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LIPOPROTEIN ASSEMBLY AND SECRETION BY HEPATOCYTES

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PERSPECTIVES AND OVERVIEW

Considerable interest in lipoprotein metabolism has arisen over the past few decades because of the causal relationship between high levels of serum cholesterol and cardiovascular disease. The main culprit is low density

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lipoprotein (LDL), which carries the major portion of cholesterol, as cholesterol ester, in human blood. Conversely, high levels of high density lipoprotein (HDL), which is also enriched in cholesterol ester, appear to lower the risk of heart attack or stroke. Significant progress has been made in understanding the metabolism of lipoproteins, and at the current pace we can expect an even more rapid elucidation of the intricacies of lipoprotein metabolism and its regulation.

The present review is restricted to the assembly and secretion of hepatic lipoproteins. Several recent reviews cover the impressive progress that has been made in understanding the structure of the apoproteins, the structure and expression of the genes that code for these apoproteins, the chromosomal location of these genes, and genetic regulation of apoprotein synthesis (8, 47). Lipoproteins are largely secreted from the liver, although the intestine is the only source of chylomicrons (85) and is a contributor to plasma HDL (24). Space limitations preclude a review of the literature on lipoprotein secretion from the intestine (see 82). Some apolipoproteins, particularly apo E, are secreted by other tissues (e.g. brain, kidney, spleen) and cells as well as the liver (51). Exactly how important these other tissues are to plasma lipoprotein metabolism is still under investigation (51). It is also beyond the scope of this article to review lipoprotein metabolism in the circulatory system and receptor-mediated uptake of lipoproteins (see Ref. 9).

Lipoprotein assembly and secretion from liver has been studied in intact animals, perfused livers, and cultured hepatocytes. The perfused liver system continues to be a useful model (34). For many experiments cultured primary hepatocytes offer certain advantages as previously noted (20). The cells from a single animal's liver can be investigated under a variety of conditions in a chemically defined medium. Primary rat hepatocytes continue to display many of the functions of liver, including secretion of lipoproteins; the uptake of newly secreted very low density lipoprotein (VLDL) is negligible (20). However, the rat is a poor model for studying some aspects of human lipoprotein metabolism because this rodent has low levels of LDL and is resistant to atherosclerosis. Yet, because it is difficult to induce cardiovascular disease, the rat is an interesting animal for comparison with human and primate lipoprotein metabolism. Recently, a human liver tumor-derived cell line, HepG2, has been utilized for studies on lipoprotein secretion (74, 75). Two other human liver-derived cell lines, Hep3B and NPLC, secrete significant amounts of lipoproteins and may be useful for future investigations (29).

The general scheme for lipoprotein assembly and secretion is well accepted (1, 27, 82). Initial assembly occurs on the endoplasmic reticulum (ER) where apoproteins, cholesterol, phospholipids, and triacylglycerols are made. Transport vesicles carry the nascent VLDL to the Golgi where modification of the oligosaccharide components of the apoproteins occurs and some exchange

of phospholipids probably takes place (41). The Golgi also has the capacity to synthesize the major phospholipids found in lipoproteins and may contribute these lipids to the nascent lipoproteins in the lumen (81). Secretory vesicles carry the lipoproteins to the cell surface, fuse with the plasma membrane, and release the lipoproteins into the space of Disse and subsequently into the plasma. It is not clear whether the rate-limiting step in VLDL secretion is movement out of the ER or transit through the Golgi. Although HDL secretion may follow a route similar to that of VLDL, such a process is not well documented.

The influence of diet and hormones on lipoprotein secretion is complex; nevertheless, a few generalizations can be made. (*a*) Fatty acids (saturated and monounsaturated) stimulate the secretion of triacylglycerol in VLDL, but in most studies they do not have a major effect on the amount of apo B secreted or synthesized. Because there is only one apo B molecule per VLDL particle (25), the increase in triacylglycerol content relative to apoprotein results in larger particles. (*b*) When certain polyunsaturated fatty acids (e.g. eicosapentaenoic acid) are substituted for saturated fatty acids, the stimulation of VLDL secretion is not observed. (*c*) Inhibition of phosphatidylcholine (PtdCho) biosynthesis decreases the secretion of VLDL, but not of HDL, by rat hepatocytes. (*d*) Inhibition of cholesterol biosynthesis also results in reduced secretion of VLDL. These data suggest that the active synthesis of both PtdCho and cholesterol is required for assembly of VLDL particles within the cell. (*e*) Fasting decreases VLDL secretion (of both lipid and apo B moieties), and a carbohydrate-rich diet stimulates the secretion of triacylglycerol in VLDL. (*f*) Hormonal effects are complex, but generally insulin inhibits, whereas the synthetic glucocorticoid hormone dexamethasone stimulates, VLDL secretion from cultured hepatocytes.

Recently studies have revealed that the liver and cultured rat hepatocytes secrete lysophospholipids (62). This secretion is strikingly stimulated by albumin or other acceptors of lysophospholipids in the medium (61).

KINETICS OF LIPOPROTEIN ASSEMBLY AND SECRETION

The time course for assembly and secretion of VLDL has been studied in several different laboratories with conflicting conclusions. In cultured hepatocytes from estrogen-treated chickens Lane and co-workers (67) demonstrated that 10 min were required for translation of apo B, the major protein of VLDL, and another 20–25 min for the secretion of newly made VLDL. Chick hepatocytes were pulse labeled for 2.5 min with [^{3}H]leucine and [^{3}H]glycerol, and the time course for secretion of ^{3}H-labeled apoproteins and glycerolipids was determined (43). The [^{3}H]triacylglycerol and ^{3}H-labeled apoproteins were secreted in parallel and appeared in the medium 30–40 min after the

pulse. In contrast, phospholipid secretion showed a characteristic biphasic pattern. The data suggested that triacylglycerol, and some phospholipid, were assembled with apoprotein early in the secretory pathway (probably in the ER) and that additional phospholipid associated with the VLDL particles just prior to secretion (probably in the Golgi). In another study, ER and Golgi fractions were isolated from estrogen-treated chick hepatocytes after they had been pulse labeled with [^{3}H]leucine (3). The time required for transport of the apoproteins through the ER and Golgi compartments was calculated. In contrast to current models of protein secretion (49), the rate-limiting step for secretion of VLDL apoproteins from the cells was transport through the Golgi, not the ER. A similar conclusion was reached in a study of HepG2 cells (7) in which the time required for the synthesis of apo B was estimated to be 14 min, for the transfer of apo B from ER to Golgi was estimated to be 10 min, and for the transfer of the protein through the Golgi to the extracellular space was estimated to be 20 min. Thus, movement through the Golgi was the slow step in secretion of apo B by these cells. In both of these studies, a large proportion of apo B in the Golgi was apparently associated with the membranes. The reason for this association cannot be readily rationalized.

In contrast, in cultured rat hepatocytes the rate-limiting step in VLDL secretion was identified as movement of apo B-containing particles out of the ER, not out of the Golgi (6). The rate of apo B secretion was one half that of albumin, suggesting that a unique processing of apo B (perhaps its association with lipid) was required in the ER for VLDL assembly and secretion.

The reasons for the different conclusions from these experiments have not yet been explained but may be due to the different experimental systems used and technical difficulties involved in these types of experiments. For example, the lipoproteins secreted by HepG2 cells are different from classical VLDL secreted by normal human or rat liver. HepG2 cells secrete mainly an LDL-sized particle containing a triacylglycerol-rich core and apo B. These cells have a greatly reduced amount of smooth ER, the major site of lipid synthesis. Inadequate amounts of ER may result in the defective assembly of lipoprotein in the ER and a shorter residence time in this organelle than in the organelles of normal liver cells (60).

ASSEMBLY OF LIPIDS INTO LIPOPROTEINS

The Roles of the Golgi and the Endoplasmic Reticulum

Synthesis of the major lipids of hepatic lipoproteins (cholesterol, cholesterol esters, triacylglycerols, and phospholipids) is generally assumed to occur primarily in the endoplasmic reticulum (ER). However, many of the enzymes of phospholipid and triacylglycerol biosynthesis have been identified in Golgi, as well as in ER, membranes (35, 44, 81). Thus, both organelles potential-

ly may be able to provide lipids for assembly into VLDL, although the quantitative contribution of these two organelles to the lipid moieties of VLDL has not been established. The above-mentioned study in chick hepatocytes (43) suggested that triacylglycerols are assembled with apo B in the ER, whereas at least some of the phospholipids [especially the "minor" phospholipids phosphatidylethanolamine (PtdEtn), phosphatidylinositol, and phosphatidylserine (PtdSer)] are added to the VLDL in the Golgi. Additional evidence that some phospholipids of VLDL were added in the Golgi was provided by a study in rat liver (36). Lipoprotein particles were isolated from the lumina of the ER and Golgi. The lipoproteins in the ER lumen were smaller than those in the Golgi and, compared with the Golgi lipoproteins, were highly enriched in triacylglycerols relative to phospholipid. The authors suggested that the majority of phospholipid was not added to the VLDL until the particles reached the Golgi and that the initial assembly of VLDL in the ER involved aggregation of triacylglycerol and apoprotein, with only a very small complement of phospholipid. How these very hydrophobic triacylglycerol-apo B particles would be stable in the aqueous lumen of the ER was not explained. Relevant to these studies may be the apparently rapid exchange of phospholipid between the luminal content lipoproteins and the membranes of the Golgi (41).

Several problems are inherent in studying nascent lipoproteins in Golgi fractions. First, the quantities are small. Second, it is difficult to isolate the luminal contents free from membrane contamination; and third, there is considerable contamination of many Golgi preparations by multivesicular bodies that contain the same lipids and apoproteins that are present in nascent VLDL (40).

Specific Pools of Lipids are Used for Lipoprotein Assembly

Studies show that specific intracellular pools of lipids are preferentially used, or even required, for assembly into hepatic lipoproteins. Cultured rat hepatocytes were incubated with either [^{3}H]choline, [^{3}H]ethanolamine, or [^{3}H]serine as precursors of PtdCho and PtdEtn (80). (PtdCho is made in liver from both the CDP-choline and PtdEtn methylation pathways, whereas PtdEtn is made from the CDP-ethanolamine pathway and also from the decarboxylation of PtdSer.) VLDL and HDL secreted into the culture medium were separated by ultracentrifugation, and the specific radioactivities (dpm/nmol) of PtdCho and PtdEtn were compared in the cells and lipoproteins. For incubations with [^{3}H]choline the specific radioactivity of PtdCho was approximately the same in the cells and the lipoproteins. For incubations with [^{3}H]ethanolamine, the specific radioactivity of PtdEtn and PtdCho (synthesized via the methylation of PtdEtn) was several-fold lower in the lipoproteins than in the hepatocytes. Surprisingly, from [^{3}H]serine the specific

radioactivities of both PtdEtn (made from PtdSer decarboxylation) and PtdCho (made by methylation of PtdEtn) were higher in the secreted lipoproteins than in the cells. These data implied that phospholipids assembled into lipoproteins were not derived from a single, uniformly labeled cellular pool; rather, a preference was given to phospholipids derived from certain biosynthetic routes (i.e. from PtdSer) versus phospholipids synthesized by other pathways (i.e. from ethanolamine).

The role of the PtdEtn methylation pathway in supplying PtdCho for lipoprotein assembly was investigated in cultured hepatocytes that were incubated with the methylation inhibitor 3-deazaadenosine (78). Because liver was the only tissue with a quantitatively significant methylation pathway for PtdCho biosynthesis (approximately 20–40% of hepatic PtdCho is thought to be synthesized by this route) and because liver was a major producer of lipoproteins (85), it was hypothesized that the methylation pathway may have been the source of the PtdCho used for VLDL assembly. Although deazaadenosine inhibited the cellular methylation of PtdEtn to PtdCho, no diminution of secretion of [^{3}H]leucine-labeled apoproteins or phospholipid mass occurred (78). Apparently the methylation pathway was not required for VLDL secretion. In addition, although the cellular methylation of both ethanolamine- and serine-derived PtdEtn was inhibited by deazaadenosine (by approximately 95 and 75% respectively), and the secretion of ethanolamine-labeled PtdCho was blocked, enigmatically the secretion of PtdCho labeled from serine was almost unaffected (79). These data indicated the possible existence of a unique PtdEtn methyltransferase involved in the production of PtdCho used for assembly into lipoproteins; this methyltransferase was believed to use specifically a pool of PtdEtn derived from PtdSer as a substrate.

Further compartmentalization of phospholipids used for lipoprotein assembly has been observed. High performance liquid chromatography and radiolabeled phospholipid precursors in combination were used to compare the molecular species composition of phospholipids of lipoproteins with that of the hepatocytes from which the lipoproteins were secreted. The percent distribution of molecular species of PtdEtn and PtdCho derived from ethanolamine was the same in cells and lipoproteins. In contrast, the percent distribution of molecular species of PtdCho labeled from choline, and PtdEtn and PtdCho labeled from serine, was different in the cells and in the medium. Discrimination against secretion of the 1-stearoyl-2-arachidonoyl species of both PtdCho and PtdEtn was evident (76). The selective secretion of certain molecular species of phospholipids is probably due to the fact that secreted phospholipids were derived from a pool of newly synthesized phospholipids, whereas the percent distribution of molecular species of the phospholipids remaining in the cells was modified by rapid deacylation-reacylation or by selective degradation of certain species (77). Thus, there appears to be a

preference for secretion of phospholipids derived from certain biosynthetic routes, as well as a preference for newly made phospholipids.

In support of the idea that new synthesis of phospholipids may be linked to lipoprotein secretion, the active synthesis of PtdCho, by either the CDP-choline or PtdEtn methylation pathways, is apparently required for secretion of VLDL by cultured rat hepatocytes (87). Hepatocytes were prepared from choline-deficient rats and were cultured in the presence or absence of choline and/or methionine. In the choline- and methionine-deficient cells (in which there was decreased synthesis of PtdCho by both the CDP-choline and methylation pathways), the level of secretion of all VLDL lipids and apoproteins was approximately 70% lower than the level found in the choline-supplemented cells. The secretion of HDL lipids and apoproteins was unaffected in the choline-deficient hepatocytes. Thus, new synthesis of PtdCho was required for VLDL secretion; PtdCho already present in the hepatocyte membranes was not sufficient. The choline moiety was specifically required as the head group of the secreted phospholipid because the secretion defect was not remedied when choline-deficient cells were supplemented with ethanolamine, monomethylethanolamine, or dimethylethanolamine instead of choline (88).

In addition to compartmentalization of phospholipids for lipoprotein assembly, specific pools of cholesterol and triacylglycerols may also be used. Lovastatin (mevinolin), an inhibitor of hydroxymethylglutaryl CoA reductase (the rate-limiting enzyme of cholesterol biosynthesis), was fed to rats (45). Both the biosynthesis of cholesterol and the secretion of all VLDL lipids into a liver perfusate were greatly reduced compared to levels found in control animals. In addition, the specific radioactivity of cholesterol was higher in the VLDL than in the liver (45). These data indicated that active synthesis of cholesterol was required for VLDL secretion and that newly synthesized, rather than preexisting, cholesterol was preferentially used. Similarly, newly made, rather than preexisting, hepatic triacylglycerol may be preferred for secretion with VLDL (42).

All available evidence suggests that specific pools of phospholipids, and probably also triacylglycerols and cholesterol, are used for assembly into hepatic VLDL. In particular, newly made, rather than preexisting, pools of lipids may be used for this purpose. An intriguing hypothesis is that synthesis of new lipid, especially PtdCho, may be coordinated with extrusion of the hydrophobic apo B into the aqueous lumen of the ER or Golgi during formation of nascent VLDL particles.

REGULATION OF LIPOPROTEIN SECRETION

The majority of studies on hepatic lipoprotein secretion have been concerned with VLDL only, although HDL particles are also secreted in smaller amounts

by the liver. The exact nature and composition of nascent HDL are not firmly established (84). Most likely these particles are secreted as a heterogeneous population containing triacylglycerol, cholesterol, and some apoprotein; additional apo AI and lipid moieties, such as cholesterol and phospholipids, probably associate with the particles in the plasma. The few studies on the mechanism of HDL secretion that are available illustrate the different effects of experimental manipulations on the secretion of VLDL and HDL. For example, in the studies with choline-deficient rat hepatocytes (87) VLDL, but not HDL, secretion was inhibited. In another study (33), perfused livers of orotic acid-fed rats secreted almost no VLDL but normal amounts of HDL. Evidence also indicates that the secretion of VLDL and HDL from cultured rat hepatocytes is differently influenced by treatment with hormones such as dexamethasone (a synthetic glucocorticoid hormone) and insulin (P. Martin-Sanz, J. E. Vance, and D. N. Brindley, unpublished data). Thus, it is becoming apparent that VLDL and HDL may be assembled and secreted by different mechanisms.

Effects of Fatty Acids

The secretion of VLDL is affected by the exogenous supply of fatty acids. When 1 mM of oleic, linoleic, linolenic, myristic, or palmitic acid bound to albumin was added to the culture medium of rat hepatocytes, a rapid two- to threefold increase in the secretion of triacylglycerol was observed and a smaller increase in the secretion of phospholipid (16). The secretion of free and esterified cholesterol was not significantly affected. Oleic acid, however, did not affect the rate of synthesis or secretion of the apoproteins. A similar result was obtained with chicken hepatocytes (55). In neither of these studies was the stimulation of triacylglycerol synthesis and secretion by fatty acids coupled to an increase in apoprotein secretion. However, the effects of exogenous fatty acid supply on the synthesis and secretion of VLDL apoproteins are not straightforward. When oleic acid was added to the medium used to perfuse livers from fed rats (65), secretion of triacylglycerols and apoproteins C and E increased, but there was no effect on the secretion of apoproteins B. In the same report, where oleic acid was added to the medium used to perfuse livers from fasted rats, the secretion of all apoproteins and lipids increased. In another study, when oleate (0.8 mM) was added to the culture medium of HepG2 cells (60) the secretion of apo B increased threefold, but there was no change in the level of apo B mRNA. The reasons for the apparent discrepancies are not clear. One possible difference in the experiments may be the fatty acid concentrations used. Pullinger et al (60) have investigated the dependence of apo B secretion on the oleic acid concentration in the culture medium of HepG2 cells. The maximum stimulation of apo B secretion occurred with 0.1 M oleate; with higher concentrations of oleate,

there was less stimulation of apo B secretion. In addition, Patsch et al (58) have suggested that in some cases there may not be an increase in the total amount of apo B secreted. Rather, the additional supply of fatty acids, which causes increased synthesis and secretion of triacylglycerols, may increase the size of the lipoprotein particles. Thus, there may be a redistribution in the sizes of the particles secreted, so that normally higher density particles are shifted to the VLDL density fraction. In no instance have researchers observed an increase in the rate of synthesis of apo B in response to increased lipid availability. Thus, the apo B gene appears be expressed constitutively. Whether or not the supply of lipid alters the rate of apo B secretion remains controversial.

Oleic acid clearly stimulates VLDL secretion in perfused rat liver (65), isolated rat (16) and chicken (55) hepatocytes and HepG2 cells (60), and the stimulation by oleic acid is greater than that with palmitic acid (16). However, an apparent anomaly arises from these studies because even though oleic acid stimulates VLDL secretion to a greater extent than does palmitic acid, the plasma lipoprotein and cholesterol levels of humans fed a diet rich in oleic acid were lower than in subjects fed a diet rich in palmitic acid (4a). Interestingly, subjects fed a diet rich in stearic acid had LDL cholesterol levels 21% lower than those on the high palmitic acid diet (4a). The authors concluded that stearic acid and oleic acid each lowered the plasma LDL cholesterol level (by 21% and 15% respectively) when either replaced palmitic acid in the diet. Moreover, human subjects were fed either a low fat diet or a diet rich in monounsaturated or saturated fatty acids. Subjects that were fed the high monounsaturated fat diet and the low fat diet had plasma LDL cholesterol levels that were 21% and 15% lower, respectively, than levels found in those subjects fed the high saturated fatty acid diet (31a).

An increased endogenous supply of fatty acids in rat liver was achieved by reduction of the rate of β-oxidation of long-chain fatty acids. Addition of the β-oxidation inhibitor 2-tetradecylglycidate to the perfusion medium increased by approximately fivefold the secretion of both triacylglycerol and cholesterol into VLDL (42). The effects on apoprotein secretion were not studied. Thus, the supply of both endogenous and exogenous fatty acids is an important determinant of the rate of production of hepatic VLDL.

There has been a great deal of interest in the last few years in polyunsaturated fatty acids, particularly those of the (n-3) series such as eicosapentaenoic acid (20:5, n-3), because they appear to lower plasma levels of triacylglycerol and VLDL. Most experimental evidence supports the hypothesis that (n-3) fatty acids decrease the hepatic production of VLDL. In lipoprotein turnover studies in humans, these (n-3) fatty acids reduced the secretion of apo B in VLDL (56). In addition, both triacylglycerol synthesis and secretion into VLDL were increased several fold by the addition of 0.6 mM saturated and monounsaturated fatty acids (such as palmitic, stearic, and oleic acids) to

the medium of cultured rat hepatocytes (64). Cells incubated with the same concentration of eicosapentaenoic acid showed no increase in triacylglycerol synthesis or secretion (64). That production of triacylglycerol is lower in the presence of eicosapentaenoic acid than in the presence of other fatty acids may be ascribed to inhibition of the enzyme acyl-CoA:1,2-diacylglycerol acyltransferase (64), which is involved in triacylglycerol synthesis. However, no significant differences in the activity of phosphatidate phosphohydrolase (possibly the rate-limiting enzyme in triacylglycerol biosynthesis under many conditions) were observed in the presence of eicosapentaenoic acid versus in the presence of other fatty acids (39).

An interesting recent finding is that apo B, newly synthesized and secreted by HepG2 cells, is acylated covalently with palmitate and stearate (37), in contrast to circulating apo B in low density lipoproteins, which is not acylated. Similarly, apo AI secreted by HepG2 cells was also acylated by palmitate (38). The authors speculate that acylation of apo B may play an important, but as yet undefined, role in lipoprotein secretion.

Effects of Cholesterol

Most recent studies have shown that cholesterol feeding elevates plasma cholesterol levels. Perfused livers (32) from cholesterol-fed guinea pigs secreted VLDL particles that contained more cholesterol ester, but less triacylglycerol, than was found in VLDL from control livers. In addition, there was an increased secretion of cholesterol-rich discoidal HDL particles, whose apo E content was increased nearly 25-fold over that from normal guinea pigs. Similarly, isolated hepatocytes from cholesterol-fed rats secreted VLDL that contained 30% less triacylglycerol, but 4 times more cholesterol ester, than did control hepatocytes (22). The mole ratio of these two core lipids in secreted VLDL varied as a linear function of the same mole ratio of these lipids in the hepatocytes. These data suggest that animals can alter the neutral lipid composition of the secreted VLDL in response to the supply of lipids in the diet and that the neutral lipid content of the hepatocyte determines the neutral lipid composition of nascent VLDL.

Studies on the effects of dietary cholesterol on the secretion of apoproteins have produced apparently conflicting observations. In cultured rat hepatocytes the rate of secretion of apo B and apo E into VLDL was unaffected by the presence of cholesterol in the culture medium (21). Yet a recent study (30) showed that exposure of HepG2 cells to cholesterol, which increased the intracellular cholesterol level, increased the secretion of apo B and at the same time reduced the receptor-mediated uptake of LDL.

A pertinent question concerning the regulation of lipoprotein secretion is, do circulating lipoproteins affect the hepatocytes' ability to secrete lipoproteins? In particular, does cholesterol supplied by exogenous lipoproteins

affect hepatic lipoprotein secretion? When rat liver was perfused with cholesterol- and apo E-rich lipoprotein (HDLc), the hepatic cholesterol content increased, and a fivefold increase was observed in the secretion of the lipid components of VLDL, i.e. triacylglycerols, phospholipids, cholesterol, and cholesterol esters, (72). Although the individual apoproteins of VLDL were not separated, the total amount of VLDL proteins secreted showed a comparable increase.

The regulation of apoprotein synthesis and secretion in HepG2 cells by exogenously added chylomicron remnants and β-VLDL has been studied (11). Chylomicron remnants are rich both in cholesterol and triacylglycerol and are thus a source of fatty acids, as well as cholesterol. In contrast, β-VLDL particles are rich in cholesterol but have a low triacylglycerol content. Hepatoma cells were incubated with either chylomicrons or β-VLDL for 24 h and then were incubated for another 24 h with medium containing [^{35}S]methionine and either of the lipoproteins. Chylomicron remnants and β-VLDL caused striking increases (14- and 7-fold, respectively) in secretion of VLDL apoproteins, with an unchanged ratio of apo B/E (11). The data support the hypothesis that apoprotein secretion is dependent on the supply of cholesterol. It is noteworthy that in both experiments using added lipoproteins as a source of exogenous cholesterol, the added lipoproteins are also a source of phospholipid. Consequently, it would be interesting to know if these phospholipids, exogenously added in the form of lipoproteins, would meet the cells' requirement of PtdCho for lipoprotein secretion in a situation (e.g. in choline-deficiency) where endogenous PtdCho synthesis was depressed. Although preexisting, cellular PtdCho cannot substitute for newly made PtdCho in choline-deficient hepatocytes (87), a pool of PtdCho added exogenously in the form of lipoproteins might be used for VLDL assembly.

Effects of Carbohydrate and Fasting

The rate of synthesis and the composition of hepatic VLDL can be altered by diet, as has already been noted in studies of fatty acid and cholesterol feeding. In hepatocytes from rats fed a diet rich in sucrose an increase occurred in secretion of triacylglycerols and apo E, but not of apo B, into VLDL (5, 73). The VLDL particles produced were larger in size and triacylglycerol-rich compared to VLDL from control hepatocytes. The enhanced production of hepatocyte apo E in the sucrose-fed rats was ascribed in part to increased transcription of the apo E gene (73), although the mechanism of the modulation is not known. In a similar study with perfused livers of sucrose-fed rats, the rates of hepatic triacylglycerol synthesis and secretion were also higher than the rates for control rats (86). The authors concluded that alterations in the processing of free fatty acids by the liver after sucrose feeding (i.e. an increase in the esterification:oxidation ratio) resulted in increased triacyl-

glycerol secretion. The effect of sucrose feeding on the secretion of apoproteins was not determined (86).

When hepatocytes were prepared from fasted rats, the level of secretion of all lipoprotein lipids was lower than that found in cells from fed rats (17). At the same time, the secretion of the low molecular weight form of apo B decreased by 50%, whereas the secretion of large apo B was unchanged and that of apo E increased two- to fourfold. The increased secretion of apo E correlated with a twofold increase in the level of translatable apo E mRNA. (19, 46). The major portion of the apo E secreted from cells of both control and fasted rats was not associated with lipid. Thus, apo E secretion was regulated independently of the secretion of the other VLDL components. Indeed, it is noteworthy that synthesis and secretion of the lipids and the individual apoproteins appear to be independently regulated.

Effects of Hormones

INSULIN There have been numerous studies on the effects of insulin on lipoprotein secretion, with differing results. Insulin is generally believed to stimulate de novo fatty acid synthesis and to inhibit fatty acid oxidation; both of these processes may stimulate the secretion of VLDL triacylglycerol. Difficulties in interpreting experiments on the effects of insulin often arise because of uncertainty about the hormonal status of the animals prior to the insulin treatment, the time frame during which the hormones were given, the use of isolated hepatocytes or perfused liver, and the age of the hepatocyte cultures (4). Under most conditions, at least in short-term studies, insulin inhibits the secretion of VLDL apoproteins and lipids by cultures of rat hepatocytes (57, 59, 68) and HepG2 cells (13, 60). In two studies (46, 52), no change was observed in the mRNA level of apo E in cells treated with insulin. Nor was the apo B mRNA level altered by incubation of HepG2 cells with insulin, even when the amount of apo B secreted was reduced by one half (60). In contrast, the output of apo AI by cultured rat hepatocytes was unaffected by insulin treatment (48), although surprisingly the levels of mRNA for apo AI and apo AIV in hepatocytes incubated with insulin were seven- and twofold greater, respectively, than in control cells (26).

The effects of developmental stage of animals on lipoprotein secretion have been investigated in hepatocytes prepared from fetal, 6-day old, and adult rats (10), whose capacity to synthesize and secrete triacylglycerol labeled from [^{3}H]glycerol followed the order: adult > 6-day old > fetal. In order to determine whether the differences in triacylglycerol secretion were associated with differences in apo B metabolism, the amounts of secreted low and high molecular weight forms of apo B were measured after immunoprecipitation. The ability of the hepatocytes to secrete VLDL paralleled their ability to synthesize and secrete small, but not large, apo B. In other studies, the levels

of mRNA for apoproteins AI, AIV, and E in rat liver were measured in relationship to the stage of development (26, 52). A sudden increase in the mRNA level for apo E in rat liver occurred immediately after birth (52) and was accompanied by a dramatic increase in the plasma glucagon level and a decrease in the insulin/glucagon ratio. Thus, it appears that apo E gene expression is regulated by plasma glucagon levels or the glucagon/insulin balance.

In general agreement with these data, administration of dibutyryl cAMP to rats resulted in an increase in the mRNA level for apo E in liver (52). However, the effect of cAMP on apoprotein synthesis is not clear, since in rat hepatocytes the cAMP analogue slightly decreased, rather than increased, apo E synthesis and mRNA levels (46).

A possible role of phosphorylation of apo B in the regulation of lipoprotein secretion by hepatocytes has been suggested. Rat hepatocytes were incubated with [^{32}P]phosphate, and apo B (both large and small forms) was immunoprecipitated from the cells and medium. The small molecular weight form of apo B was observed to be phosphorylated on a serine residue, whereas large apo B was unphosphorylated (18). The presence of phosphorylated small, but not large, apo B was confirmed in the in vivo state in rat liver (18).

Slightly different results were obtained in another study (70), probably as a result of the different metabolic states of the rats and the different amounts of insulin in the culture medium in the two experiments. Both large and small forms of apo B in the culture medium of rat hepatocytes were found to be phosphorylated (70). In lipoproteins secreted by hepatocytes of diabetic rats the specific radioactivity of ^{32}P-labeled apo B was between 18- and 31-fold greater than that from control hepatocytes. The increase in ^{32}P-label in the apo B secreted from diabetic rat hepatocytes was mainly due to an increase in phosphorylation of tyrosine residues, in addition to an increase in phosphorylation of serine residues. The role of apo B phosphorylation is not understood, but it may possibly play a role in the intracellular transport of apo B during VLDL assembly and secretion.

GLUCOCORTICOIDS Glucocorticoid hormones stimulate the secretion of hepatic VLDL. When isolated rat hepatocytes were preincubated for 19 h with 1 μM dexamethasone, and the incubation was continued for another 23 h with dexamethasone and a radiolabeled precursor of glycerolipids, no significant change was observed in the rate of triacylglycerol synthesis (53). A two- to fourfold increase in secretion of radiolabeled triacylglycerol, and a smaller, but significant, increase in secretion of radiolabeled PtdCho, were noted, however. Incubation of cultured rat hepatocytes with dexamethasone caused a four- to eightfold increase in the secretion of [^{3}H]leucine-labeled VLDL apoproteins (B large and small, E and C) with a smaller increase in apo AI

secretion, while incorporation of [^{3}H]leucine into total cellular proteins was slightly reduced (P. Martin-Sanz, J. E. Vance, D. N. Brindley, unpublished data). Conversely, in HepG2 cells 10^{-6} M dexamethasone did not affect apo B secretion (60). Similarly, Sparks and co-workers observed no stimulation of apo B secretion from hepatocytes incubated for 12 h with 10^{-7}M cortisol, a natural glucocorticoid (68, 69). However, since hepatocytes have the capacity to degrade this natural glucocorticoid, the effective hormone concentration may have been well below physiological levels. In other studies, dexamethasone (10^{-7}M) increased the mRNA levels of apo AIV of rat hepatocytes by fourfold and of apo AI by twofold (26) but had no effect on the amount of apo E mRNA (26, 46). Interestingly, a synergistic increase of VLDL secretion is noted when low levels of both insulin and dexamethasone are added simultaneously to cultured rat hepatocytes (26; P. Martin-Sanz, J. E. Vance, D. N. Brindley, unpublished data).

The consensus of most studies on the effects of insulin and glucocorticoids on VLDL secretion by cultured rat hepatocytes is that (*a*) glucocorticoids stimulate secretion of VLDL apoproteins, triacylglycerols, phospholipids, cholesterol (59), and cholesterol esters (59), (*b*) insulin inhibits the secretion of VLDL apoproteins, triacylglycerols, phospholipids, cholesterol, and cholesterol esters, and (*c*) the interrelationship among the effects of insulin and other hormones on lipoprotein secretion is complex.

THYROID HORMONES The liver of a hypothyroid rat, compared to that of a normal rat, secretes decreased amounts of triacylglycerols in the form of VLDL (23). The regulation of apo B gene expression in rat liver, under conditions of altered thyroid hormone status, has been studied (15). When pharmacological doses of 3,5,3'-triiodothyronine were given to rats the synthesis of large apo B was suppressed, but no effect on the level of small apo B was noted. Interestingly, thyroid hormone treatment increased the proportion of hepatic apo B mRNA containing a stop codon at the position that would be expected to result in the production of the small, rather than the large, form of apo B (15). In another study, the rate of synthesis of hepatic apo E increased twofold in hypothyroid rats and decreased sevenfold in hyperthyroid animals, although the mRNA abundance was the same in all instances (14).

ESTROGENS Numerous studies have demonstrated that estrogen hormones stimulate the secretion of lipoproteins. For example, secretion of VLDL triacylglycerols and cholesterol esters was greater in estrogen-treated, compared to untreated, rats (83). Similarly, after injection of estrogens into male turkeys there was a 55-fold increase in plasma triacylglycerol levels, a 3-fold increase in plasma phospholipids, and a doubling of the cholesterol content of plasma (12).

The effects of estrogens on apoprotein synthesis and secretion have been

extensively studied. When Luskey et al (50) administered estrogen to roosters, the rate of hepatic synthesis of VLDL "antigen," presumably apo B, increased fourfold. Similarly, the secretion of apo B by estradiol-treated chicken hepatocytes was elevated fourfold above the level in control cells (54), and apo B and apo II levels in turkey plasma were also increased by estrogen administration (12). On the other hand, estrogen had no effect on apo B secretion by HepG2 cells (74). Estrogen administration stimulated the secretion of apo AI by HepG2 cells (74) and also stimulated apo AI secretion into rat serum (83), but the apo AI level in the serum of turkeys decreased (12). The reasons for the apparently conflicting results are not clear. In a recent study, (71), liver mRNA levels for apo AI, apo AII, and apo E were measured in rats treated with estrogens. Although there was an increase in the apo AI mRNA level after estrogen treatment, this increase was ascribed not to a direct effect of the hormone but to changes in food intake. Likewise, estrogen treatment produced no change in the mRNA level for apoE. Administration of estrogen did cause a dose-dependent decrease in apoAII mRNA, irrespective of food intake.

An interesting study of estrogen-treated chickens showed that the level of phospholipid transfer activity in their livers was twice that found in untreated chickens (63), which would suggest that this activity might be involved in the assembly of plasma lipoproteins. However, further studies on the role of this protein are required.

In summary, developmental changes and various hormonal treatments can alter the rate of synthesis and secretion of the lipid and apoprotein components of lipoproteins, although not necesssarily in concert. The long-term, hormone-related changes in apoprotein secretion may, in some instances, be correlated with altered mRNA levels of the apoproteins. However, in instances of short-term regulation of lipoprotein secretion in response to dietary changes in cholesterol, carbohydrates, or fatty acids, or in response to changes in the endogenous supply of the lipids, there is no obvious coordinate regulation of the synthesis of apoproteins and lipids. In practically all experiments in which the rate of lipid secretion was altered there was either no change, or a much smaller change, in the rate of apo B (especially the large molecular weight form) secretion. There are no documented examples in which the synthesis of apo B (as measured by mRNA levels) is altered as a result of changes in lipid availability or even as a result of hormonal changes. When HepG2 cells were stimulated with oleate or inhibited with insulin, a sevenfold range in the output of apo B was observed, but no change occurred in the apo B mRNA levels (60). The apo B mRNA from HepG2 cells had a relatively long half-life (about 16h) (60). These data suggest that the apo B gene is expressed constitutively. On the contrary, the synthesis and secretion of apo E appear to vary greatly under different dietary or hormonal conditions.

SECRETION OF LYSOPHOSPHOLIPIDS

LysoPtdCho is the second most prevalent phospholipid (after PtdCho) in plasma of rats and several other species (2). Much of the lysoPtdCho was originally thought to be derived from PtdCho in the plasma through the action of lecithin:cholesterol acyltransferase (66). However, it is now well established that perfused rat livers (66) and cultured rat hepatocytes (2, 53) secrete significant amounts of lysoPtdCho [approximately 1 μmol per gram liver per hour; (66)]. Secreted lysoPtdCho is rich in polyunsaturated fatty acids (2, 31, 66), and these acids, when added to the medium of hepatocytes, stimulate the secretion of lysoPtdCho (31). Secreted lysoPtdCho was made from hepatocyte PtdCho derived either from the CDP-choline pathway or via the methylation of PtdEtn (62). The secreted lysoPtdCho was largely recovered in the density >1.2 g/ml fraction of the medium, presumably associated with albumin (61). Albumin or alpha-cyclodextrin (both of which bind lysoPtdCho) markedly stimulated lysoPtdCho secretion (2). Colchicine, which disrupts microtubules and inhibits lipoprotein secretion, had no effect on lysoPtdCho secretion (31). In addition, choline-deficient hepatocytes, in which PtdCho secretion was two-thirds lower than the level found in choline-supplemented hepatocytes, secreted lysoPtdCho normally (62). Hepatocytes maintained in the presence of albumin also secreted lysoPtdEtn but at about one seventh the rate of lysoPtdCho (61). Thus, it appears that the liver secretes lysophospholipids to a significant extent and this might be a particularly important mechanism for delivery of polyunsaturated fatty acids and choline to extrahepatic tissues.

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