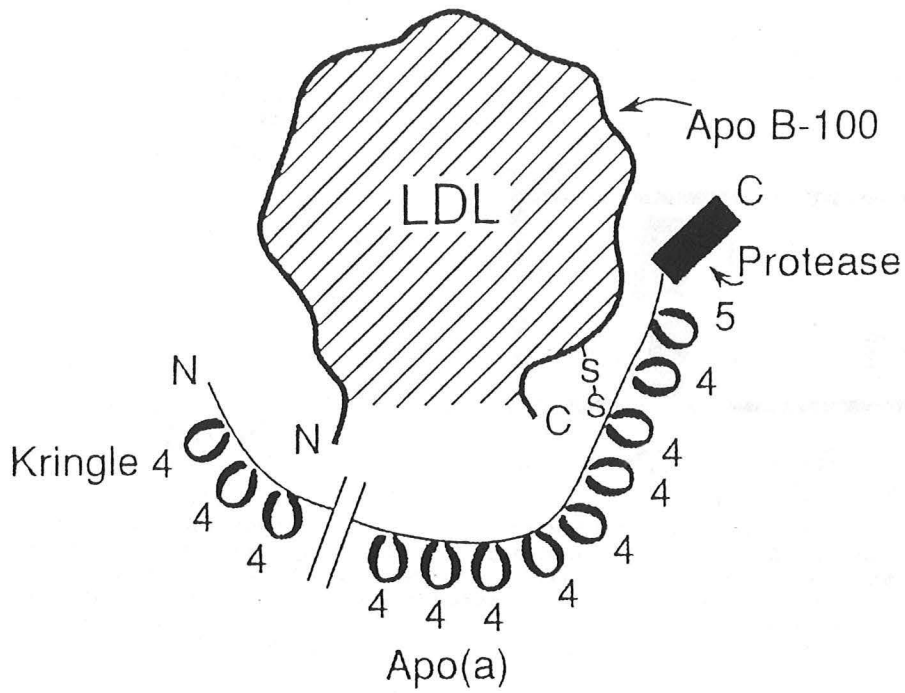
LIPOPROTEIN (a)-Lp(a)



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INTERNAL MEDICINE GRAND ROUNDS
JUNE 25, 1992

Lipoprotein(a) [Lp(a)] is a cholesterol ester-rich plasma lipoprotein which was discovered by Kare Berg in 1963 (1-4). The lipoprotein consists of a large glycoprotein, apolipoprotein(a) [apo(a)] attached to a particle of low density lipoprotein (LDL) by a putative disulfide linkage [Fig. 1] (5,6). Lp(a) has a density that is intermediate between LDL and HDL (g=1.050-1.100 gm/l). Apo(a) is a highly glycosylated protein, so the sugar content of Lp(a) is 4 times higher than LDL. Lp(a) has pre-beta mobility on agarose gel electrophoresis [Fig. 2], and thus has alternatively been referred to as the "sinking pre-beta" lipoprotein.

Figure 1

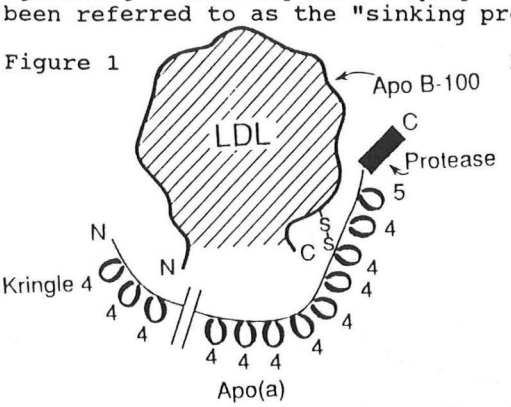
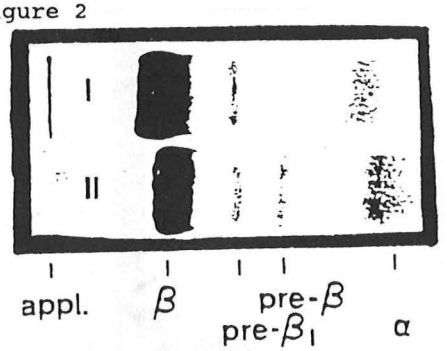
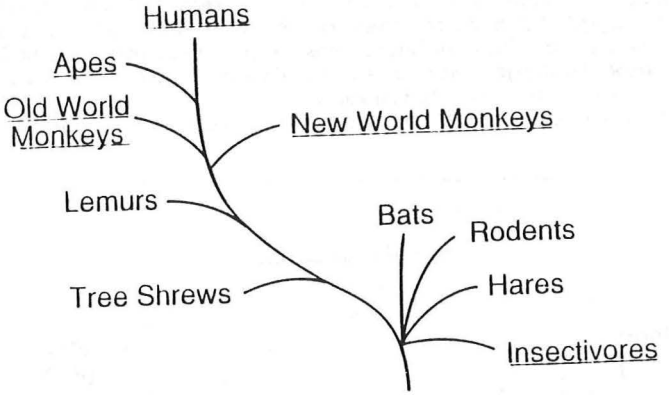


Figure 2



Lp(a) has an unusual species distribution. It is the major cholesterol-carrying lipoprotein of the hedgehog (an insectivore) (7). It is not found in any other mammals in the branch of the evolutionary tree that leads to humans until the marmoset monkey (which is a new world monkey) (8). It is found in all old world monkeys and great apes (9) [Fig. 3].

Figure 3



In humans, the plasma levels of Lp(a) vary over a wide range between individuals (10). The distribution of plasma Lp(a)

concentrations in the Caucasian population is remarkably different from that of LDL. Whereas plasma concentrations of LDL have a normal distribution, those of Lp(a) have a distribution highly skewed towards lower levels [Fig. 4]. Consequently, the majority of Caucasians have a relatively low plasma concentration of Lp(a) (10) [Fig. 5].

Figure 4

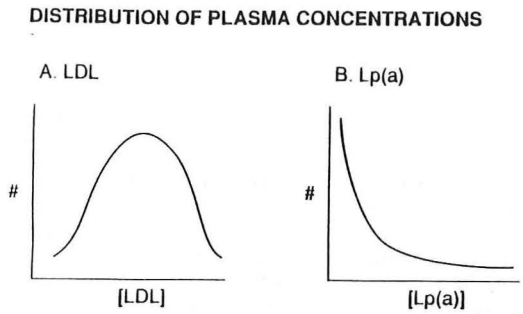
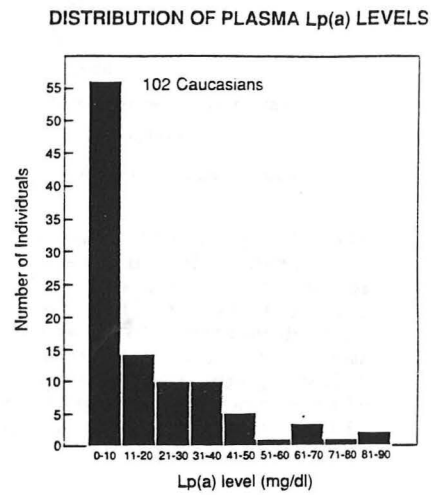


Figure 5



At birth, the plasma Lp(a) levels are universally low and increase to adult levels over the first three months of life (11) [Fig. 6]. Unlike LDL, the plasma concentrations of Lp(a) do not differ significantly between males and females and do not increase with aging [Fig. 7]. One exception is postmenopausal women who have a 50-60% higher plasma concentration of Lp(a) than premenopausal women, presumably due to the decrease in estrogen levels [Fig. 7] (12).

Figure 6

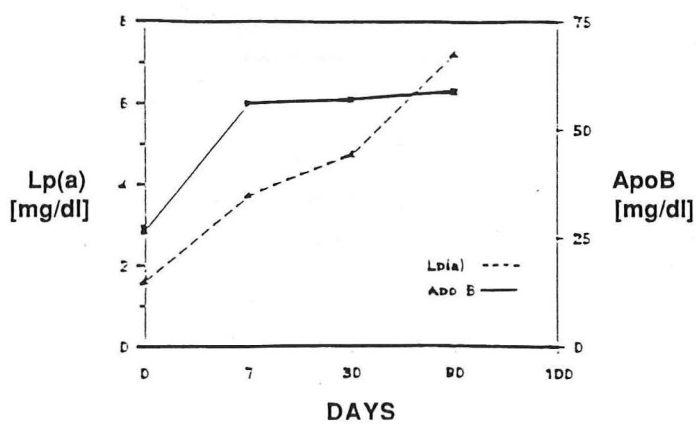
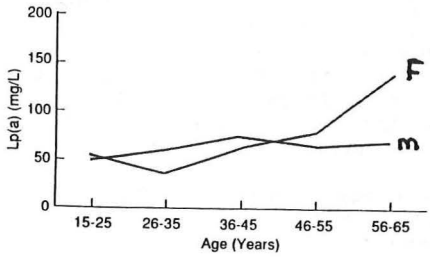


Figure 7

RELATIONSHIP BETWEEN AGE AND PLASMA Lp (a) CONCENTRATIONS



males	(n):	59	189	186	303	127
females	(n):	72	94	76	101	30

Figure 8

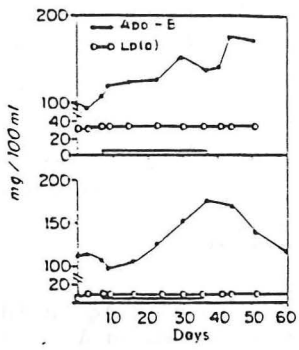
FACTORS THAT DO NOT SIGNIFICANTLY AFFECT PLASMA Lp (a) LEVELS

- Gender
- Age
- Diet
- Weight
- Most lipid lowering medications
  - Bile acid resins
  - HMG-CoA reductase inhibitors

Lp(a) differs from LDL in multiple respects [Fig. 8]. Unlike plasma LDL levels, the plasma concentrations of Lp(a) are remarkably stable in any given individual. The concentration of Lp(a) is not significantly affected by diet [Fig. 9] (13,14), changes in weight (15) or moderate exercise (16) (although very vigorous exercise may be associated with some decrease in plasma Lp(a) concentrations) (17). In addition, numerous drugs which impact importantly on the plasma concentration of LDL have little effect on that of Lp(a) [Fig. 9], as will be discussed in detail later.

Figure 9

EFFECT OF HIGH CHOLESTEROL DIET (36 g/d) ON PLASMA ApoB AND Lp(a) CONCENTRATIONS

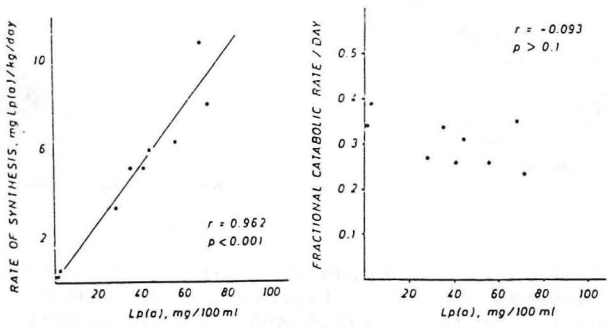


There is no correlation between the plasma concentration of Lp(a) and that of the other lipoprotein fractions, including LDL (18). Some investigators find a modest correlation between the plasma concentrations of Lp(a) and LDL or total cholesterol. Approximately one-third of the mass of the Lp(a) particle is cholesterol ester, so the contribution of Lp(a) to the plasma level of total cholesterol can be estimated by dividing the Lp(a) mass by 3. For example, if the Lp(a) concentration is 90 mg/dl, it contributes ~30 mg/dl to the total cholesterol level. Therefore, only very high plasma concentrations of Lp(a) significantly raise the plasma concentration of either LDL or total cholesterol.

Many questions remain unanswered about the normal metabolism of Lp(a). Apo(a) is synthesized almost exclusively in the liver (19) and less than 5% of the protein circulates in plasma free of lipoproteins (20). Metabolic studies suggest that, unlike LDL, Lp(a) does not have a triglyceride-rich precursor, and does not pass through the lipolytic cascade (21). Only small amounts of apo(a) are associated with triglyceride-rich particles (22). At the present time, it is not known if the Lp(a) particle is formed in the hepatocyte and then secreted, or whether apo(a) attaches to the LDL particle in the circulation. Metabolic studies suggest that differences in rates of synthesis, rather than degradation, are responsible for the observed variation in plasma concentrations of Lp(a) (23) [Fig. 10].

Fig. 10

Relationship between Lp(a) concentration, its rate of synthesis [left] and FCR [right]



Krempler et al., JCI 65: 1488, 1980

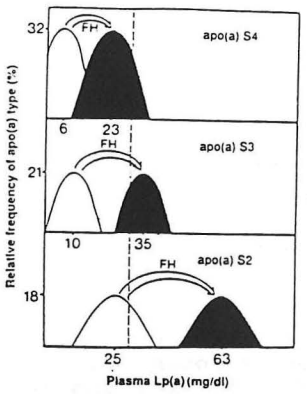
The mechanism by which Lp(a) is cleared from the circulation is also not entirely clear. A number of observations suggest that Lp(a), like LDL, is removed from plasma via the LDL receptor. *In vitro* studies have shown that Lp(a) can bind to, and be

internalized, by the LDL receptor, although at a reduced affinity when compared to LDL (24-27). Turnover studies performed in mice expressing the LDL receptor transgene have demonstrated rapid catabolism of Lp(a), as well as LDL (28). Patients with heterozygous familial hypercholesterolemic (FH) who have reduced LDL receptor activity due to a mutation in the LDL receptor gene, have plasma levels of Lp(a) that are 2-3 fold higher (29,30) [Fig. 11A] which suggests that the LDL receptor is a major route of clearance of Lp(a) from the circulation.

However, other studies are not consistent with the LDL receptor playing a major role in the catabolism of plasma Lp(a). Most importantly, pharmacological agents that significantly modify LDL receptor activity, such as bile acid resins or HMG-CoA reductase inhibitors [Fig. 11B], seem to have no appreciable effect on the plasma concentration of Lp(a) (31-34).

Figure 11A

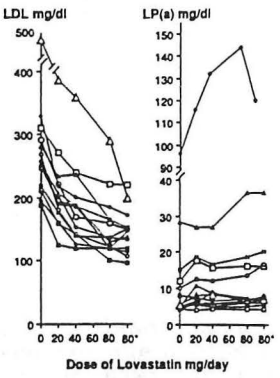
Effect of LDL receptor mutation on plasma level of Lp(a)



Utermann, Science 246: 407, 1990

Figure 11B

Effect of Lovastatin on plasma concentration of LDL and Lp(a)



Kostner et al., Circulation 80: 1315, 1989

The physiological role for this enigmatic lipoprotein remains obscure (2,3). Lp(a) is of medical interest because multiple studies have independently demonstrated that high plasma concentrations of Lp(a) are associated with coronary atherosclerotic disease (CAD), as well as peripheral vascular disease. Cross-sectional studies of Caucasian men have found that elevated plasma levels of Lp(a) (over 25-30 mg/dl) are associated with between a 1.5 and a 3 fold increased risk of coronary atherosclerosis (13,35-37). The association is independent of the contribution of other lipoproteins (38). The evidence that high concentrations of Lp(a) are associated with peripheral vascular

disease is more scanty, though compelling (39-41), so this discussion will focus on the relationship of high plasma concentrations of Lp(a) with coronary artery disease.

In 1975 Dahlèn and Berg used a qualitative assay for Lp(a) and found that a higher percentage of patients with CAD had evidence of Lp(a) in their plasma than control patients (42). Kostner et al. were the first to demonstrate that high concentrations of plasma Lp(a) were associated with CAD in normolipemic Caucasian men. He compared the plasma Lp(a) levels in 36 men (ranging in age from 40-60 years) with a history of a myocardial infarction and yet normal lipoprotein levels (i.e. VLDL, LDL, and HDL) to 55 age- and sex-matched controls. Forty-four percent of the men who had suffered a myocardial infarction had an Lp(a) level greater than 30 mg/dl in contrast to only 26% of the controls [Fig. 12] (43).

Figure 12

RELATIONSHIP OF PLASMA Lp(a) CONCENTRATION TO CAD IN NORMOLIPIDEMIC MEN (AGE 40 - 60)

	Lp(a) (mg/dl) <30	Lp(a) (mg/dl) >30
CONTROLS (55)	41 (75%)	14 (26%)
MI (36)	20 (56%)	16 (44%)

The association between elevations in plasma Lp(a) and ischemic heart disease is strongest in the younger age groups (37). Rhoads et al. screened a large population of Hawaiian men of Japanese ancestry and compared the plasma Lp(a) concentrations of 303 men with history of a myocardial infarction to 408 population-based controls (37). In the subset of men under the age of 60 years who had an Lp(a) in the top quartile, the odds ratio of having had a myocardial infarction was 2.54 higher that in the control group [Fig. 13]. The Lp(a) was not as significant a predictor of CAD in individuals over age 60. He estimated that the population attributable risk of having an MI if the Lp(a) was in the top quartile was 1 in 4 for men under 60, and one in eight for men over 60.

Figure 13

ODDS RATIO FOR HISTORY OF MI IN 303 HAWAIIAN MEN WHO HAD PLASMA Lp(a) IN TOP QUARTILE

AGE	HISTORY OF MYOCARDIAL INFARCTION	
	ODDS RATIO	POPULATION-ATTRIBUTABLE RISK, %
<60 years	2.54	28
60-69 years	1.57	13
≥70 years	1.22	5
ALL AGES	1.65	14

Two studies have shown an association between the plasma level of Lp(a) and a parental history of CAD. In one study, Lp(a) levels were measured in all 18 year-old Austrian military recruits over a 4 month period. Those that did (n=52) and did not (n=1434) have a parental history of myocardial had significantly different plasma levels of Lp(a) [Fig. 14] (44). In the second study, Durrington et al. looked at 41 men who were status-post myocardial infarction and compared them with 78 controls (45). In both groups, those with no parental history of a myocardial infarction had significantly lower levels of plasma Lp(a) than those with a parental history [Fig. 15]. In this cohort, the best predictors as to whether or not a patient had CAD were the plasma concentrations of 1) apoB, 2) apoAI, and 3) the presence of a positive parental history of MI. The plasma Lp(a) level could substitute for the parental history of myocardial infarction as a risk factor. In other words, no additional benefit was derived from knowledge of a parental history of myocardial infarctions if the plasma Lp(a) level was known.

Figure 14

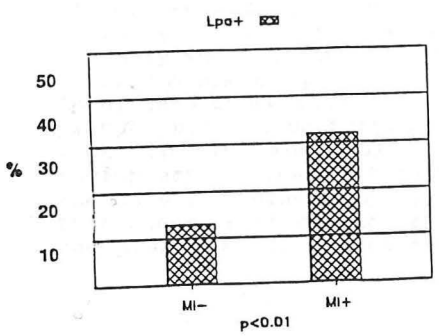
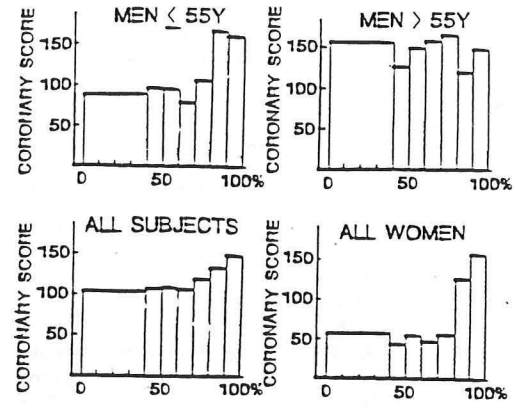


Figure 15

	No Parental History		Parental History	
	n	Lp(a)	n	Lp(a)
Controls	70	92	8 (11%)	355
MI	26	64	12 (46%)	362

Not only are high plasma concentrations of Lp(a) associated with the presence of CAD, but also are directly related to the severity of coronary atherosclerosis. Dahlèn et al. evaluated 307 Caucasians who had undergone coronary angiography and examined the relationship between the severity of coronary atherosclerosis (coronary score) vs. the plasma Lp(a) concentrations (ranked by deciles) (46). If all subjects were pooled, there was a direct relationship between the level of plasma Lp(a) and the severity of CAD, as assessed by coronary angiography [Fig. 16]. In subset analyses of women, and men less than the age of 55, the same direct relationship between plasma concentration of Lp(a) and severity of CAD held true; however, the relationship was not observed in the men over the age of 55 [Fig. 16], again pointing to a high plasma Lp(a) being a better predictor of significant CAD in younger men.

Figure 16



Elevated plasma Lp(a) levels have also been found to be associated with an increase in the incidence of re-stenosis after coronary artery bypass surgery or percutaneous transluminal coronary angioplasty (47,48). Individuals who developed restenosis after bypass surgery had significantly higher levels of plasma Lp(a) than those that did not have stenosis [Fig. 17A] (47). When plasma Lp(a) concentrations were measured in 69 patients within 1 week to 10 mos. after percutaneous transluminal coronary angioplasty (PTCA), it was found that the individuals with re-stenosis had plasma Lp(a) levels that were significantly higher than those in which the artery remained patent (48). In contrast, the plasma total cholesterol, VLDL-cholesterol, LDL-cholesterol,

and HDL-cholesterol levels were not significantly different between the two groups [Fig. 17B].

Figure 17A Lp(a) in patients with and without restenosis after coronary artery bypass surgery

Measurement	No-stenosis group (n = 32)	Stenosis group (n = 135)	p value
Lp(a) (mg/dl)	16.7 ± 22.6	32.0 ± 32.7	.002
Cholesterol (mg/dl)	231.8 ± 48.8	251.3 ± 69.1	.06
Triglycerides (mg/dl)	186.2 ± 80.0	238.2 ± 207.1	.85
Blood pressure (mm Hg)			
Systolic	132 ± 19	135 ± 23	.82
Diastolic	82 ± 13	81 ± 11	.83
Lp(a) in men ≤55 yr	16.3 ± 18.0 (n = 12)	43.4 ± 38.7 (n = 34)	.008
Lp(a) in men >55 yr	18.3 ± 28.0 (n = 16)	29.8 ± 31.3 (n = 87)	.07
Lp(a) in women	12.4 ± 11.3 (n = 4)	22.2 ± 18.1 (n = 14)	.43

Figure 17B

	RESTENOSIS 0 (n=20)	RESTENOSIS + (n=49)
CHOLESTEROL (MMOL/LITER)	5.70±1.02	5.16±1.12
VLDL	1.12±0.49	0.79±0.42
LDL	3.47±1.05	3.32±0.96
HDL	1.06±0.26	1.06±0.24
TOTAL:HDL RATIO	5.59±1.33	5.04±1.42
TRIGLYCERIDES (MMOL/LITER)	2.50±1.07	1.72±0.91
APO A-I (mg/dl)	99±15.6	99±17.7
APO B (mg/dl)	135±36.8	119±30.8
Lp(a) (mg/dl)	7.0 (0.5-44.0)	18.8 (1-120)

High plasma concentrations of Lp(a), when coupled with elevated plasma levels of LDL, appear to be particularly atherogenic. Armstrong measured the plasma Lp(a) levels in a sample of 428 Caucasian men (age 40-60) with coronary artery disease (at least one major coronary vessel occluded >50%) documented by angiography as well as 142 individuals who had no evidence of coronary artery disease (35). Armstrong calculated the odds ratio of having CAD when the plasma Lp(a) level was over 30 mg/dl compared to under 5 mg/dl. The method used to calculate the odds ratio is shown in Figure 18. Individuals who had plasma Lp(a) levels over 30 mg/dl had an odds ratio of having a coronary artery

disease of 2.7, as compared to those with an Lp(a) with less than 5 mg/dl.

Armstrong then examined whether the odds ratio was modified by other risk factors for coronary artery disease such as smoking, hypertension, HDL, VLDL, and LDL. He found that only the plasma concentration of LDL had a significant effect on the odds ratio. In individuals with plasma Lp(a) concentrations >30 mg/dl, if the LDL-cholesterol level was less than the median, the odds ratio fell to ~1.5, but if the LDL was greater than the median, then the odds ratio of having CAD jumped from 2.7 to 6.33 [Fig. 19]. In contrast, smoking, hypertension, and a low HDL had no effect on the odds ratio (35). Therefore, high Lp(a) levels coupled with high LDL levels appear to be particularly atherogenic.

Figure 18

	< 5 mg/dl	> 30 mg/dl
CAD+	a	b
CAD-	c	d

$$\frac{b \times c}{a \times d} = 2.7$$

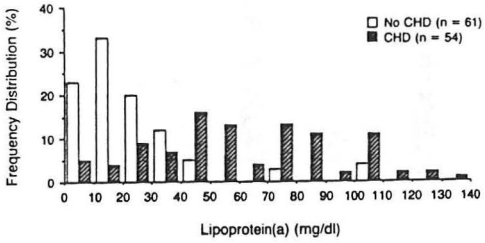
Figure 19

	Odds Ratio	
	< median	> median
Total Cholesterol	1.56	4.5
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

In support of high levels of Lp(a) and LDL together being more atherogenic then high plasma levels of Lp(a) alone is the observation made by three groups of investigators that patients with FH and CAD have significantly higher plasma Lp(a) than FH patients without clinical evidence of CAD [Fig. 20] (49-51). However, in these studies the patients were not age and sex-matched. In the only study in which the patients have been age and sex-matched, there was no significant relationship found between the plasma Lp(a) levels and the presence or absence of CAD (52; Mbewu et al., 1991). Therefore, at this time it is still debatable whether elevated levels of Lp(a) are a prognosticator for ischemic heart disease in patients with FH.

Figure 20

Frequency Distribution of Serum Lp(a) in FH Heterozygotes with and without CHD



Seed et al., NEJM 322: 1496, 1990

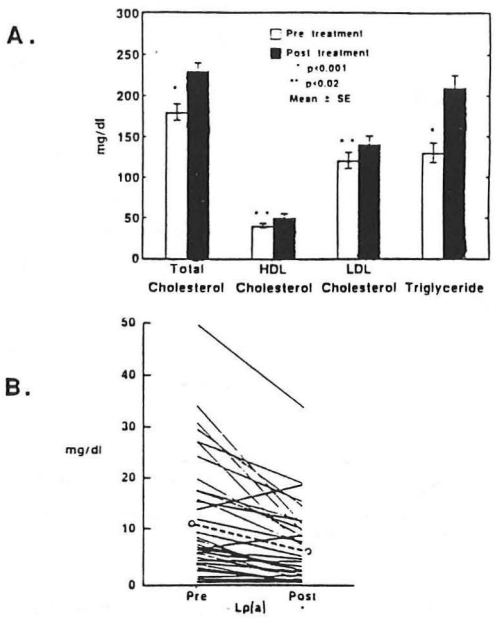
Lp(a) levels are elevated in two conditions which are associated with accelerated atherosclerosis - hemodialysis and diabetes. Patients on hemodialysis have a 3-fold elevation in their plasma Lp(a) levels when compared to controls [Fig. 21] (53,54). Patients on peritoneal dialysis actually have significantly higher plasma Lp(a) concentrations than those on hemodialysis (55). It has not been determined whether the higher concentrations of plasma Lp(a) in these patients are due to renal failure or the dialysis treatment. Interestingly, plasma Lp(a) levels fall over 50% within one week of renal transplantation in both peritoneal and hemodialysis patients (55). Whether this fall was due to the improvement in renal function or to the initiation of immunosuppressive agents has not been determined. The fact that Lp(a) also falls after cardiac transplant (despite increases in LDL and HDL) suggests that the administration of immunosuppressive agents are responsible for the reduction in plasma Lp(a) concentrations [Fig. 22] (56).

Figure 21

	Mean ± standard deviation (and median)	
	Controls (n = 71)	Hemodialysis patients (n = 71)
Age, years	44.0 ± 14.98 (44.28)	44.03 ± 15.08 (44.67)
Lp(a), mg/L	125.00 ± 172.80 (71.40)	378.00 ± 463.50 (285.70)[a]
TC, mmol/L	5.93 ± 1.08 (5.92)	5.99 ± 2.06 (5.77)
TG, mmol/L	1.11 ± 0.39 (1.09)	2.50 ± 1.47 (2.04)[a]

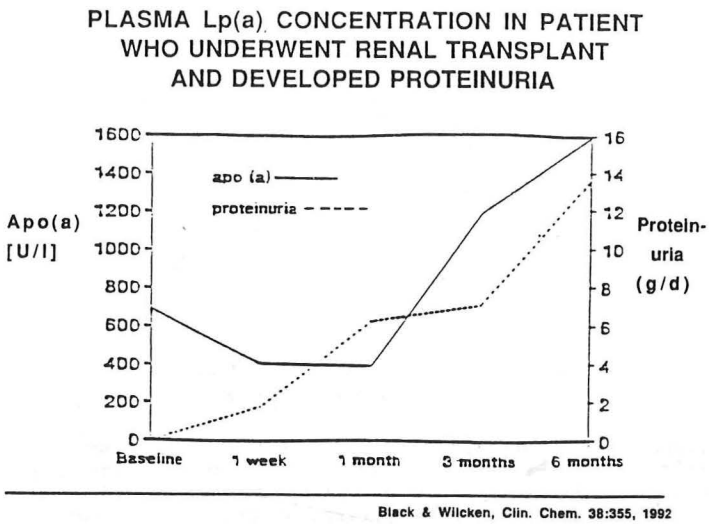
[a] Values significantly different from controls: $P < 0.0001$ (Mann–Whitney U-test).

Figure 22



For reasons that are not known, the plasma Lp(a) levels also tend to be elevated in patients with proteinuria (55,57) [Fig. 23]. In nephrotic syndrome, the increase in plasma Lp(a) levels does not correlate with the levels of the other apoB-containing lipoproteins. In a small sample of patients with minimal change disease, there was a direct relationship between the plasma Lp(a) levels and the amount of protein excreted in the urine. Conversely, there was an inverse correlation between the plasma Lp(a) and the levels of proteinuria in the patients with membranoproliferative disease (57). Unfortunately, in this study the medications the patients were taking were not provided, so it can not be ruled out that the observed differences in plasma Lp(a) levels in these two groups of patients with different pathological renal lesions were not due to the effect of medications. Interestingly, the highest reported levels of plasma Lp(a) in patients with proteinuria have been in patients with amyloidosis (57).

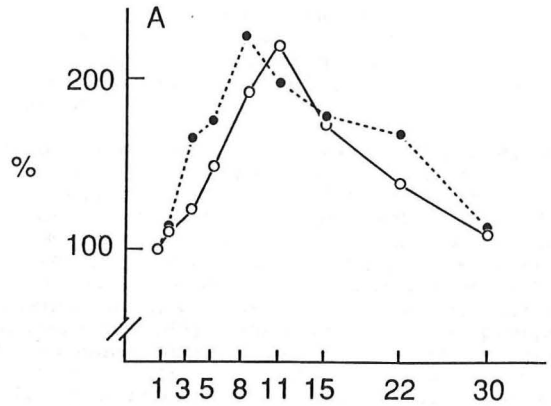
Figure 23



The elevating effect of renal disease on plasma Lp(a) levels may be a nonspecific response. A number of studies suggest that Lp(a) is an acute phase reactant. One study found that plasma levels of Lp(a) dramatically increase after a myocardial infarction or major surgery (58). The Lp(a) concentration rose to twice the baseline levels within 11 days after a myocardial infarction and Lp(a) remained elevated for up to three months [Fig. 24]. Another study found only a modest increase in Lp(a) in the 6 day period following a myocardial infarction (59). A cross-sectional study showed that patients with unstable angina and patients who received thrombolytic therapy have no acute changes in the level of plasma Lp(a) (60). Additional studies are needed to resolve this conflicting data concerning the level of Lp(a) after myocardial infarction. In support of Lp(a) being an acute phase reactant, patients with rheumatoid arthritis tend to have an increase in plasma Lp(a) levels despite decreases in the other lipoprotein fractions (61).

Figure 24

CHANGES IN SERUM Lp(a) AFTER MI (○) AND SURGERY (•)

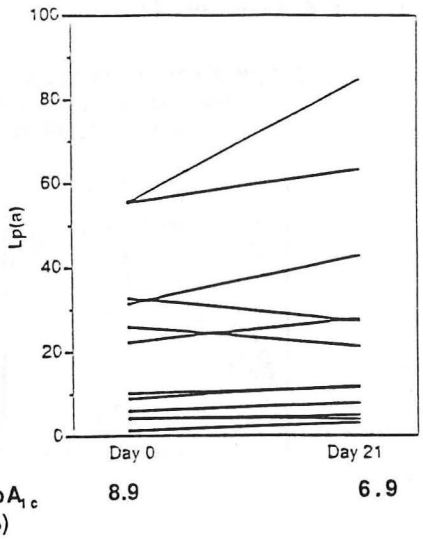


Plasma levels of Lp(a) are also elevated in poorly-controlled insulin-dependent diabetics (62-65). Improvements in diabetic control, as reflected by a lowering of glycosylated hemoglobin levels, are associated with a small, but significant drop in the plasma concentration of Lp(a) (62) [Fig. 25A]. In adult onset diabetics, there is little correlation between the plasma level of Lp(a) and diabetic control [Fig. 25B] (63).

Figure 25A

	Poorly Controlled Diabetes	Good Control
Lipoprotein(a), mg/dL	46.1 ± 6.3	29.3 ± 5.2
Fasting plasma glucose, mmol/L	12.9 ± 1.1	7.1 ± 1.7
Glycosylated hemoglobin, %	10.3 ± 2.57	8.51 ± 2.47

Figure 25B



It has not been shown that elevated levels of Lp(a) are associated with CAD in diabetics. One small 4 year prospective study, the Wisconsin Epidemiological Study of Diabetic Retinopathy, failed to find any difference in the 11 Type I and 24 Type II diabetics who had coronary events when compared to controls (64). A potential problem with this study is that the plasma samples were stored for 5 years prior to measuring the Lp(a) concentrations. Lp(a) is known to be easily degraded and the lack of difference in Lp(a) levels may be artifactual.

Three prospective studies have examined the effect of plasma Lp(a) on the incidence of coronary events. In an early study, the Lp(a) level was measured in 232 Swedish males (age 50-53) with no known atherosclerotic disease. The patients were followed for 11 years, and there was a 2-3 fold higher incidence of myocardial infarction and stroke in those with high plasma concentrations of Lp(a) [Fig. 26] (68). An association between elevated Lp(a) levels and coronary events was also observed in a prospective case-control study of middle age men (69). However, a recent prospective study failed to find an association between high plasma levels of Lp(a) and coronary events (70). In the Helsinki Heart Study, 140 individuals who had a myocardial infarction were compared with 136 controls (70). There was no significant difference in the distribution of plasma Lp(a) concentrations in these two populations [Fig. 27]. The reason for the apparent discrepancy in the findings of the Helsinki study and the other two prospective studies remain to be explained, but it appears not to be due to survival bias (that is, the lack of association of cardiac events with plasma Lp(a) concentration is not explained by a bias incurred if individuals with high Lp(a) that died before or during the course of the study and thus were not represented in the analysis). Additional prospective studies are needed to confirm that high concentrations of Lp(a) are associated with an increased risk for CAD.

Figure 26

CARDIOVASCULAR EVENTS IN 232 SWEDISH MALES (50-53 YEARS) DURING 11-YEAR FOLLOW-UP

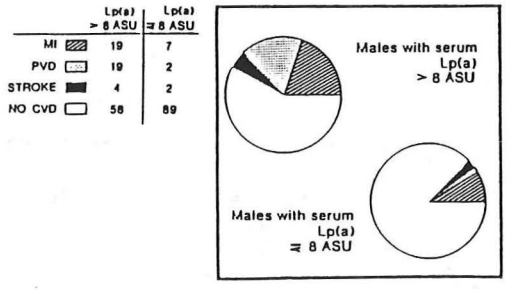
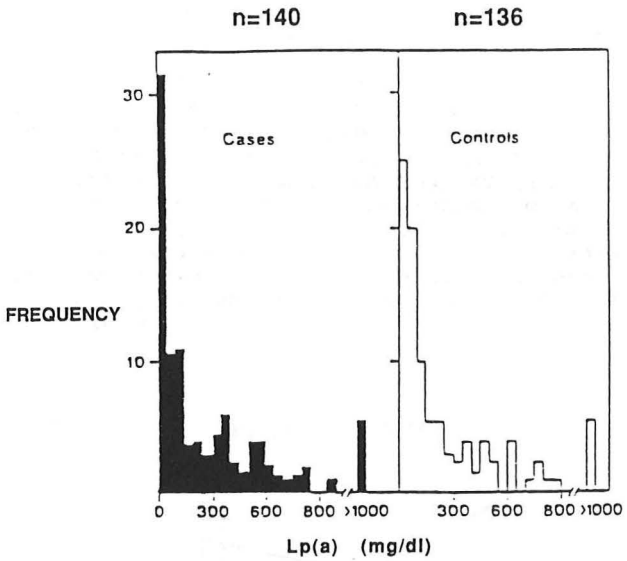
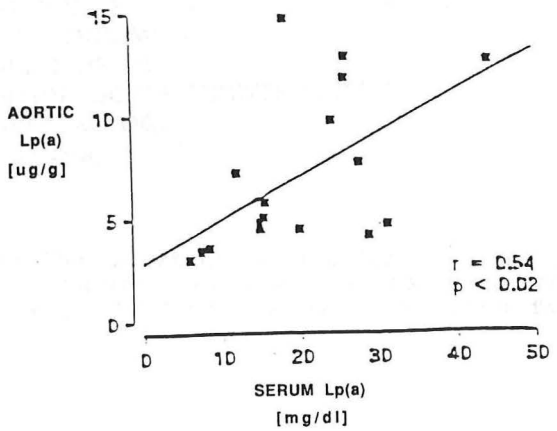


Figure 27



A number of studies have examined atherosclerotic lesions immunocytochemically and shown that Lp(a) is actually present in the lesions (71). In monkeys in which atherosclerosis was induced by a high fat diet, pathological examination of aortic atherosclerotic lesions demonstrated immunoreactive apo(a) associated with lipid staining (72). Moreover, there was a direct relationship between the serum Lp(a) level and the aortic Lp(a) concentrations in the animals [Fig. 28]. Rath et al. has shown this same association in human aortic lesions (73), and immunocytochemical studies of human venous saphenous grafts have demonstrated co-localization extracellularly of apo(a) and apoB in the intima of the grafts (74).

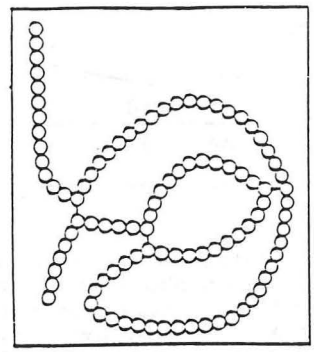
Figure 28



To probe the possible mechanisms responsible for the association between high plasma concentrations of Lp(a) and increased atherosclerosis demands a more detailed look at the structure of apo(a). Apo(a) contains multiple copies of a cysteine-rich protein motif which has been found in many other proteins and is referred to as a kringle. Each kringle contains six cysteine residues that form three intramolecular disulfide linkages. The tri-loop structure of the kringle motif is so named because it resembles a Danish "kringle" pastry [Fig. 29]. This motif is found in urokinase, tissue-plasminogen activator, prothrombin, factor XII, hepatocyte growth factor, and most importantly for this discussion, plasminogen. The plasma zymogen, plasminogen, has five copies of this cysteine-rich motif (referred to as kringles 1 through 5) which share approximately 80% sequence identity with each other.

Figure 29

KRINGLE DOMAIN

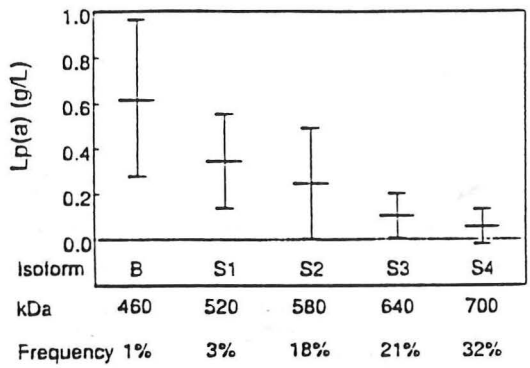


- UROKINASE
- TISSUE PLASMINOGEN ACTIVATOR (TPA)
- PROTHROMBIN
- FACTOR XII
- HEPATOCYTE GROWTH FACTOR
- PLASMINOGEN
- APO(a)

Utermann made the important observation that the size of the apo(a) glycoprotein varies over a wide range (~300-800 kDa) and this size variation is inherited in a stable Mendelian fashion (2).

He classified the apo(a) isoforms into six groups according to their relative mobility on an PAGE-SDS gel compared to apoB [F = fast, B = same size as apoB (~513 kD), S1-S4 = slow, i.e. larger than B] (2). He observed an inverse correlation between the size of the apo(a) isoform and the plasma level of Lp(a). That is, the apo(a) isoforms of smaller size were associated with a higher concentration of plasma Lp(a) [Fig. 30]. He also noted that the frequency of the different isoforms in the population partially explained the skewed distribution of plasma Lp(a) concentrations [Fig. 30]. The isoforms of highest frequency were the larger ones (S1-S4) and these isoforms tended to be associated with lower plasma levels of Lp(a). The smaller isoforms (F and B) tended to be associated with higher plasma concentrations of Lp(a).

Figure 30



The reason for the inverse correlation between plasma concentrations of Lp(a) and apo(a) isoform size is still not known, but the molecular mechanism responsible for the size variation in the apo(a) protein has been revealed. It was first suggested by the structure of the apo(a) cDNA when it was cloned in 1987 and mapped to chromosome 6 (75). The apo(a) gene is not only closely linked to the plasminogen gene, but the two neighboring genes have a remarkable resemblance (76). Fig. 31 shows a comparison between the sequence of the apo(a) and plasminogen cDNAs. There is a high degree of sequence identity over the entire coding regions of these two genes [Fig. 31]. In the 5' untranslated and signal sequence region, the two genes are identical in sequence. The tail region and kringles 1-3 of plasminogen are not present in apo(a). The kringle 4-encoding sequence of plasminogen is repeated up to 37 times in apo(a). This is followed by a kringle 5-encoding sequence and, finally, a region that shares 94% sequence identity with the protease domain of plasminogen. Apo(a), unlike plasminogen, has no fibrinolytic activity. Normally when plasminogen is activated it is cleaved at an arginine residue in kringle 5, but in apo(a) there is a substitution to serine at this position [Fig. 32].

Figure 31

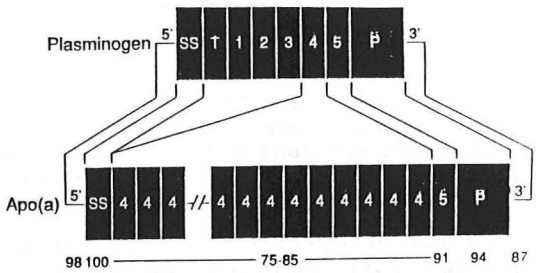


Figure 32

COMPARISONS BETWEEN HUMAN PLASMA APOLIPOPROTEIN(a) [apo(a)] AND PLASMINOGEN

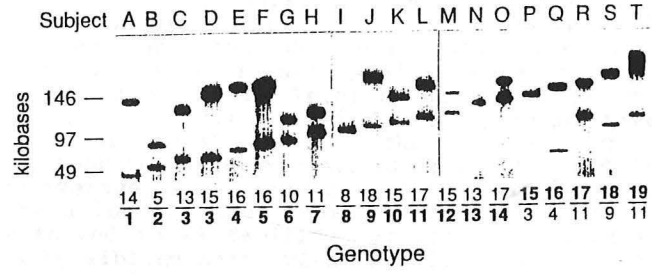
PARAMETER	Apo(a)	PLASMINOGEN
kDA	280-800	~90
Signal Sequence	19 residues	19 residues
Kringle 1-3	Absent	Present
Kringle 4, number	Multiple	1
Kringle 5, number	1	1
Activation site	Ser-Ile	Arg-Val
	4308-4309	561-562
Catalytic triad	Ser-His-Asp	Ser-His-Asp

McLean et al. hypothesized that the variations in size of the apo(a) protein were due to differences in numbers of kringle 4 repeats in the apo(a) gene (75). After the apo(a) cDNA was cloned from the rhesus monkey, it was determined that apo(a) was expressed in only three tissues - the liver, in trace amounts of the brain, and testes (19). A number of investigators examined the relationship between the size of the apo(a) mRNA and the size of the apo(a) isoforms in monkeys, baboons, and man (77-79). With few exceptions, there was a direct relation between the size of the mRNA and the size of the protein. This suggested that the apo(a) size variation was due to a variation in the apo(a) gene, but did not rule out the possibility that the variation was due to differences in mRNA splicing.

Despite the fact that the apo(a) cDNA was cloned and characterized five years ago, the apo(a) gene has yet to be completely characterized. By using the restriction enzyme *Kpn*I, a large restriction fragment which contains most of the kringle 4-encoding region of the apo(a) gene was identified (80) [Fig. 33A]. By performing Southern blotting and using an apo(a)-specific probe from the kringle 4-encoding region, the size of this restriction fragment was found to vary between individuals. Initially, 19 alleles of different sizes were identified in a population of 102 Caucasians. In each case, the size of a restriction fragment correlated with the size of the apo(a) protein isoform [Fig. 33B].

More recently, a total of 31 different sized alleles have been identified (unpublished observation). A total of 94% of the individuals studied were heterozygous for the length polymorphism which makes this locus as polymorphic as the variable number tandem repeats used for DNA fingerprinting (see H.H. Hobbs, Grand Rounds, 1990). The difference in size between adjacent alleles were 5.5 kb - the size of the single kringle 4-encoding unit. Therefore, taken together, this analysis confirmed that the size polymorphism in the apo(a) protein was due to differences in the number of kringle 4 repeats in the apo(a) gene.

Figure 33 **PULSED-FIELD ELECTROPHORESIS OF GENOMIC DNA: HYBRIDIZATION WITH KRINGLE 4-SPECIFIC PROBE**

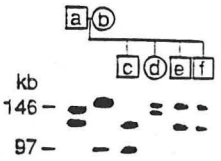


The size of the apo(a) gene was found to account for 69% of the inter-individual variation in the plasma Lp(a) concentrations in the sample (81). However, it was also found that individuals with apo(a) genes of identical size could have very different plasma levels of Lp(a). To determine if these observed differences were due to the impact of other genes, or to sequence differences at the apo(a) locus, segregation analysis of the apo(a) gene and plasma Lp(a) levels was performed in families.

Numerous family studies have shown that the heritability of plasma Lp(a) levels is very high (~90%). Segregation analysis of plasma Lp(a) levels in families has been consistent with the suggestion that there was a very small number of major genes that determine its level (82-84). Recently, the length polymorphism in the kringle 4-encoding region of the apo(a) gene was used as a genetic marker to follow the segregation of the apo(a) gene in families. In most families, all four parental alleles could be differentiated so that the apo(a) alleles inherited by each offspring could be determined. An example is shown in Figure 39. It was found that the concentration of Lp(a) tended to segregate with particular apo(a) alleles. In the family shown in Fig. 34, *apo(a)7* is associated with a high plasma concentration of Lp(a).

SEGREGATION ANALYSIS
OF Apo(a) GENE

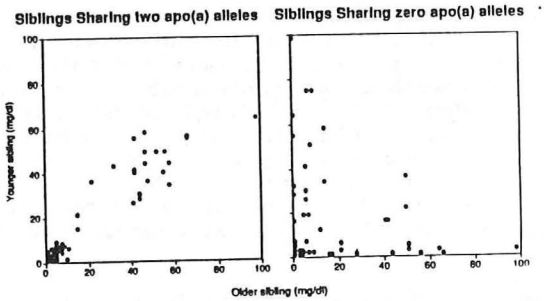
Figure 34



Apo(a) Genotype	14/12	15/7	12/7	15/14	15/12	15/12
Lp(a) (mg/dl)	<1	32	28	<1	1	1

To estimate the contribution of the apo(a) gene to the plasma level of Lp(a), the plasma Lp(a) concentrations were compared in sibling pairs who inherited identical apo(a) alleles from their parents. If the apo(a) gene was the major gene that contributed to the plasma concentration of Lp(a), then siblings who inherit identical apo(a) genes from their parent should have similar concentrations of Lp(a). If other genes are important contributors, then the plasma levels of Lp(a) should differ, since siblings, on average, share only 25% of the same parental alleles at any unlinked locus. When segregation analysis of the apo(a) gene was performed in 48 families, it was found that the plasma Lp(a) levels in sibling pairs with identical apo(a) genotypes had a correlation co-efficient (r) of 0.95, whereas those with no alleles in common actually had a negative correlation (81) [Fig. 35]. From statistical analysis of these results, it could be concluded that the apo(a) gene itself explains 90% of the inter-individual variation in plasma concentration of Lp(a).

Figure 35



What is the mechanism responsible for the association of high levels of plasma Lp(a) and coronary artery disease? As stated previously, apo(a) has no known protease activity. It has been suggested that apo(a) is atherogenic because it interferes with the physiological role of plasminogen in thrombolysis by molecular

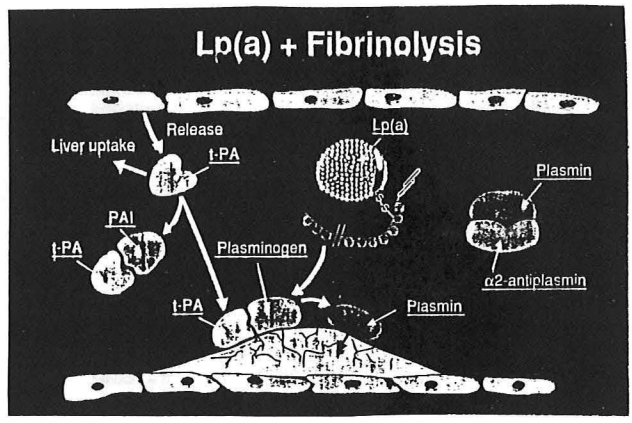
mimicry. There is *in vitro* evidence to suggest that apo(a) can compete with:

1) plasminogen binding to the surface of endothelial cells and macrophages (85,86);

2) plasminogen activation by streptokinase and tissue plasminogen activator (87);

3) plasminogen binding to fibrinogen or fibrin (87-91).

Figure 36

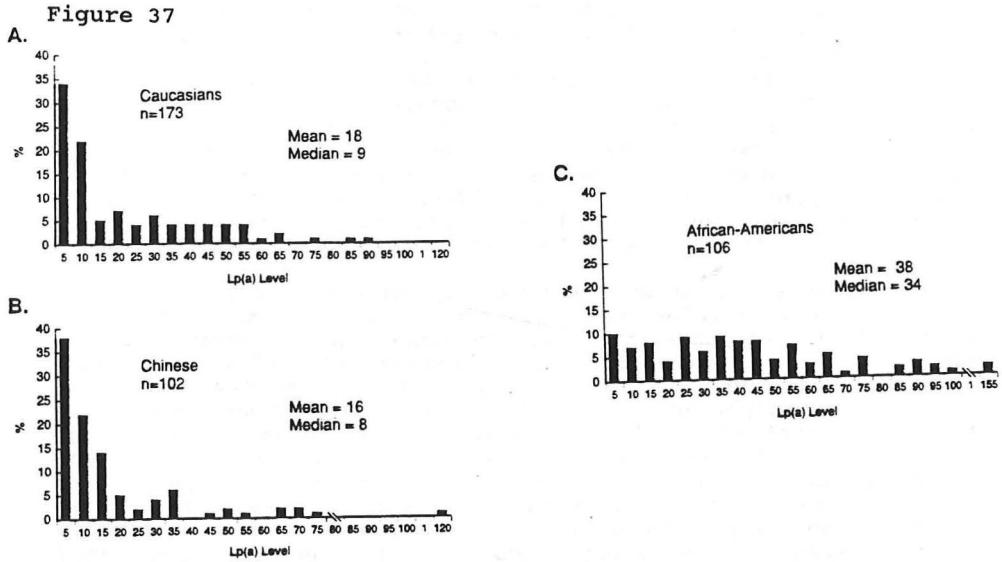


In vivo evidence that Lp(a) inhibits thrombolysis is still forthcoming. The fact that individuals in which restenosis occurs after PTCA have higher plasma levels of Lp(a) suggests this may be the case. However, to date there is no direct evidence that high plasma concentrations of Lp(a) inhibit thrombolysis *in vivo*. If high concentrations of Lp(a) interfere with the activities of plasminogen activation, it may be expected that thrombolytic therapy would be less effective in patients with high Lp(a) levels, but this appears not to be the case (92). In addition, there is no evidence that high concentrations of Lp(a) predispose to venous thrombosis.

Alternatively, apo(a) may simply be an innocent bystander in the atherosclerotic process. The Lp(a) particle may simply get trapped by the atherosclerotic lesion. It has been shown that Lp(a) can bind tightly to glycosaminoglycans extracted from human aortic tissue (93) and to cultured mouse macrophages (94).

A seeming paradox in the relationship between plasma concentrations of Lp(a) and coronary atherosclerosis is the fact that the average plasma Lp(a) level is ~2-fold higher in Africans and African-Americans (2, 95). Unlike Caucasians, African-Americans have a more normal distribution in their plasma Lp(a) levels [Fig. 37]. The reason for the higher plasma concentrations of Lp(a) in African-Americans can not be explained by differences in the distributions of apo(a) isoforms of different sizes since there is little difference in the size distribution of apo(a) isoforms in the Caucasian and African-American populations (96,97).

Figure 37



Despite the higher plasma levels of Lp(a), there is no increase in incidence of CAD in the African-American population. High plasma concentrations of Lp(a) in African-Americans may not be associated with CAD. Supporting this contention is the observation that the plasma Lp(a) levels in Caucasian children are a good predictor as to whether or not the parents had CAD, but this was not true for the African-American children (98). In a recent study at this institution, we have failed to show any difference in the plasma concentration of Lp(a) in Blacks with and without coronary artery disease as documented by coronary angiography (Moliterno et al, unpublished observation).

A possible explanation for the lack of association between high plasma concentrations of Lp(a) and CAD in blacks is that there may be different apo(a) alleles in the two populations. The apo(a) locus is exceedingly heterogenous, not only in the number of the kringle 4 repeats, but also in their sequence. Therefore, in the

African-American population there may be apo(a) alleles which generate a high concentration of plasma Lp(a) but do not contain the epitope that is responsible for the association with atherosclerosis. Alternatively, there may be another ethnic-specific factor which protects African-Americans from the atherogenic effects of Lp(a).

In light of the strong association between Lp(a) with atherosclerotic disease, should we be measuring plasma levels of Lp(a) in our patients? The answer to this question is not straightforward. Cross-sectional studies, but not all prospective studies, have demonstrated that high concentrations of plasma Lp(a) are associated with coronary atherosclerosis in Caucasians. No studies have demonstrated that by lowering the plasma concentrations of Lp(a) there is an associated reduction in the risk of coronary artery disease. And intervention studies will not be immediately forthcoming for the important reason that very few pharmacological agents have been identified that lower Lp(a). Agents which up-regulate the LDL receptor, such as bile acid resins or HMG Co-A reductase inhibitors, and significantly lower the plasma concentration of LDL, have little to no appreciable effect on the plasma concentration level of Lp(a) (31-34).

The only medications that have been shown to result in a fall in plasma Lp(a) levels include nicotinic acid, anabolic steroids and estrogens. The administration of nicotinic acid, which interferes with the secretion of lipoproteins, is not only associated with a fall in plasma VLDL and LDL concentrations, but also in plasma levels of Lp(a) (99-101). The degree to which the Lp(a) concentration falls is proportional to the fall in LDL and the initial plasma Lp(a) level [Fig. 38A and 38B] (100). Therefore, individuals with higher plasma levels of Lp(a) have a more significant response to medications than those with more moderate levels. Paradoxically, the plasma Lp(a) can actually increase in amount when hypertriglyceridemic patients take nicotinic acid [Fig. 38A]. Therefore, pharmacological agents which interfere with lipoprotein synthesis have a more pronounced effect on plasma Lp(a) levels than those that accelerate the clearance of the lipoprotein.

Figure 38A

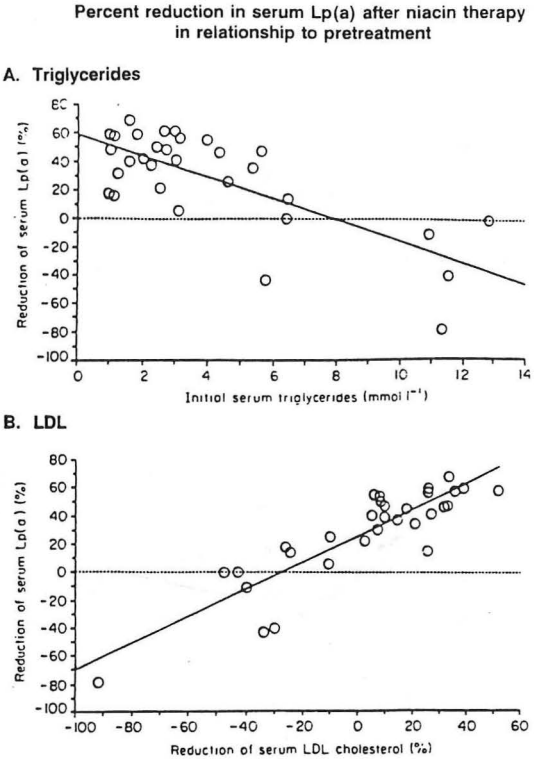
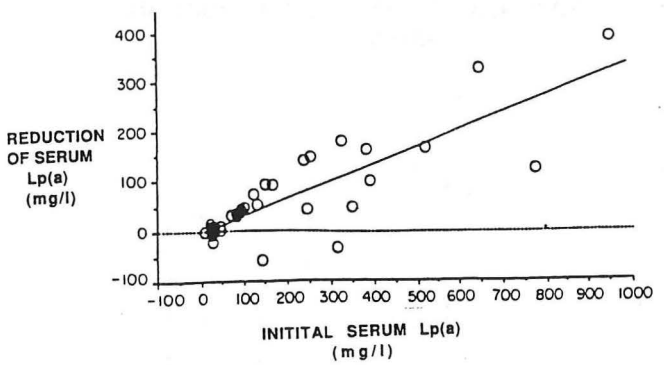


Figure 38B

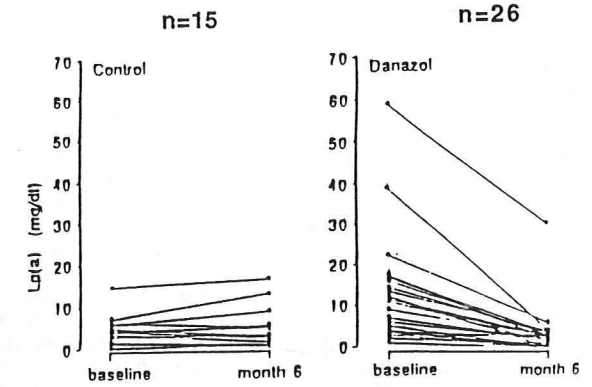


For reasons that are not clear, administration of either anabolic steroids - stanazolol and danazol - or estrogens (102-105)

[Fig. 39 & 40] is associated with up to a 50% lowering of plasma Lp(a) levels.

Figure 39

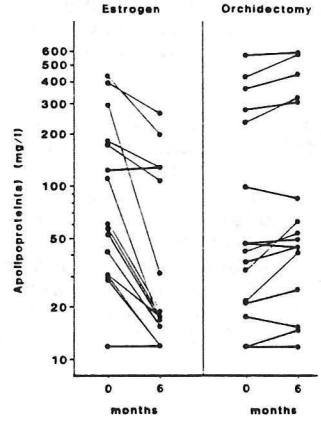
EFFECT OF DANAZOL (600mg/dl) ON PLASMA Lp(a) CONCENTRATION IN 26 PREMENOPAUSAL WOMEN WITH ENDOMETRIOSIS



Crook et al. Atherosclerosis 92:45, 1992

Figure 40

INDIVIDUAL SERUM Lp(a) LEVELS 6 MONTHS AFTER ORCHIDECTOMY OR INITIATION OF ESTROGEN THERAPY



Reducing agents have been used to dissociate apo(a) from the LDL particle in an attempt to lower plasma levels of Lp(a). When n-acetylcysteine (NAC) (MUCOMYST) was given to two patients, there was a marked reduction in plasma Lp(a) levels [Fig. 41] (106). However, a more recent study has failed to confirm this result (107). Whether or not it is advisable to use this strategy to lower plasma Lp(a) levels is not clear, since it is not known how the free apo(a) is cleared and whether it is less atherogenic than the intact Lp(a) particle.

Figure 41 **Concentrations of lipoproteins in two patients on NAC**

—	Chol	Trig	VLDL-C	LDL-C	HDL-C	LP(a)
Patient 1						
Baseline	430	117	27	375	28	60
NAC (2 g/day)	463	235	40	394	29	30
NAC (4 g/day)	463	210	20	419	24	19
Patient 2						
Baseline	193	93	16	118	59	56
NAC (2 g//day)	209	104	20	137	52	22
NAC (4 g/day)	221	74	22	139	60	14

Thus, there are only three medications which have been convincingly shown to lower the plasma level of Lp(a) in some patients - nicotinic acid, estrogens, and anabolic steroids. Since these medications all have multiple effects on the other classes of lipoproteins, it is not possible to selectively lower the plasma Lp(a) concentration without changing the levels of other lipoproteins. Therefore, it will be difficult to design a study to assess the independent effect of lowering plasma Lp(a) levels on coronary risk.

To complicate matters, there are multiple problems associated with measuring the plasma Lp(a) level. There are no universally accepted methodologies or standards for any of the assays currently used (for discussion see ref. 108). It has not been shown that the antibodies used in the assays developed to date interact to all the different apo(a) isoforms with equal affinity. The high degree of polymorphism within the apo(a) protein has made it problematic to standardize the assay.

The most sensitive and frequently used assays at the present time are enzyme-linked immunosorbent assays which employ a capture antibody and then a detection antibody (ELISA). Two general schemes are used. First, the capture antibody is frequently a polyclonal antibody to apo(a), and the detecting antibody is a mouse anti-human apo(a) monoclonal antibody. Alternatively, a polyclonal antibody is used for capture and an anti-apoB antibody is used for detection. An advantage of the latter assay system is that the Lp(a) concentrations are expressed in moles (i.e., number of particles), rather than total lipoprotein mass. The assay

available here, at both Parkland and the Aston Center, is an assay that was developed by Utermann. The first antibody is a polyclonal antibody of apo(a), and the second antibody is a monoclonal antibody to the kringle 4-encoding region. The assay is performed at Gene Screen and costs $25.00.

So, should we be measuring and then treating patients found to have high plasma concentrations of Lp(a)? Despite the numerous gaps in our knowledge and ability to treat this enigmatic lipoprotein, I think there is sufficient evidence to warrant measuring the concentration of Lp(a) in Caucasian patients with ischemic heart disease or with a strong family history of CAD. As has been reviewed, it remains controversial whether Caucasian patients who have elevated plasma Lp(a) levels and no abnormalities in their other lipoproteins should be treated with a medication to lower the plasma Lp(a) levels. If a patient with a high plasma Lp(a) is going to be initiated on lipid-lowering treatment for elevations in other lipoproteins, nicotinic acid would be preferable to the other available lipid-lowering medications. Knowledge of an elevated plasma Lp(a) level in a post-menopausal women should further encourage treatment with estrogens. Finally, in those patients found to have high plasma concentrations of Lp(a), the other known risk factors for ischemic heart disease should be aggressively treated, especially any elevations in plasma LDL levels.

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