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Lipoproteins in Liver Disease

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Summary: Liver disease is associated with profound and characteristic changes in lipoprotein composition and metabolism. The most pronounced alterations are the formation of lipoprotein-X in intra- and extrahepatic cholestasis, the decrease of apolipoproteins A-I and A-II and the increase of apolipoprotein E. These alterations impair the activities of both lipoprotein lipase and lecithin : cholesterol acyltransferase. They are also responsible for an abnormal receptor mediated uptake of the lipoproteins from plasma. The abnormal lipid and apolipoprotein composition of the lipoproteins in liver disease appears to affect various important functions of cell membranes. The understanding of how these changes occur and their significance in the pathogenesis of other metabolic disturbances secondary to the abnormal lipid metabolism are important challenges for future research.

Introduction

Lipids serve both as a major energy source and as structural components of cell membranes. Not surprisingly, their homeostasis is carefully maintained by a number of interrelated metabolic pathways, not only of the lipids but also of the carbohydrates and proteins. The insolubility of lipids in aqueous fluids adds complexity to the transport processes in plasma by creating a need for a carrier system. This carrier system of protein-lipid aggregates or lipoproteins has been the subject of intense investigation for many years. The basic achievements of the last three decades, which have led to a better understanding of the biochemistry and pathobiochemistry of lipids in health and disease, arose from the characterization of the lipoprotein system in terms of the structural properties and metabolism of the plasma lipoproteins.

The association between plasma lipid concentrations and cardiovascular risk was without doubt the major stimulus in clinical research on plasma lipids. However, lipid disturbances occurring in liver disease and in particular the hypercholesterolaemia accompanying biliary obstruction, a phenomenon recorded first by *Flint* (1) over a century ago, are among the oldest lipid disorders known to clinical medicine.

The data discussed in this review are intended to demonstrate that liver dysfunction produces complex derangements of the lipoprotein system. The basic defects seem to be a disturbed hepatic biosynthesis of lipoproteins, a disturbed hepatic removal of lipoproteins from the circulation and a disturbed plasma lipolytic activity.

Nature of Lipoproteins in Plasma

The liver plays a central role in the regulation of lipoprotein synthesis and degradation as well as in the storage of lipids in various tissues. Therefore, liver dysfunction can be associated with hyper-, hypo-, dys- or paralipoproteinaemia. Any approach to an appreciation of the pathobiochemical significance of the various normal and abnormal plasma lipoprotein patterns requires a consideration of the multifactorial control mechanisms regulating the lipoprotein system.

Lipoprotein metabolism covers a broad field and cannot be fully summarized in a review such as this (for further information see l. c. (2, 3)).

Important terms and steps in lipoprotein metabolism can be summarized as follows:

- intestinal absorption of lipids;
- rates of synthesis of lipids and apolipoproteins;
- assembly and secretion of lipoproteins;
- structure of lipoproteins and apolipoproteins;
- interconversion and intravascular catabolism of lipoproteins mediated by plasma-lipases and lecithin : cholesterol acyltransferase;
- recognition, uptake and catabolism of lipoproteins by hepatic and extrahepatic cells;
- transformation and elimination of lipids and steroids by the liver.

The plasma lipoproteins are water-soluble macromolecular complexes heterogenous in size and composition, with relative molecular weights of up to several million and a particle sizes in the range 7–1000 nm (see fig. 1). They contain specific proteins, apolipoproteins and lipids. All plasma lipoproteins possess a common structural organisation that is characterized by a hydrophobic core composed of cholesterol esters and triglycerides surrounded by a relatively hydrophilic shell consisting of proteins, phospholipids and most of the unesterified cholesterol. The most commonly adapted classification of plasma lipoproteins is based on their behaviour in the ultracentrifuge in high salt solutions (4, 5) (see fig. 2). According to their flotation characteristics the lipoproteins have been classified as very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). All three major density

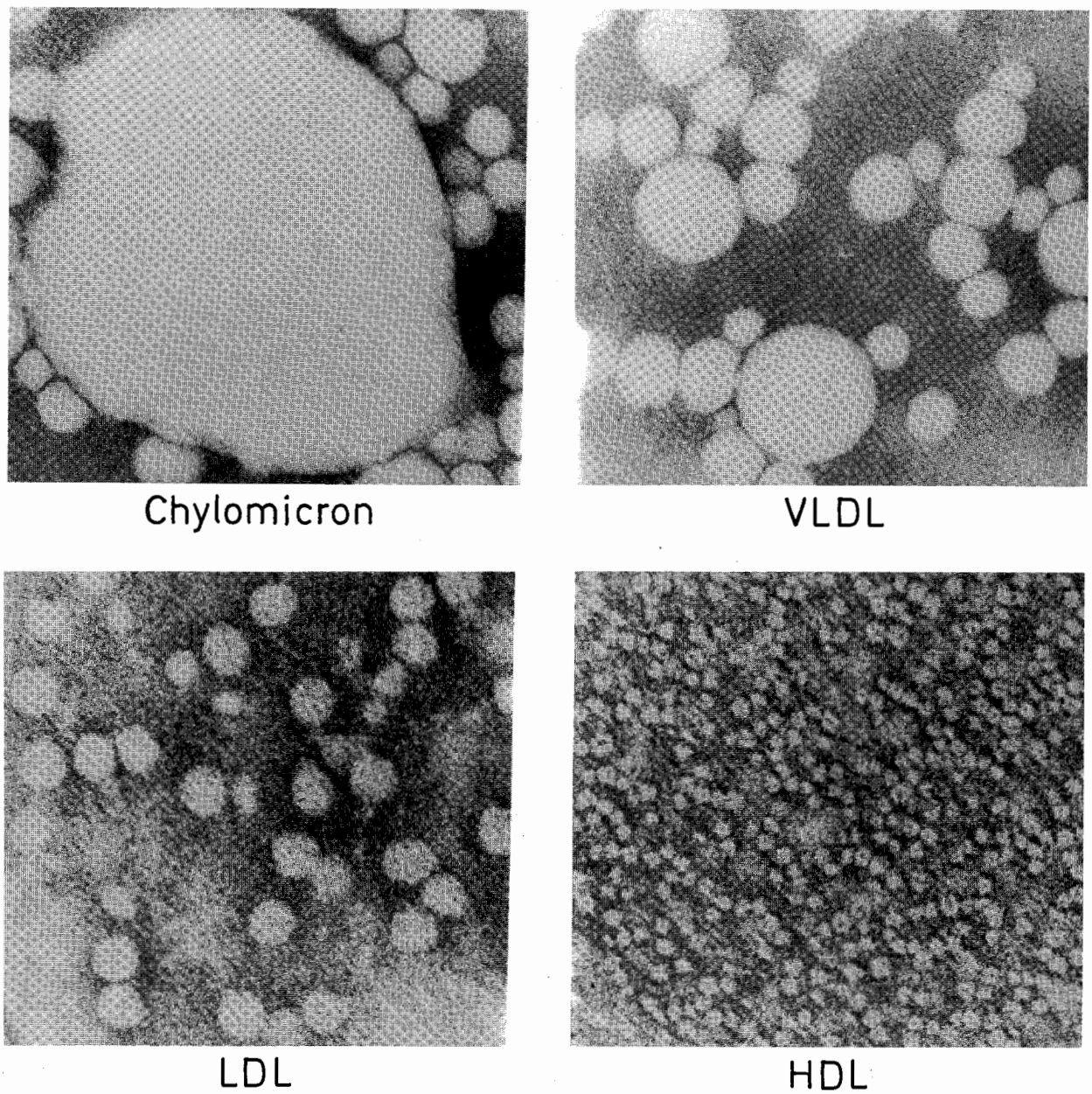

Fig. 1. Electron microscopy of the four major lipoprotein fractions (chylomicrons, VLDL, LDL and HDL). Negative staining with 1% potassium phosphotungstate.

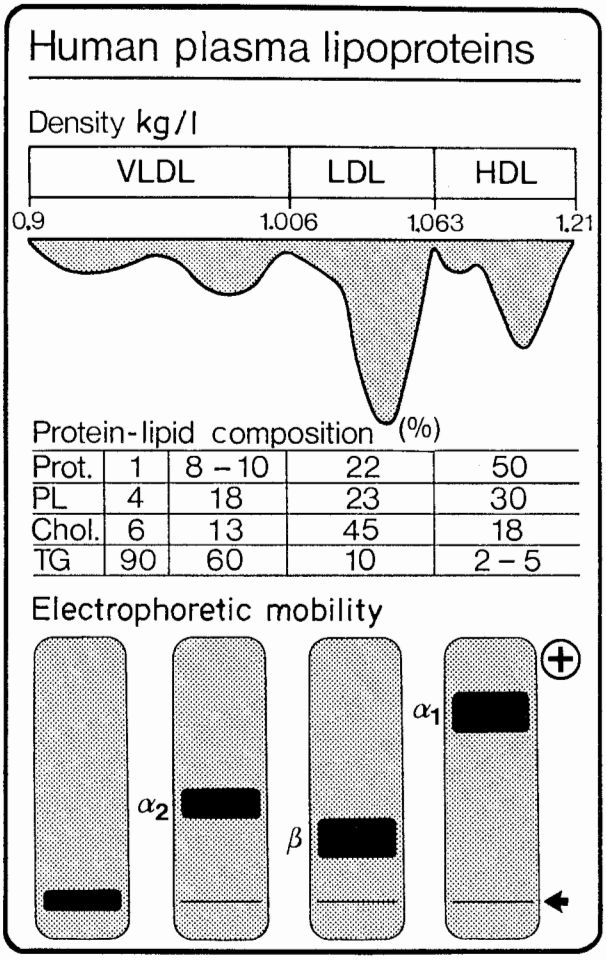

Fig. 2. Scheme for the separation of plasma lipoproteins by ultracentrifugation, protein lipid composition of isolated fractions and electrophoretic mobility of the four major lipoprotein fractions. Quantification can best be achieved by quantitative lipoprotein electrophoresis (8). Prot. = protein; PL = phospholipids; Chol. = cholesterol; TG = triacylglycerols.

fractions exhibit varying degrees of heterogeneity and differences in size and protein lipid composition. The lipoproteins can also be separated by electrophoretic methods in various supporting media (6, 7). According to their electrophoretic migration they are classified as pre-β- (VLDL), β- (LDL) and α-lipoproteins (HDL). Chylomicrons remain at the origin. In recent years a number of other techniques have been used for separating lipoproteins according to their physico-chemical properties, such as gel filtration, size exclusion high performance liquid chromatography, adsorption chromatography, heparin-sepharose affinity chromatography, immuno-absorption chromatography, polyacrylamide gel electrophoresis, isotachophoresis, isoelectrofocussing and various other techniques.

Some of the separation and classification systems have proven to be of value in establishing a relationship between lipoprotein profiles and various diseases. However, for practical purpose, isolation, characterization and measurement of plasma lipoproteins are most widely based on their electrophoretic mobility or hydrated density. Results obtained from both techniques are often used interchangeably, which may be unjustified, especially if abnormal lipoproteins occur as a result of metabolic disturbances. This is because the lipoproteins represent dynamic structures in plasma, which are subject to exchange and transfer processes as well as remodelling during their metabolism in the vascular space.

The Apolipoproteins and Lipolytic Enzymes

Various groups of proteins are important regulators of the lipoprotein system. These include the apolipoproteins, transport and transfer proteins, enzymes involved in lipoprotein metabolism and lipoprotein receptors.

Apolipoproteins are important structural constituents of the lipoprotein particles and have been shown to participate in lipoprotein synthesis, secretion, processing and catabolism. In the last 15 years advances in techniques of protein chemistry have allowed the identification, isolation and characterization of almost a dozen distinct apolipoproteins (see tab. 1). Protein sequencing techniques have allowed the assignment of the primary amino acid sequence of six of these proteins. Recently c-DNA and genomic clones have been derived for several of these apolipoproteins. The DNA sequences combined with cell-free synthesis and tissue and organ culture studies have revealed the presence of apolipoprotein precursors, including prepeptides and in some cases propeptides. In addition most of the apolipoprotein genes have been mapped in the human genome. Finally human mutations in the apolipoprotein genes have been identified at both the amino acid and DNA-level (for further detailed information see l. c. (9, 10)).

The importance of the apolipoproteins for maintaining the structural properties of the plasma lipoproteins and their functional roles in lipoprotein metabolism (some function as activators or inhibitors of lipolytic enzymes) and clearance from the plasma (some operate as ligands for specific transport receptors) show that the determination of their concentration and distribution may be of importance for a better understanding of the pathobiochemistry of lipid disturbances, mainly in secondary forms of dyslipoproteinaemia such as in liver disease.

Of the four major plasma-lipases involved in lipoprotein metabolism (lecithin:cholesterol-acyl-transferase (LCAT), lipoprotein lipase, hepatic triglyceride li-

Tab. 1. Characterization and function of human apolipoproteins.

Apolipo-protein	Approximate relative molecular mass	Major density class	Major sites of synthesis in man	Major function in lipoprotein metabolism*)
A I	28 000	HDL	liver-intestine	activates LCAT, structural
A II	18 000	HDL, Chylomicrons	liver-intestine	structural
A IV	45 000	Chylomicrons	intestine	activates LCAT
B-100	514 000	VLDL-IDL-LDL	liver	binds to LDL receptor, structural
B-48	250 000	Chylomicrons-VLDL-HDL	intestine-liver	structural
C I	6 500	Chylomicrons-VLDL-HDL	liver	activates LCAT
C II	9 000	Chylomicrons-VLDL-HDL	liver	activates lipoprotein lipase
C III_{0-2}	9 000	Chylomicrons-VLDL-HDL	liver	inhibits lipoprotein uptake by the liver
D	22 000	HDL	?	cholesteryl ester exchange protein
E_{2-4}	34 000	Chylomicrons-VLDL-HDL	liver, macrophage	binds to E receptor
F	30 000	HDL	?	–
G	75 000	VHDL	?	–
H	45 000	Chylomicrons	?	–
(a) determinant	250 000 700 000	HDL, Chylomicrons	? (liver-intestine)	–

*) LCAT = lecithin : cholesterol acyltransferase

pase, and the phospholipases), three are synthesized in the liver, i. e. lecithin : cholesterol-acyl-transferase, hepatic triglyceride lipase and phospholipase. Other important cellular enzymes involved in lipid metabolism, such as acyl-CoA: cholesteryl-acyl-transferase (ACAT), hydroxymethylglutaryl-CoA-reductase, hydroxymethylglutaryl-CoA-synthase, cholesterol-ester-hydrolase, enzymes of fatty acid and phospholipid synthesis and cholesterol 7-α hydrolase are also hepatic enzymes.

The Metabolism of Lipoproteins in Plasma

Although it has been assumed for many years that the terminal catabolism of mammalian plasma lipoproteins occurs predominantly in the liver, most detailed investigations of receptor mediated processing of macromolecules in this organ have until recently not been concerned with plasma lipoproteins, but rather with other plasma proteins, polypeptides and hormones. The first detailed studies on receptor mediated endocytosis of lipoproteins in the liver were facilitated by the discovery that administration of pharmacological amounts of oestradiol to rats induces a several fold increase in the number of LDL-receptors on the surface of hepatocytes and increases the uptake and catabolism of LDL by the liver (11). From combined biochemical and morphological approaches it was possible to show that the various steps to lipoprotein binding to receptors, internalisation, movement to the lysosomal region and lysosomal processing resemble closely those described for LDL in fibroblasts (12, 13) and other macromolecules in hepatocytes (see fig. 3). Cholesterol taken up by hepatocytes suppresses hydroxymethylglutaryl-CoA-reductase, activates acyl CoA : cholesteryl acyltransferase, inhibits expression of LDL receptors but leaves apolipoprotein E receptor activity unchanged (14, 15). LDL binds to the receptor via a single site on apolipoprotein B. Also VLDL remnants bind to this receptor but with much higher affinity than that of LDL, presumably because these particles possess apolipoprotein E and bind polyvalently to the LDL-receptor. These differences in affinity have major

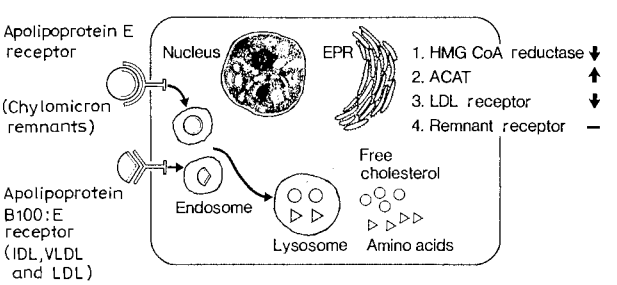

Fig. 3. Scheme for lipoprotein uptake and metabolism by hepatocytes (for detail see text).
HMGCoA = 3-hydroxy-3-methylglutaryl-CoA
ACAT = acyl-CoA:cholesteryl acyltransferase
ER = endoplasmatic reticulum

consequences for lipoprotein catabolism, since VLDL remnants are removed from the blood within minutes, whereas LDL circulates for many hours (16, 17). Since it has been estimated that the liver clears more than 80% of the structural lipids, phospholipids and cholesterol from the circulation (18) it is important to realize that more than one receptor seems to participate in hepatic lipoprotein catabolism and that processes other than receptor mediated endocytosis may also participate in hepatic lipoprotein metabolism. Studies in a mutant rabbit that lacks LDL-receptors (*Watanabe* hereditary hyperlipidaemic, WHHL, rabbit) have provided evidence that LDL-receptors are not necessary for uptake of chylomicron remnants by the liver (19). Uptake of chylomicrons by hepatic parenchymal cells in these animals seems to occur via a distinct chylomicron remnant receptor, the apolipoprotein E receptor, which so far has only been described for hepatocytes. A third lipoprotein receptor, the Scavenger receptor (Scavenger-system), present mainly in reticulo-endothelial cells and also in *Kupffer*-cells, efficiently removes certain chemically modified and abnormal lipoproteins from the blood (10, 21). The importance of this pathway for lipoprotein catabolism is uncertain because the extent to which lipoproteins are modified in vivo so as to permit them to be recognized by the Scavenger receptor is still unknown. On the other hand, LDL and possibly also VLDL remnants are also taken up by hepatocytes of WHHL-rabbits, which may indicate the existence of other, as yet undefined processes capable of clearing these lipoproteins from the circulation (22). Also binding sites for high density lipoproteins (HDL) have been postulated for hepatocytes and demonstrated in membrane preparations of rat liver (23). This process however, may not involve endocytosis, because the rates of uptake of lipids and the apolipoproteins by this system are different.

The role of the liver in the synthesis of apolipoproteins and lipids, their assembly in the *Golgi*-apparatus and the secretion of lipoproteins was established in the late 50's and then became the focus of a great deal of research (for definition see l.c. (24)). It was appreciated that the rate of synthesis and secretion of these particles by the liver is an important determinant of their circulating level. Secretion and degradation are equally important in determining lipoprotein levels because in the steady state the rate of synthesis must equal the rate of degradation, and a change in either will cause a change in the circulating level.

The role of the liver in the regulation of the plasma lipoprotein system can briefly by outlined as follows (see fig. 4):

The liver secretes two types of nascent lipoproteins: nascent VLDL which is rich in triglycerides and apolipoprotein B but contains very little apolipoprotein C and apolipoprotein E, and nascent HDL which is rich in phospholipids, unesterified cholesterol, apolipoprotein E and apolipoprotein A-II and contains some apolipoprotein C. These particles possess laminar structures and are found within the density class d 1.063 – 1.21 kg/l on ultracentrifugation. After nascent HDL particles enter the plasma they are acted upon by lecithin : cholesterol acyltransferase, an enzyme also synthesized and secreted by the liver. Cholesterol is esterified, thus rendering it hydrophobic, and it is thereby transferred into the core of the particles. This brings about a change in the shape of the particles into a globular form. At the same time there is an exchange of apolipoprotein E and apolipoprotein C with apolipoprotein A-I, the cofactor for lecithin: cholesterol acyltransferase which is primarily derived from the intestine. By this mechanism mature HDL is generated, and it is believed that these particles leave the circulation by an uptake mechanism in the liver, which, however, has not yet been fully elucidated. During the esterification of HDL-cholesterol, apolipoprotein C and apparently apolipoprotein E is transferred to the pathway leading from nascent to mature VLDL, intermediate-density lipoproteins (IDL) and finally to LDL. This metabolic cascade is regulated by lipoprotein lipase and by hepatic-triglyceride-hydrolase, the two major enzymes of postheparin plasma lipolytic activity. During triglyceride hydrolysis some apolipoprotein C and apolipoprotein E is transferred back to the HDL density class. It has been postulated that these apolipoproteins form, together with apolipoprotein A-I, apolipoprotein D, lipid transfer proteins and lecithin: cholesterol acyltransferase, the so-called Reversed Cholesterol Transport Complex (RCTC), by which cholesterol is transferred from the peripheral cells to the liver for excretion.

The Abnormal Lipoprotein Profile in Liver Disease

From the complex metabolic pathways of the plasma lipoproteins in which the liver is predominately involved it is clear that abnormal liver function may cause profound disturbances in the lipoprotein system. The abnormal patterns, however, are generally not indicative of a particular liver disease. Therefore in the following description emphasis will be placed on the pathobiochemical aspects, rather than attempting to directly link a particular lipoprotein or lipid pattern to a specific liver dysfunction.

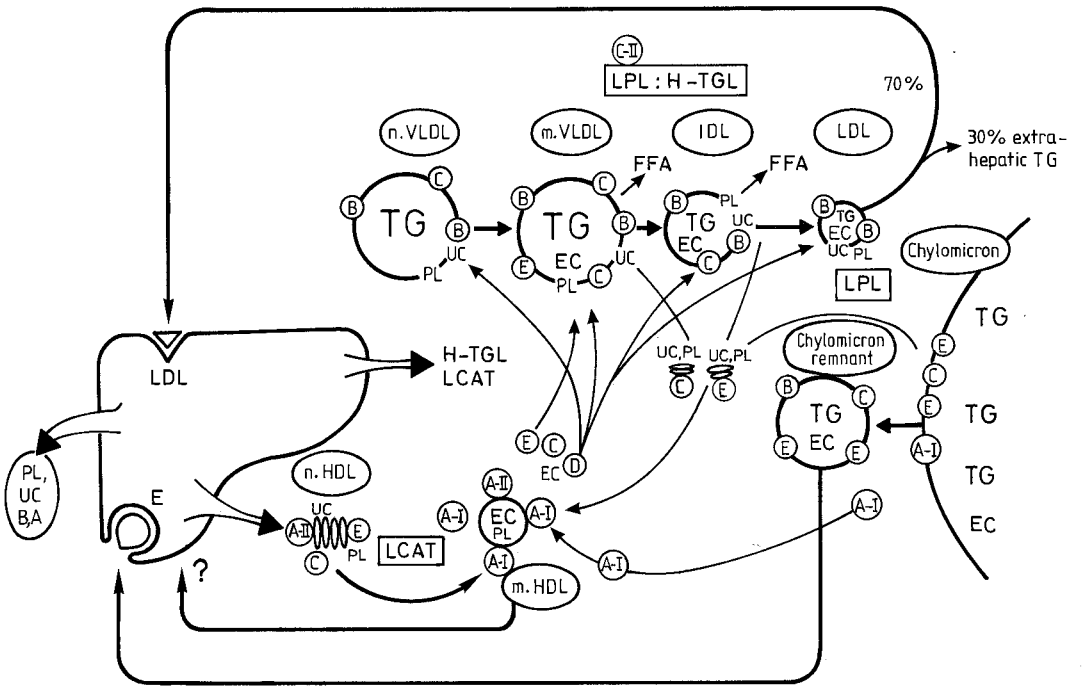

Fig. 4. Scheme to illustrate the metabolism of plasma lipoproteins in human plasma (for detail see text).

(chylomicron) etc: designation of lipoproteins

o lipoproteins

a) consisting of
- apolipoproteins (A-I , A-II etc)
- triacylglycerols (TG)
- esterified cholesterol (EC)
- unesterified cholesterol (UC)

b) releasing
- phospholipids (PL)
- free fatty acids (FFA)

enzymes, i.e.
- lipoprotein lipase (LPL)
- hepatic triacylglycerol hydrolase (H-TGL)
- lecithin : cholesterol acyltransferase (LCAT)

micelles

The following abnormalities in plasma lipids, lipoproteins and apolipoproteins may occur as a result of liver dysfunction:

- hypercholesterolaemia;
- increased ratio of free to esterified cholesterol;
- hyperphospholipidaemia;
- normal or elevated levels of plasma triglycerides;
- increased or decreased concentration of LDL;
- normal or elevated levels of VLDL, often with abnormal (β-) mobility on electrophoresis;
- normal or decreased levels of HDL with a marked heterogeneity and abnormal composition of HDL particles;
- decreased concentrations of apolipoproteins A and B-100;
- elevated concentration of apolipoprotein E;
- increased concentrations of IDL and apolipoprotein B-48;
- decreased plasma post-heparin lipolytic activity;
- elevated or reduced activity of lecithin : cholesterol acyltransferase;
- appearance of the abnormal lipoprotein-X (LP-X) in cholestasis.

Electrophoresis of lipoproteins in liver disease reveals a typical pattern of only one, often broadened, band in the β-position, which masks a heterogeneity which can only be elucidated by the employment of various analytical techniques (25). Drastic changes in the concentration of apolipoproteins often occur concomitantly with this lipoprotein pattern, but they are not disease-specific (26, 27). The normalization of these changes, however, may be helpful in following the course of the disease.

Cholestasis and Hypercholesterolaemia

Several years ago we were able to show that the lipoprotein pattern of cholestasis is mainly characterized by the appearance of an abnormal lipoprotein particle in the LDL density class. This particle has been subsequently isolated and characterized with respect to its physicochemical properties. It has been designated lipoprotein-X (28, 29). Lipoprotein-X has the characteristics of a vesicle, in which a phospholipid-cholesterol-apolipoprotein membrane structure separates an internal from an external water compartment. Its mean diameter is approximately 50 – 70 nm. The disc-formation seen on electron micrographs is not unique for this particle (30). Similar laminar structures have been described for artificial lipid-protein complexes; they have also been demonstrated in the plasma of cholesterol-fed animals, as well as in the plasma of patients with lecithin : cholesterol acyltransferase deficiency (31, 32).

The protein-lipid composition of lipoprotein-X is unique, and is characterized by a high content of phospholipids and a low content of cholesterol esters (28). Bile salts are bound to lipoprotein-X and, depending on their concentration, may exert a strong influence as a detergent on the physicochemical properties of the particle (33). Albumin accounts for up to 60% of the protein component of lipoprotein-X and is primarily located inside the vesicle (29). In contrast, the specific apolipoproteins of lipoprotein-X (apolipoprotein C and apolipoprotein D) are located on the surface. Apolipoprotein B, the major apolipoprotein of LDL and also apolipoprotein E are not present in lipoprotein-X. In addition, aggregates of various liver and bile enzymes, such as alkaline phosphatase or γ-glutamyltransferase with lipoprotein-X have been described, but have been erroneously interpreted as isoenzymes (34, 35, 36). The detection of lipoprotein-X in the serum of a patient is the most sensitive and specific clinical chemical parameter for the diagnostic confirmation or exclusion of cholestasis, as shown by a large number of clinical studies (for further information see l. c. (37)).

The appearance of lipoprotein-X in plasma has been noted after bile duct ligation in experimental laboratory animals and after insertion of the common bile duct into the venous system (38).

In addition, incubation of bile lipoproteins with serum leads to a complete conversion of the bile lipids into lipoprotein-X (33). The data from both the in vitro and in vivo experiments indicate that the bile lipids are converted into lipoprotein-X when they enter the plasma compartment, as in cholestasis. The finding that lipoprotein-X or its precursors are formed in experiments in which only biliary lipids and albumin are incubated together is of particular theoretical and pathobiochemical significance. Such precursors exhibit a high affinity for apolipoprotein C and apolipoprotein D, which in the vascular space may bring about disturbances in the balance or exchange of these apolipoproteins between VLDL and HDL (see fig. 4), a mechanism which is important for plasma lipoprotein metabolism. The hypertriglyceridaemia of cholestasis may be caused by such a mechanism, a view which is supported by the observation that these apolipoproteins are either absent or drastically reduced in VLDL particles obtained from patients with cholestasis (25, 27).

Results from *Walli* et al. in our laboratory (39) concerning the catabolism of lipoprotein-X, revealed that lipoprotein-X is like other macromolecules taken up and catabolized by the reticuloendothelial system, primarily by the spleen and not as most normal plasma lipoproteins by the liver. Moreover receptor-binding studies indicate that isolated hepatocytes or fibroblasts exhibit a low degree of binding and uptake of lipoprotein-X, whereas in lymphocytes high rates are observed and these cell types show a marked reduction in the activity of the hydroxymethylglutaryl-CoA-reductase when in contact with lipoprotein-X. Also, the reductase activity is greatly depressed in lymphocytes from cholestatic patients.

Normally chylomicron remnants are rapidly removed from the circulation by the liver and are most effective in suppressing hepatic cholesterolgenesis. Lipoprotein-X causes a marked inhibition of remnant uptake by isolated perfused livers and isolated hepatocytes, even though it is devoid of apolipoprotein B and apolipoprotein E and is not taken up by hepatocytes itself (14, 39).

A parallel clinical finding is hypertriglyceridaemia with persisting apolipoprotein B-48, which is often noted in cholestatic patients and is indicative of the accumulation of remnant-like particles (40). In contrast to the direct effects of lipoprotein-X on chylomicron remnant uptake, no such effects of lipoprotein-X on the degradation of LDL by isolated perfused livers or monolayer cultures of skin fibroblasts are noted (15, 39).

In order to understand the relevance of these in vitro results for the situation in vivo, we perfused livers from cholestatic rats which had lipoprotein-X in their serum. Such livers degraded twice as much [^{125}I]LDL as normal livers. A similar situation is observed in patients with cholestasis. The apolipoprotein B concentration in the serum of cholestatic patients is re-

duced to half of that found in normal subjects, reflecting a reduced LDL concentration. Also, in the isolated perfused liver lipoprotein-X stimulates the key enzyme of cholesterol synthesis, hydroxymethylglutaryl-CoA-reductase, by a factor of 2.5 (14, 39). Taken together, these observations are of particular interest with respect to both hepatic lipoprotein receptor activity and cholesterolgenesis in hepatocytes. Although lipoprotein-X is devoid of apolipoprotein B and apolipoprotein E, it appears to affect apolipoprotein E receptors directly, but hepatic LDL receptor activity indirectly. The mechanism by which lipoprotein-X affects the function of the hepatic apolipoprotein E receptor is as yet unknown but presents an important challenge for future research in this field.

Hypertriglyceridaemia

Clinical studies first indicated that hypertriglyceridaemia is also frequently found in severe cholestasis and in primary biliary cirrhosis (41, 42).

The main increase in triglycerides is found in the LDL-II density class. Triglyceride-rich, cholesterol-poor apolipoprotein B, apolipoprotein C and apolipoprotein E-containing intermediate density lipoproteins account for the accumulation of tryglycerides in this density fraction (43). These unusual particles are heterogeneous in size, ranging from 30 nm to 80 nm, and are thus much larger than normal LDL (20 – 25 nm). Dietary studies have indicated that these particles are remnants derived from a disturbed catabolism of chylomicrons (25). Their accumulation may be caused by reduced plasma lipolytic activity and/or by a disturbed hepatic uptake due to an inhibition of the apolipoprotein E receptor. *Jahn* et al. recently demonstrated a decreased activity of hepatic lipase in all his patients with primary biliary cirrhosis due to the presence of a heat labile enzyme inhibitor (44). In contrast, altered cholesterol esterification was only observed in subjects with advanced disease.

Influence of Alcohol Intake on the Lipoprotein System

Hypertriglyceridaemia as a consequence of liver damage by alcohol is also well known, but not fully understood. The metabolic situation is complicated, as acute and chronic intake of alcohol may bring about opposing disturbances in lipid metabolism. Moreover the quantity of alcohol consumed is important.The well-documented increase in plasma HDL concentration following a constant, but non-toxic intake of alcohol (45, 46) seems to be caused by an accelerated turn-over of very low density lipoproteins. *Nikkilä* et al. (47) found both the fractional catabolic rate of VLDL and the turn-over rate of VLDL to be high in alcoholic men, and they normalized following two weeks of abstinence. However, the removal of triglycerides from plasma is not increased by acute alcohol intake but rather impaired. Continued use causes a gradual return to normal concentrations of VLDL. This change parallels an increase in lipoprotein lipase activity (47). The reason for the decrease of plasma LDL concentration following heavy alcohol consumption remains to be elucidated (46). Both elevated HDL and reduced LDL return rapidly to normal after alcohol withdrawal.

HDL and α-Lipoproteins

The HDL fraction in liver disease contains hardly any cholesterol esters or triglycerides, while the concentration of free cholesterol and phospholipids is increased. The α-lipoproteins cannot be visualized on lipoprotein electropherograms (25, 27). Ultracentrifugal and chromatographic analysis, however, reveal the presence of lipoproteins in this density class but with an unusual degree of heterogeneity. Under the electron microscope most particles show rouleaux formation similar to lipoprotein-X or nascent HDL particles. The rates of apolipoprotein A-I catabolism and elimination are increased by 2 – 4 fold in acute alcohol hepatitis, as compared with healthy control subjects (48). From this observation it may be concluded that the diminished plasma concentration of apolipoprotein A found in liver patients is primarily due to its accelerated catabolism, rather than to a disturbed synthesis, as described for *Tangier* disease, a primary deficiency of HDL (49). The apolipoprotein A-I and apolipoprotein A-II in the plasma of patients with liver disease often show a complete dissociation and they lose much of their lipid binding capacity (25). It is possible that the dissociation of the two apolipoprotein A subunits is a major reason for their accelerated catabolism and this may well result from a disturbed generation of mature HDL from nascent HDL in the plasma of such patients, emphasizing the general importance of the structural properties of a lipoprotein with respect to its metabolism. The HDL fraction in most patients with liver disease not only shows decreased concentrations of their major apolipoprotein constituents, apolipoprotein A-I and apolipoprotein A-II, but also a marked increase in apolipoprotein E (50), a phenomenon which has also been described for familial leci-

thin : cholesterol acyltransferase deficiency when such patients are subject to a dietary cholesterol load. Recently *Owen* et al. (51) reported that a subfraction of HDL, containing primarily apolipoprotein E, strongly reduced LDL binding, internalization and degradation in cultured human skin fibroblasts. In contrast to normal HDL, these HDL particles were also taken up and degraded by the fibroblasts apparently through the LDL-receptor pathway. The particles also stimulated cellular cholesterol esterification, increased cellular cholesterol ester content and suppressed cholesterol synthesis and receptor activity (51). It was concluded from these results that this particulate HDL may deliver cholesterol to the cells via LDL-receptor mediated uptake. It should be interesting to elucidate whether in cirrhotic patients. It is reasonable to anticipate that apolipoprotein E accumulates in the HDL fraction as a consequence of plasma lecithin : cholesterol-acyl-transferase deficiency.

In collaboration with Dr. *Clemens* from the University of Tübingen, Drs. *Armstrong* and *Walli* from our laboratory were able to demonstrate lipoproteins with abnormal binding characteristics to lipoprotein receptors in all three major density classes of a patient suffering from familial lecithin : cholesterol acyltransferase deficiency. An abnormal HDL rich in apolipoprotein E with high affinity to heparin-sepharose revealed a high binding affinity to the LDL receptor in cultured fibroblasts, while a small sized LDL from this patient showed a lower than normal affinity to the LDL-receptor (52). In isolated perfused rat livers the HDL fraction rich in apolipoprotein E was degraded more rapidly than any other lipoprotein isolated from the plasma of the patient.

The data discussed in this review indicate that liver dysfunction produces complex derangements of the lipoprotein system. The basic defects seem to be a disturbed hepatic biosynthesis of lipoproteins, a disturbed hepatic removal of lipoproteins from the circulation and a disturbed plasma lipolytic activity. This suggestion is strongly supported by the finding of structural similarities between nascent VLDL and nascent HDL with the plasma VLDL and HDL accumulating in liver disease patients.

Plasma Lipoproteins and Plasma Membranes

We have described and discussed some of the most common abnormalities of lipoproteins, apolipoproteins and lipolytic enzymes, disturbances of metabolic regulation and altered lipoprotein-receptor activities found in liver disease. Since most plasma lipoproteins, normal or abnormal, interact with cell surface membranes and since cell membrane fluidity is known to alter the capacity of cells for many important biological mechanisms, it is reasonable to anticipate that alterations of the lipoprotein system in liver disease may have important consequences for a number of cellular functions including carrier mediated transport, the properties of certain membrane-bound enzymes, the binding to specific receptors, endocytosis, depolarization dependent exocytosis, immunological cytotoxicity, prostaglandin production, cell growth and possibly for many other as yet unknown cell functions (53, 54). The effects of lipid modification and cellular function were, however, very complex. They often vary from one type of cell to another and they do not exert a uniform effect on all processes in a single cell line. Therefore, it is not yet possible to make any generalizations or to predict how a given cell system may respond to a particular type of lipid modification. Many of the functional responses probably are caused directly by the changes in membrane lipid structure which affect either bulk lipid fluidity or specific lipid domains. The conformation or quaternary structures of certain transport proteins, receptors or enzymes probably are sensitive to changes in the structure of their lipid microenvironement. This may lead to changes in their biological activity. The dynamic state of lipids in membrane bilayers was first described by *Singer & Nicolson* in 1972 (55) as one of the fundamental factors of the fluid mosaic model of membrane structure. The basic question to be raised now is whether and to what extent the interaction or exchange of membrane lipids with abnormal lipoproteins in liver disease can modulate cell membrane composition and consequently the activity of membrane proteins. The immediate change in the shape of red blood cells by incubation with lipoproteins from liver disease patients (56), and the abnormal erythrocytes (57, 58) and abnormal platelet aggregation in human liver disease (59) indicate that it may be possible to investigate this problem. Also the inhibition of hepatic apolipoprotein E receptor (14, 15, 39) by lipoprotein-X which is devoid of the protein ligand to the receptor (29) may be a consequence of an altered cell membrane fluidity deriving from the interaction with lipoprotein-X. The hyperglycaemia of liver disease may be another example of such a general pathobiochemical mechanism.

It now seems important and interesting to further correlate abnormal functions of organs and cells with the abnormal composition of plasma lipoproteins in liver disease.

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