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The assembly of lipids into lipoproteins during secretion

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Summary. The process of assembly and secretion of lipoproteins is discussed with particular reference to the role of lipids. The majority of circulating lipoproteins is produced by the liver (80 %) with the remainder being supplied by the intestine. The liver secretes both very low density lipoproteins and high density lipoproteins, but the assembly and secretion of these two types of particles may follow different routes. The major lipid components of lipoproteins are triacylglycerols, cholesterol, cholesterol esters and phospholipids. The biosynthesis of these lipids occurs on membranes of the endoplasmic reticulum, with many of the enzymes also being present in the Golgi; the roles of these two subcellular organelles in the assembly of lipoproteins are discussed. There appears to be a compartmentalization of lipids in cells, such that defined pools, often those newly-synthesized, are preferred, or even required, for lipoprotein assembly. The process of hepatic very low density lipoprotein secretion appears to be regulated by the supply of lipids. Indeed, the synthesis of new lipid may be a major driving force in lipoprotein assembly and secretion.
Key words. Lipoprotein secretion; very low density lipoproteins; high density lipoproteins; lipid compartmentalization.

Introduction

Great interest in lipid and lipoprotein metabolism has been generated recently because the risk of atherogenesis is now recognised as being related to the blood levels of lipoproteins. The four major classes of circulating lipoproteins are: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), which are classified according to their densities. The basic structure of the approximately spherical lipoprotein particles consists of a hydrophobic core (mainly triacylglycerols and cholesterol esters) surrounded by a hydrophilic coat comprising a monolayer of amphipathic lipids (cholesterol and phosholipids) interspersed with a mixture of specific proteins called apo(lipo)proteins. The hydrophilic coating of lipoproteins permits the hydrophobic core lipids to be transported in the aqueous environment of blood to other tissues.

Lipoproteins are secreted by liver and intestine, with the majority of the apoproteins ($\approx$ 80 %) derived from the liver[83]. The intestine produces the large triacylglycerol-rich chylomicrons and VLDL, and some HDL, whereas the liver secretes VLDL and HDL. There are two related

forms of apo(protein) B, an important apoprotein of VLDL and LDL. Apo B-100 is a very large, single polypeptide chain (4536 amino acids) which in humans is synthesized and secreted into VLDL by the liver[41]. A truncated form of apo B, called apo B-48, consisting of the N-terminal portion of apo B-100, is secreted solely by the intestine. In the rat, however, both the large and small forms of apo B are present in VLDL made by the liver[62]. Rat and human VLDL probably contain a single molecule of apo B per particle[22].

Although the levels of circulating lipoproteins depend upon their rates of both synthesis and removal, the majority of research on lipoprotein metabolism has been focussed on the uptake and degradation of lipoproteins, chiefly due to the pioneering work of Brown and Goldstein[25]. Details of the process of lipoprotein assembly and secretion, however, are far less well understood. Newly-secreted lipoproteins are rapidly metabolized and modified in the circulation; therefore, it is difficult to dissect and study lipoprotein secretion in humans or intact animals. Several alternative model systems are routinely used, including perfused rat liver, isolated rat hepatocytes, estrogen-treated chick hepatocytes in culture and the continuous human hepatoma cell line, HepG2. Although none of these systems is physiologically the same as the intact animal, tissue culture systems do offer the advantages of being very reproducible and of having the capacity for careful manipulation of the culture conditions. Monolayer cultures of rat hepatocytes do not proliferate but retain many characteristics of liver cells in vivo, such as secretion of lipoproteins[18]. Chicken hepatocytes have also been used as a model for studying lipoprotein secretion[39]. The output of VLDL by these cells can be increased 10- to 50-fold by administration of estrogen to the chicks. In addition, the human hepatoblastoma-derived cell line HepG2 is used to study the assembly and secretion of lipoproteins[70]. Very little classical VLDL is secreted by these cells; instead, the majority of lipoprotein particles contain apo B-100 only and are the size of plasma LDL. However, their lipid composition is unlike that of plasma LDL, the major core lipid being triacylglycerol rather than cholesterol ester. HDL-like particles are also secreted by HepG2 cells.

1) Overview of assembly of apoproteins into lipoproteins

Secretion of VLDL

The assembly and secretion of lipoproteins involves the coordination of the synthesis of each of the lipid and apoprotein components, aggregation of these components in the required proportions and movement of the nascent particles from their sites of assembly through the secretory route and into the circulation. In a classic study, Alexander et al.[1] provided the first evidence for the intracellular location of apo B in liver using immunoelectron microscopy. They found apo B in rough endoplasmic reticulum (ER), in vesicles located between rough ER and Golgi apparatus, in the Golgi, in secretory vesicles and in secreted VLDL. This study, together with other evidence, has suggested that triacylglycerols and other lipid components, which are synthesized in the ER, combine at the junction of the rough and smooth ER with apo B that is synthesized in the rough ER. The nascent particles are transferred to the Golgi apparatus, where there is further processing of the apoproteins (e.g. glycosylation). Secretory vesicles are subsequently released from the Golgi, move to and fuse with the plasma membrane and nascent VLDL are released into the space of Disse.

There are conflicting conclusions about the rate of intracellular movement of apo B from the rough ER through the secretory pathway. In cultured chick hepatocytes pulsed with [^{3}H]leucine, the time taken for synthesis of a complete apo B molecule was 10 min[60] and for radiolabeled apo B to appear in the medium was 30–35 min. In an extension of these studies, after pulse-labeling with [^{3}H]leucine, Golgi and ER membranes were isolated from the hepatocytes. The data[5] indicated that the rate-limiting step in the secretion of apo B was its transport through the Golgi, not the ER. A similar conclusion was reached in pulse-chase studies in HepG2 cells[11,12]. This conclusion is in contrast to current models of protein secretion[48]. An interesting, but unexplained, finding was that approximately equal amounts of apo B were found in the lumen and in the membranes of Golgi fractions. In contrast, albumin was almost entirely located in the luminal compartment[5].

In cultured rat hepatocytes the rate of transport of apo B out of the ER, and not the Golgi, apparently determined the overall rate of secretion[10]. Moreover, the half-life for secretion of small apo B was twice that of albumin, suggesting that a process unique to apo B secretion was required in the ER. Possibly the additional time required for secretion of apo B may be due to its association with lipid components in the ER. The reasons for the apparent discrepancy about which step in apo B secretion is rate-limiting are not clear. The experiments are, however, technically very complicated and different cell types and methodologies were used in each case.

Secretion of HDL

In addition to VLDL, HDL particles are secreted by hepatocytes, although the mechanism of this process is in doubt[21,28,82]. There is even some question about the nature of nascent HDL particles secreted by liver. Discoidal, lipid-bilayer particles, composed primarily of cholesterol, phospholipids and HDL apoproteins, with only small amounts of cholesterol esters and triaclyglycerols, were secreted from livers perfused with an inhibitor (5,5′-dithionitrobenzoic acid) of lecithin:cholesterol acyltransferase (LCAT)[28]. However, discoidal HDL particles have not been detected intracellularly[21,34]. Transformation of nascent HDL into spherical HDL of circulating plasma by acquisition of cholesterol ester is

dependent on the action of LCAT, which transfers a fatty acyl chain from phosphatidylcholine (PtdCho) to cholesterol. It is likely that HDL are secreted as a heterogeneous population of particles containing triacylglycerol, cholesterol, phospholipid and some apoprotein [28,82] and that more apo Al associates with the particles in plasma. Additional lipid moieties (cholesterol and phospholipids) may also become associated with HDL after secretion, perhaps by exchange from the cell surface.

VLDL and HDL are probably assembled and secreted by different mechanisms. For example, treatment of hepatocytes with orotic acid [27] inhibited (by an unknown mechanism) VLDL, but not HDL, secretion. Similarly, the secretion of VLDL was markedly reduced in choline-deficient, compared with choline-supplemented, rat hepatocytes, whereas HDL secretion was unaffected [85]. There also appear to be differences in the hormonal regulation of VLDL and HDL secretion (P. Martin-Sanz, J. E. Vance and D. N. Brindley, unpublished).

Lipoproteins are isolated in solution at a density of less than 1.21 g/ml after ultracentrifugation. Some lipid material can also be isolated in the fraction of density greater than 1.21 g/ml, the major protein of which is albumin. In this fraction there is a high content of lysoPtdCho [3,26,59], most likely associated with albumin. There is evidence for the direct secretion of lysoPtdCho by cultured rat hepatocytes [3,26] and perfused rat liver [59]. This may provide an important mechanism by which choline and unsaturated fatty acids are delivered to extrahepatic tissues.

2) Subcellular sites of enzymes of lipid synthesis

The major lipids present in lipoproteins are generally assumed to be synthesized principally in the ER.

Production of cholesterol and cholesterol ester

All membrane-bound enzymes of cholesterol biosynthesis are present in the ER [54] but these enzymes have also been identified in rat liver peroxisomes [42,69], and in an unidentified membrane fraction [47]. The functional significance of production of independent pools of cholesterol by peroxisomes, as well as ER, is not understood. Interestingly, peroxisomes have been recently identified as a major location of the non-specific lipid transfer protein (sterol carrier protein 2), one of whose putative roles is the intermembrane transport of cholesterol and phospholipids [2,43].

Although triacylglycerol is the major non-polar core lipid of VLDL there is also some cholesterol ester. The enzyme responsible for the intracellular synthesis of cholesterol ester is the ER enzyme acyl CoA:cholesterol acyl transferase. Cholesterol ester in rat plasma VLDL is most likely derived from this source and secreted with the nascent particles [51]. In humans, on the other hand, the full complement of cholesterol ester in serum VLDL and LDL is probably acquired in plasma from HDL after secretion by the combined actions of LCAT and cholesterol ester transfer protein [21]. This process is thought to be insignificant in rats because plasma from these animals lacks cholesterol ester transfer activity.

Production of triacylglycerols and phospholipids

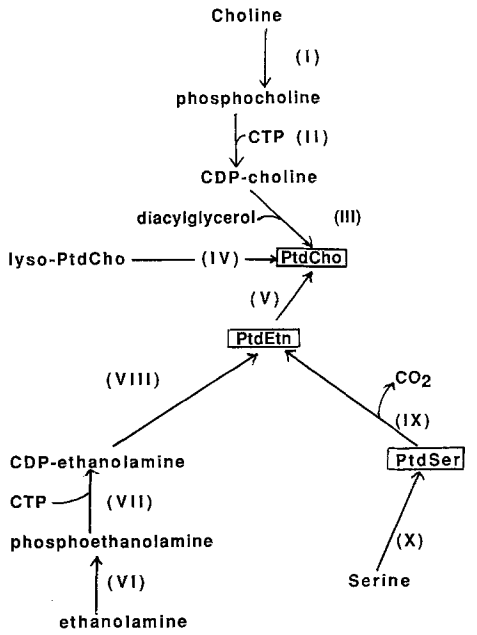
Triacylglycerols used for assembly into hepatic lipoproteins are synthesized by the following sequence: a) the conversion of glycerol-3-P into phosphatidic acid by two specific acyltransferases, b) the hydrolysis of phosphatidic acid to diacylglycerol by phosphatidic acid phosphohydrolase, and c) the acylation of diacylglycerol to triacylglycerol by diacylglycerolacyltransferase. These enzymes are associated with the ER but most are also present in other organelle membranes [13].

The choline-containing phospholipids, particularly PtdCho, are the major phospholipids of all lipoproteins; however, smaller amounts of phospholipids such as phosphatidylethanolamine (PtdEtn), phosphatidylserine (PtdSer) and phosphatidylinositol (Ptdlns), as well as sphingomyelin and lysophosphatidylcholine, are also present (table). The requirement for these "minor" phospholipids in the structure and functioning of lipoproteins has not been determined. The phospholipid composition of lipoproteins is different from that of any of the cellular membranes from which these lipids are presumably derived [34]. The general scheme and major routes of biosynthesis of PtdCho, PtdEtn and PtdSer are shown in the figure. In liver, the majority of PtdCho is synthesized via the CDP-choline pathway. Approximately 20–40 % of PtdCho might be derived from the methylation of PtdEtn [65]. The membrane-bound enzymes of PtdCho biosynthesis by the CDP-choline pathway, CTP:phos-

Phospholipid composition of human plasma lipoproteins

Phospholipid	Phospholipid composition (% of total phospholipid)			
	Chylomicrons	VLDL	LDL	HDL
Phosphatidylcholine	78.5	59.7	63.7	74.4
Phosphatidylethanolamine	5.6	4.6	2.2	3.1
Sphingomyelin	11.7	14.8	25.9	13.2
Lysophosphatidylcholine	4.2	5.0	2.7	2.9
Phosphatidylserine	–[a]	1.5	0.8	0.8
Phosphatidylinositol	–	3.6	1.6	2.4
Phosphatidic acid and (or) polyglycerolphosphatides	–	7.6	2.0	2.2
Other and (or) unidentified	–	3.2	1.1	0.9

Data are taken from Skipski [61]. [a] The minor phospholipids were not reported for chylomicrons.



Biosynthesis of phospholipids. Enzyme activities are indicated by: (I) choline kinase, (II) CTP: phosphocholine cytidylyltransferase, (III) CDP-choline: 1,2-diacylglycerol cholinephosphotransferase, (IV) lysophosphatidylcholine acyltransferase, (V) phosphatidylethanolamine-*N*-methyltransferase, (VI) ethanolamine kinase, (VII) CTP: phosphoethanolamine cytidylyltransferase, (VIII) CDP-ethanolamine: 1,2-diacylglycerol ethanolaminephosphotransferase, (IX) phosphatidylserine decarboxylase and (X) phosphatidylserine synthase (base exchange enzyme).

phocholine cytidylyltransferase and CDP-choline: 1,2-diacylglycerol cholinephosphotransferase, are located in both ER and Golgi[40,76] with approximately equal specific activities. In terms of total cellular activities, however, the Golgi has the capacity to provide only 15–20 % of the PtdCho made by the CDP-choline pathway[76]. The cytidylyltransferase occurs both in microsomal membranes and in cytosol. The membrane-associated enzyme is the active species and the cytosolic enzyme acts as a reserve supply[73]. PtdEtn-*N*-methyltransferase, a single polypeptide chain of molecular weight 18 kDA, catalyses all three methylation reactions[56]. This enzyme also resides in both ER and Golgi membranes[29,76]. Phosphatidylcholine can be made by one other important route – acylation of lysoPtdCho (fig.).

PtdEtn is made in liver by two biosynthetic pathways from ethanolamine via CDP-ethanolamine, and from PtdSer via decarboxylation (fig.). In the past, PtdSer decarboxylation had not been considered to be important in the production of PtdEtn. However, experiments in BHK and CHO cells have shown that even when these cells were cultured in the presence of ethanolamine, at least 80 % of the PtdEtn was derived from decarboxylation of PtdSer[46,81]. Experiments in our laboratory (J. E. Vance, unpublished) suggest that PtdSer decarboxylation provides a major portion of PtdEtn in cultured rat hepatocytes. PtdSer decarboxylase activity appears to be restricted to mitochondria[20,76]. For the CDP-ethanolamine pathway the membrane-bound enzyme, CDP-ethanolamine: 1,2-diacylglycerol ethanolaminephosphotransferase, has similar specific activities in both ER and Golgi of rat liver[76]. A minor pathway for PtdEtn biosynthesis is the exchange reaction of ethanolamine with the head group of another phospholipid.

Phosphatidylserine is synthesized in liver by exchange of the polar head-groups of either PtdEtn or PtdCho with serine[46]. The reactions are catalysed by PtdSer synthases that have been detected chiefly in the ER. In addition, there appears to be some PtdSer synthase activity in Golgi which cannot be accounted for by cross-contamination by ER[40,76]. Similarly, PtdIns synthase activity has been detected in both ER and Golgi membranes of mouse liver[40].

Thus, both major organelles involved in the assembly of lipoproteins, the ER and Golgi, each contain most of the enzymes required for phospholipid synthesis. The notable exception is the mitochondrial enzyme, PtdSer decarboxylase[20], which may be the source of most secreted PtdEtn. (A preference for secretion of PtdSer-, rather than CDP-ethanolamine-, derived PtdEtn has been demonstrated[77]). Hence, an interesting question arises: how is PtdEtn, made in mitochondria, directed into assembly of VLDL which takes place in the ER and/or Golgi?

There are other topographical questions involved in assembly of phospholipids into lipoproteins. Since phospholipid biosynthetic enzymes of ER and Golgi are located on the cytosolic, not luminal, surface of these membranes[4,14,74,76], the phospholipid products must presumably undergo transbilayer movement to the luminal leaflet for assembly into lipoproteins.

3) Subcellular sites of association of lipids with apoproteins

One major question concerning the association of lipids with apo B is: from which organelles are the lipids derived and at what subcellular location are these lipids added to apo B? Howell and Palade separated rat liver Golgi membranes from their luminal contents (including lipoproteins) by high pH treatment of Golgi vesicles[33]. The VLDL-like particles in the lumen had a similar, but not identical, lipid composition to that of serum VLDL[34]. No discoidal HDL precursors could be identified. From experiments using radiolabeled phospholipid precursors the authors proposed an active exchange of phospholipids between Golgi membranes and lipoproteins in the lumen[34].

Janero and Lane[38] attempted to determine the subcellular organelle(s) from which phospholipids were added to lipoproteins in chick hepatocytes using pulse-labeling experiments with both [^{3}H]glycerol and [^{3}H]leucine. They demonstrated that triacylglycerol and apoprotein moieties of VLDL followed a similar time course for secretion, with radioactivity appearing in these two secreted

components approximately 30–40 min after addition of radiolabel. The time course of secretion of phospholipids, however, was markedly different. It appeared that phospholipids were added to VLDL at two stages during assembly, which were interpreted to be during passage through the ER and the Golgi.

Additional evidence that at least some phospholipid in VLDL was added from the Golgi came from a study in rat liver[30]. The putative VLDL precursors in the lumen of the microsomes were smaller than those in the Golgi and had a mol ratio of triacylglycerol:phospholipid of ten, whereas the contents of the Golgi had a mol ratio of triacylglycerol: phospholipid of one. The authors proposed that triacylglycerol destined for secretion was sequestered in the ER lumen and that the bulk of phospholipid, cholesterol and apo B[31] were not added until the triacylglycerol reached the Golgi. However, in some of these experiments the conclusions were based on very low levels of incorporation of radioactive precursors and measurements of low mass. Moreover, it is not clear how such hydrophobic, triacylglycerol-rich, polar lipid-deficient particles would be stable in the aqueous environment of the microsomal lumen. Experiments such as these are technically very difficult due to such problems as isolating Golgi free from ER and multivesicular body[32] contamination, and ensuring that luminal contents are free of membrane fragments. The experiments did not show whether VLDL phospholipids derived from the Golgi were synthesized in the ER and subsequently transferred to the Golgi, or whether the phospholipids were synthesized in situ in the Golgi and assembled into VLDL directly.

At this juncture it appears likely that most phospholipids are added to the nascent VLDL particles during assembly in the ER and that exchange of phospholipid between nascent VLDL and Golgi membranes will occur.

4) Compartmentalization of lipids for assembly into lipoproteins

Compartmentalization of phospholipids

Convincing evidence is accumulating that phospholipids and phospholipid precursor molecules in cells do not exist in single, metabolically homogeneous pools. There appear to be defined intracellular pools of these compounds that are derived from different origins and that can potentially be used for different purposes.

One vivid example of compartmentalization of phospholipids is their assembly into hepatic lipoproteins[77]. Cultured rat hepatocytes were incubated with one of [^{3}H]choline, [^{3}H]ethanolamine or [3-^{3}H]serine as precursors of PtdCho and PtdEtn (fig.). The VLDL and HDL secreted into the culture medium were isolated by ultracentrifugation and the specific radioactivity (dpm/nmol) was measured in PtdCho and PtdEtn of both lipoproteins and cells. As anticipated, the specific activity of PtdCho derived from choline via the CDP-choline pathway was approximately equal in cells and lipoproteins. Surprisingly, from [^{3}H]ethanolamine the specific radioactivity of PtdCho and PtdEtn was several-fold *lower* in the medium than in the cells, whereas from [3^{3}H]serine the specific radioactivity in both PtdCho and PtdEtn was *higher* in lipoproteins than in cells[77]. These data suggested that phospholipids used for assembly into lipoproteins did not originate from a single, uniformly-labeled cellular pool. Instead, there was a preference for secretion of PtdCho and PtdEtn labeled from serine (fig.) and a discrimination against secretion of these lipids labeled from ethanolamine. Thus, phospholipids derived from different biosynthetic origins do not rapidly equilibrate throughout the cells' membranes and may exist in membrane domains.

Since liver is responsible for production of the majority of lipoproteins[83], and the PtdEtn methylation pathway for PtdCho biosynthesis is quantitatively important only in liver, an attractive possibility was that the methylation pathway was required for lipoprotein secretion. However, this hypothesis is probably not correct. When monolayer cultures of hepatocytes were incubated with the methylation inhibitor, 3-deazaadenosine, which blocked the methylation of ethanolamine- and serine-derived PtdEtn by 95 and 75% respectively, there was no decrease in the mass of PtdCho secreted and lipoprotein secretion was normal[80]. Secretion of ethanolamine-derived PtdCho was blocked by this adenosine analogue, whereas surprisingly, the secretion of serine-labeled PtdCho was almost unaffected[75]. These data indicated that there may be a unique PtdEtn methyltransferase involved in production of PtdCho for assembly into lipoproteins, that specifically uses PtdSer-derived PtdEtn as substrate. The existence of this putative enzyme has not yet been confirmed.

Recent work has shown an additional degree of compartmentalization of phospholipids for lipoprotein secretion. The molecular species composition of secreted phospholipids was compared with that of hepatocytes from which the lipoproteins were derived, using high performance liquid chromatography and radioabeled phospholipid precursors[78]. From [^{3}H]ethanolamine, the molecular species composition of cellular and secreted phospholipids was identical. However, for PtdCho derived from choline, and PtdCho and PtdEtn labeled from [^{3}H]serine, the percentage of the species 1-stearoyl-2-arachidonoyl PtdCho and PtdEtn was much lower in the medium than in the cells. The explanation may be that secreted phospholipids are representative of the newly-made phospholipid molecular species distribution, whereas the acylation pattern of cellular phospholipids is modified as a result of selective degradation of certain species or as a result of remodeling by deacylation-reacylation reactions[79]. Thus, not only are phospholipids from specific biosynthetic routes selected for assembly into lipoproteins but newly-made phospholipids may be se-

questered for secretion before they can mix with pre-existing phospholipids of membranes.

In support of this idea, Yao and Vance [85] have shown that *active* synthesis of phosphatidylcholine, by either the CDP-choline or the PtdEtn methylation pathway, is required for secretion of VLDL by cultured rat hepatocytes. In these experiments, hepatocytes were prepared from choline-deficient rats, then cultured in the absence (choline-deficient) or presence (choline-supplemented) of choline. The secretion of lipid and apoprotein components of VLDL from choline-deficient cells was markedly reduced compared to choline-supplemented cells. Rather surprisingly, secretion of HDL lipids and apoproteins was unaffected by choline deficiency. From these data it is apparent that secretion of VLDL requires active synthesis of new PtdCho by any route; pre-existing membrane PtdCho cannot suffice. The head-group specificity of phospholipid synthesis required for VLDL secretion was also investigated in the same choline-deficient model [84]. The choline moiety of PtdCho was specifically required and could not be replaced with ethanolamine, monomethylethanolamine or dimethylethanolamine.

The existence of specific domains of lipids within cells has previously been indicated. Only a small phospholipid pool was active in the Ca^{++}-stimulated base exchange reaction with choline, ethanolamine and serine [8]. In addition, only a small fraction ($^1/_{25}$ to $^1/_{70}$) of the PtdSer pool was used for decarboxylation to PtdEtn [7]. Likewise, the compartmentalization of PtdEtn in rat liver microsomal membranes was suggested in cross-linking studies with the reagent 1,5-difluoro-2,4-dinitrobenzene [72]. The biosynthesis of PtdEtn from [^{3}H]glycerol or [^{3}H]ethanolamine occurred in a compartment that was accessible to this surface probe. PtdEtn was transferred to another compartment where the lipid was available for cross-linking to protein but was not degraded by phospholipase A_2.

Compartmentalization of other lipids

Cellular pools of diacylglycerol may also be compartmentalized, which is not surprising when the roles of diacylglycerol as a second messenger and as an intermediate in several biosynthetic pathways are considered. For PtdCho synthesis in rat brain microsomes, diacylglycerol newly formed from glycerol-3-phosphate existed in a metabolic pool separate from the bulk of membrane diacylglycerol [6]. Similarly, for PtdCho biosynthesis in rat lung microsomes, diacylglycerols, newly synthesized in vitro from [^{3}H]glycerol-3-phosphate constituted a pool of diacylglycerol separate from the endogenous diacylglycerol pool [58].

The requirement of cholesterol for VLDL secretion was investigated in perfused rat livers [44]. Administration of lovastatin (mevinolin), a hydroxymethylglutaryl-CoA (HMGCoA) reductase inhibitor reduced secretion of VLDL compared with control animals. In addition, the specific radioactivity of cholesterol in secreted VLDL was much higher than in liver, suggesting that a small pool of highly-labeled cholesterol, distinct from the majority of cellular cholesterol, was used for VLDL assembly.

Compartmentalization of pools of triacylglycerols may also occur. In liver perfused with [^{14}C]oleic acid the specific radioactivity of secreted triacylglycerol was substantially greater than that of liver triacylglycerol [37], indicating that newly-synthesized, rather than pre-existing, triacylglycerol was preferred for secretion.

Compartmentalization of water-soluble lipid precursors

Quite surprisingly, there may also be compartmentalization of pools of water-soluble, cytoplasmic molecules, such as phosphoethanolamine, phosphocholine, choline and *S*-adenosylmethionine [23] that are used for phospholipid synthesis. When radioactive ethanolamine was injected into rats, the specific radioactivity of CDP-ethanolamine was twice that of phosphoethanolamine [64]; this was incompatible with the expected precursor-product relationship of these two compounds. Apparently, phosphoethanolamine formed from exogenous ethanolamine was not mixed with the bulk of phosphoethanolamine in the liver, before conversion to CDP-ethanolamine [64]. Similarly, there is evidence for channeling of water-soluble intermediates of PtdCho biosynthesis in cultured glioma cells [24]. Despite the rapid incorporation of [^{3}H]choline into PtdCho in permeabilized cells there was no incorporation of exogenous, radiolabeled phosphocholine or CDP-choline into PtdCho, even though the radiolabeled precursors entered the cells. Moreover, neither phosphocholine nor CDP-choline competed for incorporation of [^{3}H]choline into PtdCho. These provocative data suggest that the individual steps of the CDP-choline pathway for PtdCho biosynthesis are functionally linked, and that the individual enzymes and substrates might be arranged in an organized fashion in the membranes and cytoskeleton. It is sometimes easy to forget that the cytoplasm is not merely a random mixture of enzymes and substrates in solution. Presumably, this putative compartmentalization would be an efficient way of channeling substrates.

5) Regulation of secretion of lipids in lipoproteins

Some clues about the mechanism of assembly of lipids into lipoproteins can be obtained from numerous studies on the effects of different dietary and hormonal conditions. The rate of synthesis and the composition of hepatic VLDL can be altered by diet. In hepatocytes from rats fed a diet rich in sucrose there was an increase in secretion of triacylglycerols and apo E, but a much smaller increase in the secretion of apo B [9,63]. The VLDL produced were thus larger in size and richer in triacylglycerol than VLDL from control hepatocytes. Likewise, in hepatocytes from fasted rats, secretion of all lipoprotein lipids was lower than in cells from fed rats [16,17]. At the

same time, secretion of the low molecular weight form of apo B was decreased by 50 %, whereas secretion of large apo B was unchanged and of apo E was increased 2–4-fold. Thus, there does not appear to be any coordination of triacylglycerol and apoprotein synthesis or of secretion of the invidual apoproteins of VLDL.

The secretion of VLDL is also regulated by the supply of fatty acids and cholesterol. When oleic acid was added to the culture medium of rat hepatocytes, there was a 2–3-fold increase in secretion of triacylglycerol and a smaller increase in secretion of phospholipid[15]. The secretion of free and esterified cholesterol was not significantly affected, nor was the rate of synthesis or secretion of the apoproteins. A similar result was obtained with chicken hepatocytes[50]. In addition, both perfused livers and isolated hepatocytes from cholesterol-fed rats secreted VLDL particles that contained more cholesterol ester, but less triacylglycerol, than control livers[19]. Thus, in rats, diet can alter the neutral lipid composition of secreted VLDL but stimulation of triacylglycerol synthesis and secretion is not coupled to increased apoprotein synthesis or secretion.

In the last few years it has consistently been observed that polyunsaturated fatty acids, particularly those of the (n-3) series such as eicosapentaenoic acid (20:5, n-3), lower plasma levels of triacylglycerol and VLDL. Most experimental evidence supports the hypothesis that (n-3) fatty acids decrease hepatic production of VLDL[57] but the mechanism is not known.

Hormones can cause profound effects on lipoprotein secretion but a thorough review of this topic is beyond the scope of this article. In general terms, glucocorticoid hormones stimulate the secretion of lipids and apoproteins into hepatic VLDL[49,53] (also: P. Martin-Sanz, J. E. Vance and D. N. Brindley, unpublished data). There are conflicting reports on the effects of insulin on this process, but the consensus appears to be that insulin inhibits the secretion of all VLDL components, under most conditions[49,53].

The effects of estrogens on lipoprotein secretion are well established. In chicken hepatocytes[39] and HepG2 cells[68] estrogen treatment stimulated secretion of VLDL apoproteins, and presumably also the lipids, although there is a paucity of information on the latter.

6) Lipids in intestinal lipoproteins

Most research on lipoprotein assembly has concerned the formation of these particles by liver. Approximately 20 % of plasma apoproteins are derived from the intestine[83], which secretes chylomicrons, VLDL and HDL, and contributes more than 50 % of plasma apo Al and apo AlV. The process of lipoprotein assembly in intestinal cells is poorly understood, mainly due to difficulties in isolation of intact enterocytes. There do, however, appear to be similarities between lipoprotein assembly in liver and intestine.

The rate of progress in this area of research should be increased by the availability of a cell line of a colon adenocarcinoma, CaCo-2. These cells retain many characteristic properties of small intestinal mucosal epithelia and synthesize and secrete apoproteins Al, C, E and B[35,71]. Fatty acids increased the secretion of triaclyglycerols into intestinal VLDL[35,55,71], and although there was no effect on the overall rate of apo B secretion, apo B was redistributed from more dense fractions to VLDL[35]. Thus, as in liver, fatty acids increased the secretion of triacylglycerol, but not apo B, into intestinal lipoproteins. Cholesterol feeding also affected secretion of intestinal lipoproteins in a manner similar to that of hepatic lipoproteins. For example, VLDL synthesized by the intestine of cholesterol-fed rats were enriched in cholesterol and cholesterol ester compared to control rats[66]. Similarly, intestinal lipoproteins of monkeys fed a high cholesterol diet had a higher cholesterol ester content than lipoproteins of monkeys on a regular diet[45].

There is little information on the intracellular assembly of intestinal lipoproteins. Nascent VLDL-like particles have been isolated from Golgi of intestinal epithelial cells[67]. The lipoproteins in Golgi from hypercholesterolemic rats contained less triacylglycerol, but more cholesterol ester, than those from control rats[67].

The relative contributions of dietary and endogenously-synthesized PtdCho for intestinal lipoprotein secretion were investigated using molecular species analysis of PtdCho by high performance liquid chromatography[52]. The molecular species of lymph VLDL and HDL were very similar and also closely resembled the composition of the intestinal mucosa. The major molecular species of PtdCho from these sources were 18:0–18:2, 16:0–18:2 and 18:0–20:4. When rats were infused via the duodenum with a mixture of molecular species of [^{14}C]choline-labeled PtdCho, the specific radioactivity of all major molecular species of PtdCho in lymph lipoproteins was greater in VLDL than in HDL[52]. These data suggested that in HDL, compared to VLDL, a greater proportion of PtdCho was derived from endogenous PtdCho. In addition, the molecular species of PtdCho in secreted intestinal lipoproteins reflected the supply of fatty acids in the diet. The authors therefore concluded that PtdCho incorporated into VLDL and HDL originated from different metabolic pools.

7) Conclusion

The literature summarized above clearly indicates that lipids play a crucial role in assembly and secretion of lipoproteins. Of course, it has also been established that VLDL cannot be secreted without apo B. In the inherited disease abetalipoproteinemia there is a defect in the synthesis of apo B and a very low concentration of triacylglycerol and apo B-containing lipoproteins in the plasma, presumably as a result of a defect in VLDL secretion. However, in most instances the synthesis and supply of

apo B does not appear to limit VLDL secretion. It is more likely that apo B is synthesized constitutively (excess apo B may be rapidly degraded) and that the supply of lipid determines the rate of VLDL secretion. Several experimental studies support this hypothesis. For example, in a recent study, the secretion of apo B by Hep G2 cells was varied over a 7-fold range by incubation with oleate, insulin or albumin. However, no changes in apo B mRNA levels were detected under any of the conditions that changed the output of apo B[53a]. In addition, an increased supply of either fatty acids or sucrose to the liver increased the rate of secretion of triacylglycerol, whilst there was little change in the rate of apo B synthesis and secretion[9,15–17,50,63]. Similarly, in hypercholesterolemic animals there was increased secretion of cholesterol esters into VLDL without a corresponding increase in apoprotein secretion[19]. In fact, the core composition of VLDL is dictated by the composition of the lipids available to the cells. These data imply that when lipid supply is plentiful, larger VLDL particles are secreted containing relatively less apo B and relatively more neutral lipid. In addition, in choline-deficient rat hepatocytes where the supply of PtdCho is limited by the lack of choline, secretion of all VLDL lipid and apoprotein components is markedly reduced and triacylglycerol accumulates in the hepatocytes[85]. Moreover, when hepatic cholesterol biosynthesis is inhibited by the HMGCoA reductase inhibitor mevinolin, VLDL secretion is also decreased[44].

The time course for secretion of apo B is not the same as for other secretory proteins, such as albumin[10,38]. Presumably the extra time required for secretion of apo B compared with albumin is dependent upon the association of apo B with lipids, somewhere along the secretory route from ER to Golgi to plasma membrane. According to some studies[5,12], the slow step in secretion of apo B is transit through the Golgi, implying that some important processing, for example the addition of lipid, occurs there. In other studies[10] however, the rate-limiting step in VLDL secretion is movement of apo B out of the ER, as for most secretory proteins. The additional time taken for apo B to move from ER to Golgi, compared with albumin, is proposed to be the result of the time taken for apo B to associate with lipid. The apparent discrepancy over which step is rate-limiting in the intracellular assembly of VLDL needs to be resolved.

The basic pathway of hepatic VLDL assembly proposed by Havel and co-workers[1] is still generally accepted. That is, apo B is synthesized on the rough ER and associates with lipids (that are also synthesized on the ER) to form a pre-VLDL particle in the ER lumen. The composition of the lipoprotein particle at this stage is still not established. The particle is directed from the ER to, and through, the Golgi, presumably via a vesicle transport mechanism. In Golgi there is processing of apo B (glycosylation) and probably addition of more lipid. From the *trans*-Golgi, secretory vesicles are formed containing nascent VLDL. These vesicles move to, and fuse with, the plasma membrane, releasing their luminal contents (i.e. nascent VLDL) from the hepatocyte.

From recent information summarized above, a more detailed scheme for assembly of VLDL may now be proposed. In this model, apo B, an unusually large and hydrophobic protein, is made on ribosomes attached to the rough ER and during synthesis becomes integrated into the ER membrane. Triacylglycerols, not usually major components of lipid bilayers, are synthesized at the cytosolic surface of the ER and accumulate in 'droplets' at the hydrophobic interface between the two leaflets of the bilayer. Since apo B is hydrophobic it may begin to associate with triacylglycerol at this stage. The non-polar apo B and triacylglycerol cannot enter the aqueous luminal compartment without stabilization by PtdCho and cholesterol. These lipids may be supplied to the triacylglycerol-apo B droplet by expansion or 'budding' from the inner leaflet of the bilayer. Hence, the active synthesis of new phospholipids[85] and cholesterol[44] may be the driving force required for assembly of completed nascent VLDL and their movement into the lumen.

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Lipid transport pathways in mammalian cells

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Summary. A major deficit in our understanding of membrane biogenesis in eukaryotes is the definition of mechanisms by which the lipid constituents of cell membranes are transported from their sites of intracellular synthesis to the multiplicity of membranes that constitute a typical cell. A variety of approaches have been used to examine the transport of lipids to different organelles. In many cases the development of new methods has been necessary to study the problem. These methods include cytological examination of cells labeled with fluorescent lipid analogs, improved methods of subcellular fractionation, in situ enzymology that demonstrates lipid translocation by changes in lipid structure, and cell-free reconstitution with isolated organelles. Several general patterns of lipid transport have emerged but there does not appear to be a unifying mechanism by which lipids move among different organelles. Significant evidence now exists for vesicular and metabolic energy-dependent mechanisms as well as mechanisms that are clearly independent of cellular ATP content.
Key words. Lipids; membranes; transport; organelles; vesicles.

The major structural components of cell membranes are phospholipids, sphingolipids and cholesterol. Analysis of the total lipid content of an average nucleated mammalian cell reveals a composition of 45–55% phosphatidylcholine, 15–25% phosphatidylethanolamine, 10–15% phosphatidylinositol, 5–10% phosphatidyl-