Control of chylomicron export from the intestine

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Mansbach CM 2nd, Siddiqi S. Control of chylomicron export from the intestine. Am J Physiol Gastrointest Liver Physiol 310: G659–G668, 2016. First published February 25, 2016; doi:10.1152/ajpgi.00228.2015.—The control of chylomicron output by the intestine is a complex process whose outlines have only recently come into focus. In this review we will cover aspects of chylomicron formation and prechylomicron vesicle generation that elucidate potential control points. Substrate (dietary fatty acids and monoacylglycerols) availability is directly related to the output rate of chylomicrons. These substrates must be converted to triacylglycerol before packaging in prechylomicrons by a series of endoplasmic reticulum (ER)-localized acylating enzymes that rapidly convert fatty acids and monoacylglycerols to triacylglycerol. The packaging of the prechylomicron with triacylglycerol is controlled by the microsomal triglyceride transport protein, another potential limiting step. The prechylomicrons, once loaded with triacylglycerol, are ready to be incorporated into the prechylomicron transport vesicle that transports the prechylomicron from the ER to the Golgi. Control of this exit step from the ER, the rate-limiting step in the transcellular movement of the triacylglycerol, is a multistep process involving the activation of PKCζ, the phosphorylation of Sar1b, releasing the liver fatty acid binding protein from a heteroquatromeric complex, which enables it to bind to the ER and organize the prechylomicron transport vesicle budding complex. We propose that control of PKCζ activation is the major physiological regulator of chylomicron output.

chylomicrons; diacylglycerolacyltransferase; microsomal triglyceride transport protein; monoacylglycerololayltransferase; PKCζ

THE PROCESS OF LIPID ABSORPTION from dietary input to chylomicron output is a multifaceted and complex process that covers many scientific disciplines. Some of the major problems that the intestine needs to overcome in the process of absorbing lipid and packaging it for export are the lack of control over the dietary lipid input rate, the cellular toxicity of fatty acids (FA), and lysophosphatidylcholine (lyso-PC). Most PC delivered to the intestine, however, comes from sloughed enterocytes that have a half-life of 4 days (25).

Dietary lipid is transported from the intestine into the lymph in the intestine’s unique TAG-rich lipoprotein, the chylomicron. In addition to the chylomicron, another TAG-rich lipoprotein, the very low-density lipoproteins (VLDL), were found in lymph, which contains 47% TAG and 54% cholesterol during fasting (72). Svedberg flotation (Sf) rates for chylomicron are 400 or greater whereas Sf rates of VLDL are 20–400. Electron microscopic study demonstrated that the vesicle isolated from intestinal Golgi contained either chylomicron or VLDL particles, not a mixture (21, 46, 72). Chylomicron formations are substrate driven; in the absence of dietary input, few chylomicrons are formed. During fasting, the low level of chylomicron production is lowered to near 0 by bile diversion (72), suggesting that biliary lipids are the source of chylomicron production during fasting. By contrast, when progressively larger loads of lipid are infused into rats, chylomicron output reaches a limit (62). This review will focus on mechanisms for delivery of FA to the ER, the enzymes involved in incorporating these FA into triacylglycerol (TAG), the development of the chylomicron, molecular mechanisms for its export from the ER, and control mechanisms for the export of chylomicrons from the intestine.

The Absorption of Dietary FA into the Enteroctye

There are two transporters that have been proposed to translocate dietary FA from the apical membrane to the cytosol: CD36 and fatty acid transporter 4 (FATP4). CD36 is an
88-kDa transmembrane protein that is heavily glycosylated. It is localized mainly to the brush border of enterocytes, predominantly at the tips of villi of the proximal 2/3 of the small intestine (54, 66), the same site of maximal lipid absorption (70). It appears to play a prominent role in FA uptake (66, 67). CD36 gene disrupted mice display reduced FA uptake (67) with a consequent reduction in chylomicron output (67).

FATP4 is not in the brush border but is localized to the subapical compartment and the ER (64). Interestingly, it functions as a long-chain fatty acid CoA synthetase (99) whose actions trap the absorbed FA in the cytosol as the hydrophilic FA-CoA derivative, a process that has been named vectorial acylation (2). Knockdown of FATP4 in primary enterocyte cultures by siRNA reduced FA uptake into the cells, suggesting that FATP4 might play a major role in FA uptake (99). It should be noted that, in primary cultures of enterocytes, FA could gain entry into the cell by not only the apical but also the basolateral membrane. Differences in the mechanisms of transport and metabolic fate of the absorbed FA between FA absorbed apically and basolaterally are well known (103). However, when lipid absorption was tested in FATP4−/− mice, no effect on lipid-absorptive parameters were seen (88), calling into question the importance of FATP4 in FA absorption in vivo. The discordant data between cell culture studies and in vivo observations suggest caution in translating cell culture observations to in vivo physiology. Other data in support of this thesis have been extensively discussed elsewhere (89).

A third potential for FA absorption is FA diffusion. The pH of the microclimate next to the brush border is 5.5, suggesting that some FA would be protonated and could diffuse through the apical membrane (85, 86). On the cytosolic side of the membrane, the FA would be captured by becoming ionized again in the more basic cytosol and thus could not freely diffuse back through the apical membrane. Although this is an attractive mechanism, no evidence of its importance to FA absorption has been presented.

If diffusion, FATP4, or CD36 were the dietary FA transporter, which of these could account for the mass of FA that the absorption has been presented. If diffusion, FATP4, or CD36 were the dietary FA transporter, which of these could account for the mass of FA that the absorption has been presented. If diffusion, FATP4, or CD36 were the dietary FA transporter, which of these could account for the mass of FA that the absorption has been presented.

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A unique feature of MGAT2 is that it dimerizes with DGAT2 but not DGAT1 (42). This would provide a facile “handoff” of MGAT2’s product, DAG, to DGAT2, which utilizes it along with FA-CoA as substrates for TAG synthesis. This should provide for efficient TAG synthesis in accord with prior observations. Interestingly, in response to a high-fat diet, MGAT2 protein is increased threefold but its activity is not (20). These data were essentially confirmed subsequently (13). A potential explanation for these data is posttranslational modifications that have not yet been described. Intestinal specific MGAT2 KO mice (Mogat2IKO) have been produced (69) with several surprising results. The first is that MAG uptake by these mice is greatly reduced compared with WT mice. The mechanism of MAG uptake has not been carefully studied but MGAT is not described as an apical membrane component. MGAT2’s absence may lead to changes in the composition of the membrane, however, which may influence uptake rates. Alternatively, in Caco-2 cells, FA and MAG can be shown to compete for uptake, suggesting a common transport mechanism (33). The second unexpected finding was that the intestinally specific absence of MGAT2 led to higher proportions of FA being oxidized vs. WT mice. This may be because more FA is available for oxidation in the KO mice than WT mice since it is not utilized for acylation or it may mean that MGAT2 has a more generalized effect on metabolism than just its enzymatic function. The Mogat2IKO mice also had reduced TAG in their plasma post-fat feeding, pointing again to the importance of MGAT in chylomicron TAG production. Interestingly, MGAT2 also displays DGAT activity as well, suggesting the bifunctionality of the enzyme (12).

Diacylglycerol acyltransferase. The terminal enzyme for TAG synthesis is DGAT. It acylates DAG to TAG, using long-chain acyl-CoA as the other substrate. All TAG produced by the intestine is likely generated from apoB48-containing chylomicrons that enter the intestinal epithelium (19, 106). Both of these enzymes have been cloned (15, 16) and their topology elucidated. In humans only DGAT1 is expressed (15, 16). Of particular interest is that the terminal enzyme for TAG synthesis is DGAT. It acylates DAG to TAG, using long-chain acyl-CoA as the other substrate. All TAG produced by the intestine is likely generated from apoB48-containing chylomicrons that enter the intestinal epithelium (19, 106). Both of these enzymes have been cloned (15, 16) and their topology elucidated. In humans only DGAT1 is expressed (15, 16). Of particular interest is that the ER topology of the DGATs. DGAT2’s topology places it with its active site facing the cytosol (102) whereas DGAT1 is proposed to face the ER lumen (63) though some data suggest that it also faces the cytosol (113). Although both enzymes use acyl-CoA and DAG as substrates, they are genetically not related. DGAT1 belongs to the same family as acyl-cholesterol acyl transferase. It can acylate retinol and is also involved in the synthesis of waxes (87, 116). DGAT2 belongs to the MGAT family and is specific for TAG synthesis.

With the identification of the DGAT genes, both DGAT KO mice and overexpressing mice were developed. As expected, DGAT1 KO mice or mice whose DGAT1 was specifically inhibited had impaired chylomicronemia after a lipid bolus and retained lipid in their enterocytes (11, 83) since 89% of intestinal TAG is synthesized via DGAT1 (19). Interestingly, a human with DGAT1 gene mutation has recently been described. The defect was identified in an infant with intractable diarrhea (27). A potential cause of this is that FA, not able to be incorporated into TAG, is left to diffuse to cellular membranes and disrupt them, causing the diarrhea. By contrast to the KO mice, when DGAT1 is overexpressed in liver, the mice both synthesized more TAG in their liver and secreted more of it than WT mice (51). The phenotype of mice overexpressing only intestinal DGAT-1 has not shown significant TAG response than WT mice (48).

Whereas DGAT1 KO mice survive, DGAT2 KO mice do not. This is due not to lack of TAG production in the intestine but rather to TAG synthesis in the skin. This results in a disrupted and permeable skin barrier (79, 101), leading to an early death. Because of this, study of the phenotype of these mice has been limited. DGAT2-overexpressing mice have been created (107). These mice show no change in intestinal lipid content on a high-fat diet compared with a reduction in storage lipid in DGAT1-overexpressing mice (107). DGAT2 is an inherently unstable enzyme, being subject to ERAD degradation and ubiquitination. This process is controlled by the E3 ligase, gp78 (18).

Distribution of TAG in the ER

The end product of the DGATs is TAG. TAG is only sparingly soluble in membranes (2%) (28), but recent suggestions are that the TAG, on synthesis, is sequestered between the ER membrane bilayer. The ER-TAG has two choices: either to enter the ER lumen as substrate for the enlarging lipid particle that serves as a precursor of chylomicron formation or to exit the ER toward the cytosol as a component of enlarging lipid droplets. What influences which direction the TAG will go is as yet not completely known. In extreme cases such as a nonfunctional microsomal triglyceride transport protein (MTP), only small amounts of TAG enter the ER lumen.

In another case where progressively more triolein was added to an intraduodenal infusion in rats, at the highest level of infusion, 135 μmol/h, evidence was obtained of TAG collecting on the surface of the ER (62) that was accessible to added pancreatic lipase. It was speculated at the time that more TAG was synthesized than could cross the ER membrane resulting in the development of a second pool of TAG. We now speculate that the second TAG pool was lipid droplets, which has been shown to be attached to the ER (39).

The origin of lipid droplets is unknown (111). An attractive hypothesis places the TAG in between the lipid bilayer of membranes and, by extension, the ER (56). The intestine has no control over the quantity of lipid presented to it for absorption. Therefore, during the digestion and absorption of a lipid-rich meal, potentially large amounts of absorbed FA could be presented to the ER as substrate for TAG synthesis. It is conceivable that the newly synthesized TAG progressively enlarges the interbilayer TAG lens until it blisters out on the surface of the ER as a lipid droplet. Alternatively, TAG on the cytosolic surface of the ER could be dewetted as it enlarges, forming a lipid droplet (102) that detaches from the ER and enters the cytosol. Of interest is that the lipid droplets contain a complete set of Kennedy pathway enzymes including DGAT2 (112). In this way, lipid droplet DGAT2 could compete with the ER for FA.

TAG that does not get incorporated into lipid droplets crosses the ER membrane and is included in an apoB48-deficient lipid droplet within the ER lumen (24). Because of the insolubility of TAG in water, the TAG is ferried from the ER membrane to the cytosol as a lipid droplet (102) that detaches from the ER and enters the cytosol. Alternatively, TAG on the surface of the ER as a lipid droplet. Whereas DGAT1 KO mice survive, DGAT2 KO mice do not. This is due not to lack of TAG production in the intestine but rather to TAG synthesis in the skin. This results in a disrupted and permeable skin barrier (79, 101), leading to an early death. Because of this, study of the phenotype of these mice has been limited. DGAT2-overexpressing mice have been created (107). These mice show no change in intestinal lipid content on a high-fat diet compared with a reduction in storage lipid in DGAT1-overexpressing mice (107). DGAT2 is an inherently unstable enzyme, being subject to ERAD degradation and ubiquitination. This process is controlled by the E3 ligase, gp78 (18).
luminal side of the ER membrane to the expanding lipid droplet by the microsomal triglyceride transport protein (MTP). MTP is composed of two proteins, the microsomal triglyceride transport protein (88 kDa) and protein disulfide isomerase (57 kDa) (109, 110), but MTP will be used here to indicate the larger 88-kDa portion of the heterodimer unless indicated otherwise. Genetic modifications of MTP yield a phenotype in which the intestine is unable to transport TAG in its unique TAG-rich lipoprotein, the chylomicron, out of the intestine into the lymph. This mutation, misnamed abetalipoproteinemia, is a rare autosomal recessive disorder (108).

Another function of MTP is as a chaperone for apoB48 emerging from the translocon. The binding site on apoB for MTP has been identified in the NH2-terminal globular portion of apoB (B:430–570) (36). ApoB, as it is translated, has a relatively short window of opportunity to bind phospholipids before being degraded (40). This window is prolonged by its binding to MTP (40). The affinity of apoB for MTP is increased by first binding to phospholipids (3, 4).

Interestingly, MTP levels vary during the 24-h cycle, as do serum TAG levels potentially as a consequence. In rats, MTP levels peak when rats are eating (nocturnally) and are lowest during the day (73). A likely explanation for this was found in mice in which it was shown that MTP expression was at least under partial control of the CLOCK gene (37, 74).

Nothing is known about the proteins that target the apoB-containing dense particle to the TAG-rich one or the mechanism by which they merge. Nevertheless, the dense particle and the TAG-rich lipid particle fuse in the ER lumen to form the prechylomicron. Once the prechylomicron is formed, nothing is known about how it signals the ER to Golgi transport machinery that it is mature enough for transport. Chylomicrons vary in size depending on the TAG input rate (31), so size alone is not the variable. It should be noted that overexpression of apolipoprotein-AIV greatly increases the size of the secreted chylomicron (55). One possible signal relates to the 70-kDa carboxy fragment that is exposed to the cytosol and binds to the prechylomicron export machinery (91).

**Exit of chylomicrons from the ER.** A major question is how the chylomicrons exit the ER. The importance of this step is highlighted by the finding that this is the rate-limiting step in the movement of absorbed FA from the apical membrane to the basolateral membrane as chylomicrons (61). Since chylomicrons are very large (100–500 nm) (120), a vesicular mechanism was proposed to transport the prechylomicrons to the Golgi (94). The majority of cargoes carried from the ER to the Golgi are transported by the COPII-dependent pathway in 60-nm Golgi (94). The majority of cargoes carried from the ER to the Golgi transport vesicles (PCTV) contain prechylomicrons but no other ER-derived proteins such as calnexin (94). Since there is only one cargo, prechylomicrons, cargo selection is simplified. PCTV are sealed (150–500 nm); proteinase K treatment of PCTV spares apoB48 within PCTV but not if the vesicle is first treated with Triton X-100 to disrupt the vesicle membrane (94). This keeps the prechylomicron sequestered from both pancreatic lipase (57) and adipose tissue lipase (71), both of which are expressed in the intestine. An additional aid in cargo selection is that the carboxy terminal 70-kDa fragment of apoB48 is exposed to the cytosol (91). Therefore, cytosolic proteins that make up the PCTV budding complex can readily bind (91). The inability of proteinase K to attack apoB48 in PCTV is in contrast to proteinase K treatment of apoB48 in the ER (see above). Finally, PCTV contain the Golgi targeting protein, Sec24C (93).

Because the COPII proteins are not involved in the budding complex, what are the proteins that perform this function? There are three transmembrane proteins: VAMP7, CD36, and in the ER, apoB48. VAMP7 is predominantly a post-Golgi protein, but in the intestine it is uniquely present in the ER (95). Antibody inhibition of either VAMP7 or apoB48 or gene disruption of CD36 leads to a cessation of PCTV production (91). The only cytosolic protein member of the budding complex is the liver fatty acid binding protein (FABP1), and in its absence by gene disruption, no PCTV are generated (91). These data indicate that for adequate PCTV budding to occur, all four budding complex components must be present. The vesicle produced by the complex is present in cytosol but is not targeted to the Golgi. For Golgi targeting to occur, the COPII protein, Sec24C, is required (93). This COPII component is added at the generation of the vesicle because, once PCTV undergo a scission event from the ER membrane, Sec24C cannot be effectively added back to the vesicle. Because GTP is not required for PCTV budding, Sar1b is assumed to bind to GDP. Without GTP binding, its NH2-terminal α-helix is not able to penetrate the ER membrane as it does in the case of GDP binding (49). This suggests that the COPII proteins associated with PCTV are bound to PCTV by protein-protein interaction. ApoB48 is a likely binding partner for the COPII proteins since the COPII proteins must be added to PCTV at its inception when the COOH-terminal 70-kDa portion of apoB48 is exposed to the cytosol. This suggestion is supported by coimmunoprecipitation studies that show Sar1b, Sec23, and Sec24C to bind to apoB48 (91) and the inability of the COPII proteins to bind to PCTV once it is cut from the membrane. In the cytosol, apoB48 is completely within the interior of PCTV and is resistant to proteolysis by proteinase K (94).
Regulation of Prechylomicron Output from the ER

A major question concerning prechylomicron output is whether it can be regulated. Multiple studies suggest that the rate of PC input into the intestine correlates with chylomicron export. In bile duct diversion models, chylomicron output is low (104), and if PC is supplemented in a triolein intraduodenal infusion, chylomicron TAG output is much higher, similar to large TAG input rates (60). How are these observations rationalized with the observation that FABP1 alone can elicit PCTV generation? In the experiments we performed using FABP1, we used recombinant FABP1 and no ATP. However, when whole cytosol, which contains 2–3% cytosolic protein as FABP1, was used in a similar assay of PCTV production, no PCTV were produced unless ATP was added (90) (Fig. 1).

These studies suggested the requirement of a protein kinase that was identified as PKCζ (90). Sar1b was found to be the substrate for the kinase (90). Sar1b in intestinal cytosol is a member of a heteroquaternary complex composed of Sar1b, FABP1, Sec13, and SVIP (90). On phosphorylation of Sar1b, the heteroquaterneric complex is completely disrupted, releasing each of the components in its monomeric form including FABP1. Of particular interest is that FABP1 as a complex member cannot bind to the ER whereas FABP1 by itself can (90). Thus the rate at which FABP1 is released from the complex could represent a control point in PCTV generation (Fig. 2).

Once produced, PCTV behave as a typical transport vesicle. Transport vesicles use their vSNARE (vesicle SNARE) to direct the vesicles to their targets (80). The vSNAREs pair with the tSNAREs (target SNARE) of the target membrane to form a SNARE complex to deliver the vesicle contents to the target lumen on membrane fusion (45). The SNARE complex is composed of four helices, one from the transport vesicle and three from the target membrane (45). The energy gained by the formation of the SNARE complex is utilized to overcome the antagonistic forces of the two membranes. Ultimately, a trans complex is formed in which the opposing membranes are essentially melded into one. In the case of PCTV, the vSNARE is VAMP7 and the tSNAREs are rbet1, syntaxin5, and vti1a (96).

Once the prechylomicron enters the Golgi, two maturation events occur in preparation for its export into the mesenteric lymph. ApoAI is not added to the prechylomicron in the ER. Presumably the apoAI is transported to the Golgi with other secretory proteins. Prechylomicrons isolated from the ER lumens by HCO₃ treatment do not contain apoAI (94) and do not bind to exogenously added apoAI (Kumar S and Mansbach C, unpublished observations). By contrast, prechylomicrons isolated from the Golgi contain apoAI (94) and bind exogenously added apoAI. In addition to the accretion of apoAI, apoB₄₈ undergoes glycosylation changes (9). The result of these changes is the maturation of the chylomicron. No further lipid is added to the chylomicron in the Golgi since chylomicrons in PCTV are the same size as the average chylomicron (120).

The mature chylomicron is now ready to be exported into the mesenteric lymph. The chylomicrons leave the Golgi in a separate transport vesicle with several chylomicrons per vesicle (81). Little is known about the exit of chylomicrons from the Golgi, but microtubules are likely involved as a mechanism by which chylomicrons are targeted to basolateral membrane. These vesicles are targeted to the basolateral membrane and the chylomicrons are exocytosed into the lamina propria by reverse exocytosis and then into the mesenteric lymph (81).

A major question concerns whether chylomicron output into the lymph can be controlled. For many years it has been known that the amount of PC delivered to the intestine correlates with the rate of chylomicron export. Physiologically, the majority of PC entering the intestine comes from the bile (10). Using intraduodenal infusion techniques and bile duct diversion allowed a closer study of the effect of PC intestinal input on
Fig. 2. Control mechanisms for cytosolic FABP1 binding to the ER. A: FABP1 is a member of a heteroquatromeric protein complex in cytosol comprised of sar1b, sec13, and SVIP. FABP1 cannot bind to the ER as a member of this complex. B: PKCζ and lyso-PC (LPC) are endocytosed via the caveolin-1-containing endocytic vesicle (CEV) from the apical membrane. Both are present on the surface of the CEV. On activation of PKCζ by LPC, the PKCζ elutes off the CEV into the cytosol. C: activated PKCζ phosphorylates Sar1b in the cytosolic heteroquatromeric complex. D: phosphorylation of Sar1b completely disrupts the heteroquatromer and releases the proteins in their monomeric form. Monomeric FABP1 now can bind to the ER where it can organize the PCTV budding complex.

chylomicron output. These studies have shown that the lowest rate of chylomicron export is when the bile duct has been diverted (104). Chylomicron output was improved when lyso-PC was infused in the bile-diverted rats, less so when choline was infused, suggesting the importance of the lyso-PC pathway of PC synthesis compared with the Kennedy pathway (105). This thesis was tested by increasing amounts of PC into bile-diverted rats with the finding that, at 10 mM, almost all chylomicron PC was derived from infused PC, confirming the importance of the lyso-PC pathway (59). Furthermore, intraluminal PC was the preferred substrate for chylomicron PC compared with de novo synthesized PC as shown by infusing 32P and biliary PC was preferred to ovo-PC for chylomicron PC formation (59). These studies were originally interpreted as the requirement of exogenous PC as a surface component of chylomicrons for maximal chylomicron output since the de novo synthetic pathway could not produce the necessary amount of PC needed. Supplemental studies showed that fat-prefed rats, in which biliary PC is increased (47), and those infused with 9 mM PC put out more chylomicrons into the lymph than did chow-fed rats (60). In sum, these studies confirmed that there was a graded relationship between the PC input rate and chylomicron output.

The relationship between dietary PC and chylomicron output was brought into focus recently in studies on the regulation of PCTV export from intestinal ER. Although it was known that FABP1 organized a four-membered budding complex that selected specifically chylomicrons as cargo and completed the fission step of the transport vesicle (68), we were surprised to find that whole cytosol, which is replete with FABP1 (8), did not initiate budding. Budding occurred only when ATP was added to the cytosol (90), which, as mentioned, was utilized by PKCζ to phosphorylate Sar1b, releasing FABP1 from a heteroquatromer, and enabling it to bind to the ER (90).

PKCζ is a member of the PKC family of protein kinases and as such is not activated by DAG or Ca2+. Because the data correlating PC delivery to the intestine and chylomicron output were so clear, we tested lyso-PC, the absorbed form of intestinal PC, as a potential activator. It was found that lyso-PC was the most potent activator of intestinal cytosolic PKCζ of the lysophospholipids and phospholipids tested, including ceramide (89). To determine whether lyso-PC was an important kinase activator physiologically, we developed five different rat models of increasing amounts of PC delivery to the intestine, from bile diversion to 9 mM PC infusion. In each case, as the PC delivery increased, there was an increase in cytosolic lyso-PC concentration and a graded increase in PKCζ activity (89). Importantly, the km of PKCζ was below the highest concentration achieved in the cytosol after PC infusion, suggesting that there would be under most physiological conditions a direct relationship between PC input into the intestine and PKCζ activation.

It was further found that lyso-PC was absorbed on CEV, the same transport vesicle as transports PKCζ from the apical membrane to the cytosol (89). Since lyso-PC and PKCζ were both found to be on the surface of CEV, the geographical proximity suggests that only small amounts of activator could be effective in activating the enzyme. On activation, PKCζ undergoes a conformational change and elutes from the surface of the CEV to diffuse freely in the cytosol, enabling it to reach its substrate, Sar1b, likely more quickly than if it were still on CEV because of its smaller size (89). The elution step is vectorial; activated PKCζ cannot repopulate CEV. In sum, we propose that the normal physiological control of chylomicron output is via luminal PC delivery, causing activation of PKCζ, phosphorylation of Sar1b, release of FABP1, and generation of the PCTV budding complex.

Another well-known modulator of chylomicron output is abetalipoproteinemia. This genetic defect is named for the phenotype but the defect is in production of the microsomal TAG transfer protein (108). Although the phenotype of abetalipoproteinemia as regards the intestine can be reproduced with an intestinal-specific mtp KO mouse model (38, 114), these findings show that the lipid absorption is reduced in intestinal
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specific MTP KO mouse under physiological circumstances in the intestine. However, the CLOCK gene has been shown to affect intestinal MTP levels and plasma TAG as a consequence (73, 74). Pharmacological attacks on MTP have also been successful in lowering plasma TAG and apoB levels in humans (22, 82).

Other mechanisms of affecting intestinal chylomicron output have been described. The incretin GLP-2 has been shown to increase chylomicron delivery into the circulation (32, 35). The MTP KO mouse under physiological conditions in the intestine is controlled by the input rate of dietary fat and the biliary delivery of PC. Since fat is the greatest stimulator of biliary bile output, it is tempting to speculate that the two are correlated. This would lead to the suggestion of a feed-forward theory of dietary fat absorption. The more fat that enters the intestine, the more lyso-PC enters the circulation. The greater the PC, the more lyso-PC enters the circulation. The more the dietary fat load, the more biliary PC is secreted by the liver. This would lead to the suggestion of a feedback theory of dietary fat absorption. The more dietary fat load, the more lyso-PC enters the circulation. The greater the PC, the more lyso-PC enters the circulation. The more the dietary fat load, the more biliary PC is secreted by the liver.

Concluding Remarks

Under most physiological conditions chylomicron output by the intestine is controlled by the input rate of dietary fat and the biliary delivery of PC. Since fat is the greatest stimulator of biliary bile output, it is tempting to speculate that the two are correlated. This would lead to the suggestion of a feed-forward theory of dietary fat absorption. The more fat that enters the intestine, the more lyso-PC enters the circulation. The greater the PC, the more lyso-PC enters the circulation. The more dietary fat load, the more biliary PC is secreted by the liver. This would lead to the suggestion of a feedback theory of dietary fat absorption. The more dietary fat load, the more lyso-PC enters the circulation. The greater the PC, the more lyso-PC enters the circulation. The more the dietary fat load, the more biliary PC is secreted by the liver.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

C.M.M. and S.S. conception and design of research; C.M.M. and S.S. drafted manuscript; C.M.M. and S.S. edited and revised manuscript; C.M.M. and S.S. approved final version of manuscript; S.S. performed experiments; S.S. prepared figures.

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