Preferential Influx and Decreased Fractional Loss of Lipoprotein(a) in Atherosclerotic Compared with Nonlesioned Rabbit Aorta

Lars B. Nielsen,* Steen Stender,‡ Matti Jauhiainen,§ and Børge G. Nordestgaard‖

**Department of Clinical Biochemistry, Rigshospitalet, DK-2100 Copenhagen, and ‖Department of Clinical Biochemistry, Herlev Hospital, DK-2730 Herlev, University of Copenhagen, Denmark; §National Public Health Institute, Department of Biochemistry, FIN-00300 Helsinki, Finland; and ‡Clinical Institute, University of Odense, DK-5000 Odense, Denmark*

Abstract

The aim was to investigate the atherogenic potential of lipoprotein(a) (Lp(a)) and to further our understanding of the atherogenic process by measuring rates of transfer into the intima-inner media (i.e., intimal clearance) and rates of loss from the intima-inner media (i.e., fractional loss) of Lp(a) and LDL using cholesterol-fed rabbits with nonlesioned ($n = 13$) or atherosclerotic aortas ($n = 12$). In each rabbit, ^{131}I-Lp(a) (or ^{131}I-LDL) was injected intravenously 26 h before and ^{125}I-Lp(a) (or ^{125}I-LDL) 3 h before the aorta was removed and divided into six consecutive segments of similar size. The intimal clearance of Lp(a) and LDL was similar and markedly increased in atherosclerotic compared with nonlesioned aortas (ANOVA, effect of atherosclerosis: $P < 0.0001$). Fractional losses of labeled Lp(a) and labeled LDL in atherosclerotic aorta were on average 25 and 43%, respectively, of that in nonlesioned aortas (ANOVA, effect of atherosclerosis: $P < 0.0001$). Fractional loss of Lp(a) was 73% of that of LDL (ANOVA, effect of type of lipoprotein: $P = 0.07$). These data suggest that the development of atherosclerosis is associated with increased influx as well as decreased fractional loss of Lp(a) and LDL from the intima. Accordingly, Lp(a) may share with LDL the potential for causing atherosclerosis. (*J. Clin. Invest.* 1996. 98:563–571.)

Key words: atherosclerosis • cholesterol • efflux • fractional loss • low-density lipoprotein

Introduction

High plasma levels of LDL are causally related to development of atherosclerosis (1); LDL carries the major fraction of apo B and cholesterol in plasma. The importance for development of atherosclerosis of the lipoprotein(a) (Lp(a))[1] particle, which is another apo B and cholesterol-containing lipoprotein fraction, is less well understood (1, 2). However, in most case-control studies, high plasma levels of Lp(a) were associated with an increased risk of atherosclerosis-related disease, and in five of seven prospective studies, plasma Lp(a) levels were higher in atherosclerosis-effected individuals than in controls (for review see reference 2). The transgenic mice expressing human apo(a) also developed more severe atherosclerosis than control mice (3).

LDL- and Lp(a)–like particles have predominantly been detected in the vessel wall at sites of atherosclerosis (4, 5). The concentration of the Lp(a) apo(a) in vein grafts was 240% that of apo B, when compared with the concentrations of the two apolipoproteins in plasma (6). More Lp(a) than LDL was extractable after plasmin digestion of human arterial intima (7), and autoradiographic studies in mice suggest that Lp(a) accumulates in the arterial intima preferentially to LDL (8). Whether these observations reflect increased influx or decreased efflux in the vessel wall remains to be determined.

The bulk of evidence supports the idea that accumulation of plasma-derived atherogenic lipoproteins in the intima constitutes a fundamental event in the initiation of atherosclerotic lesions (9). It is therefore important to further our understanding of the interaction of plasma lipoproteins with the arterial intima in vivo. A specific aim of the present study was to compare the atherogenic potential of Lp(a) and LDL: rates of transfer into intima-inner media (i.e., intimal clearance) and rates of loss from intima-inner media (i.e., fractional loss) of labeled Lp(a) and LDL were determined in vivo in nonlesioned and atherosclerotic aortas of cholesterol-fed rabbits.

Methods

Animals. Male white rabbits (Danish Country Strain, Statens Serum-institut, Copenhagen, Denmark) weighing 2.8 to 4.0 kg received 100 g of chow per day containing 94 to 94.75% standard rabbit pellets (Altromin 2113; Lage, Germany), 5% corn oil (Oleum Maides; Mecobenzon, Copenhagen, Denmark) and 0.25 to 1% cholesterol (USP-cholesterol; Sigma Chemical Co., Copenhagen, Denmark).

To induce atherosclerosis in the aorta, 12 rabbits (6 used for LDL studies and 6 for Lp(a) studies) were fed this chow for 173–236 d; the amount of cholesterol in the chow was adjusted regularly to maintain a plasma cholesterol level of 30–70 mmol/liter. These 12 rabbits all had visible atherosclerotic lesions in the aorta. The severity of atherosclerosis in aortic segments was quantified by measuring the cholesterol content of the intima-inner media; the aortic cholesterol content is closely associated with other indices of atherosclerosis, in rabbits and pigs (10, 11).

To increase the plasma LDL-cholesterol level without inducing atherosclerosis, 13 other rabbits (7 used to study LDL and 6 to study Lp(a)) were fed the 1 g cholesterol/d chow for 6–10 d. None of these rabbits had visible lesions in the aorta.

The experimental protocol was approved by the Danish government body supervising animal experiments (the Animal Experiment Inspectorate).

Isolation of human Lp(a) and LDL. As described previously (12), Lp(a) concentrations in human plasma, isolated Lp(a) preparations, and rabbit plasma were determined using a turbidimetric assay or

Address correspondence to Børge G. Nordestgaard, Department of Clinical Biochemistry, Herlev Hospital, DK-2730 Herlev, Denmark. Phone: +45 44 53 53 00, ext. 3843; FAX: +45 44 53 53 32; or Lars Bo Nielsen, Gladstone Institute of Cardiovascular Disease, P.O. Box 419100, San Francisco, CA 94141-9100. Phone: 415-826-7500; FAX: 415-285-5632.

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1. *Abbreviation used in this paper:* Lp(a), Lipoprotein(a).

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rocket immunoelectrophoresis. LDL protein concentration was estimated from the absorbance at 220 nm before iodination (12).

For the isolation of Lp(a), plasma Lp(a) concentration was determined in at least 10 healthy subjects. Lp(a) was subsequently isolated from pooled plasmas from the two subjects with the highest Lp(a) concentrations (0.38–0.58 mg/ml), as described in detail elsewhere (13). The apo(a) isoforms of the donors were S2/S5 and S3 for one of the two preparations of Lp(a) and S1/S5 and S2 for the other; apo(a) isoforms were determined essentially as described by Utermann et al. (14).

LDL ($1.019 < d < 1.063$ g/ml) was isolated from the plasma of a donor with low plasma Lp(a) by the use of sequential ultracentrifugation (15). The contamination of purified LDL with Lp(a) was less than 0.2% of the total lipoprotein mass; total Lp(a) mass was compared with total LDL mass.

Lipoprotein labeling. Two separate aliquots of purified Lp(a) (2 ml, 6.2–9.6 mg total Lp(a)) or purified LDL (0.6–1.2 ml, 5 mg LDL protein ≈ 22 mg total LDL, since protein constitutes 22.4% of total LDL mass, reference 16) were labeled with 75–260 MBq ^{125}I and 90–260 MBq ^{131}I, respectively, using the iodine monochloride method (17, 18) as described previously (12, 15). To remove unbound iodine, labeled lipoprotein preparations were passed through a PD-10 column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) before 100–160 mg of rabbit albumin (Sigma Chemical Co., St. Louis, MO) was added. The iodination efficiency averaged 57% and was similar for the two lipoproteins. The specific activities were 0.2 to 1.2×10^9 cpm ^{125}I per mg Lp(a), 0.1 to 1.0×10^9 cpm ^{131}I per mg Lp(a), 0.2 to 2.5×10^9 cpm ^{125}I per mg LDL, and 0.1 to 0.8×10^9 cpm ^{131}I per mg LDL. 96 to 98% of the radioactivity in both labeled Lp(a) and LDL was TCA precipitate. Less than 3.3% of the TCA-precipitable radioactivity was extractable with chloroform-methanol (1:1, vol/vol). Labeled lipoprotein preparations were passed through 0.22- or 0.45-μm filters and used for injection within 48 h of labeling.

Lipoprotein injection. In six rabbits with nonlesioned aortas and six rabbits with atherosclerotic aortas, ^{131}I-Lp(a) ($3.4 \pm 0.9 \times 10^8$ cpm per kg body wt) was injected intravenously 26.4±0.2 h before and ^{125}I-Lp(a) ($2.7 \pm 0.7 \times 10^8$ cpm per kg body wt) 3.0±0.04 h before the aorta was removed. Similarly, in seven rabbits with nonlesioned aortas and in six rabbits with atherosclerotic aortas, ^{131}I-LDL ($2.0 \pm 0.4 \times 10^9$ cpm per kg body wt) was injected 26.1±0.3 h before and ^{125}I-LDL ($7.0 \pm 2.0 \times 10^8$ cpm per kg body wt) 3.0±0.02 h before the aorta was removed.

The rabbits used to study Lp(a) kinetics received an intravenous injection of 7–10 ml human $d < 1.12$ g/ml lipoproteins (containing 29–38 mg Lp(a)) immediately before injection of ^{131}I-Lp(a). The $d < 1.12$ g/ml lipoprotein fraction was prepared using the same batch of human Lp(a)−rich plasma as was used for isolation of Lp(a) for labeling (12).

Blood samples of 1 ml were drawn from an ear vein at regular intervals after injection of labeled lipoproteins.

Removal of aorta. Each rabbit was injected intravenously with pentobarbital (50–100 mg/kg) before the thoracic cavity was opened and a cannula was placed in the left ventricle of the heart, through which the vascular bed was perfused with 1 liter cold saline. The aorta was removed and freed of adventitial tissue. The lumen was flushed with cold saline several times during this procedure to chill the aortic tissue. The vessel was opened longitudinally, rinsed further with cold saline, and divided into the aortic arch (from the heart to the first intercostal branches), the thoracic aorta (to the celiac axis), and the abdominal aorta (to the iliac bifurcation). Each of these three parts were then divided into a proximal and a distal segment of similar size. The intima-inner media in each of the six aortic segments was stripped from the outer media and minced at 4°C.

Trichloroacetic acid precipitable radioactivity. Minced tissues and aliquots of plasma and diluted doses were TCA precipitated after the addition of albumin as described (15). The time between removal of aorta and precipitation of proteins in the intima-inner media was 30–45 min. Radioactivity was determined in a double channel gamma counter (LKB compugamma 1282; Wallac, Copenhagen, Denmark). Tissues were counted for at least 42 min; typical standard errors of counting rates of TCA-precipitable ^{125}I and ^{131}I in aortic intima-inner media were < 2%.

Analysis. Lipids in TCA-precipitates of aortic intima-inner media were extracted over a 24-h period with chloroform/methanol (2:1, vol/vol; ∼ 1 ml solvent per 50 mg tissue) followed by two further extractions with chloroform/methanol (1:1, vol/vol) before the combined lipid extract was washed by the Folch procedure (19). Cholesterol was quantified with the Liebermann-Burchard method (20) or with an enzymatic method, which measures total cholesterol (CHOD-PAP; Boehringer Mannheim, Mannheim, Germany) after evaporation of the chloroform/methanol and solubilization of the extract in isopropanol. When cholesterol in lipid extracts of 66 TCA-precipitated aortic segments was determined by both methods, similar results were obtained: on linear regression analysis, Pearson's correlation coefficient (r) for the association between values obtained by the two methods was > 0.98 and the slope and y-intercept of the regression line were not significantly different from 1 and 0, respectively. The CHOD-PAP enzymatic method was also used for determination of cholesterol in plasma and lipoprotein fractions.

Agarose gel electrophoresis (0.5% agarose), nondenaturating polyacrylamide gradient gel electrophoresis, and two-tier rocket immunoelectrophoresis were performed as previously described (12, 21). The recovery of radioactivity on the two-tier rocket immunoelectrophoresis gel was 92.2±1.2% (n = 78; 8 aliquots of doses and 70 plasma samples).

Calculations of intimal clearance and fractional loss. TCA-precipitable radioactivity in tissues, washes, plasma, and doses were used in the calculations. Intimal clearance and fractional loss of labeled Lp(a) and LDL were calculated under the assumption that the kinetics of newly entered lipoproteins in the intima-inner media can be described by a one-pool compartment model; i.e., the loss of labeled lipoproteins from the intima-inner media increases linearly with the amount of labeled lipoproteins in the compartment. This assumption has previously been tested and was found to be plausible for iodine labeled LDL in nonlesioned and atherosclerotic aortas of rabbits (22) and monkeys (23). In the one-pool compartment model, the kinetics of labeled lipoproteins can be described by the equation (23–25):

$$\frac{dA}{dt} = k_i c(t) - k_e A(t) \quad (1)$$

with two unknowns, intimal clearance (k_i, in nl/cm^2 per h) and fractional loss (k_e, in h^{-1}). $A(t)$ is the aortic radioactivity (cpm/cm^2) at time t and $c(t)$ is the concentration of radioactivity in plasma (cpm/nl) at time t. Plasma radioactivity curves were fitted to biexponential functions:

$$c(t) = C_1 e^{-b_1 t} + C_2 e^{-b_2 t} \quad (2)$$

where C_1 and b_1 are constant and slope for the initial rapid removal of radioactivity from plasma and C_2 and b_2 are constant and slope for the subsequent slow removal of radioactivity from plasma. After substitution of Eq. 2 into Eq. 1, Eq. 1 can be solved to obtain:

$$A(t) = k_i \left(C_1 \frac{(e^{-k_e t} - e^{-b_1 t})}{(b_1 - k_e)} + C_2 \frac{(e^{-k_e t} - e^{-b_2 t})}{(b_2 - k_e)} \right) \quad (3)$$

Since the rabbits were given two differently labeled aliquots of the same lipoprotein but at different time intervals before removal of the aorta, Eq. 3 can be written for each isotope: fractional loss (k_e) and intimal clearance (k_i) can then be calculated by solving two equations with two unknowns.

Plasma and arterial wall radioactivity values are subject to measurement error. Two previous studies have estimated uncertainties in calculated values of intimal clearance and fractional loss that are induced by measurement uncertainties of the order likely in practice (24, 26), using experimental conditions quite similar to the present study. Wootton et al. (24) appplied random noise with zero mean and 1.5% SD to plasma radioacitvity data and random noise of zero mean and 5% SD to arterial wall radioactivity data, which produced uncertainties of 7% SD in calculated intimal clearance and 16% SD in cal-

culated fractional loss. Schwenke and Zilversmit (26) estimated that a 5% error in arterial wall radioactivity would produce an 8.5 to 14.5% error in calculated fractional loss and a 1.5 to 10% error in calculated intimal clearance (data calculated from Table 2 in reference 26).

In the Lp(a) experiments, some of the radioactivity in plasma was in Lp(a) particles that had lost the apo(a) moiety; i.e., Lp(a)−. Accordingly, the combined transfer of Lp(a) and Lp(a)− between plasma and the arterial wall was studied when total (TCA-precipitable) radioactivity was used to solve Eq. 3. Since Lp(a)− shares properties with LDL (27, 28), the following calculations were performed to correct values of Lp(a) fractional loss and intimal clearance for the presence of Lp(a)−. The relative amount of radioactivity in Lp(a)− 10 min, 3 h and 26 h after intravenous injection of Lp(a) was determined in each rabbit using two-tier rocket immunoelectrophoresis. The relative amount of radioactivity in Lp(a)− at other time points was estimated by linear interpolation. The plasma Lp(a)− radioactivity curve was then fitted to third-degree polynomials and the contribution of Lp(a)− to the arterial wall radioactivity at both 3 and 26 h was calculated in each rabbit as previously described (25). In these calculations, it was assumed that the intimal clearance of Lp(a)− is similar to that of LDL (and Lp(a)), and that fractional loss of Lp(a)− is similar to that of LDL: the Lp(a)− intimal clearance was that determined for Lp(a) in the same rabbit on the basis of total amounts of TCA-precipitable radioactivity in plasma and aortic intima-inner media. Fractional loss of Lp(a)− in a given aortic segment of nonlesioned and atherosclerotic aortas was assumed to be the average fractional loss of LDL in that same segment of nonlesioned or atherosclerotic aortas in the LDL experiments. After subtraction of Lp(a)− radioactivity from total Lp(a) radioactivity in plasma and arterial tissue, corrected intimal clearance and corrected fractional loss for pure Lp(a) were calculated based on the arterial wall and plasma radioactivity that was attributable only to Lp(a). Since labeled free apo(a) was not detected in plasma of rabbits injected with labeled Lp(a), no corrections for the possible contribution of labeled free apo(a) to arterial wall radioactivity were, or could be, made.

Calculations of crude accumulation and crude fractional loss. Crude accumulation in aorta after 3- and 26-h exposures (in nl/mg wet wt) to the labeled lipoproteins was calculated as radioactivity in intima-inner media (in cpm/mg wet wt), divided by the mean plasma radioactivity concentration during the experimental period (in cpm/nl).

Crude fractional loss was calculated as:

$$1 - \frac{\text{crude accumulation}_{\text{long}}}{\text{crude accumulation}_{\text{short}} \times \left(\frac{t_{\text{long}}}{t_{\text{short}}} \right)} \quad (4)$$

in which t is the exposure period (in h). Suffixes "long" and "short" refer to 26- and 3-h exposure periods, respectively. The calculations of crude accumulation and crude fractional loss do not depend on the assumption of a one-pool compartment.

Statistics. Arterial wall parameters were evaluated using a two-way (aortic cholesterol in atherosclerotic aortas) or a three-way (all other parameters) layout ANOVA (29) with random effects (30). In this model, for a given variable, each rabbit has its own level: the random effects describe variations between rabbits. The model allows differences in variances between atherosclerotic and nonlesioned aortas and in the model for crude accumulation for 3 h. The model furthermore was altered to allow differences in variances between different aortic sites. The contributions of different aortic sites (segments 1–6), the type of lipoprotein (Lp(a) or LDL), and the presence or absence of atherosclerosis (nonlesioned or atherosclerotic) to the total variation were assessed using the "proc mixed" procedure in the statistical program (6.09 ed.; SAS Institute, Cary, NC). Aortic cholesterol, intimal clearance, fractional loss, crude accumulation during 3 and 26 h, and crude fractional loss were all transformed logarithmically before statistical analysis, to approximate normal distributions. Initially, an ANOVA model was built to include all main effects (aortic site, type of lipoprotein, presence or absence of atherosclerosis) and all two-factor interactions (aortic site by type of lipoprotein, type of lipoprotein by atherosclerosis, aortic site by atherosclerosis). In the two-way layout, ANOVA aortic site, type of lipoprotein, and the aortic site by type of lipoprotein interaction were included. Using a step-down procedure, the model was reduced as much as possible; reduction in −2 log likelihood determined if the main effects or interaction terms could be removed. If the reduction in −2 log likelihood was significant (x^2 distribution with 1 degree of freedom), when a term was removed, that term remained in the model. The significance level upon removal from the model determined the effect of a term; main effects were only tested when removed from a model without interaction terms involving the main effect under investigation. Differences in variances were also tested for significance using reduction in −2 log likelihood. Student's t test was used to test differences between means in two group comparisons. P values are two tailed. Values are presented as means±SEM.

Results

Table I shows plasma lipoprotein concentrations and length of the cholesterol feeding period in rabbits used to study accumulation of labeled lipoproteins in the intima-inner media.

Labeled Lp(a) and LDL, visualized with autoradiography, comigrated with their respective nonlabeled lipoproteins, visualized with Coomassie blue staining, on nondenaturating 2.5–16% polyacrylamide gradient gels and 0.5% agarose gels (Fig.

Table I. Lipoprotein Concentrations and Duration of Cholesterol Feeding Period in the Rabbits with Nonlesioned or Atherosclerotic Aortas

	Number of rabbits	Plasma cholesterol				Lp(a)*	Period of cholesterol feeding
		Total	VLDL + IDL	LDL	HDL		
		mmol/liter‡				*mg/ml*	*d*
LDL studies							
Rabbits with nonlesioned aortas	7	21.2±2.9	15.8±2.2	4.8±0.8	0.6±0.1	ND§	7±1
Rabbits with atherosclerotic aortas	6	58.3±9.4	46.6±6.5	11.0±3.3	0.8±0.1	ND	180±2
Lp(a) studies							
Rabbits with nonlesioned aortas	6	17.1±3.4	11.3±3.2	5.2±0.4	0.6±0.1	0.27±0.03	6±0
Rabbits with atherosclerotic aortas	6	31.2±3.1	24.3±2.7	6.3±0.6	0.6±0.1	0.23±0.05	229±3

Values are means±SEM. *Initial Lp(a) plasma concentration after intravenous injection of $d < 1.12$ g/ml lipoproteins. ‡Plasma total and lipoprotein cholesterol values are at the end of the experiment. §ND, not determined: rabbits used for LDL studies were not injected with $d < 1.12$ g/ml lipoproteins.

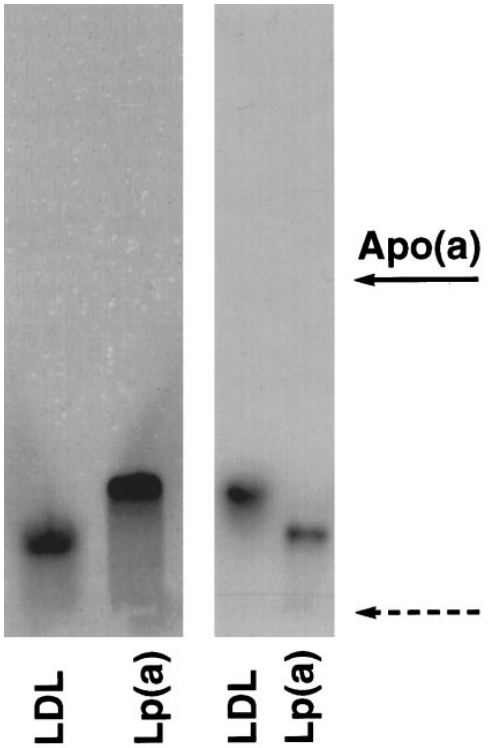

Figure 1. Autoradiographs of agarose electrophoresis gels (0.5%) (*left two lanes*) and nondenaturating polyacrylamide gradient gels (2.5–16%, Isolab Inc., Akron, OH) (*right two lanes*) of ^{131}I-Lp(a) and ^{125}I-LDL. Lipoproteins were loaded at the bottom (*dotted arrow*). The migration distance of free apo(a) (*solid arrow*) on nondenaturating polyacrylamide gradient gels was determined in another electrophoresis, but under similar conditions (21, 27).

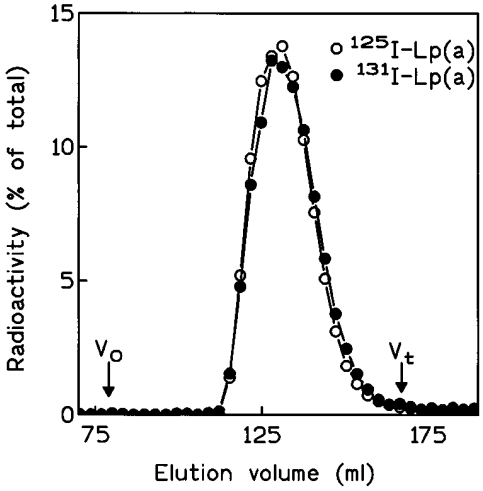

Figure 2. Gel filtration chromatography of rabbit plasma 3 h after intravenous injection of ^{125}I-Lp(a) and 26 h after injection of ^{131}I-Lp(a). The recovery was 99% for ^{125}I-Lp(a) and 100% for ^{131}I-Lp(a). V_0 and V_t are void and total volume, respectively. Iodinated LDL eluted ~ 5 ml before Lp(a) on this column (2.6 cm × 100 cm); the gel was Sephacryl S-500 HR (Pharmacia LKB Biotechnology Inc.).

1). Isolated Lp(a) and LDL migrated as two distinct bands and no labeled free apo(a) was detected. On gel filtration chromatography, no disintegration products of labeled Lp(a) were observed (Fig. 2).

26 h after injection of labeled lipoproteins, 21.6±1.8% of the initial amount of labeled Lp(a) and 42.5±2.3% of the initial amount of labeled LDL remained in plasma (Student's *t* test: $P < 0.001$). The decay in plasma of ^{125}I-labeled and ^{131}I-labeled lipoproteins were very similar, both for Lp(a) and LDL, and there was no difference in decay of labeled Lp(a) or LDL between rabbits with nonlesioned aortas and rabbits with atherosclerotic aortas (data not shown). The decay in plasma of labeled Lp(a) was almost similar to that of unlabeled Lp(a) (Fig. 3).

Formation of labeled free apo(a) in plasma after injection of labeled Lp(a) was minimal: the fraction of radioactivity in labeled Lp(a) that migrated through anti–apo B–containing gel and precipitated in anti–Lp(a)–containing gel was < 1%,10 min, 3 and 26 h after injection of labeled Lp(a) (data not shown). In contrast, the fraction of total labeled Lp(a) in plasma that was present in Lp(a)– (i.e., labeled particles that migrated through anti–Lp(a)–containing gel and precipitated in anti–apo B–containing gel) increased with time during the 26 h of circulation in vivo (Fig. 3). In accordance with this and the fact that Lp(a)– is smaller than Lp(a), ^{131}I-labeled lipoproteins in plasma 26 h after intravenous injection of ^{131}I-Lp(a) eluted slightly later than ^{125}I-labeled lipoproteins in plasma 3 h after injection of ^{125}I-Lp(a) on gel filtration chromatography (Fig. 2).

Aortic cholesterol. The cholesterol content in the nonlesioned aortas from the rabbits that were fed cholesterol for 6–10 d (Fig. 4) was similar to the aortic cholesterol content in rabbits that had never been fed cholesterol (31). In the present study, the values were 2.2±0.2, 1.9±0.3, 2.1±0.3, 1.8±0.3, 1.6±0.3, and 1.8±0.3 nmol/mg wet wt ($n = 12$) in segments 1, 2, 3, 4, 5, and 6, respectively. The cholesterol content of atherosclerotic aortas in rabbits that had been fed cholesterol for 173–236 d was markedly elevated compared with the cholesterol content of nonlesioned aortas. In atherosclerotic aortas, there was a highly significant variation in cholesterol content between aortic segments (ANOVA, effect of aortic site: $P < 0.0001$). Most cholesterol accumulated in the aortic arch (Fig.

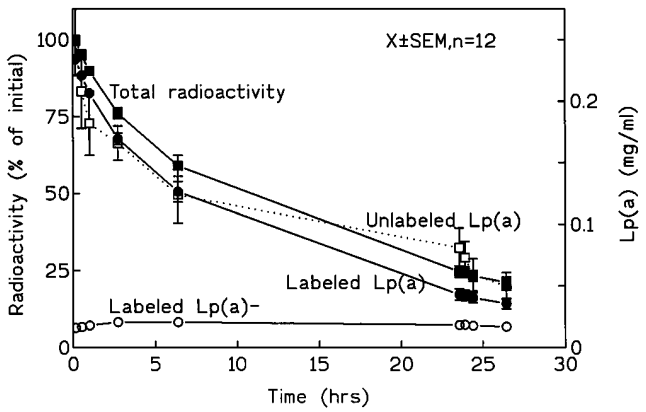

Figure 3. Plasma radioactivity decay curves (*filled lines, left y-axis*) and plasma Lp(a) mass (*dotted line*, *right y-axis*) after intravenous injection of ^{131}I-Lp(a) and $d < 1.12$ g/m lipoproteins. -■-, total radioactivity; -□-, Lp(a) plasma concentration (mg/ml); -●-, labeled Lp(a); -○-, labeled Lp(a)–. Values for labeled Lp(a)– were determined at 10 min, 3 h, and 26 h and the relative amount of radioactivity and Lp(a)– at other time points was estimated by linear interpolation in each rabbit. Values are means±SEM.

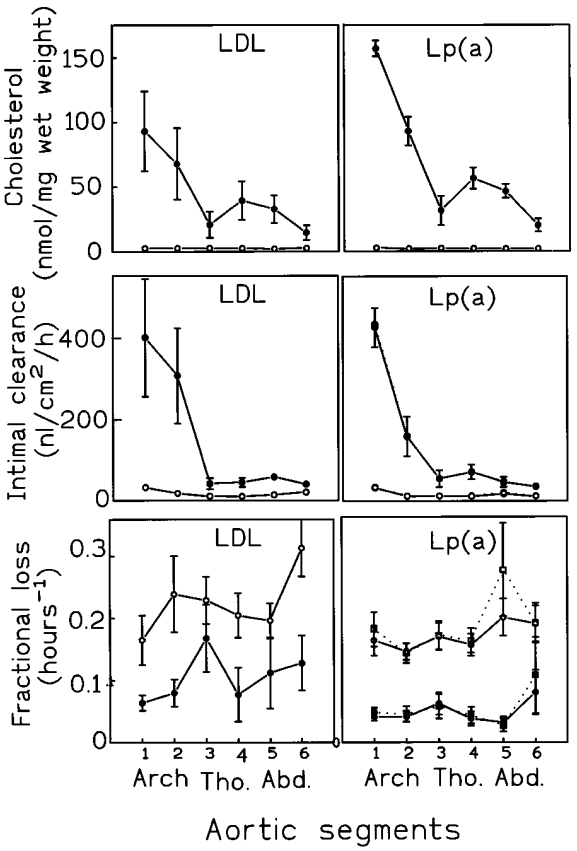

Figure 4. Cholesterol content and lipoprotein intimal clearance and fractional loss in the intima-inner media of six aortic segments of nonlesioned (○) and atherosclerotic aortas (●) of the cholesterol-fed rabbits studied. Intimal clearance and fractional loss of labeled Lp(a) after corrections for Lp(a)−contribution to total values (*circles*) (see Methods) are shown as boxes connected by dotted lines. Values are means±SEM.

4). There was no significant difference in aortic cholesterol content in atherosclerotic aortas between rabbits used to study LDL and Lp(a) (ANOVA, effect of group of rabbits: $P = 0.10$), but there was a difference in the variance between the two groups ($P < 0.01$).

Intimal clearance. Intimal clearance and fractional loss were calculated using a one-pool compartment model of the aortic intima-inner media. Intimal clearance represents the rate constant for transfer of a given species of labeled lipoproteins from plasma into the arterial wall.

The final ANOVA model to describe intimal clearance included aortic site ($P < 0.0001$), atherosclerosis ($P < 0.0001$), and the aortic site by atherosclerosis interaction ($P < 0.0001$). The intimal clearance of both lipoproteins was larger in atherosclerotic than in nonlesioned aortas (Fig. 4). The relatively larger intimal clearance in segments 1, 2, and 4 of atherosclerotic aorta compared with nonlesioned aorta was the cause of the aortic site by atherosclerosis interaction. There was no difference in intimal clearance between Lp(a) and LDL ($P < 0.90$).

Fractional loss. Fractional loss represents the rate constant for loss of newly entered lipoprotein from the arterial intima-inner media, by degradation by arterial wall cells or by transfer of labeled lipoproteins out of the arterial wall.

The final ANOVA model to describe fractional loss included aortic site ($P < 0.0001$) and atherosclerosis ($P < 0.0001$). On a logarithmic scale, the variance in fractional loss was larger in nonlesioned aortas than in atherosclerotic aortas; however, the model allows such a difference. Fractional loss in atherosclerotic intima-inner media of labeled Lp(a) and labeled LDL was on average 25 and 43%, respectively, of that in nonlesioned intima-inner media (Fig. 4). Fractional loss of labeled lipoproteins was lower in the atherosclerosis-prone aortic arch than in the more atherosclerosis-resistant abdominal aorta.

Although type of lipoprotein did not remain in the final ANOVA model for fractional loss, fractional loss of Lp(a) was borderline significantly lower than that of LDL (ANOVA, effect of type of lipoprotein; $P = 0.07$); Lp(a) fractional loss was 73% (95% confidence, interval 51 to 104%) of that of LDL. Since there was no interaction between type of lipoprotein and absence or presence of atherosclerosis, this borderline significant effect is derived both from nonlesioned and atherosclerotic aorta.

Crude accumulation. The final ANOVA model to describe crude accumulation during the 3 h included aortic site ($P < 0.0001$), atherosclerosis ($P < 0.0001$), and the aortic site by atherosclerosis interaction ($P < 0.0001$). On a logarithmic scale, the variance was larger in the aortic arch than in the thoracic and abdominal aortas ($P < 0.0001$), but the model was modified to allow this difference. The crude accumulation during the 3 h for both lipoproteins was larger in atherosclerotic than in nonlesioned aortas (Table II). The relatively larger accumulation in segments 1, 2, and 4 of the atherosclerotic aorta compared to the nonlesioned aorta was the cause of the aortic site by atherosclerosis interaction. There was no difference in accumulation during the 3 h between Lp(a) and LDL ($P = 0.90$).

The final ANOVA model to describe crude accumulation during the 26 h also included aortic site ($P < 0.0001$), atherosclerosis ($P < 0.0001$), and the aortic site by atherosclerosis interaction ($P < 0.0001$). On a logarithmic scale, the variance was larger for nonlesioned than for atherosclerotic aorta ($P < 0.0001$). The crude accumulation during the 26 h for both lipoproteins was larger in atherosclerotic than in nonlesioned aortas (Table II). The relatively larger accumulation in segments 1, 2, and 4 of atherosclerotic aorta compared to nonlesioned aorta was the cause of the aortic site by atherosclerosis interaction. There was no significant difference in accumulation during the 26 h between Lp(a) and LDL ($P < 0.10$); the accumulation of Lp(a) was 121% (95% confidence interval 95–152%) of that of LDL.

Crude fractional loss. The final ANOVA model to describe crude fractional loss included aortic site ($P < 0.0005$), atherosclerosis ($P < 0.0001$), and the aortic site by atherosclerosis interaction ($P < 0.0001$). On a logarithmic scale, the variance was larger for nonlesioned aorta than for atherosclerotic aorta ($P < 0.0005$). Crude fractional loss for both lipoproteins was smaller in atherosclerotic than in nonlesioned intima-inner media (Table II). There was no significant difference in crude fractional loss of Lp(a) and LDL ($P < 0.90$).

Discussion

A noticeable finding of the present study was a markedly reduced fractional loss of Lp(a) and LDL in atherosclerotic com-

Table II. Crude Accumulation After 3- and 26-h Exposures and Crude Fractional Loss of Labeled Lp(a) and Labeled LDL in Nonlesioned and Atherosclerotic Aorta

	Aortic segment					
	1, Arch	2, Arch	3, Thoracic	4, Thoracic	5, Abdominal	6, Abdominal
Nonlesioned aorta						
Crude accumulation during 3 h (nl/mg wet wt)						
Lp(a), $n = 6$	1.59±0.25	0.64±0.12	0.71±0.06	0.67±0.10	0.99±0.19	0.78±0.17
LDL, $n = 7$	1.99±0.30	1.21±0.25	0.81±0.17	0.71±0.16	1.18±0.37	1.48±0.34
Crude accumulation during 26 h (nl/mg wet wt)						
Lp(a), $n = 6$	2.99±0.65	1.29±0.29	1.24±0.13	1.18±0.15	1.34±0.14	1.07±0.13
LDL, $n = 7$	4.57±1.98	1.98±0.40	1.20±0.12	1.12±0.10	1.80±0.40	1.68±0.32
Crude fractional loss (per 26 h)						
Lp(a), $n = 6$	0.77±0.04	0.77±0.02	0.79±0.03	0.78±0.04	0.85±0.04	0.80±0.05
LDL, $n = 7$	0.71±0.06	0.79±0.04	0.80±0.04	0.79±0.03	0.78±0.04	0.85±0.01
Atherosclerotic aorta						
Crude accumulation during 3 h (nl/mg wet wt)						
Lp(a), $n = 6$	14.5±2.15	6.28±1.27	2.71±0.87	3.35±0.70	2.46±0.43	2.01±0.34
LDL, $n = 6$	12.2±4.00	10.8±4.06	2.29±0.61	2.36±0.44	3.02±0.29	2.51±0.34
Crude accumulation during 26 h (nl/mg wet wt)						
Lp(a), $n = 6$	71.8±8.90	30.7±5.1	12.6±4.21	17.2±3.20	14.7±2.79	10.0±3.12
LDL, $n = 6$	52.8±19.4	51.3±21.4	13.4±7.32	17.1±5.88	15.6±6.22	10.2±5.22
Crude fractional loss (per 26 h)						
Lp(a), $n = 6$	0.42±0.05	0.39±0.09	0.48±0.10	0.37±0.09	0.31±0.09	0.45±0.15
LDL, $n = 6$	0.57±0.07	0.51±0.05	0.47±0.14	0.24±0.14	0.45±0.16	0.55±0.17

Values are means±SEM.

pared with nonlesioned intima-inner media; the same conclusion was drawn when the much simpler calculation of crude fractional loss was done. This supports the idea that reduced fractional loss of both of these lipoproteins from arterial intima may play an important role during the development of atherosclerosis. The data also underscores the notion that an increased macromolecular intimal clearance, leading to an increased lipoprotein influx, contributes significantly to the accumulation of Lp(a) and LDL in atherosclerotic lesions.

In the present study, the difference in fractional loss of Lp(a) and LDL between nonlesioned and atherosclerotic aortas was similar at all aortic sites, while accumulation of cholesterol and severity of atherosclerosis in the intima was much more pronounced in the aortic arch than in the thoracic and abdominal aortas. It cannot therefore be excluded that the difference in fractional loss between normal and atherosclerotic aortas resulted from as yet uncharacterized general structural changes in the intima after long term cholesterol feeding rather than from the presence of atherosclerosis per se. The nature of such structural changes that would cause a decreased loss of lipoproteins from the intima-inner media is unknown. Increased diffusion distance due to intimal thickening may play a role. Previous in vivo studies on the effect of atherosclerosis on fractional loss of labeled LDL from the arterial wall are conflicting: one study in the pigeon found a significantly lower fractional loss of LDL from aortic sites with atherosclerosis than from adjacent nonatherosclerotic sites (32), a finding similar to the present study, whereas other studies in fat-fed monkeys (23) and genetically hyperlipidemic rabbits (22) did not detect differences between nonlesioned and atherosclerotic arteries. It cannot be excluded that differences in the composition of atherosclerotic plaques in aortas of cholesterol-fed rabbits and pigeons compared with that in aortas of fat-fed monkeys and genetically hyperlipidemic rabbits have induced the discrepant findings. It is, however, possible that the two studies with negative results (22, 23) overlooked a difference between normal and atherosclerotic aortas because of limited statistical power.

The importance of plasma contamination on the arterial surface for interpretation of the present aortic radioactivity data was estimated by considering a worst-case situation, in which as much as 50% of the radioactivity in the intima-inner media of the nonlesioned aortas after 3 h exposure to labeled Lp(a) or labeled LDL was attributable to plasma contamination (in atherosclerotic aorta, plasma contamination corrections would only have a minimal effect because the intimal clearance was very large). This estimate yielded plasma contaminations ranging from as much as an average 43 nl/cm^2 in the nonlesioned proximal segment of the aortic arch to an average 15 nl/cm^2 in the distal segment of the thoracic aorta. These plasma contaminations of aortic intima-inner media are

considerably higher than the average 6 nl/cm^2 in a previous study (31), but similar to an average 30 nl/cm^2 in another study (15), both from our laboratory. After subtraction of aortic radioactivity after 3- and 26-h exposures estimated to be due to plasma contamination in each aortic segment of each rabbit, fractional loss of labeled Lp(a) and LDL from nonlesioned aortas were recalculated: fractional loss in atherosclerotic aortic intima-inner media of labeled Lp(a) and labeled LDL was then on average 51 and 78%, respectively, of that in nonlesioned aortic intima-inner media. Accordingly, the overall conclusion of a reduced fractional loss of Lp(a) and LDL in atherosclerotic compared to nonlesioned aortas, is valid even after these plasma contamination corrections.

In the present study, fractional loss of labeled Lp(a) and labeled LDL from nonlesioned aortas was lowest in the atherosclerosis-susceptible aortic arch and highest in the atherosclerosis-resistant abdominal aorta. This is in accordance with a previous study by Schwenke and Carew (33). These findings support the idea that regional variations in rates of loss of LDL and Lp(a) in the intima may be important for the focal development of atherosclerotic lesions, at least in the initial stages of the atherogenesis (33). The extracellular matrix most likely plays a role in lipoprotein retention by aortic tissue (34), and LDL has been shown to bind more to types I and III collagen than to types IV and V collagen (35). Moreover, glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to LDL (36). Thus, variation in fractional loss of lipoproteins could be related to variation in affinity of aortic glycosaminoglycans to LDL and Lp(a) at different aortic sites.

Previous studies have suggested that Lp(a) accumulates in vessel walls preferentially to LDL (6–8) and in vitro studies suggest that Lp(a) relative to LDL has a higher affinity to fibrin (37) and to arterial wall glycosaminoglycans (38). Moreover, we have recently reported a markedly increased accumulation of Lp(a) compared with LDL in balloon-injured rabbit aorta in vivo (13). In the present study, there was a borderline significant lower fractional loss for labeled Lp(a) than for labeled LDL in nonlesioned and atherosclerotic aortas combined. However, since atherosclerotic aortas used for studying Lp(a) had (nonsignificantly) more cholesterol than aortas used for studying LDL (Fig. 4), and since atherosclerosis reduces fractional loss, it is possible that the difference in fractional loss between Lp(a) and LDL can be explained by differences in the severity of atherosclerosis.

One explanation for the present lack of a clear difference in fractional loss between Lp(a) and LDL may be that the rabbit aorta does not contain sufficient amounts of structural components, such as fibrin and glycosaminoglycans, which may be necessary for specific accumulation of Lp(a) compared with LDL in the intima. A second explanation could be that an experimental period of 26 h is too short to detect subtle differences in loss of Lp(a) and LDL from the arterial wall. Finally, the one-pool compartment model may not adequately describe the kinetics of Lp(a) in the arterial wall. Nevertheless, calculation of crude fractional loss that does not assume a one-pool compartment model also failed to demonstrate a statistically significant difference in losses of Lp(a) and LDL.

The intimal clearance was similar for Lp(a) and LDL throughout the length of aorta. This observation suggests that the transfer of Lp(a) from plasma across the endothelial barrier is by the same mechanism as LDL; i.e., presumably a lipoprotein size, lipoprotein concentration, and blood pressure-dependent transfer (15, 39, 40), independent of LDL-receptors (41). The present data are in accordance with findings from our previous study in which there was a close association between the accumulation of labeled Lp(a) and LDL during 1 and 3 h in rabbit aorta after labeled Lp(a) and LDL were injected simultaneously (21).

In accordance with our previous studies in rabbits, the plasma decay of Lp(a) was faster than that of LDL (21), which contrasts observations in humans (42–44). Importantly, using iodinated Lp(a) and LDL that had been labeled using the same protocol as the present study, we found a similar removal rate of simultaneously injected Lp(a) and LDL in humans during a 3-h observation period (data will be published elsewhere). Furthermore, in the present study, plasma decay of labeled and unlabeled Lp(a) after a bolus injection were almost similar. These data altogether support the conclusion that iodination did not severely affect the metabolism of Lp(a).

Rabbits injected with labeled Lp(a) additionally received a bolus injection of unlabeled Lp(a) at the start of the experiment to increase the plasma levels of Lp(a) to a level comparable to that in humans. The decline in plasma Lp(a) concentration during the experiment is not believed to have affected the present results, since the accumulation of labeled Lp(a) in the rabbit aortic intima-inner media was similar after a 5–10-min (13), 3-h (21), and 23-h exposure (12), irrespective of whether the rabbits had received a bolus injection of unlabeled Lp(a) together with labeled Lp(a) or not.

The fraction of total plasma radioactivity in Lp(a)− increased with time after intravenous injection of labeled Lp(a). The contribution of Lp(a)− to the total area under the plasma radioactivity concentration vs the time curve during the 26 h after injection of labeled Lp(a) was on average 16%. The contribution of this labeled Lp(a)− to the total arterial wall radioactivity was assessed under the assumption that Lp(a)− interacts with the arterial wall like LDL (see Methods). Corrections for Lp(a)− changed the values of intimal clearance and fractional loss of labeled Lp(a) only to a minimal extent and did not affect the overall results and conclusions of the present study. No labeled free apo(a) was detected in plasma, either on two-tier rocket immunoelectrophoresis or on gel filtration chromatography. This observation is similar to that in humans after an intravenous injection of iodinated Lp(a) (28, 42), and is particularly relevant for the present study since free apo(a), due to its small size similar to albumin and HDL, presumably would enter and leave the arterial wall more rapidly than Lp(a) (22, 39). We have previously estimated that 15 to 25% of the radioactivity in iodinated Lp(a) is in apo(a), with the remaining fraction in apo B (21). Formation of labeled Lp(a)− must therefore be accompanied by the formation of labeled free apo(a). Since labeled free apo(a), however, was not detectable in plasma, labeled free apo(a) must either be removed very rapidly from plasma or be dissociated from Lp(a) extravascularly. If labeled free apo(a) was dissociated from Lp(a) in the arterial wall and labeled free apo(a) was then removed from the wall faster than Lp(a)−, this may have led to an overestimation of fractional loss of the lipid/apo B moiety of Lp(a).

In conclusion, the present data suggest that decreased fractional loss of LDL and Lp(a) in the intima at atherosclerosis-susceptible sites accompanies the development of atherosclerosis. The data further suggest that Lp(a) shares with LDL the

potential for causing atherosclerosis; the increased intimal clearance and decreased fractional loss of Lp(a) in atherosclerotic aortas favors the idea of accumulation of Lp(a) in atherosclerotic lesions. This may stimulate foam cell formation (45), smooth muscle cell proliferation (46), and fibrin deposition (47).

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