysis (see below). Gastric lipase preferentially hydrolyzes the ester bond in the 3 position of triacylglycerol, generating mainly fatty acids and diglycerides.

Within the stomach, there is acid-peptic digestion of the protein component of food lipoproteins and liberation of oil droplets. The churning action of gastric contractions against the closed pylorus results in the formation of an emulsion containing small lipid particles (less than 2 nm) that can then be emptied from the stomach into the duodenum. These small oil droplets with a large surface-to-volume ratio are the preferred substrate for the **colipase-pancreatic lipase enzyme system** that is responsible for the majority of triglyceride digestion in adults (see below).

3.2 Hormonal Regulation of Biliary and Pancreatic Secretion

As the gastric fluid empties into the duodenum, there is initiation of a coordinated series of events designed to properly deliver biliary and pancreatic secretions into the small intestine to continue the process of triglyceride digestion. Exposure of the duodenal and upper jejunal mucosa to gastric acid stimulates secretion of the peptide hormone **secretin** from neuroendocrine cells in the mucosa into the portal circulation. Secretin, in turn, stimulates ***production of a bicarbonate-rich fluid*** by the pancreatic ductular cells. Delivery of this alkaline pancreatic fluid into the duodenum partially neutralizes the gastric acid secretion. The pH of the upper small intestine is therefore adjusted to approximately pH 6.5, which is optimal for the activity of the colipase-pancreatic lipase enzyme system. Fatty acids and certain amino acids in the intestinal fluid stimulate the release of a second peptide hormone, **cholecystokinin** (**CCK**) (also called **pancreozymin**) from the mucosa into the portal circulation. CCK induces ***digestive enzyme secretion*** by the pancreatic acinar cells, including the secretion of *pancreatic lipase* and *colipase*. In addition, CCK also stimulates ***contraction of the gallbladder*** and ***relaxation of Oddi's sphincter***, resulting in the delivery of concentrated bile into the small intestine. Secretin, CCK, and other hormones produced in the intestinal mucosa also ***regulate gastric motility***, preventing excessively rapid delivery of the food bolus into the intestine and overwhelming of the digestive processes. For a detailed discussion of the regulation of gastric emptying, pancreatic, and biliary secretion, see Chaps. 3, 4 and 12.

3.3 Pancreatic Lipase

The enzyme responsible for most of the triglyceride digestion in adult humans is pancreatic lipase. This 449-amino-acid glycoprotein is secreted from the pancreas in its active form. Pure pancreatic lipase has a pH optimum of pH 8–9, but in the

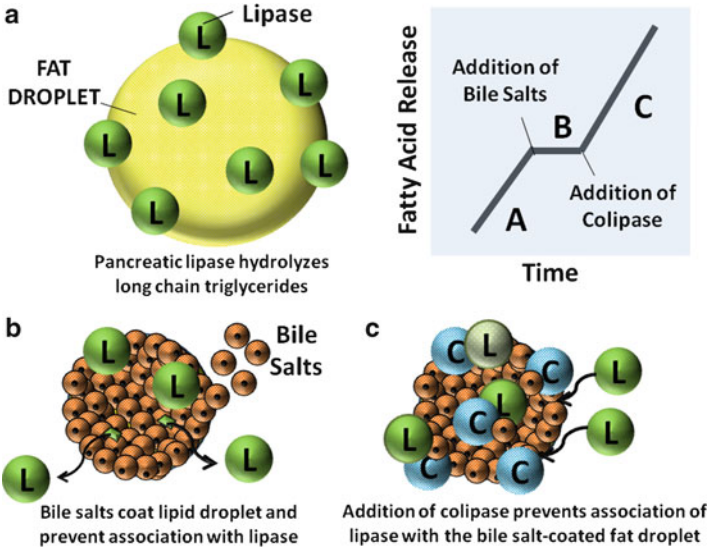

Fig. 7.2 Actions of pancreatic lipase, bile salts and colipase in fat digestion. (**a**) Pancreatic lipases break down triglycerides. (**b**) Bile salts coat lipid droplet and displace lipases. (**c**) Addition of colipases anchor lipases to bile salt-coated fat droplet (Adapted from American Gastroenterological Association Teaching Slide Collection 19 ©, Bethesda, Maryland, slide 29; Used with permission)

presence of bile salts the pH optimum is reduced to pH 6–7, the typical pH of the upper small intestine. At **acid pH** (less than pH 4.0), pancreatic lipase is ***irreversibly inactivated***; therefore, neutralization of gastric acid by bicarbonate in pancreatic juice is essential for preserving enzyme activity. Pancreatic lipase is an **interfacial enzyme** that is most active at an oil-water interface and thus efficiently hydrolyzes triglyceride present in the small lipid droplets that are emptied from the stomach into the small intestine. In the small intestine, the oil droplets are coated on their surfaces with bile salts, phospholipids and other compounds that inhibit the binding of pure pancreatic lipase (Fig. 7.2). In order to achieve effective association of pancreatic lipase with the surface of these oil droplets, a protein cofactor known as **colipase** is required which forms a complex with pancreatic lipase and bile components that associate with lipid droplets. Colipase is secreted into pancreatic fluid as a proprotein, and an amino-terminal pentapeptide is cleaved by **trypsin** in the small intestinal fluid to produce the 96-amino-acid form that can associate with the lipid droplet. In addition, fatty acids also appear to facilitate association with pancreatic lipase and the oil-water interface; therefore **partial triglyceride hydrolysis** by gastric lipase may be an important mechanism to enhance the efficiency of the colipase-pancreatic lipase enzyme system. The amounts of both pancreatic lipase and pro-colipase in pancreatic fluid are increased by dietary fat and secretin, and fat feeding increases the mRNA abundances for both pancreatic lipase and pro-colipase in the pancreatic acinar cells.

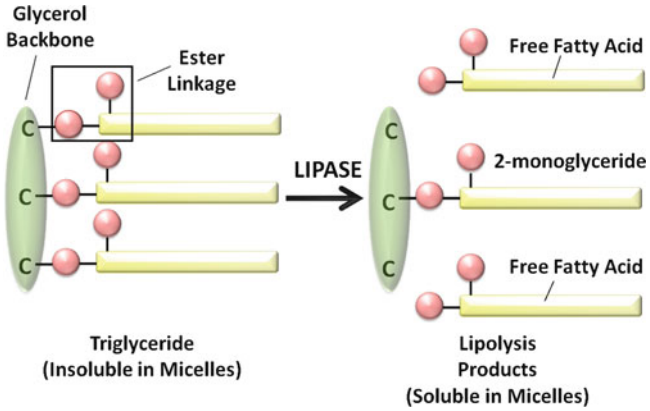

Fig. 7.3 Digestion of triglyceride by pancreatic lipase (Adapted from American Gastroenterological Association Teaching Slide Collection 5 ©, Bethesda, Maryland, slide 10; Used with permission)

Pancreatic lipase preferentially hydrolyzes the first and third ester bonds in triglyceride, forming **fatty acids** and **2- or β-monoglycerides** (Fig. 7.3). The colipase pancreatic lipase system is highly efficient, and triglyceride digestion is nearly complete within the first 100 cm of the proximal jejunum. There is a great excess of pancreatic lipase in pancreatic secretions, and lipase activity must be less than 10 % of normal for triglyceride absorption to be impaired and for steatorrhea to occur.

3.4 Other Lipases

In addition to pancreatic lipase, pancreatic secretions contain another lipase that requires bile salts for activity. In contrast to pancreatic lipase, which has a high degree of substrate specificity for triacylglycerol, **bile salt-activated lipase** will catalyze the hydrolysis of carboxyl ester bonds not only in acylglycerols, but also in other dietary fats such as cholesteryl esters, fat-soluble vitamin esters, and phospholipids. Human bile salt-activated lipase, which has a molecular weight of approximately 100 kd, represents about 4 % of total pancreatic juice protein. Bile salts likely induce conformational changes in the enzyme that enhance access of the active site to bulky lipid substrates and provide additional lipid-binding capability. **Trihydroxylated bile salts**, such as *cholate*, *taurocholate*, or *glycocholate*, are more potent activators than **dihydroxylated bile salts**, such as *taurochenodeoxycholate*; and the 7a-hydroxyl group of bile salts is extremely important for activation. Bile salt-activated lipase catalyzes the complete hydrolysis of triglyceride to fatty acids and glycerol. Patients with ***congenital deficiency of pancreatic lipase or colipase***

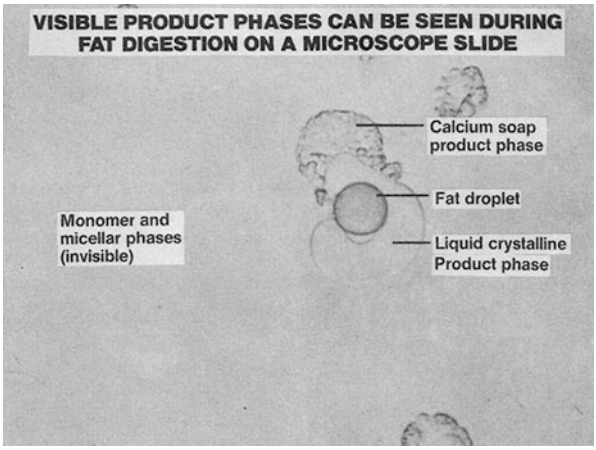

Fig. 7.4 Product phases of fat digestion seen by light microscopy (Adapted from American Gastroenterological Association Teaching Slide Collection 19 ©, Bethesda, Maryland, slide 31; Used with permission)

have been described, and although they have steatorrhea, some fat digestion and absorption continue due to the lipolytic activities of the gastric and bile salt-activated lipases.

Human milk contains a bile salt-activated lipase that is essentially identical to the pancreatic enzyme, differing principally in the glycosylation pattern. The concentration of bile salt-activate lipase in milk is approximately 100 mg/L. Because of the enzyme's bile-salt requirement, the enzyme will not catalyze hydrolysis of lipids in breast tissue or milk until it reaches the duodenum and is exposed to bile.

In adults, pancreatic lipase has 10–60 times the lipolytic activity of bile salt-activated lipase and is responsible for the majority of triglyceride digestion. In the neonate and especially in premature infants, however, pancreatic function is immature, and secretion of pancreatic lipase is inadequate to support efficient triglyceride hydrolysis. In addition, the colipase-pancreatic lipase system is relatively inefficient in digesting milk-fat globules. **Milk bile salt-activated lipase** and **gastric lipase** (which is present in neonates at about the same level as adults), therefore, play important roles in ensuring adequate triglyceride digestion during early development. Other **pancreatic lipase-related proteins** (**PLRP1 and 2**) in pancreatic juice may also play a role in neonatal triglyceride digestion. As noted above, the generation of some fatty acids by the actions of milk bile salt-activated lipase and gastric lipase on milk triglycerides will increase colipase binding to the lipid droplet and to pancreatic lipase, thereby enhancing pancreatic lipase activity.

3.5 Phase Contrast Studies

The process of triglyceride digestion has been studied *in vitro* using **phase contrast microscopy** (Fig. 7.4). Small oil droplets are suspended in buffer containing concentrations of bile salts and phospholipids similar to those in intestinal fluid, and hydrolysis is initiated by addition of pancreatic juice. Initially, one observes

the formation of calcium fatty acid soaps on the surface of the lipid droplet, which, if unchecked, would limit access of colipase and lipases to the surface of the droplet and limit further lipolysis. Formation of calcium fatty acid soaps, however, is inhibited by β-monoglycerides. This has been offered as a potential teleologic explanation for the structural specificity of pancreatic lipase, as the partial breakdown of triglyceride to fatty acids and β-monoglyceride would limit production of fatty acid soaps and favor continued digestion of the lipid droplet.

The **second**, **or viscous isotropic**, **phase** involves the formation of liquid crystals containing protonated fatty acids and monoglycerides. Continued accumulation of fatty acids and monoglycerides on the surface of the lipid droplet, however, would result in product inhibition of the lipases and would limit lipolysis. The products of triglyceride digestion, however, are removed from the droplet surface by formation of vesicles and mixed micelles (see below).

4 Triglyceride Absorption

4.1 Bile Salts and Micelle Formation

Bile salts are a family of **amphiphilic** compounds that are important for the efficient absorption of the products of triglyceride hydrolysis. At concentrations of approximately l–5 mmol/L, bile salts form **micelles**, macromolecular aggregates containing up to 20 or more bile salt molecules. These disc or spherical shaped structures have the polar aspect of the bile salts facing the outside of the structure and the hydrophobic portion pointing toward the interior of the micelle. Fatty acids and other lipids are incorporated between the bile salts and in the interior of these structures, forming **mixed micelles** (Fig. 7.5). This results in a remarkable increase

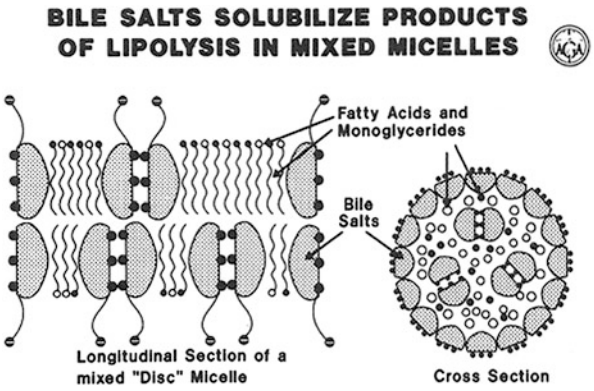

Fig. 7.5 Structure of a mixed disc micelle (Adapted from American Gastroenterological Association Teaching Slide Collection 19 ©, Bethesda, Maryland, slide 35; Used with permission)

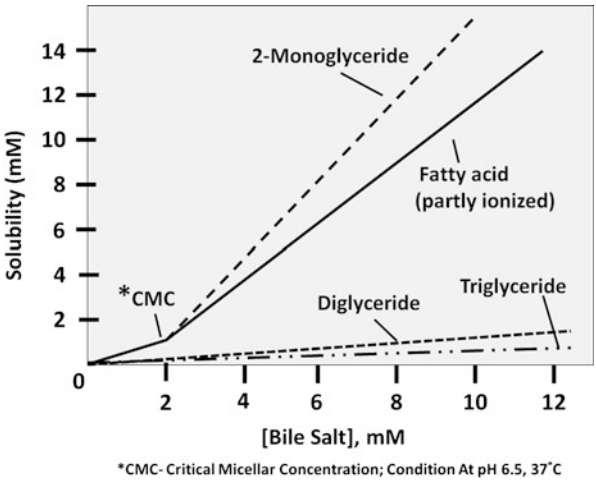

Fig. 7.6 Solubility of fat digestion products in bile salt solution (Adapted from American Gastroenterological Association Teaching Slide Collection 19 ©, Bethesda, Maryland, slide 39; Used with permission)

in the solubilization of fatty acids, monoglycerides, and other lipids in the aqueous environment of the luminal contents (Fig. 7.6). It should be noted, however, than even in the complete absence of luminal bile salts, some fatty acid absorption (approximately 50–75 %) still occurs. Some data indicate that unilamellar vesicles formed in the intestinal contents at low bile salt concentrations may be a vehicle for some fat absorption. Adequate luminal bile salt concentration for micelle formation, however, is required for the normal highly efficient (95 % or greater) absorption of dietary triglycerides.

4.2 Uptake of Fatty Acids and β-Monoglycerides into the Enterocyte

Along the apical brush-border membrane surface of the enterocytes is a layer of structured water known as the **unstirred water layer**. Because of hydrophobic nature of fatty acids and β-monoglycerides, the unstirred water layer has been considered to represent the major permeability barrier limiting the rate of uptake of the products of triglyceride hydrolysis into the intestinal cells. The **rate of flux of a lipid** across the unstirred water layer will be determined by the product of a **diffusion rate constant** and the **concentration gradient** (Table 7.1). Because of their large size, the diffusion constant of mixed micelles is somewhat smaller than that of monomeric fatty acids in solution. This, however, is more than offset by the marked increase in the concentration of fatty acids achieved in mixed micelles compared with that possible with monomeric fatty acids in aqueous solution. The flux of fatty acids across the unstirred water layer in mixed micelles is therefore

Table 7.1 Effect of micellar solubilization of fatty acid on diffusive flux through unstirred water layer[a]

From	Fatty acid concentration (mmol/L)	D × 10^4 (cm^2/s)	J × 104 (mmol/L/cm-s)	Relative flux
Molecules	0.01	7	0.07	1
Micelles	10	1	10	142

[a]Fatty acid concentration x diffusion constant (D) = flux (J). All values are approximate

estimated to be more than 100 times greater than that achieved with fatty acid monomers. The fate of the mixed micelles at the apical brush-border membrane surface is still somewhat unclear. The most accepted theory is that the micellar fatty acids are in equilibrium with monomeric fatty acids in the unstirred water layer and that the monomeric fatty acids then cross the brush-border membrane.

The uptake of fatty acids across the enterocyte brush-border membrane occurs by both **passive diffusion** and by **protein-mediated mechanisms**. Protonate long chain fatty acids diffuse across the brush-border membrane by "flip flopping" through the membrane lipid bilayer down a steep concentration gradient. Several proteins have been identified that potentially play roles in fatty acid uptake. **CD36** is a 472 amino acid heavily glycosylated transmembrane protein that appears to be involved in fatty acid uptake in many tissues. In the small intestine, it is mostly expressed on the brush-border membranes of the ***duodenum*** and ***jejunum***, with little expression in the ileum or colon. Enterocytes prepared from the jejunum of CD36 knockout mice demonstrate reduced fatty acid uptake compared with wild-type controls. CD36 knockout mice, however, do not have significant fat malabsorption, although fat absorption is shifted to the more distal small intestine. Current observations indicate that CD36 mainly plays a regulatory role in **intestinal triglyceride synthesis** and **chylomicron production** (see below). CD36 is also expressed in ***lingual taste buds*** where is mediates perception of fatty acids, and may be involved in satiety signaling. **FATP4** is high expressed in the ***small intestine***, but is localized to the endoplasmic reticulum and subapical membranes. It, therefore, does not function as a true membrane transporter, but facilitates fatty acid uptake by virtue of its **acyl CoA synthase** activity. This results in metabolic trapping of fatty acids via conversion to fatty acyl CoA products that undergo rapid conversion to complex lipids, maintaining a concentration gradient for fatty acid uptake. Other candidate proteins involved in enterocyte fatty acid uptake (FABPpm, etc.) have been identified, but their role has not been completely elucidated. Further research is needed to define the relative contributions of diffusion and protein-mediated transport to enterocyte fatty acid uptake.

Studies of the intestinal uptake of **β-monoglyceride**, the other major lipolytic product, indicate that uptake is saturable and reduced by trypsin digestion of the cell membrane, implying a role for a membrane protein in β-monoglyceride transport. β-monoglyceride has been found to inhibit fatty acid uptake, suggesting coordinated transport of these lipids.

4.3 Intracellular Triglyceride Transport

Cytosolic Fatty Acid-Binding Proteins

Within the enterocyte, the lipolytic products, **fatty acids** and **β-monoglycerides**, are directed to the smooth endoplasmic reticulum for resynthesis of triglyceride and other complex lipids, such as phospholipids and cholesteryl esters. These processes are thought to involve the association of the absorbed fatty acids with low molecular weight cytosolic **fatty acid binding proteins** (**FABPs**). The small intestine contains at least **two cytosolic FABPs**, ***I-FABP*** and ***L-FABP***, named after the organs (intestine and liver, respectively) in which they were first identified. These proteins are expressed at high levels (1–2 % of cytosolic protein) and their respective mRNAs represent about 3 % of total small-intestinal mRNA. The amino acid structures of these proteins have been deduced from cloned DNA sequences, and they belong to a large gene family of cytosolic lipid-binding proteins. The mRNA abundances for both I-FABP and L-FABP increase around birth in the rat and achieve maximal expression in the adult. Both I-FABP and L-FABP are expressed only in villus cells, and expression is highest in the jejunum and declines distally.

The roles that these cytosolic fatty acid binding proteins play in intracellular lipid transport are uncertain, but proposed **functions** include:

1. Removal of fatty acids from the brush-border membrane and maintenance of the concentration gradient for fatty acid influx;
2. Protection of cellular organelles from toxic effects of free fatty acids;
3. Direction of fatty acids to the smooth endoplasmic reticulum for triglyceride synthesis and
4. Targeting fatty acids toward oxidation.

Mouse models with knockout of either fatty acid binding protein have normal fat absorption, but L-FABP knockouts have reduced chylomicron secretion and I-FABP knockout mice show weight loss compared to wild-type animals.

Triglyceride Synthesis in Enterocytes

Fatty acids in the enterocyte are converted to their acyl-CoA derivatives that are used for complex lipid synthesis by enzymes that are specific for long-chain or very long-chain fatty acids. There is evidence that the synthetase ACLS5 delivers acyl-fatty acids for triglyceride synthesis whereas the enzyme ACLS3 channels acyl-fatty acids for phospholipid production. Within the smooth endoplasmic reticulum there is the re-synthesis of triglyceride on the smooth endoplasmic reticulum via **two metabolic pathways** (Fig. 7.7). The ***major pathway*** that accounts for approximately 75 % of triglyceride secreted from the intestine involves the generation of diglycerides from fatty acyl-CoA and β-monoglyceride by **monoglyceride acyltransferase** (**MGAT**) **enzymes**. A ***minor pathway*** for diglyceride production

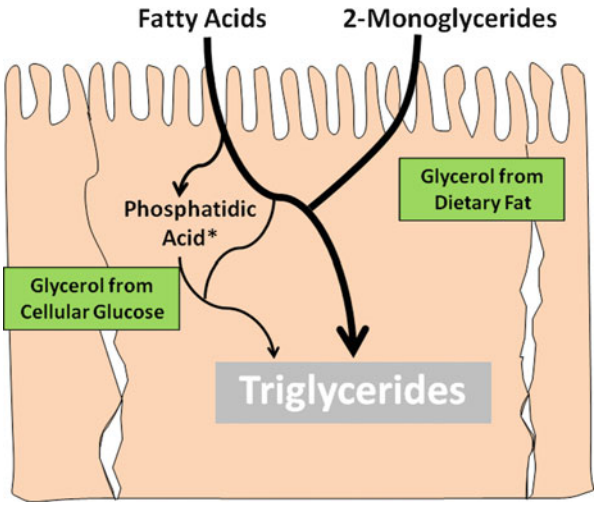

Fig. 7.7 Pathways of triglyceride synthesis in enterocytes

utilizes phosphatidic acid that is produced from α-glycerol phosphate derived from glycosis or absorbed glycerol. The phosphatidic acid is then dephosphorylated to produce diacylglycerol.

Fatty acyl-CoA is then transferred to diglyceride to form triglycerides by **diacylglycerol acyltransferases** (**DGATs**). The intestine expresses **two DGATs**, ***DGAT1*** and ***DGAT2***. DGAT1 is thought to be responsible for most of the triglyceride secreted from the intestine, and DGAT1 knockout mice have reduced chylomicron triglyceride secretion. The two DGATs have different subcellular localization, with the active site of DGAT2 facing the cytosolic side of the smooth endoplasmic reticulum, whereas the active site of DGAT1 faces the lumen of the endoplasmic reticulum. Some MGATs also have DGAT activity.

4.4 Assembly of Chylomicrons

The small intestine is the site of synthesis of several classes of **lipoproteins**, which are macromolecular aggregates of lipids (triglyceride, cholesterol, cholesteryl ester, phospholipid, etc.) and proteins known as **apolipoproteins**. These lipoproteins are characterized on the basis of their flotation density, charge, and size. The ***core*** of these lipoproteins is composed of triglyceride, cholesteryl esters, and other lipids, whereas the apolipoproteins, phospholipid, and free cholesterol are arrayed on the ***surface***. **Chylomicrons**, the largest lipoprotein class, are responsible for delivery of most of the absorbed triglyceride into the circulation. Two proteins are keys to the assembly of chylomicrons in the enterocyte, **apoB48** and the **microsomal triglyceride transfer protein** (**MTTP**). ApoB48 is the structural protein on the surface of chylomicrons. MTTP facilitates chylomicron production by shuttling triglycerides from within the endoplasmic reticulum to associate with apoB48.

ApoB48 is synthesized in the endoplasmic reticulum and is translocated to the endoplasmic reticulum lumen while still attached to the polysome. The amino terminus of apoB48 contains of region for interaction with MMTP, resulting in partial lipidation of apoB48 and organization into a primordial particle within the ER. Optimal folding of apoB48 requires physical interaction with MMTP which prevents apoB48 degradation. Binding of phospholipid to apoB48 also is important for subsequent for chylomicron secretion. Additional triglyceride is extracted from the endoplasmic reticulum membrane by MMTP, and brought to lipid droplets in the endoplasmic reticulum membrane lumen. These lipid droplets acquire phospholipids, cholesterol, and apolipoprotein AIV, and subsequently merge with the nascent apoB48-containing particle, resulting in expansion of the particle. The critical role of MMTP in transfer of triglyceride to the endoplasmic reticulum is demonstrated by the observation that MMTP knockout mice have large cytosolic lipid droplets, but no lipid droplets in the endoplasmic reticulum.

MMTP is a 97 kDa heterodimeric protein that is complexed with the endoplasmic reticulum chaperone protein disulfide isomerase. In addition to its primary role of transferring triglycerides to apoB48, MTTP also shuttles other lipid classes such as cholesteryl esters, free cholesterol, and phospholipids for lipoprotein formation. Loss of function mutations of MMTP results in the disease **abetalipoproteinemia**, an ***autosomal recessive*** disorder in which the heterozygous parents have normal plasma apoB levels. Affected children have very low plasma apo B, mild fat malabsorption, accumulation of triglyceride within enterocytes and hepatocytes, and neurologic and hematologic abnormalities secondary to vitamin E malabsorption and deficiency.

4.5 Intestinal Apolipoproteins

The term **apoB** refers to two large, hydrophobic proteins that are components of triglyceride-rich lipoproteins and their metabolic products. These two proteins, products of a single gene located on chromosome 2, are denoted on a centile scale as **apo B100** and **apo B48**. Apo B100 is produced in the liver and is the form present on **very low-density lipoproteins** (**VLDL**) and their metabolic products **intermediate-density lipoproteins** (**IDL**) and low-density lipoproteins (LDL). Apo B100 plays an important role in lipoprotein metabolism, as it is a ligand for the LDL receptor. Apo B48, which corresponds to the amino-terminal 48 % of apo B100, is produced in the intestine and is the major lipoprotein of chylomicrons.

The regulation of the tissue-specific production of **apo B48** in the ***intestine*** and **apo B100** in the ***liver*** has been extensively studied. In brief, the genomically-encoded mRNA contains as CAA codon encoding **glutamine**, which is modified in the intestinal transcript to UAA, an in-frame **stop codon**. This results in the termination of protein synthesis and the production of the smaller molecular form apo B48. This overall process is now referred to as **apoB-mRNA editing**. Apo B-mRNA editing in the intestine is mediated by a complex that contains

apobec-1, the catalytic deaminase, and an obligate RNA binding subunit, **apobec-1 complementation factor**. Aobec-1 knockout mice that have apoB100 in their chylomicrons have less efficient triglyceride absorption with fewer, larger chylomicron particles. In addition, there is evidence that apoB48 may protect the intestine from lipotoxicity when fat intake is high or MTTP content is reduced. ApoB-mRNA editing has several other important functional consequences. The region of apoB100 responsible for interaction with the LDL receptor is in the carboxyl-terminal portion that is not present in apoB48. Apo B48–containing chylomicron remnants are, therefore, not taken up via the LDL receptor, but instead are believed to undergo **receptor-mediated internalization** predominantly into hepatocytes via another receptor recognizing apolipoprotein, **apo E**. This results in significantly different plasma half-lives of LDL (2–3 days) versus chylomicrons (30 min). The carboxyl-terminal portion of apo B100 also contains the attachment site for another apolipoprotein known as **apo** (**a**). This protein is a member of the **plasminogen multigene family**, and attachment of apo (a) to apo B100 produces a lipoprotein referred to as **Lp**(**a**), which is an important, genetically determined risk factor for atherosclerotic disease.

The intestine is the site of other apolipoproteins that play crucial roles in the assembly and secretion of lipoproteins by enterocytes and are the major regulators of the metabolism and uptake of circulating lipoproteins by peripheral tissues. **Apo AI** is one of the most abundantly expressed apolipoproteins, as its mRNA represents 1–2 % of total intestinal mRNA. Apo AI is a 243-amino-acid protein with a molecular weight of approximately 28 kd. In addition to being an important protein component of **chylomicrons**, apo AI is the major protein of plasma **high density lipoproteins** (**HDL**). Apo AI is a cofactor for the enzyme **lecithin-cholesterol acyltransferase** (**LCAT**), which catalyzes the esterification of plasma cholesterol. It also has an important function in the removal of free cholesterol from cells for incorporation and esterification in HDL and transport to the liver for catabolism or secretion (**reverse cholesterol transport**). The abundance of intestinal apo AI mRNA increases at the time of birth, presumably in connection with suckling, and in adult animals there is a gradient of apo AI expression from the proximal to the distal intestine. The amount of protein, however, does not appear to be modulated by dietary fat intake. With triglyceride feeding, there is a shift of apo AI in the enterocyte to the lipoprotein-associated fraction and an increase in apo AI in intestinal lymph.

Apo AIV is a protein of 377 amino acids with a molecular weight of about 46 kd. It is abundantly expressed in the intestine, constituting about 3 % of protein synthesis. Apo AIV synthesis is highly-inducible by triglyceride feeding, and there is a gradient of expression within rat intestine, with the highest level in ***proximal villus cells***. Apo AIV mRNA increases at birth in response to the initiation of suckling and fat intake, followed by a decline during the suckling period. Apo AIV increases MMTP expression, enhances intestinal triglyceride output by increasing the size of chylomicrons, and increases apo CIII secretion. In plasma, about 25 % of apo AIV leaves chylomicrons and is found in HDL or in the lipoprotein-free fraction. Apo AIV can activate LCAT, but is less active than apo AI, and may

also participate in the transfer of **apo CII**, an **activator of lipoprotein lipase**, from HDL to triglyceride-rich lipoproteins. Apo AIV also appears to function as an **acute satiety factor**.

Apo CIII functions as an **inhibitor of lipolysis** of circulating triglyceride-rich lipoproteins, chylomicrons, and VLDL. Apo C-III displaces apo CII, an activator of lipoprotein lipase, from the surface of these particles. Apo C-II is expressed in the intestine, and synthesis of this protein is stimulated by triglyceride feeding. Evidence concerning the production of other apolipoproteins, apo AII, apo CI, and apo CII, in the intestine is somewhat conflicting, but if they are expressed, it is at very low levels. **Apo D** is found mainly in **HDL** and functions in the exchange of cholesteryl esters between lipoprotein species. The mRNA for apo D has been detected in ***small intestine***.

4.6 Secretion of Chylomicrons

The nascent chylomicron particles leave the endoplasmic reticulum and are transported to the Golgi for secretion into the intestinal lymphatics. Several proteins appear to be involved in the movement of chylomicrons from endoplasmic reticulum to the Golgi, including several ***COPII proteins***, ***L-FABP***, ***CD36***, and ***VAMP7***. **CD36 signaling** via ***Src kinases*** and ***extracellular regulated kinases*** may be important for phosphorylating proteins required for endoplasmic reticulum processing of prechylomicron vesicles. CD36 signaling via a rise in intracellular calcium may also influence multiple events in lipid procession and secretion. **Mutations of the SARA2 gene** that codes SAR1B, one of the COPII proteins, is associated with ***Anderson/chylomicron-retention disease*** in which enterocytes assemble chylomicrons in the endoplasmic reticulum, but fail to transport them through the secretory pathway, causing intestinal lipid droplet accumulation. In the Golgi, apolipoprotein AI and other lipids are added to the particle and there is glycosylation of apolipoproteins. Golgi organelles containing chylomicrons fuse into secretory vesicles, which then move toward the basolateral membrane surface of the enterocytes by a process involving microtubules. These vesicles fuse with the basolateral membrane and release their contents to the extracellular space. The chylomicrons enter the intestinal lacteals and subsequently are transported through the intestinal lymphatics and into the circulation for further metabolism.

As they circulate through the mesenteric lymph and subsequently in the plasma, chylomicrons are modified by exchanging both surface and core components with other lipoprotein classes. Apolipoproteins such as ***apo CII***, an activator of lipoprotein lipase, and ***apoE***, a regulator of cellular lipoprotein uptake, are acquired by the chylomicrons. The enzyme lipoprotein lipase, found on capillary endothelial cells in muscle, adipose tissue, and other tissues, hydrolyzes triglyceride within the core of the chylomicron, forming smaller particles known as **chylomicron remnants**. These remnants undergo receptor-mediated uptake, thought to be mediated via apo E, mainly into **hepatocytes**, completing chylomicron metabolism.

4.7 Intestinal Production of Other Lipoproteins

The small intestine synthesizes and secretes lipoproteins corresponding in size and density to VLDL. The protein and lipid content of these particles is similar to that of chylomicrons, and it is possible that only the amounts of triglycerides and other complex lipids being produced and exported determine particle size. Some investigators, however, have presented data suggesting that intestinal chylomicrons and VLDL have distinct pathways of intracellular assembly.

The small intestine contains and secretes small amounts of LDL-sized lipoproteins. Since these particles have a protein composition similar to intestinal chylomicrons and VLDL and less triglyceride than phospholipid, they are thought to represent **hypolipidated VLDL**. Because the small intestine does not produce apo B100, the characteristic apolipoprotein of LDL, the gut is not felt to significantly contribute to the circulating plasma LDL pool.

HDL particles have been visualized as 6–13-nm spherical lipoproteins within Golgi vesicles of the rat small intestine. **Two populations** were noted: one containing ***apo AI and apo AIV*** as surface components, and the other containing ***apo B48***. These particles were composed mainly of protein and phospholipid, with little triglyceride, and it has been speculated that they are the most under-lipidated precursors to intestinal chylomicrons. **Mesenteric lymph** has been demonstrated to contain both **spherical** and **discoidal HDL** that appears to arise by *de novo* synthesis in the intestine. In addition, HDL particles can be produced following lipolysis of chylomicrons or VLDL in the bloodstream. Figure 7.8 is a summary of assembly and secretion of chylomicrons.

5 Digestion and Absorption of Medium-Chain Triglycerides

Medium-chain triglycerides (**MCTs**), containing fatty acids of 6–12 carbon chain lengths, are present in only small amounts in the normal diet, but they are important components of nutritional supplements used in patients with GI disorders. They are ***useful therapeutic agents*** because the digestion and absorption of MCTs differs significantly from that of typical long-chain dietary triglycerides.

MCTs are hydrolyzed by various lipases, including gastric lipase, bile salt-dependent lipases, and pancreatic lipase, more rapidly than are long-chain triglycerides. The medium-chain fatty acids are much more water-soluble than are long-chain fatty acids and are efficiently taken up into the enterocytes even in the absence of intraluminal bile salts. Some MCTs can even be absorbed intact by the small intestine without requiring prior digestion. Within the enterocytes, medium-chain fatty acids are not utilized for the resynthesis of triglycerides and are therefore not packaged into chylomicrons. Instead, the medium-chain fatty acids are directly released from the enterocytes into the portal circulation, where they are rapidly taken

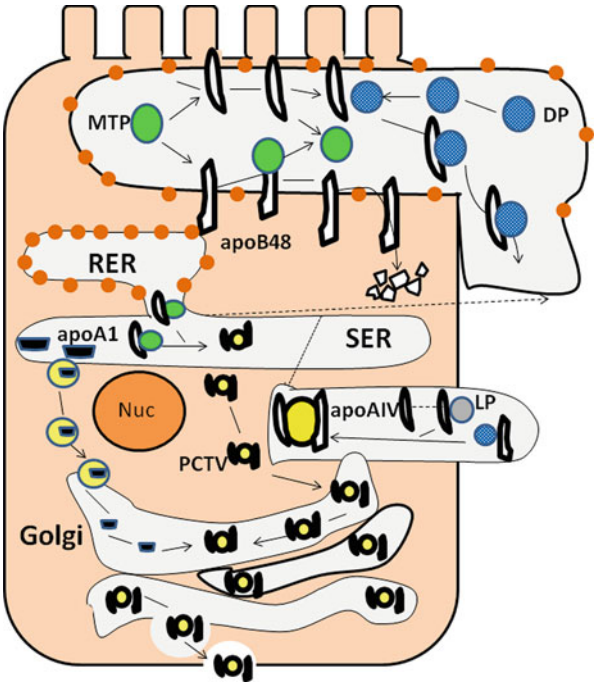

Fig. 7.8 Assembly and secretion of chylomicrons. Apo B48 is synthesized in the rough endoplasmic reticulum (RER). The microsomal triglyceride transfer protein (MTP) stabilizes apo B48, and promotes association with some lipid (DP). Triglyceride synthesized on the smooth endoplasmic reticulum (SER) membrane is brought by MTP to the SER lumen. The triglyceride droplet and apo AIV merge to form a light particle (LP), which fuses with the apo B48-containing particle to form a lipoprotein with a core of neutral lipid surrounded by a surface of apoB48, apoAIV, and phospholipid. This particle buds from the SER, and the prechylomicron transport vesicle (PCTV) fuses with the Golgi. Apo A1 associates to form a mature chylomicron that exits the Golgi and fuses with the basolateral membrane for secretion (Adapted from Mansbach and Gorelick [5])

up by the liver and other tissues and used as an energy source. The differences in digestive and absorptive mechanisms between MCTs and long-chain triglycerides are summarized in Fig. 7.9.

MCTs can therefore be employed as a ***well-absorbed source of calories*** in patients with a wide variety of GI diseases resulting in malabsorption. These disorders include **pancreatic insufficiency**; **intraluminal bile salt deficiency** due to cholestatic liver disease, biliary obstruction, or ileal disease or resection; **mucosal diseases** with impaired intracellular lipid metabolism and chylomicron assembly; and disorders causing **obstruction of intestinal lymphatics**. It must be remembered, however, that MCT preparations do not contain essential polyunsaturated fatty acids, and therefore some long-chain dietary triglycerides are required in patients with these malabsorptive disorders.

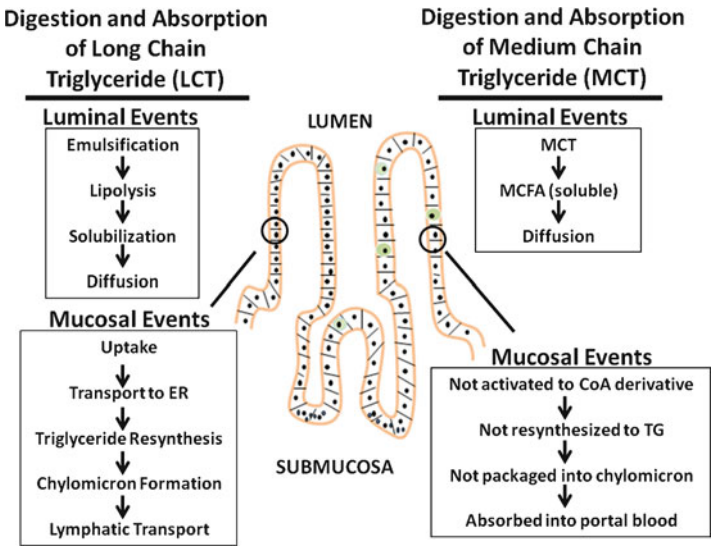

Fig. 7.9 Comparison of digestion and absorption of long-chain and medium-chain triglycerides

Clinical Correlations

Case Study 1

A 10-year-old girl with a history of recurrent pneumonias, chronic diarrhea, and poor growth is found to have cystic fibrosis. On a 100-g-fat diet, she is noted to excrete 40 g of fat in the stool. Analysis of duodenal fluid obtained after stimulation of the pancreas by injection of secretin and cholecystokinin shows undetectable pancreatic lipase. She is started on therapy with bovine pancreatic enzyme extracts that have been pressed into tablets given with meals and snacks; however, she continues to have marked steatorrhea.

Questions

1. **Why was this form of pancreatic enzyme supplementation of little therapeutic benefit?**
   Answer: At an **acid pH** (less than pH 4.0), **pancreatic lipase** is ***irreversibly inactivated***. Therefore, when enzyme supplements are taken by mouth, almost all of the lipase is inactivated by gastric acid. Because of the lack of pancreatic bicarbonate secretion in this patient, the pH of the upper intestinal contents will be low, and pancreatic lipase present in the intestinal lumen will not be functioning at its pH optimum (pH 6–7).
2. **How would you alter the enzyme therapy to achieve better results?**
   Answer: The pancreatic enzyme supplements can be combined with measures to **decrease gastric acid**, such as administration of *sodium bicarbonate* with

meals, or treatment with **antisecretory drugs** such as H_2 *blockers* or *proton-pump inhibitors*. Pancreatic enzyme preparations have been prepared in which the enzymes are contained in **pH-sensitive microspheres** that do not dissolve at acid pH but release the enzymes at neutral pH. These measures will permit adequate delivery of active lipase into the small bowel and greatly improve triglyceride absorption. **Preparations with acid-resident microbial lipases** are being developed for clinical use.

3. **Why is the patient capable of absorbing some dietary triglyceride if her pancreas is secreting minimal pancreatic lipase?**
   Answer: Lingual and gastric lipase activity will be preserved in the patient with cystic fibrosis and accounts for much of the dietary triglyceride digestion. Pancreatic bile salt-activated lipase secretion will likely also be greatly diminished in this patient.

Case Study 2

A 65-year-old man presents with right upper quadrant abdominal pain, diarrhea, weight loss, and jaundice. He is found to have a common bile duct cancer and complete biliary obstruction. On a 100-g-fat diet, he is noted to have 32 g of steatorrhea.

Questions

1. **What is responsible for this patient's fat malabsorption?**
   Answer: Biliary obstruction in this patient will result in ***lack of bile salts*** and ***phospholipids*** in the intestinal lumen. Bile salts are required for the solubilization of large amounts of the lipolytic products, fatty acids and β-monoglycerides, in mixed micelles. Micellar solubilization results in a large concentration gradient for fatty acid and β-monoglyceride flux across the unstirred water layer. Bile salts are also needed for the **activation of pancreatic bile salt-activated lipase** that participates in digestion of triglyceride and other esterified lipids. Biliary phospholipid absorption is required to supply the phospholipid component of chylomicrons.
2. **A nutrition consultant recommends that the patient be put on a supplement containing medium-chain triglycerides to encourage weight gain. What is the rationale behind this therapy**?
   Answer: Medium-chain fatty acids derived from the lipolysis of medium-chain triglycerides are quite **water-soluble**, even in the absence of bile salts. Medium-chain triglyceride supplements would therefore be **well-absorbed** in this patient with biliary obstruction and intraluminal bile salt deficiency.
3. **Would it be advisable for this patient to be on a dietary program containing only medium-chain triglycerides and no long-chain triglycerides?**
   Answer: This patient continues to require some long-chain dietary triglyceride, at least 2–5 % of caloric intake. **Medium-chain triglyceride preparations** will *not* provide essential polyunsaturated fatty acids that are the precursors for prostaglandins, leukotrienes, and other important lipid-derived regulators of cellular function. Essential fatty acid deficiency can develop within a few weeks of dietary deprivation.

Case Study 3

A 2-year-old boy presents with diarrhea, poor growth, and multiple neurologic deficits. He is found to have steatorrhea, very low plasma levels of apo B (both apo B100 and apo B48), cholesterol, triglycerides, and vitamin E. His parents have reduced plasma apoB levels, and a diagnosis of homozygous hypobetalipoproteinemia is made.

Questions

1. **Why does this child have steatorrhea?**
   Answer: Children with homozygous hypobetalipoproteinemia have a **mutation in the apo B gene**, resulting in **defective apo B48 synthesis** in the ***small intestine*** and **apo B100** in the ***liver***. Apo B48 is required for the proper assembly and secretion of chylomicrons. Intestinal biopsies of these children show enterocytes that are packed with triglyceride. Dietary triglyceride digestion, fatty acid absorption, and resynthesis of triglyceride in the intestine proceed normally; however, the triglyceride cannot be properly packaged into chylomicrons for secretion. The triglyceride is lost into the stool as the enterocytes are sloughed as part of normal mucosal turnover.
2. **Why does this child have low plasma levels of both apo B-100 and apo B-48?**
   Answer: Apo B100 and apo B-48 are both **products of a single gene**. Apo B48 is produced as a result of the process of apo B-mRNA editing, where a CAA codon is modified to UAA, an in-frame stop codon that results in the termination of protein synthesis. Children with homozygous hypobetalipoproteinemia have a **mutation in the apo B gene** that causes ***reduced synthesis of both forms of apo B***.
3. **Would medium-chain triglyceride oil be a useful nutritional supplement for this patient? Why?**
   Answer: Yes. Absorbed medium-chain fatty acids are not utilized for the resynthesis of triglyceride in the enterocytes and are therefore not packaged into chylomicron particles. Medium-chain fatty acids are **released from the intestine into the portal circulation**. This child with a defect in chylomicron assembly and secretion would digest and absorb medium-chain triglycerides well, which could **provide an important source of energy**.

Case Study 4

A 40-year-old woman presents to the doctor with chronic diarrhea, a 10 lb weight loss, and a rash on her trunk and arms. Biopsy of the skin rash is diagnostic of dermatitis herpetiformis, and biopsy of the small intestine shows partial loss of the villous architecture and inflammation in the mucosa. Analysis of her stool shows increased fecal excretion of both fatty acids and triglyceride.

Questions

1. **What is the diagnosis of her intestinal disease?**
   Answer: The patient has gluten-sensitive enteropathy (**celiac disease**), a condition in which ingestion of certain grains, such as wheat, rye, and barley,

incites inflammation of the intestine in a patient with genetic susceptibility. The inflammation damages the small intestinal mucosa, and often results in malabsorption of fat and other nutrients. All patients with the skin disease ***dermatitis herpetiformis*** have gluten-sensitive enteropathy, although it is often milder than the damage seen in patients with gluten-sensitive enteropathy who do not have associated dermatitis herpetiformis.

2. **Why does this patient have increased fecal excretion of both fatty acids and triglyceride?**
   Answer: The damaged proximal small intestine will have reduced release of the hormones secretin and CCK in response to a meal, resulting in **diminished secretion of pancreatic fluid and enzymes**. This causes "functional" pancreatic insufficiency, even though the pancreas itself is normal. **Intestinal fatty acid absorption is impaired** because of the damage to the enterocytes, resulting in a reduced small intestinal surface area and lack of the transporters, binding proteins, and enzymes involved in fat absorption. **Bacterial metabolism of malabsorbed triglyceride** in the distal bowel also contributes to the increased fatty acid excretion.
3. **What is the treatment for this patient? Does she need a fat-restricted diet?**
   Answer: This patient should be placed on a **gluten-free diet**, eliminating foods containing wheat, rye, and barley. For most individuals, this results in resolution of the intestinal inflammation and return of the intestinal structure and function to normal. A minority of patients with gluten-sensitive enteropathy will require treatment with **immunosuppressive medication** to heal the mucosal injury. **Dietary fat restriction** is only needed in a small minority of patients to decrease steatorrhea and diarrhea while the intestine heals. Dietary fat restriction is not required in patients when the intestine has healed in response to a gluten-free diet.

Further Reading

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