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The metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein (a) in human beings☆

Jennifer L. Jenner[a], Leo J. Seman[a], John S. Millar[b], Stefania Lamon-Fava[a], Francine K. Welty[a], Gregory G. Dolnikowski[c], Santica M. Marcovina[d], Alice H. Lichtenstein[a], P. Hugh R. Barrett[e,f], Carl deLuca[a], Ernst J. Schaefer[a,*]

[a]*Lipid Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, USA*
[b]*Department of Medicine, University of Pennsylvania, Philadelphia, PA 19004, USA*
[c]*Mass Spectrometry Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, USA*
[d]*Northwest Lipid Research Laboratories, University of Washington, Seattle, WA 98103, USA*
[e]*School of Medicine and Pharmacology, University of Western Australia, Perth 6847, Australia*
[f]*Western Australia Institute for Medical Research, Perth 6847, Australia*

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Abstract

The metabolism of apolipoproteins (apo) (a) and B-100 within plasma lipoprotein (a) [Lp(a)] was examined in the fed state in 23 subjects aged 41 to 79 years who received a primed-constant infusion of [5,5,5-$^2H_3$] leucine over 15 hours. Lipoprotein (a) was isolated from the whole plasma using a lectin affinity-based method. Apolipoprotein (a) and apoB-100 were separated by gel electrophoresis, and tracer enrichment of each apolipoprotein was measured using gas chromatography/mass spectrometry. Data were fit to a multicompartmental model to determine fractional catabolic rates (FCRs) and secretion rates (SRs). The FCRs of apo(a) and apoB-100 (mean ± SEM) within plasma Lp(a) were significantly different (0.220 ± 0.030 pool/d and 0.416 ± 0.040 pool/d, respectively; $P < .001$). Apolipoprotein (a) SR (0.50 ± 0.08 mg/[kg per d]) was significantly lower than that of apoB-100 SR (1.53 ± 0.22 mg/[kg per d]; $P < .001$) of Lp(a). Plasma concentrations of Lp(a) were correlated significantly with both apo(a) SR and apoB-100 SR ($r = 0.837$ and $r = 0.789$, respectively; $P < .001$) and negatively with apo(a) FCR and Lp(a) apoB-100 FCR ($r = -0.547$ and $r = -0.717$, respectively; $P < .01$). These data implicate different metabolic fates for apo(a) and apoB-100 within Lp(a) in the fed state. We therefore hypothesize that apo(a) does not remain covalently linked to a single apoB-100 lipoprotein but that it rather reassociates at least once with another apoB-100 particle, probably newly synthesized, during its plasma metabolism.

1. Introduction

Elevated plasma levels of lipoprotein (a) [Lp(a)] are associated with an increased risk of developing coronary heart disease (CHD) [1-3]. A meta-analysis of 27 prospective studies has confirmed a clear association between Lp(a) and CHD [4]. The metabolism of this lipoprotein has not yet been fully elucidated in spite of 40 years of investigation. Lipoprotein (a) contains 2 disulfide-linked proteins, apolipoprotein (apo) B-100 and apo(a). The latter is a highly glycosylated protein comprising a variable number of repeat domains that are homologous to the kringle IV structure found in plasminogen. The number of repeat domains ranges from 11 to 41 [5]. Apolipoprotein (a) is attached to cysteine at the amino acid position 4326 of apoB-100, which is a 513 kd protein found on very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low-density lipoproteins (LDL). The majority of Lp(a) is found in the LDL density range (1.019-1.063 g/mL), but some are being found in the density range of high-

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* Corresponding author. Tel.: +1 617 556 3100; fax: +1 617 556 3103.
E-mail address: ernst.schaefer@tufts.edu (E.J. Schaefer).

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density lipoprotein 2 (1.063-1.125 g/mL) and of triglyceride-rich lipoproteins (<1.019 g/mL) [6,7].

Plasma concentrations of Lp(a) mass vary widely from less than 1 mg/dL to greater than 100 mg/dL, and its distribution in white populations is highly skewed, with most individuals having concentrations 10 mg/dL or less [8,9]. A modest inverse correlation of Lp(a) concentrations with plasma triglyceride levels has been shown [8]. Most lipid-lowering medications, with the exception of niacin and neomycin, do not reduce plasma Lp(a) levels [10,11]. Hormones such as estrogen, progestins, and anabolic steroids have been shown to reduce Lp(a) levels in plasma [12,13]. To develop strategies to reduce plasma Lp(a) concentrations, a clear understanding of Lp(a) metabolism is essential. A number of investigators have examined the kinetics of Lp(a) in normolipidemic and hyperlipidemic subjects [14-21]. Many of these studies, however, were conducted with a limited number of subjects, usually in the fasted state. The purpose of the present study was to use a stable isotope methodology to examine the kinetics of apo(a) and apoB-100 within Lp(a) isolated from whole plasma in subjects with a wide range of plasma Lp(a) concentrations while in the constantly fed state. The postprandial state is the most physiological one in human beings. Our data indicate that the kinetics of postprandial apo(a) and apoB-100 within Lp(a) differ from each other, suggesting a more complex metabolism of the protein constituents of this particle than has been previously described.

2. Materials and methods

2.1. Subjects

A total of 23 subjects (16 men and 7 women) older than 40 years was studied. Women were postmenopausal. All subjects had no evidence of chronic disease, were nonsmokers, and did not take medications known to affect lipoprotein metabolism. Alcohol intake was not permitted during the study. The study protocol was approved by the Tufts University-New England Medical Center Investigation Review Board. Subjects gave written consent after having the protocol explained to them.

2.2. Experimental protocol

Subjects were provided an average American diet for 6 weeks, as previously described [22]. At the end of this diet period, subjects underwent a 15-hour primed-constant infusion with [5,5,5-$^2H_3$] leucine (99.8% atom excess) while in the fed state, as previously reported [23]. Briefly, at 6:00 AM, after having fasted for 12 hours, subjects were provided a diet equivalent to the average American diet (35% of energy as fat, 14% saturated fat, 144 mg cholesterol/4.2 MJ [1000 kcal]) in the form of 20 small hourly meals, each representing 1/20th of each subject's daily estimated energy requirement. At 11:00 AM, an intravenous bolus injection of deuterated leucine (10 μmol/kg body weight) was administered, immediately followed by a constant infusion (10 μmol/kg body weight per hour). Blood was drawn via a second intravenous line at hours 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15.

2.3. Lipoprotein fraction preparation and quantitation

Blood from each time point was collected into tubes containing EDTA (0.1% final concentration). Plasma was separated at 1100*g* for 20 minutes at 4°C. Very low density lipoprotein, IDL, and LDL fractions were separated by sequential ultracentrifugation using an L8-80 Beckman ultracentrifuge (Beckman, Palo Alto, Calif), as previously described [24].

Lipid measurements in plasma obtained at each time point were carried out using an Abbott Diagnostics Spectrum CCX (bichromatic analyzer) with Abbott enzymatic reagents (Abbott Diagnostics, Irving, Tex) [25]. High-density lipoprotein cholesterol was measured after precipitation of apoB-containing lipoproteins in plasma using dextran sulfate-Mg^{2+} [26]. In addition, fasting plasma triglycerides were measured at weeks 4, 5, and 6 of the average American diet.

Plasma total apoA-I and apoB concentrations and apoB concentrations in VLDL and IDL were measured using noncompetitive enzyme-linked immunosorbent assay, as previously described [27,28]. Low-density lipoprotein apoB concentrations were calculated by subtracting VLDL apoB and IDL apoB from total plasma apoB concentrations. Plasma Lp(a) concentrations and isoforms sizes were determined at the Northwest Lipid Research Laboratories, University of Washington, using monoclonal antibodies that do not recognize kringle IV type 2 domains [29].

2.4. Isolation of Lp(a) and separation of apo(a) and apoB-100 within Lp(a)

Lipoprotein (a) was isolated from 200 μL aliquots of plasma according to the method of Seman et al [30]. As previously reported, this method does not coisolate VLDL particles as determined by nondenaturing gradient gel electrophoresis with lipid staining [30]. Briefly, plasma was added to 75 μL of a 50% mixture of wheat germ agglutinin cyanogen bromide linked to sephacryl S-1000. Samples were incubated for 20 minutes and then washed twice with 500 μL of phosphate-buffered saline containing 200 mmol/L of L-proline for 20 minutes. The second wash was followed by a 30-minute incubation with an elution buffer (125 μL of 200 mmol/L *N*-acetyl-D-glucosamine in phosphate-buffered saline). Samples were centrifuged and the eluates were collected for protein separation.

For separation of apo(a) and apoB of Lp(a), eluates were heated at 100°C for 10 minutes with 50 μL of a sample buffer containing 2-mercaptoethanol. Proteins were separated by 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis at 50 V for 18 hours. Gels were stained with Coomassie blue. Apolipoprotein (a) and apoB bands were identified by Western blotting using a monoclonal anti-apo(a) antibody (Strategic Diagnostics Inc, Newark, Del)

and a polyclonal anti-apoB antibody [28], respectively, in all samples.

Subjects in this study had either a dominant apo(a) isoform band, defined as greater than 80% of the total apo(a) mass, or a single isoform, as determined by both analysis of gel scans (AlphaImager 2000, version 3.2, Alpha Innotech Corporation, San Leandro, Calif) and the method of Marcovina et al [29]. The dominant band was used for derivatization and kinetic analysis. A total of 6 of the 23 subjects expressed a single apo(a) isoform. The molecular weight of the dominant/single isoform was used to calculate apo(a) protein concentration for each study subject.

2.5. Measurement of [5,5,5-$^2H_3$] leucine enrichment

After identification, apo(a) and apoB-100 protein bands from each time point were excised from gels and derivatized, as previously described [23]. Briefly, gel bands were hydrolyzed with 12 N HCl for 24 hours at 110°C, dried under prepurified nitrogen, propylated, and then dried again under nitrogen. The derivatizing agent heptafluorobutyric anhydride was added to the sample tubes, which were then incubated for 1 hour at 60°C. The tubes were dried again to remove all traces of the derivatizing reagent. Ethyl acetate was used to extract the derivatized sample. Plasma leucine was isolated using Dowex Ag-50W-X8 100-200 mesh cation exchange resin columns (BioRad Labs, Richmond, Calif). Previous studies from our laboratory have reported that, under these conditions, plasma-deuterated leucine reaches its plateau after 1 hour and remains constant throughout the infusion [23].

Derivatized samples were analyzed by methane-negative chemical ionization using a Hewlett-Packard (Palo Alto, Calif) 5890/5988A gas chromatograph/mass spectrometer. Chromatographic separations were performed with a 30 m × 0.32 mm DX 4 capillary column (J&W Scientific, Inc, Rancho Cordova, Calif). The column flow rate was 2.3 mL/min; the temperature was programmed to start at 50°C and to ramp up to 250°C at a rate of 10°C/min. The ion source pressure and temperature were 0.5 mm Hg methane and 200°C, respectively.

2.6. Kinetic analysis

Isotope ratios for apo(a) and apoB-100 within Lp(a) and apoB-100 within VLDL and LDL were calculated as $R = q/Q$, where R is the isotope ratio, q is the amount of labeled leucine, and Q is the amount of unlabeled leucine. Enrichment (%E) was calculated from the isotope ratio and then converted to tracer/tracee ratios, as previously described [31]. Kinetic data were analyzed with the compartmental model shown in Fig. 1 using the SAAM-II software package (SAAM Institute, Seattle, Wash). The model includes apo(a) and apoB-100 within Lp(a) as separate compartments derived from the same precursor leucine pool. The input, delay, and output parameters are all adjustable and independent from one another. The fractional synthetic rates (FSRs) of apo(a) and apoB-100 in Lp(a) correspond to the irreversible loss from compartments 3 and 4 (Fig. 1), respectively. The average percent fractional SD of the apo(a) and apoB-100 FSRs was 10.4. Under steady-state conditions, the FSR equals the fractional catabolic rate (FCR) [23,32]. Very low density lipoprotein apoB-100 plateau enrichment was determined using a monoexponential function, also using SAAM II, and was used as a measure of the precursor pool. The mean VLDL apoB-100 plateau enrichment value is about 75% of the mean plasma leucine enrichment value [23].

Plasma apolipoprotein pool size was determined by multiplying the apolipoprotein plasma concentration

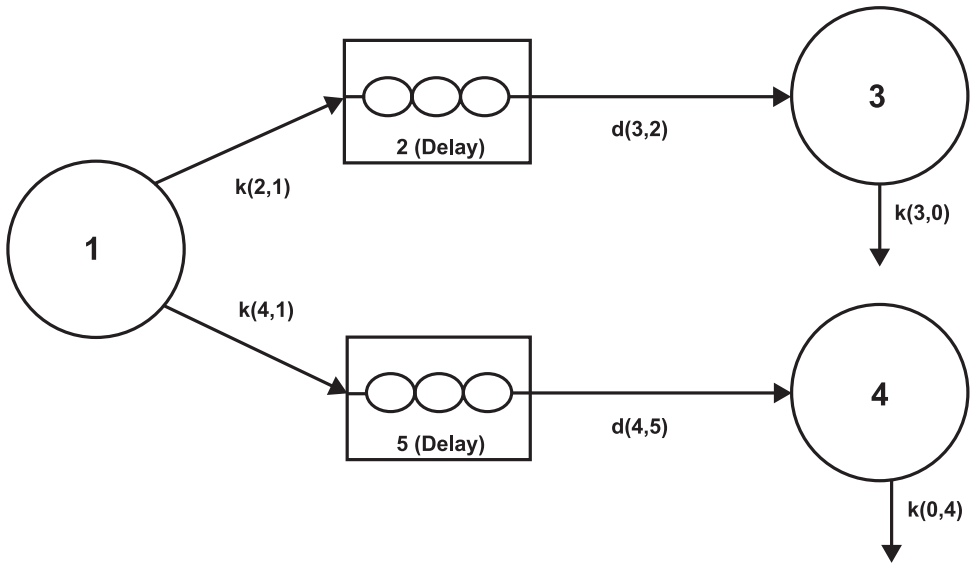

Fig. 1. Model used to determine the FCRs of apo(a) and apoB-100 within Lp(a). Compartment 1 signifies the precursor pool. Compartments 2 and 5 represent the delays for the hepatic synthesis and secretion of apo(a) and apoB-100, respectively. Compartment 3 represents the apo(a) pool; compartment 4, the Lp(a) apoB-100 pool. The arrows indicate movement of label out of and into these compartments.

Table 1
Age, body mass index, and nonfasting plasma lipid and lipoprotein concentrations (milligrams per deciliter)[a] in subjects grouped according to plasma Lp(a) concentrations

	Low (n = 6)	Intermediate (n = 8)	High (n = 9)
Age (y)	65 ± 8.0	62 ± 12	57 ± 11
Body mass index (kg/m^2)	25 ± 1.7	27 ± 2.5	25 ± 2.7
Fasting triglyceride (mg/dL)	136 ± 36	115 ± 63	99.8 ± 15
Fed triglyceride (mg/dL)	246 ± 84	166 ± 89	154 ± 42
Total cholesterol (mg/dL)	184 ± 21	215 ± 48	224 ± 48
VLDL cholesterol (mg/dL)	22 ± 9.0	20 ± 13	17 ± 10
LDL cholesterol (mg/dL)	118 ± 30	148 ± 47	150 ± 43
HDL cholesterol (mg/dL)	42.7 ± 8	43.4 ± 7	45.2 ± 8
Total apoB-100 (mg/dL)	107 ± 12	102 ± 18	125 ± 38
VLDL apoB-100 (mg/dL)	12.5 ± 5.5*	6.62 ± 3.3	5.62 ± 2.1*
IDL apoB-100 (mg/dL)	1.87 ± 0.66	2.11 ± 1.1	2.06 ± 1.1
LDL apoB-100 (mg/dL)	89.6 ± 16	92.6 ± 24	117 ± 38
Total apoA-1 (mg/dL)	114 ± 14	124 ± 10	131 ± 15
Lp(a) mass	5.9 ± 6.1**	21 ± 6.5**	81 ± 36**
Lp(a) isoform number	23 ± 6	25 ± 4**	19 ± 2**

[a] Values are expressed as mean ± SD.
* Significantly different from each other, $P < .05$.
** Significantly different from each other, $P \leq .01$.

(mg/dL) by the estimated plasma volume (4.5% body weight [kg]) [23]. The apolipoprotein secretion rate (SR) (mg/kg per day) was calculated by the following equation [23]:

$$SR = [FCR\ (\text{pools/d}) \times \text{apo pool size (mg)}] / \text{body weight (kg)}$$

We assumed that a primed-constant infusion provides a steady enrichment of plasma leucine to be incorporated into apolipoproteins. We also assumed that each subject was studied under steady-state conditions with regard to their lipid and apolipoprotein concentrations; the identical energy content and composition of each hourly meal were designed to achieve these conditions [23,33].

2.7. Statistical analyses

The SPSS version 10 for Windows (SPSS, Inc, Chicago, Ill) was used for all statistical analyses. There were 6 subjects with low levels of Lp(a) (mean plasma Lp(a), 5.92 mg/dL) in whom we were unable to adequately measure enrichment of individual Lp(a) because of lack of sensitivity. For the purpose of this paper, the group of these 6 individuals is identified as the low Lp(a) group. For the kinetic studies, plasma from these 6 individuals was pooled and the isotopic enrichment in apo(a) and apoB-100 within Lp(a) was measured as a single specimen. All other data for this group with low Lp(a) levels were generated using individual (uncombined) specimens. The remaining 17 subjects exhibited a wide range of Lp(a) concentrations that enabled us to divide them into 2 clinically relevant groups: one group containing 8 subjects with plasma Lp(a) concentrations greater than 10 mg/dL and less than 30 mg/dL (intermediate Lp(a) group) and 9 with Lp(a) concentrations of 30 mg/dL or higher (high Lp(a) group). A plasma Lp(a) concentration of 30 mg/dL or higher is considered a risk factor for CHD [34].

Statistically significant differences in plasma lipid, lipoprotein, and apolipoprotein levels among the 3 groups with different Lp(a) concentrations were assessed by 1-way analysis of variance. Post hoc Tukey's honestly significant differences test was used to compare groups. For kinetic parameters, the low Lp(a) group was not included in the statistical analyses, and differences between the intermedi-

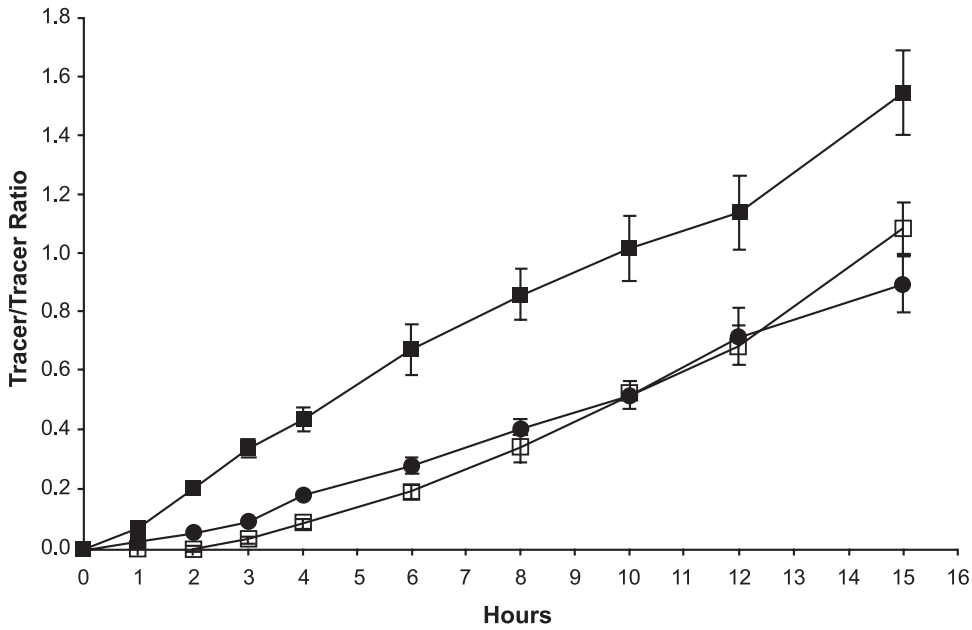

Fig. 2. The filled circle indicates the mean (±SEM) of tracer/tracee ratios of 10 infusion time points for apo(a) within Lp(a); filled square, apoB-100 within Lp(a); and open square, apoB-100 within LDL.

Table 2
Kinetic parameters of apo(a) and apoB-100 within Lp(a) for the low, intermediate, and high Lp(a) groups[a]

	Low	Intermediate[b]	High[b]	P
Apo(a) pool (mg)	21.7	99.5 ± 16.7	307 ± 46.5	.001
Apo(a) FCR (pools/d)	0.430	0.285 ± 0.0476	0.140 ± 0.0121	.006
Apo(a) SR (mg/kg per day)	0.140	0.370 ± 0.0837	0.649 ± 0.118	.079
ApoB-100 pool (mg)	37.5	144 ± 18.8	526 ± 77.2	.001
ApoB-100 FCR (pools/d)	0.756	0.483 ± 0.0428	0.310 ± 0.0429	.009
ApoB-100 SR (mg/kg per day)	0.422	0.932 ± 0.157	2.19 ± 0.284	.002

[a] Group 1 not included in statistical analysis.
[b] Mean ± SE.

ate and the high Lp(a) groups were assessed using the Student t test for normally distributed variables and Mann-Whitney U test for skewed variables. Pearson's correlation coefficients were used to test for correlations between kinetic and lipid variables. Skewed variables were tested using Spearman's ρ correlations. Forward multiple linear regression was used to determine the kinetic variables that best predict Lp(a) concentrations. Skewed variables were log transformed, and in the case of Lp(a) concentration (the dependent variable), where the log-transformed data were still skewed, the data were instead squared to achieve a normal distribution.

3. Results

The characteristics of subjects in each Lp(a) group are presented in Table 1. Lipid, apolipoprotein, and Lp(a) concentrations are the average values obtained from 10 blood samples taken during the metabolic studies (fed state). There were no significant differences between the groups with respect to age, body mass index and lipid and lipoprotein levels, except that the mean VLDL apoB-100 concentration was 2-fold higher in the low Lp(a) group than in the high Lp(a) group ($P < .05$). There were significant differences in Lp(a) mass concentration and apo(a) isoform number among the groups.

Fig. 2 illustrates the mean (±SEM) infusion tracer/tracee ratios of apo(a) and apoB-100 in Lp(a) and of apoB-100 in LDL in the 17 individual subjects and in the pooled sample (n = 18). The appearance of deuterated leucine in the apoB-100 of Lp(a) preceded its appearance in both the apoB-100 of LDL and the apo(a) of Lp(a).

The kinetic parameters of apo(a) and apoB-100 of Lp(a) for the low, intermediate, and high Lp(a) groups are shown in Table 2. As noted previously, the values for the low Lp(a) group were obtained using a pooled plasma specimen. Therefore, this group was not included in the statistical analysis and is only shown for comparison purposes. Apolipoprotein (a) FCR was significantly lower in the high Lp(a) group than in the intermediate Lp(a) group. There was a nonsignificant trend for higher apo(a) SR values in the high Lp(a) group than in the intermediate Lp(a) group. For Lp(a) apoB-100, FCR values were significantly lower and SR values were significantly higher in the high Lp(a) group than in the intermediate Lp(a) group.

The overall (n = 18) mean (±SEM) Lp(a) apoB-100 FCR (0.416 ± 0.040 pools/d) was significantly higher than the apo(a) FCR (0.220 ± 0.030 pools/d; $P < .001$). Similarly, the Lp(a) apoB-100 SR (1.53 ± 0.219 mg/kg per day) was significantly higher than the apo(a) SR (0.497 ± 0.078 mg/kg per day; $P < .001$). Significant differences ($P < .01$) between these parameters were also maintained within each Lp(a) group.

Correlations between plasma Lp(a) mass, apo(a) isoform number, and kinetic parameters for apo(a) and apoB-100 and apo(a) within Lp(a) are provided in Table 3. Apolipoprotein (a) and apoB-100 FCRs were negatively associated with Lp(a) mass concentrations ($P < .01$), whereas SRs were strongly and positively associated with Lp(a) mass concentrations ($P < .0001$). Isoform number was positively associated with FCRs and negatively associated with SRs of both Lp(a) apolipoproteins.

In addition, correlation coefficients were calculated between the kinetic parameters in Table 3 and apoB-100 kinetic parameters of VLDL and LDL in 8 of the 17 subjects studied [33] (data not shown). No association was observed between VLDL apoB-100 FCR and Lp(a) apoB-100 FCR ($r = -0.028$; $P = .95$). Similarly, no association was observed between LDL B-100 FCR and Lp(a) apoB-100 FCR ($r = -0.096$; $P = .82$). Low-density lipoprotein apoB-100 SR was significantly correlated with apo(a) and Lp(a) apoB-100 pool size ($r = 0.727$, $P < .05$ and $r = 0.723$, $P < .05$, respectively).

Table 3
Correlation coefficients between kinetic parameters of Lp(a) for 17 individual subjects and the pooled specimen

	Apo(a) FCR[a] (pools/d)	ApoB-100 FCR (pools/d)	Apo(a) SR (mg/kg per day)	ApoB-100 SR (mg/kg per day)	Lp(a) mass[a] (mg/dL)
ApoB-100 FCR (pools/d)	**0.491** (.038)	–			
Apo(a) SR (mg/kg per day)	**−0.069** (.785)	**−0.464** (.052)	–		
ApoB-100 SR (mg/kg per day)	**−0.383** (.117)	**−0.308** (.214)	**0.671** (.002)	–	
Lp(a) mass[a] (mg/dL)	**−0.547** (.006)	**−0.717** (.001)	**0.837** (.000)	**0.789** (.000)	–
Isoform number	**0.588** (.010)	**0.537** (.022)	**−0.478** (.045)	**−0.694** (.001)	**−0.716** (.001)

Correlation coefficients are in bold; P values are in parentheses.
[a] Spearman's ρ; all others, Pearson's coefficients.

In a forward multiple regression analysis, apo(a) FCR, Lp(a) apoB-100 FCR, apo(a) SR, and Lp(a) apoB-100 SR demonstrated significant linear associations with plasma Lp(a) concentration. All variables were retained in the model, which explained 95.6% (adjusted $R^2 \times 100$) of the variation in plasma Lp(a) concentration (analysis of variance, $P < .0001$).

4. Discussion

Initial studies of Lp(a) metabolism had been carried out with radiolabeling of the whole particle [14,15]. In these studies, Lp(a) SR was shown to be a major determinant of Lp(a) mass [15].

Subsequently, endogenous labeling with stable isotopes has been used in kinetic studies analyzing apo(a) and apoB-100 within Lp(a) separately. Morrisett et al [16] studied 5 normolipidemic subjects on a liquid diet providing 126 kJ (30 kcal)/kg body weight and containing 22% of calories as fat during a 16-hour infusion with $^3H_4$-Lysine. Lipoprotein (a) was isolated by sequential ultracentrifugation and immunoprecipitation. The mean apo(a) FCR was 0.162 pools/d and Lp(a) apoB-100 FCR was 0.139 pools/d. The correlation coefficient between plasma apo(a) concentration and apo(a) FCR was −0.448, indicating a role of Lp(a) catabolism in determining Lp(a) plasma concentrations.

Su et al [20] have studied the metabolism of Lp(a) in 12 postmenopausal women during a 12-hour infusion with $^2H_3$-leucine. A fat-free and leucine-free lunch and dinner, providing 60% of daily energy requirement, were provided to the subjects after 4 and 10 hours of infusion. In this study, Lp(a) was isolated by ultracentrifugation at $1.05 < d < 1.15$ g/mL. In the 6 subjects with only one apo(a) isoform, mean apo(a) FCR was 0.277 pools/d and Lp(a) apoB-100 FCR was 0.270 pools/d.

Demant et al [21] studied 7 normolipidemic subjects (5 men and 2 women) in the fasted state. The infusion with $^2H_3$-leucine lasted for 10 hours and Lp(a) was isolated using a modified version of our methodology, which uses wheat germ agglutinin and 200 mmol/L proline. These authors have used a complex multicompartmental model that includes separate and parallel compartments for apo(a) and apoB-100 in Lp(a) in addition to VLDL, IDL, and LDL apoB-100 compartments. The apo(a) FCR was 0.266 pools/d and Lp(a) apoB-100 FCR was 0.256 pools/d.

In our study, the FCR of apo(a) is approximately half that of Lp(a) B-100. This is in contrast with other studies using stable isotope methodology to study the protein components of Lp(a) separately. There are notable differences in design and methodology between our study and the previous stable isotope studies [16,20,21]. One possible contributing factor for the different results is that other studies were carried out either in the fasted state or with different feeding regimens, whereas in our study, subjects consumed hourly meals throughout the infusion to achieve a steady-state situation in terms of lipoprotein secretion by the liver. We have chosen to carry out the metabolic studies in the fed state because human beings spend the greater part of each day in the postprandial state, and, therefore, our subjects were studied under a more physiological condition. Another important consideration is the difference in methodologies used to isolate Lp(a). Morrisett et al [16] and Su et al [20] used ultracentrifugation to isolate Lp(a). Seman and Breckenridge [7] have demonstrated that Lp(a) exists across the density range of lipoprotein particles; therefore, separation at a density of 1.05 to 1.15 g/mL does not isolate all particles. Demant et al [21] used our method to isolate Lp(a) but modified it by subjecting the Lp(a) eluate to additional manipulations by ultracentrifugating at a d of 1.15 g/mL and then subjecting the supernatant to B-mercaptoethanol before a second ultracentrifugation at a d of 1.15 g/mL. Also, the models used to assess the kinetic parameters differ among the 4 stable isotope Lp(a) studies [16,20,21]. Our apo(a) FCR data, with a mean of 0.220 pools/d, are consistent with those of other studies, which also indicate a slow turnover for this apolipoprotein. The fact that all studies, regardless of study design and methodology, have consistently shown similar apo(a) FCRs may be an indication that the metabolism of apo(a) is not affected by feeding. In our study, the mean apo(a) SR was 0.497 mg/kg per day, and this parameter was significantly and strongly associated with the plasma Lp(a) mass as also shown previously [15,18,19].

As stated above, in our study, the apoB-100 of Lp(a) had a mean FCR that was approximately twice that of apo(a) FCR, indicating a faster catabolism of the apoB-100 component of Lp(a). It has been previously shown that VLDL does not coisolate with Lp(a) with this methodology. Our data show that the appearance of the labeled leucine in apo(a) and in apoB-100 of Lp(a) is different, with incorporation of leucine in apoB-100 occurring much earlier than in apo(a), at approximately 30 minutes compared with 1 hour. We are confident that the early appearance of label in apoB-100 is not the result of contamination of our samples by VLDL apoB-100 because 200 mmol/L proline was used in the isolation procedure. This concentration of proline has clearly been shown to inhibit the nonspecific association of VLDL with Lp(a) [35]. In addition, there was no correlation between VLDL apoB-100 FCR and Lp(a) apoB-100 FCR, also suggesting no VLDL contamination of our samples. It is recognized that the assembly of apo(a) with an apoB-containing lipoprotein occurs extracellularly [36]. It is thought that preformed apo(a) is bound to the hepatocyte surface via its kringle domains and then can first form a noncovalent interaction with an apoB-containing lipoprotein, followed by formation of disulfide bonds [37]. It has been shown in primary baboon hepatocytes that there is a preformed, unlabeled pool of apo(a) on the hepatic cell surface [38]. This is consistent with our results of earlier labeling of Lp(a) apoB-100 than of apo(a).

With respect to Lp(a) catabolism, our data show significant negative correlations of both apo(a) and Lp(a) apoB-100 FCR with serum Lp(a) mass concentrations, indicating that

Lp(a) concentrations in plasma are also regulated by the rate of its catabolism. Morrisett et al [16] reported a moderate ($R = -0.448$; P = NS) negative association of apo(a) FCR with plasma Lp(a) in 5 subjects. An early report by Krempler et al [15] showed an inverse relationship ($R = -0.50$; P = NS) between Lp(a) serum and Lp(a) FCR in 9 subjects. Although these relationships are not statistically significant, the magnitude of the correlations is very similar to that reported in the present study ($R = -0.547$) (n = 18). This suggests that apo(a) FCR may, in addition to apo(a) SR, play a role in determining Lp(a) concentration.

Currently, a clear pathway of Lp(a) catabolism is not known, but several mechanisms have been implicated in the removal of Lp(a) from the circulation. In transgenic mice expressing the human LDL receptor, injected Lp(a) was catabolized much faster than it was in control mice [39]; however, Rader et al [17] demonstrated that Lp(a) clearance in human beings occurs independently of this receptor. Knight et al [40] suggested that the LDL receptor may be involved in the clearance of the apoB-100 portion of Lp(a) after the removal of apo(a). In addition, apo(a)-specific receptors such as the plasminogen receptor or the VLDL receptor may be responsible for a significant portion of Lp(a) or apo(a) clearance [41-43]. The kidney has also been implicated as a route for Lp(a) catabolism; apo(a) fragments that are generated in the plasma are found in urine and plasma and Lp(a) concentrations are elevated in patients with renal insufficiency [44-46]. Patients with renal impairment have elevated concentrations of LDL-unbound apo(a) in the circulating plasma, indicating that the dissociation of apo(a) from apoB-100 is physiologically plausible [47]. It is not known if this mechanism is affected by feeding.

Our data are consistent with the fact that, in the fed state, apo(a) can recombine with apoB-100 particles twice on average. It has been shown that changes in the metabolism of apoB-100–containing lipoproteins occur as a result of being in the fed vs the fasted state. Feeding leads to a significant increase in VLDL size and triglyceride content and to a 50% increase in VLDL apoB-100 SR [23]. In the context of our results, the following hypothesis is suggested to explain the metabolism of apo(a) and apoB-100 of Lp(a) in the fed state. Apolipoprotein (a), attached to triglyceride-rich lipoprotein apoB-100, is released from the hepatocyte surface. This newly formed Lp(a) particle releases apo(a) as the triglyceride-rich lipoprotein portion is catabolized via receptor-mediated clearance. The free apo(a) then recombines with another apoB-100 particle, most likely of triglyceride-rich lipoprotein origin. Because about 50% of triglyceride-rich lipoprotein is converted to LDL in the fed state, the “second” Lp(a) particle may survive catabolism. Previous work in subjects with familial defective apoB-100 has shown that although the defective apoB-100 represented the majority of total apoB-100 in the LDL fraction, the distribution of the defective and the wild-type apoB-100 was the same in Lp(a) particles isolated in the LDL range [48]. This observation supports our hypothesis that apo(a) can dissociate before Lp(a) reaches the LDL density range and can possibly reassociate with a newly synthesized triglyceride-rich lipoprotein particle.

Apolipoprotein (a) isoform size is negatively correlated with both Lp(a) concentration and Lp(a) SR. Not surprisingly, we found that Lp(a) apoB-100 and apo(a) SR had a significant inverse association with apo(a) isoform size. White et al [36] have shown that secretion of larger-sized apo(a) isoforms was delayed by 1 hour in primary baboon hepatocytes compared with that of smaller isoforms. This was caused by prolonged residence time of larger apo(a) isoforms in the endoplasmic reticulum and, probably, greater intracellular apo(a) degradation. Another study using the same cell system showed that the avidity of apo(a) for the hepatocyte surface increased with increasing numbers of kringle IV type 2 repeats [38]. Recombinant apo(a) studies have demonstrated that the fewer kringles the apo(a) protein contains, the more readily it associates with apoB-100 [49]. In the present study, smaller isoforms, having less avidity for the hepatocyte surface, may have bound more rapidly to apoB-100 than larger isoforms. This and the possibility that they are also less likely to be degraded intracellularly suggest a mechanism for the inverse correlation seen between isoform size and both apo(a) and apoB-100 SRs. The significant positive correlations between apo(a) isoform size and the FCRs of both apoB-100 and apo(a) suggest that larger isoforms are turned over at a higher rate than smaller isoforms.

In conclusion, our data are consistent with the following concepts: (1) the plasma FCR of apo(a) at 0.220 pools/d is significantly less that that of apoB-100 within Lp(a) (0.416 pools/d); (2) apo(a) within plasma Lp(a) appears to dissociate from Lp(a) at least once during its plasma metabolism and to reassociate with a second apoB-100–containig lipoprotein, and; (3) the concentration of Lp(a) is determined by both the SR and the FCR of these 2 proteins within Lp(a). The kinetic behavior of apo(a) and apoB-100 of Lp(a) in the constantly fed state may be different from that in the fasting state.

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