Mechanisms Involved in the Intestinal Digestion and Absorption of Dietary Vitamin A

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Abstract Dietary retinyl esters are hydrolyzed in the intestine by the pancreatic enzyme, pancreatic triglyceride lipase (PTL), and intestinal brush border enzyme, phospholipase B. Recent work on the carboxylester triglyceride lipase (CEL) knockout mouse suggests that CEL may not be involved in dietary retinyl ester digestion. The possible roles of the pancreatic lipase-related proteins (PLRP) 1 and 2 and other enzymes require further investigation. Unesterified retinol is taken up by the enterocytes, perhaps involving both diffusion and protein-mediated facilitated transport. Once in the cell, retinol is complexed with cellular retinol-binding protein type 2 (CRBP2) and the complex serves as a substrate for reesterification of the retinol by the enzyme lecithin:retinol acyltransferase (LRAT). Retinol not bound to CRBP2 is esterified by acyl-CoA acyltransferase (ARAT). The retinyl esters are incorporated into chylomicrons, intestinal lipoproteins that transport other dietary lipids such as triglycerides, phospholipids, and cholesterol. Chylomicrons containing newly absorbed retinyl esters are then secreted into the lymph. J. Nutr. 131: 1405–1408, 2001.

Key Words: • retinoids • lipid absorption • pancreatic enzymes • chylomicrons • lipases

The major sources of vitamin A in the diet are the provitamin A carotenoids in fruits and vegetables and retinyl esters found in foods of animal origin. In humans, carotenoids are either cleaved to generate retinol or absorbed intact. In contrast, retinyl esters are completely hydrolyzed in the intestinal lumen and free retinol is taken up by enterocytes (1,2). This review focuses on the mechanisms involved in the digestion of retinyl esters in the intestinal lumen, the uptake and reesterification of retinol in enterocytes, and the incorporation of the resulting retinyl esters into chylomicrons and secretion of these lipoproteins from the enterocytes.

Hydrolysis of Retinyl Esters in Intestine. Carboxylester lipase (CEL)
and pancreatic triglyceride lipase (PTL) effectively hydrolyze retinyl palmitate in vitro. CEL also catalyzes the hydrolysis of cholesteryl esters, triglycerides and lysophospholipids. CEL knockout (CELKO) mice were generated to study the functions of CEL (2–4). CELKO mice absorbed ~50% less cholesterol provided as cholesteryl ester compared with wild-type mice. In contrast, CELKO mice absorbed the same amount of retinol, when provided as retinyl ester, as did wild-type mice. On the other hand, neither strain absorbed retinyl hexadecyl ether (2,4). These data suggested that retinyl ester hydrolysis was required for absorption and that CEL was not the enzyme involved (at least in this study in which 100 μg of retinyl esters were delivered in 100 μL of peanut oil). Therefore, one or more other retinyl ester hydrolase (REH) enzymes must be present in the gut lumen or in the enterocyte.

We sought to identify the non-CEL, pancreatic REH activity that was present in CELKO mice, as well as to investigate this activity in wild-type mice and in rats. Several lines of evidence suggest that the activity is due to PTL (5). First, when pancreatic homogenates of wild-type mice and rats were assayed with different bile salts, cholesteryl ester hydrolase activity was detected only in the presence of trihydroxy bile salts, consistent with previous results (6). Pancreatic REH activity, however, is not absolutely dependent on trihydroxy bile salts and was detected not only in the presence of trihydroxy bile salts, but also in the presence of dihydroxy bile salts and CHAPS, a bile salt analog, and in the absence of bile salts. Second, when pancreatic homogenates obtained from rats, and wild-type and CELKO mice were used to assay REH activity, a considerable stimulation of the REH activity by colipase was observed, indicating that PTL was contributing to the bile salt-dependent REH activity. Third, when pancreatic homogenates were applied to DEAE-chromatography, the majority of REH activity coeluted with PTL activity. Fourth, the enzymatic characteristics of purified human PTL suggested that retinyl palmitate was a substrate. Hydrolysis of both retinyl esters and triglycerides by the enzyme were completely dependent on the presence of colipase, and other enzymatic properties were similar for both substrates.

Although our data strongly suggest that PTL is a major REH in rat and mouse intestinal lumen, they do not provide final proof. For example, some triglyceride hydrolysis was observed in the absence of colipase in pancreatic homogenates, which may point to the presence of other related enzyme activities such as pancreatic lipase related protein 2 (PLRP2) and PLRP2 is 65% identical to PTL and shows activity toward triglycerides in the classical PTL assay (7). At present, we do not know the percentage contribution of PLRP2 to pancreatic bile salt–dependent REH activity. Also, another pancreatic lipase related protein (PLRP1) has been cloned, which is 68% homologous to PTL, but whose substrate remains unknown (8). Thus, more than one enzyme may be responsible for the complete hydrolysis of retinyl esters in the intestinal lumen.
In addition to pancreatic bile salt-dependent REH activities, an REH activity intrinsically located in the brush border membrane of enterocytes was shown in rat and human intestines (9,10). This activity was suggested to be due to an intestinal phospholipase B. The authors showed that rat brush border membrane, isolated from rats in which the common pancreatic duct had been ligated for 2 d (thus prohibiting contamination of brush border membrane with enzymes secreted by pancreas such as CEL or PTL), had a greatly decreased hydrolytic activity against short-chain retinyl esters (in the presence of trihydroxy bile salts), and a smaller (30%) decrease in activity against long-chain retinyl esters (such as retinyl palmitate) compared with sham-operated rats. Therefore, they suggested that short-chain REH was due mainly to enzymes of pancreatic origin, whereas the majority (70%) of long-chain REH was intrinsic to the brush border. The remaining 30% of REH activity could be due to PTL because this REH activity was detected in the presence of both trihydroxy and dihydroxy bile salts. It is important to point out that the relative activities observed in vitro may not reflect the relative contributions of the various enzymes in vivo. To determine which of the above-mentioned enzymes is the most critical in intestinal RE digestion and absorption, it will be necessary to perform RE absorption experiments in the appropriate knockout mouse strains and in mice deficient in more than one enzyme. The enzymes potentially involved in hydrolysis of dietary retinyl esters are outlined in Figure 1.

**Uptake of Retinol by Enteroctyes.** Studies of the uptake of retinol by the human colon carcinoma cell line, Caco-2, indicated that retinol at physiologic and pharmacologic concentrations was taken up by a saturable, carrier-mediated process and a nonsaturable, diffusion-dependent process, respectively (11). The retinol taken up by these cells was esterified, and the retinyl esters contained mainly palmitic and oleic acids (11,12). Our studies showed that retinol uptake is rapid and not affected by the presence of high concentrations of free fatty acids (13).

Early studies using intestinal segments also suggested that the unesterified retinol was taken up by protein-mediated facilitated diffusion and passive diffusion mechanisms at physiologic (150 nmol/L) and pharmacologic concentrations (450–2700 nmol/L), respectively (14,15). Recently, some evidence for protein-mediated uptake of retinol has been presented using intestinal segments (16,17). Until now, no protein has been identified and characterized that might be involved in the uptake of retinol (Fig. 1). However, three different membrane-bound proteins, CD36, membrane-bound fatty acid–binding protein and a fatty acid transport protein that might be involved in fatty acid uptake have been iden-

**FIGURE 1** Overview of digestion and absorption of vitamin A. Dietary retinyl esters (RE) are hydrolyzed in the lumen by the pancreatic enzyme, pancreatic triglyceride lipase (PTL), and the intestinal brush border enzyme, phospholipase B (PLB). Recent work on the carboxylester lipase (CEL) knockout mouse suggests that CEL may not be involved in dietary RE digestion. The possible roles of the pancreatic lipase-related proteins (PLRP) 1 and 2 and other enzymes require further investigation. Unesterified retinol (ROH) is taken up by the enterocyte, perhaps facilitated by a yet unidentified retinol transporter (RT). Once in the cell, retinol is complexed with cellular retinol-binding protein type 2 (CRBP2) and the complex serves as a substrate for reesterification of the retinol by the enzyme lecithin:retinol acyltransferase (LRAT). It is possible that “excess” retinol not bound to CRBP2 serves as a substrate for acyl-CoA acyltransferase (ARAT) to form RE that are retained in the cell. The RE are then incorporated into chylomicrons, intestinal lipoproteins containing other dietary lipids such as triglyceride (TG), phospholipid (PL), and cholesterol (Ch) and apolipoprotein B (apoB). Chylomicrons (CM) containing newly absorbed retinyl esters (CMRE) are then secreted into the lymph. Assembly of CM requires apoB and microsomal triglyceride transfer protein (MTP).
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Reesterification and Incorporation into Chylomicrons.
Early studies in intact rats and humans clearly demonstrated that after uptake of newly absorbed retinol, the retinol was largely reesterified with long-chain fatty acids (mostly palmitate) and secreted into the lymphatic system along with other dietary lipids in chylomicrons. More recently, Caco-2 cells have been used to study mechanisms of vitamin A absorption that are difficult to study in intact animals. Differentiated Caco-2 cells were shown to express CRBP2, ARAT, LRAT and retinal reductase (11,12). Studies on retinol secretion revealed that Caco-2 cells supplemented with no fatty acids secreted only free retinol. However, cells incubated with oleic acid were shown to secrete retinyl esters in addition to free retinol (12). On the basis of these observations, it has been suggested that lipoprotein particles secreted by these cells may contain retinol and retinyl esters (12). However, no data about the secretion of either free or esterified retinol as part of different lipoprotein particles were reported in these studies.

In enterocytes, two enzymes, LRAT and ARAT, have been identified that are involved in the esterification of free retinol (Fig. 1). It has been suggested (but not shown) that retinyl esters formed by LRAT and ARAT may be targeted for secretion with chylomicrons and storage, respectively (19). It is generally believed that retinol is secreted into the lymph mainly as retinyl palmitate. During metabolic studies, analysis of the plasma revealed that most of the retinyl esters are present in small chylomicrons (34). Substantial amounts of retinyl esters are also found in large chylomicrons followed by smaller amounts in VLDL (34). In contrast to triglycerides, cholesterol esters and other lipids, retinyl esters are not present in other lipoproteins such as intermediate density lipoproteins, LDL or HDL. These studies indicate that retinyl esters are present mainly in large and small chylomicrons and behave very differently from other neutral lipids such as triglycerides and cholesterol esters. What is the molecular basis for this specificity? How do intestinal cells incorporate retinyl esters into chylomicrons?

To understand the mechanism of secretion of RE by the intestine during fasting and postprandial states, we conducted studies in which differentiated Caco-2 cells were supplemented with radiolabeled retinol under conditions that support (postprandial) or do support (fasting) chylomicron secretion (13). After uptake, cells store retinol in both esterified and unesterified forms. Under fasting conditions, cells secrete variable amounts of free retinol, mainly unassociated with lipoproteins. However, under postprandial conditions, these cells secreted significant amounts of retinyl esters, mainly with chylomicrons. The secretion of retinyl esters with chylomicrons was independent of the rate of uptake of retinol and intracellular free and esterified retinol levels, and was dependent on the assembly and secretion of chylomicrons.

The secretion of retinyl esters was correlated with the secretion of chylomicrons and not with total apolipoprotein B secretion. Inhibition of chylomicron secretion by Pluronic L81 decreased the secretion of retinyl esters and did not result in their increased secretion with smaller lipoproteins. These data strongly suggest that retinyl ester secretion by intestinal cells is a highly specific and regulated process that is dependent on the assembly and secretion of chylomicrons. Our data also indicate that retinyl ester incorporation into chylomicrons is not a passive process but is an exquisitely orchestrated event. Retinyl ester secretion does not occur at all times. It is induced when cells can assemble and secrete chylomicrons. Thus, it appears that intestinal cells may have a specific mechanism for the targeting of retinyl esters to nascent chylomicrons. These
cells appear to wait for the assembly of chylomicrons before secreting retinyl esters. Chylomicron assembly requires apoB48, microsomal triacylglyceride transfer protein (MTP), phospholipids and triglycerides and occurs in the endoplasmic reticulum (35–37). ApoB48 is a structural protein for the assembly of these lipoproteins, whereas MTP is required for the lipidation of apoB48. We have provided evidence to suggest that different lipids are added onto apoB48 in discrete events during the formation of these lipoproteins (13,36–38). First, large amounts of preformed phospholipids, probably from the membranes of the endoplasmic reticulum, are added onto nascent apoB polypeptides to form “primordial lipoproteins.” In the second event, newly synthesized triglycerides are added in bulk to form “nascent lipoproteins.” It appears that retinyl esters are added after chylomicron assembly as a final event of lipoprotein maturation just before secretion. Due to the specificity of the secretion of retinyl esters, we propose that retinyl esters cannot be used as surrogates for the final stages of chylomicron assembly (36,37).

The literature reviewed above suggests that the intestinal digestion and absorption of vitamin A is a highly complex process, and that a number of enzymes and other proteins participate in the process. Mice deficient in one or more proteins may define the redundancy and absolute requirement of various proteins in retinyl ester metabolism. In addition, overexpression of specific proteins might lead to better understanding of the role of individual proteins in vitamin A absorption. The development of highly specific inhibitors could shed light on these issues. Furthermore, understanding the mechanisms of retinyl ester absorption may shed light on the mechanisms involved in the assembly and secretion of chylomicrons. Knowledge about the absorption of vitamin A may be valuable in studying the absorption of other fat-soluble micro-nutrients.

LITERATURE CITED


