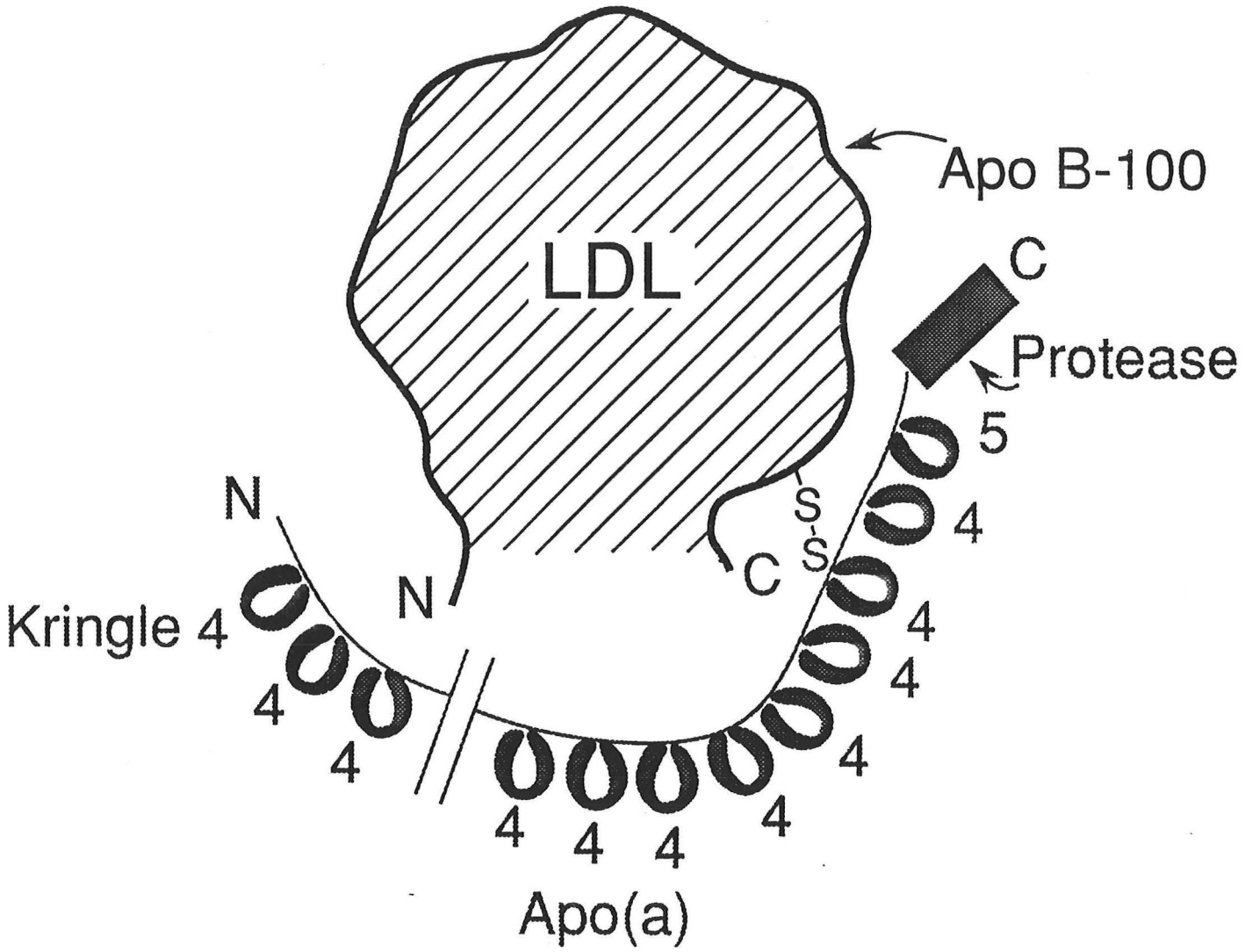
Lp(a) - TO MEASURE OR NOT TO MEASURE?



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Areas of interest: 1. Human genetics
2. Lipoprotein metabolism

This is to acknowledge that Helen H. Hobbs has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

INTRODUCTION

High plasma levels of lipoprotein(a) [Lp(a)], a cholesterol ester-rich plasma lipoprotein, are an independent risk factor for the development of atherosclerosis (1). Lp(a) is of medical interest because individuals with plasma levels over 25-30 mg/dl, which include 20% of Caucasians, have a 1.5- to 3-fold increased risk of coronary artery disease (CAD) (1). Elevated plasma levels of Lp(a) are also associated with peripheral vascular disease and cerebrovascular disease. The impetus to once again review the role of Lp(a) in human disease is the recent availability of a reliable, well-standardized assay to measure plasma levels of Lp(a) at Parkland Memorial Hospital. The specific questions that will be addressed in this protocol will be:

1. What determines plasma levels of Lp(a)?
2. What is the relationship between plasma levels of Lp(a) and cardiovascular disease?
3. In whom should we measure plasma levels of Lp(a)?
4. What are the therapeutic options for treating high plasma levels of Lp(a)?
5. What is the physiological role of this enigmatic lipoprotein?

GENERAL CHARACTERISTICS OF Lp(a)

Lp(a) was discovered in 1963 by Kare Berg in his search for antigenic differences in LDL that might exist among individuals (2). By injecting human LDL into rabbits, he developed an antiserum that reacted with an antigen present in only one-third of plasma samples tested; he called this antigen Lp(a).

Lp(a) has two components - a low density lipoprotein (LDL) particle and a large glycoprotein, apolipoprotein(a) [apo(a)], which is attached to the apoB-100 of LDL by a disulfide linkage. The lipid composition of Lp(a) is nearly identical to LDL but its density is greater due to the presence of apo(a) (d=1.050-1.100 g/L vs 1.019-1.063 g/L). Apo(a) is hydrophilic, which distinguishes it from all other apolipoproteins, which are amphipathic in structure. Apo(a) is richly endowed with N- and O-linked sugars so ~28% of its mass is carbohydrate. Lp(a) migrates between LDL and VLDL on agarose gel electrophoresis and has thus been referred to as the "sinking pre-beta" lipoprotein.

Distribution of plasma levels of Lp(a) in the population

In contrast to other lipoproteins which have a normal (bell-shaped) distribution within populations, the distribution of plasma concentrations of Lp(a) is skewed with most individuals having a low plasma concentration of Lp(a) (<10 mg/dl) (10) [Fig. 1]. In individuals of African descent, the distribution of plasma levels is relatively flat up to a plasma level of ~50 mg/dl. As a consequence, the median plasma level of Lp(a) is 2-4 fold higher in Africans than in either Caucasians or Orientals. The distribution of plasma levels of Lp(a) in Hispanics is similar to that of Caucasians, whereas Asian Indians have a distribution resembling that of African-Americans.

Another major difference between the distribution of plasma levels of Lp(a) and the other classes of lipoproteins is the greater range in its plasma values. Plasma levels of Lp(a) vary over a 1000-fold range (from 0.1 mg/dl to >100 mg/dl), whereas levels of VLDL, HDL and LDL vary over only a 3-5 fold range (3).

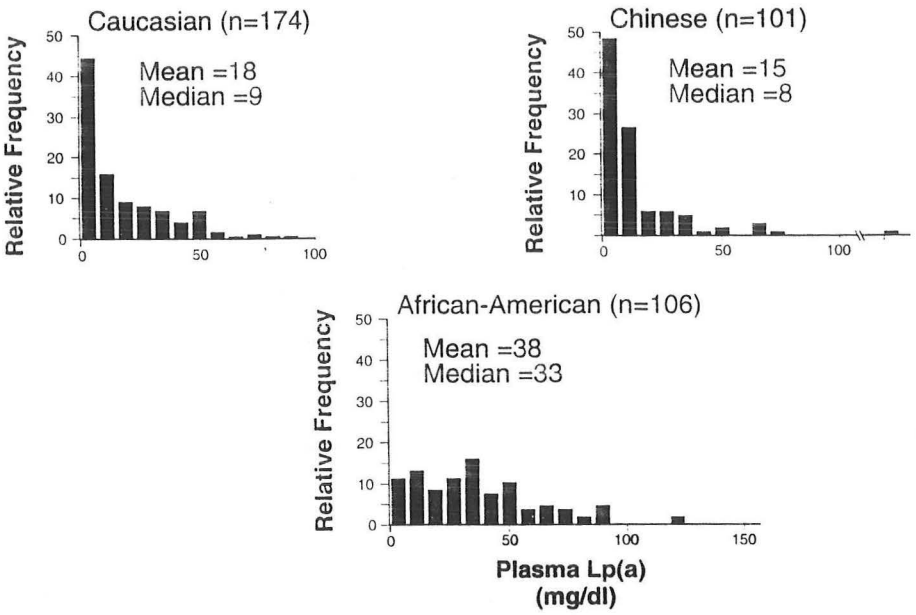


Fig. 1. Distribution of plasma Lp(a) levels in three ethnic groups.

Plasma levels of Lp(a) - relationship to levels of other lipoproteins

Lp(a) contributes modestly to the plasma concentration of cholesterol since only one-third of the total mass of Lp(a) is cholesterol ester. In a person with a plasma Lp(a) of 90 mg/dl, ~30 mg/dl of the cholesterol in his plasma circulates as part of Lp(a).

In routine fasting lipid profiles, the plasma cholesterol in Lp(a) is included in the LDL-cholesterol. If a subject has a plasma LDL-cholesterol of 150 mg/dl and Lp(a) level of 90 mg/dl, then the actual LDL-cholesterol level is 120 mg/dl, since 30 mg/dl of cholesterol in the LDL fraction is circulating as Lp(a). On average, approximately 10% of plasma apoB-100 is in Lp(a). Thus, only very high plasma concentrations of Lp(a) contribute significantly to the plasma levels of cholesterol, LDL-C and apoB-100.

Less than 5% of apo(a) circulates with triglyceride-rich lipoproteins, either chylomicrons or VLDL. Overall, there is a modest inverse relationship between plasma levels of triglyceride and Lp(a) (R = -0.15) (4), but the relationship is more pronounced (R= -0.69) in very hypertriglyceridemic individuals (TG > 400 mg/dl) (5).

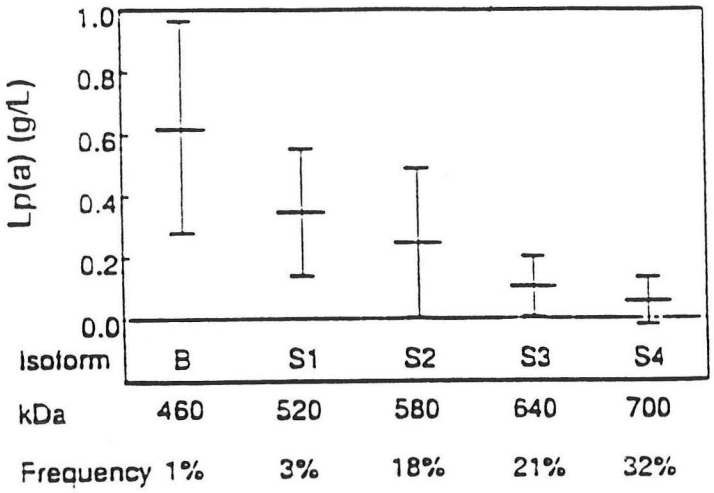
APO(a) PROTEIN AND GENE

Apo(a) contains multiple copies of a cysteine-rich protein motif called a kringle (K). Each kringle contains six cysteine residues that form three intra-molecular disulfide linkages resulting in a tri-loop structure that resembles a Danish "pretzel-like" pastry known as a kringle. This motif is found in several proteins including urokinase, tissue-plasminogen activator (tPA), prothrombin, factor XII, hepatocyte growth factor, angiostatin and plasminogen. The crystal structure of a kringle from tPA has been solved and it is an oblate ellipsoid (i.e., has a shape resembling a hockey puck). Between each kringle is a spacer consisting of a 36- residue sequence that is rich in O-linked sugars. Electron micrograph studies of Lp(a) reveal that all but last few kringles in the tandem array extend as a flexible arm away from the particle and into the aqueous phase (6).

The size of the apo(a) glycoprotein varies from ~300-800 kDa and there is an inverse relationship between apo(a) isoform size and plasma level of Lp(a) (1); the smaller apo(a) isoforms (250-500 kDa) tend to be associated with higher concentrations of plasma Lp(a) and the larger isoforms (>650 kDa) with lower plasma Lp(a) levels. The frequency distribution of apo(a) isoforms by size partially explains the skewed distribution of plasma

Lp(a) concentrations in the population [Fig. 2]. The larger isoforms, which tend to be associated with lower plasma levels of Lp(a), are more common than the smaller isoforms, which tend to be associated with higher plasma levels. The molecular mechanism responsible for the variation in apo(a) isoform size was revealed by the characterization of the apo(a) cDNA.

Fig. 2. Relationship between apo(a) isoform size and plasma levels of Lp(a).
B=migrates same distance as apoB-100
S = migrates slower than apoB-100.



Apo(a) cDNA and gene - comparison with plasminogen

The structures of the apo(a) and plasminogen cDNAs are compared in Fig. 3 (7). The two cDNAs share a high degree of sequence identity. Plasminogen has five kringles (K1-K5). The pre-activation region (T) and K1-K3 of plasminogen are not present in apo(a). Apo(a) is distinguished by having multiple copies of a 342-bp sequence that are 75-85% identical to the K4 of plasminogen. These repeats are tandemly arrayed in a head-to-tail fashion and are followed by a sequence similar to the K5 domain of plasminogen. Finally, the C-terminus of apo(a) contains a region sharing 94% sequence identity with the protease domain of plasminogen.

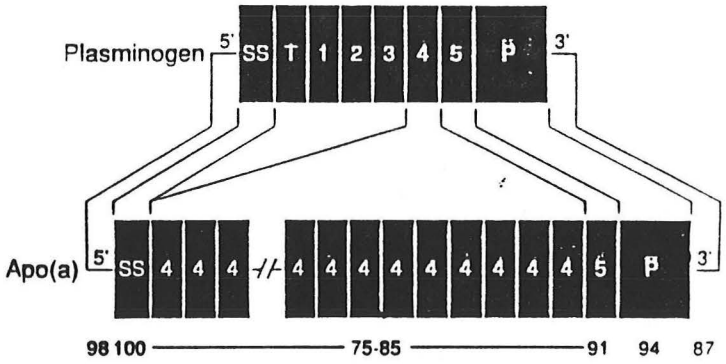


Fig. 3. Comparison of apo(a) and plasminogen cDNAs. Percent sequence identity between domains is provided.

Plasminogen circulates in plasma as a proenzyme. The kringle domain of plasminogen mediates its localization to fibrin and plasminogen binding sites in tissues. Plasminogen is cleaved by tissue plasminogen activator (tPA) at an arginine residue immediately downstream of the K5 domain, and plasmin is released. Plasmin dissolves fibrin clots and extracellular matrix components; it also activates some growth factors, including transforming growth factor β-1 (TGFβ-1). An amino acid substitution at the corresponding site in apo(a) (arginine to serine) prevents its cleavage by tPA or urokinase and apo(a) does not have any protease activity.

Size variation in apo(a) is due to variation in K4-type 2 repeats

The size variation in apo(a) isoforms is due to a variation in number of a subset of K4 repeats (8,9). Not all K4 repeats in the apo(a) genes are identical in sequence [Fig. 4]. The first K4 repeat (K4-type 1) and last eight K4 repeats (K4-types 3-10) are unique. The only K4 repeats that vary in number between apo(a) alleles are the K4-type 2 repeats and it is the variation in the number of these repeats that is responsible for the variation in size of apo(a) glycoprotein (9).

The integrity of the last six K4 repeats (types 5-10) is required for apo(a) to associate with apoB-100 (10). A single free cysteine residue in the penultimate K4 repeat, K4-type 9, forms a disulfide linkage with a cysteine residue in the C-terminal region of apoB-100, at amino acid 4326 (11,12). The last repeat (K4-type 10) is the only K4 repeat that contains all the amino acid residues predicted to confer high affinity lysine (and fibrin) binding. Lower affinity lysine binding sites are present in K4-type 5 to 8, but these sites are only accessible in free apo(a) or after mild reduction of the Lp(a) particle (13). Apo(a) binds a receptor on lipid-laden macrophage via K4-type 6 and type 7 (14). Thus, the downstream K-4 repeats are required for 1) the proper coupling of apo(a) to apoB-100, 2) to mediate lysine binding, and 3) for the uptake and degradation of Lp(a) by foam cells.

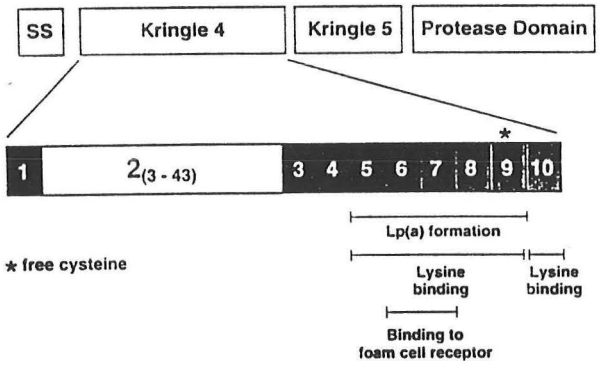


Fig. 4. K-4 repeat region of apo(a).

The apo(a) gene is one of the most polymorphic genes in man

More than 95% of individuals have two apo(a) alleles that differ in the number of K4 repeats contained within their sequence. This length polymorphism makes apo(a) as polymorphic as the repeat sequences used for DNA fingerprinting. However, in contrast to these latter sequences, the length polymorphism in apo(a) involves coding sequences. Moreover, more than 95% of apo(a) alleles of the same size differ in sequence (15). The high degree of length and sequence polymorphism in the apo(a) gene makes apo(a) one of the most polymorphic genes (and perhaps proteins) in man.

The apo(a) gene resides next to the plasminogen gene on chromosome 6q 2.6-2.7

The apo(a) gene is found ~40 kb from the plasminogen gene at the end of chromosome 6 [Fig. 5] (16). The two genes are oriented in a head-to-head fashion and are flanked by an apo(a)-like gene and a plasminogen pseudogene. The mRNA for the apo(a)-like gene is present only in the liver and, although this gene contains sequences for a series of K4 repeats, followed by a protease domain, it has a mutation in a splice donor site that changes the reading frame and creates a premature stop codon. The gene is predicted to encode a signal sequence (which is required for secretion) followed by a single K4 repeat (17), but there is no evidence that the mRNA transcript generated from this gene is translated. Presumably, this apo(a)-related gene is a pseudogene.

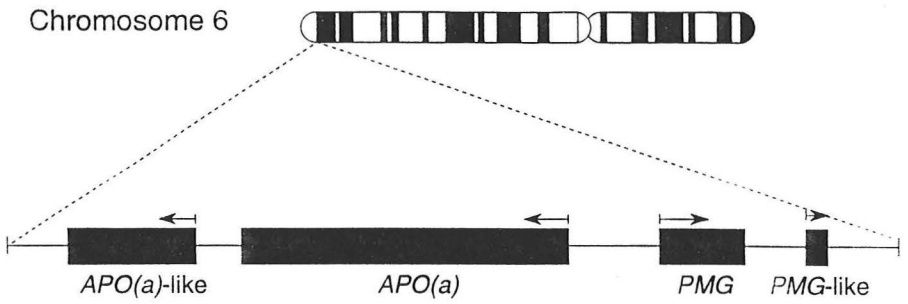


Fig. 5. Apo(a)-plasminogen gene complex on chromosome 6

GENETICS OF PLASMA LEVELS OF Lp(a)

The apo(a) gene is the major determinant of plasma levels of Lp(a)

The heritability of plasma Lp(a) levels, which includes that portion of variation in plasma levels due to shared genes and/or shared environment, is very high (h^2 = 85-95%) (1). The size polymorphism of the apo(a) gene accounts for ~40-70% of the inter-individual variation in the plasma Lp(a) concentrations in Caucasians (1,8). However, individuals with apo(a) alleles of identical size can have very different plasma levels of Lp(a) (8,18). This variation in plasma levels is much greater among individuals with smaller apo(a) alleles (18). By comparing plasma Lp(a) concentrations in sibling pairs who inherited identical apo(a) alleles from their parents, we showed most of the differences in plasma levels of Lp(a) among individuals with apo(a) alleles of the same size are due to sequence differences in the apo(a) gene (and not to the impact of other genes) (19). If the apo(a) gene is the major gene that contributes to plasma concentrations of Lp(a), then siblings who inherit identical apo(a) alleles from their parents should have similar plasma concentrations of Lp(a). If other genes are important contributors, then plasma levels of Lp(a) would also be correlated in siblings who share no apo(a) alleles in common, since siblings share ~50% of their genetic material in common. Plasma levels of Lp(a) in sibling pairs with identical apo(a) alleles have very similar plasma Lp(a) levels (r= 0.95), unlike those with no shared apo(a) alleles (r=-0.23) (19). From this analysis, we estimate that sequence differences linked to the apo(a) gene explains ~90% of the inter-individual variation in plasma concentration of Lp(a). No other gene has been identified that contributes so substantially to the variation in a quantitative trait.

This finding that the apo(a) gene is the major determinant of variation in plasma Lp(a) levels needs to be interpreted with care. This does not mean that a given apo(a) allele will result in a fixed level of Lp(a) in an invariant manner; rather it means that two siblings with the same apo(a) alleles will have Lp(a) levels within a similar range, relative to the rest of the population. The apo(a) gene determines where, within the 1000-fold range of possible Lp(a) values, a particular individual's plasma level will fall; it does not predict the exact value. For example, if two individuals inherit the same apo(a) alleles, and one has a plasma level of 1 mg/dl, the other could have a plasma level of ~0.5, 1 or even 2 mg/dl (i.e., up to a 100% difference), but will not have a plasma level of Lp(a) of 15, 50 or 100 mg/dl. Thus, the apo(a) gene is the major determinant of the relative plasma levels of Lp(a), but plasma levels of Lp(a) can vary significantly (in percentage terms) around that level.

Plasma levels of Lp(a) differ from the levels of other classes of lipoproteins in that large percent variations in levels can be of minor significance. For example, a 50% change in Lp(a) (from 1 mg/dl to 1.5 mg/dl) is not clinically significant, whereas a similar percent difference in either LDL or HDL is a major change.

METABOLISM OF APO(a) AND Lp(a)

Apo(a) is secreted independently of apoB-100

Many questions remain unanswered regarding the normal metabolism of Lp(a). The apo(a) circulating in plasma is synthesized almost exclusively in the liver (20) [Fig. 6]. Trace amounts of apo(a) mRNA are detected in adrenal glands, lung, pituitary, brain and testes of monkeys (21), but there is no evidence that the protein is made in these tissues. Lp(a), unlike LDL, does not have a triglyceride-rich precursor, and does not pass through the lipolytic cascade (22). Only a small amount (<5%) of the apo(a) in plasma is non-covalently associated with triglyceride-rich particles, either VLDL or chylomicrons (23).

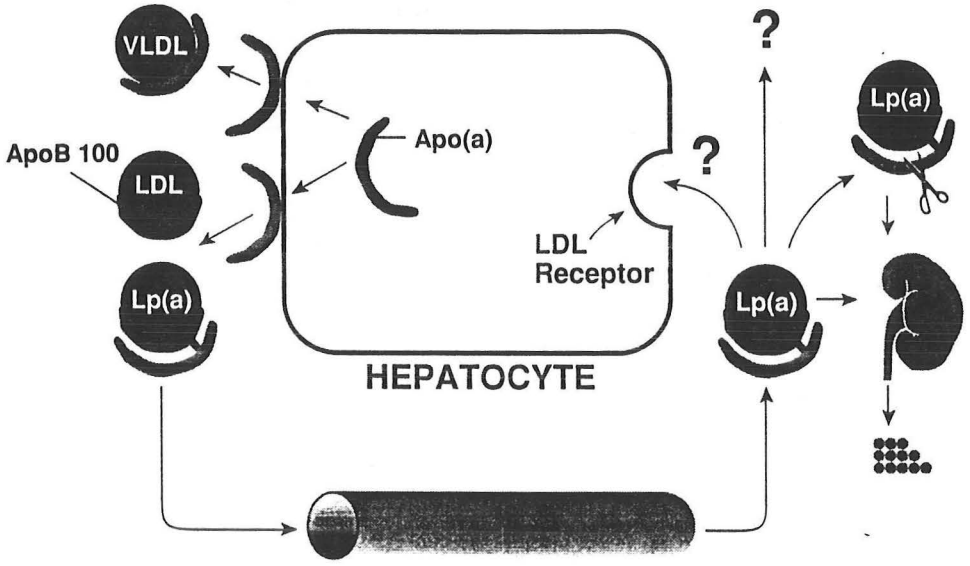


Fig. 6. Overview of the metabolism of Lp(a).

Formation of Lp(a) - a two-step process

The bulk of evidence suggests apo(a) is secreted from the hepatocyte and then attaches to LDL particles either on the surface of the hepatocyte or in the circulation (24-26). The formation of Lp(a) is a two-step process [Fig. 7]. The initial association is non-covalent and subsequently, the free cysteine in the K4-type 9 repeat forms a disulfide linkage with a cysteine residue in the C-terminal region of apoB-100. Once the disulfide bond is formed, the linkage cannot be dissociated, except by biochemical reduction.

Alterations in the conformation of apoB-100, either due to sequence variations in apoB-100 or to perturbations in the lipid composition of LDL, can interfere with the formation of Lp(a). Mutations in the C-terminal region of apoB-100, such as a missense mutation at amino acid 3500, which is responsible for familial defective apoB-100 (FDB), can adversely affect the affinity of apo(a) for apoB-100 (27). Consequently, individuals with heterozygous FDB appear to have more variation in their plasma levels of Lp(a) than is usual, perhaps due to greater sensitivity to differences in LDL particle composition (28).

Patients with lecithin:cholesterol aclytransferase (LCAT) deficiency uniformly have very low plasma levels of Lp(a). These individuals have very abnormal LDL particles since they are deficient in the formation of cholesterol esters, which comprise the hydrophobic core of LDL. Apo(a) fails to make a covalent linkage with these abnormal particles, resulting in very low plasma levels of Lp(a) (29).

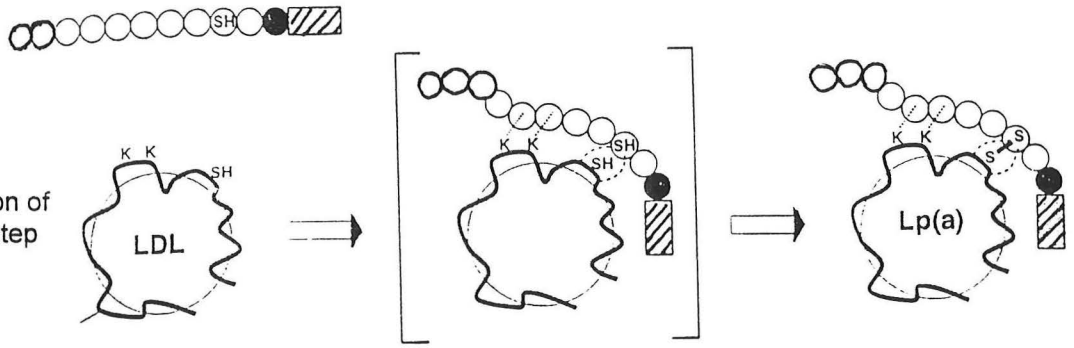


Fig. 7. Formation of Lp(a) is a two-step process (11).

Differences in plasma levels of Lp(a) are due to differences in Lp(a) synthesis

Variations in plasma levels between individuals are due to differences in the rate of apo(a) (and Lp(a)) synthesis, not its degradation (30). The inverse relationship between the size of the apo(a) protein and plasma level of Lp(a) is not due to size-dependent differences in the clearance of Lp(a) from the circulation. Individuals with apo(a) isoforms of different sizes have a similar fractional catabolic rate of Lp(a) (32). Also, individuals with apo(a) isoforms of the same sizes that have different plasma levels of Lp(a) have similar rates of clearance of Lp(a) (33).

Since apo(a) is only synthesized in the liver and is not made in significant quantities in any cultured hepatocyte cell lines, it required the development of a special *in vitro* model to examine the synthesis of apo(a). Ann White (who is in the Center for Human Nutrition) used primary cultures of baboon hepatocytes to study the biosynthesis of apo(a). The synthesis and intracellular processing of apo(a) isoforms of different sizes were compared using hepatocytes from different baboons (24,25). Apo(a) is a huge glycoprotein and its synthesis requires the formation of many disulfide bonds so it takes 30-60 minutes to achieve a fully oxidized, properly folded state (34). The movement of apo(a) from the endoplasmic reticulum (ER), where it is synthesized, to the Golgi complex, where sugars are added and modified, can be monitored by the associated increase in apparent molecular mass. The efficiency of apo(a) secretion from the hepatocyte is inversely related to the size of the apo(a) isoforms. Thus, a higher portion of the smaller apo(a) isoforms, compared to large apo(a) isoforms, is transported out of the ER to Golgi complex, and then secreted (25). This phenomenon contributes to the inverse relationship between apo(a) isoform size and plasma level of Lp(a).

The LDL receptor does not play a major role in the clearance of Lp(a) from plasma

The mechanism by which Lp(a) is removed from the circulation has not been completely defined. *In vitro* studies have shown that Lp(a) binds to (and is internalized by) the LDL receptor, although at a reduced affinity when compared to LDL (1). Transgenic mice expressing high levels of human LDL receptors rapidly catabolize Lp(a) (35). However, mice have very low plasma levels of LDL, so there are few particles that compete with Lp(a) for uptake via the LDL receptor. In contrast, humans have >20-fold more LDL than Lp(a) particles, as well as numerous apoE-containing lipoproteins, which have an even higher affinity for the LDL receptor, and all these particles compete for uptake. Lp(a) turnover studies in individuals with homozygous familial hyperchol-esterolemia (FH) (who have little to no functional LDL receptors) reveal that Lp(a) is cleared from the plasma at a rate that is indistinguishable from normal controls (36) [Fig. 9]. Administration of HMG-CoA reductase inhibitors, which increase hepatic LDLR activity, does not affect plasma levels of Lp(a), indicating, again, that the LDL receptor does not play a major role in Lp(a) clearance from plasma (37).

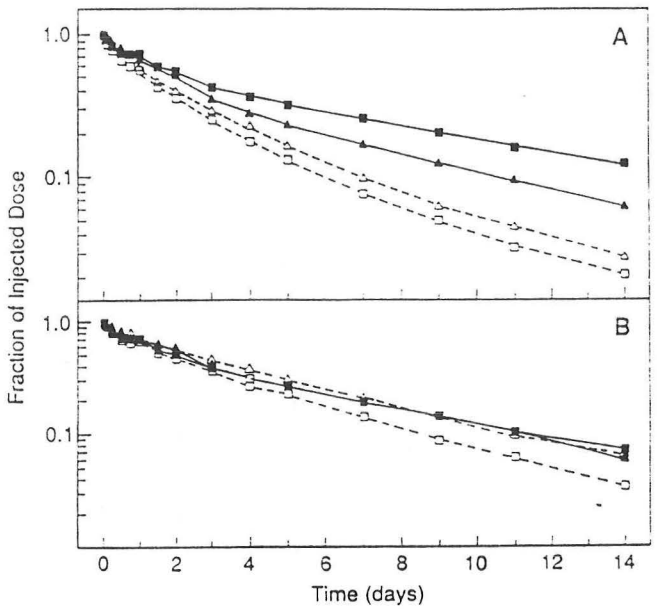


Fig. 8. Catabolism of ^{131}I-LDL (top)and ^{125}I-Lp(a) (bottom) in FH heterozygotes (■▲)and in normal controls (□△).

Do individuals with FH have higher plasma levels of Lp(a)?

Although the LDL receptor, at most, plays a minor role in removing Lp(a) from plasma, it is still controversial whether individuals with FH have higher plasma levels of Lp(a), perhaps due to an effect on Lp(a) production. An initial report suggested heterozygous FH was associated with 2- to 3-fold higher plasma levels of Lp(a) (38). However, the FH patients in this study were obtained from lipid clinics whereas the non-FH controls came from the general population. This study is flawed by selection bias because FH subjects with high plasma levels of Lp(a) are more likely to be seen in a lipid clinic since elevated plasma levels of Lp(a) contribute to CAD. When plasma levels of Lp(a) are compared within families, no significant differences are found between the FH and non-FH relatives (39, 40, Miserez and Hobbs, unpublished observations). Thus, it appears that mutations in the LDL receptor are not associated with a systematic increase in plasma levels of Lp(a).

FH homozygotes appear to have higher plasma levels of Lp(a), although sampling bias may also contribute to this observation (40).

Proteolytic processing of Lp(a)

Between 10%-25% of plasma Lp(a) is converted to LDL by removal of apo(a) and the LDL receptor clears the Lp(a)$^-$ particles (36,42). We have shown that some apo(a) on Lp(a) undergoes proteolytic cleavage in the circulation by an unknown mechanism (43,44). The apo(a) protein is cleaved between the K4-type 4 and K4-type 5 and a series of K4-containing fragments are released from the NH_2-terminus of apo(a). These fragments circulate in the plasma and are excreted into the urine. Exactly how they enter the urine is a mystery since their large size (85-215 kDa) and high sialic acid content (and resultant negative charge) likely precludes their entry via glomerular filtration. Even individuals with no detectable Lp(a) in plasma have apo(a) in the urine [Fig. 9], suggesting that these kringle-containing fragments may be biologically important. The release of kringle-containing fragments from apo(a) resembles the generation of angiostatin from plasminogen by elastase cleavage [Fig. 10] (45). Angiostatin, which consists of K1-3 of plasminogen, inhibits capillary proliferation in tissue culture and angiogenesis *in-vivo* (45). Elastase may also be responsible for the proteolytic cleavage event that releases apo(a) fragments from Lp(a) (46). Although the apo(a) fragments resemble angiostatin in structure, they do not affect blood vessel formation, at least in tissue culture studies (unpublished observations).

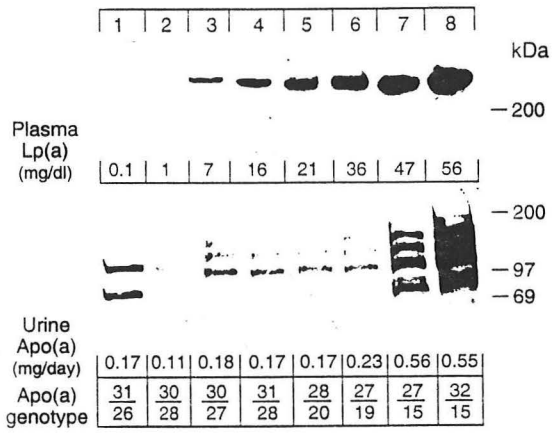


Fig. 9. Apo(a) immunoblot analysis of plasma and urine from eight individuals (43).

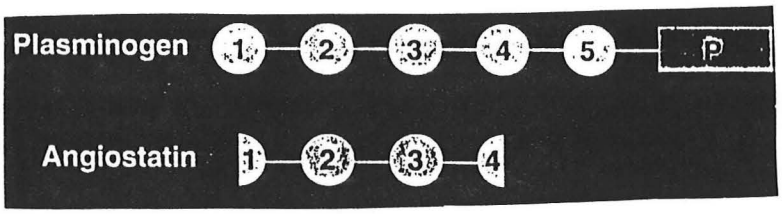


Fig. 10. Angiostatin is derived from kringle domain of plasminogen. 1-5=kringles; P= protease domain

FACTORS THAT MODIFY PLASMA LEVELS OF Lp(a)

Problems associated with comparative studies of plasma Lp(a) levels

The literature is riddled with contradictory papers regarding the effect of various diseases, drugs and physiological perturbations on plasma levels of Lp(a). Some causes of the confusion in the Lp(a) literature are as follows:

1. Sample storage: Prolonged storage of plasma samples yields spuriously low plasma levels of Lp(a). Plasma levels decrease by ~37% if kept at -20°C for three years and ~20% if maintained at -70°C (47). Therefore, comparisons of plasma levels of Lp(a) in samples obtained at different time points can be problematic, even if they are measured in the same assay.

2. Lp(a) quantification: The assays used to measure plasma levels of Lp(a) are not standardized and most utilize antibodies that do not react with all isoforms of apo(a) with equal affinity. In addition, some assays are not specific for Lp(a) (to be discussed in detail later).

3. Population heterogeneity: Due to the marked differences in the frequency distribution of plasma Lp(a) levels in different ethnic groups, study subjects should not be from different racial populations, unless each subject serves as its own control. Samples should not include related individuals due to the major role of genetics in defining plasma levels. Post-menopausal women have higher plasma levels of Lp(a), so it is important to control for the estrogen status of women.

4. Sample size: Sample sizes are often too small to reliably reflect the degree of polymorphism in the apo(a) protein and the wide range of plasma Lp(a) levels in the population. Observed differences between mean plasma levels of Lp(a) between groups may be due to the chance inclusion of a few individuals with very high or very low plasma levels of Lp(a) in one sample. If small samples are used, then intra-individual comparisons of plasma levels of Lp(a) should be shown.

Some investigators claim that if no significant difference in the frequency of apo(a) isoforms (by size) is found between two samples, any observed differences in plasma levels of Lp(a) are due to the effect of what they are testing. This is not necessarily true. As alluded to previously, apo(a) isoforms of the same size can be associated with a 1000-fold difference in plasma levels of Lp(a).

5. Statistical analysis: The statistical analyses of Lp(a) studies are frequently flawed. Comparisons of mean plasma levels of Lp(a) are often misleading, especially in small

samples. Since plasma levels of Lp(a) are not normally distributed, comparisons should be made between median, rather than mean plasma levels of Lp(a). Nonparametric statistical methods should to be used to compare plasma levels of Lp(a) between groups.

Plasma levels of Lp(a) remain relatively stable in healthy individuals

Plasma levels of Lp(a) are low at birth and increase over the first few months of life, reaching adult levels within two years (49). Breast milk has a higher fat content than formula, so it is not surprising that babies maintained on breast milk have a higher plasma level of LDL. But what is surprising is that breast-fed babies have ~40% lower plasma levels of Lp(a) than bottle-fed counterparts, and that weaning is associated with a significant increase in plasma Lp(a) levels (50). In adults, plasma levels of Lp(a) are not significantly affected by the usual dietary differences in fat composition and content (3), suggesting that some factor(s) in breast milk acts to lower plasma Lp(a) levels, or conversely, that a component in formula causes an increase in plasma levels.

In adults, changes in body weight or moderate exercise do not significantly affect plasma concentrations of Lp(a) (although very vigorous exercise has been associated with a decrease in plasma Lp(a) levels in one study and an increase in another!). Unlike plasma concentrations of LDL, plasma levels of Lp(a) do not differ significantly between men and women and do not increase significantly with age, except in post-menopausal women, who have higher plasma levels of Lp(a) (1).

Elevated plasma levels of Lp(a) in post-menopausal women and the Lp(a)-lowering effect of estrogen

Menopause is associated with a 15-30% increase in plasma levels of Lp(a) and replacement doses of oral estrogen reverses the increase (51). The addition of progesterone slightly attenuates the Lp(a)-lowering effects of estrogen [Fig. 11]. Intramuscular injection of estrogen does not affect plasma levels of Lp(a) despite reducing plasma LDL-C and increasing HDL-C levels (52). When estrogens are given orally, they are delivered directly to the liver, so the liver is exposed to a much higher level of estrogen than if the drug is given peripherally. The concentration of estrogen in the liver that is required to lower plasma levels of Lp(a) must be higher than that needed to either increase plasma HDL-C or lower plasma LDL-C. Transdermal estrogen lowers plasma levels of Lp(a), but not as much as oral estrogens (16% vs. 31%) (53).

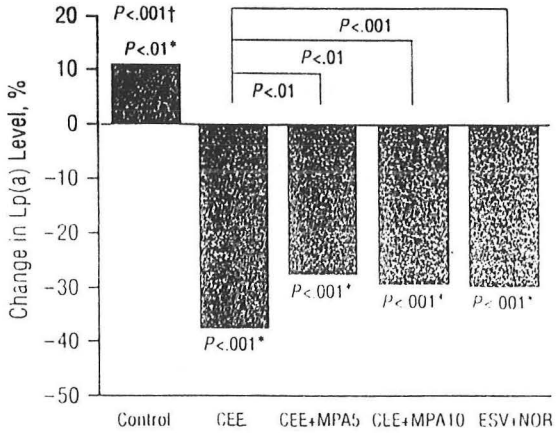


Fig. 11. Decreases in plasma Lp(a) levels after 12 months on .625 mg conjugated equine estrogen (CEE) +/- medroxyprogesterone (MPA)

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Tamoxifen, an estrogen receptor agonist/antagonist, lowers plasma levels of Lp(a) up to a 50% (54).

Androgens lower plasma levels of Lp(a)

Androgens have a variable effect on plasma levels of Lp(a). Orchiectomy results in a ~20% increase in plasma Lp(a) (55). Replacement doses of testosterone in hypogonadal men are not associated with any significant changes in plasma levels of Lp(a) (56), but when supplemental testosterone (250 mg every week) is given to normal men, there is a 20% fall in plasma Lp(a) (57); this fall in plasma Lp(a) levels may in part be due to conversion of some testosterone to estradiol. However, administration of anabolic steroids, which cannot be converted to estrogens is associated with an even more profound Lp(a)-lowering (58) effect, as will be discussed later.

Thyroid hormonal status and Lp(a)

Plasma levels of Lp(a) are modulated by thyroid hormone. Treatment of hypothyroidism is associated with a fall in plasma levels of Lp(a) (59) whereas treatment of hyperthyroidism results in a 30-60% increase in plasma levels of Lp(a) (60). Normalization of plasma Lp(a) levels after treatment for hyperthyroidism requires 4-6 months (61).

Growth hormone (GH) administration raises plasma levels of Lp(a)

Acromegalics have significantly higher plasma levels of Lp(a) (62). There is a dose-dependent increase in plasma levels of Lp(a), as well as fall in plasma LDL-C levels, associated with GH administration. Thus, unlike estrogen or thyroid hormone, which have similar effects on plasma levels of LDL and Lp(a), GH raises plasma Lp(a) despite up-regulating hepatic LDL receptors and lowering plasma concentrations of LDL (63,64).

It is not known how GH increases plasma levels of Lp(a). Many effects of GH are mediated through IGF-1. Ironically, IGF-1 administration results in a fall, rather than increase in plasma Lp(a) levels (65). IGF-1 does not affect plasma LDL-C levels (65).

Elevated levels of Lp(a) in renal failure: evidence some Lp(a) is cleared by the kidney

Chronic renal failure is associated with increased plasma levels of Lp(a) (66), but only in those subjects with large-sized apo(a) isoforms (67). Patients on hemodialysis have 1.5 to 3-fold higher plasma Lp(a) levels than controls (66,67); subjects on peritoneal dialysis have even higher plasma Lp(a) concentrations (68). The elevations in plasma levels of Lp(a) in subjects on peritoneal dialysis are directly related to the loss of albumin associated with peritoneal dialysis, and not to the plasma levels of albumin (68).

Part of the reason renal failure is associated with higher plasma levels of Lp(a) is because the kidneys play a role in its clearance. Dieplinger and his colleagues measured plasma Lp(a) levels in the ascending aortas and renal veins of 100 subjects and determined the difference in levels of Lp(a) across the kidney. After correcting for hemoconcentration, they found that ~10% of the apo(a) entering the kidney is cleared by the organ (69).

Whether the higher plasma Lp(a) levels contribute to vascular disease in subjects with renal failure remains to be conclusively shown.

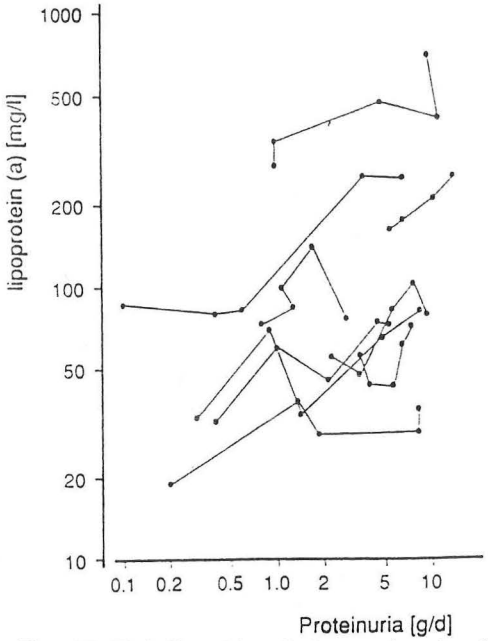

Fig. 12. Relationship of plasma levels of Lp(a) to 24 h urinary excretion of albumin in individual patients (71).

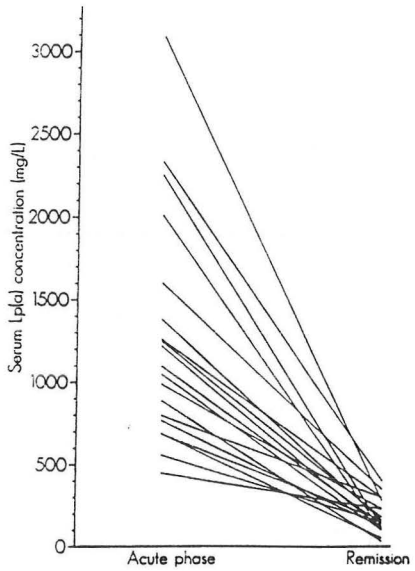

Fig. 13. Fall in plasma Lp(a) associated with treatment of proteinuria (72).

Elevated plasma levels of Lp(a) in nephrotic syndrome

Plasma levels of Lp(a), as well as LDL and VLDL, are increased in subjects with nephrotic syndrome (70). Nephrotic syndrome is associated with an increase in LDL and VLDL production, which is proportional to the increase in plasma levels of Lp(a), suggesting there is also an increase in Lp(a) production (71). As is the case for peritoneal dialysis, the amount of the increase in plasma Lp(a) level is directly related to urinary excretion of albumin [Fig. 13] (70). Successful reduction in proteinuria using an angiotensin converting enzyme inhibitor, or other therapy, is associated with a dramatic fall in plasma levels of Lp(a) [Fig. 14] (72).

The role of dyslipidemia in the progression of renal disease remains controversial. Lp(a) binds with lower affinity than LDL to mesangial cells but with higher affinity to the extracellular matrix component of the mesangium (73). Immunoreactive Lp(a), as well as LDL, is easily detected in the mesangium of kidneys from patients with glomerulosclerosis (74), but it is not known if deposition of these lipoproteins is a cause or effect of the renal disease.

Subjects with mild to moderate essential hypertension who develop end-organ damage have been reported to have higher plasma levels of Lp(a) (75). However, hypertensive individuals with more end-organ damage also have more renal insufficiency, which independently increases plasma levels of Lp(a). In another study (76), subjects with hypertension and CAD had higher plasma levels of Lp(a) than those without CAD, but again, no information is provided regarding renal function. A prospective study is needed to determine if high plasma levels of Lp(a) influence the course of hypertension-associated end-organ damage.

Renal transplantation results in a fall in plasma levels of Lp(a)

Plasma Lp(a) levels fall more than 50% within one week of renal transplantation and the fall is proportional to the improvement in renal function (77). Reductions in plasma levels of Lp(a) are more pronounced in individuals with larger apo(a) isoforms (77). Initially, it was thought that cyclosporin may contribute to the Lp(a)-lowering effect of renal transplantation, but longitudinal studies in cardiac transplant subjects fail to show any changes in plasma Lp(a) levels associated with cyclosporin administration over a six month period (78).

Diabetes has almost no effect on plasma levels of Lp(a)

Early reports suggested plasma levels of Lp(a) were elevated in poorly-controlled diabetics. Unfortunately, many of these reports were flawed by selection bias and by the relatively small numbers of subjects studied (for review, see ref. 79). Subjects with significant renal insufficiency and proteinuria, which are both associated with elevated plasma levels of Lp(a), were included in many of these studies. Improved diabetic control in Type I diabetes is associated with a small, but significant drop in the plasma concentration of Lp(a) (80). In adult-onset diabetics, there is no correlation between plasma levels of Lp(a) and diabetic control (79). Microalbuminuria appears not to be associated with a significant increase in Lp(a) (81).

Lp(a) undergoes glycation in diabetics. In normal individuals, ~1-2% of Lp(a) is glycated, but in diabetics this increases to 4-7% (82). The relationship between the amount of glycated Lp(a) in plasma and vascular disease has not been investigated.

Liver disease is associated with decreased plasma levels of Lp(a)

Since all the Lp(a) that circulates in plasma is synthesized in the liver, it is not surprising that severe liver disease and acute viral hepatitis are both associated with reduction in plasma Lp(a) levels in proportion to the severity of the disease (83,84).

Is Lp(a) an acute phase reactant?

A number of investigators have suggested Lp(a) may be part of the acute phase response. The results of these studies are contradictory, probably because some assays used to measure Lp(a) have not been specific. One study found that plasma levels of Lp(a) increase to twice the baseline levels within 11 days after a myocardial infarction, and remained elevated for up to three months (85). Another found no significant change in plasma levels of Lp(a) in the 42 days following a myocardial infarction (86); in these same patients there was a 7-fold increase in C-reactive protein by day three (86). Thus, the time course of the increase in plasma level of Lp(a) after major stress is not typical for an acute phase response protein. Moliterno and Cohen, at this institution, found no change in plasma Lp(a) levels associated with coronary artery bypass surgery up to 24 hours after the operation (87). A recent study confirmed these results but extended the time points of sampling and found a significant decrease (54%) in plasma levels of Lp(a) three days after the operation, mimicking the decrease in plasma LDL-C (87a).

Some of the confusion regarding plasma Lp(a) levels in acute stress may be due to the plasma levels of this lipoprotein being less stable after major stress. Additionally, much larger studies need to be performed that follow intra-individual changes in plasma Lp(a) levels to resolve the conflicting data regarding the effect of acute stress on plasma level of Lp(a).

Plasma levels of Lp(a) are elevated in rheumatological disorders

Subjects with rheumatoid arthritis and systemic lupus erythematosus tend to have increased plasma levels of Lp(a) (88,89). Patients with Behcet's disease have 25% higher plasma levels of Lp(a) when the disease is active than when it is quiescent (90). It has been suggested that high plasma levels of Lp(a) may predispose to arterial and venous thrombosis in these disorders, although this has not been convincingly demonstrated.

Individuals with lupus who have antiphospholipid antibodies (APL) had elevated plasma levels of Lp(a) in some (91,92), but not all studies (93). The subset of individuals with APL antibody syndrome who have arterial thrombosis may have higher plasma levels of Lp(a)

(94), although, again, the number of subjects analyzed has been small. Interestingly, it has been shown that β_2-glycoprotein1 (or apoH), which is part of the epitope for APL antibody binding to cardiolipin (95), binds the common K4 repeat of apo(a) (96).

Ethnic differences in plasma levels of Lp(a) - Why do blacks have higher plasma levels of Lp(a)?

As discussed previously, different ethnic groups have significantly different distributions of plasma Lp(a). Shown in Figure 14 are the relative median plasma levels of Lp(a) in different ethnic groups. Individuals of African descent have 2-4 fold higher median plasma levels of Lp(a) than Caucasians [Fig. 14].

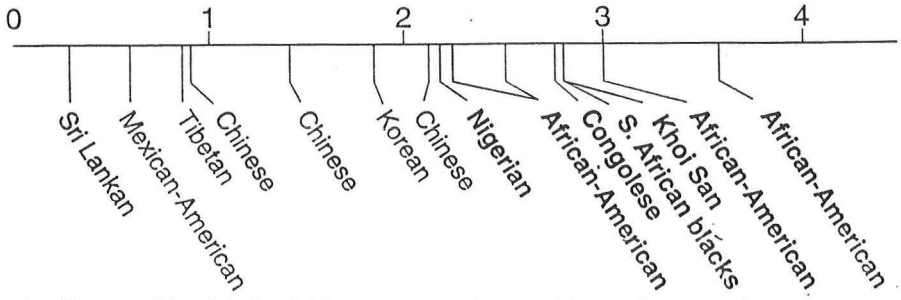

Fig. 14. Ratios of median plasma levels of Lp(a) in different ethnic groups compared to Caucasians.

The higher plasma concentrations of Lp(a) in African-Americans than Caucasians are not due to differences in the size distribution of apo(a) isoforms (1,97). In African-Americans, like Caucasians, the apo(a) gene is the major determinant of inter-individual differences in plasma Lp(a) levels (98), although it accounts for somewhat less of the variability in plasma levels (85% vs. 91%). We have found no molecular evidence for the existence of a common ethnic-specific sequence variant in the apo(a) gene that is responsible for the higher plasma levels in individuals of African descent. We suspect that the higher levels of plasma Lp(a) are due to a *trans*-acting factor that drives either transcription or translation of the apo(a) gene or, alternatively, increases the efficiency of its secretion. African-Americans have higher levels of GH (99), and since GH increases plasma Lp(a) levels, this may contribute to the higher levels in African-Americans.

It is of interest that despite having higher plasma levels of Lp(a), Africans do not have an increased incidence of CAD, a seeming contradiction that will be discussed presently.

Lp(a) AND ATHEROSCLEROTIC DISEASE

Hundreds of studies have been performed to examine the relationship between plasma levels of Lp(a) and atherosclerotic disease. Due to time constraints, I will focus on cardiovascular disease, although the evidence that high plasma concentrations of Lp(a) are associated with peripheral, as well as cerebrovascular disease is equally compelling (100-102). Valentine, at this institution, has shown that high plasma levels of Lp(a) is a common finding in men with premature symptomatic peripheral vascular disease (102).

Apo(a) is present in the atherosclerotic lesion

Immunoreactive apo(a) is easily detected in fatty streaks and fibrous plaques, but not in non-atherosclerotic vessels (103-105). Apo(a) co-localizes with apoB within vascular lesions (103,104) and the amount of apo(a) in lesions is directly proportional to the plasma levels of Lp(a) (103). Lp(a) appears to accumulate preferentially to LDL in atherosclerotic lesions; the ratio of apo(a) to apoB is ~2-3 fold higher in lesions than in plasma (104).

Pathological evaluation of cerebral vessels from the circle of Willis demonstrates that

most of the apo(a) in the early plaque is found within endothelial cells (105). This suggests that initially Lp(a) may traverse the endothelium and enter the lesion by vesicular transport; the VLDL receptor, is expressed at high levels in capillaries and small arteries (106) and binds Lp(a) *in vitro* (107) so may mediate this transport. In later, more mature atherosclerotic lesions, the apo(a) is predominantly located extracellularly within the thickened intima.

Apo(a) binds with high affinity to many components of the subendothelial extracellular matrix, including fibrin, proteoglycans, glycosaminoglycans, fibronectin, tetranectin, and collagen. Lp(a) binding to fibrin is enhanced with the addition of homocysteine, or other reducing agents, probably by exposing the lower affinity lysine binding sites located in the downstream K4 repeats. Apo(a) is so tightly bound to tissues that only 20% can be extracted unless strong denaturing agents are employed; this is in contrast to apoB, which can be easily extracted from lesion using simple buffers (108). Fragments of apo(a), similar in size to those found circulating in plasma, are present in atherosclerotic lesions (108). Lesions also contain intact, oxidized Lp(a) particles, which are ligands for the macrophage scavenger receptor (109).

Cross-sectional and retrospective studies - plasma levels of Lp(a) are 1.5- to 3- fold higher in subjects with CAD

The same problems that plague other studies of Lp(a) are also common in the retrospective and cross-sectional studies that have examined its relationship to CAD: problems with sample storage, the Lp(a) assay, small sample size, selection bias, mixing of ethnic groups, and poor statistical methodology. Some investigators repeatedly re-analyze the data using increasingly high thresholds of plasma Lp(a) to classify individuals as "high" or "low" until a significant difference is found between groups, which can be very misleading. Most of the studies consist of samples culled from catheterization laboratories, lipid clinics or other selected groups, which often bias the results. Many of these studies fail to take into account the problem of survival bias. Moreover, plasma levels of Lp(a) are often measured during an acute illness, which may influence their level.

Despite these many shortcomings, almost all the cross-sectional and retrospective studies of Caucasian men have found that plasma levels of Lp(a) greater than the 80th percentile (over 25-30 mg/dl) are associated with a 1.5- to 3-fold increase in risk of coronary atherosclerosis (for review, see ref. 1). High plasma levels of Lp(a) are a common lipid abnormality in familial premature CAD (116). Not only are high plasma concentrations of Lp(a) associated with the presence of CAD, but also its severity (111). This association is independent of the contribution of other lipoproteins (112), and the effect is greater in individuals <60 years of age (113). In fact, high plasma levels of Lp(a) may not be associated with CAD in the elderly.

It is still not clear if high plasma levels of Lp(a) increase the risk of restenosis after angioplasty or bypass surgery. In many of these studies, only the individuals who became symptomatic after the cardiac procedure were re-catheterized. Many of the studies are too small (114) and not analyzed properly. Most, but not all, of these studies suggest there is an association between restenosis and Lp(a), but definitive proof awaits the performance of better-designed and executed studies.

Prospective studies and Lp(a)

Although the results of cross-sectional and retrospective studies strongly implicate high plasma levels of Lp(a) as an independent risk factor for CAD, the results can be

misleading, due to selection bias, survival bias and the possibility that the disease itself affects levels of plasma Lp(a). The relationship between high plasma levels of Lp(a) and CAD has been more variable in prospective studies, and this has generated significant controversy. In 1993 the Physician's Health Study, a large (n=14,916) prospective study, found no increase in plasma levels of Lp(a) in the 296 physicians who had a cardiac event (115). Nor was any increase in plasma Lp(a) levels found in the 198 physicians who had a stroke in a 7.5-year period (116). Ensuing editorials appeared with titles such as "Has Lipoprotein "Little"(a) Shrunk?" (117) and "Lipoprotein(a): Important Risk Factor or Passing Fashion" (118). The pendulum has swung back in favor of plasma Lp(a) levels being an important risk factor for CAD, since an additional seven prospective studies, some of which are strongly positive, have been published. All the prospective studies examining the effect of high plasma levels of Lp(a) on the incidence of coronary events are listed in Tables 1 and 2.

Table 1. Prospective studies finding no relationship between plasma Lp(a) excess and CHD risk

Study, y	Mean/Range of Follow-up, y	Cases (m/f)		No. of (95% CI)	Age, y RR Limitations
Helsinki, 1991* (hyperchol.)	5	138/	40-55	1.3 (0.8-2.0)	Assay problem[1] Parametric statistics
Guernsey, 1992*	~5-8	/51	40-64	2.1 (1.0-4.7)	Assay problem[1] Vague definition or CHD No follow-up
Physician's Health, 1993*	5.2	296/	40-84	1.1 (0.7-1.8)	Aspirin use
North Korelia, 1994*	9	134/131	40-64	1.01 (0.88-1.16)	Assay problem[1]
Uppsala, 1996*	19	180/	50	---	Some lost to follow-up Immunoradiometric assay

[1]samples stored at 20°C; immunoradiometric assay used
*no CHD at baseline

Adapted from Stein et al. *Arch.Intern.Med.* 157:1172

Table 2. Prospective studies showing a relationship between plasma Lp(a) excess and CHD risk*

Study, y	Mean follow -up (y)	No. of Cases(m/f)	Age, y (95% CI)	RR	Comments Regarding Risk	Limitations
Gothenberg, 1980	6	26/	50	...	Inconsistent resultsamong quartiles	Sample size too small
Reykjavik, 1992	8.6	104/	45-72	1.2	Similar to apoB, systolic BP Weaker than chol	Assay problem[1]
LRC-CPP T, 1994 (LDL>190)	7-10	233/	35-59	1.1 (1.0-1.2)	Similar to LDL (RR=1.25),age Weaker than smoking	
BUPA, 1994 (premature CAD or positive fam. hist.)	5-12	229/	35-64	2.3 (1.5-3.6)	Similar to apoAI Weaker than apoB	
GRIPS, 1994*	5	107/	40-60	2.4 (1.5-3.9)	Weaker than LDL, fam. hist., fibrinogen and HDL	
Framingham, 1996*	15.4	129/	20-54	1.9 (1.2-2.9)	Similar to LDL, HDL, diabetes Weaker than smoking	Qualitative assay: Sensitivity 42.5% Specificity 93.3%
Framingham, 1994*	12	/174	45±14	1.6 (1.3-2.3)		" "

*no CAD at baseline
[1]samples stored at -20°C; radioimmunometric assay used

Adapted from Stein et al. *Arch.Intern.Med.* 157:1172

The GRIPS study (see Table 2) extended the follow-up (from 5 to 10 years total) of the 5790 men (age 40-59.9 years) who entered the study at baseline without CAD (120). The endpoints for analysis included myocardial infarction (n=299) and/or sudden coronary death (n=25). Multivariate analysis revealed that the plasma LDL-C level was the best predictor of risk (by a landslide), followed by a positive family history of CAD, and then the plasma level of Lp(a). Shown in Figure 15 is the relationship between the plasma LDL-C, Lp(a) levels and family history. For any given level of plasma LDL, a high plasma level of Lp(a) increased the risk of CAD by ~2-fold (126). As expected, age, blood pressure and a very low HDL-C (<35 mg/dl), were also good predictors; high plasma levels of VLDL-C had no predictive value.

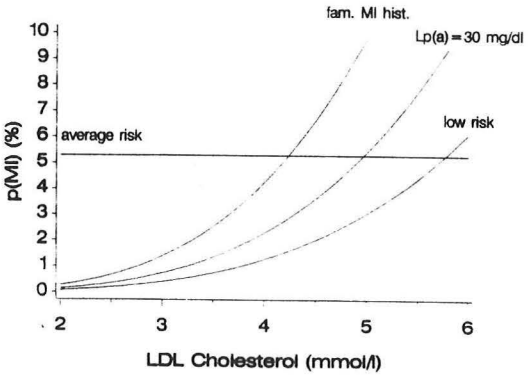

Fig. 15. Ten year probability of MI in low risk individuals (i.e., no other risk factors); potentiation of effect of high plasma LDL-C on risk in individuals with a family history of CAD or an elevated plasma level of Lp(a) [>30 mg/dl].

Are individuals with a high Lp(a) and high LDL at increased risk?

The results of the GRIPS study suggest that high plasma concentrations of Lp(a), when coupled with elevated plasma levels of LDL, are particularly atherogenic. Armstrong was the first to recognize the marked increase in risk associated with high plasma levels of LDL and Lp(a) (121). He measured plasma levels of Lp(a) in a sample of 428 Caucasian men (age 40-60) with CAD documented by angiography, as well as 142 individuals who had no evidence of CAD (137). The odds ratio of having CAD when the plasma level of Lp(a) was more than 30 mg/dl was 2.7 higher that when the plasma level of Lp(a) was less than 5 mg/dl. The odds ratio was significantly influenced by the plasma level of LDL. In individuals with plasma Lp(a) concentrations >30 mg/dl, the odds ratio fell to ~1.5 if the LDL-cholesterol level was less than the median, but if the plasma LDL-cholesterol was greater than the median, the odds ratio jumped from 2.7 to 6.33. In contrast, smoking, hypertension, and a low HDL did not affect the odds ratio.

Other studies have confirmed that the combination of high plasma levels of Lp(a) and LDL is especially atherogenic (122). Moreover, subjects with FH and CAD have significantly higher plasma Lp(a) than FH patients without clinical evidence of CAD in some studies (123-125). In one study, however, in which the patients were matched for age and sex, no significant relationship was found between plasma levels of Lp(a) and the presence or absence of CAD in individuals with FH (126). Thus, the evidence is suggestive but not conclusive that elevated plasma levels of Lp(a) is a poor prognostic indicator for ischemic heart disease in patients with FH.

The observation that the risk of CAD associated with high plasma levels of Lp(a) is greater in individuals who also have high plasma levels of LDL-C, suggests that Lp(a) may play a role later in the atherosclerotic process. High plasma levels of LDL may be required for initiation of lesions, with Lp(a) subsequently augmenting the atherosclerotic process.

Why don't African-Americans have more CAD since they have higher levels of Lp(a)?

A paradox in the relationship between plasma concentrations of Lp(a) and coronary atherosclerosis is the fact that the median plasma Lp(a) level is ~2-4-fold higher in Africans and African-Americans and yet African-Americans do not have an increased incidence of CAD. A possible explanation is that there may be ethnic differences in the distribution of apo(a) alleles. That is, within the African-American gene pool there may be apo(a) alleles that generate a high concentration of plasma Lp(a) but do not contain the epitope that make apo(a) atherogenic. Alternatively, there may be another ethnic-specific factor which protects African-Americans from the atherogenic effects of Lp(a).

Santica Marcovina has suggested another possible explanation. If the atherogenicity of Lp(a) is related more to the size of the apo(a) isoform than to its plasma levels, as some have suggested (1), the similar incidence of CAD in blacks and whites may be due to the fact they have a similar proportion of small (atherogenic) apo(a) isoforms. The small apo(a) isoforms (<16 K4 repeats) in both backs and whites are associated with high plasma levels of Lp(a) [Fig. 16] (127). The two groups also have a similar proportion of large isoforms, which are invariably associated with low plasma levels of Lp(a). What distinguishes African-Americans from Caucasians are the higher plasma levels of Lp(a) associated with apo(a) alleles of intermediate size. Perhaps these individuals are not at increased risk of CAD because of having apo(a) isoforms of larger size. This hypothesis could be tested by measuring the plasma levels of Lp(a) and determining the apo(a) isoform sizes in a large prospective study that examines CAD risk in blacks.

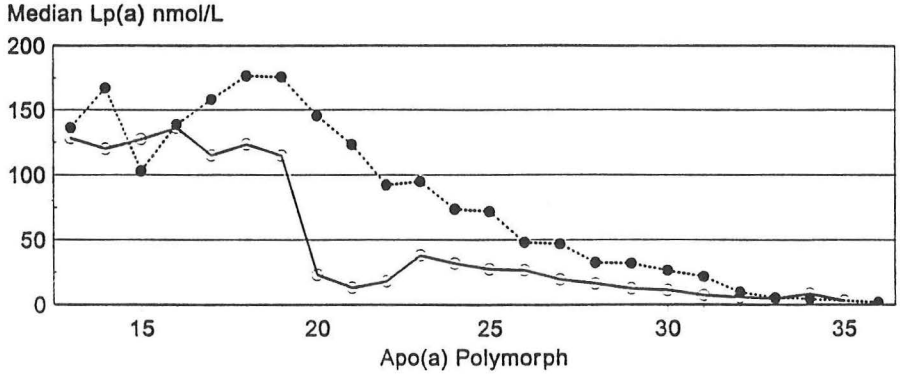

Fig 16. Relationship between median plasma Lp(a) levels and apo(a) size in 1336 blacks (closed circles) and 1370 whites (open circles).

THE PATHOPHYSIOLOGICAL LINK BETWEEN Lp(a) AND ATHEROSCLEROSIS?

Apo(a) and Lp(a) transgenic mice

There is conflicting data regarding the development of vascular lesions in mice expressing a human apo(a) transgene. Some studies have shown a greater than 20-fold increase in the mean lesion area of fat staining in the proximal aorta in apo(a) transgenic mice fed a high-fat diet, when compared to controls (128). Whereas other studies, using the same mice, failed to find any effect of apo(a) expression on the development of vascular lesions (129). In the apo(a) transgenic mice, the apo(a) does not circulate covalently attached to apoB-100, as it does in humans (presumably because of sequence differences between humans and mouse apoB-100), but rather is non-covalently associated with mouse LDL (26). In mice expressing both human apoB-100 and apo(a) transgene, the apo(a) circulated as part of Lp(a) (i.e., covalently linked to apoB-100) (130,131); these mice do not have an increase in fatty lesions in their aortas, when compared to mice expressing similar amounts of only human apoB-100 (131,132). Therefore, in the transgenic mouse, Lp(a) has not been consistently shown to be atherogenic and, thus, this animal model has not been as useful as was originally

anticipated. Recently rabbits that express both a human apo(a) and apoB-100 transgene has been developed and these animals may be a better (but more expensive!) animal model in which to study the link between Lp(a) and vascular lesions (133).

Is Lp(a) atherogenic because it interferes with plasminogen activation and thrombolysis?

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 surfaces of endothelial cells and macrophages (134,135), 2) plasminogen activation by streptokinase and tissue plasminogen activator (136), and 3) plasminogen binding to fibrinogen or fibrin (137). In support of these points, mice expressing an apo(a) transgene have been shown to have a decrease in the conversion of plasmin to plasminogen in the aortic vessel wall (138) as well as a decreased rate in the dissolution of clots infused into the pulmonary vasculature (139). However, in these mice, apo(a) does not circulate covalently linked to LDL, as it does in humans, and thus the results are of uncertain physiological significance.

In this regard, there is no *in vivo* evidence that Lp(a) inhibits thrombolysis in humans. We showed that higher plasma levels of Lp(a) are associated with a decrease in spontaneous recanalization of coronary arteries occluded with a thrombus (in subjects who had a myocardial infarction and were not treated with thrombolytic agents) but this was a retrospective study (140). There is no relationship between plasma levels of Lp(a) and the amount of plasminogen in clots (141), as might be expected if Lp(a) competed with plasminogen for binding to fibrin. Nor do plasma levels of Lp(a) correlate with any indices of thrombolysis, such as the euglobulin clot lysis time, or plasma levels of fibrin split products. If high concentrations of Lp(a) interfered with the activities of plasminogen activation, it might be expected that thrombolytic therapy would be less effective in patients with high Lp(a) levels, but this is not the case (142). In addition, there is no evidence that high concentrations of Lp(a) predispose to venous thrombosis.

Perhaps the most dramatic studies showing a prothrombotic effect of Lp(a) *in vitro* were performed in the cynomolgus monkey. In these studies, the carotid artery was traumatized until there was a significant reduction in blood flow. An occlusive thrombus developed in 5 of the 6 animals with a high plasma level of Lp(a) (>35 mg/dl), and in only a single animal with a low plasma level (143).

In tissue culture studies, as well as in the transgenic apo(a) mouse model, apo(a) and Lp(a) inhibit activation of TGFβ-1 by plasmin (144,145). TGFβ-1 is a cytokine that inhibits smooth muscle cell proliferation, so a decrease in TGFβ-1 is associated with an increase in smooth muscle proliferation. Apo(a) transgenic mice were reported to have a reduction in active TGFβ-1 in their aortic wall(138), which supports this scenario, although no evidence has been provided that there is an increase in smooth muscle proliferation in the vascular lesions of these mice. Therefore, it remains uncertain whether high plasma levels of Lp(a) contribute to the atherosclerotic process by interfering with the activation of TGFβ-1.

Effect of Lp(a) on vascular reactivity

High plasma levels of Lp(a) may also adversely affect vascular tone. High plasma levels of Lp(a) (>40 mg/dl) are associated with an increase in vasoconstriction in the coronary arteries in response to the acetylcholine and the cold pressor test (146). Sorensen et al. showed that endothelium-dependent vasodilatation of the femoral artery

was dramatically decreased in children with FH (1.2 vs. 7.5%), and was further impaired by high levels of plasma Lp(a) (147). However, a recent paper failed to find any effect of high plasma levels of Lp(a) on endothelium-dependent vasodilatation in the brachial artery in healthy middle-aged men (148); they found higher basal levels of nitrous oxide in individuals with elevated plasma levels of Lp(a).

Is Lp(a) atherogenic because it transports lipids into the lesion?

Alternatively, apo(a) may be an innocent bystander in the atherosclerotic process, and may simply get trapped by the atherosclerotic lesion. In this regard, Lp(a) binds tightly to glycosaminoglycans extracted from human aortic tissue (149), as well as other components of the extracellular matrix of the arterial wall, as reviewed above.

Lp(a) - TO MEASURE OR NOT TO MEASURE?

Considering the strong association between high plasma levels of Lp(a) and atherosclerotic disease in cross-sectional, retrospective and in most prospective studies, should we be measuring plasma levels of Lp(a) in our patients? The answer to this question is not straightforward. No studies have shown that lowering levels of plasma Lp(a) is associated with a reduction in risk of CAD. Moreover, intervention studies to examine the effect of lowering plasma Lp(a) levels on CAD will not be immediately forthcoming since few pharmacological agents are available that lower plasma levels (as will be reviewed below).

The results of the prospective studies do not support population-wide screening for high plasma levels of Lp(a). Despite the sometimes sizeable differences in the median and mean plasma levels of Lp(a) in individuals with CAD when compared to normals, there is a substantial overlap in plasma levels of Lp(a) between the two groups. Therefore, the population-attributable risk of high plasma Lp(a) levels is much lower than might be expected based on a comparison of the differences in median plasma levels.

Despite the many gaps in our knowledge regarding Lp(a), I think there is sufficient evidence to warrant measuring its plasma levels in individuals with:

- premature CAD, peripheral vascular disease, or cerebrovascular disease
- a strong family history of premature CAD
- CAD and no identifiable cardiac risk factors

Also, a case can be made to measure plasma levels of Lp(a) in individuals who have elevated plasma levels of LDL-C but no other risk factors. The finding of a high plasma level of Lp(a) in such a patient may tip the scale in favor of initiating LDL-lowering therapy earlier, and influence the selection of lipid-lowering agent(s).

At this time there is no evidence to support the measurement of plasma Lp(a) levels in individuals of African descent, since we do not know the risks associated with elevated plasma levels in this population. Also, additional prospective studies need to be performed in women to ensure that the risk associated with high plasma levels of Lp(a) is similar to the risk in men.

Lp(a) assays

As reviewed previously, there are many problems with the assays commonly used to measure plasma levels of Lp(a). No universally accepted methods or standards are available (for discussion, see ref. 150). The high degree of polymorphism within the apo(a) protein has made it difficult to standardize the assay. Some antibodies used in commercial

assays do not react with all apo(a) isoforms with equal affinity. As discussed previously, temperature and duration of storage of plasma samples affect the immunoreactivity of Lp(a).

The most sensitive and reliable assays are enzyme-linked immunosorbent (ELISA) assays. Two general schemes are used. The plate is coated with either a polyclonal or monoclonal anti-apo(a) antibody. The plasma sample is added and the Lp(a) is captured by the antibody. Next the detection antibody is added to the plate, which is either an anti-human apo(a) or apoB-100 antibody. If the detection antibody is directed against the common K4-type 2 repeat, the assay systematically overestimates the levels of Lp(a) associated with large apo(a) isoforms (since these isoforms contain multiple copies of the K4 epitope) and underestimates the plasma Lp(a) levels in individuals with small isoforms. It is, thus, preferable to use either an anti-apoB-100 antibody or an antibody to a unique epitope in apo(a) [i.e., outside the K4-type 2 repeat] as the detection antibody so the Lp(a) concentrations can be expressed in moles (i.e., number of particles).

The Lp(a) ELISA assay available at Parkland and the Aston Center to measure plasma Lp(a) uses a monoclonal antibody to an epitope in the K4-type 2 repeat of apo(a) as the capture antibody and a monoclonal antibody to a unique epitope in the type 9 K4 repeat as the detection antibody. This assay, which is performed at Northwest Lipid Laboratory, is the only assay available that has been shown to measure all apo(a) isoforms equally (151). The results can be reported either in moles (nmol/L) or as Lp(a) mass (mg/dl) or in moles. The percentile of the value, compared to a reference population of 2000 individuals, is also provided (with > 80% being associated with risk). The cost of the assay is $30.00.

The biological variation in plasma levels of Lp(a) ranges from 3% at high levels of Lp(a) to 51% at low levels (48). Although the biological variation is high at low plasma levels, it translates into very small differences in the absolute amount of plasma Lp(a). For example, if an individual has a plasma level of 1 mg/dl, the level may range between .5 and 1.5 mg/dl, which are minor changes, given the 1000-fold range in levels in the population. Often, only a single measurement of plasma Lp(a) suffices if the level is <10 mg/dl or >40 mg/dl. But if the level is near the threshold value (20-40 mg/dl) and treatment is being contemplated, the plasma Lp(a) level should be measured 2-3 times.

Plasma Lp(a) levels are not significantly influenced by food ingestion, so fasting before sample collection is not required.

PHARMACOLOGICAL REDUCTION OF Lp(a)

As discussed previously, most conventional lipid-lowering drugs do not affect plasma levels of Lp(a). HMG-CoA reductase inhibitors, gemfibrozil (in the usual doses) and bile acid resins have little to no effect on its plasma levels. Bezafibrate, which is widely used in other parts of the world but not in the USA, lowers Lp(a) by as much as 39% (152).

High-dose nicotinic acid decreases Lp(a) synthesis (153) and is associated with up to a 38% fall in plasma levels of Lp(a) (154). The degree to which the Lp(a) concentration falls is proportional to the initial plasma Lp(a) level and to the percent reduction in plasma LDL levels (154). Therefore, individuals with higher plasma levels of Lp(a) have a greater response to niacin. Paradoxically, the plasma Lp(a) can increase in hypertriglyceridemic subjects treated with niacin (154).

Oral estrogen and tamoxifen therapy also lower plasma Lp(a), presumably by decreasing its synthesis (51-54). Administration of anabolic steroids - either stanozolol or danazol- is associated with up to a 50% lowering of plasma Lp(a) levels, but these agents also dramatically decrease HDL-C so are not therapeutic options (58,155).

Oral neomycin treatment (2 gm/d) results in a 24% fall of plasma Lp(a) (156). Neomycin sulfate lowers plasma LDL-C levels by inhibiting intestinal absorption of cholesterol. *In-vitro* studies show that neomycin inhibits release of apo(a) from the hepatocyte cell surface (157), but since neomycin is poorly absorbed, it is unlikely to lower Lp(a) by this mechanism *in-vivo.*

Diets very rich in fish oil may be associated with lower plasma levels of Lp(a) (158). High doses of fish oils (>3 gm per day) were associated with up to a 40% fall in plasma levels of Lp(a)(159) in one study, although this has not been a universal finding.

An attempt to dissociate apo(a) from the LDL particle using a reducing agent such as n-acetylcysteine (NAC;MUCOMYST) was initially reported to dramatically reduce plasma Lp(a) levels (160) but this has not been confirmed (161). The advisability of lowering plasma Lp(a) levels by dissociating apo(a) from the LDL particle is questionable since "free" apo(a) may be more atherogenic. When apo(a) is not coupled with LDL, additional low affinity lysine binding sites in apo(a) are accessible(11,13); this may be why subjecting Lp(a) to reducing agents, such as homocysteine, *in-vitro* is associated with an increase in the binding affinity of apo(a) to fibrin (162). (It would be interesting to investigate whether high plasma levels of homocysteine increase the risk associated with high plasma levels of Lp(a)).

Thus, the only two agents that have been proven to significantly lower plasma levels of Lp(a) and are readily available are nicotinic acid and estrogens. Since both drugs have major effects on the other classes of lipoproteins, it is not possible to assess the independent effect of lowering plasma Lp(a) levels on coronary risk at this time.

The most effective, but also most expensive and invasive method to lower plasma levels of Lp(a) is by using LDL apheresis, which reduces levels by up to 70% (163).

TREATMENT OF ELEVATED PLASMA LEVELS OF Lp(a)

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 the preferred lipid-lowering medications (if it can reduce plasma LDL-C concentrations sufficiently). Estrogen therapy should be strongly considered in post-menopausal women with elevated plasma levels of Lp(a), especially they are also hypercholesterolemic. In patients with an isolated high plasma level of Lp(a), all other known risk factors for ischemic heart disease should be aggressively treated. 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; these individuals probably should not be treated unless they have other risk factors for CAD.

Three studies suggest that lowering plasma levels of LDL-C may remove the atherogenic effect of Lp(a). In the Familial Atherosclerosis Treatment Study (FATS), 146 hypercholesterolemic men (age <62) with CAD were treated with various lipid-lowering agents for 25 years and then recatheterized (164). Elevated plasma Lp(a) levels were the best predictors of the severity of CAD at the start of the study. However, if subjects were treated with an agent that lowered plasma LDL-C >10%, the effect of plasma Lp(a) on the progression of CAD was eliminated. In those individuals in whom the plasma levels of LDL-C did not significantly change on treatment, high plasma levels of Lp(a) was a strong predictor of events and disease progression.

Two studies in FH subjects have compared the effect of LDL apheresis, which lowers both plasma LDL-C and Lp(a), to HMG-CoA reductase inhibitor +/- bile acid resin therapy,

which only lowers plasma LDL-C levels, on coronary artery lesion progression. No benefit was accrued by lowering plasma levels of Lp(a) if LDL was significantly lowered (165,166). The results of these studies suggest that the best therapeutic option for a high plasma level of Lp(a) is to lower the plasma level of LDL, but how aggressive one should be in lowering plasma LDL-C levels in individuals with elevated plasma Lp(a) levels remains to be determined.

WHAT IS THE PHYSIOLOGICAL ROLE OF Lp(a)?

The highly polymorphic nature of apo(a), and the lack of any phenotype associated with its near absence in plasma of some individuals raises the question as to why we have this distinctive lipoprotein in our plasma. Apo(a) has a very unusual species distribution and perhaps an examination of the evolution of this lipoprotein will provide insights into its physiological role.

Evolution of apo(a)

The apo(a) and plasminogen genes are paralogous; i.e., they are both descended from the same gene. The plasminogen gene is ancient; it is present in very primitive species like the lamprey, as well as all birds, fish and mammals. In contrast, apo(a) is not universally expressed, even among mammals. Lp(a) is the major cholesterol-carrying lipoprotein of the hedgehog (*Erinaceus europaeus*), an insectivore (167) that diverged from other placental mammals about 90 million years ago. Remarkably, antibodies to human apo(a) cross-react with hedgehog apo(a). The apo(a) in hedgehogs varies in size, just as it does in humans, and circulates covalently attached to LDL.

Apo(a) is not present in the plasma of any mammals extending from the hedgehog to Old World monkeys (168). Apo(a) is present in the plasma of all lesser and great apes. Did the apo(a) gene evolve in the hedgehog and then accumulate mutations that resulted in loss of expression of the gene in other species? To answer this question, Richard Lawn cloned the hedgehog cDNA (169). This was no small feat since European hedgehogs are not available in the United States, and cannot be imported. To make his initial cDNA library, he obtained hedgehog livers from roadkills collected by St. Tiggwinkles, which is the part of the Wildlife Hospital Trust in Britain. He ultimately cloned the full-length hedgehog apo(a) cDNA from a library he made from livers of African hedgehogs, which are now popular pets in the USA.

Comparison of the hedgehog and human apo(a) cDNAs is shown in Figure 17. Like human apo(a), hedgehog apo(a) contains multiple repeats (n=31) of a kringle domain that shares 74-100% identity. However, the kringles resemble the K3 domain of plasminogen (69-78% sequence identity) rather than K4 domain. Interestingly, these K3 repeats in plasminogen contain seven cysteines (and thus have an extra, unpaired cysteine), whereas all but the last K3 of hedgehog apo(a) has six cysteine residues. Presumably, the single free cysteine residue in the last K3 repeat connects apo(a) to the apoB-100 of LDL by a disulfide linkage. The amino acid sequences required for kringles to bind lysine are also present in the hedgehog K3 repeat.

Therefore, it appears that the apo(a) gene has arisen not once, but at least twice, from the plasminogen gene. This is an example of convergent evolution.

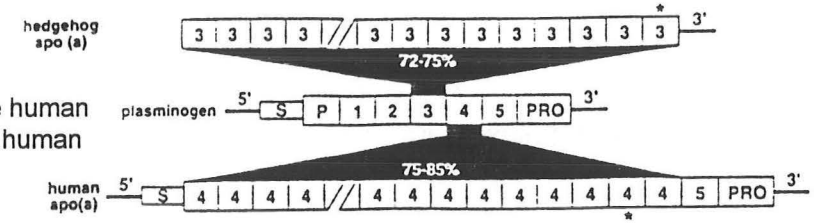

Fig. 17. Comparison of the human and hedgehog apo(a) and human plasminogen cDNAs

Based on the percentage of sequence identity in the rhesus monkey and human apo(a) cDNA, it has been estimated that the human form of apo(a) evolved ~80 million years ago from a duplication of the plasminogen gene (170). This corresponds to the time of the evolutionary branching that led to the radiation of the mammalian species. Therefore, it would be expected that the apo(a) gene would be present in other mammals. No evidence of apo(a) sequences have been found in the genome of any species between hedgehogs and Old World monkeys, although a more exhaustive molecular search for apo(a)-like sequences will be required before ruling out the existence of an apo(a) gene in any of these intervening species.

Does evolution provide any insights into the physiological role of Lp(a)? Comparison of the hedgehog, rhesus monkey and human apo(a) cDNAs reveal a number of common features. All three cDNAs contain multiple copies of a kringle domain. Although the apo(a) cDNA of the rhesus monkey, which is an Old World monkey, is 93% identical to the human apo(a) cDNA, it contains no sequences resembling the kringle 5 domain of plasminogen, which is found in human (and chimpanzee) apo(a) (171). Hedgehog and human apo(a) can bind fibrin but rhesus apo(a) cannot. Rhesus apo(a) has an amino acid substitution in the K4-type 10 repeat (Arg 72 Trp) that is required for lysine binding. (Interesting, the same mutation has been identified in two humans, but the plasma levels of Lp(a) in these individuals were too low to assess the effect on atherosclerosis (13)). All three proteins have a single free cysteine residue within the kringle domain that connects apo(a) to the apoB of LDL. None of the proteins have any protease activity and one apo(a) (hedgehog) has even relinquished having a protease domain.

Based on these comparisons, the critical common features of apo(a) are 1) the presence of multiple kringle repeats (which in other proteins are responsible for targeting proteins) and 2) the coupling of apo(a) to LDL. These shared properties allow us to speculate on a possible physiological role for this enigmatic lipoprotein.

Possible physiological role of Lp(a)

Perhaps the best idea regarding a possible role for Lp(a) was put forth by Brown and Goldstein, who proposed that Lp(a) may play a role in wound healing (172). Upon tissue injury, the extracellular matrix becomes exposed to the bloodstream. Perhaps apo(a) gets trapped in the lesion, and with it the LDL particle, which provides lipids for wound repair.

Immunocytochemical studies of wounds support the notion that Lp(a) participates in the wound healing process. Apo(a) is present in lesions during the early stages of healing, when fibrin clots encase the wound and the wound is infiltrated with inflammatory cells (173). At this point, Lp(a) coats the fibrous cap and is present within and surrounding the endothelial cells of newly-infiltrating small blood vessels. The pattern of apo(a) and apoB staining is similar, suggesting that Lp(a), and not just apo(a), is present in the wound. If apo(a) has a role in wound healing, it is in the early phases since apo(a) cannot be detected in the healing lesion after the epithelium had regenerated (173). Lp(a) is also found in association with the microvasculature of tissues involved in inflammatory disorders, such as Crohn's disease and thrombotic thrombocytopenic purpura (174). Angiogenesis is an important component of inflammation and wound repair and it is possible that Lp(a) plays a role in angiogenesis, given its sequence similarity to

plasminogen and angiostatin.

Another possible mechanism responsible for the accumulation of Lp(a) in wounds involves defensins. Defensins are a family of cationic peptides present in granules of neutrophils that are released during inflammation. When defensins are released during degranulation, they insert into the membranes of adjacent cells and perturb ion transport, eventually resulting in lysis of the cell. Defensins bind endothelial and vascular smooth muscle cells as well as fibrin. A recent report demonstrated that in cultured cells defensins enhance the binding and internalization (but not degradation) of Lp(a) by endothelial and smooth muscle cells (175). Thus, the recruitment and degranulation of polymorphonuclear leukocytes may enhance the localization of Lp(a) to lesions. Lp(a) in turn, may promote the influx of other inflammatory cells; in tissue culture studies, Lp(a) has a chemotactic effect on monocytes (176).

Lp(a) may act as a scavenger of oxidized lipids and other debris that accumulates in lesions. Lp(a) is rich in platelet activating factor acetylhydrolase activity (7-fold higher levels per particle than LDL) (177), which inactivates platelet activating factor (a potent vasoactive compound) as well as oxidized lipids. It is of interest that the K4 domain of apo(a) binds β_2-glycoprotein-1, which in turn binds oxidized phospholipids. In this setting, Lp(a) may act as a detoxifier, accumulating and deactivation biologically active lipids by swinging the long, repetitive K4 domain of apo(a) through tissues much like a pool sweeper collects debris in a pool. According to this scenario, the potentially damaging oxidized lipids that bind the K4 repeats of apo(a) are delivered to the LDL particle where they can be degraded by PAF-acetyl hydrolase, or perhaps neutralized by free radical scavengers such as vitamin E.

The striking difference in the frequency distribution of plasma Lp(a) levels between populations raises the question as to whether there is (or was) selection for high plasma levels of Lp(a) in Africa. Lp(a) may protect against some tropical infectious agent or environmental factor and, thus, provide a survival advantage. For example, Lp(a) may accelerate the removal of oxidized lipids generated during a hemolytic crisis, perhaps precipitated by malaria or another environmental challenge.

SUMMARY

Questions remain unanswered about Lp(a) in regard to its normal physiology and to its role in atherosclerosis. The evidence that high plasma levels of this unusual lipoprotein are associated with vascular disease is overwhelming, although the constellation of risk factors required for Lp(a) to enhance atherosclerosis has not been clearly defined. Individuals with high plasma levels of Lp(a), coupled with high plasma levels of LDL, appear to be at particular risk, and efforts should be directed to aggressively lowering plasma levels of LDL in these individuals until better agents become available to reduce plasma concentrations of Lp(a).

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