Increased Degradation of Lipoprotein(a) in Atherosclerotic Compared With Nonlesioned Aortic Intima–Inner Media of Rabbits

In Vivo Evidence That Lipoprotein(a) May Contribute to Foam Cell Formation

Lars B. Nielsen, Klaus Juul, Børge G. Nordestgaard

Abstract—To investigate a potential role of lipoprotein(a) [Lp(a)] in foam cell formation, we have measured the degradation rates of Lp(a) and LDL in the rabbit aorta in vivo. Lp(a) (or LDL) was labeled with both 131I-TC and 125I and injected into 17 rabbits with extensive aortic atherosclerosis and into 16 rabbits without atherosclerosis. As the protein moiety of the doubly labeled lipoproteins is degraded, 131I-TC is trapped in the cell, whereas 125I diffuses out of the cell. Twenty-four hours after injection, 12 samples of the aorta and biopsies from 9 other tissues were removed. The degradation rate of Lp(a) (percent of plasma pool per gram tissue per day) was less than that of LDL in the adrenals and in the intestine. In contrast, degradation rates of Lp(a) and LDL were similar in liver, spleen, kidney, heart, lung, skeletal muscle, and adipose tissue. In nonlesioned aortic intima–inner media, the degradation rate of Lp(a) was 39% of that of LDL (t test: P <.05 in aortic arch and thoracic aorta), whereas the degradation rates of Lp(a) and LDL were similar in atherosclerotic aortic intima–inner media. Lp(a) degradation rates were markedly increased in atherosclerotic compared with nonlesioned aortic intima–inner media: 28.2±9.2×10−7% and 5.0±0.6×10−7% of the plasma pool per gram tissue per day in the intima–inner media of the proximal segment of atherosclerotic and nonlesioned aorta, respectively (t test: P <.01). These results suggest that the metabolism of Lp(a) is different from that of LDL in nonlesioned arterial intima, possibly reflecting that Lp(a) is degraded by LDL receptors in arterial intima less efficiently than LDL. The results also indicate that the degradation rate of Lp(a) is markedly increased in atherosclerotic lesions of rabbits, supporting the idea that Lp(a) contributes to foam cell formation during the development of atherosclerosis. (Arterioscler Thromb Vasc Biol. 1998;18:641-649.)

Key Words: atherosclerosis ■ cholesterol ■ low-density lipoprotein ■ macrophages

Like LDL,1 Lp(a) contains cholesterol, cholesterol esters, phospholipids, triglycerides, and apoB.1 In epidemiological studies, high plasma levels of Lp(a) have been associated with an increased risk of cardiovascular disease.2 The mechanism by which Lp(a) may promote atherothrombotic disease is unclear, but several possibilities are suggested by the structure of Lp(a). Lp(a) contains apo(a), which is attached to apoB through a disulfide bridge.3 Apo(a) has a high degree of structural similarity to plasminogen,4 and Lp(a) has been shown to inhibit plasmin formation in vitro.5 As a result of this effect, Lp(a) inhibits fibrinolysis5 and accelerates growth of smooth muscle cells6 in vitro. Although these effects could promote the development of atherothrombotic disease, the significance needs to be determined in vivo.

Because Lp(a) contains cholesterol and cholesterol esters, uptake and degradation of Lp(a) by monocyte-macrophages in the arterial intima could promote foam cell formation, the pathologic hallmark of early atherosclerosis.7 In vitro evidence suggests that Lp(a) may be taken up and degraded by macrophage-derived foam cells via a cellular receptor distinct from the LDL receptor, scavenger receptors, the LDL receptor–related protein, or plasminogen receptors.8-10 Other studies have shown that Lp(a) can be degraded by monocyte-macrophages via the LDL receptor and “nonspecific” pathways.11-16 Complex formation by Lp(a) with glycosaminoglycans or modification of Lp(a) with malondialdehyde results in a markedly enhanced uptake and degradation of Lp(a) by monocyte-macrophages via scavenger receptors.15,17 Demonstration of Lp(a) immunoreactivity within the foam cells of human atherosclerotic lesions supports the theory of Lp(a) uptake by arterial wall macrophages.18 However, it remains unknown whether degradation of Lp(a) by arterial wall cells in vivo occurs at a rate sufficient for foam cell formation.

In a previous study, we detected human apo(a) immunoreactivity within foam cells of atherosclerotic lesions in
cholesterol-fed rabbits that had received an intravenous injection of human Lp(a) 3 hours before the removal of the aorta.\(^1\) This observation suggested that Lp(a) is taken up by rabbit arterial wall cells and prompted us to investigate, in a quantitative fashion, rates of degradation of Lp(a) in atherosclerotic lesions. In the present study, we used a sensitive method to determine the tissue sites and rates of Lp(a) degradation and LDL degradation in rabbits with atherosclerotic lesions in the aorta and in rabbits without atherosclerosis. Degradation rates of Lp(a) and LDL in 12 parts of the aorta and in 9 other tissues were determined after an intravenous injection of Lp(a) (or LDL) that had been doubly labeled with \(^{125}\text{I}\) and \(^{131}\text{I}\)-TC.\(^{20,21}\)

### Methods

**Animals**

Male white rabbits (Danish Country Strain, Statens Seruminstitut, Copenhagen, Denmark) weighing 2.9 to 4.0 kg were used. To induce atherosclerosis in the aorta, 17 rabbits were fed a 0.25% to 1% cholesterol-enriched chow for 5 to 6 months; the chow was prepared as described elsewhere.\(^{22}\) The amount of cholesterol in the chow was adjusted regularly to maintain a plasma cholesterol level of about 10\,000 mg/dL. Sixteen other rabbits were fed a 1% cholesterol-enriched chow for 6 days; this short cholesterol-feeding period was chosen to increase the plasma cholesterol concentration without inducing atherosclerosis. The experimental protocol was approved by the Danish government body supervising animal experiments (Dyreforsøgstilsynet).

**Isolation of Human Lp(a) and LDL**

For each isolation of Lp(a), plasma from two donors with a mean plasma Lp(a) concentration of 0.5 mg/mL was pooled and added to N\(_2\)-EDTA, chloramphenicol, gentamycin sulfate, benzamidine, aprotonin, and \(\epsilon\)-amino-\(\delta\)-caproic acid as previously described.\(^{22,23}\) After fixed-density ultracentrifugation, the d\(_{<1.12}\)-g/mL fraction was passed over a lysine Sepharose column. Purified Lp(a) was eluted with \(\epsilon\)-amino-\(\delta\)-caproic acid. The sizes of apo(a) isoforms of the donors were, respectively, 640 kD/820 kD and 580 kD for one of the two preparations of Lp(a) and 640 kD/760 kD and 580 kD for the other.\(^{24}\) The apo(a) isoforms were determined by Dr Matteu Jauhiainen (National Public Health Institute, Helsinki, Finland).\(^{25}\) LDL (1.019<d<1.063 g/mL) was isolated by sequential ultracentrifugation from plasma of a donor with low plasma Lp(a).

The concentrations of Lp(a) (Lp(a) mass per mL) in human plasma and batches of purified Lp(a) were measured using a turbidimetric assay (DAKO A/S, Glostrup, Denmark).\(^{25}\) The polyclonal apo(a)-specific rabbit anti-Lp(a) antiseraum that is used in this assay consists of a mixture of sera from >200 rabbits that were each immunized several times with pools of Lp(a) from 6 to 20 donors (Dr Kim Christoffersen, DAKO, Copenhagen, Denmark, personal communication, October 1, 1997). The concentration of LDL protein in purified LDL was estimated from the absorbance at 220 nm before iodination. This method has been extensively validated by Zilversmit and Shea,\(^{27}\) and in control experiments we found that it gave results similar to those obtained by the method of Lowry et al.\(^{28}\)

**Lipoprotein Labeling**

Lp(a) (4.5 to 7.4 mg total Lp(a) mass) or LDL (5 mg protein, 30 mg total LDL mass) was labeled with 150 to 185 MBq \(^{125}\text{I}\) (Amersham, Birkerød, Denmark) using ICl,\(^{29,31,32}\) TC (50 nmol) was labeled with 150 to 185 MBq \(^{131}\text{I}\) using Iodogen (Pierce Chemical Company, Aarhus, Denmark).\(^{20}\) I-TC was transferred to a vial containing 10 \(\mu\)L NaHSO\(_3\) (0.1 mol/L) and 5 \(\mu\)L NaI (0.1 mol/L) and activated by addition of 20 \(\mu\)L cyanuric chloride (2.5 mmol/L in acetone) followed by 5 \(\mu\)L NaOH (20 mmol/L) and 10 \(\mu\)L acetic acid (15 mmol/L). Lp(a) or \(^{125}\text{I}\)-Lp(a) [4.5 to 7.4 mg Lp(a)], or LDL or \(^{131}\text{I}\)-LDL (5 mg protein, 30 mg total lipoprotein mass) was adjusted to pH 9 to 10 by addition of 50 to 100 \(\mu\)L borate buffer (0.3 mol/L) immediately before addition of the activated \(^{131}\text{I}\)-TC. On average, 14% of the \(^{131}\text{I}\)-TC was bound to Lp(a) and LDL. After 15 to 40 minutes, unbound \(^{131}\text{I}\)-TC was removed using a PD-10 column equilibrated with PBS containing NH\(_4\)HCO\(_3\) (0.1 mol/L).\(^{20}\) Rabbit albumin (100 mg) (Sigma, Copenhagen) was added to the labeled lipoproteins before residual unbound \(^{131}\text{I}\)-TC was removed by dialysis against excess volumes of PBS with NH\(_4\)HCO\(_3\) (0.1 mol/L) for 18 to 20 hours. Mean specific activities were 0.8\times10^8 cpm \(^{125}\text{I}\) and 0.2\times10^8 cpm \(^{131}\text{I}\) per milligram Lp(a), and 0.6\times10^8 cpm \(^{125}\text{I}\) and 0.1\times10^8 cpm \(^{131}\text{I}\) per milligram LDL protein (0.1\times10^8 cpm \(^{131}\text{I}\) and 0.1\times10^8 cpm \(^{131}\text{I}\) per milligram total LDL mass). Less than 5% of the total radioactivity in the labeled Lp(a) and LDL was extractable with chloroform-methanol (1:1, vol/vol). Labeled lipoprotein preparations were passed through 0.22-\(\mu\)m or 0.45-\(\mu\)m filters and used for injection within 48 hours of labeling.

**Lipoprotein Injection**

Three rabbits with nonlesioned aortas and three rabbits with atherosclerotic aortas received an intravenous injection of labeled Lp(a) in which the same aliquot of Lp(a) had been labeled twice: first with the ICl method and then with TC. Two rabbits with nonlesioned aortas and four rabbits with atherosclerotic aortas received an intravenous injection of labeled Lp(a) in which the same aliquot of Lp(a) had been labeled with the ICl method and another aliquot with TC. Similarly, five rabbits with nonlesioned aortas and five rabbits with atherosclerotic aortas received an intravenous injection of labeled LDL in which the same aliquot of LDL had been labeled twice, and six rabbits with nonlesioned aortas and five rabbits with atherosclerotic aortas received an intravenous injection of labeled LDL in which one aliquot of LDL had been labeled with the ICl method and another aliquot with TC. The arterial wall degradation rates were similar with the two labeling protocols. The average amount of radioactivity injected was 1.0\times10^8 cpm \(^{125}\text{I}\) and 0.8\times10^8 cpm \(^{131}\text{I}\).

To ensure that degradation rates of Lp(a) were not severely affected by the presence of endogenous Lp(a) in the rabbit, four rabbits with atherosclerotic aortas and two rabbits with nonlesioned aortas received a intravenous injection of 9.5 mL human d<1.12-g/mL lipoproteins [containing 25 mg Lp(a)] immediately before injection of labeled Lp(a).\(^{21}\) The fractional catabolic rate and the degradation rates of Lp(a) in aortic tissues were similar in the rabbits that received the intravenous injection of Lp(a) and in the comparable rabbits with and without atherosclerotic lesions that did not (data not shown). The data from the rabbits that did and the rabbits that did not receive unlabeled Lp(a) were therefore combined.

Blood samples (1 mL) were drawn from an ear vein at 10 minutes and 1, 3, 6, 11, and 24 hours after injection of labeled lipoproteins.

**Removal of Aorta**

Twenty-four hours after injection of the labeled lipoproteins, each rabbit was injected intravenously with pentobarbital (50 to 100 mg/kg) before the thoracic cavity was opened and a cannula was placed in the left ventricle of the heart. The circulation was perfused with 500 mL NaCl (0.15 mol/L) at 4°C followed by perfusion with 500 mL half-strength Karmowsky’s fixative.\(^{33}\) After removal of adventitial tissue, the aorta was opened longitudinally and fixed for an additional 20 to 24 hours in half-strength Karmowsky’s fixative, which was removed before counting. This fixation regimen results in

### Selected Abbreviations and Acronyms

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>apo</td>
<td>apolipoprotein</td>
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<tr>
<td>*I</td>
<td>either (^{125}\text{I}) or (^{131}\text{I})</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>lipoprotein(a)</td>
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<tr>
<td>TC</td>
<td>tyramine cellobiose</td>
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in the tissue of protein-bound *I and of all *I-TC. The aorta was divided into the aortic arch, thoracic aorta, and abdominal aorta, and each of these three segments were then subdivided into a proximal and distal part of similar size, as previously described. The intima–inner media was separated from the outer media in each of these six aortic segments. There was no difference in the weight of aortic tissues from rabbits used for studying Lp(a) or for studying LDL. Other selected tissues were also removed and fixed for at least 20 hours in half-strength Karnovsky’s fixative; the fixative was removed before counting.

Determination of Radioactivity

Aliquots of plasma and diluted doses of labeled lipoproteins were precipitated with trichloroacetic acid after the addition of albumin. Radioactivity in tissues, plasma, and doses was determined in a double-channel gamma counter (LKB Compugamma 1282, Wallac). Tissues were counted for at least 42 minutes and radioactivity measures were corrected for decay of *I during counting. Standard errors for counting rates of *I and *I were less than 2%.

Analysis

Because the aortic cholesterol content is closely associated with other indices of atherosclerosis in rabbits and pigs, we chose to assess the severity of atherosclerosis in aortic segments by measuring the cholesterol content of the intima–inner media. After determining radioactivity, lipids in aortic intima–inner media were extracted over a 24-hour period with chloroform/methanol (2:1 vol/vol) followed by two further extractions with chloroform/methanol (1:1, vol/vol) before the combined lipid extract was washed by the procedure of Folch et al. Total cholesterol content was determined by an enzymatic method (CHOD-PAP, Boehringer Mannheim) after evaporation of the chloroform/methanol and solubilization of the extract in isopropanol. In control experiments, the addition of half-strength Karnovsky’s fixative to plasma samples did not interfere with subsequent extraction and quantification of cholesterol, supporting the idea that fixation of aortic tissues before lipid extraction did not affect the cholesterol measurement (data not shown).

Nondenaturing polyacrylamide gradient gel electrophoresis, gradient density ultracentrifugation, and a two-tier rocket immunoelectrophoresis assay were used to evaluate the intactness of labeled Lp(a) and LDL in labeled preparations used for injections and in plasma after intravenous injection into the rabbits. In two-tier rocket immunoelectrophoresis, 5 µL plasma was loaded on an agarose gel (1.25%) that consisted of three portions. The lower third of the gel, where samples were applied, consisted of pure agarose. The middle third contained anti-Lp(a) antiserum (DAKO A/S), and the upper third contained anti-apoB antiserum (DAKO A/S). Alternatively, the middle third of the gel contained anti-apoB antiserum and the upper third anti-Lp(a) antiserum. After electrophoresis and staining of the gel with Coomassie blue, the application spot and rockets were cut out and assayed for radioactivity.

Calculations

All calculations were based on protein-bound radioactivity (ie, trichloroacetic acid–precipitable radioactivity in plasma and total radioactivity in fixed tissues).

The accumulation of undegraded Lp(a) (or LDL) (in microliters per gram per day) in aortic intima–inner media was calculated as the amount of *I-Lp(a) (or *I-LDL) radioactivity per gram aortic tissue divided by the mean plasma concentration of *I-Lp(a) (or *I-LDL) and the duration of the experiment.

The calculation of degradation rates was performed exactly as described by Pittman, Carew, and coworkers, who have thoroughly discussed and validated the assumptions for these calculations. On cellular uptake and degradation of Lp(a), the protein moiety is degraded by lysosomal enzymes into amino acids. In contrast to the free amino acids, TC is trapped in the lysosomal compartment of cells after degradation of the protein moiety of a TC-labeled protein. After a 24-hour exposure of the arterial wall to intravenously injected *I-TC–Lp(a), a fraction of the radioactivity in the arterial wall represents *I-TC trapped in lysosomes after uptake and degradation of *I-TC–Lp(a). The remaining *I-Lp(a) in the arterial tissue represents accumulation of undegraded *I-TC–Lp(a) mainly in the extracellular space. *I-Lp(a) is used to assess the amount of undegraded *I-TC–Lp(a). *I-Lp(a) is bound to tyrosine residues. When *I-Lp(a) is degraded, *I-tyrosine rapidly diffuses out of the cells and is removed from the intima–inner media. After removal of the tissue biopsies, any remaining *I-tyrosine will diffuse out of the arterial tissue during fixation with the modified Karnovsky’s fixative. Therefore, *I-Lp(a) in the fixed-tissue samples represents intact *I-Lp(a) mainly in the extracellular space.

When *I-TC–Lp(a) and *I-Lp(a) are coincjected, the contribution of undegraded *I-TC–Lp(a) to the total amount of *I-Lp(a) in the tissue can be assessed from the amount of *I-Lp(a) in that tissue [after taking into account the relative plasma concentrations of *I-Lp(a) and *I-TC–Lp(a)]. The amount of *I-TC–Lp(a) degraded by the whole body during 24 hours after intravenous injection is calculated from the plasma decay of *I-TC–Lp(a) using a two-compartment model. The Lp(a) degradation rate (% of plasma pool per gram tissue per day) in a given tissue is then calculated as the ratio between the amount of *I-TC–Lp(a) that is degraded in that tissue and the amount of *I-TC–Lp(a) degraded in the whole body multiplied by the fractional catabolic rate of Lp(a). These calculations can also be performed for Lp(a) labeled with *I-TC and *I-LDL, and for double labeled LDL. The mass of Lp(a) (or LDL) degraded per gram tissue per day would be calculated by multiplication of the degradation rate by the total plasma pool of Lp(a) (or LDL).

Statistics

The contributions of different aortic sites (segments 1, 2, 3, 4, 5, or 6), lipoprotein type [Lp(a) or LDL], and presence or absence of aortic atherosclerosis (nonlesioned or atherosclerotic) to the total variation in aortic cholesterol content, accumulation of undegraded lipoproteins, or degradation rates in the intima–inner media were assessed with ANOVA with random effects using the “proc mixed” procedure in the SAS statistical program as described in detail previously. Briefly, the initial model included all main effects and all possible two-factor interactions. Using a step-down procedure, the model was then reduced as much as possible to include only significant (P < 0.05) main effects and interactions. The contributions of aortic site, lipoprotein type, and aortic layer (intima–inner media and outer media) to the total variation in degradation rates in nonlesioned aorta (or atherosclerotic aorta) were analyzed similarly. In each of the ANOVAs, the variance was significantly different at different aortic sites and/or in nonlesioned compared with atherosclerotic aortas; accordingly, the ANOVA models were modified to allow for this.

Student’s t test was used to test differences between means in two-group comparisons. All values are presented as mean ± SEM. A probability value of < 0.05 on two-sided tests was considered significant.

Results

Labeled Lipoproteins

Two different methodologies were used to assess the integrity and homogeneity of labeled Lp(a) and LDL. First, on nondenaturing polyacrylamide gel electrophoresis, Lp(a) and LDL labeled with *I-TC or *I comigrated with their respective nonlabeled lipoproteins (Fig 1). Second, by density gradient ultracentrifugation, *I-TC–Lp(a) and *I-Lp(a) had similar density profiles (Fig 2). These observations demonstrate that the TC-labeling procedures did not induce aggregation of lipoproteins or separation of apo(a) from the Lp(a) particle, although they do not exclude fragmentation of the apolipoproteins within the labeled lipoproteins.

Malondialdehyde derivatization can induce cross-linking of apo(a) to apolipoproteins within the labeled lipoproteins. A priori, we suspected that
coupling of *I-TC to Lp(a) might also induce cross-linking of apo(a) to apoB. To investigate this possibility, *I-TC–Lp(a) and *I-Lp(a) were treated with DTE before density gradient ultracentrifugation. DTE reduces the disulfide bridge between apoB and apo(a) in Lp(a), resulting in the formation of “Lp(a)−” [i.e., Lp(a) that has lost the apo(a) moiety and therefore resembles LDL] and free apo(a). After reduction with DTE, the density profiles of *I-TC–Lp(a)− and *I-Lp(a)− were similar; both profiles were shifted to a lesser density compared with the density profiles of “intact” labeled Lp(a). Also, density gradient ultracentrifugation of reduced *I-TC–Lp(a) and reduced *I-Lp(a) displayed radioactivity in the bottom fraction where free apo(a) would be found (Fig 2). Nondenaturing gradient gel electrophoresis of the *I-TC–Lp(a)− fraction followed by autoradiography revealed that all radioactivity in the *I-TC–Lp(a)− fraction was in LDL-sized particles and that there was no radioactivity in Lp(a)-sized particles (data not shown). The absence of radioactivity in Lp(a)-sized particles in the *I-TC–Lp(a)− fraction suggests that DTE reduction yielded total separation of apo(a) from apoB in the labeled Lp(a). Thus, TC labeling of Lp(a) did not appear to induce cross-linking of apo(a) to apoB under the present experimental circumstances. The fraction of the total radioactivity in *I-TC–Lp(a) in the apo(a) moiety was on average 22 ± 7% (n = 3).

To estimate the formation of labeled free apo(a) or labeled fragments of apo(a) separated from labeled Lp(a) in plasma, we measured the amount of labeled particles in plasma that migrated through anti-apoB antiserum containing gel and precipitated in anti-Lp(a) antiserum containing gel. Even 24 hours after injection of labeled Lp(a), less than 1% of the total plasma radioactivity was in labeled free apo(a). We also used two-tier rocket immunoelectrophoresis to assess formation of *I-TC–Lp(a) particles that lose ability to precipitate in anti-Lp(a) antiserum containing agarose gel. The fraction of *I-TC in the plasma of rabbits injected with *I-TC–Lp(a) that did not precipitate in the anti-Lp(a) antiserum containing gel (but did precipitate in anti-apoB antiserum containing gel) increased with time during the 24 hours after injection of *I-TC–Lp(a) and was on average 5%, 16%, and 28% after 10 minutes and 3 and 24 hours, respectively.

Tissue Sites for Degradation of Lp(a) and LDL
The rate of degradation of Lp(a) per gram of tissue was highest in the spleen; moderately high in the liver, adrenal, and kidney; and low in heart, lung, intestine, adipose tissue, and skeletal muscle (Fig 3). However, on a per-organ basis, most Lp(a) was degraded in the liver. Compared with LDL, the rate of degradation of Lp(a) was smaller in the adrenal and intestine (t test for both tissues, P < .05). Degradation rates of Lp(a) and LDL were similar in the remaining tissues examined. These results are consistent with the notions that LDL...
uptake and degradation in the adrenal and intestine are predominantly via the LDL receptor and that Lp(a) is degraded less efficiently than LDL via the LDL receptor. Thus, the intrinsic metabolic properties of LDL and Lp(a) presumably were not altered by the labeling procedures.

**Aortic Atherosclerosis**

Degradation rates of Lp(a) and LDL were studied in rabbits with atherosclerotic aortas and in rabbits with nonlesioned aortas; the basic characteristics of the rabbits are shown in the Table. All rabbits that had been fed a cholesterol-enriched chow had visible atherosclerotic lesions in the aorta. The variation in severity of atherosclerosis throughout the length of the aorta showed the expected pattern: the cholesterol content of the intima–inner media was largest in the aortic arch (segments 1 and 2) and at the level of the celiac axis (segments 4 and 5) (Fig 4). The cholesterol content of the intima–inner media of atherosclerotic aortas compared with nonlesioned aorta was most pronounced in aortic segments 1 and 2. Accordingly, there was a significant aortic site by atherosclerosis interaction in the final ANOVA model to describe the accumulation of undegraded lipoproteins in the intima–inner media (ANOVA, aortic site by atherosclerosis interaction: *P*<.0001). In accordance with previous findings, there was no difference in accumulation of undegraded Lp(a) and undegraded LDL in the intima–inner media of atherosclerotic and nonlesioned aorta combined (ANOVA, effect of lipoprotein type: *P*=.11).

**Increased Accumulation of Undegraded Lp(a) and LDL in Atherosclerotic Compared With Nonlesioned Aortic Intima–Inner Media**

The accumulation of undegraded Lp(a) and LDL in the intima–inner media was increased in atherosclerotic aortas compared with nonlesioned aortas (ANOVA, effect of atherosclerosis: *P*<.0001) (Fig 5). There was a large variation in accumulation of undegraded lipoproteins in the intima–inner media between different aortic segments (ANOVA, effect of aortic site: *P*<.0001). The difference in accumulation of undegraded lipoproteins between the atherosclerotic and nonlesioned aorta was most pronounced in aortic segments 1 and 2. Accordingly, there was a significant aortic site by atherosclerosis interaction in the final ANOVA model to describe the accumulation of undegraded lipoproteins in the intima–inner media (ANOVA, aortic site by atherosclerosis interaction: *P*<.0001). In accordance with previous findings, there was no difference in accumulation of undegraded Lp(a) and undegraded LDL in the intima–inner media of atherosclerotic and nonlesioned aorta combined (ANOVA, effect of lipoprotein type: *P*=.11).

**Increased Degradation Rates of Lp(a) and LDL in Atherosclerotic Compared With Nonlesioned Aortic Intima–Inner Media**

The rates of degradation of Lp(a) and LDL were increased in the intima–inner media of atherosclerotic aortas compared with nonlesioned aortas (ANOVA, effect of atherosclerosis: *P*<.03) (Fig 5). There was a large variation in lipoprotein degradation rates between aortic segments (ANOVA, effect of aortic site: *P*<.0001). The variation in lipoprotein degradation rate between aortic segments in the atherosclerotic intima–inner media closely resembled the variation in atherosclerosis severity (compare Figs 4 and 5). The differences in Lp(a) and LDL degradation rates between nonlesioned and atherosclerotic aortic intima–inner media were most pronounced in the aortic arch. Accordingly, there was a significant interaction between aortic site and atherosclerosis in the

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**Figure 3. Rates of degradation of Lp(a) and LDL in selected tissues of rabbits that had been fed a 1% cholesterol-enriched chow for 6 days. The rabbits received an intravenous injection of doubly labeled Lp(a) (n=5) or LDL (n=11) 24 hours before the tissues were removed and assayed for radioactivity content. Values are mean±SEM. *P*<.05, t test.**

**Figure 4. Cholesterol content in the intima–inner media of nonlesioned and atherosclerotic aortas. Lp(a) degradation (A) and LDL degradation (B) in rabbits are shown. Rabbits with atherosclerotic aortas had been fed a 0.25% to 1% cholesterol-enriched chow for ~6 months. Rabbits with nonlesioned aortas had been fed a 1% cholesterol-enriched chow for 1 week; the aortic cholesterol content in nonlesioned aorta was determined in another study that was performed under similar conditions.**

**Basic Characteristics of Rabbits Used for Studying Degradation Rates of Lp(a) and LDL**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Period of Cholesterol Feeding, d</th>
<th>Plasma Cholesterol, mmol/L</th>
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<td>LDL studies</td>
<td></td>
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<tr>
<td>Rabbits with nonlesioned aortas</td>
<td>11</td>
<td>6±0.1</td>
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<tr>
<td>Rabbits with atherosclerotic aortas</td>
<td>10</td>
<td>165±5</td>
</tr>
<tr>
<td>Lp(a) studies</td>
<td></td>
<td></td>
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<tr>
<td>Rabbits with nonlesioned aortas</td>
<td>5</td>
<td>6±0</td>
</tr>
<tr>
<td>Rabbits with atherosclerotic aortas</td>
<td>7</td>
<td>156±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Plasma cholesterol values are at the end of the experiment.
Different Degradation Rates of Lp(a) and LDL in Nonlesioned But Not in Atherosclerotic Aortic Intima–Inner Media

Fig 6 depicts the degradation rates of Lp(a) and LDL in the intima–inner media and in the outer media of nonlesioned and atherosclerotic aortas. ANOVAs were performed for nonlesioned and atherosclerotic aortas separately.

The degradation rates of Lp(a) were on average 39% of those of LDL in intima–inner media of nonlesioned aortas (t tests, P<.05 in segments 1 to 4) (Fig 6). Moreover, in nonlesioned aortas, the degradation rates of Lp(a) in the intima–inner media were smaller than the degradation rates of Lp(a) in the outer media in all six aortic segments (paired t tests, P<.05). This observation was opposite of the findings for LDL: the degradation rates of LDL were larger in the intima–inner media than in the outer media in aortic segments 1 to 5 (paired t tests, P<.05). The final ANOVA model to describe degradation rates of Lp(a) and LDL in nonlesioned aorta included lipoprotein type by aortic layer interaction (P<.0001), aortic site by aortic layer interaction (P<.0001), and aortic layer by lipoprotein type interaction (P=0.3). The aortic layer by lipoprotein type interaction was caused by a larger degradation rate of Lp(a) compared with LDL in the outer media but not in the intima–inner media.

Discussion

In this study, we quantified degradation rates of human Lp(a) and LDL in nonlesioned and atherosclerotic aortas of rabbits. For this purpose, Lp(a) or LDL were doubly labeled with both 1-T and 1-I and injected intravenously into conscious rabbits 24 hours before the aorta was removed. This dual isotope method provides a very sensitive means for detecting low rates of lipoprotein degradation in vivo and has previously been extensively validated.

Several major findings that provide novel insight into the metabolism of Lp(a) in the arterial wall were apparent from the present studies. One striking observation was that the rate of degradation of Lp(a) was markedly increased in atherosclerotic compared with nonlesioned aortic intima–inner media. Several lines of evidence support the extrapolation that this indicates that Lp(a) is degraded by foam cells in atherosclerotic lesions. First, it is well established that foam cells

![Figure 5](https://example.com/fig5.png)

**Figure 5.** Accumulation of undegraded Lp(a) (A) and LDL (B) and rates of degradation of Lp(a) (C) and LDL (D) in the intima–inner media of nonlesioned and atherosclerotic aortas. Each rabbit received an intravenous injection of Lp(a) (or LDL) that was doubly labeled with 1-T and 1-I 24 hours before the aorta was removed. The aorta was separated into six consecutive segments that were each divided into the intima–inner media and the outer media and assayed for radioactivity. Tho indicates thoracic aorta and Abd, abdominal aorta. Values are mean±SEM.

![Figure 6](https://example.com/fig6.png)

**Figure 6.** Rates of degradation of Lp(a) and LDL in aortic intima–inner media and outer media. Degradation rates of Lp(a) (A) and LDL (B) in nonlesioned aorta and of Lp(a) (C) and LDL (D) in atherosclerotic aorta are shown. Each rabbit received an intravenous injection of Lp(a) (or LDL) that was doubly labeled with 1-T and 1-I 24 hours before the aorta was removed. The aorta was separated into six consecutive segments that were each divided into the intima–inner media and the outer media and assayed for radioactivity. Tho indicates thoracic aorta and Abd, abdominal aorta. Values are mean±SEM.
cells constitute the most abundant cell type in aortic atherosclerotic lesions of cholesterol-fed rabbits. Second, we have previously shown that Lp(a) is taken up by foam cells in the aortic intima of cholesterol-fed rabbits 3 hours after an intravenous injection of human Lp(a). In that study, the major fraction of Lp(a) immunoreactivity in the intima was detected within macrophage-derived foam cells rather than within smooth muscle cells or between cells. Third, the increased rate of degradation of Lp(a) in atherosclerotic compared with nonlesioned aorta was almost exclusively due to an increase in degradation rates of Lp(a) in the intima-ininner media where foam cells are located, whereas the degradation rates of Lp(a) in outer media were similar in nonlesioned and atherosclerotic aortas. Finally, in the intima-ininner media of atherosclerotic aortas, the pronounced regional variation in Lp(a) degradation rates between aortic segments closely resembles the regional variation in cholesterol content (and therefore presumably foam cell abundance). Thus, several lines of indirect evidence all support the idea that the increased degradation rate of Lp(a) in atherosclerotic compared with nonlesioned aortas is secondary to uptake of Lp(a) by foam cells in the intima. In future studies, this hypothesis may be tested directly by isolating macrophages from atherosclerotic lesions to assess whether the TC label is mainly associated with these cells. Preferably, such studies should be done in humans.

A second major finding of the present study was that the degradation rate of Lp(a) in atherosclerotic aortic intima–inner media was as high as that of LDL. We have recently demonstrated that increased Lp(a) influx from plasma into the arterial intima, as well as decreased efflux of Lp(a) from the intima, results in specific accumulation of undegraded Lp(a) in atherosclerotic lesions. Such excess accumulation of undegraded Lp(a) in atherosclerotic lesions, as also confirmed in the present study, may cause increased proliferation of smooth muscle cells and deposition of fibrin in the intima. Both these effects can potentially accelerate the development of atherosclerotic lesions. The present study indicates that accumulation of Lp(a) in atherosclerotic lesions may have another important impact in developing atherosclerotic lesions. Because multiple lines of evidence have established that LDL can cause foam cell formation, the similarity in degradation rates of Lp(a) and LDL in atherosclerotic aortas suggests that Lp(a) has the potential to cause (or accelerate) foam cell formation in vivo. In comparing the atherogenic potentials of Lp(a) and LDL, it should be kept in mind that in most humans the plasma Lp(a) concentration of Lp(a) is lower than that of LDL. For instance, a typical individual might have a plasma Lp(a) concentration of 0.2 mg/mL and a plasma LDL cholesterol of 2.5 mmol/L (total LDL mass concentration = 2 mg/mL). If the degradation rates of Lp(a) and LDL (expressed as percent of plasma pool per gram tissue per day) are similar in atherosclerotic lesions of such an individual, the mass of Lp(a) degraded in the lesions would be 10 times lower than that of LDL.

It is possible that the uptake and degradation of Lp(a) by foam cells are mediated by receptors that recognize native Lp(a) particles. However, other pathways may also be important. Malondialdehyde modification of Lp(a) produces avid uptake of Lp(a) by monocyte-macrophages, and Lp(a), like LDL, is susceptible to oxidation by Cu. Since recent evidence indicates that the residence time of Lp(a) in the arterial intima is increased in atherosclerotic lesions compared with nonlesioned intima, it is conceivable that Lp(a) can undergo oxidative modifications that result in scavenger receptor–mediated uptake by foam cells in the lesion. Another possible scenario is that Lp(a) binds to the extracellular matrix in the arterial intima and that the complexes between Lp(a) and arterial wall matrix components are taken up by macrophages in the intima. The idea of a specific binding of Lp(a) to extracellular components in the arterial intima has gained in vivo support from the finding of a preferential accumulation of Lp(a) compared with LDL in balloon-injured rabbit aorta.

A third major finding of the present study was that the degradation rate of Lp(a) was only on average 39% of that of LDL in nonlesioned intima–inner media. This observation indicates that the metabolism of Lp(a) is quite different from that of LDL in normal arterial intima. It is conceivable that this smaller degradation rate of Lp(a) compared with LDL can be explained by the low efficiency of Lp(a) degradation via the LDL receptor. Carew et al showed that LDL degradation in nonlesioned arterial intima of the rabbit aorta predominantly is mediated by the LDL receptor. These authors also showed that the degradation rate of LDL was larger in the intima than in the media of the thoracic aorta. The latter finding is in accordance with the larger degradation rate of LDL in the intima–inner media compared with the outer media in the aortic arch and in the thoracic aorta in the present study. In striking contrast to this finding for LDL, the degradation rates of Lp(a) in nonlesioned aorta were larger in the outer media than in the intima–inner media. This observation suggests that the LDL receptor was of minor importance for Lp(a) degradation in nonlesioned aorta. The rabbits used to study lipoprotein degradation in nonlesioned aorta had been fed a cholesterol-enriched diet for 6 days before the injection of labeled lipoproteins. Cholesterol feeding leads to a reduction in the number of β-VLDL binding sites in the liver but not in the adrenals of rabbits. The degradation rates of LDL in nonlesioned aortic intima–inner media were threefold less in the presently studied cholesterol-fed rabbits than in the normcholesterolemic rabbits that we studied previously. This result may reflect that cholesterol feeding leads to reduced LDL receptor activity in the arterial intima of rabbits. Therefore, the difference in degradation rates between Lp(a) and LDL in nonlesioned aortic intima could be more pronounced in normcholesterolemic rabbits than in the cholesterol-fed rabbits that were used in the present study. The idea that Lp(a) degradation is mediated only to a small extent by the LDL receptor was also supported by the lower degradation rate of Lp(a) compared with LDL in the adrenals and in the intestine, simply because LDL catabolism in these tissues is highly dependent on LDL receptors. Since there are no or only very few macrophages present in the nonlesioned aorta of rabbits after only 6 days of cholesterol feeding, cell types in the arterial wall other than monocyte-macrophages (ie, endothelial cells and/or smooth muscle cells) seemingly are capable of degrading Lp(a) in...
vivo. Uptake and degradation of Lp(a) in nonlesioned aortas may have occurred by the low density receptor-related protein,^{44} plasminogen receptors,^{45,46} as yet unidentified receptor(s), and/or nonspecific mechanisms.

Some potential caveats regarding the direct comparison of Lp(a) and LDL degradation rates should be mentioned. Obviously, the interpretation of the present data is dependent on the integrity of the tracer molecules. In 1991, Knight et al^{47} found that after intravenous injection of *I-Lp(a), a small fraction of the radioactivity in plasma appeared in LDL-like particles. More recently, two comprehensive studies have demonstrated apo(a)–kringle 4 fragments in human urine and in urine of mice that had received an intravenous injection of human Lp(a).^{48,49} These findings suggest that a small fraction of Lp(a) might lose the NH₂-terminal portion of apo(a) after intravenous injection of labeled Lp(a) into animals. If labeled free apo(a) or labeled fragments of apo(a) were formed on intravenous injection of labeled Lp(a) and such particles remained in plasma to a large extent, they conceivably would enter the arterial wall directly from plasma and then be taken up by arterial wall monocyte-macrophages.^{9,11} This occurrence could lead to an overestimation of the degradation rate for the lipid/apoB moiety of Lp(a). However, we suspect that the presence of labeled free apo(a) or fragments of apo(a) in plasma was minimal because two-tier rocket immunoelectrophoresis revealed that less than 1% of *I-TC in plasma was in particles that escaped precipitation in the anti-apoB antiserum–containing agarose gel. This finding is in accordance with previous results.^{22,23,47} Even if labeled free apo(a) or fragments of apo(a) were formed by dissociation from the Lp(a) particle within the arterial wall, density gradient ultracentrifugation studies revealed that, on average, only 22% of the total radioactivity in TC-labeled Lp(a) was in apo(a). Thus, we anticipate that the contribution of labeled free apo(a) to the measured overall degradation rate of Lp(a) was minor.

After intravenous injection of labeled Lp(a), a fraction of the labeled particles lost ability to precipitate in anti-Lp(a) antiserum–containing agarose gel but did precipitate in anti-Lp(a) antiserum containing agarose gel. This finding is in accordance with previous results.^{22,23,47} Even if labeled free apo(a) or fragments of apo(a) were formed by dissociation from the Lp(a) particle within the arterial wall, density gradient ultracentrifugation studies revealed that, on average, only 22% of the total radioactivity in TC-labeled Lp(a) was in apo(a). Thus, we anticipate that the contribution of labeled free apo(a) to the measured overall degradation rate of Lp(a) was minor.

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References


Increased Degradation of Lipoprotein(a) in Atherosclerotic Compared With Nonlesioned Aortic Intima—Inner Media of Rabbits: In Vivo Evidence That Lipoprotein(a) May Contribute to Foam Cell Formation

Lars B. Nielsen, Klaus Juul and Børge G. Nordestgaard

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