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review

Intestinal lipoprotein metabolism

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INTRODUCTION

The intestine has long been recognized as an organ active in lipoprotein formation. This is hardly surprising when one considers the large quantities of dietary and endogenous lipids that traverse the intestinal mucosa daily and are absorbed into the mesenteric lymph as lipoproteins. Until recently, the major focus of research on intestinal lipoprotein metabolism was concerned with the intestine as an absorptive organ, exploring triglyceride absorption and secretion during a variety of experimental and clinical situations. In recent years an additional focus has emerged, namely, that of the intestine as an important source of lipoprotein constituents for plasma lipoproteins.

The rapid modifications which lipoproteins undergo after secretion have served to disguise the contribution of a given organ since plasma lipoproteins may differ markedly from precursor lipoproteins analyzed closer to the synthetic source. This is particularly true of intestinal lipoproteins such as chylomicrons which are rapidly metabolized, distributing constituents such as phospholipids and apoproteins to other plasma lipoproteins. It has therefore become increasingly important to define the composition and factors influencing the secretion of lipoproteins as close to the synthetic source as possible. In the case of the intestine, such analyses can best be carried out on intestinal mesenteric lymph lipoproteins or within the intestinal mucosa directly. It is not the intent of this review to provide a comprehensive review of plasma lipoproteins. Several reviews (1–3) have appeared over the last few years summarizing the current state of knowledge of the composition, structure, distribution, and function of the lipoproteins and their apoproteins. Rather, this review will concentrate on the available evidence for the intestinal synthesis and secretion of lipoproteins with emphasis on lipoprotein apoproteins and the factors influencing their secretion. In addition, the peripheral metabolism of intestinal lipoproteins will be briefly reviewed to indicate mechanisms by which lipoproteins of intestinal origin participate in systemic lipoprotein metabolism.

ANATOMIC AND STRUCTURAL FEATURES OF THE INTESTINAL EPITHELIUM (4)

The small intestine is a large organ which, in man, measures some 10–22 feet in the adult. The proximal 10 inches, which is retroperitoneal, represents the duodenum. There is no specific anatomical feature which marks the junction between the jejunum and ileum. Arbitrarily, the proximal two-fifths of mobile intestine is designated jejunum and the distal three-fifths designated ileum. Most studies of lipid absorption and lipoprotein formation in man have only studied the duodenum or proximal jejunum (ligament of Treitz), areas from which tissue can be obtained by oral biopsy techniques. Limited data are available in animals concerning lipoprotein formation in more distal portions of the gastrointestinal tract and will be reviewed below. These considerations may be of importance since discrete specialized functions exist in different portions of the intestine. Most nutrient absorption including lipid is thought to occur in the upper half of the intestine while the distal intestine (ileum) contains other specific absorptive functions (i.e., bile salt and vitamin B_{12} absorption). It is probable that lipid absorption and lipoprotein formation differ along the length of the intestine.

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; 4-APP, 4-aminopyrazolo(3,4-d)pyrimidine; TG, triglyceride; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid.

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In addition to functional differences between proximal and distal intestine, the intestinal villus is composed of a heterogeneous mixture of cells (**Fig. 1**). Mitotically active cells comprise the base of the crypt zone. The cells are functionally immature and lack a well developed microvillus membrane and disaccharidase enzyme activity. These cells divide and move to the upper portion of the crypt zone where they differentiate morphologically and biochemically. Progressive movement occurs toward the villus tip with full differentiation achieved when cells reach the upper third of the villus. When epithelial cells reach the villus tip, they degenerate, become detached from the villus, and are sloughed into the lumen. In man, the entire process of cell division, maturation, and migration from crypt to villus tip takes 5 to 7 days in jejunum and 4 to 5 days in the ileum. In the rat, cell migration takes approximately 36 hr. While it is known that most triglyceride absorption takes place in epithelial cells populating the upper third of the villus, less is known concerning the capacity of less differentiated cells (crypt cells, lower villus cells) to synthesize lipoproteins. This possibility is discussed below. Ultrastructurally, the villus epithelial cell is highly differentiated and displays a marked polarity (4) (**Fig. 2**). The apical (lumenal) surface membrane is thrown up into numerous projections or microvilli which form the brush border of the cell. A continuous glycoprotein coat is applied directly to the outer surface of the microvillus membrane. It is actively synthesized by the cells and is an integral part of the microvillus membrane. The microvillus membrane is also biochemically distinctive, containing specific hydrolytic enzymes for peptides and disaccharides, specific transport proteins for sugars and amino acids, and differences in lipid composition. The microvillus membrane contains proportionally more cholesterol and glycolipids than other portions of the intestinal cell plasma membrane (5). The core of each microvillus contains actin, a contractile protein which, in conjunction with contractile elements in the terminal web, is responsible for the contractile properties of the microvillus surface. In addition to specific absorptive functions, the microvillus membrane provides an immense surface amplification for absorption.

Various organelles are distributed in the cytoplasm beneath the terminal web. As in most cells these include mitochondria, lysosomes, a well developed granular and smooth surface endoplasmic reticulum, a well defined supranuclear Golgi complex, and microtubules. As discussed below, many of these organelles are intimately involved in triglyceride absorption and chylomicron formation.

The basolateral plasma membrane is biochemically distinct from the microvillus membrane. It lacks the hydrolases characteristic of the microvillus membrane, is enriched in sodium-potassium ATPase, and has a characteristic lipid composition (5). This region of the plasma membrane is the site of secretion of intestinal lipoproteins. The specific compositional

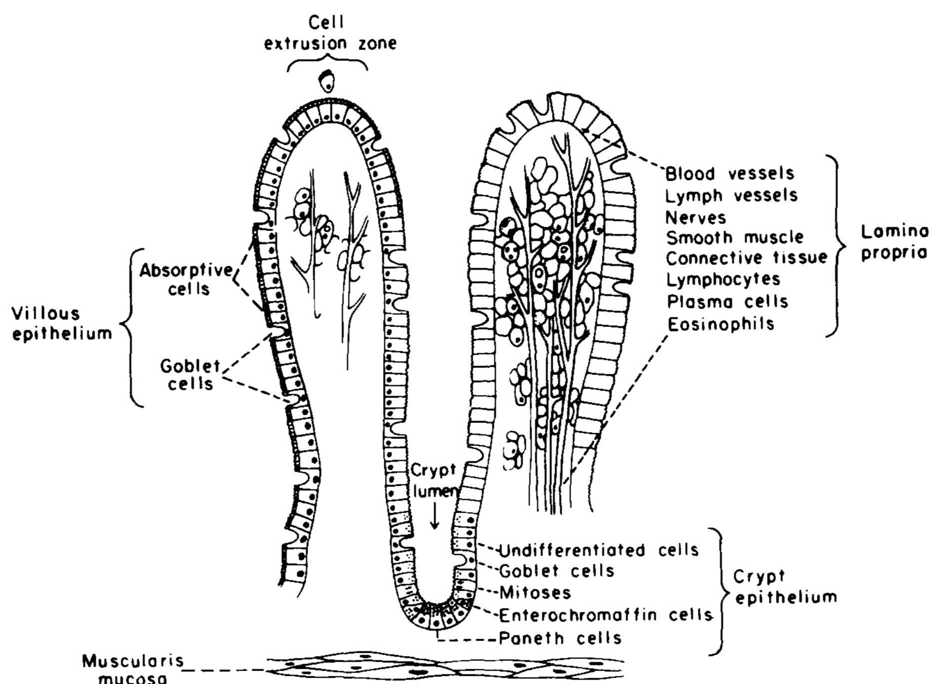

Fig. 1. Histologic organization of the intestinal villus.

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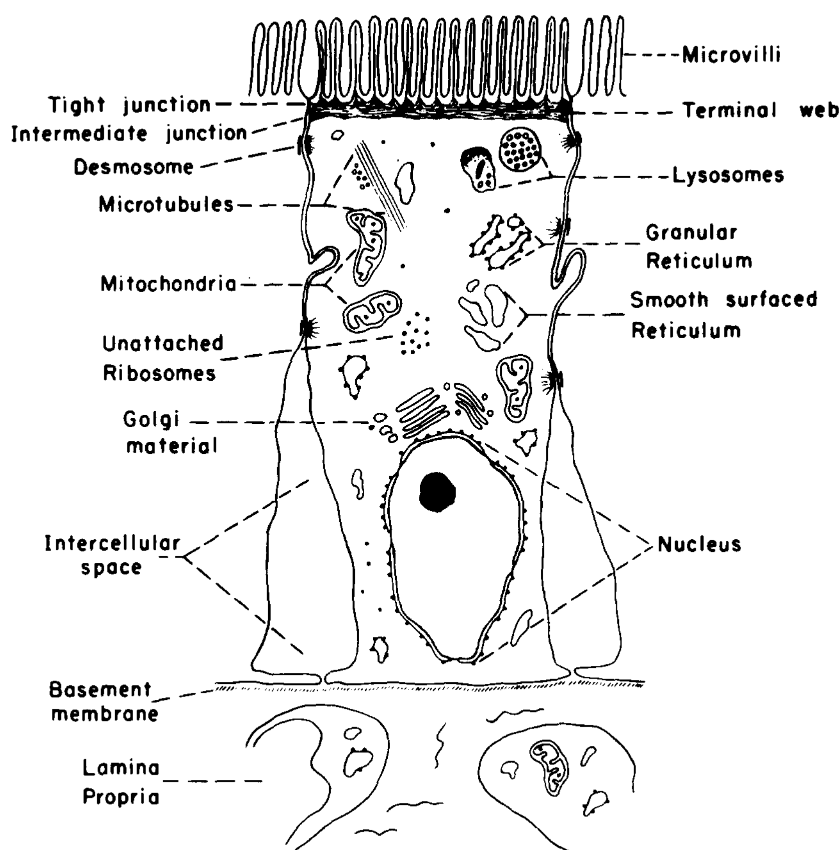

Fig. 2. Intestinal epithelial ultrastructural features.

features which confer this specialized function to this region of the plasma membrane are largely unknown.

CHYLOMICRONS

Much of what is known about intestinal lipoprotein formation has resulted from studies of triglyceride absorption and chylomicron secretion into lymph.

Lymph Chylomicrons

Physical Properties

Chylomicrons are triglyceride-rich particles formed in the intestine during lipid absorption. The term chylomicron was originally coined by Gage (6) in 1920 to describe the large particles seen in plasma after lipid absorption. Chylomicrons have been isolated from intestinal mucosa (7, 8) and mesenteric (9) and thoracic duct lymph (10) of experimental animals. Newly secreted human chylomicrons are more difficult to obtain; however, human chylomicrons have been isolated from thoracic duct lymph (11–13), chylous pleural effusions (14), chylous ascites (15–17), and chylous urine (18), as well as plasma from normal (12) and hypertriglyceridemic subjects (19). Alteration in the composition of chylomicrons occurs due to interaction with plasma components; composition will therefore depend somewhat on the site of isolation.

Chylomicrons (S_f 400–10,000) are heterogeneous with a size range from 750–6000 Å and a mean diameter of ~1200 Å (20, 21). Chylomicron size is largely determined by the flux of triglyceride through the intestinal cell. Chylomicrons are small at the beginning and larger during the peak of lipid absorption. When lymph triglyceride is greater than 1.5–3.0 mg/ml, most triglyceride is transported in larger chylomicron-sized particles; while at lower rates, smaller chylomicrons or intestinal VLDL predominate in lymph (22). Chylomicron size appears dependent on the rate of lipid absorption and triglyceride resynthesis compared to the availability of surface constituents. During maximal lipid absorption the formation of larger lymph particles would allow more triglyceride transported while surface constituents would be conserved. Rabbits chronically fed a high cholesterol diet produced larger lymph chylomicrons than animals fed a low cholesterol diet (23), however, acute

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intraduodenal infusion of cholesterol did not alter chylomicron size (24). The degree of saturation of absorbed fatty acids may also determine chylomicron size (25).

Composition and Structure

The lipid compositions of chylomicrons from experimental animals and man are similar. The major lipid is triglyceride, reflecting the role of chylomicrons in lipid absorption. Triglyceride comprises 86–92%, cholesteryl ester 0.8–1.4%, free cholesterol 0.8–1.6%, phospholipids 6–8%, and protein 1–1.5% of the mass (1, 3, 18). The fatty acids of chylomicron triglyceride reflect the fatty acid composition of the ingested triglyceride (26).

Chylomicron phospholipids are mainly lecithin (70–100%) with smaller amounts of sphingomyelin and phosphatidylethanolamine. The fatty acids of phospholipids differ from those present in the diet and remain relatively constant despite large variations in the composition of dietary fatty acids (27). Similarly, cholesteryl ester fatty acid composition bears little relationship to dietary fatty acids (28).

Structurally, chylomicrons consist of an oily core which contains the bulk of the triglyceride and cholesteryl ester and about 30% of the free cholesterol but no phospholipid (29). Phospholipid is found in the surface membrane which is considered to consist of a monolayer of phospholipid, apoproteins, free cholesterol, and some saturated triglyceride. The amount of phospholipid present is sufficient to cover 80–100% of the surface and sufficient protein to cover 10–20% (29).

Chylomicron composition varies depending upon the site of isolation. Chylomicrons isolated from intestinal mucosa ("prechylomicrons") have greater amounts of free fatty acid, free cholesterol, and protein, and less phospholipid than lymph chylomicrons (7). These alterations may have been due to contamination with intracellular components. Chylomicrons isolated from plasma have more protein and less phospholipid than lymph chylomicrons. In vitro incubation of chylomicrons with plasma resulted in a gain of protein and free cholesterol and a loss of phospholipid which was recovered in the HDL fraction (30, 31).

Chylomicron Apoproteins

Similarities exist between the apoprotein composition of chylomicrons isolated from intestinal lymph of experimental animals and thoracic duct lymph or chylous urine of man (**Fig. 3**). The characteristics of the lipoprotein apoproteins are shown in **Table 1.**

Rat chylomicron apoproteins

Lymph duct cannulation of the main mesenteric lymphatic of the rat has enabled detailed studies of the apoprotein composition of rat mesenteric lymphatic lipoproteins.

The SDS polyacrylamide gel pattern of chylomicron apoproteins resembles that of plasma HDL more closely than other rat plasma lipoproteins (31–33). Chylomicrons however, contain apoB, which represents approximately 10% of chylomicron protein when estimated by either gel chromatography or tetramethylurea (TMU) insoluble protein (31). Chylomicron apoB can be distinguished from apoB of plasma LDL. It has a lower molecular weight (240,000 vs 353,000) as assessed by 3.5% polyacrylamide gels (34). This lower molecular weight apoB is also present in lymph VLDL and LDL and plasma VLDL of fed rats but not in fasting plasma LDL (34). These observations suggest that it is derived from the intestine. In more recent studies by Elovson et al. (35), three forms of rat apoB were identified: a high and low molecular weight apoB as well as a minor form with an intermediate molecular weight. Low molecular weight apoB, the major form of apoB in chylomicrons, was

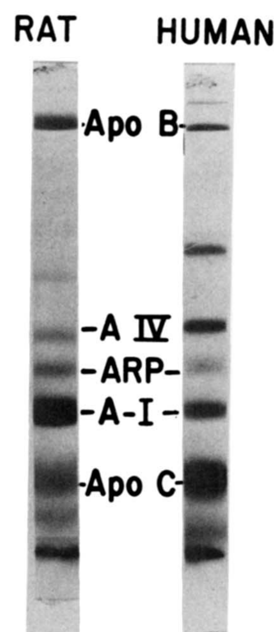

Fig. 3. SDS polyacrylamide gels (5.6%) of rat and human chylomicron apoproteins. Rat chylomicrons were obtained from rat mesenteric lymph and human chylomicrons from chylous urine (18). ARP = apoE. ApoA-II migrates with the C proteins. An ink marker is seen at the bottom of each gel.

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TABLE 1. Lipoprotein apoproteins

Apo-protein	Molecular[a] Weight	Function	Components of Lipoproteins in: Lymph	Plasma	Sites of Synthesis
A-I	28,300	LCAT activation	CM, VLDL, HDL	HDL	intestine, liver
A-II	17,000	?	CM, VLDL, HDL	HDL	intestine, liver
A-IV	46,000	?	CM, VLDL	CM, d > 1.21	intestine > liver
B — B100	459,000	CM and VLDL secretion, triglyceride transport	CM, VLDL, LDL	CM, CM-remnants, VLDL, LDL	B48 intestine
B — B48	264,000				B100 liver
C-I	6,331	inhibits hepatic CM remnant uptake	CM, VLDL	HDL, CM	?
C-II	8,837	activates lipase, inhibits hepatic CM remnant uptake	CM, VLDL	HDL, CM	liver ≫ intestine
C-III	8,764	inhibits lipase? inhibits hepatic remnant uptake	CM, VLDL	HDL, CM	liver ≫ intestine
D	22,100	?	CM	HDL	?
E	33,000	binds to hepatic CM-remnant receptor	CM, VLDL	CM, CM-remnants, VLDL, HDL	liver

[a] Values for human apoproteins (1, 2, 3, 48).
CM, chylomicrons; CM-remnant, chylomicron remnant; VLDL, plasma very low density lipoproteins.

also found in plasma LDL (7% of apoB) and found to be synthesized in the liver along with high molecular weight apoB. Intestinal apoB has a similar amino acid composition to plasma LDL apoB which is presumed to be synthesized in the liver.

The major apoprotein of chylomicrons is apoA-I (mol wt ~ 28,000) comprising 38–50% of chylomicron protein (31, 32, 36). Within rat plasma, apoA-I is found almost entirely in the HDL fraction (37), comprising ~60% of HDL apoprotein (33). ApoA-I from chylomicrons and HDL share immunological identity and have similar amino acid compositions (31, 32). Differences have, however, been noted in the polymorphic components of apoA-I in isoelectric focusing polyacrylamide gels of apoA-I from HDL and lymph chylomicrons (31). Other middle molecular weight chylomicron apoproteins include apoA-IV and apoE (ARP) (31). ApoA-IV (mol wt ~ 46,000) comprises 7–13% of chylomicron apoprotein (31, 36). Chylomicron apoA-IV has a similar amino acid composition and immunological identity to plasma HDL apoA-IV (31). ApoA-IV is present in mesenteric lymph in chylomicrons, VLDL, HDL, and the d > 1.21 g/ml fractions (38, 39). It is known to be synthesized in the intestine and to a lesser extent in the liver (40). To date, no metabolic role has been determined for apoA-IV. ApoE (mol wt 35,000) comprises only 5% of chylomicron protein (31).

Rat mesenteric lymph chylomicrons contain apoC representing ~40% of chylomicron protein (31). Isoelectric focusing studies have demonstrated that apoC-II, C-III-0, -2, and -3 are present on chylomicrons (31, 36, 39). Recent evidence suggests that apoC-II may be synthesized in the intestine (36, 39, 41); however, the liver is a more important site of synthesis (40).

ApoA-II is not a major rat apoprotein and is variably present in mesenteric lymph chylomicrons (31, 39).

Studies by Roheim, Gidez, and Eder (42) in hepatectomized dogs showed radioactive amino acid incorporation into plasma HDL, suggesting non-hepatic sites of synthesis of HDL apoproteins. Windmueller, Herbert, and Levy (43), using the isolated perfused intestine confirmed that the intestine synthesized the high and middle molecular weight apoproteins of lymph VLDL and HDL. More recent isotopic labeling studies have demonstrated that apoB, apoA-I, apoA-IV and, probably to a lesser extent, apoC-II (and apoC-III-0) (31, 32, 36, 39) are actively synthesized in the rat intestine during lipid absorption (**Fig. 4**). Little or no label was incorporated into apoE in normal (31) or chronic cholesterol fed animals (44), indicating apoE is not synthesized in the intestine. In order to investigate the contribution of filtered plasma HDL apoproteins to chylomicrons, Imaizumi et al. (45) infused radioiodinated HDL intravenously into lymph fistula rats. The specific activity of apoA-I in chylomicrons was only 3–6% of apoA-I in plasma HDL, indicating a minimal contribution of filtered

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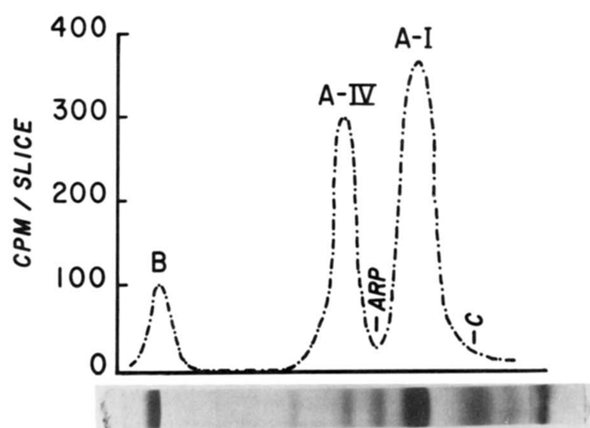

Fig. 4. [H^3]Leucine incorporation into rat mesenteric lymph chylomicron apoproteins from a lymph fistula rat during lipid absorption. Individual radioactivity of each band was determined by counting 1-mm gel slices. Radioactivity was incorporated into apoB, A-IV, and A-I but not in apoE (ARP) or the C proteins.

HDL apoA-I to chylomicrons. The bulk of the radioactivity transferred from plasma HDL to chylomicrons in lymph was in the low molecular weight proteins (mainly apoC), indicating extensive transfer of these apoproteins to chylomicrons in lymph.

The studies of Imaizumi et al. (45) indicate that 130–140 μg of apoA-I is transported in the mesenteric lymph of saline- or glucose-fed animals per hr. This value rises almost twofold during triglyceride absorption and chylomicron transport in lymph. Similar values were reported by Riley et al. (44) in glucose-infused and fat-fed animals. The values reported by Glickman and Green (32) using a rocket immunoelectrophoresis were several-fold higher. Animals chronically fed cholesterol and olive oil transported less apoA-I in lymph after a fat challenge than control animals (44).

Isotopic incorporation studies performed by Wu and Windmueller (40) provided evidence that the intestine and the liver were the sole source of synthesis of plasma apolipoproteins in the rat. They found that 19% of the total apoprotein pool was synthesized in the intestine. The percent contribution of the intestine to individual apoproteins was apoA-IV, 59%; apoA-I, 56%; apoB, 16%; apo-C-II, 10%; apo C-III-0, 7%; apo C-III-2-(-1), <10%; apo-C-III-3, <1%; and apoE, <1%. These studies indicate that the rat intestine is a major source of apoA-I and apoA-IV, two apoproteins found in plasma HDL.

Human chylomicron apoproteins

Alaupovic et al. (11) immunochemically identified apoA, B, and C in human thoracic duct chylomicrons and VLDL. Earlier findings of Rodbell and Fredrickson (46) demonstrated that at least one of the chylomicron proteins resembled the major plasma HDL apoproteins. Studies by Kostner and Holasek (47) on thoracic duct chylomicrons separated the apoproteins by polyacrylamide gel and chromatographic techniques. These studies revealed the presence of the C peptides comprising 60% of the protein; apoC-I, C-II, and C-III were present. ApoB comprised 22% and apoA comprised about 12%, with apoA-I and apoA-II present in equal amounts. The presence of these apoproteins was confirmed by Schaefer, Jenkins, and Brewer (14) who also demonstrated the presence of apoD. These studies, however, used repetitive centrifugation to remove albumin from the chylomicrons, which may have resulted in the loss of apoA proteins.

Green et al. (18) isolated human chylomicrons from chylous urine and human thoracic duct (12) lymph by a single ultracentrifugation and purified them by agarose column chromatography. By this method, chylomicrons were free of albumin with minimal ultracentrifugation. The apoprotein pattern of chylomicrons from both sources was similar The gel pattern closely resembled that of human chylomicrons published by Weisgraber, Bersot, and Mahley (13) and Utermann and Beisiegel (15). The SDS gels of chylomicrons isolated from chylous urine are shown in **Figs.** 3 and **5.** The percentage distribution of chylomicron protein (determined densitometrically) from 5.6% SDS gels was apoB (3.4%), apoA-I (15%), apoE (4.4%), apoA-IV (10%), and apoC and A-II (47.3%). ApoA-I and A-II content determined immunologically re-

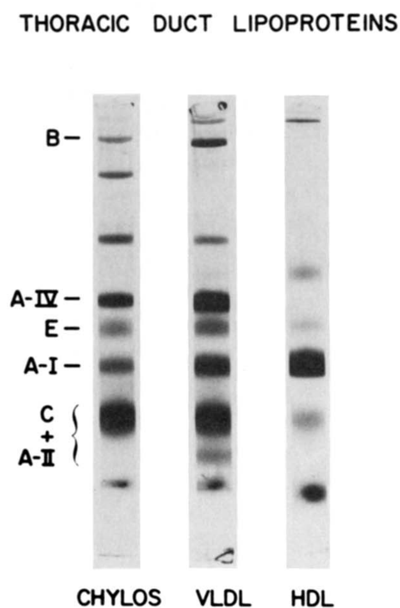

Fig. 5. SDS polyacrylamide gels of human thoracic duct lipoproteins. Chylomicrons contain the lower molecular weight apoB while intestinal VLDL contains both species of apoB. The protein bands between apoB and apoA-IV have not been identified.

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vealed an A-I/A-II ratio of 5.2 for one subject and 13 for another. Chylomicron apoA-I is immunologically identical to plasma HDL apoA-I and is present in chylous urine and thoracic duct lymph in all lipoprotein fractions.

Heterogeneity of apoB has also been demonstrated for human apoB. Kane, Hardman, and Paulus (48) isolated four species of apoB with unique molecular weights and amino acid composition. LDL apoB from normal volunteers consisted of a single protein band (mol wt 459,000) called B100. In 50% of volunteers, the B100 form of apoB was the sole apoB component. LDL from some normal donors contained two extra bands of molecular weight 407,000 (B74) and 144,500 (B26). Chylomicrons, whether isolated from thoracic duct lymph or plasma, contained a lower molecular weight form of apoB (mol wt 264,000) called B48 with a distinct amino acid composition. Chylomicrons also contained the B100 form of apoB. However, the B48 form or intestinal form of apoB was absent from plasma LDL. The nomenclature proposed by Kane et al. (48) is an attempt to normalize the nomenclature for different methods of isolation and characterization of apoB. It arbitrarily assigns value 100 to the major form of LDL apoB and others are expressed in relation to it. These findings in man suggest that the liver and the intestine produce lipoproteins with unique and characteristic forms of apoB. Whether these organs in man are capable of producing the other form of apoB is not known.

Human apoA-IV (mol wt 46,000) has been recognized in chylomicrons from chylous urine (18), thoracic duct lymph (12, 13), and chylous ascites (15). It is present in plasma chylomicrons isolated from lipemic postprandial plasma (12) and from patients with fasting hypertriglyceridemia (49). ApoA-IV is, however, mainly found free in plasma unassociated with the major lipoproteins, whether plasma lipoproteins are separated by ultracentrifugation or column chromatography. Plasma levels are 14–16 mg/dl and rise postprandially. The rise is mainly accounted for by a rise in the plasma chylomicron fraction. The function and metabolic fate of apoA-IV has not been determined.

Recently Polz and Kostner (50) have described the association of β_2 glycoprotein -I (β_2 GI) with human lipoproteins. β_2 GI is an amphipathic protein (mol wt 40,000) present in all plasma lipoproteins, especially chylomicrons. Lipid feeding is associated with a rise in plasma levels.

The proline-rich peptide (PRP, mol wt 74,000) (51) has been identified in chylomicrons from pleural fluid and from two patients with endogenous hyperlipemia 4 hr after an oral fat load. PRP exists mainly in plasma bound to little or no lipid; however, it binds to triglyceride emulsions (Intralipid). The function or significance of the association with chylomicrons is not known.

Considerable alteration in the protein composition of chylomicrons occurs after exposure to plasma. This occurs in vivo (18, 52) and in vitro (18, 31). Chylomicrons ($S_f > 400$) isolated from lipemic plasma have, as their major middle molecular weight apoprotein, apoE with only small traces of apoA-I and apoA-IV variably detected in SDS gels (12, 18, 52). ApoC and apoB are the other major apoproteins. Similar apoprotein patterns are produced by in vitro incubation of chylomicrons from chylous urine with plasma (18). This apoprotein exchange occurs at 4°C and is considered to be non-enzymic.

When human chylomicrons were incubated with plasma or lipoprotein fraction, there was loss of apoA-I and apoA-IV when HDL was present in the incubation mixture, and a gain in apoE (18). Using a radioimmunoassay, Imaizumi et al. (31) demonstrated that rat mesenteric lymph chylomicrons gained apoE when incubated with plasma. There was, however, no loss of apoA-I. The reason for the difference between the rat and human studies is unclear. Chylomicrons also gain apoC after exposure to plasma.

While the apoprotein pattern of human chylomicrons when isolated from either thoracic duct lymph or chylous urine appears constant, it is probable that apoprotein transfer has already occurred in lymph. Similarly, chylomicrons isolated from chylous effusions may have intermediate apoprotein patterns with apoE, A-I, and A-IV present in varying amounts[2] probably depending on the duration of exposure to plasma or its components.

Intestinal Mucosal Aspects of Chylomicron Formation

Ultrastructural Aspects of Chylomicron Formation

Considerable insight into chylomicron formation has been gained through careful morphologic studies of triglyceride absorption. The studies of Palay and Karlin (53), and Cardell, Badenhaussen, and Porter (54) and most recently Sabesin and Frase (55) demonstrate that newly absorbed fatty acid is first visible morphologically as triglyceride droplets within profiles of the smooth endoplasmic reticulum (SER) in the apex of the enterocyte (**Fig. 6**). Strauss (56), using ultrastructural, radioautographic techniques, showed that newly absorbed fatty acid appeared as triglyceride

[2] Green, P. H. R., and R. M. Glickman. Unpublished observations.

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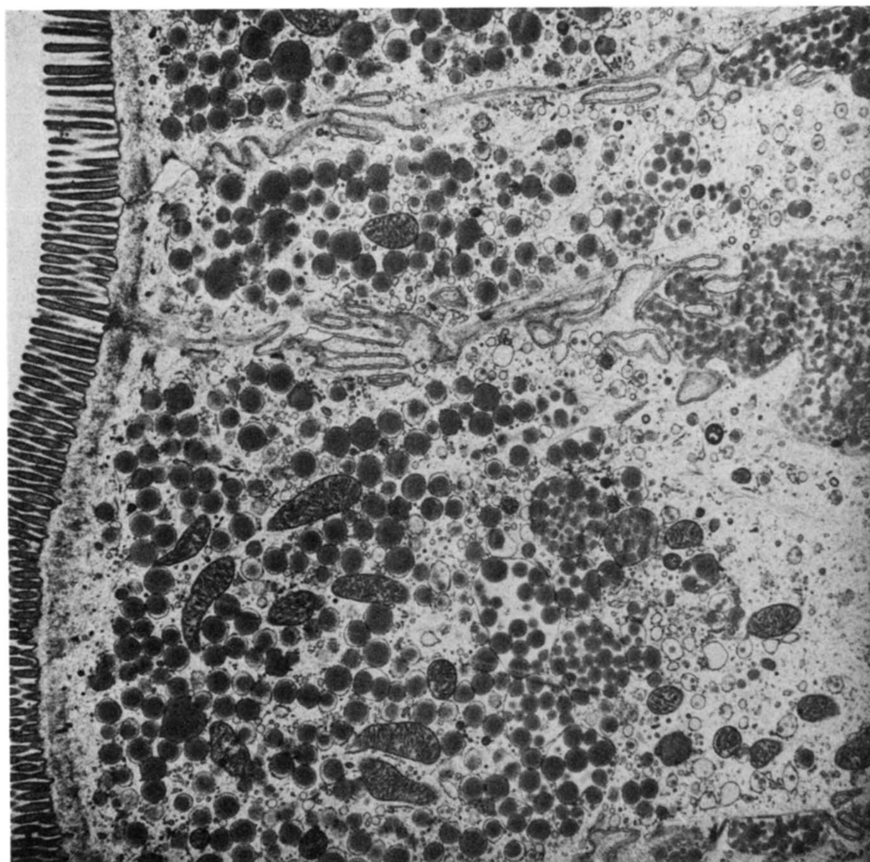

Fig. 6. Electron micrograph of the supranuclear region of rat intestinal epithelial cells 15 min after the instillation of triglyceride into the lumen. Triglyceride droplets fill Golgi vesicular structures and are seen in intercellular spaces.

droplets in the SER. Subsequently, lipid droplets appear in profiles of the endoplasmic reticulum and accumulate in the Golgi apparatus in the supranuclear region of the cell. Migration of Golgi vesicles to the lateral basal aspect of the cell occurs. Discharge of chylomicrons into the intercellular space occurs morphologically by reverse pinocytosis with fusion of Golgi membranes with the basolateral cell membrane. The factors responsible for this directed intracellular movement of membrane-bound triglyceride are largely unknown. Microtubules have been implicated in the process since colchicine, an inhibitor of microtubule polymerization, decreases chylomicron secretion from the intestine (57, 58). Lipid droplets appeared to accumulate in the Golgi suggesting that post-Golgi triglyceride transport may involve microtubules. Additional studies by Reaven and Reaven (59) showed a depolymerization of microtubules during fat absorption. There are no studies, however, directly involving microtubules in chylomicron transport. It has also been suggested by Redgrave (60) that intracellular phospholipases may also function in chylomicron secretion; however, the precise mechanisms have not been elucidated.

The rapidity of the process of chylomicron formation has been shown by the time sequence studies of Jersild (61). These studies showed that as early as 12 min after lipid was placed in the intestinal lumen, chylomicrons were seen in the intercellular spaces. Thus the various synthetic events in chylomicron formation proceed rapidly.

Synthesis of Triglyceride, Phospholipid, and Cholesteryl Esters

The products of triglyceride hydrolysis by pancreatic lipase—fatty acids and 2-monoglycerides—enter the microvillus membrane by passive diffusion. Fatty acid is taken up from micellar solutions at low temperatures (62) and metabolic activity of the tissue is not required. Such uptake is reversible as demonstrated by incubation of intestinal mucosa with albumin-containing solutions that results in removal of the fatty acids

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from the cell membrane (63). Translocation of fatty acids from the inner surface of the microvillus membrane to the cytosol of the enterocyte is also obligatory. The work of Ockner et al. (64–66) has clarified how this may occur. They isolated a cytosolic protein (mol wt 12,000) which bound unsaturated long chain fatty acids with high affinity and showed weaker affinities for saturated long chain fatty acids. Long chain fatty acid analogs, cholesterol, and medium chain triglycerides showed almost no binding. This protein, fatty acid binding protein (FABP), is present in highest concentrations in proximal intestinal mucosa, is increased by a high fat diet, and is present in higher concentration in villus epithelial cells when compared to crypts. FABP has also been found in many other tissues (64). In further studies, it was shown that compounds that bound to FABP (flavaspidic acid, bromopalmitate) decreased the incorporation of fatty acids into triglycerides, suggesting that FABP-fatty acid complexes may be a transport route to the smooth endoplasmic reticulum, the site of triglyceride resynthesis. Within the endoplasmic reticulum two distinct pathways have been demonstrated for the synthesis of triglyceride and phospholipid. The α-glycerophosphate pathway (67) utilizes *sn*-glycerol-3-phosphate with the subsequent addition of two molecules of fatty acyl-CoA to form phosphatidic acid. Triglycerides can be synthesized by the addition of another fatty acyl CoA. Lecithin can be synthesized through the action of cholinephosphotransferase, which adds a molecule of phosphocholine to the diglyceride molecule. The other pathway, the monoglyceride pathway, synthesizes triglyceride directly from 2-monoglyceride, a major product of lumenal triglyceride hydrolysis. Thus, direct acylation with two molecules of fatty acyl-CoA yields triglyceride (68–71). Similarly, lysolecithin, generated in the lumen by the action of pancreatic phospholipase A, can be absorbed and directly acylated to form lecithin. The monoglyceride pathway is thought to be the major pathway by which triglyceride is reesterified in the intestine. Similarly, Mansbach (72) has demonstrated that, when lumenal phospholipids are readily available (i.e., biliary lecithin), direct reacylation of absorbed lysolecithin is the source of most chylomicron phospholipid. Under experimental conditions of reduced availability of biliary phospholipid, de novo synthesis of lecithin from *sn*-glycerol-3-phosphate or plasma phospholipid may be of greater importance as a lecithin source within the intestinal mucosa (72). In addition, crypt cells with a high requirement for new membrane synthesis utilize the α-glycerophosphate pathway for lecithin synthesis to a greater degree than villus epithelial cells (73). Fatty acids for lecithin synthesis in crypt cells are derived from plasma to a greater extent than in villus cells, perhaps due to limited availability of lumenal fatty acids to the crypt cell (74). Thus the microsomes contain the necessary enzymes for triglyceride resynthesis and lecithin synthesis, two major components of chylomicrons. Cholesterol, another major lipid component of chylomicrons, is present largely in the esterified form. Two possible enzymes involved in cholesterol esterification have been found in intestinal microsomes. Acyl-CoA:cholesterol acyltransferase (ACAT) esterifies cholesterol with fatty acid-CoA with a marked preference for oleate (75). It has also been suggested that cholesterol esterase, presumably derived from pancreatic fluid, functions within the intestinal mucosa to esterify cholesterol (76). Definitive evidence has not yet been presented to determine which of these enzymes is responsible for chylomicron cholesteryl esters.

Chylomicron Apoprotein Synthesis

The human genetic disorder abetalipoproteinemia (discussed below) first indicated that specific intestinal apoprotein synthesis was required for triglyceride transport and chylomicron formation. This prompted a series of studies designed to develop experimental models of impaired chylomicron formation resulting from impaired apoprotein synthesis. Sabesin and Isselbacher (77) administered puromycin to rats and observed that the lipemic response in plasma was decreased after an oral fat load. Furthermore, intestinal epithelial cells were engorged with triglyceride droplets, suggesting an impairment of chylomicron formation. Studies by Friedman and Cardell (78) demonstrated ultrastructural accumulation of triglyceride in the endoplasmic reticulum and triglyceride droplets in the cytoplasm with few lipoproteins present in the Golgi. These studies were challenged when further studies showed that triglyceride transport in mesenteric lymph proceeded despite a significant pharmacological inhibition of protein synthesis (79, 80). Subsequent studies by Glickman and Kirsch (9) showed that, although triglyceride appeared in lymph during intestinal protein synthesis inhibition, the chylomicrons had an altered morphology and composition. These chylomicrons were significantly larger in size and contained decreased amounts of apoproteins. The interpretation suggested for these results was that, in the face of a decreased ability to synthesize surface constituents of chylomicrons (i.e., apoproteins), the formation of larger particles would require less surface material for triglyceride transport. It has also been shown that protein synthesis inhibition is

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associated with impaired intestinal phospholipid synthesis, another major chylomicron surface constituent (81). These studies, coupled with the demonstration that radioactive amino acids are rapidly incorporated into mesenteric lymph chylomicron apoproteins, provided evidence for de novo apoprotein synthesis by the intestinal mucosa. There have been fewer studies, however, that have directly quantitated chylomicron apoproteins within intestinal epithelial cells and have directly demonstrated their synthesis.

ApoB

Kessler et al. (82), using immunoprecipitation techniques and anti-LDL antisera, demonstrated apoB synthesis in microsomes from rat intestine. In a further study using cell fractionation techniques, chylomicron apoprotein synthesis was demonstrated in a microsomal fraction of rat intestine with carbohydrate addition to the apoproteins demonstrated in a Golgi fraction (83). Recently, Blue, Protter, and Williams (84) also demonstrated apoB synthesis in vitro in slices of small intestine. Further validation for the synthesis of apoB derives from measurement of the apoB content of rat intestine before and after triglyceride feeding. Studies by Schonfield, Bell, and Alpers (85) determined that the content of apoB in mucosal scrapings of fasting rats was 80 ng/ per mg of cellular protein and rose 2- to 3-fold, 1.5 hr after lipid administration. In these experiments corn oil was administered intragastrically to rats and variable gastric emptying precluded an exact statement concerning the time course of apoB synthesis during triglyceride absorption. Several lines of evidence suggest that the stimulus to apoB synthesis is more rapid. Glickman et al. (86, 87) visualized apoB in isolated rat villus epithelial cells using fluorescent antibody techniques. In these experiments, in situ intestinal loops were exposed to oleic acid-monolein emulsions, thus avoiding gastric emptying and triglyceride lipolysis. Under these conditions, apoB was demonstrated in the apical portion of enterocytes with a marked increase in cellular fluorescence filling the apical portion of the cell as early as 10 min after lipid exposure. Since no mass measurements of apoB accompanied these studies, the results are qualitative. Supporting a rapid synthesis of apoB after lipid absorption are studies of lymph chylomicrons that demonstrate incorporation of apoB into lymph chylomicrons within 30 min of administration of the isotope (9).

Few data are available on the possibility of a preformed pool of apoB within intestinal epithelial cells or on the relationship of this pool to active chylomicron formation. While immunologic localization of apoB within enterocytes as well as content measurements indicate that a pool of apoB is present in the fasting state, endogenous triglyceride absorption and triglyceride-rich lipoprotein secretion does occur (25, 88). It is possible that a portion of the mucosal apoB measured in the fasting state is newly synthesized. This would be supported by data demonstrating lymph apoB synthesis during duodenal saline infusions (35). Biliary diversion known to deplete the intestinal mucosa of triglyceride-rich lipoproteins (88) was associated with a persistence of apoB immunofluorescence, suggesting that a pool of apoB exists in the enterocyte despite the absence of active triglyceride absorption. While no quantitative estimates of the apoB pool exist, it is probable that it is of limited size. Glickman et al. (87) administered protein synthesis inhibitors to rats, followed by lipid instillation into in situ loops, and followed apoB immunofluorescence sequentially. While protein synthesis inhibitors had no effect on preformed apoB, within 15 min of a maximal lipid challenge, cells contained no apoB immunofluorescence and large cytoplasmic lipid droplets accumulated. Recovery from protein synthesis inhibition was associated with a return of apoB immunofluorescence and an apparent mobilization of triglyceride. These studies suggest that preformed apoB may be mobilized for initial chylomicron formation but that de novo synthesis of apoB is required for continued triglyceride transport.

While it is clear that microsomes synthesize apoB, there is little data available on the ultrastructural localization of apoB or where this apoprotein is added to the chylomicron surface. Although there have been no cell fractionation studies determining the apoB content of various cell organelles, it is probable that it is membrane-bound, in view of the extreme hydrophobicity of apoB. Fluorescent antibody studies on the light microscopic level show a distribution of apoB in the apex of the enterocyte (86). Rubin et al. (89), reporting in abstract form, have studied the electron microscopic localization of apoB using anti-LDL antisera. They indicate that apoB is visualized in the rough endoplasmic reticulum and on triglyceride droplets in profiles of the smooth endoplasmic reticulum, demonstrating an addition of apoB early in chylomicron formation. Similar conclusions were reached by Alexander, Hamilton, and Havel (90) in rat liver where apoB addition was visualized in a similar location.

As discussed above, recent studies in the rat (34, 35) and man (47) have described two major forms of apoB. The higher molecular weight form is the principal form present in plasma LDL, while the lower molecular weight form comprises the major apoB species isolated from lymph chylomicrons. These two

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forms appear to share common antigenic determinants; however each form may have unique antigenic determinants, as evidenced by nonparallel displacement in radioimmunoassays against anti-LDL antiserum. Most studies of apoB in intestinal epithelium have employed anti-LDL antiserum. To date, antisera uniquely recognizing determinants of intestinal apoB have not been applied to intestinal apoB quantitation (85, 86).

Studies of apoB in human intestine are more limited. Rachmilewitz et al. (91, 92), using per oral duodenal biopsies, showed the in vitro incorporation of radioactive amino acids into apoB, A-I, and A-II, thus demonstrating de novo synthesis of these apoproteins. Measurements of apoB content in human intestinal biopsies in the fasting state and 45 min after the introduodenal administration of corn oil were carried out by Rachmilewitz, Albers, and Saunders (93). Fasting biopsies contained 1.3 μg of apoB per mg cell protein. Postprandial biopsies, unexpectedly, showed a decrease in apoB content to 0.3 μg. It was suggested that this decrease in apoB content represented depletion of a preformed pool of the apoprotein that exceeded the rate of de novo synthesis. Glickman et al. (94), using fluorescence antibody techniques, demonstrated a marked increase in apoB immunofluorescence when compared to fasting biopsies in epithelial cells from human biopsies 60 min after lipid instillation into the duodenum. Explanations for these seemingly contradictory results will require further studies on the time course of apoB synthesis in response to lipid feeding.

Several studies have attempted to visualize apoB in intestinal biopsies from patients with abetalipoproteinemia. In this genetic disorder, there is an inability to secrete triglyceride-rich lipoproteins and apoB is immunochemically absent from plasma. Glickman et al. (94) studied two such patients and found an absence of apoB immunofluorescence in the fasting state as well as in postprandial biopsies. These observations have been confirmed by Schwartz et al. (95) using immunoperoxidase localization of apoB. These authors also found that apoA-I was present. We have not been able to localize apoA-I in the intestine of three other patients with abetalipoproteinemia.[2] There are no other data, however, on the synthetic interrelationships of chylomicron apoproteins. The morphological features of abetalipoproteinemia suggest that apoB addition to the triglyceride droplet is an early event, since lipid droplets are not seen in the Golgi apparatus but rather accumulate in the apical cytoplasm. The finding of discrete forms of apoB arising from liver and intestine may be of importance in regard to chylomicron formation. An interesting patient investigated by Malloy et al. (96) showed an apparent inability to secrete hepatic apoB as evidenced by absent plasma LDL, low cholesterol, and severely reduced, but not absent, plasma apoB. Of great interest was the preservation of chylomicron formation as evidenced by postprandial rise in triglycerides and the appearance of small amounts of apoB immunoreactivity. These findings suggest an impairment of hepatic apoB synthesis with a preservation of gut apoB synthesis. No direct studies of intestinal mucosal apoB measurement have been reported. It is also probable that patients will be discovered with an inability to synthesize intestinal apoB but with intact hepatic apoB synthesis. Such patients would have normal levels of LDL and plasma apoB but present clinically as a malabsorption syndrome. Their intestinal biopsies would presumably be engorged with triglyceride and there would be an absence of postprandial lipemia.

ApoA-I

Direct demonstration of apoA-I within intestinal mucosal cells has been provided by immunochemical localization and direct quantitation using specific radioimmunoassay. Mucosal cells from fasting rats contain apoA-I in the apical region that increases markedly with fat absorption (32, 85, 87). This increase can be prevented by protein synthesis inhibitors. The content of apoA-I in fasting rat intestinal mucosa was found by Schonfield, Bell, and Alpers (85) to be 80 ng/mg homogenate protein and to rise after fat feeding. In studies by Riley and Glickman,[3] the apoA-I content of isolated epithelial cells was measured and similar values were found. An additional finding was that apoA-I was also present in intestinal crypt cells as determined by radioimmunoassay and immunochemical localization. It is not clear whether crypt cell apoA-I is merely a precursor of villus cell A-I or whether it could participate in lipid transport from crypt cells, an active site of cholesterol synthesis. In this regard preliminary studies show that 4-APP, known to reduce plasma cholesterol and stimulate intestinal cholesterol synthesis (97), results in a threefold increase in crypt cell apoA-I while villus cell A-I levels are largely unchanged. It is possible that circulating lipoproteins may influence the rate of apoprotein synthesis and the state of intestinal cholesterol synthesis. In this regard, biliary diversion known to deplete the intestinal mucosa of triglyceride, was associated with no reductions of apoA-I content in either villus or crypt cells (98). Similarly, biliary diversion is associated with an increase in intestinal cholesterol synthesis. We have also found in the rat

[3] Riley, J. W., and R. M. Glickman. Unpublished results.

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that both crypt and villus cells from the ileum contained similar amounts of apoA-I per cell as found in the jejunum. While it is known that the ileum can readily form chylomicrons when perfused with triglyceride (99, 100) under normal conditions, it does not participate in triglyceride absorption. There are no data directly analyzing the lipoproteins secreted by the distal intestine since mesenteric lymph is derived from the entire small intestine. It is possible, but not proven, that this portion of the intestine secretes apoproteins in lipoproteins other than triglyceride-rich lipoproteins, i.e., HDL. Direct studies of apoprotein synthesis in the different portions of the intestine and in crypt and villus cells will be required to answer these questions. The finding of apoA-I in crypt cells and in distal intestine and the preservation of mucosal apoprotein levels in the absence of active triglyceride absorption raise the possibility that there are stimuli in addition to triglyceride absorption that may affect apoprotein synthesis and secretion. The recent findings of Windmueller and Wu (101) suggest that apoA-I synthesis occurs despite the absence of active triglyceride absorption.

Studies by Zannis, Breslow, and Katz (102) indicate that isoforms of apoA-I, differing in charge, exist within the intestinal mucosa; however, their synthetic interrelationships are unknown.

ApoA-I has similarly been demonstrated in human intestinal biopsies using immunochemical localization (52, 95, 103) and isotopic incorporation (104). Localization in the fasting state is confined to the apex of villus cells with a marked increase of apoA-II with triglyceride feeding (**Fig. 7**). Preliminary data on human intestinal biopsies[2] indicate that the content of jejunal apoA-I is ~200 ng/mg protein. Distal intestinal (ileal) biopsies give similar values.

ApoA-I has been demonstrated in intestinal biopsies from patients with Tangier disease (52, 95) characterized by extremely low plasma levels of HDL and apoA-I. Current evidence indicates that no major defect in A-I synthesis exists in this disease but rather rapid catabolism of apoA-I occurs (105).

ApoA-IV

As discussed above, apoA-IV is a major apoprotein of triglyceride-rich lipoproteins in rat and man. Studies of lymph chylomicron formation in rats (31, 45) and quantitation of apoA-IV secretion in chyluric man (12) indicate an active synthesis of apoA-IV. The marked increase in mucosal immunofluorescence for A-IV during lipid absorption is consistent with active mucosal synthesis (12). The role of apoA-IV in chylomicron formation is not yet defined.

Other chylomicron apoproteins

ApoA-II is a minor apoprotein in rat chylomicrons. Human intestinal biopsies incorporate radioactive amino acids into apoA-II (104) indicating intestinal synthesis.

ApoC-II and apoC-III have been demonstrated by immunocytochemical techniques in human and rat enterocytes. Fat feeding resulted in an alteration in the localization of these apoproteins from the supranuclear region to between cells, suggesting synthesis by the enterocyte during lipid absorption (41).

INTESTINAL VLDL

VLDL-size particles (280–750 Å) of S_f 20–400 are present in rat mesenteric lymph from fasting animals (106). Particles of similar size are present in intestinal

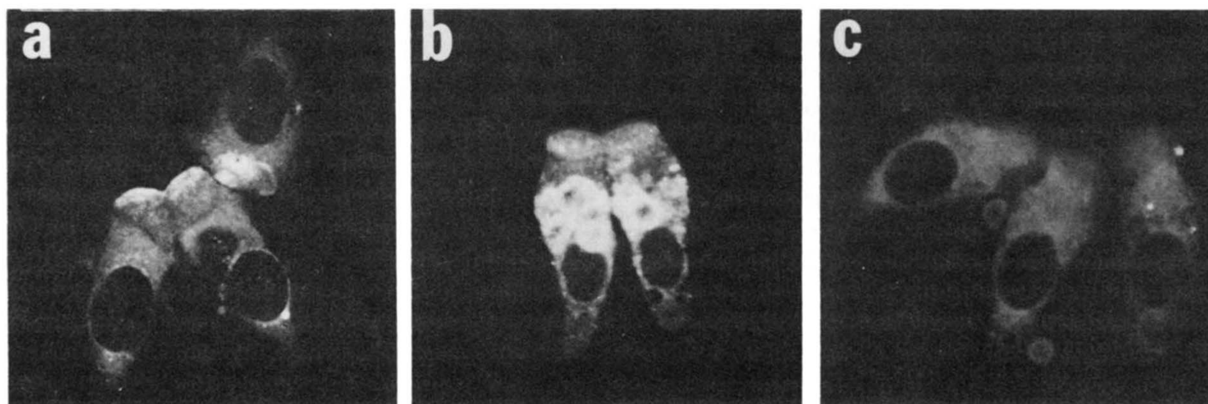

Fig. 7. Immunofluorescence for apoA-I in isolated human epithelial cells from a normal volunteer: (a) fasting, minimal fluorescence is seen beneath the brush bonder; (b) 45 min after corn oil was instilled intraduodenally, a marked increase in supranuclear fluorescence; and (c) using antisera absorbed with pure apoA-I, demonstrating specificity of the fluorescence.

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mucosal cells from fasting animals and in mesenteric lymph (88) when hepatic lipoprotein secretion is blocked by orotic acid (107), indicating that they are synthesized in the intestine. Bile diversion or the administration of cholestyramine in the rat results in depletion of VLDL from intestinal mucosal cells and mesenteric lymph, indicating that intestinal VLDL production in the fasting state provides a mechanism for reabsorption and secretion of endogenous (biliary lipids) from the intestine (88, 106). Rat mesenteric lymph VLDL transports about 50% of cholesterol and triglyceride in fasting lymph (106).

Intestinal VLDL is also formed during lipid absorption. VLDL-size particles are formed at low rates of lipid absorption. The findings of Ockner, Hughes, and Isselbacher (25), that absorption of micellar concentrations of saturated fatty acid (palmitate) resulted in formation of VLDL-size particles while unsaturated fatty acids resulted in larger chylomicron-size particles, were not confirmed by Redgrave and Dunne (22) who infused intraduodenally larger concentrations of unsaturated lipid. This discrepancy is probably explained by the slower rates of absorption of saturated fatty acids and the low (micellar) concentrations infused by Ockner et al (25).

The human intestine also produces VLDL. Fasting and postabsorptive human jejunal mucosa contain VLDL-size particles (108). Intestinal VLDL is also present in fasting chylous urine (18), thoracic duct (11), and mesenteric lymph (109). The lipid composition of human and rat intestinal VLDL is similar to chylomicrons (18, 106); however, as they are smaller particles, there is an increased content of surface constituents (phospholipid 15%, free cholesterol 2.3%, protein 4%). Cholesteryl ester content is also increased to 6% of the mass, reflecting the role of this particle in intestinal cholesterol transport.

The apoprotein content of intestinal VLDL resembles chylomicrons rather than plasma VLDL (18). The apoproteins of human intestinal VLDL include apoB, apoA-IV, apoE, apoE-I, apoA-II, and the C peptides. ApoA-IV (20–30%) and apoB content is greater in VLDL than chylomicrons and the apoC content is lower. Intestinal VLDL also gains apoE and C-protein and loses apoA-I and A-IV when incubated with plasma resulting in an altered mobility in agarose from α_1 to α_2 migration (18). After incubation with plasma, intestinal VLDL thus acquires characteristics of plasma VLDL. As intestinal very low density lipoproteins more closely resemble chylomicrons than plasma VLDL, they have been regarded as small chylomicrons, at the lower end of the size spectrum of triglyceride-rich intestinal lipoproteins. There is, however, evidence that at least a portion of intestinal VLDL may be derived by a different process. Glickman and Kirsch (17), in studies of intestinal lipoproteins from chylous ascitic fluid, demonstrated a decrease in chylomicron apoB content with decreasing chylomicron size; however, intestinal VLDL contained more apoB than small chylomicrons suggesting that intestinal VLDL are not just small chylomicrons. This is supported by the findings of Mahley et al. (8) who demonstrated limited mixing of chylomicron and VLDL-size particles in secretory vesicles from isolated rat epithelial cells. This suggests subcellular compartmentalization of these particles. Thus, some VLDL may be produced in the intestine by a different process or in a different location from that involved in chylomicron formation.

MESENTERIC LYMPH LDL

Only small amounts of lipid have been isolated in the 1.006–1.063 g/ml density range of rat mesenteric lymph (44). This is consistent with the formation of normal LDL from VLDL within the plasma compartment and a low rate of filtration into lymph (1, 2). The recent finding of both plasma LDL apoB (mol wt 335,000) and intestinal apoB (mol wt 240,000) in rat thoracic duct LDL suggests that it is an admixture of intestinally derived particles and filtered plasma LDL secreted by the liver (31). However, recent studies (35, 110) have demonstrated hepatic synthesis of both major forms of apoB.

Cholesterol feeding results in the appearance of abnormal lipoproteins in plasma. There is an increase in the VLDL and LDL fractions and a reduction in HDL (111–113). These abnormal plasma lipoproteins may be secreted directly by the liver (114) or be the products of altered catabolism of intestinal VLDL resulting in accumulation of remnant particles in plasma, as demonstrated by Ross and Zilversmit (115). Riley et al. (44) examined intestinal lymph from rats chronically fed a 1% cholesterol–10% olive oil diet. Lymph contained lipoproteins in the density range 1.006–1.030 g/ml which transported 19% of lymph cholesterol compared to 4% in control animals. These lipoproteins contained CE 24%, TG 36%, FC 8% and PL 35%, resembling the lipid composition of the plasma lipoprotein fraction of this density. However, apoA-I was the major apoprotein of the lymph particle while apoE was the major protein of the plasma particle, suggesting that the apoA-I particle may be secreted by the intestine. Further evidence for the intestinal origin of this particle was provided by the presence of labeled retinyl ester after intraluminal infusion of retinol. Plasma LDL from rats fed a high

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cholesterol and fat diet contained the lower molecular weight apoB characteristic of intestinal lipoproteins, suggesting an intestinal origin of these abnormal particles (34).

LDL-size particles have been observed in negatively stained human mesenteric lymph (109) and have been isolated from human thoracic duct lymph (11). Thoracic duct LDL transported 24% of total lymph cholesterol (11). The lipid composition of lymph LDL resembled that of plasma LDL except for increased triglyceride content: 20% compared to 11% for the plasma LDL. A detailed study of the apoproteins of this lipoprotein fraction has not been performed. Unlike plasma LDL, thoracic duct LDL did react with an anti-HDL antibody, suggesting the presence of apoA. It is possible that a portion of mesenteric LDL may therefore be derived from the intestine.

HIGH DENSITY LIPOPROTEINS

Rat mesenteric lymph contains HDL (32, 38, 43, 44). The percent of mesenteric lymph apoA-I recovered in the d > 1.006 g/ml fraction, which contains HDL, is variable. Glickman and Green (32) reported 85% of apoA-I in fasting lymph and 50% in fatty lymph, while Imaizumi et al. (45) reported less in this fraction: 30–40% in fasting lymph and 4–20% in fatty lymph. In order to characterize lymph HDL, Green, Tall, and Glickman (38) isolated HDL from fasting and fatty lymph in the presence of an LCAT inhibitor and studied the lipid and protein composition and structure by electron microscopy. Lymph HDL contained discoidal particles (190 × 55 Å) that were not present in control sera. Spherical HDL, similar to serum HDL, was also present in the lymph HDL fraction. Lymph HDL was enriched in PL and deficient in CE. The PL/CE ratio was greatest in basal fasting lymph HDL which was composed of about 50% discoidal particles. Fatty lymph HDL collected during triglyceride absorption had a lower PL/CE ratio and contained about 30% discoidal particles. The greater percentage of spherical HDL particles in fatty lymph was considered to be due to increased filtration of plasma HDL into lymph during lipid absorption. Mesenteric lymph HDL contained discoid particles which resembled hepatic nascent HDL secreted by the isolated perfused rat liver, suggesting that they were secreted by the intestine (116). Lymph HDL differed from hepatic HDL in that the major apoprotein was apoA-I and not apoE and it was more phospholipid-rich (PL/FC = 7) compared to hepatic HDL (PL/FC = 3). Intestinal HDL would potentially have greater capacity to acquire free cholesterol from other lipoproteins and cell membranes.

Bile diversion, which depletes rat intestinal epithelial cells and mesenteric lymph of triglyceride-rich lipoproteins (88), resulted in sustained mesenteric apoA-I and HDL output (98). The HDL fraction from these bile-diverted animals contained discoidal particles which were absent from plasma. The presence of these particles at a time when lymph contained greatly reduced chylomicrons and VLDL confirms that these particles are not derived from triglyceride-rich particles in lymph.

Alaupovic et al. (11) demonstrated the presence of HDL in human thoracic duct lymph. This fraction carried 16% of total lymph cholesterol. The lipid composition was similar to plasma HDL except for an increase in triglyceride content. HDL isolated from chylous urine in the presence of an LCAT inhibitor had a lipid composition similar to that of plasma HDL except for increased TG content (11.1%) of total HDL lipid compared to plasma HDL (2.8%) (18). The apoproteins of chylous urine HDL (18) and thoracic duct lymph HDL (12) resembled plasma HDL, with apoA-I as the major apoprotein. In two subjects with chyluria (18), the A-I/A-II ratio in urinary HDL was increased to 5.6 and 7.1, compared to the value of 3 for plasma HDL. Negative stain electron microscopy revealed only spherical HDL; no discoid particle was seen. Spherical HDL-size particles have also been visualized in human mesenteric lymph with negative staining techniques (109). Anderson et al. (117) failed to find discoidal HDL in human thoracic duct lymph. HDL_{2A} predominated in lymph HDL whereas HDL_{2A} and $_{2B}$ were equally represented in plasma HDL. Lymph HDL was more TG-rich than plasma HDL.

Approximately 85% of apoA-I and apoA-II in chylous urine was present in the d > 1.006 g/ml fraction, reflecting the presence of comparatively large quantities of HDL in mesenteric lymph (18). HDL in chylous urine and thoracic duct lymph is probably from several sources including the intestine, although this has not been demonstrated in man. Possible sources include the intestine, filtration from the plasma compartment at the level of the intestine, and the liver. The large amount of HDL present in mesenteric and thoracic duct lymph provides an opportunity for chylomicron/HDL interactions as reflected in the recent report in which most chylomicron cholesterol in chylous urine was found to originate from plasma lipoproteins (118).

While there is suggestive evidence in the rat that chemically and morphologically distinct forms of HDL exist in mesenteric lymph, there is no evidence directly

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demonstrating the secretion of these particles by the intestine. Specifically, discoidal particles have not been identified in intestinal epithelial cells in rat or man. Discoidal HDL has not been demonstrated in lymph of intestinal origin in man.

METABOLIC FATE OF INTESTINAL LIPOPROTEINS

Chylomicrons

The metabolic fate of chylomicron components is diverse. The lipid components are metabolized in two or three phases. Core triglyceride is hydrolyzed in the peripheral circulation (119, 120) by lipoprotein lipase while surface phospholipids are transferred in the circulation to the HDL fraction (121, 122). The result of this peripheral metabolism is the formation of the chylomicron remnant which is rapidly removed by the liver (123). The metabolism of chylomicron lipid components is intimately associated with alteration in the apoprotein composition of chylomicrons.

Upon entering plasma, chylomicrons acquire apoE and apoC (18, 31) associated with an increase in protein content from 1% to 3% of mass (124). ApoC is gained from the HDL fraction (125) while apoE is derived mainly from the HDL fraction (126). The presence of apoC serves two functions, *1*) activation of lipoprotein lipase by apoC-II (127) and *2*), inhibition of chylomicron removal by the liver enabling peripheral catabolism to occur. Lipoprotein lipase is found bound to the capillary endothelium and can be detected in most extrahepatic tissues. Chylomicron triglyceride hydrolysis results in the formation of partial glycerides which may be taken up by tissue (128) or hydrolyzed in plasma (129), and fatty acids which are removed by albumin (127). Lipoprotein lipase has greater affinity for larger triglyceride-rich particles than for smaller chylomicrons, most likely related to their greater apoC content (125). Chylomicrons are markedly altered after in vitro incubation with lipoprotein lipase (130). Early changes associated with core depletion result in peripheral indentation of the intact surface membrane suggesting that lipoprotein lipase acts at the triglyceride surface. After more prolonged action, negative stain electron microscopy reveals the presence of marked alterations in size and shape with the appearance of protrusions of surface material, lamellar surface constituents, dumbbell-shaped particles, and collapsed vesicles. The product of lipoprotein lipase action is a particle rich in phospholipid. Similar phospholipid-enriched particles have been seen in the plasma of animals pretreated with 4-APP (131) and in the plasma of patients with Tangier disease (132) and LCAT deficiency (133), all associated with low circulating HDL levels.

Subsequent to lipolysis and loss of surface constituents, the chylomicron remnant is formed. This particle was originally isolated from the functionally hepatectomized rats by Redgrave (123). Remnants are characterized by a smaller spherical size comprising ~4% of the original chylomicron mass. The remnant is depleted in triglyceride and more enriched in cholesteryl ester, phospholipid, and protein, as a percent of total mass, then chylomicrons. However, it must be emphasized that during remnant formation, there is considerable loss of chylomicron mass (~96%) which involves loss of core triglyceride as well as surface phospholipid and protein. The major apoproteins of the remnant are apoB (intestinal) and apoE. Loss of apoA-I, apoA-IV, and apoC occurs during chylomicron and remnant metabolism, as discussed below. Chylomicron remnants are rapidly removed by the liver (123). Uptake is by a specific receptor-mediated, high velocity process (134, 135). Several studies suggest that apoE is the major chylomicron determinant for uptake by the chylomicron receptor (136–139). Studies by Windler, Chao, and Havel (139) using α-ethinyl estradiol-treated animals, which have extremely low apoE levels, have demonstrated that chylomicrons that lack apoE are also rapidly removed, suggesting that intestinal apoB may also be a recognition factor. ApoC inhibits the hepatic uptake of chylomicrons (136, 137).

As mentioned above, there is evidence that a portion of chylomicron phospholipid is transferred to HDL. Chylomicron phospholipid transfers to HDL during in vitro incubation of dog and rat lymph chylomicron with serum (30, 31). Havel (140) demonstrated an increase in HDL phospholipid during alimentary lipemia that was accounted for by an increase in the HDL_2 fraction. Similarly, transfer of chylomicron phospholipid radioactivity to HDL was noted after chylomicron infusions into dogs (120). Mass transfer of chylomicron phospholipid into HDL after labeled chylomicrons were infused into intact animals has recently been demonstrated by Redgrave and Small (121). In similar studies, Tall et al. (122) demonstrated that loss of chylomicron phospholipid was accompanied by an increase in HDL phospholipid:cholesteryl ester ratio due to both an increase in HDL phospholipid and a loss of cholesteryl ester to lighter density lipoproteins. Chylomicron phospholipid is potentially a major source of HDL phospholipid. The ingestion of 50–100 g of fat (the average daily intake in the United

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States) results in the secretion of chylomicrons containing 3–6 g of phospholipid (18). If one considers that the fasting HDL phospholipid pool in man is ~1.5 g (141), only a fraction of the chylomicron phospholipid mass entering the HDL fraction could raise the HDL phospholipid pool considerably.

In order to explore the possible mechanism of phospholipid transfer to HDL, Forte et al. (142) studied the effect of heparin-induced lipolysis on lipoprotein structure in hypertriglyceridemic subjects. Chylomicron surface configuration was altered and vesicular structures appeared in the LDL region. There was an increase in larger, lighter HDL particles (HDL_{2a}) and a decrease in HDL_3 suggesting that with lipolysis, HDL_3 is transformed into HDL_{2a} by incorporation of chylomicron components. In vitro studies by Tall and Green (143) in which phospholipid vesicles were incubated with HDL_3 resulted in formation of an HDL_{2a}-like particle. The factors responsible for the transfer of chylomicron surface components into HDL, however, remain to be determined.

Plasma chylomicrons lose apoC, apoA-I, and apoA-IV during remnant formation. ApoC is transferred back to the HDL fraction (125), allowing the remnant to be removed by the liver. When rat chylomicrons labeled in vivo in apoB, apoA-I, and apoA-IV were infused into intact animals, only apoB remained associated with chylomicrons and apoA-I and apoA-IV were transferred to HDL (122).

In man, intravenous infusion of labeled human chylous fluid chylomicrons resulted in transfer of chylomicron apoA-I and apoA-II into plasma HDL (14). Lipid feeding in man results in a significant rise in plasma apoA-I which is recovered wholly in the HDL fraction (52). Thus, available evidence indicates that, in man, chylomicron apoA-I is a source of plasma HDL apoA-I. Estimates of the quantitative importance of intestinal apoprotein secretion are provided by studies in chyluric man (18). Calculations based on the amount of apoA-I secreted in the chylomicron and VLDL fraction of chylous urine after the ingestion of corn oil indicated that between 30–40% of the calculated daily synthesis of apoA-I is secreted into plasma associated with these particles. The figures for apoA-II were 20–30% of the daily synthesis. These results suggest that the human intestine contributes significantly to the HDL apoprotein pool, findings confirmed in the studies performed by Anderson et al. (117).

The intestine may also secrete lipoproteins directly into the portal venous system, bypassing the lymphatic route. Windmueller et al. (43) identified newly labeled HDL apoproteins in the venous effluent of the isolated perfused intestine. McDonald et al. (144) demonstrated that a substantial proportion of unsaturated long chain fatty acids was transported from the rat intestine via the portal vein, especially at low rates of fat absorption. More recently, Windmueller and Wu (101) have provided evidence that, in the absence of triglyceride absorption, a greater proportion of apoproteins synthesized in the intestine is secreted directly into the portal system than in periods of lipid absorption. However, the significance of this portal route as a source of plasma lipoproteins secreted by the intestine remains to be determined.

The fate of chylomicron apoA-IV is less clear. In the rat, apoA-IV is transferred to HDL (122). Studies performed by Fidge (145) suggest that apoA-IV may enter the HDL fraction after residing in the d > 1.21 g/ml fraction of plasma. In man, plasma apoA-IV levels rise after lipid ingestion (12); however, apo-IV is recovered mainly in the lipoprotein-free fraction. In subjects with alcoholic hepatitis and LCAT deficiency, apoA-IV exists in the HDL fraction associated with a discoidal lipoprotein (146).

Intestinal VLDL

Intestinal VLDL or small chylomicrons probably are metabolized as chylomicrons. Incubation of rat or human intestinal VLDL increases their mobility in agarose from α to $\alpha 2$ mobility, similar to plasma VLDL (18, 106). This is probably related to alteration in the apoprotein composition. Human intestinal VLDL gained apoE and apoC on incubation with plasma (18).

Several studies have attempted to quantitate the contribution that intestinal VLDL makes to the plasma lipoprotein pool while fasting. These studies have concentrated on the contribution to plasma triglyceride levels. Byers and Friedman (147) performed hepatectomy in rats, resulting in a fall of plasma triglyceride to 20% of control values, suggesting that the intestine contributed as much as 20% of plasma triglyceride. A similar figure of 20% was arrived at by Ceredella, Crouthamel, and Mengoll (148) who studied orotic acid-treated animals. Ockner et al. (106), however, estimated the intestinal contribution to be as high as 40% of the total plasma VLDL turnover. More recently Risser, Reaver, and Reaver (149) addressed this problem by calculating intestinal lymph triglyceride output and hepatic and total VLDL secretion rates by measuring the accumulation of plasma VLDL after the injection of Triton WR 1339. The intestinal contribution to plasma VLDL was only 11% in the fasting state and 14–17% in non-lipid fed rats. Triton WR 1339 blocks the removal of triglyceride lipoproteins; however, the recent studies of Ishikawa and Fidge (150) have demonstrated a profound alteration in plasma HDL structure in Triton-treated rats.

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Further studies using Triton must take into account the marked changes in lipoprotein structure and apoprotein content that result from its use.

HDL

There have been no studies to determine the metabolic fate of lymph HDL. It is presumed that discoidal HDL, known to be an excellent substrate for LCAT (116), will acquire cholesteryl ester and contribute to plasma HDL.

CONCLUSION

It is apparent that the past few years have resulted in substantial progress in understanding intestinal lipoprotein formation. There is now strong evidence that the intestine is a synthetic site of several apoproteins during chylomicron formation, notably apoB, A-I, A-II, A-IV and, to a lesser degree, apoC. Initial efforts indicate that the intestine synthesizes quantitatively important amounts of apoproteins such as apoA-I, A-II, and A-IV during triglyceride transport. Firm evidence exists that apoprotein and phospholipid constituents of triglyceride-rich lipoproteins are distributed to the HDL fraction of plasma subsequent to lipolysis. While this is felt to be an important source of plasma HDL constituents, further studies of the magnitude of this contribution are required. Additional studies are necessary to define the precise mechanisms by which chylomicron surface constituents interact with plasma HDL. Do alterations in the lipid or apoprotein composition of chylomicrons affect their subsequent metabolism? Specifically, can diet-induced alterations in chylomicron composition (i.e., cholesterol, saturated fat) influence subsequent HDL formation or the metabolism of chylomicron remnants?

Great gaps in our knowledge exist concerning the mucosal aspects of intestinal lipoprotein formation. Further information is required to elucidate the precise mechanisms of apoprotein synthesis. Are there stimuli in addition to triglyceride absorption which stimulate apoprotein synthesis and secretion? Is mucosal cholesterol synthesis linked to apoprotein synthesis? The recent findings that circulating lipoproteins may influence intestinal cholesterol synthesis (97, 151) suggest the possibility that plasma levels of lipoproteins may also influence intestinal lipoprotein formation. In addition, do different portions of the intestine differ in their pattern of lipoprotein secretion?

It is only through a more complete understanding of the factors that influence the synthesis, secretion, and subsequent metabolism of intestinal lipoproteins that the effects of diets and drugs on lipoprotein metabolism can be understood. From the information developed to date, the role of the intestine in lipoprotein metabolism is likely to be an important one.

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