Europ. J. clin. Invest. 4, 419—428 (1974)

Hypertriglyceridaemia Secondary to Liver Disease

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Received: December 13, 1973 and in revised form: March 18, 1974

Abstract. Some patients with liver dysfunction show a marked hypertriglyceridaemia, a phenomenon most frequently accompanied by cholestasis.

In this report we have identified, isolated and characterized an abnormally large (300–700 Å), triglyceride rich, low density lipoprotein, (d 1.019–1.063 g/ml) designated β_2-lipoprotein (β_2-LP), from the plasma of patients with hypertriglyceridaemia secondary to liver disease. The β_2-LP differs significantly in its percent composition and protein moiety from the unique lipoprotein-X (LP-X) specific for cholestasis and also from normal β-lipoproteins, both of which are also present in the patients LDL fraction. Furthermore, some evidence is provided suggesting that the β_2-LP is likely to represent an intermediate particle of chylomicron metabolism, which accumulates in this disease due to a markedly diminished hepatic lipase activity.

Key words: Plasma lipoproteins, hypertriglyceridaemia, lipolysis, enzymes, liver disease.

Abnormal serum lipid patterns are often associated with abnormal liver function. In the past substantial evidence has accumulated indicating that the characteristic elevation of unesterified cholesterol and phospholipids in patients with cholestasis is due to the presence of a low density lipoprotein (LDL) of abnormal composition and properties [1–4], designated LP-X. Recently, it has also been demonstrated that various liver disorders are often associated with decreased concentrations of serum α- and pre-β-lipoproteins, when lipoprotein electrophoresis is applied [5–7]. Further studies on these two lipoprotein fractions [7] provided evidence that the decreased concentration of high density lipoproteins (HDL) is primarily due to an impaired lipid binding capacity of apo-lipoprotein A (apo-A), the major protein portion of HDL, resulting in an abnormally high protein/lipid ratio of the fraction, which does not stain with lipid stain. Very low density lipoprotein (VLDL) concentration in these patients is generally not reeduced but lacking its apo-A protein moiety, causing an alteration of electrophoretic mobility from the pre-β- to the β-position [7]. Only recently particular attention has been paid to the hypertriglyceridaemia, which may be found in some patients with liver dysfunction [8–11], a phenomenon most frequently accompanied by severe cholestasis [8–11]. It has been demonstrated that major amounts of triglycerides in such patients are found within the LDL fraction [8, 10, 11] and it was suggested [8, 10, 11] that this may be due to the presence of an abnormal LDL, different from LP-X and rich in triglycerides. In the present study we have identified, isolated and characterized an abnormally large, triglyceride rich LDL in patients with liver dysfunction and provided some evidence for its source and for a possible mechanism, responsible for the accumulation of this abnormal plasma lipoprotein.

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Material and Methods

Patients

Blood samples were obtained from 32 hospitalized patients (aged 19–66 years) with various forms of liver disease. The diagnosis was confirmed by clinical evidence, liver function tests and liver biopsy or laparotomy (see Table 1). 10 patients showed plasma triglyceride levels within the normal range (50–150 mg/100 ml), 22 patients were hypertriglyceridaemic at the time their serum was investigated. The serum was prepared by low speed centrifugation from fresh blood samples after an overnight fast. 1.7 mM EDTA was added to all serum samples and lipoprotein fractions.

Chemical analyses

Lipoprotein-X (LP-X) was determined as previously described [12, 13] using the "Rapidophor all in for LP-X" (Immuno AG, Vienna, Austria). Agarose-agar electrophoresis was performed by a modification [14] of Noble's method [15]; the lipoprotein bands were visualized by the use of polyanionic compounds (0.2 M $CaCl_2$: 0.6% Na-dextransulphate 500) [12]. The immunochemical properties of isolated lipoproteins and lipoproteins in whole serum were studied by double diffusion [16] and immunoelectrophoresis [17] in 1% agar- (Difco Bacto Agar, Detroit, Mich.) or 0.8% agarose (Serva, Heidelberg) gels employing a barbital buffer; pH 8.6, ionic strength 0.05. The plates were developed as previously described [4]. Rabbit anti-sera to apo-lipoprotein A (apo-A; consisting of the A I and A II peptides), apo-lipoprotein B (apo-B) and apo-lipoprotein C (apo-C; consisting of the C I, C II and C III peptides) were prepared in our own laboratory [4] and carefully tested for their specifity,

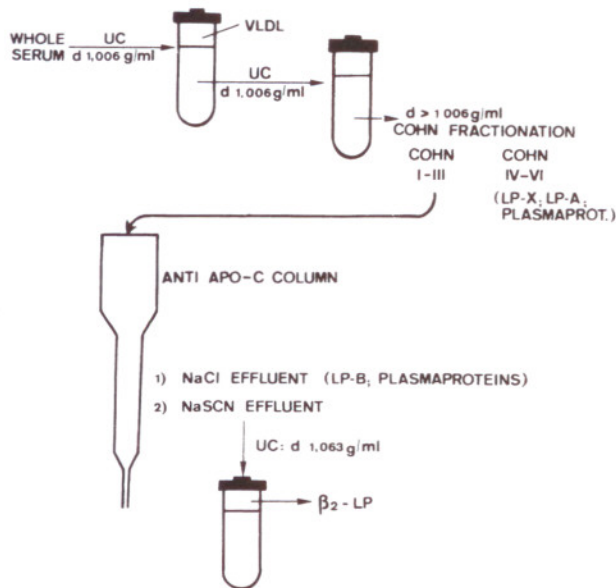

Fig. 1. Procedure for the isolation of β_2-lipoprotein from the serum of patients with severe cholestatic liver dysfunction

employing immunoelectrophoretic techniques on human plasma, isolated homogeneous lipoprotein fractions and apo-lipoprotein preparations, characterized by polyacrylamide electrophoresis. Anti-bodies to human albumin and human gamma globulins were purchased from Behring-Werke AG; Marburg a.d. Lahn. The protein portion of some lipoprotein fractions was also studied by polyacrylamide electrophoresis after treatment with tetramethylurea (TMU) according to a previously described method (18). Esterified and unesterified cholesterol, phospholipids and protein were determined as described earlier (3). Triglycerides and free glycerol were measured enzymatically using a standard procedure (Boehringer GmbH, Mannheim). To determine the relative amount of triglycerides present in the VLDL fraction ($d < 1.006$ g/ml) and in the $d > 1.006$ g/ml serum fraction, triglycerides were measured in whole fresh serum and in the corresponding d 1.006 g/ml top and bottom fraction after preparative ultracentrifugation (Spinco Model L 2 65 B Ultracentrifuge; type Ti 60 Rotor; 55000 rpm; 22 h at 4°C). The recovery of triglycerides in the isolated fractions varied between 81 and 95%. For the calculation of the [VLDL-TG]/[> 1.006 g/ml-TG] ratio the measured values were used, taking the fraction volumes into consideration. To express the absolute amount of VLDL-TG an individual correction factor was applied for the loss of triglycerides in each preparation. Total bilirubin, alkaline phosphatase, *AST*, *ALT*, sodium, potassium, calcium, inorganic phosphorus, uric acid, creatinine and urea were analysed employing a Technicon Autoanalyzer (SMA) 12/60, Technicon Inst. corp. Tarrytown, N.Y.). Determination of lipolytic activity:

The plasma lipolytic rate, that is, the rate of hydrolysis of plasma triglycerides, was determined in two healthy volunteers and two patients with severe cholestatic hepatitis. The assessment of lipolytic activity was based on the properties of intravenously applied heparin to release lipolytic activity into the plasma where it acts upon triglyceride rich lipoproteins at a rate that can be measured *in vitro* by the production of free glycerol. Heparin (Liquemin 5000, Hoffmann-La Roche AG, Grenzach) was given in a single dose of 50 U/kg body weight intravenously to the fasting subjects. 10 min. after heparin administration blood samples (50 ml) were obtained and the plasma recovered by low speed centrifugation at room temperature (8000 rpm for 10 min.). Immediately after separation of the plasma, previously delipidized bovine serum albumin (Behring-Werke, Marburg/Lahn) in a final concentration of 10% was added to the serum and to one aliquot protamine sulphate (Hoffmann-La Roche AG, Grenzach) was added to a final concentration of 3 mg/ml. Following 10 min. of incubation at 27°C the concentration of free glycerol was determined in both aliquots and taken for O-values. The rate of lipolysis was obtained by measurement of free glycerol produced during further incubation. Plasma lipolytic rate was expressed as mol free glycerol formed during incubation per ml of serum.

On the same subjects total postheparin lipolytic activity (PHLA) was also determined in the laboratory of Dr. H. Greten, Heidelberg as previously described in detail (19), using [1-^{14}C] trioleate as substrate. In the experiment the final concentration of NaCl was 0.15 M that of Tris-HCl 0.2 M (pH 8.4) in both aliquots, that of protamine sulphate 3 mg/ml in one aliquot. The preincubation time with protamine sulphate (3 mg/ml) was 10 min. at 27°C. Enzyme activity was calculated as nmol FFA released per ml/h.

Electron microscopy

Electron microscopy was performed on various lipoprotein fractions after negative staining at 0°C and at 25°C with a 1% aqueous solution of phosphotungstic acid, adjusted to pH 7.1 with KOH according to standard procedures, previously described in detail (20). Grids were examined in a Siemens Elmiskop 101 electron microscope (Siemens AG, Berlin) equipped with a cooling device and a double condensor illumination. Pictures were taken at magnifications ranging from X 20000 to 50000.

Isolation and Fractionation of Lipoproteins

The separation scheme is outlined in Fig. 1. The VLDL fraction ($d < 1.006$ g/ml) was separated by layering the serum samples under equal volumes of NaCl solutions (d 1.006 g/ml) containing 1.7 mM EDTA and by centrifugation in a type Ti 60 rotor of a Spinco Model L2 65 B Ultracentrifuge (Beckman Instruments, Fullerton, Calif.) for 22 h at 55000 rpm and

4°C. The top fraction was removed by a tube-slicing technique and the bottom fraction recentrifuged under identical conditions. The d > 1.006 g/ml density class was further fractionated using a cold (−5°C) ethanol precipitation technique according to Cohn (21) as previously described in detail (3). The filtrate corresponding to Cohn fractions IV-VI contained plasma proteins, α-lipoproteins (LP-A) and LP-X which was isolated according to our method described earlier (3). The dissolved precipitate (3) after ethanol precipitation corresponding to Cohn fractions I-III contained plasma proteins, normal β-lipoproteins (LP-B) and an abnormally large lipoprotein, rich in triglycerides with β-mobility on agarose electrophoresis and therefore designated β_2-lipoprotein (β_2-LP). β_2-lipoprotein was purified from Cohn fractions I-III, devoid of VLDL by affinity chromatography (22) on Sepharose 4 B (Pharmacia, Uppsala) containing a covalently linked monospecific rabbit anti-human apo-C globulin fraction. The non-bound normal β-lipoproteins together with other plasma proteins were removed from the column by excessive elution with 0.15 M NaCl, pH 7.2, containing 1.7 mM EDTA. The normal β-lipoproteins (LP-B) were then isolated by ultracentrifugation at the density d 1.063 g/ml under standard conditions. The bound β_2-lipoproteins were then obtained by elution of the column with 3 M NaSCN. Immediately after recovery this fraction was dialysed exhaustively for 24 h at 4°C against 0.01 M tris-buffer, pH 7.2 containing 0.15 M NaCl and 1.7 mM EDTA. After dialysis the solution density was adjusted to d 1.063 g/ml by adding KBr in appropriate amounts and the fraction submitted to ultracentrifugation (Rotor type 65, 55000 rpm, 22 h, 4°C). The top fraction containing the isolated β_2-LP was recovered by a tube-slicing technique and prior to all analyses dialysed for 24 h at 4°C against 0.15 M NaCl containing 1.7 mM EDTA. To establish the exact hydrated density in separate experiments, isolated β_2-LP was recentrifuged at densities d 1.019 and 1.063 g/ml and was found to be within that range.

Results

The majority of patients in our study (15 out of 22) with hypertriglyceridaemia and liver disease showed an abnormal low density lipoprotein (β_2-lipoprotein; β_2-LP) in their fasting serum different from LP-X and normal β-lipoproteins. On the basis of the immunoelectrophoretic pattern described in this paper this lipoprotein compound cannot be identified in liver patients without hypertriglyceridaemia (Table 1). In general, the occurrence of β_2-LP is accompanied by either intra-or extrahepatic cholestasis, although cholestasis as judged on the basis of LP-X as the most specific parameter (23, 24) may exist without β_2-LP. This correlation holds for patients either with or without hypertriglyceridaemia and liver disease (Table 1). Although, the absolute amount of triglycerides present in the VLDL fraction of patients with β_2-LP is generally slightly increased, the relative triglyceride concentration of VLDL is always markedly decreased (Table 2) indicating triglyceride rich lipoproteins in the d > 1.006 g/ml serum fraction.

Immunoelectrophoretic analysis of the patients LDL fraction in 1% agar gel (Fig. $2_{(2)}$) clearly indicates 3 different lipoprotein compounds in contrast to one lipoprotein in the serum LDL fraction of healthy controls (Fig. $2_{(1)}$). While normal LDL reacts with one arc against anti-apo B serum and shows no immunoreaction with anti-apo C serum, the patients LDL fraction shows one compound migrating towards the cathode reacting only with anti-apo C serum, a second compound at the origin reacting with both anti-apo B and anti-apo C serum and a third compound comparable

Table 1. Incidence of β_2-lipoprotein and clinical-chemical data of 22 patients with hypertriglyceridaemia and liver dysfunction and of 10 patients without hypertriglyceridaemia and liver dysfunction. Range—Values are given in parenthesis

Diagnosis	*n*	Serum-TG mg/100 ml	VLDL-TG / > 1.006 g/ml-TG	n with β_2-LP	n with LP-X	Total bilirubin mg/100 mg	AST, U/L EC No. 2.6.1.1	ALT, U/L EC No. 2.6.1.2	ALP, U/L EC No. 3.1.3.1	GMT, U/L EC No. 2.3.2.1
Hypertriglyceridaemia										
Acute hepatitis	11	386 (250–577)	0.37 (0.10–0.72)	11	10	10.3 (1.2–17.0)	369 (95–640)	390 (85–660)	232 (130–465)	127 (40–375)
	6	335 (255–400)	2,91 (1.90–3.90)	0	2	12.0 (0.6–24.0)	387 (40–820)	519 (120–940)	231 (150–390)	66 (28–120)
Chronic hepatitis	2	480; 345	0.21 (0.19; 0.23)	2	2	2,3 (2.0; 2.5)	149 (117; 180)	105 (100; 110)	237 (225; 250)	69 (68; 70)
	1	220	2,1	0	0	1,8	122	99	125	33
Extrahepatic biliary obstruction	2	470; 191	0.39 (0.31; 0.47)	2	2	15.7 (14.2; 17.1)	71 (30; 112)	103 (35; 170)	433 (325; 540)	303 (275; 330)
Acute hepatitis without hyper-triglyceridemia	10	122 (52–148)	1,90 (1.88–2.41)	0	3	4.9 (0.7–13.0)	177 (24–720)	183 (88–600)	158 (45–450)	72 (20–137)

Table 2. Serum triglyceride concentration, triglyceride distribution and clinical—chemical data of all patients in whom the presence of β_2-LP was demonstrated

No.	Diagnosis	Serum-TG mg/100 ml	VLDL-TG mg/100 ml	VLDL-TG > 1.006 g/ml-TG	Total bilirubin mg/100 ml	AST, U/L EC No. 2.6.1.1	ALT, U/L EC No. 2.6.1.2	ALP, U/L EC No. 3.1.3.1	GMT, U/L EC No. 2.3.2.1	LP-X
1	acute	380	159	0.72	12.0	450	240	210	210	pos.
2	hepatitis	390	115	0.42	8.8	210	250	140	250	pos.
3		484	106	0.28	10.3	520	650	195	40	pos.
4		577	128	0.28	15.0	640	560	465	110	pos.
5		350	107	0.44	17.0	320	450	130	100	neg.
6		290	90	0.45	1.2	95	135	205	69	pos.
7		353	102	0.40	9.1	220	660	180	60	pos.
8		250	28	0.12	11.1	580	560	350	42	pos.
9		280	97	0.53	6.0	275	85	295	375	pos.
10		500	113	0.29	14.1	540	450	190	48	pos.
11		395	36	0.10	8.8	210	250	190	100	pos.
12	chronic	480	90	0.23	2.0	180	110	250	70	pos.
13	hepatitis	345	57	0.19	2.5	117	100	225	68	pos.
14	extrahepatic	470	113	0.31	17.1	112	170	325	275	pos.
15	biliary obstruction	191	61	0.47	14.2	30	35	540	330	pos.
Mean		382	96	0.34	10.0	300	314	259	143	
Normal		50–150	66[40]	2.0[40]	<1.0	<17	<33	<170	<20	neg.

to that of normals migrating toward the anode and reacting only with anti-apo B serum. These three lipoprotein compounds can be separated and isolated as outlined under "Methods" using a combination of ultracentrifugation, cold ethanol fractionation and affinity chromatography with specific antibodies. The lipoprotein with a migration toward the cathode (Fig. $2_{(3)}$) was identified as the lipoprotein characteristic of cholestasis (LP-X). The lipoprotein that migrates toward the anode and reacts only with anti-apo B serum (Fig. $2_{(5)}$) represents normal β-lipoprotein as judged by its protein lipid composition and immunological properties. The lipoprotein without electrophoretic mobility in 1% agar gel, pH 8.6, reacting with anti-bodies to apo B and apo C will be designated β_2-lipoprotein (β_2-LP). It shares common characteristics with normal β-lipoproteins such as the hydrated density (d 1.019–1.063 g/ml); the electrophoretic mobility on agarose gel (Fig. 3) (it migrates only slightly ahead of normal β-lipoproteins in this medium) and furthermore it contains apo B as part of its protein moiety.

Because of its characteristic electrophoretic behaviour in 1% agar gel and its specific immunological properties the β_2-LP may be identified already in whole serum before isolation (Fig. 4). Fig. $4_{(1)}$ represents the serum pattern of a patient with intrahepatic cholestasis due to acute hepatitis without hypertriglyceridaemia. LP-X but not β_2-LP can be identified. Fig. $4_{(2)}$ and Fig. $4_{(3)}$ are immunoelectrophoretical patterns obtained from different patients with cholestasis, hypertriglyceridaemia and a marked decrease of their [VLDL-TG]/[> 1.006 g/ml-TG] ratio. In both cases LP-X and β_2-LP (indicated by the arrow) were identified. The absolute amount of VLDL-TG in sample Fig. $4_{(2)}$ was low (36 mg/100 ml; Patient No. 11, Table 2) and that of Fig. $4_{(3)}$ high (106 mg/100 ml; Patient No. 3, Table 2) which corresponds to a weak (Fig. $4_{(2)}$) or to a stronger (Fig. $4_{(3)}$) precipitin band toward the anode with anti-apo C serum.

The immunological properties of isolated β_2-LP were tested by double immunodiffusion and immunoelectrophoresis. This lipoprotein never reacted with anti-bodies to human albumin or human globulins and no constant immunoreaction was obtained with anti-apo A serum (2 out of 5 preparations develop a very faint line). Each of 5 different preparations however, showed a precipitin line in identity with anti-bodies to apo B and apo C in both double immunodiffusion (Fig. 5) and immunoelectrophoresis (Fig. 3) clearly indicating that these two apo-lipoproteins take part in the structure of this plasma lipoprotein. The polyacrylamide pattern of tetramethylurea treated β_2-LP revealed all bands typical for the three apo-lipoprotein C peptides. Studies characterizing the precise chemical nature of the different polypeptides present in β_2-LP and the determination of their relative amounts are in progress.

The percent composition of β_2-LP (Table 3) differs significantly from VLDL, normal β-lipoproteins, α-lipoproteins and lipoprotein-X. An unusually high content of triglycerides (35%) and a low content of cholesterol (18%) with a decreased free cholesterol/total cholesterol ratio in comparison to normal β-lipoproteins represents the unique characteristic of the chemical composition of this low density lipoprotein.

Table 3. Percent protein lipid composition of isolated β_2-lipoprotein in comparison to normal lipoproteins and to lipoprotein—X

The data for β_2-LP represent mean values of five different preparations. The values in parenthesis represent the highest and lowest value obtained.

Fraction	Protein	TG	Ch/E	UE/Ch	PL
VLDL (41) d < 1.006	10	55–65	5	10	15–20
LDL (41) d 1.006–1.063	25	10	37	8	22
LP-X (3) d 1.006–1.063	6	3	2	23	66
HDL (41) d 1.063–1.21	50	3	15	3	30
β_2-LP	30	35	10	8	16
d 1.019–1.063	(29–31)	(32–37)	(9–12)	(7–9)	(15–17)

In the electron microscope preparations of Cohn fractions I-III from five different patients with hypertriglyceridaemia and β_2-LP present in their plasma appeared to consist of more or less round particles of different size. As it is shown in Fig. 6 (A) from a typical case, most particles reveal a mean diameter of about 200 Å, which is the range of that of normal β-lipoproteins. However, several large particles representing the β_2-LP (arrows) with a diameter ranging from 300 to 700 Å, are also visible. The latter are surrounded by the smaller particles but no aggregation or fusion of individual β_2-LP particles can be observed. Storing of a Cohn fraction I-III for several days usually resulted in breakdown products of the larger particles. Ellipsoidal or disk like configurations or formation of stacks of flattened particles like those described for LP-X in patients with cholestatic jaundice (20, 25) could never be detected in this fraction. Fig. 6 (B) represents an electron micrograph of normal β-lipoproteins isolated from the Cohn fractions I-III (Fig. 6(A)) of a patient with liver disease. The preparation displays a fairly homogeneous population of round compounds quite similar to β-lipoproteins of healthy controls (26). Fig. 6 (C) shows a purified preparation of β_2-LP particles isolated from Cohn fractions I-III (Fig. 6(A)), which was negatively stained shortly after isolation. It is apparent that upon contact with each other β_2-LP particles (300–700 Å in diameter) undergo some distortion in shape. However, the individual particles are well outlined like the components of normal β-lipoproteins. As for these the fine granular appearance cannot definitely be attributed to distinct surface features or any subunit structure.

It has been known for some time that triglyceride rich plasma lipoproteins are partially catabolised by lipoprotein lipase (27, 28), an enzyme which is rapidly released into the plasma pool following intravenous administration of heparin. More recently strong evidence has been accumulated indicating that part of this lipolytic activity derives from the liver (29, 30, 19) and very lately a method of selectively measuring two different triglyceride lipase activities in post-heparin plasma was described (31). In the same report one lipase activity relatively resistant to protamine sulphate was demonstrated to originate from the liver. Since a follow-up study (Fig. 7) of a patient with acute cholestatic hepatitis showed a hypertriglyceridaemia, an altered [VLDL-TG]/[> 1.006 g/ml-TG] ratio and the presence of β_2-LP parallel with the severity of the disease as judged by the concentration of total bilirubin, we assumed that the presence and

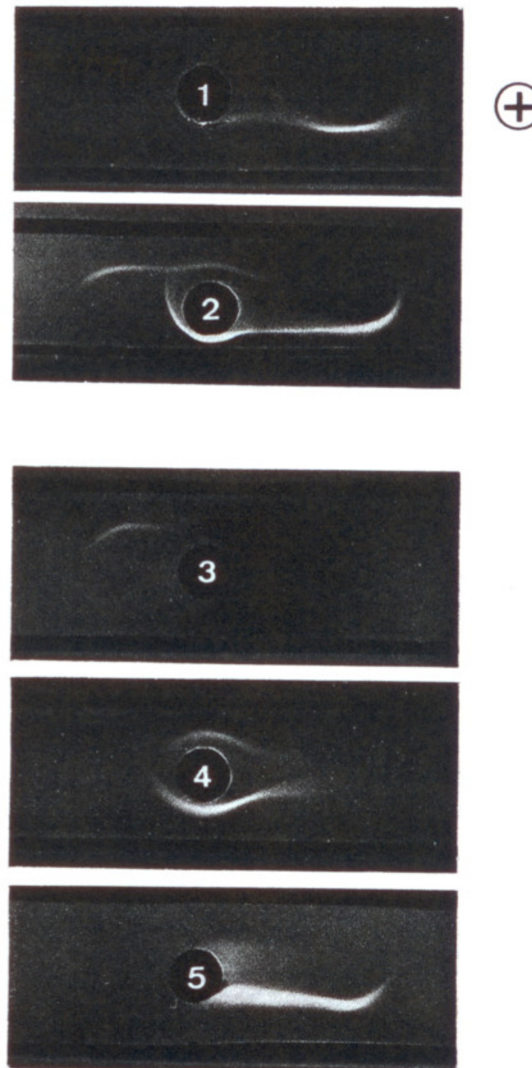

Fig. 2. Immunoelectrophoresis patterns in 1% agar gel (*1*) control LDL; (*2*) LDL of a patient with acute cholestatic hepatitis (Patient. No. 11, Table 2); (*3*) isolated LP-X from sample (*2*); (*4*) isolated β_2-lipoprotein from sample (*2*); (*5*) isolated normal β-lipoproteins from sample (*2*)

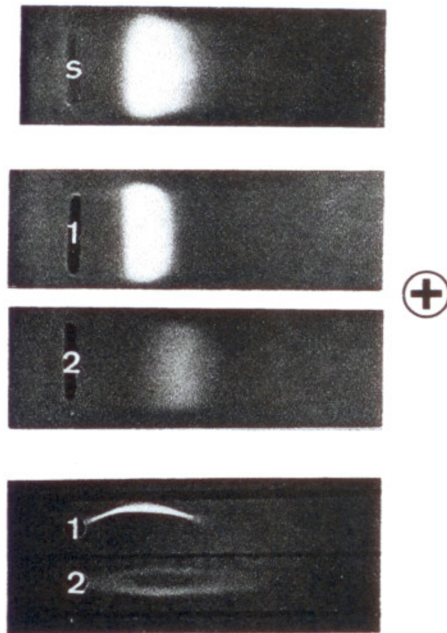

Fig. 3. Lipoprotein electrophoresis and immunoelectrophoretic patterns in 1% agarose gel. (*S*) whole serum of a patient with acute cholestatic hepatitis (Patient No. 11, Table 2). (*1*) isolated normal β-lipoproteins and (*2*) isolated β_2-lipoproteins from the same patient. The upper and lower trough in the immunoelectrophoresis contains specific anti-bodies to apo-lipoprotein B; the middle trough contains specific anti-bodies to apo-lipoproteins C

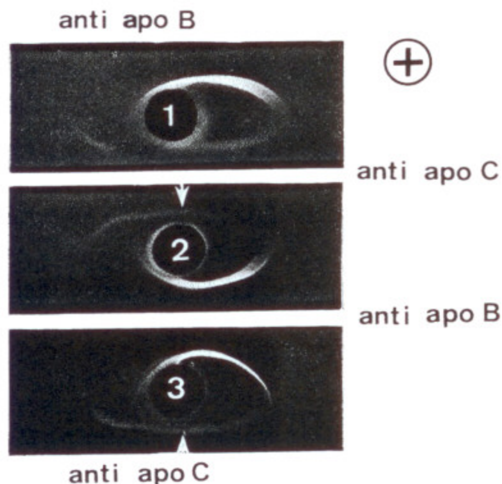

Fig. 4. Immunoelectrophoresis patterns in 1% agar-gel, pH 8.6 of whole serum from various patients with cholestatic liver disease. (*1*) sample of a patient without β_2-lipoprotein or hypertriglyceridaemia; (*2*) sample of a patient with β_2-lipoprotein, hypertriglyceridaemia and decreased VLDL concentrations (Patient No. 11, Table 2); (*3*) sample of a patient with β_2-lipoprotein, hypertriglyceridemia and increased VLDL concentration (Patient No. 3, Table 2)

accumulation of β_2-LP in the fasting plasma of such patients may be the result of a diminished lipase activity of liver origin.

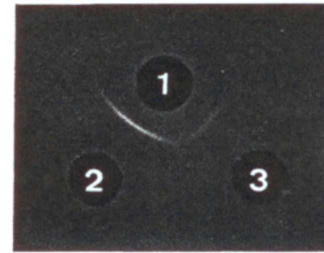

Fig. 5. Immunodiffusion pattern of isolated β_2-lipoprotein, (*1*). (*2*) anti-apo-lipoprotein B serum; (*3*) anti-apolipoprotein C serum

To explore this possibility post-heparin lipolytic activity was determined with and without protamine sulphate in two healthy volunteers ([VLDL-TG]/[>1.006 g/ml-TG] ratio; 1.9 and 1.6) and in two patients with liver disease, both carrying the β_2-LP in their plasma (acute cholestatic hepatitis; [VLDL-TG]/[>1.006 g/ml-TG] ratio; 0.28 and 0.29; Patient No. 3 and 10, Table 2). Results of the experiments are presented in Table 4 and Fig. 8.

Using [1-^{14}C] trioleate as substrate (Table 4) it is apparent that total lipolytic activity is markedly decreased in the patients with liver disease and β_2-LP present in their plasma as compared to healthy controls. After protamine sulphate incubation, this difference is even more pronounced, indicating a moderate decrease of extrahepatic lipase but a striking decrease of hepatic lipase in the patients. In a previous study (31) using similar assay conditions it was calculated that in the rat $^2/_3$ of total post-heparin lipolytic activity derives from the liver. These results are in good agreement with the data reported here. Since an artificial substrate used for the measurement of post-heparin lipolytic activity may influence the result and may not quite reflect the biological situation we have also determined the post-heparin lipolytic rate employing the subjects own plasma lipoproteins as the source of triglycerides. In the experiment, the relase of free glycerol was determined with or without incubation of protamine sulphate in both controls and patients with acute cholestatic hepatitis (Patient No. 3 and 10; Table 2). Results are presented in Fig. 8 and are in fair agreement with those obtained using trioleate as substrate, also strongly suggesting a diminished hepatic lipase activity in the patients post-heparin serum. Incubation of postheparin plasma from normals with pre-heparin plasma from liver patients did not significantly affect the total post-heparin lipolytic rate of the control serum, indicating that the diminished hepatic lipase activity of patients with liver disease is probably not due to a circulating inhibition factor.

Since the fasting serum of our patients studied with hypertriglyceridaemia secondary to severe liver disease was usually not turbid as one could expect and never showed any traces of chylomicrons as judged by

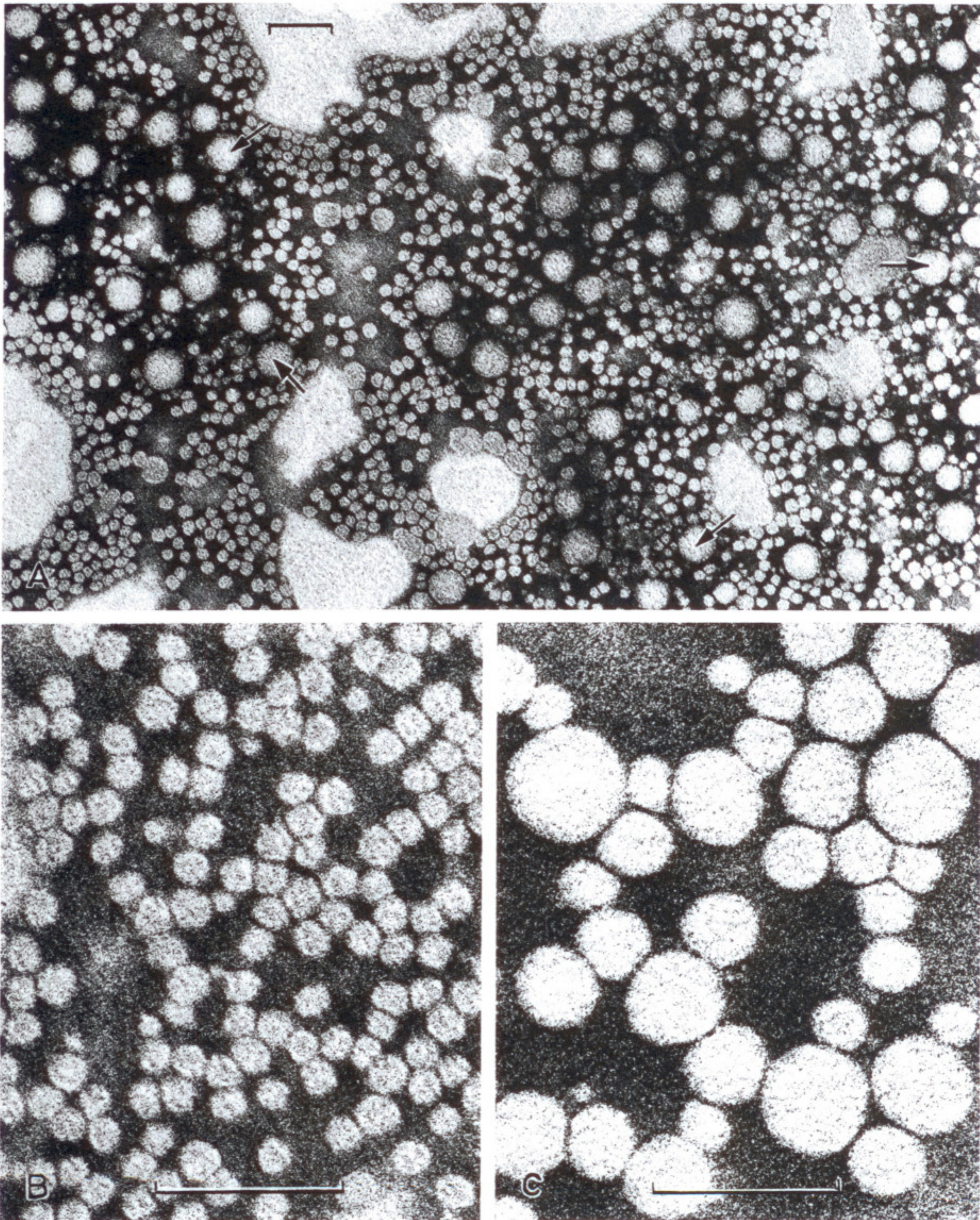

Fig. 6A—C. Electron micrographs of Cohn fractions I–III isolated from a patient with liver disease (A); of normal β-lipoproteins (B) and of β_2-lipoprotein particles (C). B and C were isolated from the same fraction represented in (A). Arrows in (A) show β_2-LP particles. Negative staining at 0°C with 1% potassium phosphotungstate, pH 7.1. Bars in Figures indicate 0.1 μm

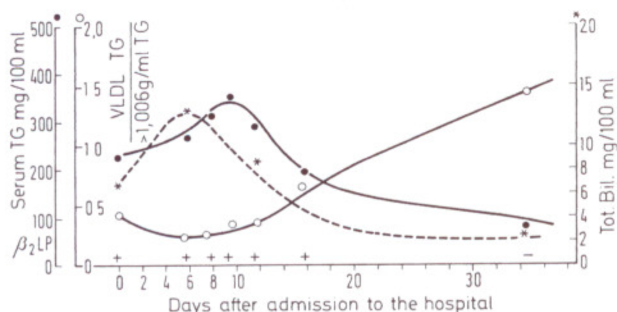

Fig. 7. Serum triglyceride concentrations; [VLDL-TG]/[> 1.006 g/ml-TG] ratio, total bilirubin concentration and β_2-lipoprotein during a follow-up study of a patient with acute hepatitis and hypertriglyceridaemia at the beginning fo the disease

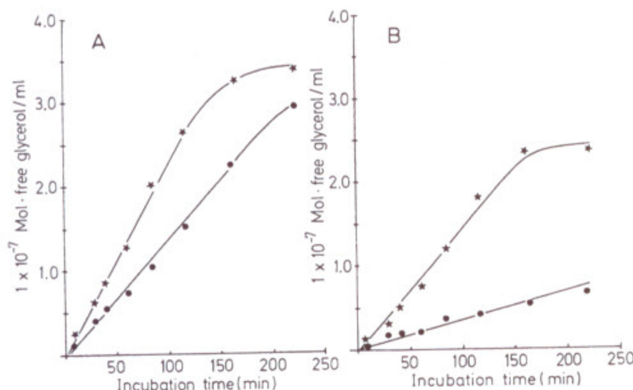

Fig. 8A and B. Post-heparin plasma lipolytic rate expressed as release of free glycerol *in vitro* (for details see methods) of healthy controls and of patients with secondary hypertriglyceridaemia due to acute cholestatic hepatitis. A: Values obtained without protamine incubation. (*) controls; (○) patients with liver disease. B: Values obtained under protamine sulphate inhibition. (*) controls; (○) patients with liver disease

Table 4. Post-heparin lipolytic activity using [1-^{14}C] trioleate as substrate. NaCl concentration of the assay was 0.15 M. Values of two controls and two patients with severe cholestatic liver disease in comparison to their triglyceride distribution

	VLDL-TG / > 1.006 g/ml-TG	Post heparin lipolytic activity nmol FFA/ml/h	
		without protamine	protamine 3 mg/ml
Controls	1.9	13560	9174
	1.6	10828	6720
Patients	0.28	4385	680
	0.29	3530	669

lipoprotein electrophoresis (Fig. 3) the question of the source of the abnormal β_2-LP occurred.

In order to explore the possibility that β_2-LP represents an "intermediate lipoprotein" due to a disturbed catabolism of triglyceride rich lipoproteins such as chylomicrons, two patients with severe intrahepatic cholestasis and hypertriglyceridaemia, showing β_2-LP as reflected by an abnormally low [VLDL-TG]/[> 1.006 g/ml-TG] ratio and by immunochemical means, were examined on a regular diet (percent calory intake 20/40/40/-protein/fat/carbohydrate) and on an almost fat free diet (percent calorie intake 20/5/75-protein/fat/carbohydrate). Both types of diets were isocaloric (1800–2200 cal/day); the carbohydrates on the low fat diet consisted of up to 70% polysaccharides and the rest of mono and dissacharides. Results of this experiment are given in Table 5. While the serum triglyceride concentration decreased on the low fat diet and increased again on a regular diet, the [VLDL-TG]/[> 1.006 g/ml-TG] ratio as a marker of β_2-LP concentration, showed an opposite behaviour. Serum bilirubin concentrations, AST, ALT, and alkaline phosphatase activity remained practically unchanged during the dietary study.

Table 5. Serum triglyceride concentration and triglyceride distribution in a dietary follow—up study of two patients with acute cholestatic hepatitis

	Regular diet	Low fat for 3 days	Low fat for 5 days	Regular diet for 4 days
VLDL-TG / > 1.006 g/ml-TG	0.44	0.73	0.82	0.48
	0.29	0.81	0.91	0.52
Serum-TG mg/100 ml	350	310	260	328
	500	410	320	390

Discussion

The liver is a major site of synthesis and degradation of plasma lipoproteins (34) and it releases lipolytic activity into the plasma pool. Since abnormal serum lipid patterns are often associated with abnormal liver function it is reasonable to anticipate that liver disease will result in alterations of plasma lipoproteins, the protein-lipid particles in which form all serum lipids circulate. In the past, one such abnormal lipoprotein has been identified with the isolation and characterization of lipoprotein-X, which, because of its unique protein lipid composition is primarily responsible for the high content of unesterified cholesterol and phospholipids in the plasma of patients with cholestasis (2–4). Alterations of the α- and pre-β-lipoprotein fractions in liver disease have been attributed to the presence of an apo-lipo-protein A with impaired lipid binding properties (7).

Only recently a marked hypertriglyceridaemia in some patients, most pronounced in those with cholestic liver dysfunction, has been reported (8–11). It was also demonstrated that the majority of triglycerides of such patients is found within the LDL lipoprotein fraction (8, 10, 11).

Isolation of a large (300–700 Å in diameter), triglyceride rich low density lipoprotein (d 1.019–1.063 g/ml) different from LP-X and normal β-lipoproteins may now be achieved by the described separation procedure which combines ultracentrifugation, cold ethanol fractionation and affinity chromatography. This lipoprotein has be designated β_2-lipoprotein (β_2-LP), because it shares certain physico-chemical characteristics and chemical properties with normal β-lipoproteins.

Our study indicates that the increased concentration of LDL-triglycerides as well as the decreased [VLDL-TG]/[> 1.006 g/ml-TG] ratio and thereby the hypertriglyceridaemia of such patients with liver disease is primarily due to the presence of β_2-LP. Since the protein moiety of this LDL-lipoprotein consists of apo B and apo C and possibly also of trace amounts of apo A it is likely that β_2-LP derives from lipoproteins of lower density, which are known to contain all three major apo-lipoproteins. This possibility is supported by our dietary experiment. The decrease of its plasma concentration on a low fat diet may indicate its nature as a degradation product or intermediate particle of chylomicron metabolism, although an additional relationship to VLDL catabolism cannot be ruled out by this study.

One significant mechanism responsible for the accumulation of this low density lipoprotein is the markedly reduced hepatic lipase activity which we first demonstrated in this study.

It seems relevant at this point to speculate on the physiological metabolism of chylomicrons. On a regular diet patients with primary type I hyperlipoproteinaemia are unable to catabolise chylomicrons, transporting the exogeneous triglycerides (32).

Recently it was demonstrated that these patients are lacking lipoprotein lipase (which is the portion of post-heparin lipolytic activity sensitive to protamine sulphate) but do release hepatic lipase following heparin administration (33). As demonstrated in this study patients suffering from hypertriglyceridaemias secondary to liver dysfunction and accumulating the β_2-LP revealed a markedly reduced hepatic lipase activity.

From this it may be concluded that chylomicrons are predominatly catabolised by the action of extrahepatic lipoprotein lipase resulting in an intermediate particle such as the β_2-LP described here which is then further catabolised by a lipase of a liver origin. These mechanisms may be altered in the patients described in this study causing their hypertriglyceridaemia. Other forms of intermediate lipoproteins have been found to accumulate postprandial or in hyperlipoproteinaemia without liver disease (for rev. see 34–36). However, all these "intermediates" differ to some degree in their physico-chemical and chemical properties and therefore a clear distinction is needed for a better understanding of the biodynamics of lipoprotein metabolism. It is noteworthy that most of the lipoprotein abnormalities described in liver disease such as the alteration of pre-β- and α-lipoproteins, the formation of the abnormal lipoprotein-X as well as the β_2-lipoprotein described here have recently also been found in patients suffering from familial lecithin:cholesterol acyltransferase (LCAT) deficiency without apparent liver dysfunction (37–39). Therefore, an important interrelated mechanism between the various forms of lipolytic enzymes present in post-heparin plasma and the LCAT system seems to occur and to regulate the metabolism of plasma lipoproteins.

Acknowledgement. We should like to thank Professor Dr. B. Kommerell (Head of Department of Gastroenterology, Medizinische Universitätsklinik, Heidelberg) for valid discussions and his help in performing this study.—The work was supported by Grants from the Deutsche Forschungsgemeinschaft (se 199/3 and SFB 90).

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