Variation in Lipoprotein(a) Concentrations among Individuals with the Same Apolipoprotein (a) Isoform Is Determined by the Rate of Lipoprotein(a) Production

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Abstract

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein which is similar in structure to, but metabolically distinct from, LDL. Factors regulating plasma concentrations of Lp(a) are poorly understood. Apo(a), the protein that distinguishes Lp(a) from LDL, is highly polymorphic, and apo(a) size is inversely correlated with plasma Lp(a) level. Even within the same apo(a) isoform class, however, plasma Lp(a) concentrations vary widely. A series of in vivo kinetic studies were performed using purified radiolabeled Lp(a) in individuals with the same apo(a) isoform but different Lp(a) levels. In a group of seven subjects with a single S4-apo(a) isoform and Lp(a) levels ranging from 1 to 13.2 mg/dl, the fractional catabolic rate (FCR) of ^{131}I-labeled S2-Lp(a) (mean 0.328 day^{-1}) was not correlated with the plasma Lp(a) level ($r = -0.346$, $P = 0.45$). In two S4-apo(a) subjects with a 10-fold difference in Lp(a) level, the FCR's of ^{125}I-labeled S4-Lp(a) were very similar in both subjects and not substantially different from the FCRs of ^{131}I-S2-Lp(a) in the same subjects. In four subjects with a single S2-apo(a) isoform and Lp(a) levels ranging from 9.4 to 91 mg/dl, Lp(a) concentration was highly correlated with Lp(a) production rate ($r = 0.993$, $P = 0.007$), but poorly correlated with Lp(a) FCR (mean 0.304 day^{-1}). Analysis of Lp(a) kinetic parameters in all 11 subjects revealed no significant correlation of Lp(a) level with Lp(a) FCR ($r = -0.53$, $P = 0.09$) and a strong correlation with Lp(a) production rate ($r = 0.99$, $P < 0.0001$). We conclude that the substantial variation in Lp(a) levels among individuals with the same apo(a) phenotype is caused primarily by differences in Lp(a) production rate. (*J. Clin. Invest.* 1993. 91:443–447.) Key words: atherosclerosis • cholesterol • metabolism • kinetics • apolipoprotein

Introduction

Lipoprotein(a) [Lp(a)][1] is an LDL-like lipoprotein containing apolipoprotein B (apoB) as well as an additional apolipoprotein termed apo(a) (1). Epidemiologic studies have demonstrated that the risk of premature coronary heart disease (CHD) is associated with plasma Lp(a) concentration (1–5). Lp(a) levels are predictive of the extent of angiographically documented CHD independently of LDL cholesterol levels (6), although the relative risk of elevated Lp(a) concentrations is significantly increased in patients who also have high levels of LDL cholesterol (7, 8). In addition to CHD, Lp(a) has been shown to be an independent risk factor for cerebrovascular atherosclerosis (9) and saphenous vein bypass graft stenosis (10).

Previous studies have demonstrated that apo(a) is genetically polymorphic (11) with the apo(a) isoproteins ranging in approximate size from 420 to 840 kD (12). Apo(a) isoforms are inherited in an autosomal codominant fashion (13). The apo(a) phenotype is thought to be an important factor in determining plasma Lp(a) concentration, with an inverse correlation between the size of the apo(a) isoprotein and the plasma Lp(a) concentration (13). The apo(a) size variation has been estimated to be responsible for ~ 40% of the variation in plasma Lp(a) concentrations (11, 14).

However, Lp(a) concentrations also vary substantially within each apo(a) isoform class (5, 8, 13, 15–17), indicating that factors other than apo(a) phenotype affect Lp(a) level. Evidence indicates that genetic factors linked to the apo(a) gene but distinct from the apo(a) phenotype have an important effect on plasma Lp(a) levels (17). To determine whether these factors affect the rate of Lp(a) production or catabolism, we performed a series of in vivo Lp(a) kinetic studies in normolipidemic subjects with the same apo(a) isoform but a wide range of plasma Lp(a) concentrations.

Methods

Study subjects. The study subjects were all healthy young adults who were admitted to the Clinical Center of the National Institutes of Health. Subjects were selected for the presence of a single detectable S2 or S4 apo(a) isoform on apo(a) immunoblotting (see below) and for a wide range of plasma Lp(a) concentrations. 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. Clinical data on the study subjects are presented in Table 1. Values are the mean of five fasting determinations made during the metabolic study.

Apo(a) immunoblotting. Apo(a) isoform determination was performed on whole plasma using a sensitive immunoblotting technique previously described (18). Briefly, plasma samples were delipidated twice in chloroform/methanol 8:5 (vol/vol) and washed twice with PBS. Samples were reduced with 100 mM DTT in 8 M urea, incubated at 37°C for 30 min, and solubilized in 40 μl 0.02 M ethylmorpholine

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1. *Abbreviations used in this paper:* CHD, coronary heart disease; FCR, fractional catabolic rate; FH, familial hypercholesterolemia Lp(a), lipoprotein(a); PR, production rate.

Table I. Characteristics of Study Subjects

Subject	Sex	Age	BMI	Chol	TG	ApoB
		yr	*kg/m²*		*mg/dl*	
1	M	21	26.2	146	95	115
2	M	22	22.6	148	68	68
3	F	21	19.7	136	53	54
4	M	20	28.5	145	101	86
5	F	20	21.1	134	44	48
6	M	22	25.0	167	109	123
7	F	21	22.5	147	69	80
8	M	21	28.2	209	145	106
9	M	23	27.9	177	100	89
10	M	20	20.6	175	125	89
11	M	39	27.8	206	108	136

BMI, body mass index.

containing 10% SDS. Samples were applied to 7.5% PAGE with 0.1% crosslinker (19) and run for ~ 4.5 hours at 20 mA. After electrotransfer of the proteins to Immobilon polyvinyldifluoride 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, Inc., Burlingame, CA). Several plasma samples of known apo(a) isoform were used as calibration standards.

Isolation and iodination of Lp(a). Lp(a) was isolated from the fasting plasma of two subjects with only one detectable apo(a) isoform (one S2 and one S4) according to the procedure described by Fless et al. (20). The subjects were healthy, had no risk factors for viral infection, and were tested serologically for hepatitis B, hepatitis C, and HIV several times before Lp(a) isolation. Plasma was obtained after a 12-h fast and NaEDTA (0.01%), sodium azide (0.05%), and difluorophosphonate (1 mM) were immediately added. 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, Uppsala, Sweden) within a pH range of 7.0–4.0. Isolated Lp(a) was analyzed for purity by nonreducing SDS-PAGE and by 0.6% agarose electrophoresis (Helena Laboratories, Beaumont, TX). Samples were extensively dialyzed against PBS with 0.01% EDTA after chromatofocusing and before iodination.

Purified Lp(a) was dialyzed against a 1-M glycine (pH 10) buffer before iodination using a modification of the iodine monochloride method (21). Briefly, 5 mCi ^{125}I or ^{131}I were added to the Lp(a) solution, then ICl was added rapidly without vortexing. Approximately 1 mol iodine was incorporated per mole of Lp(a). Samples were dialyzed extensively against PBS/0.01% EDTA to remove free iodine. HSA 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. Autoradiography of SDS-PAGE of iodinated Lp(a) confirmed that both apo(a) and apoB were radioiodinated.

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 Kallekrein inhibitor units/ml, respectively. Radioactivity in 4-ml plasma aliquots was quantitated in a Packard Cobra gamma counter (Packard Instrument Co., 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.

Analytical methods. The fractional catabolic rates (FCRs) were obtained from the plasma radioactivity curves using a multiexponential curve-fitting technique (22). Production rates (PR) were determined using the formula PR = [Lp(a) concentration × FCR × plasma volume]/body weight. Plasma volume was assumed to be 4% of body weight.

Plasma cholesterol and triglycerides were quantitated by automated enzymatic techniques on an analyzer (VP Super System; Abbott Laboratories, North Chicago, IL). Plasma apoB concentrations were determined by ELISA as previously described (23). Plasma Lp(a) concentrations were determined by a differential ELISA based on the method of Fless et al. (24). Briefly, a monoclonal antibody against apo(a) (2D1; Cappel) was used to coat microtiter plates at a concentration of 10 μg/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 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, Vienna, Austria). Two controls were run with each assay. Intra- and interassay coefficients of variation were < 3% and < 10%, respectively.

Results

Apo(a) immunoblots demonstrating the apo(a) isoforms of the study subjects are shown in Fig. 1. The apo(a) isoforms, plasma Lp(a) concentrations, and Lp(a) kinetic parameters in all study subjects are presented in Table II. Seven subjects with single S4-apo(a) isoforms and Lp(a) concentrations ranging from 1 to 13.2 mg/dl were injected with ^{131}I-labeled S2-Lp(a) isolated from an S2-apo(a) individual. There was no correlation between the S2-Lp(a) FCR and Lp(a) level ($r = -0.346$, $P = 0.45$), indicating that the differences in Lp(a) levels among these seven S4-apo(a) subjects were not caused by differences in the rate of Lp(a) catabolism. Two of the S4-apo(a) subjects (2 and 7) were also injected simultaneously with an ^{125}I-labeled S4-Lp(a) particle. The plasma curves of the radiolabeled S4-

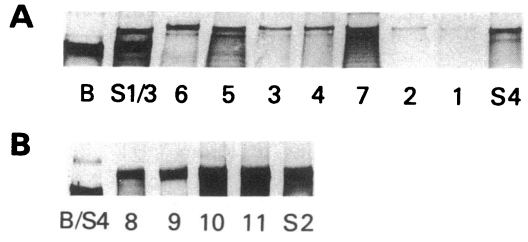

Figure 1. Apo(a) immunoblots of plasma from study subjects, which are numbered below each lane according to Tables I and II. (*A*) includes the seven subjects with S4-apo(a) and (*B*) includes the four subjects with S2-apo(a). Standards are indicated by the appropriate apo(a) isoform classification. The faint bands seen just below the major bands are a result of the apo(a) phenotyping procedure and do not represent a second apo(a) isoform.

Table II. Kinetic Parameters of Lp(a) Metabolism

Subject	Apo(a) phenotype	Lp(a) Concentration	Lp(a) FCR	Lp(a) PR
		mg/dl	*d^{-1}*	*mg/kg-d*
1	S4	1.0	0.374	0.15
2	S4	1.3	0.222*	0.13
3	S4	3.5	0.393	0.55
4	S4	3.6	0.312	0.45
5	S4	5.3	0.348	0.74
6	S4	8.6	0.361	1.24
7	S4	13.2	0.246*	1.30
8	S2	9.4	0.394	1.48
9	S2	19.0	0.281	2.13
10	S2	48.0	0.298	5.67
11	S2	91.0	0.244	8.88
Mean		18.5	0.316	2.07
SD		27.5	0.062	2.74

* FCR of S4-Lp(a); all other FCRs are of S2-Lp(a).

Lp(a) particle were very similar in these two S4-apo(a) subjects (Fig. 2), indicating that the 10-fold difference in their plasma Lp(a) levels was not caused by a difference in the catabolic rate of Lp(a). The fractional catabolic rates of the S2-Lp(a) and S4-Lp(a) particles in these two subjects were not substantially different (0.242 vs. 0.222 d^{-1} for subject 1 and 0.266 vs. 0.246 d^{-1} for subject 7, respectively), indicating that apo(a) isoform did not have a significant impact on Lp(a) catabolic rate. Therefore, in the five other S4-apo(a) subjects the FCR of the S2-Lp(a) particle was used for further analysis.

Radiolabeled ^{131}I-S2-Lp(a) was also injected into four subjects with single S2-apo(a) isoforms in order to investigate the mechanism for the 10-fold difference in their Lp(a) concentrations. In these four subjects, the FCR of S2-Lp(a) had only a weak inverse correlation ($r = -0.766$, $P = 0.23$) with the plasma Lp(a) level, whereas the Lp(a) production rate was strongly correlated ($r = 0.993$, $P = 0.007$) with plasma Lp(a) concentration.

Analysis of Lp(a) kinetic parameters in all 11 subjects revealed no correlation of Lp(a) level with Lp(a) fractional catabolic rate (Fig. 3), and a strong direct correlation with Lp(a) production rate (Fig. 4). The Lp(a) PRs in both the S4-apo(a) and S2-apo(a) subjects fit the same regression line.

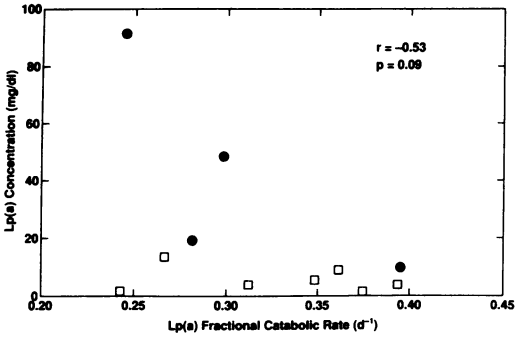

Figure 3. Correlation of plasma Lp(a) level with Lp(a) fractional catabolic rate in seven S4-apo(a) subjects (*open squares*) and four S2-apo(a) subjects (*closed circles*).

To determine whether there may be differences among Lp(a) particles from different individuals of the same apo(a) isoform which may affect their catabolic rates, we simultaneously isolated S2-Lp(a) from subject 10, with a plasma level of 48 mg/dl, and from subject 11, with a plasma level of 91 mg/dl. Purified S2-Lp(a) from subject 10 was labeled with ^{125}I and that from subject 11 with ^{131}I and both labeled Lp(a) particles were injected simultaneously into three study subjects (4, 5, and 10). The FCRs of ^{125}I-S2-Lp(a) from subject 10 were an average of only 12% faster than those of the ^{131}I-S2-Lp(a) from subject 11. This small difference in FCR between these two S2-Lp(a) particles cannot explain the twofold difference in plasma Lp(a) levels between these two homozygous S2-apo(a) subjects. Therefore, the major reason for the difference in Lp(a) concentration between these two subjects was not a structural difference between the two S2-Lp(a) particles affecting their rate of catabolism.

Discussion

Because plasma concentrations of Lp(a) are associated with risk of premature CHD, it is important to determine the factors that control Lp(a) levels. The apo(a) size polymorphism accounts for ~ 40% of the variation in Lp(a) plasma levels (14)

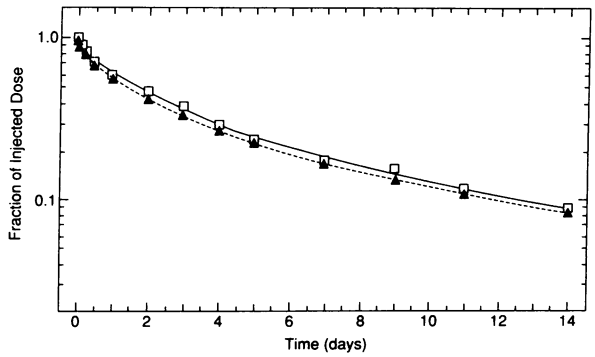

Figure 2. Plasma radioactivity curves of ^{125}I-S4-Lp(a) in two S4-apo(a) homozygotes with Lp(a) levels of 1.3 and 13.2 mg/dl (subjects 2 and 7, respectively).

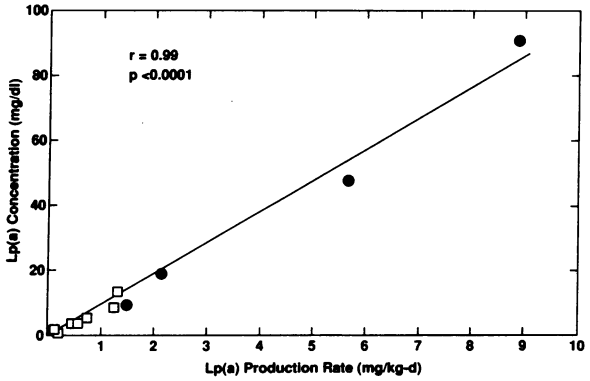

Figure 4. Correlation of plasma Lp(a) level with Lp(a) production rate in seven S4-apo(a) subjects (*open squares*) and four S2-apo(a) subjects (*closed circles*).

by a mechanism that is not known. Therefore, 60% of the variation in Lp(a) levels is caused by factors unrelated to apo(a) isoform size, as evidenced by the large variation of Lp(a) levels within the same apo(a) isoform class (5, 8, 13, 15–17). Some of this variation may be explained by known metabolic factors. Patients with familial hypercholesterolemia (FH) have been reported to have elevated Lp(a) levels (15, 25, 26), although a study in one large kindred did not support this relationship (16). Patients with chronic renal failure (27) and nephrotic syndrome (reference 28 and Wanner et al., unpublished data) have increased Lp(a) levels. Finally, recent evidence suggests that estrogens may have an influence on Lp(a) levels (29–32). Nevertheless, most of the variation in Lp(a) levels cannot be explained by these additional metabolic factors. A recent report by Lackner et al. (17) indicated that there are heritable factors linked to the apo(a) gene but distinct from apo(a) isoprotein size that strongly influence Lp(a) levels. However, it has not been determined whether these additional genetic factors affect the rate of Lp(a) production or catabolism. The purpose of this investigation was to determine the metabolic basis for the substantial variation in plasma Lp(a) concentrations among individuals with the same apo(a) phenotype.

Two other studies of the in vivo metabolism of Lp(a) in humans have been reported. Krempler et al. (33) reported the turnover of autologous Lp(a) in a series of nine subjects of undefined apo(a) phenotype, seven of whom had Lp(a) levels > 25 mg/dl. These investigators found a correlation between Lp(a) level and production rate, but an effect of apo(a) isoform could not be excluded. Knight et al. (34) reported the turnover of autologous Lp(a) in four heterozygous FH and four non-FH hyperlipidemic subjects of variable apo(a) phenotype, all of whom had Lp(a) levels > 40 mg/dl. These investigators also found a correlation between Lp(a) level and production rate, but the study included only hyperlipidemic subjects, all of whom had very high Lp(a) levels. The mean Lp(a) FCRs in these two reports (0.306 and 0.293 d^{-1}, respectively) were very comparable to the mean FCR of S2-Lp(a) in the current study (0.316 d^{-1}).

The present study was designed to directly investigate the major metabolic determinant of Lp(a) concentration in a group of subjects with the same apo(a) phenotype but a broad range of Lp(a) levels. Study subjects were selected to control not only for apo(a) phenotype, but also for other genetic and environmental variables that may affect Lp(a) metabolism. All subjects were of similar age, normolipidemic, and used no alcohol or medications. The results establish that the large differences in plasma Lp(a) levels among study subjects of the same apo(a) isoform were caused by differences in Lp(a) production rates, and not by differences in rates of Lp(a) catabolism. Our results also suggest that the apo(a) size polymorphism does not affect Lp(a) catabolic rate, since there was no significant difference in FCR between ^{125}I-S2-Lp(a) and ^{131}I-S4-Lp(a) particles studied simultaneously in two S4-apo(a) individuals. However, this must be confirmed by further studies.

It remains to be determined whether this genetic variation in Lp(a) production rate is caused by differences in apo(a) gene transcription, apo(a) protein translation, or Lp(a) particle assembly and secretion. Azrolan et al. (35) reported that in a cynomolgus monkey model, plasma Lp(a) levels correlated with hepatic mRNA abundance; however, upon multivariate analysis, apo(a) size and hepatic apo(a) mRNA levels together accounted for only 58% of the variation in plasma Lp(a) levels, suggesting that both transcriptional and posttranscriptional mechanisms may be involved. Regardless of the cellular mechanism, the demonstration that Lp(a) production rate is the major determinant of plasma Lp(a) levels independent of apo(a) isoform directs further investigation to factors affecting Lp(a) production and may have important implications for the pharmacologic modulation of elevated Lp(a) concentrations in individuals at risk for premature CHD.

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