Intestinal lipid absorption

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Dietary fats consist of a wide array of polar and nonpolar lipids (32, 33). Triacylglycerol (TAG) is the dominant fat in the diet, contributing 90–95% of the total energy derived from dietary fat. Dietary fats also include phospholipids (PLs), sterols (e.g., cholesterol), and many other lipids (e.g., fat-soluble vitamins). The predominant PL in the intestinal lumen is phosphatidylcholine (PC), which is derived mostly from bile (10–20 g/day in humans) but also from the diet (~1–2 g/day). The predominant dietary sterols are cholesterol (mostly of animal origin) and β-sitosterol (the major plant sterol). Although β-sitosterol accounts for 25% of dietary sterols, it is not absorbed by humans under physiological conditions.

Researchers have been investigating the various steps involved in lipid digestion, absorption, and metabolism to identify factors that could serve as targets for the development of drugs capable of reducing the risk of lipid-associated disorders, including dyslipidemias and cardiovascular disease. In so doing, they have explored key aspects of lipid digestion hydrolysis, emulsification, and micelle formation. They have also explored key issues of absorption, e.g., the uptake of the products of lipid hydrolysis by enterocytes (the epithelial cells lining the walls of the intestinal lumen) and their transport to intracellular compartments, where fatty acids and sterols are transformed into neutral lipids. Efficient absorption ensures that dietary fat is available to be used as a source of energy that supports various cellular functions or to be stored and serve as reservoirs for lipoprotein trafficking, bile acid synthesis, steroidogenesis, membrane formation and maintenance, and epidermal integrity in various mammalian cells. Excess fat is stored in cytosolic lipid droplets until it is needed to support intracellular processes.

In this review, we will concentrate on the biochemical processes involved in the digestion and absorption of the most common dietary lipids: TAGs, PLs, cholesterol, and fat-soluble vitamins.

Digestion and Absorption of Lipids: A Brief Overview

The digestion of lipids begins in the oral cavity through exposure to lingual lipases, which are secreted by glands in the tongue to begin the process of digesting triglycerides. Digestion continues in the stomach through the effects of both lingual and gastric enzymes. The stomach is also the major site for the emulsification of dietary fat and fat-soluble vitamins, with peristalsis a major contributing factor. Crude emulsions of lipids enter the duodenum as fine lipid droplets and then mix with bile and pancreatic juice to undergo marked changes in chemical and physical form. Emulsification continues in the duodenum along with hydrolysis and micellization in preparation for absorption across the intestinal wall. (For details about the emulsification, hydrolysis, and micellization of fats, see Refs. 131 and 144.)
Bile and pancreatic juice provide pancreatic lipase, bile salts, and colipase, which function cooperatively to ensure the efficiency of lipid digestion and absorption. The importance of bile to the efficiency of these processes is indicated by the decreased rate of lipid absorption in humans with bile fistulas. The greatly reduced concentration of bile acid in the duodenum of such individuals suggests that bile salts, although possibly not absolutely necessary for digestion, are essential for the complete absorption of dietary fats (150). Elevated concentrations of bile salts have been shown to inhibit pancreatic lipase activity in the duodenum (190). Such inhibition is offset, however, by colipase, which has been shown in vitro to restore pancreatic lipase activity under such circumstances (112, 128).

The importance of colipase in the digestion of fat was indicated in a clinical report of steatorrhea in two brothers with a congenital absence of colipase, which indicated that the steatorrhea decreased with the administration of colipase (76). It has also been demonstrated in colipase-deficient mice (41), which, when placed on a high-fat diet, developed steatorrhea that was so severe that undigested lipids could be seen in their feces. When these mice were placed on a low-fat diet, their ability to digest fat returned to normal. These findings suggest that colipase plays a critical, but not essential, role in the digestion of dietary lipids by pancreatic lipases.

**Triacylglycerides**

Digestion and absorption of TAGs. TAG is digested primarily by pancreatic lipase in the upper segment of the jejunum. This process generates a liquid-crystalline interface at the surface of the emulsion particles (13, 161). The activity of pancreatic lipase on the sn-1 and sn-3 positions of the TAG molecule results in the release of 2-monoglycerol (2-MAG) and free fatty acids (FFAs) (122–124); 2-MAG is the predominant form in which MAG is absorbed from the small intestine. The formation of 2-MAG (and 1-MAG) through isomerization in a aqueous medium occurs more slowly than the uptake of 2-MAG from the small intestine (20). Further hydrolysis of 1- or 2-MAG by pancreatic lipase results in the formation of glycerol and FFAs (78); cholesterol esterase can also hydrolyze the acyl group at the sn-2 position to form glycerol and FFAs (111).

Free fatty acids are taken up from the intestinal lumen into the enterocytes and used for the biosynthesis of neutral fats. A protein-independent diffusion model and protein-dependent mechanisms have been proposed for the uptake and transport of fatty acids (FAs) across the apical membrane of the enterocyte (for details, see Ref. 119). A number of candidate proteins have been proposed to take part in protein-dependent uptake mechanisms. FAT/CD36 plays a key role in the uptake of FAs (1, 19). FAT/CD36 is highly expressed in the intestine (37), and its expression is upregulated by the presence of dietary fat (148), genetic obesity, and diabetes mellitus (64). Studies in CD36-null mice suggest that it is intimately involved in the uptake and transport of FAs targeted for transport to the lymphatic system through their use in the assembly of chylomicrons (45). This relationship is suggested by the finding that the uptake of FAs is not impaired in CD36-null animals. However, lipids tend to accumulate in the proximal small intestine of these animals primarily because of decreased transport of FAs to the lymphatic system (45). FA transport proteins (FATPs) are well represented in the small intestine by their FATP4 isoform, which is thought to help facilitate the uptake of FAs by the enterocytes (162).

**TAG synthesis.** Once inside the enterocyte, the products of TAG hydrolysis must traverse the cytoplasm to reach the endoplasmic reticulum (ER), where they are used to synthesize complex lipids. Specific binding proteins carry FAs and MAG to the intracellular site, where they will be used for TAG biosynthesis. The two major fatty acid-binding proteins (FABPs) found in enterocytes are liver FABP and intestinal FABP (2, 16, 69). Most TAG biosynthesis in the enterocyte occurs along the MAG pathway, in which MAG and fatty acyl-CoA are covalently joined to form diacylglycerol (DAG) in a reaction catalyzed by monoaoylglycerol acyltransferases (MGATs) (40, 197). Further acylation of DAG by diacylglycerol acyltransferase (DGAT) leads to the synthesis of TAG. Two DGATs have been identified and characterized: DGAT1 and DGAT2. [For additional information about DGATs, refer to the recent review (197).] DGAT2 is expressed mainly in the liver and intestine. Its importance in the absorption of fat has been demonstrated by a reduction in fat absorption in DGAT2-knockout (KO) animals. DGAT1, which is expressed in several tissues (including liver, intestine, and skin), differs from DGAT2 in that defective fat absorption is not seen in DGAT1-KO mice. In recent studies, investigators have found that MGAT2 (29, 31) and MGAT3 (30) possess DGAT activity (26, 170). Some TAG synthesis also occurs through the dephosphorylation of phosphatidic acid and acylation of the resultant DAG. Thus, several enzymes take part in the biosynthesis of TAG in intestinal cells.

**Use of TAG in lipoprotein assembly.** The existence of multiple pools of DAG (159, 160) and TAG (51, 55, 60, 104, 175, 183, 195) has been known for some time. Using differentiated human colon carcinoma (Caco-2) cells, Luchoomun and Hussain (115) showed that nascent TAG is used preferentially for chylomicron assembly. TAG is also synthesized de novo from fatty acyl chains and glycerol phosphate. When generated through this pathway, however, TAG is only partly used to assemble nascent apolipoprotein (apo)B-containing lipoproteins. Most of the TAG synthesized through this pathway enters the cytosolic pool to be used to generate a distinct pool of DAG. (For a review of this topic, see Ref. 202.) DAG esterification also occurs in the ER lumen (142), where the resultant TAG binds to the microsomal triglyceride transport protein (MTP), which participates in the assembly and secretion of neutral lipids in chylomicrons. This process is described further in a subsequent section in this review. (For additional information, also see Refs. 18 and 119.)

**Phospholipids**

The predominant PL in the lumen of the small intestine is PC, which is found in mixed micelles that also contain cholesterol and bile salts. The digestion of PLs is carried out primarily by pancreatic phospholipase A2 (pPLA2) and other lipases secreted by the pancreas in response to food intake. These lipases interact with PLs at the sn-2 position to yield FFAs and lysophosphatidylcholine (21, 182). These products of lipolysis are removed from the water-oil interface when they are incorporated into the mixed micelles that form spontaneously when they interact with bile salts. Having both hydro-
philic and hydrophobic components, bile salts are able to facilitate micelle formation; MAG and PL enhance their ability to form mixed micelles. pPLA2-KO mice are indistinguishable from wild-type controls when fed regular chow, except for their resistance to diet-induced obesity (84). Increased excretion of TAG, on a high-fat diet, in the feces of pPLA2-KO mice indicates that pPLA2 deficiency has a greater effect on the digestion of TAG than that of PL hydrolysis (84). It does not affect PL hydrolysis and absorption, possibly because its activity is compensated for by other PLA2 enzymes (158).

Cholesterol

Cholesterol in the body represents both endogenous sources (produced in the liver and peripheral tissues) and dietary sources absorbed from the intestine (186). The human diet provides ~400 mg of cholesterol daily, and the liver secretes ~1 g daily (66, 194). Approximately 50% of the cholesterol in the intestine is absorbed; the remainder is excreted in feces (10, 39).

Digestion. Only nonesterified cholesterol can be incorporated into bile acid micelles and absorbed by enterocytes. Most dietary cholesterol exists in the form of the free sterol, with only 10–15% existing as the cholesteryl ester. The latter must be hydrolyzed by cholesterol esterase to release free cholesterol for absorption. Cholesterol is only minimally soluble in an aqueous environment (82, 177) and thus must be partitioned into bile salt micelles prior to absorption. It usually enters these micelles along with TAGs and PLs, ionized and nonionized FAs, MAGs, and lysophospholipids to form mixed micelles (74, 196). These micelles are transported to the brush border of the enterocyte, where cholesterol is absorbed. Its absorption depends on the presence of bile acids in the intestinal lumen (191) and correlates directly with the total bile acid pool (149).

Absorption: cholesterol uptake. Cholesterol must pass through a diffusion barrier at the intestinal lumen-enterocyte membrane interface before it can interact with transporter proteins responsible for its uptake and subsequent transport across the cellular brush border. Bile salt micelles facilitate the transfer of cholesterol across the unstirred water layer. The mechanism by which the cholesterol in micelles is taken up by the cell and crosses the brush-border membrane is still under investigation. Cholesterol absorption had long been considered an energy-independent, simple, passive diffusion process. However, it is taken up by the enterocyte with relatively high efficiency compared with structurally similar phytosterols (130). New evidence strongly suggests that a transporter-facilitated mechanism is involved. Interindividual differences and interstrain variations in the efficiency of intestinal cholesterol absorption (195, 196) support this suggestion, as does the discovery that multiple genes (4, 14, 106, 113) participate in the regulation of cholesterol absorption and several molecules appear to inhibit it (59).

Absorption: cholesterol transport. Several proteins have been investigated for their potential roles as intestinal cholesterol transporters, but evidence for their direct role in the uptake of cholesterol remains elusive. Studies in genetically modified animal models have helped investigators gain important insights into the mechanisms of transport and identity of transporter proteins. Through these studies, investigators have identified Niemann-Pick C1 like 1 (NPC1L1) as a cholesterol uptake transporter (4) and the ATP-binding cassette (ABC) proteins ABCG5 and ABCG8 as cholesterol efflux transporters (14, 106, 113). These three molecules appear to be key players in the control of the cholesterol absorption from the intestinal lumen.

ABCG5 and ABCG8, which function as a heterodimer (62), are critical for the control of sterol absorption. Mutations in the genes encoding human ABCG5 and ABCG8 transporters cause β-sitosterolemia (14, 106, 113), which is characterized by the accumulation of plant sterols in blood and other tissues as a result of their enhanced absorption from the intestines and decreased removal in bile. These proteins are localized at the canalicular membrane of hepatocytes and at the brush border of enterocytes. Abcg5/g8 deficiency in mice results in reduced biliary cholesterol secretion (199) and enhanced phytosterol absorption (102, 146, 199) but has only minimal effects on the efficiency of cholesterol absorption (146, 199). The pharmacological induction or overexpression of Abcg5 and Abcg8 in mice (199–201) results in a reduction in fractional cholesterol absorption (i.e., the percentage of cholesterol absorbed from the intestine, which is determined using a dual-isotope feeding technique) and indicates that ABCG5 and ABCG8 play a role in the control of cholesterol absorption under certain conditions.

The identification of NPC1L1 as a putative cholesterol transporter in the enterocytes (4) was facilitated by the discovery of the cholesterol absorption inhibitor ezetimibe (4, 59), which reduces diet-induced hypercholesterolemia (49, 56, 103, 187, 203). NPC1L1 is a glycosylated protein localized at the brush-border membrane of the enterocyte (95). The deletion of Npc1l1 in mice results in a reduction in fractional cholesterol absorption (4). Ezetimibe has been shown to bind to NPC1L1-expressing cells and to the intestinal brush border (59). Deletion of Npc1l1 also results in the elimination of the binding capacity of the brush border (59), which indicates that NPC1L1 is a target of ezetimibe.

A sterol regulatory element in the promoter and a sterol-sensing domain of NPC1L1 appear to regulate cholesterol absorption in response to cholesterol intake. Expression of Npc1l1 is enhanced in the cholesterol-depleted porcine intestine and suppressed in mice placed on a cholesterol-rich diet (83). Most of the NPC1L1 in the body is found in intracellular membranes. However, cholesterol deprivation induces its translocation to the plasma membrane, where it can pick up cholesterol and transport it to the ER for esterification and packaging into nascent lipoproteins (50, 198).

Reducing the expression of NPC1L1 at the level of transcription may reduce cholesterol absorption. Activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)α by the synthetic agonist GW610742 has been shown to reduce cholesterol absorption by decreasing Npc1l1 expression without altering the expression of Abcg5 and Abcg8 (185). A decrease in Npc1l1 expression has also been observed following treatment of human colon-derived Caeco-2 cells with ligands for PPARα/β but not for PPARγ or PPARδ (185).

Absorption: other regulatory factors. The nuclear liver X receptors (LXRs) LXRα (expressed mainly in the liver, kidney, intestine, spleen, and adrenals) and LXRβ (expressed ubiquitously) regulate pathways involved in the metabolism of cholesterol and in lipid biosynthesis. LXR target genes have been shown to be involved in cholesterol and lipid homeostasis. (For
a list of target genes and their regulation, see Ref. 174.) After activation by natural ligands (e.g., oxysterols), the LXR forms a heterodimer with the retinoid X receptor (96, 97) and binds to specific LXR response elements in the promoter regions of their target genes to activate gene transcription. LXR target genes include those that express proteins involved in the efflux of cholesterol from the cell (155, 157, 174, 189) as well as bile acid synthesis (174) and lipogenesis (174). Thus, global LXR activation by synthetic agonists has a plethora of effects, including elevated high-density lipoprotein (HDL) levels (25, 28, 98, 126, 147, 163, 178), hypertriglyceridemia (156, 163), hepatic steatosis (65), increased excretion of cholesterol in bile (147, 201), reduced efficiency of cholesterol absorption from the intestine (103, 157, 159, 191, 206), and increased loss of neutral sterols in feces (201).

Other transporters, such as scavenger receptor class B type I (SR-BI), which is localized both at the apical and basolateral membranes of enterocytes (27), have also been suspected of having a role in the control of cholesterol absorption. The possibility that SR-BI is a cholesterol transporter is suggested by the observation that intestine-specific overexpression of SR-BI in mice leads to an increase in cholesterol and TAG absorption in short-term absorption experiments (17). The importance of the FA translocase CD36 (which is also expressed in epithelial cells lining the small intestine) in FA absorption is well established. CD36 has also been implicated as the cholesterol transporter in brush-border membranes. Additionally, overexpression of CD36 in COS-7 cells has been shown to enhance cholesterol uptake from micellar substrates (184). In another study, CD36-null mice showed a significant reduction in cholesterol transport from the intestinal lumen to the lymphatic system (133). Decreased cholesterol uptake has been shown in brush-border membrane vesicles prepared from the proximal (but not distal) intestine of SR-BI-KO mice (184) and in brush-border membrane vesicles and Caco-2 cells preincubated with antibodies to SR-BI (71). However, targeted disruption of SR-BI in mice has little effect on in vivo intestinal cholesterol absorption (3, 120, 192), which suggests that SR-BI might not be essential for absorption of cholesterol from the intestine.

Another potential step in the regulation of cholesterol absorption involves two ER membrane-localized enzymes, acyl-CoA:cholesterol acyltransferase 1 (ACAT1) and ACAT2, that catalyze the esterification of intracellular cholesterol (107). ACAT1 is expressed in many tissues (36, 61), but its level of expression in the mouse small intestine is very low (125). By contrast, ACAT2 expression is restricted to the small intestine and liver (6, 35, 137). ACAT2 is highly specific for cholesterol and does not esterify plant sterols. It is the predominant enzyme responsible for the synthesis of cholesterol esters and their subsequent secretion with lipoproteins. ACAT2 deficiency results in a considerable decrease in the rate of cholesterol absorption (26). The rate of cholesterol esterification in the presence of these enzymes is notably enhanced by substrate availability, but, as we demonstrated recently, it is inhibited by product accumulation. This inhibition is relieved by MTP (94), which transfers cholesterol esters from the ER membranes to nascent apoB-lipoproteins. The importance of MTP in cholesterol absorption has been well documented (85, 86, 89).

Approximately 70–80% of cholesterol entering the lymphatic system is esterified, which suggests that esterification is important for bulk entry of cholesterol into nascent chylomicrons. Reesterification of the absorbed cholesterol within the enterocyte enhances the diffusion gradient to favor the entry of intraluminal cholesterol into the cell and is therefore an important regulator of cholesterol absorption from the intestine. Thus, inhibition of ACAT by pharmacological intervention (38, 73) or deletion of Acat2 (26) significantly reduces the rate of cholesterol absorption.

It has also been suggested that ATP-binding cassette transporter A1 (ABCA1) plays a role in the control of cholesterol absorption. ABCA1 was initially thought to be localized to the apical membrane (157); more recent studies have provided evidence suggesting that it is present in the basolateral membrane of chicken enterocytes (132) and human Caco-2 cells (138). Our studies in Caco-2 cells and in the apoA1-KO mouse model showed for the first time that basolateral efflux (secretion) of cholesterol occurs in high-density apoB-free/apoA-I-containing lipoproteins (Fig. 1) (91, 93). The importance of ABCA1 in the biogenesis of HDL in the intestine was demonstrated by deleting intestinal Abca1, which resulted in a 30% decrease in plasma HDL cholesterol levels (24). Enterocytes deficient in ABCA1 absorb smaller amounts of cholesterol (24). Thus, HDL contributes considerably to cholesterol absorption from the intestine. The origin of HDL particles in mesenteric lymph fluid has been a subject of considerable controversy (11, 53, 117, 139, 152, 169, 188). The measurement of cholesterol in lymph led to the hypothesis that HDL is not important in cholesterol transport. However, Brunham et al. (24) recently demonstrated that the HDL in lymph is dependent on the activity of hepatic ABCA1 and enters the lymph from plasma. Thus, quantification of HDL in lymph may not provide an accurate assessment of the extent of cholesterol absorption via this pathway.

**Vitamin E**

Vitamin E is one of the most abundant lipid-soluble antioxidants found in the plasma and somatic cells of higher mammals. Vitamin E exists in two forms, as tocopherols and tocotrienols, that vary dramatically in terms of biological activity due to variations in bioavailability and in intrinsic antioxidant properties. The hydrophobic nature of vitamin E creates a major challenge to living organisms in maintaining adequate uptake, transport, and delivery of this vitamin to various tissues.

**Vitamin E absorption.** Micelle formation is required for the absorption of vitamin E (57, 171). Pancreatic enzymes may also aid in its absorption (121); this is suggested by the finding of vitamin E deficiency in patients with cystic fibrosis, who do not secrete pancreatic enzymes (12, 48, 70, 172, 193).

The mechanism of absorption of vitamin E from the intestine is similar to the mechanism involved in the transport of other lipid molecules in vivo and involves molecular, biochemical, and cellular mechanisms closely related to overall lipid and lipoprotein homeostasis (22, 67, 75, 100, 101, 179–181). The uptake of vitamin E from the intestine has traditionally been assumed to be a simple process of passive diffusion. However, in a study in Caco-2 cells, it was shown to be a rapid, saturable, temperature-dependent process (7). Recent studies suggest that SR-BI plays a role in vitamin E absorption (154). Vitamin E absorption from the intestine is thought to occur predominantly...
through the secretion of chylomicrons into the lymphatic system. Consistent with this hypothesis is the observation that vitamin E absorption is dependent on the availability of oleic acid for triglyceride synthesis and chylomicron assembly and is inhibited by MTP inhibitors (7). The importance of alternative pathways for vitamin E absorption has also been suggested by the observation that symptoms of vitamin E deficiency are ameliorated after treatment with high doses of vitamin E in patients deficient in chylomicrons. Indeed, intestinal vitamin E absorption may also occur via direct secretion from epithelial cells into the portal venous circulation by HDL efflux. This HDL-dependent mechanism was recently characterized in Caco-2 cells (7). Whether intestinal ABCA1 or other ABC transporters are also critical for the absorption of vitamin E from the intestine and for steady-state plasma α-tocopherol levels remains to be seen.

Vitamin A. The de novo synthesis of compounds with vitamin A activity is limited to plants and microorganisms. Higher animals must obtain vitamin A from the diet. The main dietary forms of preformed vitamin A are carotenoids in fruits and vegetables and long-chain FA esters of retinol in foods of animal origin (145). Carotenoids are either cleaved to generate retinol or absorbed intact, whereas retinyl esters must be completely hydrolyzed within the intestinal lumen to release free retinol before retinol can be taken up by enterocytes. Hydrolysis of the retinyl esters requires lipase activity. The products of TAG hydrolysis provide a better milieu for the solubilization of vitamin E in mixed micelles.

Free retinol is taken up by intestinal mucosal cells (44). In studies conducted in Caco-2 cells (151) and intestinal segments, investigators have found that saturable carrier-mediated processes at physiological concentrations (81) and nonsat-
urable diffusion-dependent processes at pharmacological concentrations (80) of free retinol are involved in retinol uptake. After cellular uptake, the intercellular retinol-binding proteins (CRBPs) CRBP-I and CRBP-II immediately sequester free retinol. CRBP-I is expressed in many tissues, whereas CRBP-II is expressed primarily in the absorptive cells of the small intestine. CRBP-II is one of the most abundant soluble proteins in the jejunal mucosa, which indicates that it may be uniquely suited for the absorption of retinol from the intestine (109, 136, 140, 141). In studies conducted in retinoid-deficient rats (153) and in rats placed on diets containing long-chain FAs (176), investigators found that CRBP-II mRNA expression increased in the small intestine. In studies carried out in Caco-2 cells, investigators found that overexpression of CRBP-II or treatment with retinoic acid (which is associated with increased CRBP-II mRNA expression) resulted in increased absorption and intracellular esterification of retinol. In CRBP-II-KO mice, investigators found that CRBP-II plays an important (albeit not essential) role in the absorption of vitamin A from the intestine (47). Some of the free retinol in the enterocytes remains associated with CRBP, but much of the retinol is usually esterified by lecithin:retinol acyltransferases (LRATs) and stored within the cell (116). The recent characterization of the LRAT-KO mouse [in which no detectable tissue retinyl esters were found (9)] has largely resolved the question of whether enzymes such as ARAT are physiologically involved in retinol esterification in the intestine.

Metabolic studies have revealed that most of the retinyl esters in plasma are present in small chylomicrons; significant amounts are found in large chylomicrons, and smaller amounts are found in very-low-density lipoproteins (VLDL) (108). Studies have been carried out in differentiated Caco-2 cells under postprandial conditions to determine the mechanism of retinol secretion by the intestine. In these studies, investigators found that a significant amount of retinyl ester was secreted mainly with chylomicrons independent of the rate of retinol uptake and intracellular levels of free or esterified retinol (134). Pluronic L81, which inhibits the secretion of chylomicrons, decreased the secretion of retinyl ester and did not result in their increased secretion with smaller lipoproteins. This suggests that intestinal retinyl ester secretion is a highly specific and regulated process that is dependent on the assembly and secretion of chylomicrons. A significant amount of retinol is also secreted into the portal circulation, probably as free retinol; this is expected to be physiologically significant in pathological conditions that affect the secretion of chylomicrons (79). However, very little is known about the regulation of the retinol secretion by this pathway. Under fasting conditions, Caco-2 cells secrete mainly free retinol unassociated with lipoproteins (134). The secretion of free retinol may require facilitated transport. This notion is supported by the marked inhibition of the free retinol efflux into the basolateral medium by glyburide, a known inhibitor of the ABCA1 transporter (46). Mechanisms regulating retinol output through these pathways are not well understood.

ASSEMBLY, SECRETION, AND REGULATION OF INTESTINAL LIPOPROTEINS

The major lipoproteins secreted by the intestine are VLDL and chylomicrons. Of these, the chylomicrons are synthesized exclusively in the intestine to transport dietary fat and fat-soluble vitamins into the blood (Fig. 1). Chylomicrons are primarily very large, spherical TAG-rich particles (5, 58) that also contain PLs, cholesterol, vitamin E, vitamin A, and protein. The lipoprotein core contains TAG, cholesteryl esters, and fat-soluble vitamins, whereas the surface contains a monolayer of PLs (mainly phosphatidylcholine), free cholesterol, and protein (127, 204, 205). A key structural component of the chylomicron is the huge, hydrophobic, nonexchangeable protein apoB48. Other proteins associated with nascent chylomicrons (which are synthesized in enterocytes) are apoA-I, apoA-IV, and apoC. apoB48 synthesis is generally believed to be constitutive (88). However, the amount of lipids transported during the postprandial state is severalfold greater than that transported during the fasting state. Increased transport of fat with similar amounts of apoB48 occurs because of an increase in the size of the particles during the postprandial state (42, 43, 54, 63, 72, 173). Fat feeding also increases the expression of apoA-IV, which serves as a surface component for apoB48 particles in the enterocyte (77, 135).

It has been proposed that chylomicron assembly involves the synthesis of “primordial lipoproteins” followed by their core expansion, which results in the formation of “nascent lipoproteins” (88). This basic proposal was later expanded to suggest that chylomicron assembly may involve three independent steps: assembly of primordial lipoproteins, formation of lipid droplets, and core expansion (85). Subsequently, different biochemical signposts for various biosynthetic milestones were articulated (89). The association of a preformed PL with nascent apoB was proposed to determine the assembly of primordial lipoproteins. An increase in the amount of nascent TAG would serve as an indicator of the synthesis of larger lipoproteins, and retinyl esters were proposed as markers of the completion of chylomicron assembly.

Chylomicron assembly occurs mainly in the ER. These particles are then transported to the cis-Golgi in prechylomicron transport vesicles (PCTVs) (18, 105, 119). Budding of the PCTVs has been shown to require liver FABP (135). Prechylomicrons are very large and carry a unique cargo compared with vesicles that carry nascent proteins (205). Siddiqi et al. (165) showed that chylomicrons are exported from the ER in large vesicles (250 nm) by a coat protein complex II (COPII)-independent mechanism, although COPII proteins are found on PCTVs. These investigators demonstrated further that COPII-interacting proteins (Sec13, Sec32/24, and Sec13/31) are needed to form lipid vesicles that can fuse with the Golgi complex. The unique presence of vesicle-associated membrane protein 7 in the intestinal ER and on PCTVs has been suggested to play a role in the export of chylomicrons from the ER to the cis-Golgi (166). Recently, it was shown that an isoform of protein kinase C (PKC), PKCζ, is required for PCTV budding (167). Thus, chylomicrons are transported on unique particles, different from those used for protein transport, by intestinal cells. Similarly, unique particles have been shown to transport hepatic lipoproteins in the liver (164).

Various mechanisms have been shown to regulate the secretion of lipoproteins from the intestine. Intestinal lipoprotein production is affected by the transcription of apoB, which has generally been believed to be constitutive. Previously, it was known that apoB levels change primarily through co- and posttranslational mechanisms; however, Singh et al. (168)
showed that apoB secretion is affected by a modest change in its transcription. In recent studies, investigators have found that apoA-IV levels also affect intestinal lipoprotein assembly and secretion. Increased secretion of nascent TAG and PLs with chylomicrons has been shown in intestinal porcine epithelial cells (a newborn swine enterocyte cell line) overexpressing apoA-IV (114). Similarly, apical supplementation of Caco-2 cells with lipid micelles has been shown to increase apoA-IV mRNA and protein levels, which, in turn, facilitates lipid secretion (34).

Lipoprotein assembly and secretion is also regulated by MTP, which transfers several lipids and assists in the formation of primordial apoB lipoproteins (87, 90). MTP is modulated by various factors and is typically regulated at the transcriptional level (68). The initial incorporation of lipids into apoB by MTP prevents it from proteosom-mediated degradation. Genetic deficiency or pharmacological inhibition of MTP allows continued synthesis of apoB, but the protein is misfolded and is thus destroyed by ER-associated degradation (52, 110). Changes in MTP activity affect plasma apoB lipoprotein levels significantly. In an in vitro cell-free system, investigators found that MTP plays a pivotal role in facilitating lipid recruitment by acting as a chaperone to assist in apoB folding (99). They also found evidence suggesting that PL recruitment is intrinsic in the NH2-terminal domain of apoB during the translational process and may facilitate protein folding. Recently, we showed that a deficiency in inositol-requiring enzyme 1B (IRE1β) in mice placed on a high-fat, high-cholesterol diet enhances intestinal MTP expression, which leads to increased lipid absorption and chylomicron secretion (92). IRE1β was shown to cause endolytic cleavage of MTP mRNA between exons 2 and 7. The cleaved products are subsequently degraded by 3′-5′ Ski2 nuclease and 5′-3′-exoribonuclease (XRN1) and XRN2 exonucleases. Thus, IRE1β decreases MTP mRNA levels by enhancing its posttranscriptional degradation. Three potential mechanisms of MTP mRNA degradation have been proposed: 1) because both IRE1β and MTP mRNA translation are translated within the ER membrane, it is possible that, under the conditions that increase MTP mRNA synthesis (e.g., high cholesterol and lipid levels), IRE1β and MTP become juxtaposed and thus facilitate the specific degradation of MTP mRNA; 2) IRE1β may activate or recruit another nuclease that initiates MTP mRNA degradation; or 3) under the conditions of increased chylomicron assembly, the protein translational machinery is temporarily stalled, leading to the recognition of the MTP mRNA by endoribonucleases and its subsequent degradation. IRE1β is a mammalian ER stress sensor protein with prominent expression within intestinal epithelial cells. Within intestine, IRE1β protein is detectable in the stomach, small intestine, and colon. Furthermore, it is expressed mainly in the intestinal epithelial cells (15). By modulating MTP mRNA levels and, consequently, the extent of apoB lipidation, IRE1β may protect the ER from the consequences of rapid fluctuations in membrane PL, neutral lipids, or vitamin E levels or 2) mediate downregulation of chylomicron secretion in response to satiety signals or respond to endocrine factors released by the liver and adipose tissue in the presence of excess accumulation of lipid throughout the body. Thus, modulation of IRE1β activity can provide a way to modulate intestine-specific regulation of lipoprotein assembly and lipid absorption. In this mechanism, upregulation of IRE1β may be useful for avoiding a diet-induced hyperlipidemia.

Intestinal lipid absorption has been shown to exhibit diurnal variations in rodents and humans (8, 23, 118, 129, 143). Although plasma lipid concentrations are maintained within a narrow physiological range, several factors (e.g., plasma clearance, cholesterol biosynthesis, and hormonal changes) can lead to changes in plasma lipids. It was recently demonstrated that diurnal variations in plasma lipid levels in rodents are due to changes in MTP levels (143). Lipid absorption was higher at 2400 than at 1200 because of the rodents’ nocturnal feeding behavior. Using in situ loops and isolated enterocytes, it was demonstrated that circadian variations were due to changes in intestinal activities and not because of variations in gastric functions. Further studies have revealed that intestinal expression of MTP also has diurnal variations. Consistent with this finding, the transcription rate for microsomal triglyceride transfer protein (mttp) was high at 2400 compared with 1200. Thus, it appears that MTP expression and lipid absorption are maximized to absorb more lipids at mealtime. The diurnal variations in MTP, in turn, may be responsible for the change in total plasma lipids and apoB lipoproteins (143).

CONCLUSIONS

The absorption of lipids from the intestinal lumen into the enterocytes and their subsequent secretion into circulation is a complex process. Membrane transporters regulate lipid uptake on the apical surface of the brush border of the enterocyte. An essential role for NPC1L1 in cholesterol absorption and for the coordinated activities of ABCG5 and ABCG8 in limiting excess cholesterol uptake is well established. Several proteins involved in FA uptake have also been identified. Transport of lipids from the plasma membrane to the ER involves the activity of intracellular trafficking proteins. Lipids that have been absorbed into the ER are resynthesized and packaged into chylomicrons; this process is dependent on apoB and MTP activity. Specialized vesicles carry these chylomicrons to the basolateral membrane of the cell for secretion. Posttranscriptional degradation of MTP by IRE1β provides a way to modulate intestine-specific regulation of lipoprotein assembly and absorption and may be useful for avoiding diet-induced hyperlipidemias. Recent findings concerning the basolateral eflux of cholesterol as high-density apoA-I-containing lipoproteins will help us identify additional targets for the development of drugs that may suppress cholesterol absorption to reduce hypercholesterolemia and the risk for cardiovascular disease.

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Review


