Development and Physiological Regulation of Intestinal Lipid Absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons

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Mansbach CM 2nd, Gorelick FS. Development and Physiological Regulation of Intestinal Lipid Absorption. III. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons. Am J Physiol Gastrointest Liver Physiol 293: G645–G650, 2007. First published July 12, 2007; doi:10.1152/ajpgi.00299.2007.—Research in dietary fat absorption has developed urgency because of the widely recognized epidemic of obesity in the United States. Despite its clinical importance, many controversies exist over some of the basic aspects of this process from the mechanisms of fatty acid uptake to the control of triacylglycerol export in chylomicrons. Recent advances have included the identification of a number of fatty acid transporters, the discovery of families of acyl-CoA synthetase long chains and acyltransferases, a physiological function for liver-fatty acid binding protein, and the characterization of a number of fatty acid transporters, the discovery of families of acyl-CoA synthetase long chains and acyltransferases, a physiological function for liver-fatty acid binding protein, and the characterization of prechylomicron transport vesicle transporting chylomicrons from the endoplasmic reticulum to the Golgi.

lipid absorption; chylomicrons; transport vesicles; acyl-transferases; lipoproteins

Overall View of Intestinal Lipid Absorption

Lipid absorption is traditionally divided into three components; absorption into the enterocyte, intracellular processing, and export into the mesenteric lymph. Prior to absorption, dietary lipids, mostly triacylglycerols (TAG), are hydrolyzed within the lumen of the small intestine to fatty acids (FA) and sn-2-monoacylglycerol (MAG) by pancreatic TAG lipase. Intracellularly, these hydrolysis products are carried by specific binding proteins to the endoplasmic reticulum (ER), where they are resynthesized to TAG either by acylating MAG with 2 FA or by dephosphorylating phosphatidic acid and acylating the resultant sn-1,2-diacylglycerol (DAG). Acylation only occurs using the activated form of FA, FA-coenzyme A. If the TAG is synthesized on the cytosolic side of the ER hemileaflet, then it traverses the ER membrane where it is bound to microsomal triglyceride transport protein (MTP), which transports the water insoluble TAG to an enlarging lipid droplet. This droplet combines with a high-density, protein-rich particle, which is budded off the ER membrane and is transported vectorially to the Golgi. The chylomicron progresses through the Golgi stack and is carried to the basolateral membrane by a second transport vesicle that carries several chylomicrons to the basolateral membrane for exocytosis into the lamina propria and finally into the mesenteric lymph.

Movement of FFA Across the Enterocyte Apical Membrane

The ability of rats and humans to absorb prodigious amounts of fat is unquestioned. Rats can absorb at least 405 pmol oleate per hour and within 30 s convert ~80% of the absorbed FA into TAG (9). Similarly, humans can absorb up to 600 g fat with 95% efficiency. The first step in lipid absorption, the movement of FA across the apical membrane of the enterocyte, is not well understood; both a protein-independent diffusion model and protein-dependent mechanisms have been proposed.

Favoring the diffusion (flip-flop) model, the uptake of FA by Caco2 cells is unchanged by protease treatment (trypsin and pronase). Similar results have been reported in both model membranes and adipocytes. Importantly, the adipocyte work showed that the flip-flop mechanism could supply enough FA to the esterification machinery of the adipocytes so that the delivery of the FA was not limiting (6).

By contrast, equally convincing studies have shown that FA uptake by cells is saturatable and competitive with other FA, suggesting a protein-mediated model for FA uptake (18). A number of candidate proteins have been proposed. The fatty acid transport proteins (FATP) are a family of six different proteins of which FATP2–4 are expressed in the intestine. Of these, FATP4 is predominantly expressed and was proposed to mediate FA uptake by the small intestine based on a reduction in FA uptake by 40 to 50% after small interfering RNA knockdown. More recently, FATP4 has been localized to intestinal ER and to have a CoA acylase function, similar to FATP1, the best characterized family member (11). Indeed, the correlation of FA uptake with FATP4 expression has been proposed to be due to the intracellular trapping of the FA as acyl CoA, not to an apical transport function. FAT/Cd36 has also been shown to be important in FA uptake by cells (2). The role of Cd36 in FA absorption is controversial. Initial data in Cd36 gene-disrupted mice (5) suggested normal absorption of FA. However, more recent evidence suggests that Cd36 does have a role in FA uptake by the intestine (12). Fabp5 is another FA-transporting protein which has been localized to the brush border membranes of enterocytes. Antibodies to Fabp5 reduce FA uptake into enterocyte brush border membranes and whole enterocytes, but not Caco2 cells. In addition to these data, Kampf and Kleinfeld (7) have presented infor-
mation suggesting that an unknown ATP-dependent protein pump plays a major role in FA uptake by adipocytes and thus potentially enterocytes. Together, there is strong evidence that both lipid transporters and diffusion are important for FA uptake. Further studies are needed to assign the proportion of each mechanism to the overall rate of FA uptake by the intestine.

**Importance of the Topology of Acylating Enzymes in Intestinal ER Membranes**

The enzymes responsible for neutral lipid synthesis and the intracellular site of this synthesis are important. For example, TAG synthesized on the cytosolic hemileaflet of the ER membrane must traverse the membrane to gain entry to the ER lumen, where chylomicrons are formed. Although there is some solubility (~3%) of TAG in isolated membranes, their transit rates are slow. The slow rate of TAG traversing the ER membrane is supported by results in intestinal ER isolated from rats receiving large doses (135 µmol/h) of glyceryl trioletioate (triolein) intraduodenally. The ER contained considerable TAG that was susceptible to lipolytic attack, suggesting that it remained on the cytosolic face of the ER (9). The lipase-sensitive TAG is thought to detach from the ER to become cytosolic lipid droplets, the so-called TAG storage pool. Another model which favors lipid entering the TAG storage pool after synthesis in excess of what can cross the ER membrane is the DGAT2 overexpressing mouse (20). In this scenario, the DGAT2, thought to reside on the cytosolic face of the ER, presumably produces TAG more rapidly than can cross the ER membrane. This TAG then detaches and enters the cytoplasmic TAG storage pool. After a 14-h fast, the TAG in the storage pool is mostly removed presumably via the portal vein as little enters the mesenteric lymph.

**Resynthesis of TAG from Dietary Substrates**

Most dietary TAG is absorbed as FA and MAG that require reassembly to TAG on the ER membrane, predominantly through the progressive acylation of MAG. TAG can also be synthesized by a separate route by acylating glycerol-3-phosphate (G-3-P) to phosphatic acid, dephosphorylating the phospholipid to DAG, and acylating the DAG to TAG. It is important to note that the DAG synthesized from MAG and G-3-P are metabolically inequivalent; the DAG synthesized from MAG is directed to TAG synthesis only whereas the DAG derived from G-3-P may be used to synthesize either TAG or phospholipids.

How might the differences in DAG metabolism occur? The MAG to TAG acyl synthetases are tightly associated and can be coisolated during cellular fractionation. Although there are no similar data on the enzymes involved in synthesizing TAG from G-3-P, they may be separately geographically such that the products of FA utilized by one pathway cannot be captured by the other. One potential mechanism for accessing the different TAG synthetic pathways is by the route of FA entry. For example, oleate entering from the apical membrane is shunted preferentially into the MAG to TAG pathway whereas oleate entering from the basolateral membrane from the circulation is shunted into the G-3-P acylation pathway. The enzyme required to activate the FA prior to its incorporation into MAG or G-3-P may account for the delivery of FA to separate TAG synthetic pathways. This enzyme is one of five members of the acyl-CoA synthetase long chain family (ACSL 1 and 3–6). Of these ACSLs, only ACSL3 and 5 are significantly expressed in the intestine (10). In this proposed scenario, for example, oleate-CoA delivered by ACSL5 would be directed to the MAG pathway and by ACSL3 to the G-3-P pathway. Additional differences between these pathways will be discussed later.

Several monoacylglycerol acyltransferase (MGAT) isoforms may participate in MAG acylation. Of the three isoforms, MGAT (1–3), only MGAT2 and 3 are expressed in the intestine. Of these two, MGAT2 correlates best with lipid absorption (4). This conclusion is based on the findings that MGAT2 is an ER-localized enzyme that is maximally expressed in the proximal intestine. Furthermore, MGAT2 protein expression and activity increases in response to lipid feeding. In contrast, MGAT3 is expressed in the more distal intestine and is not responsive to lipid feeding. MGAT preferentially acylates sn-2-MAG vs. sn-1-MAG. On acylation of sn-2-MAG, sn-1,2-DAG is produced and on acylation of sn-1-MAG, sn-1,3-DAG is produced. sn-1,3-DAG is a metabolic dead end unless there is acyl migration to sn-1,2 DAG. Consideration of the fate of sn-1-MAG, the energetically preferred form of MAG, is worthwhile because of the spontaneous acyl migration that occurs from sn-2-MAG to sn-1-MAG, albeit at a slow rate (10% per hour), which would produce sn-1,3-DAG on acylation.

The sn-1,2-DAG produced is acylated to TAG by diacylglycerol acyltransferase (DGAT). There are two isoforms of DGAT (DGAT1, 2), both of which are expressed in the intestine. Mice with genetic disruption of DGAT1 survive but have a grossly impaired chylomicronemia after fat loading plus an increase in mucosal TAG appearing as cytosolic lipid droplets (3). By contrast, the DGAT2 knockout mice die shortly after birth with an abnormality in the barrier function of their skin. Whether they also exhibit defective intestinal lipid metabolism is unknown. Another major issue concerning the two DGAT isoforms is their localization on the ER membrane. Recently, a model for DGAT2 has been proposed in which the majority of the enzyme, including the NH2 and COOH termini, are cytosolically disposed with the enzyme anchored in the membrane by two transmembrane domains joined by a short intra-ER luminal bridge located nearer the NH2 terminus than the COOH terminus (19). Although DGAT1 has not yet been similarly investigated, suggestive data place this isoform on the luminal side of the ER membrane. First, studies have shown that TAG synthesis occurs on the luminal side of the ER membrane. These data were confirmed both in studies using the specific carnitine acyltransferase inhibitor etomoxir (22), in which TAG output into the lymph was greatly reduced despite normal TAG synthesis from dietary TAG, and in the DGAT1 gene-disrupted mouse, in which TAG output into the lymph was significantly attenuated (3). The proposed isoform distribution is also supported by data from liver in which the overexpression of DGAT1 leads to increased VLDL secretion and modest liver TAG accumulation whereas the overexpression of DGAT2 leads only to increased liver TAG but not VLDL secretion (25). Furthermore, the DGAT1 activity was expressed only in the presence of the membrane permeabilizing agent, alamethacin, indicating that enzyme activity was latent, i.e., within the ER lumen. If chylomicron-TAG is indeed
synthesized predominantly within the ER lumen, that would lessen the requirement for rapid movement of TAG across the ER membrane. In this proposed and more likely scenario, FA-CoA, synthesized on the ER cytosolic face, would be transported across the ER membrane as an acyl-carnitine mediated by carnitine acyltransferase or by a FA-CoA transporter, ultimately resulting in ER luminal FA-CoA. DAG has been shown to move rapidly across membranes and so would be available, along with the acyl-CoA as substrates for DGAT1. The TAG produced would then be on the correct side of the ER membrane for incorporation into chylomicrons.

**Formation of the Chylomicron Within the ER Lumen**

The formation of chylomicrons occurs in a two-step process within the ER lumen (Fig. 1). In the first step, a high-density particle consisting of apolipoprotein B48 (apo B48, which is unique to the intestine), phospholipid, apolipoprotein AIV (apo A-IV), cholesterol, and small amounts of TAG is formed. In the second step, this primordial chylomicron fuses with a large TAG-cholesterol ester mass that does not have apo B48 on its surface to form the prechylomicron, ready for transport to the cis-Golgi.

ApoB48 synthetic rates are not responsive to acute or chronic dietary maneuvers that alter the synthetic rates of other proteins that participate in the formation of chylomicrons such as apo A-IV. Newly synthesized apo B48 must be translocated into the ER lumen, but unlike other secretory proteins it can continue on the pathway toward secretion only if it associates with primordial chylomicrons. ApoB48 that does not is degraded by the ubiquitin-proteasome system. ApoB48 is translated from the same gene as apo B100 but translation is stopped at codon 2153 by RNA editing. ApoB48 retains 2 α-helices and only the first of the two β-sheets present in apo B100. Furthermore, it also lacks the LDL receptor binding domain present in apo B100. ApoB48 has a number of pause transfer sequences in which its transit through the translocon slows or stops. However, translocation is limited not by these pause sequences but by the β-sheet (24). As apo B48 exits the translocon, it binds to the MTP, a heterodimer composed of an “M” subunit of 97 kDa and protein disulfide isomerase (55 kDa). The M subunit is the protein that is mutated in abetalipoproteinemia. In addition to MTP, the emerging apo B48 also binds to phospholipids (15) that are crucial for its eventual secretion. Another chaperone is HSP70, which accompanies apo B100, and perhaps apo B48 as well, all the way to the Golgi. A small amount of TAG is added to the nascent high-density particle as is cholesterol. The entire primordial chylomicron (termed “dense particle” in Fig. 1) has a density similar to HDL and may be isolated separately from a low-density, TAG-rich particle that does not contain apo B48. The TAG-rich particle accretes TAG through MTP that takes TAG from its site of synthesis on the ER membrane and delivers it to the growing particle. Support for this speculation comes from mice whose gene encoding the essential M subunit of MTP is disrupted. These mice do not develop the low-density lipoproteinemia. In the rough endoplasmic reticulum (RER, see expanded portion of the figure), newly synthesized apolipoprotein B48 (apo B48, in purple) is chaperoned by the luminal protein, microsomal triglyceride transfer protein (MTP, in red). The apo B48 then follows 1 of 2 itineraries: it forms stable complexes with dense particles (DP, in gold) containing mostly phospholipids, cholesterol, and small amounts of triacylglycerol (above) or is rapidly degraded in the absence of association with lipoprotein complexes (below). In the smooth endoplasmic reticulum (ER) (SER) (see expanded portion), the neutral lipids (triacylglycerol and cholesterol esters, in orange) are ferried from their site of synthesis on the ER membrane to an enlarging particle by MTP (not shown). This particle is merged with apoprotein AIV (apo A-IV, in green) to form a large light particle (LP). The 2 chylomicron precursors merge in the SER to form lipid particles that have a core of neutral lipid surrounded by a monolayer composed of phospholipids and both apo A-IV and apo B48. This particle then buds from the SER surrounded by a membrane. The budding step is mediated by liver-fatty acid binding protein, but not COPII proteins. It also requires ATP. The prechylomicron transport vesicle (PCTV) then translocates to and fuses with the Golgi complex. At the Golgi, apoprotein A1 (apoA1, light blue) arrives in different transport vesicles than the PCTV. Within the Golgi lumen, apoA1 attaches to the prechylomicron to form a mature chylomicron containing apoA1, apo A-IV, and apo B48. The mature chylomicrons exit the Golgi complex in large transport vesicles that fuse with the basolateral membrane and are secreted. Nuc, nucleus.

This comes from apo B48 gene-disrupted mice. Without apo B48 on the TAG-rich particle, the particle is unable to leave the ER, suggesting that apo B48 on the surface of the TAG-rich particle is a signal that the prechylomicron is ready for export.

**Export of Prechylomicrons from the ER**

Because prechylomicrons are very large (150–500 nm) and have a unique cargo compared with vesicles that carry nascent

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*Fig. 1. Biogenesis of chylomicrons. The formation of chylomicrons is a sequential multistep process that begins in the endoplasmic reticulum. In the rough endoplasmic reticulum (RER, see expanded portion of the figure), newly synthesized apolipoprotein B48 (apo B48, in purple) is chaperoned by the luminal protein, microsomal triglyceride transfer protein (MTP, in red). The apo B48 then follows 1 of 2 itineraries: it forms stable complexes with dense particles (DP, in gold) containing mostly phospholipids, cholesterol, and small amounts of triacylglycerol (above) or is rapidly degraded in the absence of association with lipoprotein complexes (below). In the smooth endoplasmic reticulum (ER) (SER) (see expanded portion), the neutral lipids (triacylglycerol and cholesterol esters, in orange) are ferried from their site of synthesis on the ER membrane to an enlarging particle by MTP (not shown). This particle is merged with apoprotein AIV (apo A-IV, in green) to form a large light particle (LP). The 2 chylomicron precursors merge in the SER to form lipid particles that have a core of neutral lipid surrounded by a monolayer composed of phospholipids and both apo A-IV and apo B48. This particle then buds from the SER surrounded by a membrane. The budding step is mediated by liver-fatty acid binding protein, but not COPII proteins. It also requires ATP. The prechylomicron transport vesicle (PCTV) then translocates to and fuses with the Golgi complex. At the Golgi, apoprotein A1 (apoA1, light blue) arrives in different transport vesicles than the PCTV. Within the Golgi lumen, apoA1 attaches to the prechylomicron to form a mature chylomicron containing apoA1, apo A-IV, and apo B48. The mature chylomicrons exit the Golgi complex in large transport vesicles that fuse with the basolateral membrane and are secreted. Nuc, nucleus.*
proteins (80–90 nm), the question of how they are exported out of the ER is pertinent. Obvious physicochemical constraints would prevent the flow of chylomicrons through the ER membrane, and even if they were able to get out, how would they then be directed to the next stop on their intracellular itinerary, the cis-Golgi? One possibility is that a vesicular transport system performs this function. Although there is a well-characterized system for the transport of newly synthesized proteins from the ER to the Golgi, there are major differences between this system and the chylomicron transport system (Table 1). The information gathered from a number of studies suggests that the coatomer II proteins (COPII proteins) are able to select protein cargo for inclusion in the vesicle (called herein the protein vesicle), deform the ER membrane, and perform a fission step that cuts the budding vesicle from the ER membrane to form the nascent protein transport vesicle. These events occur at specific ER exit sites marked by Sec16 and Sec23 proteins. These membrane-bound transport vesicles protect their cargo from cytosolic proteases by providing an enclosed space. In addition, the vesicles contain on their surface information that enables them to be targeted to the cis-Golgi.

These protein transporting vesicles are constrained in their size, perhaps by the curvature of Sec23, to 60–80 nm, which is too small to contain cargo as large as prechylomicrons. To determine how chylomicrons exit the ER, the potential vesicles that perform this function had to be isolated and characterized. Because of their large TAG content, it was thought that their buoyant density would be similar to that of chylomicrons and relatively light, enabling their separation from the more dense Golgi, ER, lysosomes, and the protein transporting vesicles. The isolated PCTV exhibited the anticipated density and had the predicted morphological characteristics including a diameter of 250 nm. To function as transport vesicles, PCTV had to meet the following criteria: 1) contain prechylomicrons as defined by their TAG content as well as apo B48, an apolipoprotein specific for chylomicrons, and apo A-IV, known to be present on chylomicrons; 2) be sealed within a membrane; 3) be large enough to contain prechylomicrons; and 4) be able to fuse with the cis-Golgi, i.e., deliver their cargo prechylomicrons to the Golgi lumen. All these criteria were met by PCTV (16).

In addition to their importance in transporting chylomicrons to the Golgi, PCTV have characteristics that differentiate them from protein transport vesicles and thus make them of more general biological interest. Studies in which the COPII protein Sar1 was depleted from intestinal cytosol revealed that, unlike protein vesicles, not only did PCTV continue to be budded from ER membranes, but the budding activity was significantly enhanced. Thus inhibition of COPII function resulted in a 6- to 10-fold increase in TAG budding from ER membranes. By contrast, protein vesicles were no longer formed under these conditions. Restoration of Sar1 by adding recombinant Sar1 to Sar1-depleted cytosol reduced PCTV budding activity to normal and enabled protein transport vesicles to be formed again. Consistent with these findings, GTP removal did not affect PCTV budding. These data indicate that, unlike vesicles carrying nascent protein from the ER, the budding of PCTV does not require COPII proteins.

A second major difference between PCTV and protein vesicles is the presence of the vesicle or v-soluble N-ethylmaleimide-sensitive factor attachment protein receptor (v-SNARE), v-soluble associated membrane protein 7 (VAMP7) on PCTV but not on protein vesicles. VAMP7 functions normally as a post-Golgi v-SNARE but uniquely in the intestine is also concentrated in the ER, where it is incorporated into PCTV to function as the v-SNARE in the SNARE fusion complex that forms with the cis-Golgi. On fusion with a target membrane, the v-SNARE forms part of a very stable four-α-helical complex with three target- or t-SNAREs on the target membrane. This SNARE complex enables the two membranes to fuse, allowing the interior contents of the transport vesicle to mix with the luminal contents of the target membrane. Identification of the SNARE complex can be difficult because it dissociates as soon as fusion occurs. However, before the SNARE complex forms, the vesicle docks with the target membrane, becoming attached to the membrane without sharing interior contents. To identify the PCTV-SNARE components, conditions were developed that docked PCTV with Golgi membranes without fusion. This was done by excluding Mg$^{2+}$ and ATP and incubating the membranes at cold temperature. Docking was defined by two criteria. First, PCTV were required to shift from the light part of the gradient to become isodense with the heavier Golgi. Second, docked PCTV chylomicrons would not contain apoAI, a protein that is only found in mature chylomicrons after they have fused with the Golgi (17). We used PCTV that met these docking criteria to identify the v-SNARE as VAMP7 and the t-SNAREs as syntaxin5, vti1a, and bet1. These SNAREs formed a 112-kDa complex that on boiling became dissociated into their monomer components. Antibodies to each of the SNAREs blocked the association of the PCTV with the Golgi. To show that the SNARE complex promoted fusion, we incubated PCTV with Golgi at warm temperature with ATP and Mg$^{2+}$ and found that Golgi chylomicrons now contained apoAI whereas they had not prior to incubation.

An obvious question was that if the COPII proteins did not initiate PCTV budding, what protein complex did? To examine this issue (13), intestinal cytosol was fractionated on a gel permeation column and fractions were assayed for PCTV budding activity. Since protein vesicles require a large protein complex for budding from the ER, we were surprised to find that the budding activity was confined to the small Mr (molecular weight) range of the column and eluted just prior to cytochrome c (Mr = 12,000). After several chromatographic steps, an active fraction was produced that had one major and two minor bands on SDS-PAGE. These were submitted to tandem mass spectroscopy and were found to contain six different proteins including liver and intestinal fatty acid

| Table 1. Differences between PCTV and protein transport vesicles |
|---------------------|-----------------|-----------------|
| Characteristics     | PCTV            | Protein Transport Vesicle |
| Budding requirements| L-FABP, ATP     | GTP, ATP, COPII proteins |
| Vesicle size        | 200–500 nm      | 60–90 nm         |
| v-SNARE             | VAMP7           | Yk6, r22b        |
| Cargo               | Neutral lipids, lipoproteins | Newly synthesized proteins |

PCTV, prechylomicron transport vesicle; L-FABP, liver fatty acid binding protein; v-SNARE, v-soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VAMP7, v-soluble associated membrane protein 7.
binding proteins (L-FABP, I-FABP). Recombinant proteins for all six were tested for budding activity, but only L-FABP replicated the activity of native cytosol, a conclusion supported by the hypochylomicronemia found in L-FABP gene-disrupted mice (14). However, I-FABP had only 20% the activity of L-FABP. The vesicles produced by L-FABP met the criteria for bona fide PCTV in that they contained TAG, apo B-48, and apo A-IV, excluded resident ER proteins, were of the same size as PCTV generated in native cytosol, were sealed, and contained VAMP7. However, these rL-FABP-produced vesicles could not fuse with the Golgi. One possibility for this is that since the vesicle is produced in the absence of cytosol, neither Sar1 nor other COPII are present. We postulate that although the COPII proteins are not required for budding PCTV, they or proteins with which they interact are required for fusion with the Golgi. This conclusion is supported by the observation that individuals with chylomicron retention disease, who accumulate lipid-filled vesicles in their enterocytes, lack the COPII proteins with which they interact are present. We postulate that although the COPII proteins are not required for budding PCTV, they or proteins with which they interact are required for fusion with the Golgi. This conclusion is supported by the observation that individuals with chylomicron retention disease, who accumulate lipid-filled vesicles in their enterocytes, lack the COPII protein, Sar1b. The finding implies that distinct COPII coats might regulate PCTV and protein vesicles budding from the ER. It remains unclear how L-FABP, a 14,000 Mr protein, can perform all of the functions required of the five COPPII proteins whose combined Mr is ~576,000. One potential is that L-FABP interacts with integral membrane proteins whose function is yet to be defined. This potential protein-protein interaction could help with cargo selection. It is also possible that the wide lipid binding range of L-FABP could be utilized to bring lysophospholipids to the ER membrane where, as inverted cones, they might induce the membrane deformation required for budding.

ApoAI secretion is known to be uncoupled from chylomicron secretion in the intestine. How this occurs is unclear, but the current studies on PCTV provide a potential mechanism. Because PCTV are devoid of apoAI (16), it seems clear that the transport vesicle that exports apoAI from the ER to the Golgi is different and likely to be the COPII-dependent protein transport vesicle. Studies documenting this have not yet been done. Because chylomicrons acquire apoAI in the Golgi, the studies on PCTV fusion establish for the first time that apoAI becomes associated with chylomicrons in the Golgi. This is another piece of evidence that transport to the Golgi is physiologically important for chylomicrons.

**Physiological Regulation of Chylomicron Secretion**

The percentage of diet-derived intestinal TAG that is delivered into the lymph is variable, depending on the amount of phosphatidylcholine (PC) in the intestinal lumen, the amount of fat in the diet, the expression of apo A-IV by the enterocytes, and the hydration state of the mucosa. Chylomicron output into the lymph correlates with the amount of dietary PC; the greater the amount of PC, the greater the chylomicron output. Since most of the luminal PC is delivered by the bile, in the bile diversion rat model chylomicron output is reduced by comparison with bile intact rats. However, TAG output in this model can be partially restored with the inclusion of choline in the intraduodenal lipid infusion and completely restored if lyso-PC is included. These data suggest that the CDP-choline-dependent PC synthetic pathway is not as effective in supplying PC for chylomicron formation as is the acylation of lyso-PC. These data are supported by the preferential use of bile PC in the formation of chylomicrons and data obtained in Mdr2-/- mice that are unable to secrete PC into their bile.

Under these conditions, the knockout mice have a greatly reduced chylomicron output (21). Prefeeding rats a high (24%) fat diet also significantly increases chylomicron-TAG output into the lymph compared with chow (4% fat wt/wt) fed rats. A potential reason for this is that the high-fat diet increases the delivery of biliary PC nearly twofold (7.3 vs. 3.9 µmol/h). The overexpression of apo A-IV in IPEC cells also significantly increases chylomicron-TAG output mainly by increasing the size of the chylomicrons (1, 8). Finally, the hydration state of the intestine positively correlates with the ability of rats to export chylomicrons into the lymph.

While the data linking PC availability to chylomicron-TAG output into the lymph are convincing, the mechanism of this link is unclear. The intestine has only two PC synthetic pathways, the CDP-choline-dependent pathway and the lyso-PC acylation pathway. It lacks the phosphatidylethanolamine N-methyltransferase pathway present uniquely in liver. It is evident that when substrate is lacking for the lyso-PC pathway, the CDP-choline pathway cannot fully compensate. One possibility for this is that the DAG substrate segregated to the CDP-choline-dependent pathway for PC synthesis is preferentially utilized for TAG synthesis and there is no mechanism to direct more of it to the PC synthetic pathway.

In sum, lipid absorption is efficient, preserving caloric resources, with the hydrolytic products of dietary TAG quickly converted intracellularly to TAG to maintain cellular integrity. The production of the TAG transport vehicle, chylomicrons, is necessitated to target the TAG to appropriate organs for cargo delivery. Finally, a specialized ER-to-Golgi transport vesicle for chylomicrons is utilized perhaps in response to the intermittent nature of dietary lipid intake compared with the more constant requirement for newly synthesized proteins to be exported from the ER.

**REFERENCES**


