Distribution of Apolipoprotein(a) in the Plasma from Patients with Lipoprotein Lipase Deficiency and with Type III Hyperlipoproteinemia

No Evidence for a Triglyceride-rich Precursor of Lipoprotein(a)

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Abstract

Lipoprotein(a) consists of a low-density lipoprotein containing apolipoprotein (apo) B-100 and of the genetically polymorphic apo(a). It is not known where and how lipoprotein(a) is assembled and whether there exists a precursor for lipoprotein(a). We have determined the phenotype, concentration, and distribution of apo(a) in plasma from patients with lipoprotein lipase (LPL) deficiency (type I hyperlipoproteinemia, $n = 14$), in apo E 2/2 homozygotes with type III hyperlipoproteinemia ($n = 12$) and in controls ($n = 16$). In the two genetic conditions, there is grossly impaired catabolic conversion of apo B-100–containing precursor lipoproteins to low-density lipoproteins. Considering apo(a) type, the plasma concentration of apo(a) was normal in type III patients but significantly reduced in LPL deficiency. Despite the defects in the catabolism of other apo B–containing lipoproteins, the distribution of apo(a) was only moderately affected in both metabolic disorders, with 66.7% (type I) and 74.7% (type III) being present as the characteristic lipoprotein(a) in the density range of 1.05–1.125 g/ml (controls 81.6%). The remainder was distributed between the triglyceride-rich lipoproteins (type I 12.4%, type III 8.5%, controls 4.7%) and the lipid-poor bottom fraction (type I 19.3%, type III 15.3%, controls 12.6%). In all conditions most apo(a) (57–88%) dissociated from the triglyceride-rich lipoproteins upon recentrifugation and was recovered as lipoprotein(a). These data suggest that lipoprotein(a) is not generated from a triglyceride-rich precursor. Lipoprotein(a) may be secreted directly into plasma or may be formed by preferential binding of secreted apo(a) to existing low-density lipoprotein. (*J. Clin. Invest.* 1992. 90:1958–1965.) Key words: apolipoprotein(a) • lipoprotein(a) • lipoprotein lipase deficiency • type III hyperlipoproteinemia

Introduction

Lipoprotein(a) [Lp(a)][1] is a quantitative genetic trait in human plasma (1). High levels of Lp(a) are associated with premature myocardial infarction and cerebrovascular disease (2). Lp(a) is composed of a low-density lipoprotein (LDL)-like particle carrying all the lipids of that complex and of the Lp(a) specific glycoprotein termed apolipoprotein (a) [apo(a)]. Plasma apo(a) is almost entirely synthesized by the liver (3). Apo(a) has been sequenced on the protein and cDNA level and is highly homologous to plasminogen (4, 5). In particular it contains a kringle 4 motif that is repeated several times in the apo(a) gene and protein. Apo(a) exhibits a genetic size polymorphism with individual isoforms ranging in apparent M_r from $\sim$ 400 to > 800 kD (6). Originally six genetic apo(a) isoforms, designated LpF, LpB, LpS1, LpS2, LpS3, and LpS4, which are determined by alleles at the apo(a) structural gene locus (1, 7), were described. The existence of more than 20 different isoforms, which are caused by genetic variation in the number of kringle 4 repeats in the apo(a) gene (9, 10), has recently been reported (8). The genetic isoforms are associated with plasma Lp(a) levels in Caucasian, Asian, and African populations (1, 11) and are a significant predictor of coronary heart disease risk (12). The LDL moiety of Lp(a) is similar to authentic LDL in chemical composition and contains apo B-100 (1, 2).

LDL are the catabolic end products in plasma of triglyceride-rich very low-density lipoproteins (VLDL). In normolipidemic healthy subjects VLDL are secreted from liver parenchymal cells into plasma and are converted to intermediate-density lipoproteins (IDL) at the endothelia of capillaries through hydrolysis of core triglycerides by lipoprotein lipase with transfer of surface components to high-density lipoprotein (HDL) (13). IDL are further degraded to LDL in a process that is believed to require the action of hepatic triglyceride lipase (14, 15) and apo E (16). Different mutations affect the catabolism of apo B–containing precursor lipoproteins. Autosomal recessive lipoprotein lipase (LPL) deficiency results in the accumulation in plasma of the triglyceride-rich chylomicrons and VLDL and in a low LDL concentration (equaling type I hyperlipoproteinemia). Mutations in the apo E gene that affect binding of apo E to the LDL receptor and also affect lipolysis cause accumulation of chylomicron remnants and IDL in plasma owing to impaired cellular uptake and a defect in the interconversion of these lipoproteins to LDL (type III hyperlipoproteinemia) (17). These genetic conditions are valuable natural models that allow one to study the question of whether or not Lp(a) is formed in a process that is analogous to the formation of LDL, and whether or not this process requires the same machinery. We have determined the phenotype, concentration, and distribution of apo(a) in the plasma lipoproteins and lipoprotein-free fractions from patients with these genetic defects in the interconversion of triglyceride-rich lipoproteins to LDL. The results demonstrate that both genetic

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Received for publication 4 March 1992 and in revised form 6 June 1992.

1. *Abbreviations used in this paper:* Lp(a), lipoprotein(a); LPL, lipoprotein lipase.

J. Clin. Invest.

0021-9738/92/11/1958/08 $2.00
Volume 90, November 1992, 1958–1965

defects only moderately affect the distribution of apo(a) in plasma. This is in sharp contrast to the distribution of apo B-100 that is markedly affected by mutations in the LPL- and apo E genes.

Methods

Subjects

Control EDTA plasma was obtained from 16 healthy volunteers of known apo(a) phenotype with Lp(a) concentrations of 2.9–115 mg/dl covering the normal range. All were Caucasians living in Innsbruck, Austria. Mean cholesterol levels were 222±35 mg/dl and mean triglycerides were 136±50 mg/dl. EDTA plasma was obtained from 14 patients with familial LPL deficiency at the Division of Metabolism of the Department of Medicine of the University of Washington in Seattle. These patients had postheparin LPL activity below the 99% confidence interval of 34 normal Caucasians. They were on a very low fat diet and were receiving no medications. Mean LPL activity in the 14 patients was 9.9±7.5 nmol FFA/min·ml plasma (controls 220±59) (18). Their hepatic triglyceride lipase activity was within the normal range (females 101±24 nmol FFA/min·ml plasma, males 154±46 nmol FFA/min·ml plasma (19). Plasma samples were frozen at −70°C and shipped to Innsbruck on dry ice by air. The plasma from 12 patients with type III hyperlipoproteinemia were recruited from the Division of Endocrinology and Metabolism of the Department of Internal Medicine, University of Heidelberg. These patients had not received any hypolipidaemic therapy for at least 4 wk before the Lp(a) analysis. Mean cholesterol level of the type III patients was 435±193 mg/dl and mean triglycerides were 801±670 mg/dl. All these patients were apo E 2/2 homozygotes and had β-VLDL upon agarose gel electrophoresis. They were all of German ancestry. The plasma from these subjects was sent to Innsbruck on wet ice by an express delivery system the same day the sample was obtained.

Informed consent was obtained from all patients and controls participating in the study. All subjects were fasting for 12 h before blood was taken.

In order to control for possible effects on Lp(a) levels and apo(a) distribution of frozen storage of the LPL-deficient plasma fresh plasma samples were obtained from two Austrian patients with LPL deficiency (kind gift of Dr. F. Sandhofer, Salzburg) and aliquots were analyzed directly. A second aliquot was frozen at −70°C and stored for 6 mo before analysis. Likewise aliquots from three of the type III patients were analyzed fresh and after frozen storage. No differences in Lp(a) concentration or apo(a) distribution were noted between any of the pairs.

Methods

Apo(a) quantification. Apo(a) was quantified in human serum, or in fractions from density gradient ultracentrifugation by a sandwich ELISA essentially as described (20). In brief, affinity-purified polyclonal anti-human Lp(a) antibody (5 μg/ml) was bound to the wells of a microtiter plate. The blocking buffer used was 0.1% casein in PBS (pH 7.3). 10-μl or 50-μl sera or fractions were added in appropriate dilutions (ranging from 1/10 to 1/2,000). The apo(a) antigen was detected with the anti-(a) monoclonal antibody $1A^2$(20) conjugated with horseradish peroxidase. This antibody does not cross-react with plasminogen (H. Dieplinger and G. Utermann, unpublished observation). A commercially available Lp(a) standard (Immuno, Vienna, Austria) was used throughout. Concentrations were either expressed as milligrams per deciliter Lp(a) lipoprotein or as milligrams per deciliter apo(a) using the compositional data from Gaubatz et al. (21) and Fless et al. (22) as outlined (20). The assay has a sensitivity of 2 ng of Lp(a).

Apo(a) phenotyping. Ap(a) phenotypes were determined by immunoblotting exactly as described (23) using the monoclonal antibody $1A^2$ as the first and a horseradish peroxidase–conjugated anti–mouse IgG (Dakopatts, Glostrup, Denmark) as the second antibody. Isoforms were designated according to our original nomenclature (1, 6). Since this study was initiated, improved separation techniques have enabled us to distinguish more than 10 apo(a) isoforms by SDS-PAGE (11, 24) and more than 20 isoforms were separated by SDS-agarose-gel electrophoresis (8). Because better separation of isoforms does not affect the linear relationship between apo(a) isoform size and Lp(a) concentration, no higher resolution of isoforms was required for this study. We therefore used the same methodology and the same apo(a) standards as in previous studies. Isoforms that did not comigrate exactly with one of the isoform standards were binned with the closest respective isoform in the standard (25).

Table I. Mean Apo(a) Concentration in Plasma and Distribution (Mean and Range) of Apo(a) in Density Gradient Fractions

Group	Apo(a) concn.	Apo(a) distribution			
		TG-rich	LDL/IDL	Lp(a)	Bottom
	mg/dl		%		
Type I*	0.51	9.1	3.3	66.7	19.3
(n = 14)		(2.4–16.2)	(0.9–9.9)	(58.4–75.7)	(11.8–23.8)
Type III	2.28	3.5	5.0	74.7	15.3
(n = 12)		(0.8–6.7)	(1.9–10.9)	(68.3–87.5)	(4.7–19.5)
Controls	5.4	2.5	2.2	81.6	12.6
(n = 16)		(0.6–15.6)	(0.9–3.6)	(75.3–90.3)	(0.6–16.7)
Population‡	2.35	—	—	—	—
(n = 279)					

* Density gradient ultracentrifugation was performed on five patients (see text).
‡ From Sandholzer et al. (11).

Ultracentrifugation. Density gradient ultracentrifugation of human plasma was done according to Redgrave et al. (26), with minor modifications. Three KBr-density solutions of 3 ml each (d = 1.006, 1.019, 1.063 g/ml) were layered sequentially into the tubes of an SW41.Ti rotor (Beckman Instruments, Inc., Fullerton, CA). Finally, 4 ml plasma of d = 1.21 g/ml (adjusted with solid KBr) were applied at the bottom. Centrifugation was then performed in a SW.41-Ti rotor at 40,000 rpm at 10°C for 24 or 48 h. After centrifugation, 0.5-ml fractions were collected from below using a fractionating system (Beckman Instruments, Inc.) and a fraction collector (LKB Produker, Bromma, Sweden). Since lipoproteins are not in equilibrium after this 24-h density gradient ultracentrifugation, two control samples with a high percentage of apo(a) in the triglyceride-rich and the bottom fractions were run comparatively for 24 and 48 h (Tables I and II). As expected this resulted in a slight difference (~ 0.002 g/ml) in Lp(a) peak density (data not shown). The distribution of apo(a) between fractions was however not changed (Table II). Moreover, five controls that were centrifuged for 48 h had a distribution of apo(a) that was not significantly different from the distribution in 11 controls run for 24 h and

Table II. Distribution of Apo(a) in Density Gradient Fractions from Controls at Different Centrifugation Times

Group/Subject	Time	Apo(a) distribution			
		TG-rich	LDL-IDL	Lp(a)	Bottom
	h		%		
Control I	24	11.9	3.5	82.6	1.1
Control I	48	10.5	2.5	85.8	0.6
Control II	24	13.4	2.5	81.1	1.0
Control II	48	15.0	1.6	81.4	1.1
Controls (n = 11)	24	2.1	1.8	83.9	11.3
Controls (n = 5)	48	2.8	5.1	79.0	11.1

the total group of controls (Table II). Therefore, all other density gradients were centrifuged for 24 h. The apo(a) distribution in gradients was evaluated by ELISA (see above) and in selected gradients by densitometric scanning of immunoblots using a (Hirschmann Gerätebau, Unterhaching, FRG) densitometer.

Apo E phenotyping. Apolipoprotein E phenotyping was performed by isoelectric focusing of delipidated VLDL exactly as described (27).

Lipid and LPL activity measurements. Cholesterol and triglycerides were determined by commercially available test kits (Boehringer Mannheim GmbH, Mannheim, FRG). LPL activity in plasma (after 60 U heparin/kg body weight) was measured with triolein-phosphatidylcholine emulsion (28). The LPL activity was that precipitated from whole plasma by the 5D2 monoclonal antibody, the remaining activity was hepatic lipase activity.

Statistical procedures. The distribution of apo(a) between groups was compared by the nonparametric Kruskal-Wallis test (29). Pairwise comparisons were done by the Wilcoxon test (29).

Results

Concentration of Lp(a) in the plasma from patients with LPL deficiency and with type III hyperlipoproteinemia. The concentrations of Lp(a) in plasma from 14 patients with LPL deficiency were very low when compared to controls (Tables III and IV). If only Caucasian patients ($n = 9$) are considered the mean Lp(a) level in the patients was 1.1 mg/dl as compared to 11.8 mg/dl in a population sample representing the common apo(a) phenotypes present in LPL-deficient and type III patients (Table IV). This low concentration is obviously different from what is expected by chance (Wilcoxon rank sum test P 5.4×10^{-5}). Seven of the LPL-deficient patients had Lp(a) plasma concentrations too low for determination of the apo(a) type (null phenotypes). With only one exception (see Table III) all the remaining patients were of phenotype S2 and Lp(a) concentrations were very low for this phenotype (1.2 mg/dl in Caucasian patients vs. 24.5 mg/dl in the controls, see Table IV). Overall the low Lp(a) concentrations in the S2 phenotype and the high frequency of "null" types resulted in the low Lp(a) concentration in the total group of LPL-deficient patients. The low concentration of Lp(a) in patients with LPL deficiency might be the consequence of random differences in allele frequencies between patients and controls or of an effect of the LPL-deficiency gene on Lp(a) levels. There was an overrepresentation of "null" phenotypes in the LPL-deficient patients. Although the number of LPL-deficient subjects is small and we do not know their apo(a) DNA phenotypes, it seems unlikely that the observed difference is a consequence of random differences in allele frequencies between patients and controls. The high frequency of "null" types more likely is a consequence of the effect of the defective LPL alleles on Lp(a) levels rather than a true overrepresentation of this type in the sample. An Lp(a) lowering effect of the LPL gene would explain both the low concentrations of Lp(a) in S2 patients and the overrepresentation of the "null" phenotype.

Table III. Apo(a) Phenotypes and Lp(a) Plasma Concentrations in Patients with Familial LPL Deficiency and with Type III Hyperlipoproteinemia

Patient	Apo(a) type	Lp(a) concn.	Ethnic group
		mg/dl	
LPL deficiency			
1	S2	1.2	Caucasian
2	S2	1.4	Caucasian
3	S2	1.0	Caucasian
4	0	1.8	Caucasian
5	0	0.4	Caucasian
6	0	1.7	Caucasian
7	0	0.3	Caucasian
8	0	0.9	Caucasian
9	0	0.9	Caucasian
10	S2	3.2	Arabian
11	S2	1.0	Indian*
12	S4	15.0	Vietnamese
13	0	1.4	Black
14	S2	13.0	Black*
Type III HLP			
1	S2	1.5	Caucasian
2	S3S4	6.6	Caucasian
3	S2	16.0	Caucasian
4	S4	8.4	Caucasian
5	S4	5.4	Caucasian
6	S2	43.0	Caucasian
7	0	2.0	Caucasian
8	S4	5.6	Caucasian
9	S4	4.0	Caucasian
10	S2S4	62.0	Caucasian
11	0	1.8	Caucasian
12	S4	7.5	Caucasian

* Mean Lp(a) concentration in Black and Indian S2 subjects is 53.6 and 30.9 mg/dl, respectively (11).

Table IV. Mean Lp(a) Plasma Levels in Patients with Type III Hyperlipidemia, LPL Deficiency, and in a Caucasian Reference Population

Apo(a) phenotype*	LPL deficiency‡ ($n = 9$)	Type III HLP ($n = 12$)	Population§ ($n = 190$)
		mg Lp(a)/dl	
S2	1.2	20.2	24.5
S4	—	6.2	5.7
0	1.0	1.9	0.4
S2/S4	—	62.0	34.1
S3/S4	—	6.6	8.8
Total	1.1	13.7	11.8

* Only phenotypes that were present in patients. ‡ Only Caucasian patients. § From Sandholzer et al. (11).

The concentration of apo(a) was further determined in 12 untreated, unrelated apo E2 homozygotes with type III hyperlipoproteinemia. Upon SDS-PAGE the apo E protein of all patients had the characteristic mobility of the apo E2 ($arg^{158} \rightarrow cys$) mutation (30). All patients had excessive accumulation of remnants and IDL in plasma and exhibited the density gradient profiles characteristic for this disorder (Fig. 1). Lp(a) plasma concentrations in the patients were not significantly different from those of controls of identical apo(a) phenotype (Table IV).

Distribution of apo(a) in the plasma from normolipidemic subjects. Plasma from 16 healthy normolipidemic individuals was subjected to density gradient ultracentrifugation. The dis-

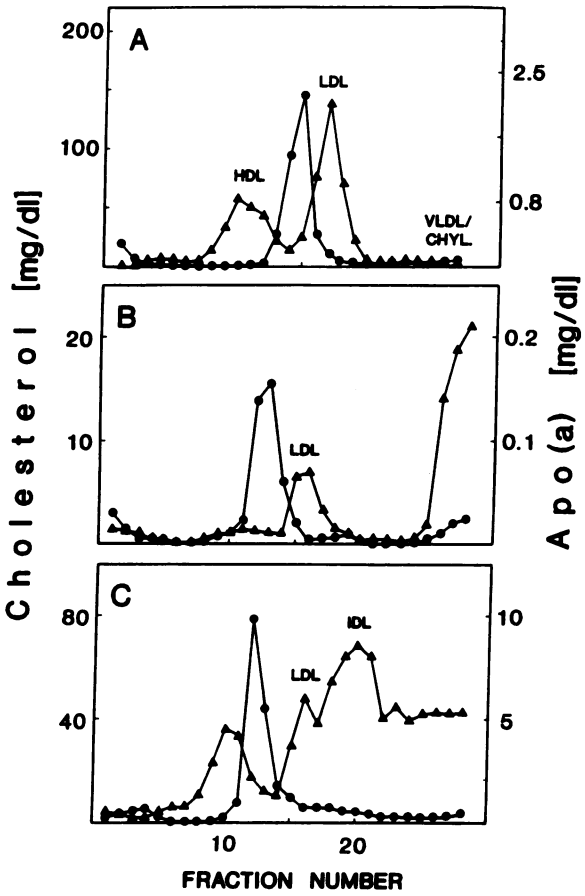

Figure 1. Density gradient ultracentrifugation of plasma samples from a control subject (*A*), a patient with LPL-deficiency (*B*), and an apo E 2/2 homozygous patient with type III hyperlipoproteinemia (*C*). The distributions of cholesterol (▲) and of apo(a) (●) are shown. The positions of HDL, LDL, IDL, and VLDL/chylomicrons are indicated in the gradients. The differences in peak densities of Lp(a) between subjects are due to different apo(a) isoforms (40). The apo(a) isoform of the control is apo(a)S1 and the isoforms of the LPL-deficient and of the type III patients are apo(a)S2.

tribution of apo(a) in the density gradient fractions was determined by a double-antibody ELISA using the apo(a) specific monoclonal antibody $1A^2$ for detection. The plasma samples represented the common apo(a) phenotypes and a wide range of plasma Lp(a) concentrations (2.9–115 mg/dl). More than 75% (range 75.3–90.3%) of apo(a) was present as Lp(a) lipoprotein in the characteristic density range of 1.05–1.125 g/ml in all subjects (Tables I and II and Fig. 1). The remainder was distributed among triglyceride-rich lipoproteins that floated at the top of the gradients (0.64–15.6%) or the lipid-poor bottom fractions of the gradient (0.6–16.7%). The authenticity of the immunoreactive material in the bottom and in the triglyceride-rich lipoproteins was demonstrated by immunoblotting (Fig. 2). The protein in the bottom and triglyceride-rich fractions had the same apparent M_r as the genetic isoform(s) in total plasma or in the Lp(a) lipoprotein fraction.

Distribution of apo(a) in plasma from patients with LPL deficiency and with type III hyperlipoproteinemia. The distribution of apo(a) in plasma fractions from patients with type I and type III hyperlipoproteinemia was determined by density

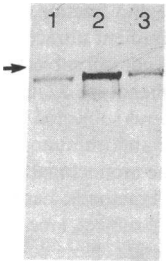

Figure 2. Immunoblot of apo(a) from density gradient fractions of a control subject with a single-band apo(a) phenotype (F-type). Lane *1*, bottom fraction; lane *2*, Lp(a) peak fraction; lane *3*, triglyceride-rich top fraction. 1 μl of the peak fraction and 5 μl of the top and bottom fractions were subjected to SDS-PAGE under reducing conditions. Proteins were blotted onto nitrocellulose filters and immunostained with the monoclonal apo(a) antibody $1A^2$. Arrow indicates position of apo B-100.

gradient ultracentrifugation and compared to the distribution in the controls (Fig. 1). Because Lp(a) levels were too low in nine of the type I patients the plasma distribution of apo(a) could be determined in only five of them. The overall distribution of apo(a) in the density gradient was significantly different among the three groups (Tables I and II). Comparisons between all three groups of the amount of apo(a) in the triglyceride-rich, the LDL/IDL, the Lp(a), and the bottom fractions demonstrated significant differences for each single fraction (Kruskal-Wallis test $P < 0.05$ for each fraction). Pairwise comparisons of apo(a) in fractions from type I, type III, and control groups showed that both type I and type III subjects differed significantly from controls (Wilcoxon test $P < 0.05$ for each fraction) but not among each other. The differences were characterized by a higher relative mean concentration of apo(a) in the triglyceride-rich and IDL fractions and in the bottom fractions from patients with type I and type III hyperlipoproteinemia and a slightly lower concentration of apo(a) in Lp(a) (Tables I and II). It is noteworthy that the higher concentration of apo(a) in triglyceride-rich lipoproteins was paralleled by a higher concentration of apo(a) in the bottom of the gradient. The differences between patients and controls, though statistically significant were, however, only moderate and there was wide overlap between the groups. There were subjects in the control group that had > 10% of apo(a) in the triglyceride-rich lipoproteins (Tables I and II) which is above the mean in type I and type III patients. As in the controls the majority (66.7%) of apo(a) was present as Lp(a) in the LPL-deficient patients. On the average, < 10% was associated with the triglyceride-rich lipoproteins which are the major lipoprotein class in this condition (see Fig. 1 and Table I), while 19.3% of apo(a) was found in the bottom fraction from type I patients. Likewise each single type III patient had at least 68% of apo(a) in the Lp(a) fractions. Only 2.7–17.6% were present in the less dense fractions. There was no accumulation of apo(a) paralleling the accumulation of cholesterol in IDL or the triglyceride-rich/remnant-rich top fractions of the gradients (Fig. 1).

Apolipoprotein(a) in triglyceride-rich lipoproteins. In all subjects investigated, including normotriglyceridemic controls there was apo(a) immunoreactivity in the triglyceride-rich fractions. The relative concentrations varied considerably among subjects ranging from < 1% to > 16% (Table I). Even within the same subject apo(a) concentrations in the triglyceride-rich lipoproteins varied severalfold at different times (data not shown). Immunoblotting experiments demonstrated that the apo(a)-reactive material in this fraction has the same apparent molecular mass as apo(a) in the plasma of the same subject and hence represents the same genetic isoform(s) (Figs. 2 and 3). This suggests that this material is authentic apo(a) and not

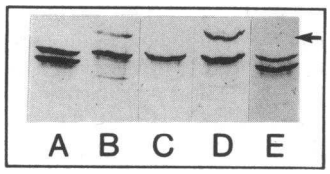

Figure 3. Immunoblot of a plasma sample and fractions from density gradient ultracentrifugation of a control subject with apo(a) phenotype S2/S4. 4 μl of plasma was subjected to SDS-PAGE and immunoblotted with the monoclonal apo(a) antibody 1A2 as outlined under materials and methods. Lanes *A* and *E*, S1/S2 standard; lane *B*, plasma from control subject, lane *C*, Lp(a) peak fraction, lane *D*, triglyceride-rich fraction. Note occurrence of the high M_r apo S4 isoform (*arrow*) in the triglyceride-rich fraction.

any cross-reacting material. In apo(a) heterozygotes both isoforms were present in the triglyceride-rich lipoproteins (Fig. 3), but the higher M_r band was frequently more intense or even exclusively present in the triglyceride-rich fraction as compared to the Lp(a) lipoprotein from the same subject (Fig. 3). This preferential association of the high M_r isoform with triglyceride-rich lipoproteins was seen in type III and control subjects (Fig. 3 and data not shown). In the context of this study it was important to clarify the nature of the apo(a) associated with the triglyceride-rich lipoproteins. To this end triglyceride-rich lipoprotein fractions from the density gradients were subjected to recentrifugation under identical conditions. The outcome of

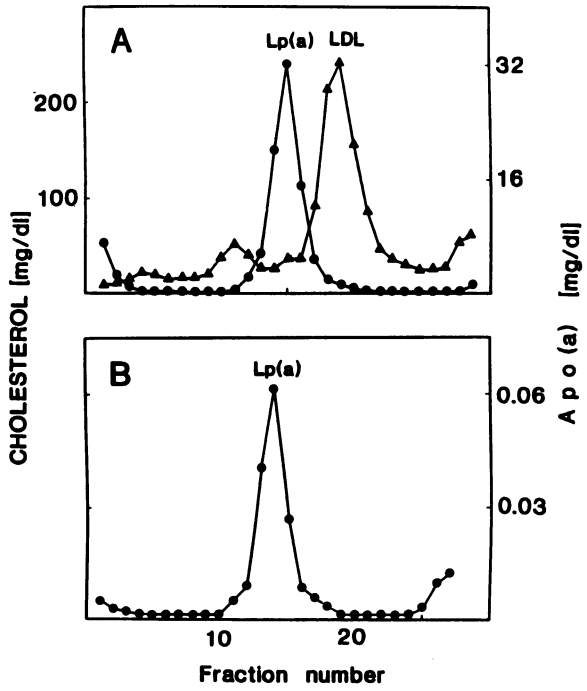

Figure 4. (*A*) Density gradient ultracentrifugation of plasma from a control subject with a high Lp(a) concentration (75 mg/dl). (*B*) The triglyceride-rich top fractions (No. 28/29) from gradient *A* were recentrifuged under identical conditions. The distribution of cholesterol (▲) and apo(a) (●) is indicated. Cholesterol could not be measured in gradient *B* owing to the very low concentration. Note the low concentration of apo(a) in fractions 28/29 from gradient *A* and in gradient *B*.

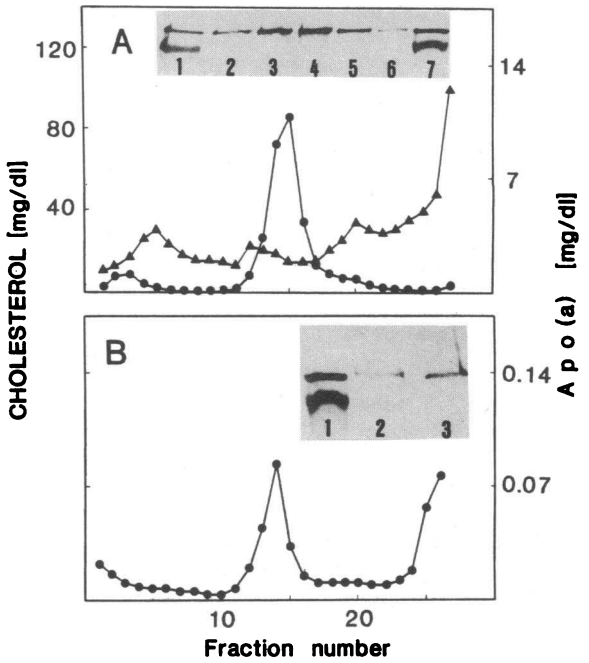

Figure 5. Density gradient ultracentrifugation of plasma from a type III patient (*A*) and of the triglyceride-rich top fractions from gradient *A* (*B*). An immunoblot analysis of selected fractions is shown in the inserts. Plasma from the patient was subjected to density gradient ultracentrifugation and fractions were analyzed for cholesterol (▲) and apo(a) by ELISA (●). Fraction 27 from gradient *A* was subjected to recentrifugation under identical conditions (*B*). Aliquots from both gradients (4 μl) were subjected to SDS-PAGE and immunostained with the monoclonal apo(a) antibody 1A2. Inset *A:* lanes *1* and *7*, S1/S2 standard; lane *2*, fraction 2 ["bottom" apo(a)], lanes *3–5*, fractions 19–21 [Lp(a)]; lane *6*, fraction 27 (triglyceride-rich fraction). Inset *B:* lane *1*, S1/S2 standard; lane *2*, fraction 14; lane *3*, fraction 26.

two such experiments, one in a control and one in a type III subject is shown in Figs. 4 and 5. Upon recentrifugation, 57–88% of apo(a) dissociated from TG-rich lipoproteins and the majority was recovered as Lp(a) lipoprotein in the typical density range (Figs. 4 and 5).

Discussion

The occurrence of apo(a) in triglyceride-rich lipoproteins and the significance of this finding has remained controversial. The first report on the occurrence of apo(a) immunoreactivity in VLDL was published by Rittner and Goenechea in 1969 (31). Later apo(a) protein was demonstrated in triglyceride-rich postprandial "chylomicrons" (32). Since then several in vivo and in vitro studies have suggested an association of Lp(a)/apo(a) with triglyceride-rich lipoproteins (33–36). Bersot et al. (32) have speculated that apo(a) might be of intestinal origin. However, studies on patients undergoing liver transplantation clearly demonstrated that most (> 95%) of apo(a) in plasma and hence of Lp(a) is derived from this organ and not from the intestine or other sources (3). The transplantation data did however not answer the questions whether Lp(a) is assembled in the liver or in the plasma compartment and

whether Lp(a) has a triglyceride-rich precursor or not. The present study was undertaken to address the latter question. Conditions that impair the conversion of triglyceride-rich lipoproteins to LDL are expected also to impair the interconversion of a putative triglyceride-rich Lp(a) precursor to Lp(a), if such a precursor exists and if the same machinery for interconversion is used. Therefore we have analyzed apo(a) in two genetic conditions that grossly impair the intravascular catabolism of triglyceride-rich lipoproteins and their remnants to LDL, namely LPL-deficiency and type III hyperlipoproteinemia.

The fairly normal distribution of apo(a) in the plasma from patients with LPL deficiency and type III hyperlipoproteinemia observed here is in sharp contrast to the grossly impaired distribution of cholesterol and apo B which reflect the defects in lipoprotein catabolism in these pathological conditions. As in the normolipidemic controls most apo(a) was present in the Lp(a) lipoprotein complex in the plasma from both patient groups (Fig. 1). Apo(a) may be hidden or present in a different conformation in non-Lp(a) fractions (triglyceride-rich lipoproteins, > 1.21 g/ml bottom fraction) and apo(a) might be detected differently in the triglyceride-rich lipoproteins from type I and type III patients by our apo(a) ELISA assay. Therefore the apo(a) distribution was also evaluated in selected density gradients by SDS-PAGE followed by densitometric scanning of the blots. A very good correlation between the two methods was obtained ($r = 0.96$, data not shown). This excellent agreement between the results from immunoblotting and the quantification by the ELISA is also demonstrated in Fig. 5. Fig. 5 *B*, moreover, demonstrates that apo(a) in triglyceride-rich particles and in Lp(a) is recognized identically by the antibody. Hence apo(a) accumulates neither with the chylomicrons or VLDL when LPL is deficient nor with chylomicron remnants or IDL when apo E is defective. This suggests that metabolic steps that are essential for the generation of LDL in plasma do not operate for Lp(a).

LDL is extremely low in patients with LPL deficiency and most apo B is associated with chylomicrons and VLDL in this genetic disorder (37). Likewise LDL concentrations are usually low in patients with type III hyperlipoproteinemia and apo B is mainly associated with remnants and IDL. This is believed to reflect a defect in the interconversion of IDL to LDL in the absence of functional apo E (16). The absence of any gross effect on Lp(a) of two different genetic defects in the pathway that results in LDL formation suggests that Lp(a) is not a product of the same metabolic cascade. Lp(a) may either be secreted from the liver as an intact native particle or parenchymal liver cells secrete apo(a) that then is preferentially assembled with LDL. Krempler et al. (38) have shown that radioiodinated apo B from VLDL, that itself did not contain apo(a), is not a precursor of apo B in Lp(a). Together with the results presented here this suggests a direct synthesis of Lp(a) that bypasses the catabolic cascade of the other apo B–containing lipoproteins. This interpretation is consistent with results in Hep G2 cells that were transfected with recombinant apo(a) containing 17 kringle 4-like domains. These cells secreted recombinant apo(a) which was found complexed with apo B in the medium and floated with the density of Lp(a) (39).

The distribution of apo(a) in the plasma from type I and type III patients was, however, not exactly identical with that in controls. Indeed the percentage of apo(a) associated with the triglyceride-rich and IDL fractions from LPL-deficient and type III patients was measured two- to three-fold higher than in the controls. This might be taken as an indication for a triglyceride-rich precursor of Lp(a). However, in terms of absolute amounts of apo(a) this slight increase was only moderate and might be explained by the affinity of Lp(a) particles to triglyceride-rich lipoproteins that has been demonstrated in vitro (33–36). In apo(a) heterozygotes the two apo(a) isoforms are in two distinct Lp(a) particles which have slightly different densities (40). Our data suggest that there is a preferential association of Lp(a) particles containing high M_r isoforms with the triglyceride-rich lipoproteins (Fig. 3 and data not shown). The preferential association of higher M_r isoforms of apo(a) with the triglyceride-rich lipoproteins seen here in fasting subjects was also observed by Bersot et al. (32) in type III patients and by Pfaffinger et al. (41) in the postprandial phase. The latter authors have suggested that this material represents apo(a)/apo B complexes which are de novo synthesized by the liver. Trieu and McConathy (35) have shown that in vitro up to 72% of purified Lp(a) added to Lp(a)-negative hypertriglyceridemic plasma floated with apo B–containing lipoproteins. The binding of Lp(a) to other apo B–containing lipoproteins is mediated by the kringle IV domain of apo(a) as shown by recombinant apo(a)/LDL complexes (36). Our experiments show that up to 80% of apo(a) can be removed from the triglyceride-rich fraction by simple ultracentrifugation and is recovered as Lp(a) in the density gradient. Together the published in vitro data and the results presented here imply that the presence of apo(a) in triglyceride-rich fractions can not be taken to indicate a triglyceride-rich precursor. Though Lp(a) does not have for a triglyceride-rich precursor, the presence of Lp(a) in association with triglyceride-rich lipoproteins may have pathophysiological consequences. Kostner and Grillhofer (42) have recently shown that apo(a)/Lp(a) stimulates the binding of LDL to fibroblasts severalfold by an LDL receptor-independent mechanism. Likewise apo(a)/Lp(a) might also trigger binding of IDL or triglyceride-rich lipoproteins to cells. The presence of Lp(a) in these fractions might mediate binding of the triglyceride-rich lipoprotein-Lp(a) complexes to various tissues and cells including macrophages (43).

The percentage of apo(a) found in the "bottom" fractions from LPL-deficient patients was also about twice as high as in controls. The reason for this is unclear as is the nature, origin, and relation to Lp(a) of apo(a) in the lipid-poor fractions. In patients with autosomal recessive abetalipoproteinemia apo(a) is almost exclusively present in this fraction as a lipid-poor complex with apo B-100 (20). An apo(a)–apo B complex has also been demonstrated in the lipoprotein-free fractions of supernatant from Hep G2 cells that were transiently transfected with recombinant apo(a) (39). Previous investigators have reported a range of 1.8–27.2% of apo(a) to occur in the lipoprotein-free bottom (21, 44–46). The reason for these discrepant results are not clear but may relate to small numbers of subjects analyzed and/or technical problems, e.g., differential detection of apo(a) isoforms in various fractions by the antibodies used. In our sample the amount of apo(a) in the "bottom" ranged from < 1% to > 29% with a mean in controls of 12%. This covers the range reported in the previous studies. Our immunoblotting experiments not only confirmed the quantitative data from the ELISA but moreover demonstrate that the immunoreactive material in this fraction is authentic

apo(a) having the same apparent M_r as the respective genetic isoform(s) in plasma or Lp(a). This suggests that the apo(a) immunoreactivity detected in the lipoprotein-free fractions of the gradient by our ELISA is not a proteolytic cleavage product of apo(a).

The very low levels of Lp(a) in patients with LPL deficiency are remarkable. They parallel the low concentration of LDL in this pathological condition. The patients with LPL deficiency were from different ethnic groups (two Black, one Arab, one Vietnamese, nine Caucasian). Therefore apo(a) type specific Lp(a) levels are given only for the Caucasian patients in Table IV. However, the plasma Lp(a) concentrations of the three subjects of Black and Indian origin were also low in comparison to their respective reference populations which have much higher Lp(a) concentrations than Caucasians. (11). No reference data are yet available for the Arab and Vietnamese patients. Individuals with Lp(a) concentrations < 2 mg/dl occur with a frequency of less than one in five in Caucasian populations. Thus the chance that nine independent subjects all have Lp(a) concentrations < 2 mg/dl is $\sim 2 \times 10^{-6}$. The low Lp(a) in LPL deficiency might reflect an increased removal capacity for Lp(a) either because LDL is low alone, or due to an increased LDL receptor activity. This effect of the LPL-gene on Lp(a) levels might be a further example for an interaction of two genes determining Lp(a) levels as has been previously demonstrated for the apo(a) and LDL receptor gene loci (23), and for abetalipoproteinemia (20).

Acknowledgments

We thank Linda Fineder and Ulrike Scheidle for their skillful technical assistance and Eva Dollinger for preparing the manuscript.

This work was supported by grants from the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (S-4602) to Dr. Utermann, the Dr. Legerlotz-Stiftung to Dr. Sandholzer, and the National Institute of Health to Dr. Brunzell, grant DK-02456.

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