

The Low Density Lipoprotein Receptor Is Not Required for Normal Catabolism of Lp(a) in Humans

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Abstract

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein which is similar in structure to low density lipoproteins (LDL). The role of the LDL receptor in the catabolism of Lp(a) has been controversial. We therefore investigated the in vivo catabolism of Lp(a) and LDL in five unrelated patients with homozygous familial hypercholesterolemia (FH) who have little or no LDL receptor activity. Purified ¹²⁵I-Lp(a) and ¹³¹I-LDL were simultaneously injected into the homozygous FH patients, their heterozygous FH parents when available, and control subjects. The disappearance of plasma radioactivity was followed over time. As expected, the fractional catabolic rates (FCR) of ¹³¹I-LDL were markedly decreased in the homozygous FH patients (mean LDL FCR 0.190 d⁻¹) and somewhat decreased in the heterozygous FH parents (mean LDL FCR 0.294 d⁻¹) compared with controls (mean LDL FCR 0.401 d⁻¹). In contrast, the catabolism of ¹²⁵I-Lp(a) was not significantly different in the homozygous FH patients (mean FCR 0.251 d⁻¹), heterozygous FH parents (mean FCR 0.254 d⁻¹), and control subjects (mean FCR 0.287 d⁻¹). In summary, absence of a functional LDL receptor does not result in delayed catabolism of Lp(a), indicating that the LDL receptor is not a physiologically important route of Lp(a) catabolism in humans. (*J. Clin. Invest.* 1995; 95:1403–1408.) Key words: apolipoprotein • atherosclerosis • kinetics • cholesterol • hypercholesterolemia

Introduction

Lipoprotein(a) [Lp(a)]¹ is a lipoprotein particle in human plasma related in structure to low density lipoproteins (LDL)

(for review see reference 1). Elevated plasma Lp(a) concentrations have been associated with an increased risk of premature coronary heart disease (CHD) in most cross-sectional studies and some, but not all, prospective studies (for review see reference 2). In family studies, Lp(a) levels in children are highly predictive of premature CHD in parents (2). The relative risk of elevated Lp(a) concentrations is significantly increased in patients who also have high levels of LDL cholesterol (3, 4).

Lp(a) is an LDL-like lipoprotein consisting of lipids and apoB-100, but differs from LDL in that it contains an additional protein called apo(a). Apo(a) is thought to be covalently linked to apoB via a disulfide bridge (1), but can also associate noncovalently with apoB (5, 6). Lp(a) concentrations are strongly genetically determined (7), with at least 90% of the variation determined by variation within the gene for apo(a) (8). The size of the apo(a) protein is highly polymorphic due to genetic variation in the number of kringle repeats in the apo(a) gene. One important factor in determining plasma Lp(a) concentration is the size of the apo(a) protein: the apo(a) protein size is inversely correlated with the plasma Lp(a) concentration (7). We recently demonstrated that apo(a) isoform size does not influence Lp(a) catabolic rate, but rather Lp(a) production rate (9). In addition, genetic factors linked to the apo(a) gene other than apo(a) size affect Lp(a) levels, again primarily by modulating the rate of Lp(a) production (10).

Because Lp(a) contains apoB-100 and bears a structural similarity to LDL, it has been proposed that Lp(a) may be removed from the plasma by the LDL receptor. Despite intensive investigation, this hypothesis remains unproven. Utermann et al. (11) first reported that patients with familial hypercholesterolemia (FH), a genetic disorder caused by molecular defects in the LDL receptor, had plasma Lp(a) levels higher than expected for their respective apo(a) phenotypes. Two other studies supported this observation (12, 13). The ability of the LDL receptor to bind Lp(a) was supported by studies in transgenic mice overexpressing the human LDL receptor, which catabolized human Lp(a) much faster than control mice (14). However, several lines of evidence do not support a role for the LDL receptor in the catabolism of Lp(a) in humans. Lp(a) levels in one large FH kindred were no different in affected and unaffected members of the kindred (15), and another study comparing siblings in FH kindreds found no effect of heterozygous FH on Lp(a) levels (16). In a rhesus monkey model of FH, affected monkeys do not have higher levels of Lp(a) than controls (17). Finally, drugs that upregulate the LDL receptor

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1. Abbreviations used in this paper: CHD, coronary heart disease; FH, familial hypercholesterolemia; Lp(a), lipoprotein(a).

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do not generally result in a decrease in plasma Lp(a) levels (18–22).

To establish whether the LDL receptor plays a physiologic role in the catabolism of Lp(a) in humans, we investigated the metabolism of Lp(a) in five unrelated patients with homozygous FH who have little or no functional LDL receptor activity. We found that radiolabeled Lp(a) was catabolized at a similar rate in the FH homozygotes as in normal subjects studied at the same time, establishing that the LDL receptor is not necessary for normal Lp(a) catabolism and probably plays little role in the metabolism of Lp(a) in humans.

Methods

Study subjects. Five unrelated patients with homozygous FH participated in Lp(a) kinetic studies. The first was a 26-yr-old male with cerebrovascular, peripheral, and coronary atherosclerosis. He had a myocardial infarction at age 7, ileal bypass surgery at age 9, and bilateral carotid endarterectomies at age 25. The second patient was a 7-yr-old female with tendinous and tuberoeruptive xanthomas and evidence of coronary atherosclerosis by coronary arteriography. Her sister, also an FH homozygote, died at age 3 of a myocardial infarction. Her mother, an FH heterozygote with tendon xanthomas and elevated LDL cholesterol, also participated in the study. The third patient was a 12-yr-old male with severe tendinous and tuberoeruptive xanthomas and evidence of coronary atherosclerosis by coronary angiography. His mother, an FH heterozygote with tendon xanthomas and elevated LDL cholesterol, also participated in the study. The fourth patient was a 41-yr-old woman with tendon and tuberoeruptive xanthomas who had previously undergone coronary artery bypass surgery. The fifth patient was an 11-yr-old female with tuberous and tendon xanthomas and coronary artery disease. Both of her heterozygous parents participated in the study. All subjects were taken off of any lipid-lowering therapy for at least 6 wk before the kinetic study.

A total of eight normal control subjects participated in the kinetic studies, two for each study. All subjects had normal fasting plasma glucose levels and normal thyroid, liver, and renal function. They were free of illness and were on no medications. All subjects gave informed consent, and the study protocol was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute.

Isolation and iodination of Lp(a) and LDL. Lp(a) and LDL were isolated from the fasting plasma of healthy subjects who had no risk factors for viral infection and were tested serologically at least twice before lipoprotein isolation. One donor was used for Lp(a) isolation in the first study and another donor was used for the other four studies. Plasma was obtained after a 12-h fast and NaEDTA (0.01%), sodium azide (0.05%), and diisopropyl fluorophosphate (1 mM) were immediately added. For the first study, Lp(a) was isolated from an apo(a)-S2/S4 heterozygote by the method of Eaton et al. (23). Briefly, plasma lipoproteins were isolated by sequential ultracentrifugation, the fraction of density 1.05–1.10 g/ml was dialyzed against PBS/0.01% EDTA, and Lp(a) was purified by lysine-Sepharose affinity chromatography (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). For the other four studies, Lp(a) was isolated from an apo(a)-S2 homozygote according to the procedure described by Fless et al. (5) as reported previously (10). Plasma was adjusted to a density of 1.21 g/ml using solid NaBr and ultracentrifuged for 48 h to isolate total plasma lipoproteins. The $d < 1.21$ g/ml fraction was adjusted to $d = 1.4$ g/ml with NaBr and ultracentrifuged on a 0–30% NaBr density gradient to remove HDL. The fraction containing Lp(a) was then adjusted to a concentration of 7.5% CsCl and ultracentrifuged for 30 h to separate Lp(a) from VLDL and LDL. Residual LDL was eliminated by chromatofocusing on a PBE94 column (Pharmacia AB, Uppsala, Sweden) within a pH range of 7.0–4.0. Samples were extensively dialyzed against PBS with 0.01% EDTA after chromatofocusing. LDL were prepared from fasting plasma

by preparative ultracentrifugation (d 1.03–1.05 g/ml) and dialyzed against PBS/0.01% EDTA. Isolated Lp(a) and LDL were analyzed for purity by SDS-PAGE, agarose electrophoresis (Helena Laboratories, Beaumont, TX), and Ouchterlony immunodiffusion using anti-apoB and anti-apo(a) antibodies.

Purified Lp(a) and LDL were dialyzed against 1 M glycine (pH 10) before iodination using a modification of the iodine monochloride method (9, 10). Briefly, 5 mCi ^{125}I or ^{131}I was added to the Lp(a) solution, then ICl was added rapidly without vortexing. Approximately 1 mol of iodine was incorporated per mol of Lp(a) and LDL. Samples were dialyzed extensively against PBS/0.01% EDTA to remove free iodine. Both lipoprotein samples contained $< 4\%$ lipid label by Folch extraction. Human serum albumin was added to a final concentration of 5% (wt/vol), samples were sterile-filtered through a 0.22- μm filter and tested for pyrogens and sterility before injection.

Study protocol. Subjects were permitted to eat a normal diet but were instructed not to drink alcoholic beverages for 1 wk before and during the study. 1 d before injection, the subjects were given potassium iodide at a dose of 900 mg/d in divided doses, and this was continued for the duration of the study. Radioiodinated Lp(a) was injected after a 12-h fast. Blood samples were obtained 10 min after injection and then at 1, 3, 6, 12, and 24 h, and at 2, 3, 4, 5, 7, 9, 11, and 14 d. Blood was drawn into tubes containing EDTA at a final concentration of 0.1%, immediately placed at 4°C, and plasma was separated by low-speed centrifugation in a refrigerated centrifuge. Sodium azide and aprotinin were added to plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivity in 4-ml plasma aliquots was quantitated in a gamma counter (Cobra; Packard Instruments, Downers Grove, IL). Plasma curves were constructed by dividing the plasma radioactivity at each time point by the plasma radioactivity at the initial 10-min time point. The fractional catabolic rates were obtained from the plasma radioactivity curves using a computer-assisted curve-fitting technique (24).

Ultracentrifugation. Plasma from selected time points after injection was subjected to sequential ultracentrifugation at $d < 1.006$ (VLDL), $d = 1.006$ –1.05 (LDL), $d = 1.05$ –1.21 [Lp(a)], and $d > 1.21$ (lipoprotein deficient) g/ml. Lipoprotein fractions were obtained by tube slicing, and the top and bottom fractions were counted after each centrifugation. Density gradient ultracentrifugation of plasma was also performed as previously described (25).

Apo(a) immunoblotting. Apo(a) isoform determination was performed on whole plasma using a sensitive immunoblotting technique previously described (25). Briefly, plasma samples were delipidated twice in chloroform-methanol 8:5 (vol/vol) and washed twice with phosphate-buffered saline. Samples were reduced with 100 mM dithiothreitol in 8 M urea, incubated at 37°C for 30 min, and solubilized in 40 μl 0.02 M ethylmorpholine containing 10% SDS. Samples were applied to 7.5% polyacrylamide gel electrophoresis with 0.1% cross-linker and run for ~ 4.5 h at 20 mA. After electrotransfer of the proteins to Immobilon PVDF transfer membranes (Millipore Corp., Bedford, MA), membranes were incubated with a 1:2,000 dilution of a monoclonal anti-apo(a) antibody (2D1; Cappel, Durham, NC) and detected with the Vectastain ABC anti-mouse IgG test kit (Vector Laboratories, Burlingame, CA). Several plasma samples of known apo(a) isoform were used as calibration standards.

Analytical methods. Plasma cholesterol and triglycerides were quantitated by automated enzymatic techniques on an Abbott VPSS analyzer (Abbott Laboratories, North Chicago, IL). Plasma apoB concentrations were determined by ELISA as previously described (26). Plasma Lp(a) concentrations were determined by a differential ELISA based on the method of Fless et al. (27) as reported previously (10). Briefly, a monoclonal antibody against apo(a) (2D1; Cappel) was used to coat microtiter plates at a concentration of 10 $\mu\text{g}/\text{ml}$. After blocking with 5% sucrose and 2% BSA, plasma samples at a 1:5,000 dilution were added to wells and incubated for 60 min at 37°C. A sheep polyclonal anti-apoB (BIODESIGN International, Kennebunkport, ME) labeled with horseradish peroxidase was added to the wells at a 1:5,000 dilution

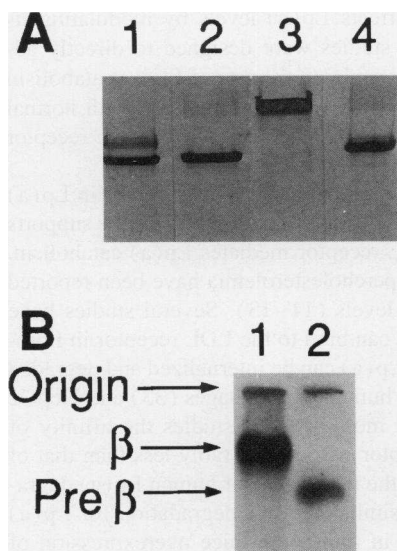


Figure 1. (A) Representative SDS-PAGE of purified Lp(a) (lanes 1 and 3) and LDL (lanes 2 and 4) under reducing (lanes 1 and 2) and nonreducing (lanes 3 and 4) conditions. The single bands in lanes 2 and 4 represent apoB. (B) Autoradiograph of a representative agarose electrophoresis gel of ^{131}I -LDL (lane 1) and ^{125}I -Lp(a) (lane 2).

and incubated for 60 min. Substrate was then added and absorbance read at 450 nm. The standard was a secondary plasma standard calibrated against two commercial Lp(a) standards (Terumo, Elkton, MD and Immuno, Graz, Austria). Two controls were run with each assay. Intra- and interassay coefficients of variation were $< 3\%$ and $< 10\%$, respectively.

Results

Analysis of radiolabeled LDL and Lp(a). For each study, purified LDL and Lp(a) were analyzed by reducing and nonreducing 5–15% SDS-PAGE. A representative SDS gel is shown in Fig. 1 A, demonstrating that the LDL contained only apoB, whereas the labeled Lp(a) contained both apoB and apo(a). In the absence of reducing agents, there was no evidence of free apoB in the Lp(a) preparations. There was also no evidence of apoB degradation, indicating lack of significant oxidative modification to the lipoproteins. Nondenaturing agarose electrophoresis was also performed on the radiolabeled lipoproteins. A representative agarose electrophoresis of radiolabeled LDL and Lp(a) is shown in Fig. 1 B. In all cases, radiolabeled LDL was found only in the beta region and Lp(a) only in the prebeta region, with no evidence of contaminating LDL or HDL in the Lp(a) preparations.

Metabolism of LDL and Lp(a). Fig. 2 A illustrates representative plasma LDL decay curves in the homozygous FH patient 2, her heterozygous mother, and two controls. In all five homozygous FH patients, the catabolism of LDL was markedly delayed, consistent with a defect in LDL receptor activity. The four heterozygous parents had moderately delayed catabolism compared with the normal subjects.

In contrast, the catabolism of Lp(a) was not significantly different in the five homozygous FH patients compared with the heterozygous parents and controls studied at the same time (Fig. 2 B). The urinary excretion of radioactivity, a marker of protein degradation, confirmed the markedly delayed catabolism of LDL and the unaffected Lp(a) catabolism in the FH homozygotes. The kinetic parameters of LDL and Lp(a) in the study subjects are provided in Table I.

We investigated whether Lp(a) may be converted to LDL

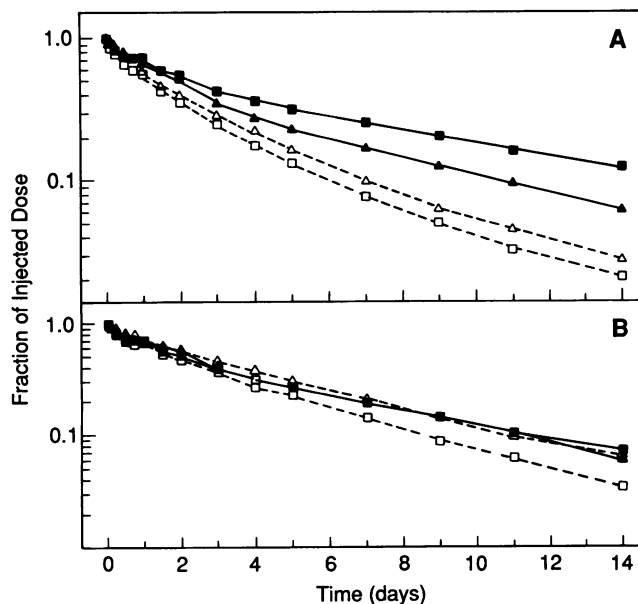


Figure 2. (A) Catabolism of ^{131}I -LDL in homozygous FH patient 2 (filled squares), her heterozygous mother (filled triangles), and two normal control subjects (open squares and triangles). (B) Catabolism of ^{125}I -Lp(a) in the same study subjects.

in the plasma by performing separation of LDL and Lp(a) at several time points after injection using both sequential ultracentrifugation and density gradient ultracentrifugation. In all studies, there was appearance of ^{125}I [originally associated only with Lp(a)] in LDL density over time, consistent with conversion of some Lp(a) to LDL. In Fig. 3, the distribution of radioactivity across the density spectrum over time in FH homozygote 3 is shown. Over time, ^{125}I counts originally associated with Lp(a) appeared in the LDL density range, accounting for a gradually increasing fraction of the total ^{125}I remaining in the plasma. Furthermore, the ^{125}I counts appearing in the LDL density range behaved kinetically like LDL; the catabolism was very slow in the FH homozygotes, intermediate in the heterozygotes, and fastest in the normal subjects (Fig. 4 A). In contrast, the catabolism of the injected ^{125}I -Lp(a) which remained in the Lp(a) density range was virtually identical in the FH homozygotes, heterozygotes, and controls (Fig. 4 B). The total amount of radiolabeled Lp(a) converted to LDL cannot be definitively quantitated without a full compartmental model, but it was estimated to be $< 10\%$ of the total injected Lp(a).

Discussion

Because plasma concentrations of Lp(a) are associated with risk of premature CHD, it is important to determine the factors which regulate Lp(a) levels. The apo(a) size polymorphism accounts for much of the variation in Lp(a) plasma levels (7) due to an effect on Lp(a) production rate, not catabolic rate (9). Other heritable factors unrelated to apo(a) isoform size also affect Lp(a) levels (8); variation in the rate of Lp(a) production is also the most important determinant of variation in Lp(a) levels independent of apo(a) phenotype (10). However, it has been proposed that LDL receptor expression may

Table 1. Kinetic Parameters of Lp(a) Metabolism

Subject	LDL FCR	LP(a) FCR
	d^{-1}	d^{-1}
FH homozygotes		
1	0.233	0.254
2	0.178	0.256
3	0.187	0.249
4	0.171	0.265
5	0.182	0.233
Mean	0.190	0.251
SD	0.025	0.012
FH heterozygotes		
1	0.308	0.270
2	0.263	0.250
3	0.318	0.228
4	0.289	0.269
Mean	0.294	0.254
SD	0.024	0.020
Control subjects		
1	0.413	0.292
2	0.452	0.303
3	0.465	0.277
4	0.415	0.297
5	0.427	0.314
6	0.365	0.236
7	0.323	0.275
8	0.349	0.301
Mean	0.401	0.287
SD	0.050	0.024

FCR, fractional catabolic rate.

be another factor that affects Lp(a) levels by modulating its catabolism (11). These studies were designed to directly address this question by determining the rate of Lp(a) catabolism in patients who lack the LDL receptor compared with normal subjects. Our results establish that absence of the LDL receptor does not impair the catabolism of Lp(a) in humans.

The data concerning the role of the LDL receptor in Lp(a) uptake and degradation are conflicting. Some evidence supports the concept that the LDL receptor mediates Lp(a) catabolism. Patients with familial hypercholesterolemia have been reported to have elevated Lp(a) levels (11–13). Several studies have demonstrated that Lp(a) can bind to the LDL receptor in fibroblasts (28–32) and that Lp(a) can be internalized and degraded via the LDL receptor by human macrophages (33) and HepG2 cells (34). However, in most of these studies the affinity of Lp(a) for the LDL receptor was considerably less than that of LDL. In vivo studies on the tissue sites of human Lp(a) degradation in rats indicated similar sites of degradation for Lp(a) and LDL (35). Finally, in transgenic mice overexpression of human LDL receptors resulted in accelerated catabolism of Lp(a) (14).

However, there is also considerable evidence against a role for the LDL receptor in Lp(a) catabolism. Studies within FH kindreds have not indicated that Lp(a) levels were elevated in heterozygous individuals compared with unaffected family members (15, 16). In a rhesus monkey model of FH, affected monkeys do not have higher levels of Lp(a) than controls (17). Lp(a) isolated from patients with familial defective apoB does not contain a higher proportion of defective apoB than normal apoB (36). Finally, drugs that upregulate the LDL receptor do not generally result in a decrease in plasma Lp(a) levels (18–22).

Two kinetic studies of the in vivo metabolism of Lp(a) in humans have not conclusively settled the question of the role of the LDL receptor in the catabolism of Lp(a) in humans. Krempler et al. (30) reported that the catabolism of Lp(a) in one putative homozygous FH patient was ~ 20% slower than the mean of 12 control subjects and were unable to make a

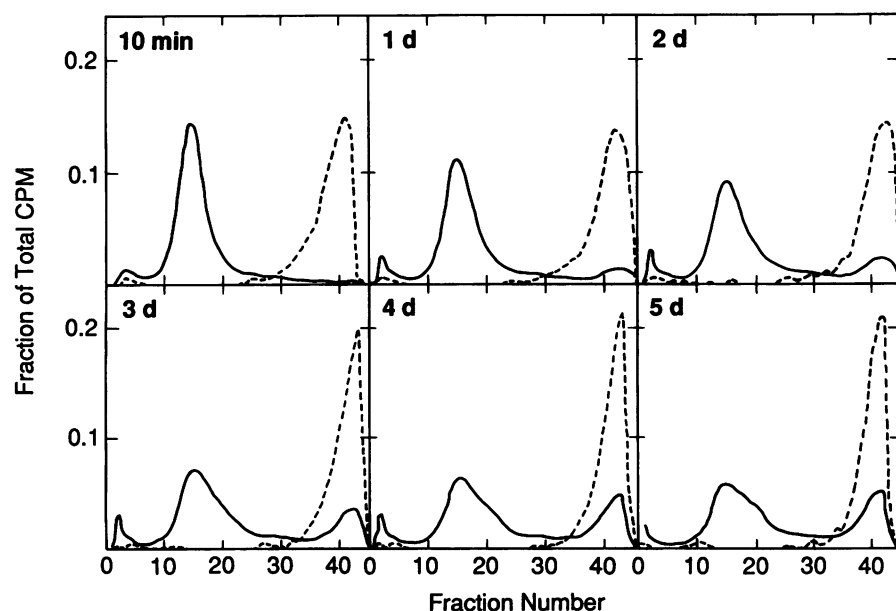


Figure 3. Representative density gradient ultracentrifugation of plasma from FH patient 3 at several time points after injection. Solid lines represent the injected ^{125}I -Lp(a), and broken lines represent the injected ^{131}I -LDL. Over time, the fraction of ^{125}I counts in the LDL density range increases.

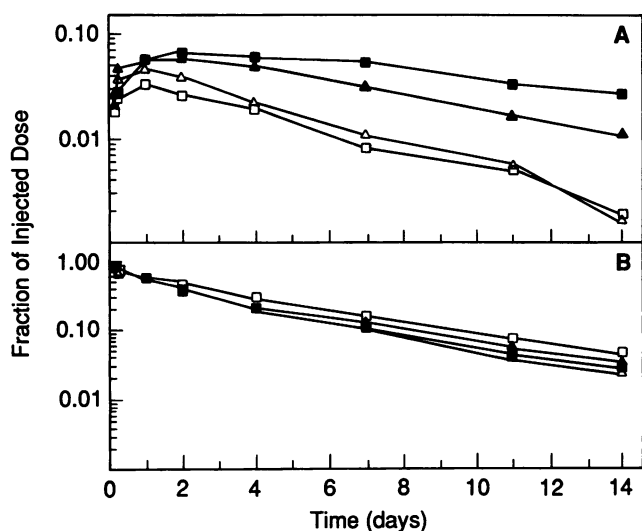


Figure 4. Comparison of injected ^{125}I -Lp(a) reisolated in the LDL density range (A) and in the Lp(a) density range (B) in homozygous FH patient 3 (filled squares), his heterozygous mother (filled triangles), and two control subjects (open squares and triangles).

definitive conclusion about the role of the LDL receptor. Knight et al. (37) reported that four heterozygous FH patients did not have delayed catabolism of Lp(a) compared with four hyperlipidemic controls, although the interpretation of these studies was limited by the fact that the subjects were heterozygous and therefore had functional LDL receptors.

The results of our studies in FH homozygotes establish that the LDL receptor is not required for normal catabolism of Lp(a), as patients lacking LDL receptors had no difference in the catabolism of Lp(a) despite a marked delay in the catabolism of LDL. Our kinetic results suggest that, if Lp(a) levels are elevated in homozygous FH, the cause is not delayed Lp(a) catabolism but rather increased Lp(a) production. This would be consistent with the fact that LDL apoB production rates are increased in homozygous FH (38).

Several theoretical limitations to this interpretation of the data should be mentioned. First, we studied only two different apo(a) isoforms, potentially limiting the ability to generalize the conclusions to other isoforms. However, this is unlikely, given the fact that apo(a) isoform size does not affect Lp(a) catabolic rate in vivo (9). Second, we used lysine-Sepharose affinity chromatography for isolation of some of the Lp(a) particles. However, most of the Lp(a) bound to the lysine-Sepharose and, furthermore, we obtained similar kinetic results when Lp(a) was isolated using an alternate method. Finally, the isolation and radioiodination of Lp(a) could theoretically influence its in vivo metabolism by causing oxidative modification. However, we had no evidence by SDS-PAGE or nonreducing agarose electrophoresis that the Lp(a) or LDL had been oxidized. Therefore, it is highly likely that the results presented here are consistent with the primary conclusion that the LDL receptor is not required for normal catabolism of Lp(a).

An additional finding in this study was that a small portion (< 10%) of the injected Lp(a) appeared to be converted to LDL in vivo. We demonstrated that the LDL which was generated from Lp(a) in vivo had markedly delayed catabolism in

the homozygous FH patients compared with the control subjects, thus confirming it metabolically to be LDL. In contrast, the catabolism of Lp(a) which was not converted to LDL was virtually identical in the FH homozygotes and controls. Conversion of Lp(a) to LDL, though not a major metabolic pathway based on these data, could be an additional metabolic source of plasma LDL in FH patients. The mechanism by which this occurs is presumably dissociation of apo(a), although these studies do not permit further investigation of the mechanism.

We conclude that the LDL receptor is not required for catabolism of Lp(a) and probably plays little if any physiological role in mediating Lp(a) catabolism in humans. A small fraction of Lp(a) appears to be converted to LDL in vivo, and the LDL which results is then dependent on the LDL receptor for normal catabolism. The disproportionately elevated Lp(a) levels found in some homozygous FH patients are probably not due to delayed catabolism of Lp(a), but may be due to increased rates of Lp(a) production.

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