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Liver in Metabolic Diseases

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Lipoprotein metabolism in liver disease

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The basic achievements of the last 30 years, which have led to a better understanding of the biochemistry of lipids in health and disease, arose from the characterization of the lipoprotein system in terms of its structural properties and the metabolism of the plasma lipoproteins. It is now evident that the regulatory mechanisms for maintaining normal concentrations of plasma lipoproteins are dependent on a normal metabolism not only of the lipids, but also of the carbohydrates and proteins. Furthermore the pathobiochemical mechanisms of lipid metabolism ensuing in liver disease can only be understood if attention is focused on the plasma lipoproteins, those macromolecular complexes with molecular weights of up to several million daltons.

The association between plasma lipid concentrations and cardiovascular risk was without doubt the major stimulus in clinical research for studying the plasma lipids. However, the lipid disturbance occurring in liver disease, which is most familiar to the physician, is the hypercholesterolaemia accompanying biliary obstruction, a phenomenon recorded over a century ago[1].

Besides the intestine, the liver is the only organ capable of synthesizing plasma lipoproteins; it also plays a central role in the regulation of the metabolism and catabolism of the plasma lipids. Therefore disturbances of liver function are often associated with hyper-, hypo-, dys- or paralipoproteinaemia. Any approach towards an appreciation of the pathobiochemical significance of the various normal and abnormal plasma lipoprotein patterns requires a consideration of the multi-factorial control mechanisms regulating the lipoprotein system. These comprise:

(1) the intestinal absorption of lipids;
(2) the rates of synthesis of lipids and apoproteins;
(3) the assembly and secretion of the lipoproteins;
(4) the structure of the lipoproteins and apoproteins;
(5) the enzymes involved in the interconversion and intravascular

catabolism of lipoproteins (lipases and lecithin : cholesterol acyltransferase, LCAT);

(6) the recognition, uptake and catabolism of lipoproteins by cells; and

(7) the transformation and elimination of lipids and steroids by the liver.

The role of the liver in the regulation of the plasma lipoprotein system can be briefly outlined as follows (Figure 11.1). The liver secretes two types of nascent lipoproteins: nascent VLDL which is rich in triglycerides and apoB but contains very little apoC and apoE, and nascent HDL which is rich in phospholipids, unesterified cholesterol, apoE and apoA II and contains some apoC. These particles possess a laminar structure and are found within the density class d 1.063–1.21 g/ml on ultracentrifugation. After nascent HDL particles enter the plasma they are acted upon by LCAT, 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 apoE and apoC with apoA-I, the latter being the cofactor for LCAT and being 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 has not yet been elucidated. However, in the rat, partial hepatectomy by two-thirds does not result in a reduction of the fractional catabolic rate of HDL[2]. During the esterification of HDL, apoC and apparently apoE are transferred to nascent VLDL. Through the mediation of apoD cholesterol ester is transferred to the pathway leading from nascent

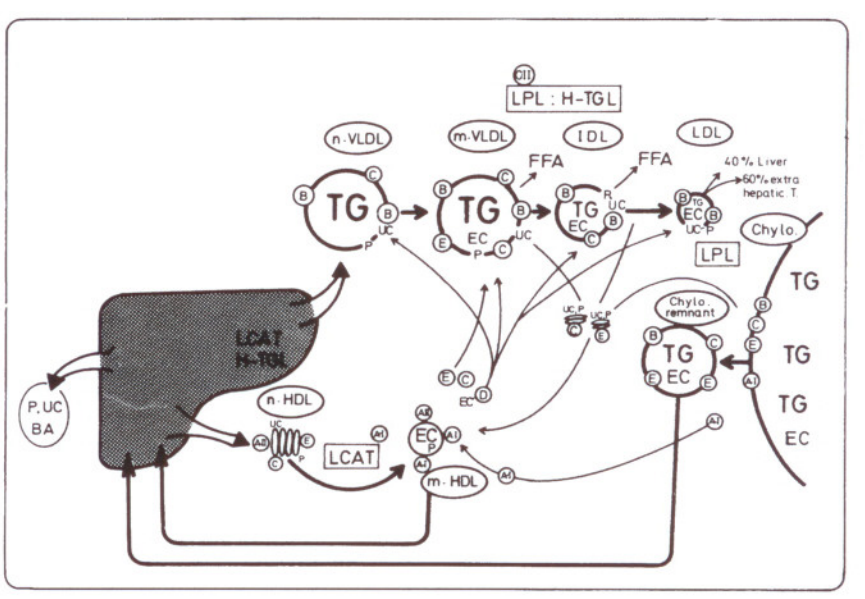

Figure 11.1 Scheme for the intravascular metabolism of plasma lipoproteins

to mature VLDL intermediate-density lipoprotein (IDL) particles and finally to LDL. This metabolic cascade is regulated by lipoprotein lipase and possibly by hepatic triglyceride hydrolase, the two major enzymes of post-heparin plasma lipolytic activity. The major portion of LDL is taken up and catabolized by the liver by means of an apoB receptor. During triglyceride hydrolysis some apoC and apoE is transferred back to the HDL density class. It has been postulated that these apoproteins form, together with apoB and LCAT, the so-called reverse cholesterol transport complex (RCTC), by means of which most of the cholesterol is transferred back from the periphery to the liver for excretion. This complex functions through an exchange of cholesterol and cholesterol esters with LDL and chylomicron remnants[3].

From these considerations it is clear that abnormal liver function may cause complex disturbances of the lipoprotein system. The abnormal patterns, however, are generally not indicative of a particular liver disease. Therefore in the following presentation emphasis will be placed on the pathobiochemical aspects, rather than attempting to directly link a particular lipoprotein or lipid pattern with a specific liver dysfunction.

The following abnormalities in lipids and lipoproteins may occur with liver dysfunction:

(1) hypercholesterolaemia;
(2) an increased ratio of free to esterified cholesterol;
(3) hyperphospholipidaemia;
(4) normal or elevated levels of plasma triglycerides;
(5) increased or decreased concentration of LDL;
(6) normal or elevated levels of VLDL, often with abnormal β-mobility during electrophoresis;
(7) normal or decreased levels of HDL, and also abnormal composition of HDL particles;
(8) decreased concentrations of apoA and apoB;
(9) elevated concentration of apoE;
(10) diet-dependent increased concentrations of IDL;
(11) decreased post-heparin plasma lipolytic activity;
(12) elevation or diminution in the activity of LCAT; and
(13) the appearance of 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 other variations from normal lipoprotein patterns (Figure 11.2). Several factors are usually responsible for these alterations, and their recognition often requires the employment of various analytical techniques. Drastic changes in the concentration of apolipoproteins often occur concomitantly with this lipoprotein pattern, but they are not disease-specific (Table 11.1). However the normalization of these changes may be helpful in clinical evaluation, especially in following the course of a disease.

About 15 years ago it was shown that the lipoprotein pattern of cholestasis is mainly characterized by the appearance of an abnormal lipoprotein particle of the LDL density class. This particle has been subsequently isolated and characterized with respect to its physicochemical properties[4–6].

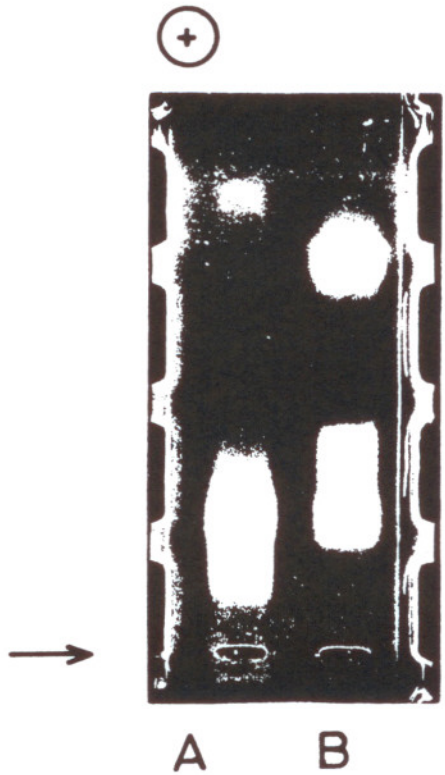

Figure 11.2 Plasma lipoprotein electrophoresis on 1% agarose. **A:** Serum of a patient with intrahepatic cholestasis. **B:** Serum of the same patient after recovery

It has been designated lipoprotein X or LP-X. It seems to have the characteristics of a vesicle, in which a phospholipid–cholesterol–apoprotein membrane structure separates an internal from an external water compartment. Its mean diameter approximates 500–700 Å (Figure 11.3). The formation of rouleaux seen on electron micrographs is not unique for this particle. Similar laminar structures have been described for artificial lipid–protein complexes[7]; they have also been demonstrated in the plasma of cholesterol-fed animals, as well as in the plasma of patients with LCAT deficiency[8].

The protein–lipid composition of LP-X is unique, and is characterized by a high content of phospholipids and a low content of cholesterol esters. Bile salts are bound to LP-X and, depending on their concentration, may exert a strong influence as a detergent on the physicochemical properties of the particle. Albumin accounts for up to 60% of the protein component of LP-X and is primarily located inside the vesicle. In contrast, the specific apoproteins of LP-X (apoC and apoD) are located on the surface. ApoB, the major apoprotein of LDL, is not present in LP-X. In addition, association of

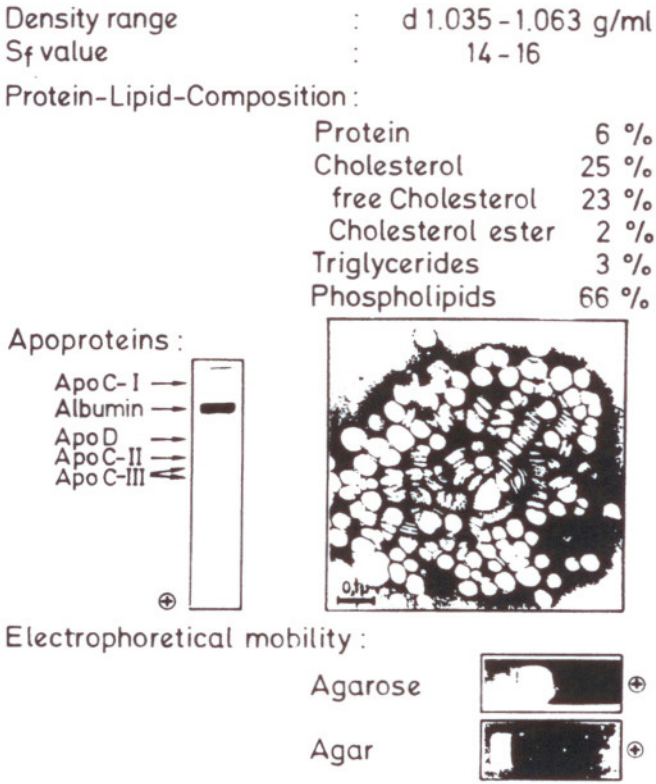

Figure 11.3 Characteristics of lipoprotein-X (LP-X), the characteristic plasma lipoprotein vesicle of cholestasis

various liver and bile enzymes, such as alkaline phosphatase and γ-glutamyl transpeptidase, with LP-X have been described, and have been falsely interpreted as isoenzymes. The detection of LP-X in the serum of a patient is the most sensitive and specific clinical chemical parameter for the diagnostic confirmation or exclusion of cholestasis[9,10].

The appearance of LP-X in plasma has been noted after bile duct ligation in experimental laboratory animals and, as expected, after insertion of the common bile duct into the venous system[11]. In addition, incubation of bile lipoproteins with serum leads to a complete conversion of the former into LP-X[11]. The bile lipoproteins, together with the bile salts themselves, can therefore be regarded as the biliary components necessary for the formation of LP-X[11]. The data from both *in vitro* and *in vivo* experiments indicate that the lipids of bile are converted into LP-X when they enter the plasma compartment, as in cholestasis.

The finding that LP-X or its precursors are formed in experiments, in which only biliary lipids and albumin are incubated together, is of particular theoretical and pathophysiological significance. Such precursors do exhibit a

Table 11.1 Concentration of serum apoproteins in various liver diseases

Disease	*Sex*	*ApoA-I* (90 ± 22 mg/dl)	*ApoA-II* (35 ± 7 mg/dl)	*ApoB* (80 ± 15 mg/dl)	*LP-X* 0	*Total cholesterol* (150–220 mg/dl)	*Bilirubin* (< 0.2 mg/dl)
Extrahepatic obstruction	M	1.1	0	75	+ +	134	22
	F	98	32	49	+	239	1.5
Acute hepatitis-B	M	17	13	52	+	132	18
Acute hepatitis-A	F	59	40	52	0	170	1.4
Cirrhosis	M	0	0	20	0	54	36

The values in the table are from determinations made in the serum of individual patients. The normal ranges are given in parentheses

high affinity for apoC and apoD, which may bring about disturbances in the balance or exchange of these apoproteins between VLDL and HDL, a regulation which is necessary for the hydrolysis of the lipoproteins rich in triglycerides. This may be, at least partly, a cause of the hypertriglyceridaemia occurring in cholestasis. This suggestion is supported by the observation that these apoproteins are either absent or drastically reduced in VLDL particles obtained from patients with cholestasis[12]. Furthermore the universal disturbances of the lipoprotein system in the rarely encountered familial LCAT deficiency[13], result in hypertriglyceridaemia without a diminution in the activity of lipoprotein lipase, but with the appearance of LP-X in the plasma of these patients[14]. As in cholestasis, the formation of these vesicles may be due to particular physicochemical conditions. A secondary LCAT deficiency in cholestasis is neither a cause nor a necessity for the formation of LP-X. This is already apparent from the finding that in acute cholestasis the activity of this enzyme may be increased, although LP-X is present and the concentration of total cholesterol is normal. It is now established that LP-X is neither a substrate for LCAT nor for post-heparin plasma lipolytic activity[15].

Results of the latest unpublished studies from our laboratory concerning the catabolism of LP-X reveal that LP-X is, like other macromolecules, but in contrast to normal plasma lipoproteins, taken up and catabolized in the reticuloendothelial system, primarily in the spleen (Table 11.2). It is further-

Table 11.2 Distribution of [^{125}I]albumin labelled LP-X in various tissues of rats after intravenous administration (cpm/g tissue ($\times 10^5$))

Tissue	*1 h*	*3 h*	*6 h*	*24 h*
Liver	6.3	4.7	2.6	0.6
Spleen	42.4	24.4	16.2	3.1
Other tissues	0.3–2.0	0.2–1.0	0.2–0.6	0.1–0.3

Table 11.3 Effect of LDL or LP-X on the HMG-CoA reductase activity in isolated perfused livers or isolated human lymphocytes

	% HMG-CoA reductase activity at 210 min
Perfused livers	
Control	200
LDL	250
LP-X	500
Isolated lymphocytes	
Fetal calf serum	100
LDS	300
LDL	80
LP-X	120

The activity of HMG-CoA reductase was measured in the microsomes from livers perfused with LDL or LP-X (40 mg/dl cholesterol concentration). The activity at 0 min represents 100%. Isolated human lymphocytes were incubated for 24 h in 15% fetal calf serum or lipoprotein-deficient serum (LDS). LDL or LP-X was added to culture dishes (~125 μg cholesterol/ml). After 24 h incubation the enzyme activity was assayed. The activity in fetal calf serum represents 100%

Table 11.4 Serum clinical chemistry and the activity of HMG-CoA reductase in lymphocytes from cholestatic patients

Patient	*Total cholesterol* (mg/dl)	*Serum* *Triglyceride* (mg/dl)	*LP-X*	γ-GT (U/l)	AP (U/l)	GOT (U/l)	GPT (U/l)	*Lymphocytes HMG-CoA reductase* (pmol. mg protein^{-1}. h^{-1})
W.L.	215	259	+ +	372	369	78	120	0
B.M.	75	54	+	106	258	35	74	11.5
B.E.	469	139	+ + +	918	1500	44	72	1.3
Controls ($n = 10$)	150–260	50–170	–	≤18 F 28 M	≤190	≤18	≤22	54.8 ± 6.7

more of importance that LP-X does not exert the negative feedback regulation of hepatic cholesterol synthesis found with apoB and apoE containing lipoproteins. In contrast, in the isolated perfused liver LP-X even stimulates the key enzyme of cholesterol synthesis, hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) by a factor of 2.5 (Table 11.3). However incubation of human lymphocytes with LP-X or LDL inhibits the activity of this enzyme. In accordance with this, the lymphocytes isolated from patients with cholestatic liver disease show a markedly reduced activity of this enzyme, as compared to those isolated from healthy controls (Table 11.4). Moreover studies on receptor binding indicate that isolated hepatocytes or fibroblasts exhibit a low degree of binding and uptake of LP-X, whereas in lymphocytes high rates are observed.

At least four different factors may be responsible for the elevated activity of HMG-CoA reductase seen in cholestasis:

(1) interruption of the enterohepatic circulation of the bile salts and cholesterol;
(2) elevation in the concentration of bile salts in the liver cells;
(3) a depletion of hepatocellular cholesterol by LP-X because of its high ratio of lecithin to cholesterol; and
(4) inhibition of the uptake of chylomicron remnants by the liver.

The information obtained so far from the various studies reported does not provide any clear understanding of this problem[16–18]. With regard to point (1) it is established that the enterohepatic circulation of steroids influences hepatic cholesterol synthesis. It is, however, difficult to estimate to what extent an interruption, as in cholestasis, may cause an increase in hepatic cholesterol synthesis in this situation.

With regard to point (2) it is not clear whether increased concentrations of bile salts in the liver cells affect cholesterol synthesis. However the results obtained by incubating isolated microsomes with LP-X or in the presence of bile salts do not support this idea (Table 11.5).

Table 11.5 Effect of LP-X or bile salt mixtures in varying concentrations on HMG-CoA reductase activity in isolated liver microsomes

LP-X cholesterol (μg in test)	*Bile salt concentration in LP-X* (nmol in test)	*Percentage change in enzyme activity*
22.9	1.07	(−) 24
91.6	4.28	(−) 43
114.5	5.35	(−) 46

LP-X cholesterol (μg in test)	*Bile salt mixture* (nmol in test)	*Percentage change in enzyme activity*
—	10.0	(+) 3
—	20.0	(+) 4
—	30.0	(−) 7

With regard to point (3), the presence of LP-X deserves attention, because of the high phospholipid content of this particle. Phospholipids are well known for leaching of cellular cholesterol, and thus may be partly responsible for the hypercholesterolaemia of cholestasis. In the case of the erythrocytes, a fusion of LP-X with the cell membrane may even take place, leading to a change in the fluidity of the membrane[19]. This expands the surface area of these cells and hence they are termed target cells[19-21].

With regard to point (4) we were recently able to show that the diet-induced negative feedback of hepatic cholesterol synthesis, which is most likely mediated by the apoE receptors of the hepatocytes, is disturbed by LP-X. The uptake of chylomicron remnants by isolated hepatocytes is significantly inhibited by LP-X in a concentration-dependent manner (Table 11.6). These findings are important for the understanding of hypercholesterolaemia in cholestasis.

Table 11.6 Binding or uptake of [^{125}I]albumin-labelled LP-X by isolated hepatocytes, lymphocytes or fibroblast cultures (ng LP-X protein bound/mg cell protein)

Concentration of LP-X in the medium (μg LP-X protein/ml)	*Hepatocytes*	*Fibroblasts*	*Lymphocytes*
5	75	200	450
10	150	400	925
15	225	562	1350

Isolated hepatocytes from rat liver, isolated human lymphocytes or fibroblast cultures were incubated with [^{125}I]albumin-labelled LP-X for 2 h at 37°C; at the end of the incubation period cells or cultures were thoroughly washed and radioactivity was measured in the cells

Hypertriglyceridaemia occurring as a consequence of liver damage by alcohol is well known, but not fully understood. The metabolic situation is complicated, as acute and chronic intake of alcohol may bring about opposing disturbances in metabolic regulation. Moreover the quantity of alcohol consumed seems to be very important. The well-documented increase in the concentration of plasma HDL following a constant, but non-toxic, intake of alcohol may be caused by a reduction in the activity of hepatic triglyceride lipase, which might be responsible for HDL removal from the vascular space through its phospholipase activity[22]. Elevated concentrations of α-lipoprotein due to high alcohol intake return rapidly to normal after alcohol withdrawal (Figure 11.4). It should be emphasized that no other exogenous factor influences HDL cholesterol concentrations in the same manner or magnitude as alcohol. Unfortunately this phenomenon is often underestimated in the evaluation of HDL cholesterol as a risk indicator for coronary heart disease.

The existence of hypertriglyceridaemia due to liver dysfunction not associated with alcohol has been recognized only in the last few years. Clinical studies have indicated that hypertriglyceridaemia is frequently accompanied by severe cholestasis[23]. The fasting plasma of these patients

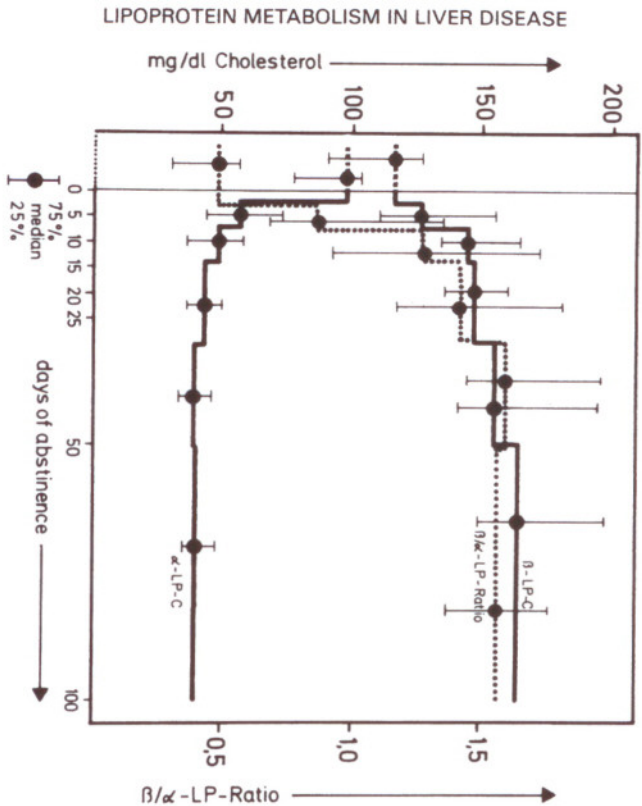

Figure 11.4 Follow-up of lipoprotein concentrations in days of abstinence after heavy alcohol drinking (120 g/d); $n = 15$

does not contain chylomicrons. The main increase in the triglyceride concentration is found in the LDL II density class. Triglyceride-rich, cholesterol poor apoB, apoC and apoE containing intermediate-density lipoproteins account for the accumulation of triglycerides in this density fraction. These particles are heterogeneous in size, ranging from 300 to 800 Å, and are thus much larger than normal LDL (200–250 Å). Dietary studies have indicated that these particles are remnants derived from a disturbed catabolism of chylomicrons. This accumulation may be caused by diminution in the activity of hepatic triglyceride lipase or to a disturbed hepatic uptake of the particles, as described above.

The HDL density class 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, but chromatographic analysis of this density class reveals an unusual degree of heterogeneity of the particles[22]. Under the electron microscope the particles show formation of rouleaux, similar to LP-X or nascent HDL particles. The rates of catabolism and elimination of apoA-I are

increased by 2–4-fold in acute alcohol hepatitis, as compared with healthy control subjects[24]. From this it is concluded that the diminished plasma concentration of apoA found in such patients is primarily due to its accelerated catabolism, rather than to a disturbed synthesis, a situation which was similarly described for Tangier disease[25]. The apoA-I and apoA-II in the plasma of patients with liver disease often show a complete dissociation[26]. It is possible that this dissociation of the two apoA subunits is a major reason for its accelerated catabolism. If this is true, it emphasizes the importance of the structural properties of a lipoprotein with respect to the processes regulating its metabolism.

In this regard the results obtained from the quantification of apoA-I during the course of hepatic disease are of interest (Figure 11.5). Measure-

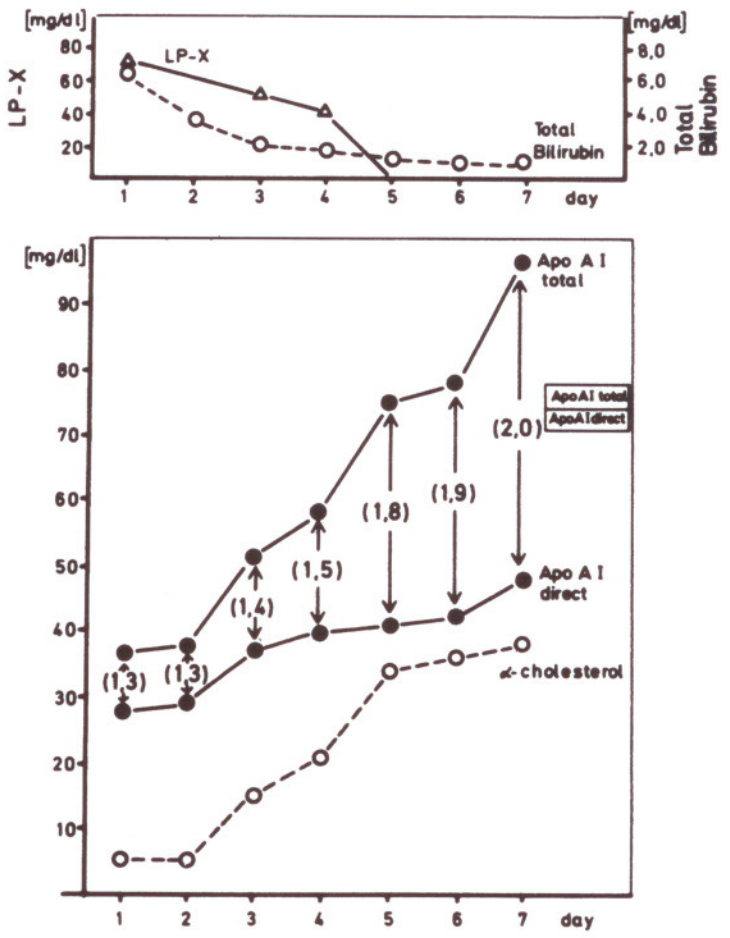

Figure 11.5 Apolipoprotein A-I concentration during the course of intrahepatic cholestasis

ment of apoA-I using sheep anti-sera and rate nephelometry[27] reveals a significant decrease in both α-lipoprotein cholesterol and total apoA-I during the severe stage of the disease. Also the ratio of directly measurable apoA-I to total (after delipidation) measurable apoA-I is decreased from about 2.4, the value established for healthy subjects, to about 1.3, indicating a high degree of accessibility of the protein to the antibody. This is in agreement with structural alteration of the α-lipoprotein particles in the disease. Under normal conditions the major portion of apoA-I is not situated on the outer part of the surface of the particle, and is therefore not fully accessible to the antibody produced against isolated apoA-I. With increasing concentrations of α-lipoprotein cholesterol the ratio of total to directly measurable apoA-I also increases during the course of the disease (Figure 11.5). This may reflect an improvement in the synthetic function of the liver and/or a recovery of the intravascular lipolytic system, which seems to be important for the generation of mature α-lipoprotein from nascent HDL[28].

SUMMARY

Liver dysfunction is followed by complex disturbances affecting the total lipoprotein system. The basic defect may not be in the disturbed hepatic biosynthesis of the lipoproteins, but rather in the lipolytic activity of the vascular space. This in turn may be influenced by the rates of both synthesis and secretion of lipolytic enzymes by the liver and the unphysiological reservoir, primarily for apoC and apoD in the form of LP-X, which may interfere with the necessary exchange of these apoproteins between the various lipoproteins during their interrelated catabolism. This suggestion is supported by the finding that there are structural similarities between nascent VLDL and nascent HDL with the characteristics of VLDL and HDL accumulating in patients with liver disease. The effect of a primary and secondary diminished activity of lipase and LCAT may well be closely and causally linked to these pathobiochemical mechanisms.

The characteristic stimulation of hepatic cholesterol synthesis in cholestasis seems to be largely brought about by changes in the lipid composition of the membrane, caused by depletion of cellular cholesterol by LP-X due to its high ratio of phospholipid to cholesterol and/or by an inhibition of the cellular uptake of remnants, mediated by LP-X.

Most reported abnormalities of the lipoprotein system in liver disease are secondary, and return to normal with clinical improvement. Most of them are not disease-specific, but may be useful in monitoring the course of the disease. A careful analysis of the lipoprotein system, together with a critical interpretation of such data, may add not only to a better understanding of the pathobiochemistry of the disease, but also to an elucidation of the mechanisms regulating normal lipid transport.

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References

1. Flint, A., Jr. (1862). Experimental researches into a new excretory function of the liver, consisting in the removal of cholesterine from the blood, and its discharge from the body in the form of stercorine. *Am. J. Med. Sci.*, **44**, 305
2. van Tol, A., van Gent, T., van 'T Hooft, F.M. and Vlaspoldr, F. (1978). High density lipoprotein catabolism before and after partial hepatectomy. *Atherosclerosis*, **29**, 439
3. Fielding, C.J. and Fielding, P.E. (1982). Cholesterol transport between cells and body fluids. In Havel, R.J. (ed.), *The Medical Clinics of North America*. Vol. 66, no. 2, pp. 363–373. (Philadelphia: W.B. Saunders)
4. Switzer, S. (1967). Plasma lipoproteins in liver disease. I. Immunologically distinct low-density lipoproteins in patients with biliary obstruction. *J. Clin. Invest.*, **46**, 1855
5. Seidel, D.C., Alaupovic, P., Furman, R.H. and McConathy, W.J. (1970). A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. *J. Clin. Invest.*, **49**, 2396
6. Seidel, D., Agostini, A. and Müller, P. (1972). Structure of an abnormal plasma lipoprotein (LP-X) characterizing obstructive jaundice. *Biochim. Biophys. Acta*, **260**, 146
7. Papahadjopoulos, D. and Miller, N. (1967). Phospholipid model membranes. I. Structural characteristics of hydrated liquid crystals. *Biochim. Biophys. Acta*, **135**, 624
8. Seidel, D., Gjone, E., Blomhoff, J.P. and Geisen, H.P. (1974). In Greten, H., Levine, R., Pfeiffer, E.F. and Renold, R.E. (eds.), *Hormone and Metabolic Research*. Suppl. 4, pp. 6–11. (Stuttgart: Thiéme)
9. Seidel, D. (1977). Studien zur Charakterisierung und zum Stoffwechsel des Lipoprotein-X (LP-X), des abnormen Lipoproteins der Cholestase. *Klin. Wochenschr.*, **55**, 611
10. Mordasini, R.C., Berthold, S., Schlumpf, E., Keller, H. and Riva, G. (1975). Lipoprotein-X bei hepatobiliären Erkrankungen. *Schweiz. Med. Wochenschr.*, **105**, 863
11. Manzato, E., Fellin, R., Baggio, G., Neubeck, W. and Seidel, D. (1976). Formation of lipoprotein-X: its relationship to bile compounds. *J. Clin. Invest.*, **57**, 1248
12. Seidel, D., Greten, H., Geisen, H.P., Wengeler, H. and Wieland, H. (1972). Further aspects on the characterization of high and very low density lipoproteins in patients with liver disease. *Eur. J. Clin. Invest.*, **2**, 359
13. Gjone, E. (1983). LCAT deficiency. This volume, Chapter 8
14. Torsvik, H., Berg, K., Magnani, H.N., McConathy, W.J., Alaupovic, P. and Gjone, E. (1972). Identification of the abnormal cholestatic lipoprotein (LP-X) in familial lecithin : cholesterol acyltransferase deficiency. *FEBS Lett.*, **24**, 165
15. Bartholomé M., Niedmann, D., Wieland, H. and Seidel, D. (1981). An optimized method for measuring lecithin: cholesterol acyltransferase activity, independent of the concentration and quality of the physiological substrate. *Biochim. Biophys. Acta*, **664**, 327
16. Kattermann, R. and Creutzfeldt, W. (1970). The effect of experimental cholestasis on the negative feed-back regulation of cholesterol synthesis in rat liver. *Scand. J. Gastroenterol.*, **5**, 337
17. Weiss, J.H. and Dietschy, J.M. (1971). Presence of an intact cholesterol feed back mechanism in the liver in biliary stasis. *Gastroenterology*, **61**, 77
18. Cooper, A.D. and Ockner, R.K. (1974). Studies of hepatic cholesterol synthesis in experimental acute biliary obstruction. *Gastroenterology*, **66**, 586
19. Cooper, R.A., Diloy-Puray, M., Lando, P. and Greenberg, M.S. (1970). An analysis of lipoproteins, bile acids, and red cell membranes associated with target cells and spur cells in patients with liver disease. *J. Clin. Invest.*, **51**, 3182
20. Owen, J.S., Bruckdorfer, K.R., Day, R.C. and McIntyre, N. (1982). Decreased erythro-

cyte membrane fluidity and altered lipid composition in human liver disease. *J. Lipid Res.*, **23**, 124
21. Okano, Y., Iida, H., Takahashi, M., Sekiya, T., Hasegawa, I. and Nozawa, Y. (1981). Erythrocyte membranes in biliary obstruction: Phospholipase A_2-induced alterations in lipid composition and morphology. *Biochem. Med.*, **26**, 314
22. Sabesin, S. M., Hawkins, H. L., Kuiken, L. and Ragland, J. B. (1977). Abnormal plasma lipoproteins and lecithin : cholesterol acyltransferase deficiency in alcoholic liver disease. *Gastroenterology*, **72**, 510
23. Müller, P., Fellin, R., Lambrecht, J., Agostini, B., Wieland, H., Rost, W. and Seidel, D. (1974). Hypertriglyceridemia secondary to liver disease. *Eur. J. Clin. Invest.*, **4**, 419
24. Nestel, P. J., Tada, N. and Fidge, N. H. (1980). Increased catabolism of high density lipoprotein in alcoholic hepatitis. *Metabolism*, **29**, 101
25. Schaefer, E. J., Blum, C. B., Levy, R. I., Goebel, R., Brewer, H. B. and Berman, M. (1976). High density lipoprotein metabolism in Tangier disease. *Circulation*, **54**, suppl. II, 11
26. Seidel, D., Greten, H., Geisen, H. P., Wengeler, H. and Wieland, H. (1972). Further aspects on the characterization of high and very low density lipoproteins in patients with liver disease. *Eur. J. Clin. Invest.*, **2**, 359
27. Weinstock, N., Bartholomé, M. and Seidel, D. (1981). Determination of apolipoprotein-A I by kinetic nephelometry. *Biochim. Biophys. Acta*, **663**, 279
28. Havel, R. J. (1982). Approach to the patient with hyperlipidemia. In Havel, R. J. (ed.), *The Medical Clinics of North America*, Vol. 66, no. 2, 319–333. (Philadelphia: W. B Saunders)