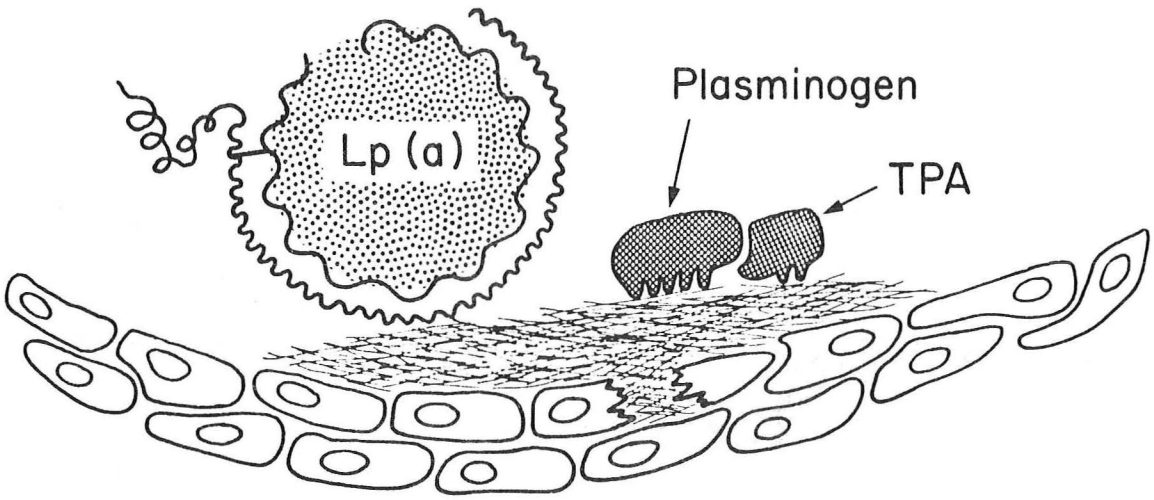
Endo.

Lp (a)
An Atherogenic Lipoprotein



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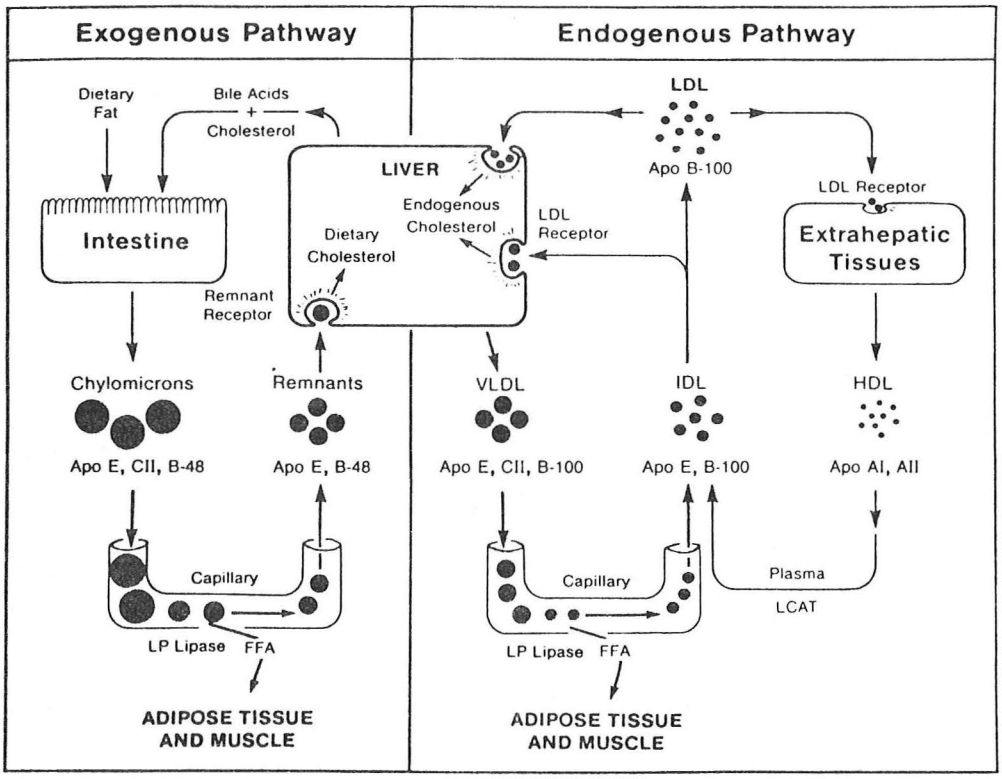
For the last several decades investigations have been directed at defining the mechanisms by which lipoproteins contribute to the development of atherosclerosis. The pathways by which different lipoprotein species are synthesized and catabolized have been defined and approaches to the diagnosis and treatment of hypercholesterolemia have been developed (1,2). The "classic" pathways of lipoprotein transport on which these models are based are shown in figure 1. Today the lipoprotein story seems to be the standard "good guy, bad guy" routine. Low density lipoprotein is the villain. Genetic and environmental factors that result in higher circulating levels of LDL result in the more rapid progression of atherosclerosis. In contrast, HDL is Wyatt Earp, wearing a white hat and offering some degree of protection against premature coronary disease.

Figure 1. Classic Pathways of Lipoprotein Metabolism



from Brown, Kovanen, and Goldstein, 1981.

However, this is not the whole story. Other factors are clearly involved in the development of atherosclerosis. Today I will discuss another lipoprotein species, known as Lp(a) or lipoprotein (a), that is another dastardly villain. Lp(a) is an independent risk factor in the development of vascular disease. Structural similarities exist between Lp(a) and LDL; however, the metabolism and functions of these two lipoproteins are quite different. Lp(a) is not derived from any of the lipoprotein pathways shown below. The circulating level of Lp(a) is quite variable and is primarily determined by genetic factors. Probably most interesting are recent studies that have defined the structure of apo(a), a unique protein associated with the

Lp(a) particle. This protein is a large protein composed of a repeating unit that is found in many proteins involved in clotting and fibrinolysis. It is most similar to plasminogen, a protease that helps dissolve clots in arteries. Such information suggests that Lp(a) might exert its deleterious effect either through deposition of cholesterol at the site of vascular injury or inhibition of the normal process of fibrinolysis that would dissolve a thrombus.

Early Studies of Lp(a)

In 1963, Berg first described the Lp(a) system. His initial studies were directed at finding a antigenic marker of LDL that could be used to study patients. Animals were immunized with LDL isolated from one individual and the antiserum was then absorbed with LDL from another individual. This approach identified an antigenic determinant that he at first thought was associated with LDL. The Lp(a) determinant appeared to be inherited in an autosomal dominant fashion. Individuals were typed with this antiserum as either Lp(a+) or Lp(a-) based on the presence or absence of a reaction by immunoelectrophoresis. He postulated that two alleles existed at the Lp(a) locus - the Lp^a and Lp^o alleles - encoding either a positive or null allele, respectively (3).

Table I. Lp(a) alleles.

Phenotype	*Possible Genotypes*
Lp(a+)	Lp^a/Lp^a or Lp^a/Lp^o
Lp(a-)	Lp^o/Lp^o

Frequency of gene Lp^o = .81

Frequency of gene Lp^a = .19

from Berg, 1963

Family studies showed that the Lp(a) phenotype was inherited in a manner consistent with this model. Some children of matings in which one or both of the parents were Lp(a+) might be Lp(a-), having inherited the silent null allele from each of Lp(a+) parents; however, all of the children of matings in which both parents were Lp(a-) were also Lp(a-).

Table II. Lp(a) Phenotypes in Families

Parents Matings		*Children* no.	Lp(a+)	Lp(a-)
Lp(a+) x Lp(a+)	4	13	9	4
Lp(a+) x Lp(a-)	9	27	19	8
Lp(a-) x Lp(a-)	10	25	0	25

from Berg, 1963.

Table III. Association of Lp(a) antigen and pre-β_1-lipoprotein.

	Number of individuals		
	Lp(a+)	Lp(a-)	Total
Healthy males			
Pre-β_1-lipoprotein present	17	2	19
Pre-β_1-lipoprotein absent	5	51	56
Healthy females			
Pre-β_1-lipoprotein present	9	7	16
Pre-β_1-lipoprotein absent	0	35	35
CHD patients			
Pre-β_1-lipoprotein present	16	1	17
Pre-β_1-lipoprotein absent	11	18	29

from Dahlen, et al, 1974 and Berg, et al, 1974.

Further studies have shown that the Lp(a) determinant is not a property of the LDL particle but actually represents a lipoprotein particle distinct from LDL in the plasma.
Lp(a) is the same lipoprotein that was studied by Dahlen as pre-β_1-lipoprotein because of its migration pattern on agarose gel electrophoresis and paper electrophoresis (4).
Other investigators have referred to this particle as sinking pre-β-lipoprotein due to its heavier density (5). When both Lp(a) and pre-β_1-lipoprotein were studied in the same individuals a strong association was found between Lp(a+) and the presence of pre-β_1-lipoprotein. This association was observed both in young healthy adults as well as patients with coronary heart disease (4-8).

Both the immunoassay for Lp(a) and electrophoresis to assess pre-β_1-lipoprotein recognize the same particle, the Lp(a) determination perhaps being slightly more sensitive than the electrophoretic assay. More recent studies have used sensitive immunoassays to detect Lp(a) and have shown that Lp(a) is detectable in essentially all subjects with a skewed distribution (9). The distinction made by Berg between Lp(a+) and Lp(a-) distinguishes those individuals with high concentrations of Lp(a) from those with lower levels of the lipoprotein. Thus the simple genetic analysis suggested by Berg with either a Lp^a or Lp^o allele is incorrect; however, quantitative variation in the Lp(a) level seems to be a property of a single autosomal locus (10).

Purification of the Lp(a) particle has shown that this lipoprotein is similar in size and lipid composition to LDL (11-14). Lipid constitutes approximately two-thirds of the mass of the Lp(a) partcle and cholesterol ester accounts for almost half of the lipid. This is similar to LDL where lipid is about three-fourths of the mass and cholesterol ester is also the major lipid component. It has a greater molecular weight primarily related to an increased amount of protein associated with each particle. Associated with the protein is a significantly greater amount of carbohydrate than that present in LDL. The density of Lp(a) is greater than that of LDL, accounting for its "sinking" during ultracentrifugation.

The plasma concentration of Lp(a) is determined independently of circulating levels of LDL. The amount of Lp(a) particles in the circulation is substantially less than the amount of LDL particles.

Table IV. Comparison of Lp(a) and LDL particles

	Lp(a)	LDL
Chemical Composition	%	%
Lipoprotein		
Protein	26	22
Sugar	8	2
Lipid	66	76
Lipid		
Phospholipid	30	31
Free Cholesterol	17	15
Cholesterol Ester	48	46
Triglyceride	5	8
Physicochemical Properties		
Density	1.085	1.033
Electrophoretic Migration	pre-β1	β
Isoelectric Point	4.5	5.5
Diameter	25 nm	21 nm
MW ($x10^6$)	5.5	2.4

from Kostner, 1976.

Association of Lp(a) with coronary heart disease

Several studies in the early 1970's suggested that high levels of Lp(a) were associated with coronary heart disease. Both Berg and Dahlen (Table V) found that Lp(a+) or the presence of pre-β_1-lipoprotein was more common in patients with suspected CHD in the Scandinavian population (6-8). Studies in other populations have supported the association of Lp(a) with vascular disease. Murai studied a Japanese population and found that levels of Lp(a) >17 mg/dl were significantly associated with either coronary heart disease or cerebral infarction in the distribution of the cortical artery (15). In an American population, significantly higher levels of Lp(a) were found in individuals that had suffered an MI when they were less than 50 years of age than those subjects who had their MI in the 6th and 7th decades (9).

Rhoads has studied a population of Hawaiian men of Japanese ancestry with a history of a prior myocardial infarction (16). A substantial increase in the odds for having had an MI was apparent in those subjects in the upper quartile of Lp(a) levels. When the risks for individuals in the top quartile of Lp(a) were examined, the odds ratio for history of an MI was 2.54 in individuals under the age of 60. They estimate that 28% of the MI's in this age group are accounted for by the excess risk

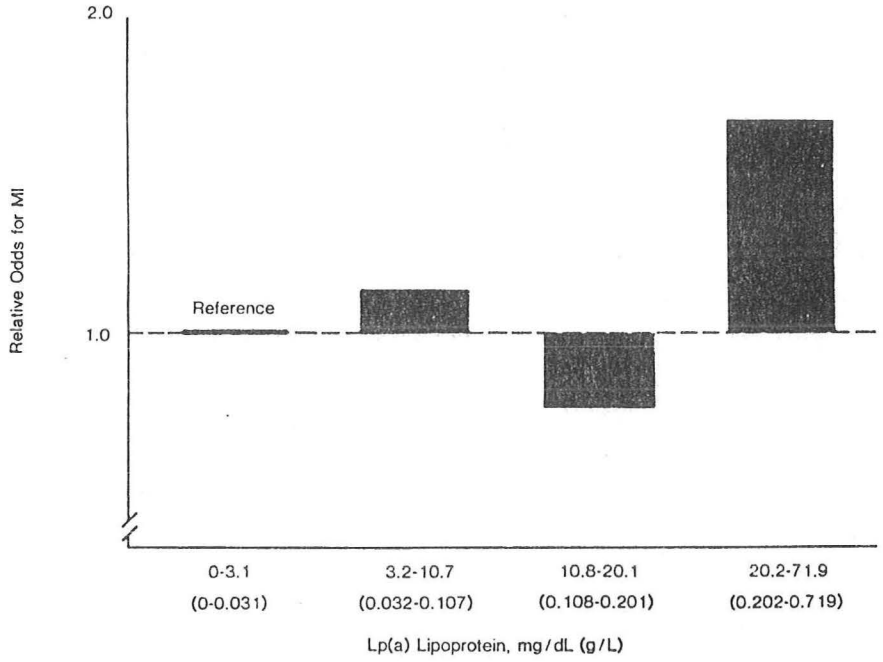
Table V. Distribution of Lp(a) phenotypes in different Scandinavian populations.

Population Sample	Lp(a+) %	Lp(a-) %
Swedish males	29	71
Healthy Finns	31	69
Swedish CHD patients	41	59
Finnish CHD patients	55	45

From Dahlen, et al, 1975.

associated with high levels of Lp(a). The association of a history of MI with Lp(a) levels could not be explained by HDL, LDL, high blood pressure, history of smoking or age. Lp(a) was as important as HDL in assessing the relative risk for an MI.

Figure 2. Relative Odds for Myocardial Infarction by Quartile of Lp(a) level.



from Rhoads, et al, 1986.

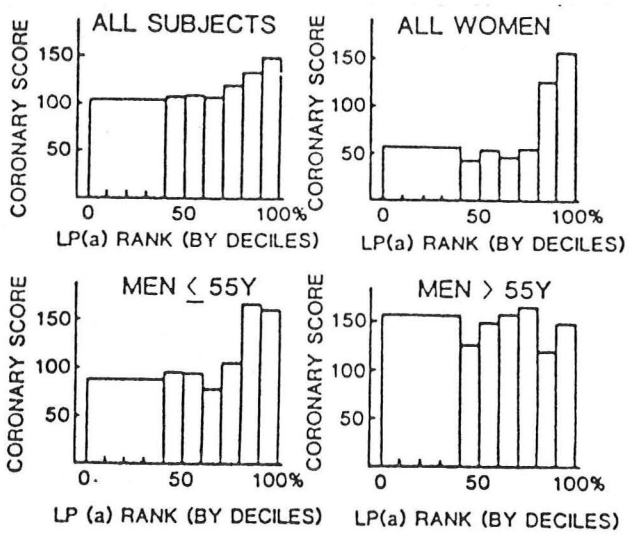
Table VI. Odds Ratio for History of Myocardial Infarction and Population-Attributable Risk for men in Top Quartile of Lp(a).

Age at Examination	Odds Ratio	Population-Attributable Risk, %
<60	2.54	28
60-69	1.57	13
>70	1.22	5
All	1.65	14

from Rhoads, et al, 1986.

Lp(a) is also linked to the severity of the coronary disease. In 307 patients who underwent coronary angiography, the relationship of coronary artery disease to plasma levels of Lp(a) was examined. As shown in figure 3, especially among women and men under the age of 55, levels of Lp(a) in the top quartile were associated with more severe coronary disease (17). Once again Lp(a) was an independent risk factor with a weight comparable to total cholesterol and HDL cholesterol.

Figure 3. Mean Coronary Lesion Scores within deciles of patient population ranked by Lp(a) levels.



from Dahlen, et al, 1986.

Does Lp(a) act alone or can high levels of LDL promote the atherosclerosis associated with high levels of Lp(a)? In patients who underwent coronary angiography Frick found a higher proportion of Lp(a+) patients in the groups with coronary disease (18). In those patients with the most severe coronary disease, 20 out of 58 patients also had hypercholesterolemia (type IIa or IIb). This was substantially greater than the groups with no CAD or moderate disease.

Table VII. Lp(a) and Lipoprotein phenotypes in patients undergoing coronary angiography.

			Lipoprotein Type					
CAD	n	Lp(a+)	N	IIa	IIb	III	IV	V
None	45	11,24%	30	3	2	0	8	2
Moderate	50	21,42%	31	3	4	0	11	1
Severe	58	33,57%	21	10	10	0	15	2

from Frick, et al, 1978.

In another study, high levels of Lp(a) in the presence of hypercholesterolemia were more frequently associated with a history of myocardial infarction than in normolipemic patients (19). Both of these studies suggest that the coexistence of high levels of both LDL and Lp(a) can promote more rapid atherosclerosis.

Table VIII. Distribution of Lp(a) levels in control and post-MI patients.

	Lp(a) concentration		
	<25 mg/dl %	25-50 mg/dl %	>50 mg/dl %
Normolipemic			
Control	69	20	11
MI	53	22	25
Type IIa			
Control	53	47	0
MI	44	37	19

from Kostner, et al, 1981.

The influence of factors such as lipid levels, smoking and hypertension on coronary artery disease associated with high levels of Lp(a) was directly addressed by Armstrong and his associates (20). They found an odds ratio of 2.7 for the presence of coronary artery disease between elevated Lp(a) levels (>30 mg/dl) versus low levels of Lp(a) (<5 mg/dl). This odds ratio was not influenced by triglycerides, HDL-cholesterol, smoking, hypertension or diabetes. However, when the LDL-cholesterol level was greater than the median value, the odds-ratio increased to 6. These data show that high levels of LDL markedly increase the risk of atherosclerosis associated with elevated levels of Lp(a).

Table IX. Influence of other variables on the association between Lp(a) and CAD as estimated by the odds ratio for high and low Lp(a) levels.

	Odds ratio	
	≤ median	> median
Total cholesterol	1.56	4.50
Total triglycerides	2.89	2.66
LDL-cholesterol	1.67	6.00
HDL-cholesterol	2.96	2.72
	Yes	No
Smoker	2.24	2.20
Hypertension	2.59	2.16
Diabetes	_	2.14

from Armstrong, et al, 1986.

All of these studies have examined the association of Lp(a) with preexisting disease - either history of a myocardial infarction or coronary artery disease assessed by coronary angiography. The progression of coronary disease was studied in a prospective manner in a group of patients under the age of 45 that had already suffered one myocardial infarction (21). Patients that had an uneventful course for the next three years were compared to those that had at least one reinfarction. The primary difference noted was a decrease in the fibrinolytic capacity due to increased plasma levels of plasminogen activator inhibitor; however a substantial difference was apparent in the Lp(a) levels of the two groups.

Table X. Risk factors for recurrent myocardial infarction.

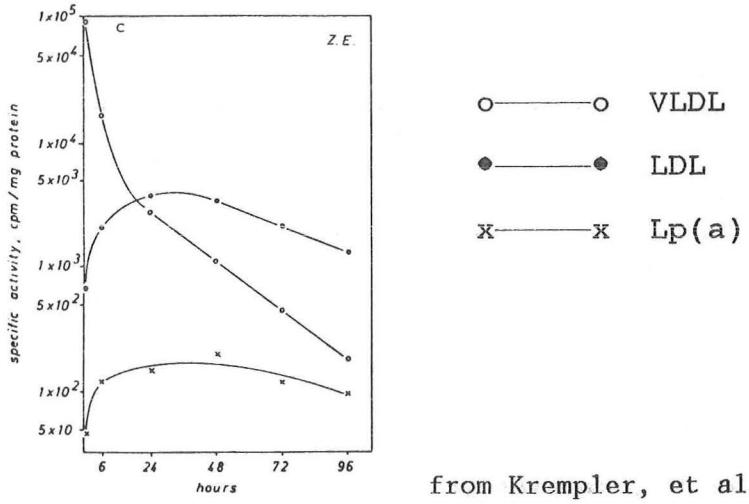
	Uneventful Course n=81	Reinfarction n=16	p
Plasminogen Activator Activity (PU)	0.32	0.08	<0.001
Plasminogen Activator Inhibitor (AU/ml)	20.50	33.00	<0.01
VLDL Cholesterol (mmol/l)	1.09	1.61	<0.05
LDL Cholesterol (mmol/l)	4.79	4.97	NS
HDL Cholesterol (mmol/l)	1.14	0.99	<0.05
Lp(a) (mg/dl)	28.30	56.90	<0.01

from Hamsten, et al, 1987.

Metabolism of Lp(a)

Lp(a) levels are not correlated with LDL levels. Metabolic turnover studies also show that these two lipoproteins are independently regulated. When turnover studies are performed in which radioactively labelled Lp(a) is injected into the circulation, essentially all the radioactivity remains with the Lp(a) particle (22). No conversion to other lipoprotein fractions is observed.

Figure 4. Apo-B Specific Activity Following the injection of labeled VLDL.



from Krempler, et al, 1979.

Following the injection of radioactive VLDL, LDL is produced in a product-precursor relationship whereas only trace amounts of radioactivity appear in the Lp(a) fraction. This was accounted for by low level contamination with LDL (23). These experiments show that conversion of LDL to Lp(a) within the circulation does not occur.

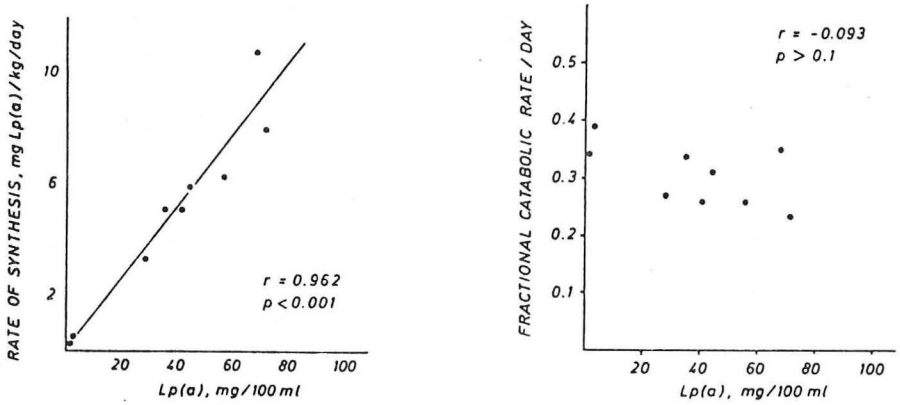
Krempler evaluated the turnover of labelled Lp(a) in eight individuals (24). The biologic half-life of Lp(a) was 3.32 days with a fractional catabolic rate of .306 pool/day. A correlation was found between the synthetic rate for Lp(a) and the serum concentration. No relationship was observed between the FCR and the serum level of Lp(a).

Table XI. Kinetic parameters of Lp(a) turnover.

Subject no.	Serum Lp(a)	FCR	Synthetic Rate
	mg/dl	pool/d	mg/kg/d
1	<1	0.343	0.16
2	3	0.392	0.51
3	44	0.308	5.89
4	41	0.258	5.03
5	71	0.236	7.97
6	35	0.336	5.11
7	28	0.272	3.31
8	68	0.350	10.75
9	56	0.257	6.26
Mean		0.306	5.00
SD		0.054	3.37

from Krempler, et al, 1980.

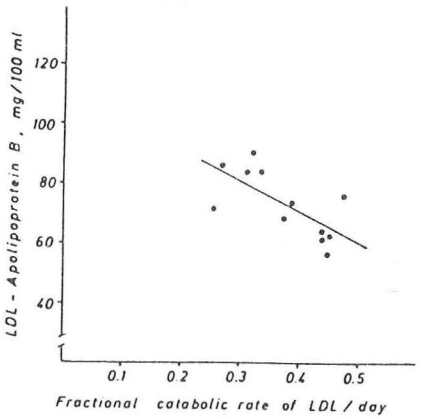
Figure 5. Relationship between serum Lp(a) concentration and rate of synthesis of Lp(a) (left) and FCR of Lp(a) (right).



from Krempler, et al, 1980.

Further studies by this group compared the turnover of LDL and Lp(a) in twelve normolipemic individuals (25). In each instance the fractional catabolic rate for Lp(a) was lower than that of LDL with a mean difference of 31%. As in the previous study, serum Lp(a) levels were correlated with the synthetic rate of Lp(a) and not the FCR, whereas the levels of LDL-Apoprotein B were correlated with the FCR of LDL.

Figure 6. Relationship between FCR of LDL and the concentration of LDL-apoprotein B in the serum of normolipemic subjects.



from Krempler, et al, 1983.

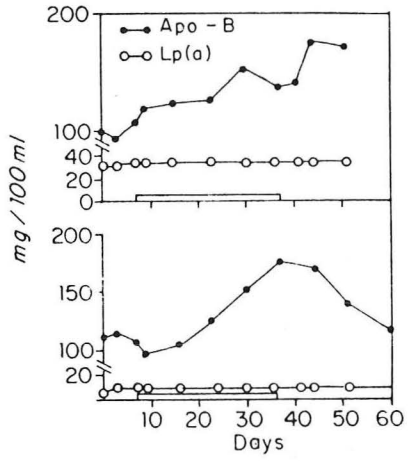
One patient with homozygous familial hypercholesterolemia was also studied by these techniques (25). Although this patient had a serum cholesterol in excess of 500 mg/dl, serum Lp(a) was only 17 mg/dl. In this patient the FCR of LDL was .205 pool/d compared to a mean of .377 pool/d in the normolipemic population. The synthetic rate of LDL was also increased two-fold with respect to normolipemic subjects. In contrast to the clearance of LDL, the FCR of Lp(a) was only slightly lower than the normals and was still within the standard deviation of the normal.

Interaction with the LDL receptor has been studied in direct binding studies. Lp(a) appears to bind poorly, if at all, to the LDL receptor of human fibroblasts. Some investigators have found low affinity binding (25-27) whereas others have found no specific binding to the LDL receptor (28). Thus, the LDL receptor pathway does not appear to be the primary route of clearance of Lp(a) from the circulation.

These studies show that the mechanisms that control the concentrations of Lp(a) and LDL are different. Whereas the clearance of LDL via the LDL receptor pathway is a major factor in controlling the level of serum LDL, Lp(a) concentrations are primarily regulated at the level of synthesis. The rate of clearance is not a major factor in regulating the amount of Lp(a). These observations may be important in understanding how different drug and dietary manipulations affect both LDL and Lp(a) levels. In some individuals cholesterol feeding results in increases in the plasma LDL level. This rise in LDL is mediated by suppression of hepatic LDL receptors that results in a delayed clearance of LDL. In contrast to LDL levels, serum Lp(a) levels

did not change during cholesterol feeding at a time when apolipoprotein B levels rose almost two-fold (5,9).

Figure 7. Changes in apolipoprotein B(•) and Lp(a)(o) during dietary supplementation (open bar) with 36 g cholesterol/day.



from Albers, et al, 1977.

Drugs that lower LDL levels by regulating LDL receptor activity don't significantly affect serum Lp(a) levels. Treatment with the bile-acid binding resin colestyramine did not change Lp(a) levels (29). In contrast neomycin alone or in combination with niacin lowered Lp(a) levels 24% and 45%, respectively (30). These changes were comparable to the changes observed in LDL levels with these drugs. Presumably these drugs do not lower these lipoproteins through effects on catabolism but perhaps effect a step common to the production of both lipoproteins.

Table XII. Reduction in Lp(a) and LDL levels with drug therapy.

	Diet Only	Neomycin Only	Neomycin +Niacin
Total Cholesterol (mg/dl)	352	286	212
VLDL-C (mg/dl)	33	34	26
LDL-C (mg/dl)	271	209	141
HDL-C (mg/dl)	49	41	58
Lp(a) (mg/dl)	18.2	13.8	10.0

from Gurakar, et al, 1985.

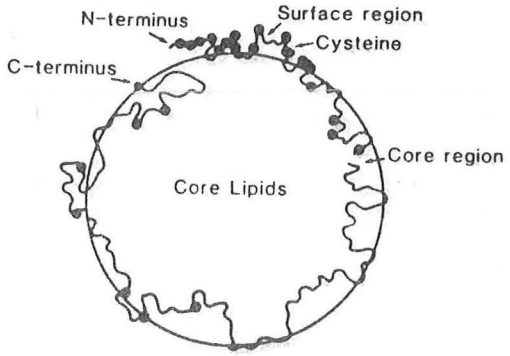
The anabolic steroid stanozolol has been shown to reduce

Lp(a) approximately 65% in ten normolipemic postmenopausal women (31). The effectiveness of such therapy in men and other women has not been investigated. The mechanism of this effect is unknown.

The Apoproteins of Lp(a)

The main difference between Lp(a) and LDL resides in the protein composition of these particles. Early studies in the 1970's showed that Lp(a) contained five to six times more protein-bound sialic acid than did LDL (11-13). More recent studies have dissected the protein structure of Lp(a) and LDL particles more precisely. The only apoprotein associated with LDL is called apoprotein B-100. This protein is quite large. It contains 4536 amino acids and is 514,000 daltons in molecular weight (32-34). Apo-B associates with the lipid core through hydrophobic regions located throughout the protein. The amino terminal end of the protein is probably a globular region not tightly associated with the lipid core of the lipoprotein. Most of the cysteines within the protein are found in this part of the molecule. A single region between amino acids 3100 and 3400 is probably the other binding site to the LDL receptor.

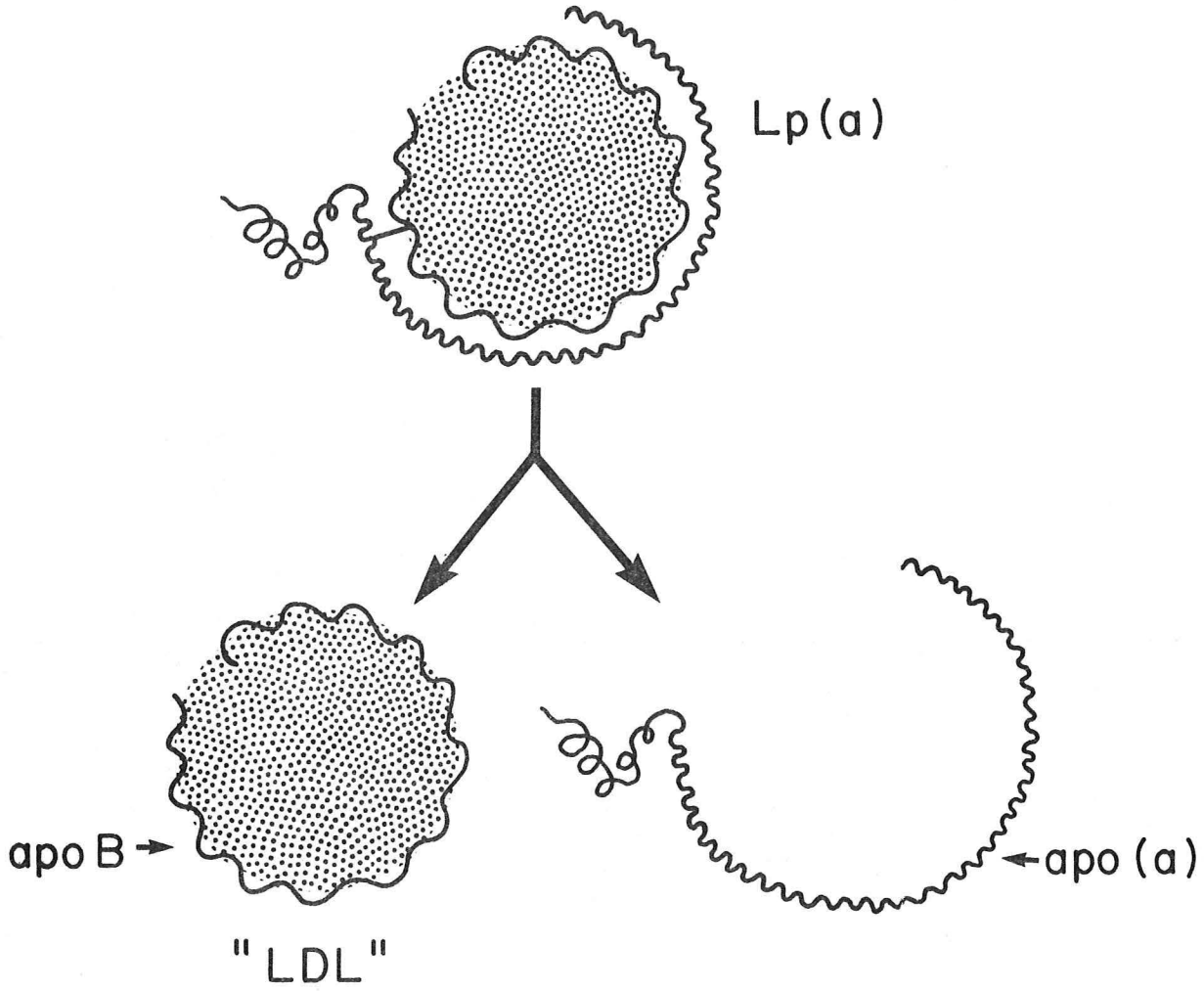
Figure 8. Structural Model of Apoprotein B-100 associated with LDL.



from Yang, et al, 1986.

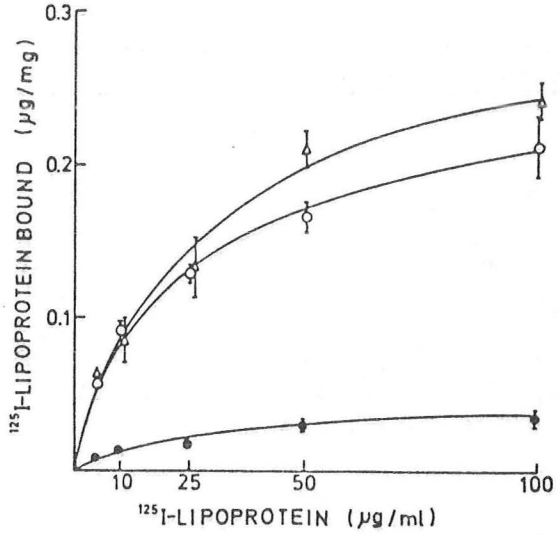
In the Lp(a) particle a large molecular weight protein has been found that is greater than 1,000,000 daltons. However, this large complex can be dissociated into two different proteins by reducing agents(35-40). One of these proteins is identical in size to apoprotein B-100 and is recognized by antibodies against apoprotein B-100. The other protein is larger than apoprotein B-100 and is stained by dyes specific for carbohydrate. This protein, known as apolipoprotein (a), is recognized by the antibody specific for Lp(a). When the Lp(a) particle is treated with a reducing agent in solution, a free protein species is released as well as a lighter lipoprotein product. The free protein is apo(a); whereas the lipoprotein particle is quite similar to native LDL.

Figure 9. Structure of Lp(a) and effect of reducing agents.



The behavior of native Lp(a) and the lipoprotein derived from Lp(a) after reduction have been compared in binding studies to human fibroblasts (40). The native Lp(a) particle binds with a low affinity to the LDL receptor on fibroblasts. In contrast, the lipoprotein particle recovered after reduction is bound with high affinity by the LDL receptors. Thus the Lp(a) particle consists of a "LDL"-like core containing apoB-100 and a lipid core that is linked to apolipoprotein(a) by one or more disulfide linkages. The apolipoprotein(a) component masks the binding site to the LDL receptor normally present on the apoB-100 molecule. After dissociating the apo(a) from the Lp(a) particle, the remaining lipoprotein particle is essentially indistinguishable from normal LDL and binds normally to the LDL receptor.

Figure 10. Binding studies to fibroblasts with Lp(a) and reduced Lp(a).



from Armstrong, Walli and Seidel, 1985

The Lp(a) particle is quite heterogeneous between individuals. Using single-spin density gradient centrifugation Fless found that Lp(a) exhibited both inter- and intraindividual density heterogeneity (37). Three different size apo(a) species were observed in different subjects, the apo(a) either being smaller, equal to or greater than apoB-100. Recently, Utermann has characterized the variation in size and concentration of Lp(a) in several individuals and families (41). Apo(a) molecules were observed that migrated faster than apoB-100, designated F; the same as apoB-100, designated B; or slower than apoB-100, designated S1 through S4. The differences in the observed molecular weights was not related to differences in the glycoprotein structure. Individuals were observed who either had a single apo(a) band or a double band.

Figure 11. Different Lp(a) glycoprotein phenotypes detected by immunoblotting.

Lane 1, F-type; lanes 2-3, B-type; lanes 4,5, and 7, S1-type; lane 6, S1/S2-type; lane 8, S2-type; lane 9, S3-type; lane 10, S4-type. Arrows indicate the relative position of apoB-100.

from Utermann, et al, 1987.

The Lp(a) concentration was correlated with the phenotype observed by immunoblotting. The smaller, faster migrating forms of apo(a) were associated with higher concentrations of Lp(a). The larger variety were associated with lower concentration of Lp(a).

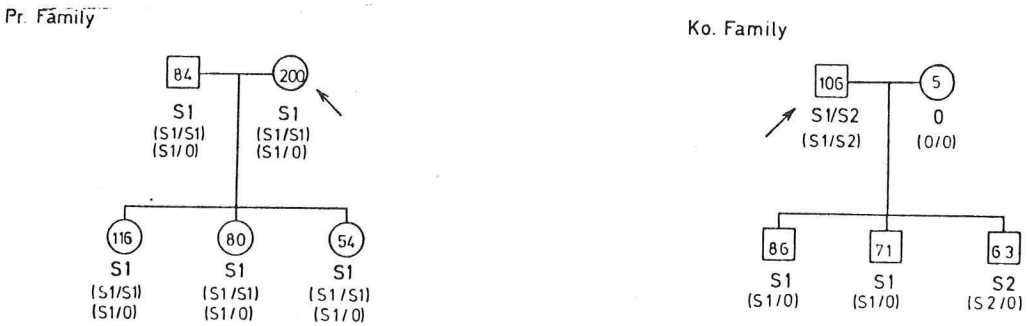
Table XIII. Lp(a) Concentrations in Individuals with Different Single-Band Lp(a) Phenotypes.

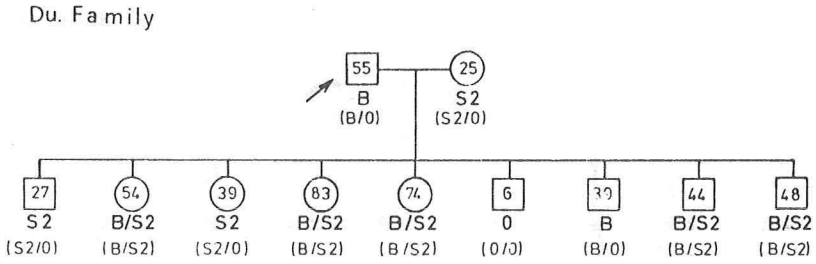
Lp(a) Phenotype	No.	Mean	SD	Median	Range
B	4	28.0	13.4	31.0	10-40
S1	18	16.3	14.1	13.5	0-46
S2	33	15.5	9.8	12.0	0-44
S3	33	8.2	4.2	8.0	0-22
S4	25	8.0	5.4	8.0	0-28

from Utermann, et al, 1987.

Studying families by Lp(a) phenotyping showed that the pattern of immunoreactivity was inherited in an autosomal codominant fashion. These studies show that the size heterogeneity as well as variations in the plasma level of Lp(a) are inherited as a result of a single autosomal locus. The size heterogeneity appears to be related to variations in the protein structure for apo(a). Variations in the plasma level of Lp(a) are related to the structure of the apo(a) allele and suggest that these different alleles lead to differing rates of synthesis of the Lp(a) particle.

Figure 12. Pedigrees of Three Families. Lp(a) phenotypes and possible genotypes are listed below each individual. Plasma concentrations(mg/dl) of Lp(a) are indicated within the symbols.



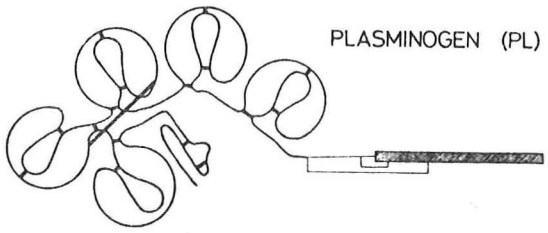


from Utermann, et al, 1987.

The structure of the apolipoprotein(a) has just been determined by Angelo Scanu and Gunther Fless at the University of Chicago together with Richard Lawn and colleagues at Genentech with surprising results. At first the amino acid sequence of several peptides derived from apo(a) were determined and found to be almost identical to sequences in plasminogen, a protease involved in the degradation of fibrin (42). The complete structure of apolipoprotein(a) has now been derived by cloning a full-length cDNA for the molecule from the liver of an Lp(a) positive subject and the homology with plasminogen is striking (43).

The structure of plasminogen is shown in figure 13. This protease precursor is 791 amino acids in length and contains several functional domains (44). At the amino-terminal end of the molecule is a short segment known as the "tail" that is released by proteolysis to expose regions that bind to fibrin with high affinity. These binding sites are located within the **kringle** structures that are present five times in the plasminogen molecule. The kringle domains are triple-loop structures that contain 3 internal disulfide bridges. They are found in many different proteins involved in different aspects of coagulation. These proteins include prothrombin, plasminogen, tissue plasminogen activator (tPA) and urokinase (45). They are 80-114 amino acids in length. In plasminogen, kringles 1 and 4 are responsible for its high affinity interaction with fibrin (46). At the carboxy-terminal end of the molecule is the protease domain. This region shares homology with serine proteases such as trypsin and can be activated by cleavage at a specific arginine residue.

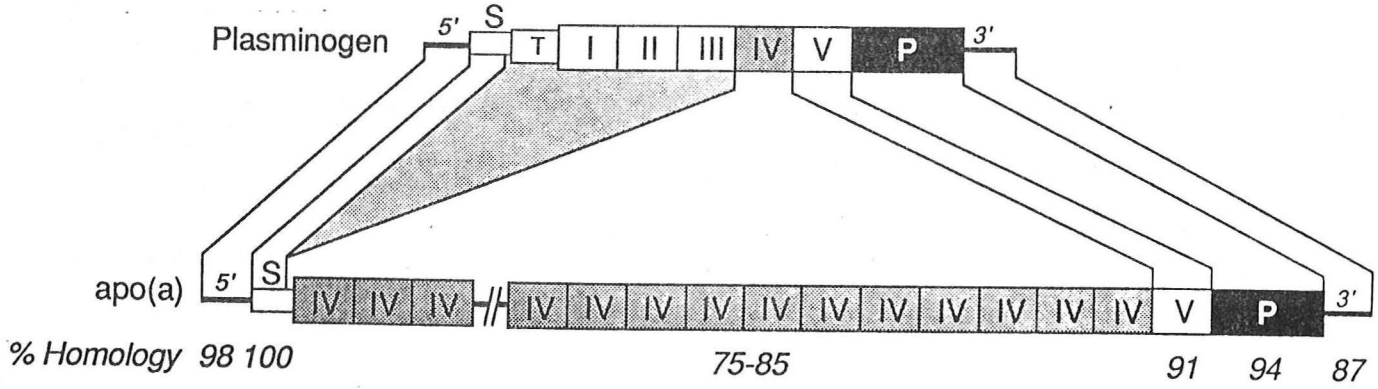
Figure 13. Structural Model for Plasminogen.



from Patthy, 1985.

Although apolipoprotein(a) is much larger than plasminogen, itis quite similar in structure with a large degree of internal replication (43). At the amino-terminal end the apo(a) protein has a signal sequence that is identical to that present in plasminogen. The signal sequence is cleaved from these proteins asthey are secreted into the endoplasmic reticulum within the cell. Apolipoprotein(a) lacks the tail region and kringles 1 through 3 found in plasminogen, but begins with a sequence highly homologous to the fourth kringle of plasminogen. This kringle structure that is homologous to the fourth kringle of plasminogen is repeated thirty-seven times in apolipoprotein(a), thus accounting for the larger size of apo(a). A single copy of a kringle homologous to the fifth kringle of plasminogen is next followed by a region homologous to the protease domain of plasminogen. This protease is probably inactive as the arginine residue in plasminogen where proteolysis activates the protease is substituted by a serine in apolipoprotein(a).

Figure 14. Comparison of the Structures of Plasminogen and Apolipoprotein(a).



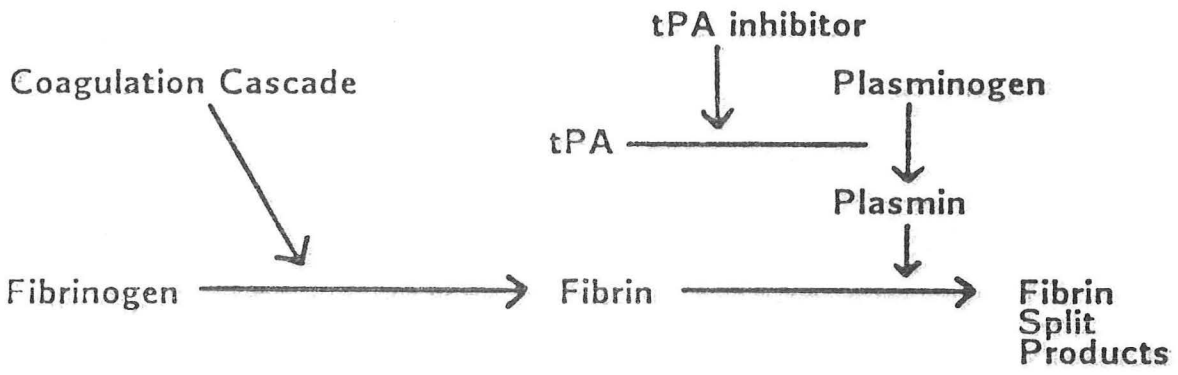
from McLean, et al, 1987.

How Does Lp(a) Lead to Atherosclerosis

The structure of Lp(a) in which an large protein stikingly similar to plasminogen is linked to apoB-100 via disulfide bridges suggests several interesting possibilities regarding the mechanism by which the Lp(a) particle can predispose to the development of atherosclerosis. The repeated kringle structures of apolipoprotein(a) should allow the Lp(a) to bind to fibrin just as molecules such as plasminogen are able to bind to fibrin. Although the fourth kringle in plasminogen is not the highest affinity binding site to fibrin, the presence of multiple copies of this structure in apo(a) should promote its association with fibrin. The Lp(a) particle can bind to lysine-Sepharose and be eluted with ε-aminocaproic acid, binding behavior that mimics the association with fibrin *in vivo* (43).

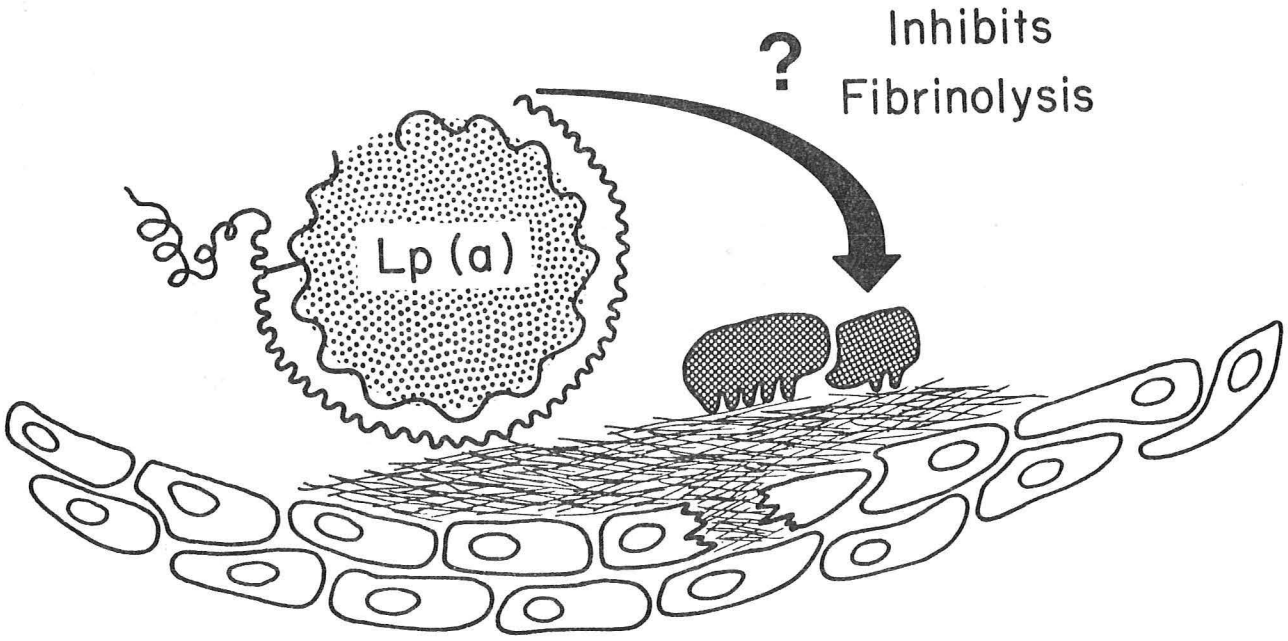
Consider the scenario in which the wall of a blood vessel has just been injured. A wrench in the endothelium triggers the coagulation cascade and ultimately fibrinogen is converted to fibrin. A fibrin clot is assembled at the site of the injury to protect against further injury and aid in healing. However, in order to prevent progressive thrombosis of the artery the fibrinolytic system is activated to dissolve the clot. The activation of the fibrinolytic pathway is brought about by the conversion of plasminogen to plasmin (47). This activation is strongly dependent on the interaction of plasminogen with tissue plasminogen activator, or tPA, and requires the presence of fibrin (48). In solution plasminogen activation is slowly catalyzed by tPA. However, when both tPA and plasminogen are bound to fibrin, plasmin is efficiently produced (48).

Figure 15. The Fibrinolytic System



The presence of the kringle structures on Lp(a) and the ability of this lipoprotein to bind fibrin, also allow it to be localized to the fibrin mesh. *In situ* studies of atherosclerotic plaques have shown that Lp(a) and apoB can be detected in the lesions. Based on the protein structure of the Lp(a) molecule, both of these antigenic determinants could have been derived from the Lp(a) particle (49).

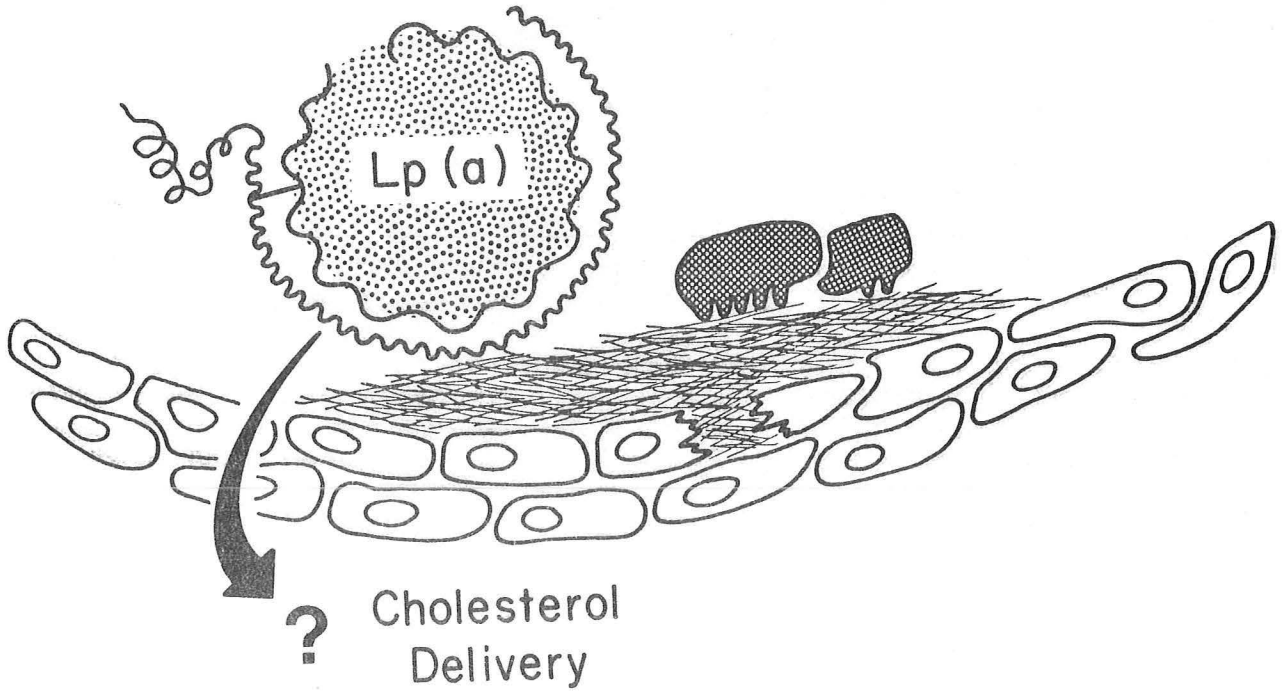
Figure 15.



Lp(a), plasminogen and tPA are all preferentially concentrated at the site of vascular injury by their specific association with fibrin. What are the potential consequences of the presence of Lp(a) at this site that might account for its atherosclerosis promoting effects? On the one hand, Lp(a) might inhibit the fibrinolytic process. This would prevent the dissolution of the clot and lead to thrombosis of the vessel. Lp(a) might compete for the binding sites available on fibrin, if there number is limited, and decrease the amount of plasminogen and tPA bound to fibrin. Alternatively, the apo(a) moiety has a protease domain that is almost identical to the protease domain of plasminogen. However, the replacement of the arginine residue required for activation with a serine prevents it from being functionally active in degrading fibrin (43). As an inactive protease, Lp(a) could bind to tPA directly and prevent it from activating plasminogen. So far it is unknown whether any of these possibilities are real. *In vitro* studies suggest that Lp(a) alone or with tPA is not an active protease and Lp(a) does not appear to inhibit *in vitro* fibrinolysis (43). Whether such effects are important *in vivo* remains is unclear; however the

studies of Hamsten shown in Table X suggest that effects on fibrinolytis might be significant. In young MI patients that had a reinfarction within three years, a significant decrease in plasminogen activator activity and an increase in plasminogen activator inhibitor activity were found. In this group, the levels of Lp(a) were substantially higher than those patients with an uneventful course. It is possible that the increased plasminogen activator inhibitor they were measuring was Lp(a) (21).

Figure 16.



It is also possible that Lp(a) exerts its deleterious effects through the LDL-like core associated with the particle. Although Lp(a) in its native state is not efficiently internalized into cells by the LDL receptor, it carries the signals necessary for internalization masked by the apo(a) component. The apo(a) component can be stripped from the LDL-like core by reducing the disulfide linkage that connects apo(a) and apoB-100. Macrophages can secrete cysteine and glutathione in response to a phagocytic challenge (50). Perhaps activated macrophages at the site of injury convert the Lp(a) into a molecule that can be effectively internalized and degraded by other cells. This process might be beneficial to a certain extent, providing an efficient route to provide cholesterol to cells involved in the wound healing process; however, if excessive amounts of cholesterol were delivered this could promote cholesterol deposition and promote formation of an atherosclerotic plaque.

Conclusion

The Lp(a) particle has shown us that we certainly do not know everything there is to know about lipoproteins. It is clearly a significant risk factor in heart disease and should become a routine part of the assessment for coronary risk. Unfortunately, at this date this assay is not yet available for routine use. Hopefully, in the near future appropriate assays will be developed for clinical use. The unusual structure and metabolism suggest many unique mechanisms that might lead to vascular disease. Future research will have to address these mechanisms and determine therapies that can either lower the amount of circulating Lp(a) or prevent the damage secondary to this lipoprotein.

REFERENCES

1. Brown, M.S., P.T. Kovanen, and J.L. Goldstein. (1981) Regulation of plasma cholesterol by lipoprotein receptors. Science 212: 628-635.

2. Brown, M.S., and J.L. Goldstein. (1986) A receptor-mediated pathway for cholesterol homeostasis. Science 232:34-47.

3. Berg, K. (1963) A new serum type system in man - The Lp system. Acta Path 59.3: 369-382.

4. Dahlen, G., K. Berg, U-B. Ramberg, and A. Tamm. (1974) Lp(a) lipoprotein and pre-β_1-lipoprotein in young adults. Acta Med. Scand. 196: 327-331.

5. Albers, J.J., G. Vereracion, C.R. Warnick, and W.R. Hazzard. (1975) Lp(a) lipoprotein: Relationship to sinking pre-β lipoprotein hyperlipoproteinemia, and apolipoprotein B. Metabolism 24: 1047-1054.

6. Berg, K., G. Dahlen, and M.H. Frick. (1974). Lp(a) lipoprotein and pre-β_1-lipoprotein in patients with coronary heart disease. Clinical Genetics 6: 230-235.

7. Dahlen, G., M.H. Frick, K. Berg, M. Valle, and M. Wiljasalo. (1975) Further studies of Lp(a) lipoprotein/pre-β_1 lipoprotein in patients with coronary heart disease. Clin. Genet. 8: 183-189.

8. Dahlen, G., K. Berg, and M.H. Frick. (1976). Lp(a) lipoprotein and pre-β_1-lipoprotein, serum lipids and atherosclerotic disease. Clinical Genetics 9: 558-566.

9. Albers, J.J., J.L. Adolphson, and W.R. Hazzard. (1977) Radioimmunoassay of human plasma Lp(a) lipoprotein. J. Lipid Res. 18: 331-338.

10. Hasstedt, S.J., D.E. Wilson, C.Q. Edwards, W.N. Cannon, D. Carmelli, and R.R. Williams. (1983) The genetics of quantitative plasma Lp(a): Analysis of a large pedigree. Am. J. Med. Genet. 16: 179-188.

11. Simons, K., C. Ehnholm, O. Renkonen, and B. Bloth. (1970) Characterization of the Lp(a) lipoprotein in human plasma. Acta Path. Microbiol. Scand. Sect. B. 78: 459-466.

12. Ehnholm, C., H. Garoff, O. Renhonen, and K. Simons. (1972). Protein and carbohydrate composition of Lp(a) lipoprotein from human plasma. Biochemistry 11: 3229-3232.

13. Utermann, G., K. Lipp, and H. Weigandt. (1972) Studies on the Lp(a)-liproprotein of human serum. IV. The disaggregation of the Lp(a)-lipoprotein. Humangenetik 14: 142-150.

14. Kostner, G.M. (1976) Lipoprotein (a). In Low Density Lipoproteins (eds. Day, C.E. and Levy, R.S.) 229-269, Plenum Press, New York, NY.

15. Murai, A., T. Miyahara, N. Fujimoto, M. Matsuda, and M. Kameyama. (1986) Lp(a) lipoprotein as a risk factor for coronary heart disease and cerebral infarction. Atherosclerosis 59: 199-204.

16. Rhoads, G.G., G. Dahlen, K. Berg, N.E. Morton, A.L. Annenberg. (1986) Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA 256: 2540-2543.

17. Dahlen, G.H., J.R. Guyton, M. Attar, J.A. Farmer, J.A. Kautz, and A.M. Gotto, Jr. (1986) Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 74: 758-765.

18. Frick, M.H., G. Dahlen, K. Berg, M. Valle, and P. Hekali. (1978) Serum lipids in angiographically assessed coronary atherosclerosis. Chest 73: 62-65.

19. Kostner, G.M., P. Avogaro, G. Cazzolato, E. Marth, G. Bittolo-Bon, and G.B. Qunici. (1981) Lipoprotein Lp(a) and the risk for myocardial infarction. Atherosclerosis 38: 51-61.

20. Armstrong, V.W., P. Cremer, E. Eberle, A. Manke, F. Schulze, H. Wieland, H. Kreuzer, and D. Seidel. (1986) The association between serum Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Dependence on serum LDL levels. Atherosclerosis 62: 249-257.

21. Hamsten, A., G. Walldius, A. Szamosi, M. Blomback, U. de Faire, G. Dahlen, C. Landou, and B. Wiman. (1987) Plasminogen activator inhibitor in plasma: Risk factor for recurrent myocardial infarction. The Lancet 2: 3-8.

22. Krempler, G., G. Kostner, K. Bolzano, and F. Sandhofer. (1978) Studies on the metabolism of the lipoprotein Lp(a) in man. Atherosclerosis 30: 57-65.

23. Krempler, F., G. Kostner, K. Balzano, and F. Sandhofer. (1979) Lipoprotein (a) is not a metabolic product of other lipoproteins containing apolipoprotein B. Biochimica et Biophysica Acta 575: 63-70.

24. Krempler, F., G.M. Kostner, K. Bolzano, and F. Sandhofer. (1980) Turnover of lipoprotein (a) in man. J. Clin. Invest. 65: 1483-1490.

25. Krempler, F., G.M. Kostner, A. Roscher, F. Haslauer, K. Bolzano, and F. Sandhofer. (1983) Studies on the role of specific cell surface receptors in the removal of lipoprotein (a) in man. J. Clin. Invest. 71: 1431-1441.

26. Floren, C.H., J.J. Albers, and E.L. Bierman. (1981) Uptake of Lp(a) by cultured fibroblasts. Biochemical and Biophysical Research Communications 102: 636-639.

27. Havekes, L., B.J. Vermeer, T. Brugman, and J. Emeis. (1981). Binding of Lp(a) to the low density lipoprotein receptor of human fibroblasts. FEBS Letters 132: 169-173.

28. Maartmann-Moe, K. and K. Berg. (1981) Lp(a) lipoprotein enters cultured fibroblasts independently of the plasma membrane low density lipoprotein receptor. Clinical Genetics 20: 352-362.

29. Vessby, B., G. Costner, H. Lithell, and J. Thomis. (1982) Diverging effects of cholestyramine on apolipoprotein B and lipoprotein Lp(a). A dose-response study of the effects of cholestyramine in hypercholesterolaemia. Atherosclerosis 44: 61-71.

30. Gurakar, A., J.M. Hoeg, G. Kostner, N.M. Papadopoulos, and H.B. Brewer, Jr. (1985) Levels of lipoprotein Lp(a) decline with neomycin and niacin treatment. Atherosclerosis 57: 293-301.

31. Albers, J.J., H.Mca. Taggart, D. Applebaum-Bowden, S. Haffner, C.H. Chesnut III, and W.R. Hazzard. (1984). Reduction of lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanozolol. Biochimica et Biophysica Acta 795: 293-296.

32. Chen, S-H., C-Y. Yang, P-F. Chen, D. Setzer, M. Tanimura, W-H. Li, A.M. Gotto, Jr., and L. Chan. (1986) The complete cDNA and amino acid sequence of human apolipoprotein B-100. J. Biol. Chem. 261: 12918-12921.

33. Knott, T.J., R.J. Pease, L.M. Powell, S.C. Wallis, S.C. Rall, Jr., T.L. Innerarity, B. Blackhart, W.H. Taylor, Y. Marcel, R. Milne, D. Johnson, M. Fuller, A.J. Lusis, B.J. McCarthy, R.W. Mahley, B. Levy-Wilson, and J. Scott. (1986) Complete protein sequence and identification of structural domains of human apolipoprotein B. Nature 323: 734-738.

34. Yang, C-Y., S-H. Chen, S.H. Gianturco, W.A. Bradley, J.T. Sparrow, M. Tanimura, W-H. Li, D.A. Sparrow, H. DeLoof, M. Rosseneu, F-S. Lee, Z-W. Gu, A.M. Gotto, Jr., and L. Chan. (1986) Sequence, structure, receptor-binding domains and internal repeats of human apolipoprotein B-100. Nature 323: 738-742.

35. Utermann, G., and W. Weber. (1983) Protein composition of Lp(a) lipoprotein from human plasma. FEBS Letters 154: 357-361

36. Gaubatz, J.W., C. Heideman, A.M. Gotto, Jr., J.D. Morrisett, and G.H. Dahlen. (1983) Human plasma lipoprotein [a] structural properties. J. Biol. Chem. 258: 4582-4589.

37. Fless, G.M., C.A. Rolih, and A.M. Scanu. (1984) Heterogeneity of human plasma lipoprotein (a). Isolation and characterization of the lipoprotein subspecies and their apoproteins. J. Biol. Chem. 259: 11470-11478.

38. Fless, G.M., M.E. ZumMallen, and A.M. Scanu. (1986) Physicochemical properties of apolipoprotein(a) and lipoprotein(a-) derived from the dissociation of human plasma lipoprotein (a). J. Biol. Chem. 261: 8712-8718.

39. Gaubatz, J.W., M.V. Chari, M.L. Nava, J.R. Guyton, and J.D. Morrisett. (1987) Isolation and characterization of the two major apoproteins in human lipoprotein[a]. J. Lipid Res. 28: 69-79.

40. Armstrong, V.W., A.K. Walli, and D. Seidel. (1985) Isolation, characterization, and uptake in human fibroblasts of an aop(a)-free lipoprotein obtained on reduction of lipoprotein(a). J. Lipid Res. 26: 1314-1323.

41. Utermann, G., H.J. Menzel, H.G. Kraft, H.C. Duba, H.G. Kemmler, and C. Seitz. (1987) Lp(a) glycoprotein phenotypes: Inheritance and relation to Lp(a)-Lipoprotein concentrations in plasma. J. Clin. Invest. 80: 458-465.

42. Eaton, D.L., G.M. Fless, W.J. Koher, J.W. McLean, Q-T. Xu, C.G. Miller, R.M. Lawn, and A.M. Scanu. (1987) Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. Proc. Natl. Acad. Sci. USA 84: 3224-3228.

43. McLean, J.W., J. Tomlinson, W-J. Kuang, D.L. Eaton, E.Y. Chen, G.M. Fless, A.M. Scanu, and R.M. Lawn. (1987) Structure of apolipoprotein(a). Nature, in press.

44. Patthy, L. (1985) Evolution of the proteases of blood coagulation and fibrinolysis by assembly from modules. Cell 41: 657-663.

45. Patthy, L., M. Terxler, Z. Vali, L. Banyai, and A. Varadi. (1984) Kringles: modules specialized for protein binding. Homology of the gelatin-binding region of fibronectin with the kringle structures of proteases. FEBS Letters 171: 131-136.

46. Trexler, M., Z. Vali, and L. Patthy. (1982) Structure of the ω-aminocarboxylic acid-binding sites of human plasminogen. J. Biol. Chem. 257: 7401-7406.

47. Wilam, B., and D. Collen. (1978) Molecular mechanism of physiological fibrinolysis. Nature 272: 549-550.

48. Hoylaerts, M., D.C. Rijken, H.R. Lijnen, and D. Collen. (1982) Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J. Biol. Chem. 257: 2912-2919.

49. Walton, K.W., J. Hitchens, H.N. Magnani, and M. Khan. (1974) A study of methods of identification and estimation of Lp(a) lipoprotein and of its significance in health, hyperlipidaemia and atherosclerosis. Atherosclerosis 20: 323-346.

50. Rouzer, C.A., W.A. Scott, O.W. Griffith, A.L. Hamill, and Z.A. Cohn. (1982) Glutathione metabolism in resting and phagocytizing peritoneal macrophages. J. Biol. Chem. 257: 2002-2008.