Development and Physiological Regulation of Intestinal Lipid Absorption.
I. Development of intestinal lipid absorption: cellular events in chylomicron assembly and secretion

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Black DD. Development and Physiological Regulation of Intestinal Lipid Absorption. I. Development of intestinal lipid absorption: cellular events in chylomicron assembly and secretion. Am J Physiol Gastrointest Liver Physiol 293: G519–G524, 2007. First published May 10, 2007; doi:10.1152/ajpgi.00189.2007.—The newborn mammal must efficiently absorb dietary fat, predominantly as triacylglycerol, and produce chylomicrons to deliver this lipid to peripheral tissues. The cellular mechanisms involved in enterocyte chylomicron assembly have recently been elucidated, and data on their regulation in the immature gut are beginning to emerge. This review focuses on key proteins involved in chylomicron assembly: apolipoprotein B-48, microsomal triglyceride transfer protein, and apolipoprotein A-IV. Recent studies support a role for apolipoprotein A-IV in enhancing chylomicron secretion by promoting production of larger particles. These proteins are regulated in a manner to maximize the lipid absorptive capacity of the newborn intestine.

apolipoprotein A-IV; apolipoprotein B-48; microsomal triglyceride transfer protein; newborn; small intestine

THE SUCKLING PERIOD OF DEVELOPMENT challenges the neonatal small intestine with a daunting task. A switch from a maternal nutrient source via the placenta to an enteral high-fat breast milk source of nutrition characterizes the period of transition from the intrauterine fetal to extrauterine neonatal environments. The newborn mammal must be equipped to efficiently absorb dietary fat, predominantly in the form of triacylglycerol (TG), and produce chylomicrons to deliver this lipid to peripheral tissues. A high-fat diet in the form of breast milk (>50% of calories as lipid) is delivered to the intestinal lumen and enters a complex scheme of events orchestrated to digest and solubilize dietary fat for delivery into the enterocyte, where the products of lipolysis are reesterified. The next step, chylomicron assembly, is a complex process designed to package dietary lipid into thermodynamically stable particles containing neutral lipid (TG and cholesteryl ester) in the core and polar lipids (phospholipid and free cholesterol) and apolipoproteins on the surface. This unique assembly process produces a “package” that is stable in the aqueous environment of the bloodstream as it delivers dietary lipid to targeted peripheral tissues and, ultimately, the liver. Details of this process are shown in Fig. 1. As the secreted chylomicron circulates, the core TG undergoes hydrolysis by endothelial-bound lipoprotein lipase with entry of fatty acids into muscle for energy production and adipocytes for storage. The resultant TG-depleted, cholesterol-enriched chylomicron remnant particle is eventually cleared by the liver via a receptor-mediated process.

Developmental deficiencies in luminal lipid digestion by pancreatic lipases and solubilization by bile acid micelles that result in “physiological” fat malabsorption in the newborn, as well as compensatory mechanisms such as gastric lipase digestion, have been well studied and are reviewed elsewhere (9). Products of TG digestion, fatty acid and sn-2-monooacylglycerol (MAG), may cross the enterocyte apical membrane via diffusion or by a carrier-mediated process. The relative contributions from each mechanism have been the source of controversy, as has been the identity of such a putative transporter. However, there is no compelling evidence that fatty acid uptake is a limiting factor in neonatal lipid absorption. In fact, suckling rat enterocytes take up lipids more efficiently than weanling rats, possibly due to increased fluidity of the brush-border membrane (6).

Once the products of lipid digestion enter the enterocyte, they must traverse the cytoplasm to the endoplasmic reticulum (ER), where the resynthesis of complex lipids occurs. Fatty acid binding proteins (FABPs) in the enterocyte exist in two forms: liver FABP (L-FABP) and intestinal FABP (I-FABP). In particular, L-FABP may function to translocate long-chain fatty acids and MAG from the apical membrane to the ER. Experimental evidence from intestinal isografts implanted subcutaneously into adult mice suggests that spatial and temporal induction along the small intestine is genetically programmed and not triggered by dietary lipid, although dietary lipid may modulate the degree of expression (17). Fatty acids and MAG are utilized to resynthesize first to diacylglycerol and then TG by acyl-CoA:monooacylglycerol acyltransferase (MGAT) and acyl-CoA:diacylglycerol acyltransferase (DGAT), respectively, in the ER. This monooacylglycerol pathway predominates in the small intestine, including that of the neonate. A recent advance has been the cloning and characterization of mouse and human MGAT and DGAT isoforms and their regulation (4). Previous studies, mainly in the developing rat, have suggested that enterocyte fatty acid esterification is not limiting in the newborn and suckling period (19). Furthermore, dietary lipid appears to be the most important modulator for the development and maintenance of intestinal fatty acid esterification.

The focus of this review will be the recent elucidation of the developmental aspects of the intracellular processes associated with chylomicron assembly in the ER and regulation of the key proteins involved in this complex process.
**Key Proteins in Chylomicron Assembly**

Recent advances in our knowledge of chylomicron assembly have focused on the roles of three key players in this process: apolipoprotein B-48 (apo B-48), microsomal triglyceride transfer protein (MTP), and apolipoprotein A-IV (apo A-IV). The essential roles of apo B-48 and MTP in intestinal lipid absorption have been well established. Studies have also defined the regulation of these proteins in the neonatal gut by dietary lipid. However, experimental evidence directly supporting an important role for apo A-IV in enhancing the secretion of chylomicrons and associated lipids in newborn enterocytes has only recently been established (14, 15) and will be a major focus of this review.

**Apo B-48.** Apo B exists as two distinct forms. Apo B-100 is the larger protein, produced primarily by the pre- and postnatal liver and prenatal small intestine, and contains the low-density lipoprotein receptor-binding domain. The smaller form, apo B-48, is primarily synthesized by the postnatal small intestine and lacks the low-density lipoprotein receptor-binding domain. Both are produced from the same gene by a unique posttranscriptional mRNA editing complex, apobec-1, that creates a premature stop codon by deamidation of a single base (C→U) in the apo B-100 mRNA transcript that allows coding for apo B-48 that correlates with the amino-terminal half of apo B-100. Apo B-100 is essential for very-low-density lipoprotein (VLDL) assembly in hepatocytes, and apo B-48 is requisite for chylomicron assembly in enterocytes.

**MTP.** MTP, a heterodimeric protein complex possessing lipid transfer activity, functions in the small intestine and liver to transport ER membrane-bound lipid, primarily newly synthesized TG, to newly translated apo B in the ER lumen as the first step in TG-rich lipoprotein biogenesis (Fig. 1). This initial lipidation of apo B prevents proteosome-mediated degradation. In the small intestine, MTP may also facilitate the further lipidation of nascent chylomicrons beyond the first apo B rescue step. MTP is a heterodimer consisting of a large subunit (97 kDa), which possesses the lipid transfer activity, and a smaller subunit identical to protein disulfide isomerase (PDI; 55 kDa). Mutations in the MTP large subunit are responsible for the disease abetalipoproteinemia, which is characterized by an inability to secrete intestinal chylomicrons or hepatic VLDL. As a component of MTP, PDI appears to maintain the large subunit in soluble form and may play a role in targeting MTP to the ER lumen. Since PDI is ubiquitous and abundant in the ER, it is not thought to be a limiting factor in MTP function.

**Apo A-IV.** Apo A-IV is a lipid binding protein that is expressed predominantly in the mammalian small intestine. Although numerous extraintestinal functions have been ascribed to apo A-IV (21), the preponderance of evidence points to a primary role in intestinal lipid absorption. Of all the intestinal genes associated with lipid absorption, the apo A-IV gene is the most responsive to intestinal lipid flux. In the enterocyte, apo A-IV is incorporated into nascent chylomicrons at an early stage of biogenesis in the ER and is secreted on the surface of chylomicrons at the basolateral membrane. Thereafter, most apo A-IV dissociates from the chylomicron surface and, in humans, circulates predominantly as a lipid-free protein.

**Chylomicron Assembly**

Chylomicron assembly is currently thought to proceed through two steps, as shown in Fig. 1. The first step involves the translation of apo B-48 with extrusion of the amino-terminal portion of the protein into the ER lumen. If not lipidated, apo B-48 is targeted for proteosome-mediated degradation. However, MTP functions to lipidate apo B-48 and rescue it from degradation, resulting in the formation of a primordial chylomicron. MTP may transfer lipid from the ER membrane and other sites directly to apo B-48 or may bind to...
apo B-48 to promote proper folding and lipid acquisition. In the second step, MTP mediates additional bulk lipidation of the primordial particle. During this process, apo A-IV is added to the particle surface and, as discussed below, appears to play a role in the assembly process.

The nascent chylomicron exits the ER in a specialized vesicle, the prechylomicron transport vesicle (PCTV), for transport to the cis-Golgi. Data from the adult rat have suggested that budding of the PCTV is the rate-limiting step in lipid absorption (20). The ER to Golgi trafficking of chylomicrons appears to be unique from that of other cargo, such as proteins. Recent data from Neeli et al. (16) have indicated that L-FABP can select cargo for and bud the PCTV from the ER membrane. The budding is independent of coating protein II (COPII) proteins, but trafficking and fusion with the cis-Golgi are dependent on COPII proteins, Sar1 and Sec23/24, as well as a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) fusion complex composed of vesicle-associated membrane protein 7 (VAMP7), syntaxin 5, Bet1, and vit1. In the Golgi, apo B-48 glycosylation is modified, lipid composition is altered, and the particle acquires apo A-I. Subsequently, the particles undergo exocytosis at the basolateral membrane.

Chylomicron Assembly in the Newborn Intestine

Only recently have the key proteins and processes involved in chylomicron assembly in neonatal small intestine been studied in detail. For the most part, it appears that the neonatal enterocyte is well adapted to handle absorption of a large lipid load through both dietary induction and preprogrammed regulation of gene expression in a coordinated manner to maximize chylomicron packaging. One reason for the paucity of data in this area has been the lack of suitable in vitro and in vivo model systems. Over the past several years, our laboratory has focused on the neonatal swine as a model system for studies of the regulation of intestinal and hepatic apolipoprotein expression and lipid synthesis by dietary lipid during development. Advantages of this model include intestinal development in the preocial newborn that is similar to that of the human infant and significant homology with human apolipoprotein and lipoprotein metabolism.

Developmental regulation of apo B-48. Apo B-48 in the developing small intestine is regulated in a manner that optimizes chylomicron formation. Apobec-1 expression is upregulated before parturition in the small intestine in rodents, swine, and humans to switch from apo B-100 production in the fetal gut to apo B-48 in the neonatal intestine (23). Interestingly, in apobec-1 knockout mice, in which the gut produces only apo B-100, there is a subtle defect in chylomicron secretion that may be of particular importance in the face of high lipid flux in the neonate, thus making the timing of the switch important (24).

In the adult rat, the mesenteric lymphatic output of apo B-48 is relatively constant regardless of the amount of absorbed lipid. Given that there is one apo B molecule per chylomicron, it appears that the adult rat secretes the same number of chylomicrons regardless of lipid load but can increase chylomicron size with increasing fat absorption. In contrast, we have observed in both the suckling swine (2) and in cultured newborn pig intestinal epithelial (IPEC-1) cells (8) that with fatty acid absorption, especially unsaturated 18-carbon fatty acids, jejunal apo B-48 mass increases, likely due to decreased targeted posttranslational degradation rather than increased synthesis, leading to increased apo B-48 secretion. This increase would make more apo B-48 available for a greater number of particles to enhance lipid export capacity. In animal studies, this effect was observed in both the jejunum and ileum, suggesting the participation of the distal small intestine in dietary lipid absorption in the newborn, perhaps to accommodate absorption of the large lipid load that may escape proximal absorption. In vivo, the magnitude of this induction diminishes near weaning. Biliary diversion strikingly downregulated apo B mass below baseline nonlipid-fed levels in both the jejunum and ileum, underscoring the importance of biliary secretion in the maintenance of apo B-48 production in the newborn. This may have clinical relevance in infants with cholestatic liver diseases that lead to decreased bile flow.

Developmental regulation of MTP. To date, there has been a paucity of information on the developmental expression of MTP in the small intestine. MTP large subunit expression has been analyzed in the fetal mouse using in situ hybridization (18). These studies suggest an important role for MTP in mobilization of yolk sac lipid early in gestation prior to liver and gut expression. Also, MTP expression has been studied in the early gestation human small intestine (12). In these studies, MTP large subunit mRNA, protein, and activity were detected in a decreasing proximal to distal gradient, but enzymatic activity was low in vitro and unresponsive to increasing substrate lipid.

To define the developmental pattern of expression of MTP large subunit mRNA and protein in the developing swine model, samples of the small intestine and liver were collected from 40-day-old gestation fetal, 2-day-old newborn, 3-wk-old weaning, and 2-mo-old weaned female swine (13). In fetal animals, MTP mRNA expression was high in the intestine and liver. However, an unexpected finding was a relatively high level of intestinal MTP large subunit mRNA expression in the fetus in the face of low levels of protein of the appropriate apparent molecular weight. The dissociation of mRNA and protein levels suggests that MTP large subunit protein levels may be regulated at the translational level in the fetal pig intestine.

Postnatally, jejunal MTP large subunit expression paralleled the intake of a high-fat breast milk diet and declined after weaning. Ileal expression was comparable with that of the jejunum in 2-day-old animals but declined to low levels afterward. Postnatal levels of MTP large subunit protein generally paralleled mRNA expression. The 24-h intraduodenal infusion of a high-TG diet in 2-day-old animals acutely increased jejunal and ileal MTP large subunit mRNA levels compared with a low-TG diet. The induction of ileal expression may have been due to some degree of ileal lipid absorption in newborn animals receiving a high-fat diet, which may have exceeded the absorptive capacity of the proximal small intestine. Thus, early increased ileal MTP large subunit expression may represent an important compensatory mechanism to scavenge proximally unabsorbed lipid.

We have studied the roles of glucocorticoids and fatty acids in MTP regulation in a newborn swine enterocyte cell line (IPEC-1) (13). Dexamethasone did not play a major role in the regulation of MTP expression in differentiating IPEC-1 cells. In contrast, fatty acids either upregulated or downregulated
faces. During chylomicron assembly, these hydrophilic helices may be recruited into the expanding nascent particle surface and, by maintaining constant interfacial elasticity, would stabilize particle growth and enable the packaging of more core lipid molecules per particle.

To test the hypothesis that expression of apo A-IV enhances enterocyte chylomicron secretion in response to fatty acid absorption, we developed a stably transfected newborn swine intestinal epithelial cell line that synthesizes and secretes excess swine apo A-IV. We chose the IPEC-1 cell line for this purpose specifically because these cells have relatively low lipid transport efficiency and low background apo A-IV expression compared with native enterocytes, enabling us to observe marked increases in lipid export should they occur. Using this cell line, we demonstrated that when cultured with oleic acid (OA; C18:1\n9), high-level constitutive overexpression and secretion of apo A-IV enhanced radiolabeled basolateral TG and cholesteryl ester secretion by fivefold and phospholipid by twofold, mainly as lipoproteins in the chylomicron and VLDL density range (15).

A limitation of this approach was the fact that the level of constitutive apo A-IV overexpression was far higher than the normal physiological range. To address this issue, IPEC-1 cells were transfected with a tetracycline-regulatable transactivator and full-length swine apo A-IV cDNA driven by the tetracycline response element, allowing regulation of apo A-IV expression and basolateral secretion in a physiological range by culture medium doxycycline concentration (14). To identify protein structural components essential for this enhancement, we also performed similar experiments with human apo A-IV as well as truncated mutants of human apo A-IV resembling swine and chicken apo A-IV.

In cells incubated with OA, a dose-response relationship was observed between medium doxycycline concentration and basolateral apo A-IV and TG secretion. Similarly, regulated expression of another apolipoprotein produced in the small intestine, apo A-I, did not enhance lipid secretion. The mean diameter of TG-rich lipoproteins secreted from doxycycline-treated cells was larger than from untreated cells (87.0 vs. 53.4 nm). Using the same expression system, full-length human apo A-IV (376 amino acids); a “pig-like” human apo A-IV, lacking the carboxy-terminal EQQQ repeats found in human apo A-IV (361 amino acids); and a “chicken-like” apo A-IV, further truncated to 343 amino acids, were expressed in IPEC-1 cells. With increasing protein secretion, cells expressing the full-length human apo A-IV displayed a 2-fold increase in TG secretion; in sharp contrast, cells expressing the pig-like human apo A-IV displayed a 25-fold increase in TG secretion and a corresponding increase in lipoprotein diameter. When human apo A-IV was further truncated to yield a chicken-like protein, TG secretion was inhibited.

These experiments demonstrated that swine apo A-IV enhances basolateral TG secretion in a dose-dependent manner by increasing the size of secreted lipoproteins. These data suggest that the region in the human apo A-IV protein from residues 344 to 354 is critical to its ability to enhance lipid secretion, perhaps by enabling the packaging of additional core TG into chylomicron particles. Since this region is not a lipid binding domain, this enhancement may be due to interaction of this domain with other regions of apo A-IV or other proteins that may influence the tertiary structure of apo A-IV and the lipid binding affinity of its α-helical domains. The EQQQ-rich carboxy terminus of human apo A-IV may play an inhibitory or modulatory role in chylomicron packaging in humans. The truncated human apo A-IV is probably more efficient in promoting chylomicron secretion than swine apo A-IV due to its more hydrophilic α-helices.

These studies established that physiological levels of native swine and human apo A-IV secretion enhance TG transport by inducing the secretion of larger chylomicron/VLDL particles, either by biophysically stabilizing their surface or possibly by interacting with apo B to slow their passage through the cellular compartments where second-step expansion occurs (7). Taken together, these data suggest that although, unlike apo B, apo A-IV is not obligatory for intestinal lipoprotein assembly, it may facilitate maximally efficient lipoprotein secretion. This function may be especially important in the newborn, where the intestine must rapidly and efficiently absorb the large lipid load provided by breast milk. Human apo A-IV mutant studies have established the importance of the carboxy-terminal sequences of apo A-IV in the enhancement of chylomicron secretion, probably by influencing particle assembly and/or lipidation in the ER. The exact biophysical properties of apo A-IV and the intracellular mechanisms by which it mediates this effect require further study.

These data may appear to conflict with the observations from transgenic mouse models indicating that lipid absorption was unaffected either by overexpression of human apo A-IV or targeted disruption of the apo A-IV gene. However, these animals were not evaluated under conditions of sustained lipid absorption that approached the rate-limiting capacity of the enterocyte. Moreover, inactivation of apo A-IV synthesis in transgenic animals may induce alternate compensatory pathways that maintain normal levels of intestinal lipid absorption. In this regard, the suckling rat does not efficiently transport dietary lipid in lymph chylomicrons, and indirect evidence has suggested that the portal venous route may be important in this species and possibly in all rodents, including mice, during the suckling period (5). In contrast, lymphatic transport is important for lipid absorption in the suckling swine (1), a species
whose intestinal development more closely resembles that of the human.

Chylomicron Trafficking in Newborn Enterocytes

No studies have yet been performed to specifically evaluate enterocyte PCTV budding from the ER, which appears to be the rate-limiting step in enterocyte lipid transport in the adult, or subsequent PCTV trafficking to the Golgi in the immature small intestine. However, indirect evidence of the importance of the proteins involved in PCTV chylomicron trafficking in neonatal lipid absorption has been provided by recently identified mutations in the SAR2A gene that encodes the COPII protein Sar1b in patients with Anderson disease and chylomicron retention disease (10). Both diseases are characterized by impaired chylomicron secretion and severe fat malabsorption early in life.

Some insight into the trafficking process in the immature enterocyte has been gained from in vitro studies of trafficking of labeled lipid from ER to Golgi vesicles performed in IPEC-1 enterocyte has been provided by recently identified mutations in the SAR2A gene that encodes the COPII protein Sar1b in patients with Anderson disease and chylomicron retention disease (10). Both diseases are characterized by impaired chylomicron secretion and severe fat malabsorption early in life.

Some insight into the trafficking process in the immature enterocyte has been gained from in vitro studies of trafficking of labeled lipid from ER to Golgi vesicles performed in IPEC-1 cells (25). These studies demonstrated that trafficking efficiency varies with different fatty acids with stearic acid (C18:0) and the long-chain polysaturated fatty acid (LC-PUFA) eicosapentaenoic acid (EPA; C20:5n-3), adversely influencing ER to Golgi TG trafficking compared with OA. Furthermore, EPA inhibited ER lipid synthesis and transfer of TG from the ER membrane to ER luminal particles. These results suggest that fatty acid composition of infant formula should be balanced between nutrient needs and enterocyte absorptive efficiency. In particular, these results may have relevance to the recent addition of the LC-PUFAs to infant formula for their positive effects on brain and eye development.

Role of HNF-4 in Neonatal Lipid Absorption

Although a comprehensive discussion of the transcriptional regulation of the genes involved in small intestinal lipid absorption during development is beyond the scope of this review, recent evidence has pointed to an important role for the nuclear receptor hepatocyte nuclear factor (HNF)-4α. This transcription factor regulates numerous genes involved in glucose and lipid metabolism, including apo A-IV and MTP, and is capable of ligand-dependent and -independent transactivation, although the identity of physiological ligands is controversial. We recently demonstrated coordinate upregulation of both HNF-4α as well as apo A-IV and MTP expression by acute lipid absorption in the newborn swine jejunum and by fatty acid treatment in IPEC-1 cells (11). Fatty acid-independent overexpression of HNF-4α in IPEC-1 cells induced apo A-IV and MTP expression at the transcriptional level, and fatty acid treatment did not further enhance this transcriptional activation. These data suggest that the acute induction of the apo A-IV and MTP genes by dietary lipid in the newborn intestine may occur, at least in part, via ligand-independent transactivation by HNF-4α, which is itself induced by a lipid-mediated mechanism. Thus, HNF-4α may serve as a “master switch” for coordinating chylomicron assembly in the newborn.

Summary and Directions for Future Research

For many years, the details of enterocyte chylomicron assembly and trafficking were unknown with these components of the lipid absorptive pathway comprising a virtual “black box.” Now, as the details of this process in the mature enterocyte are being defined, information is beginning to emerge from the immature cell. Overall, studies of the key protein components involved in chylomicron assembly, apo B-48, MTP, and apo A-IV, suggest a remarkable ability of the neonatal gut to adapt to the absorption of the high lipid load essential for growth and development. The likely important role of apo A-IV in enhancing chylomicron transport of lipid in the neonate is particularly relevant.

Rather than static descriptive studies of gene expression during development, more studies of the active physiological regulation by dietary factors in neonatal lipid absorption are needed. In particular, studies of the developmental and dietary regulation of PCTV budding and ER to Golgi trafficking are needed. This is especially true if the budding process is also the rate-limiting step in neonatal lipid absorption. The neonatal swine model, which provides “industrial-strength” quantities of cells and tissue, and whose gut development is close to that of the human infant, is an ideal model for such studies. As we learn more of the regulation of these processes in the immature intestine, especially at the transcriptional level, agents that induce expression of apo B-48, MTP, and apo A-IV as well as key molecules involved in PCTV budding and trafficking would have potential therapeutic value in increasing the lipid absorptive efficiency of the small intestine in premature infants or those with diseases such as short bowel syndrome. Also, greater knowledge of the fatty acid specificity of these processes would guide the optimal lipid composition of infant formula. Finally, determining whether these processes are susceptible to metabolic imprinting early in life may have implications for later diseases such as hyperlipidemia and obesity.

GRANTS

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