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## Lipoprotein(a) Metabolism: Potential Sites for Therapeutic Targets

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### Abstract

Lipoprotein(a) [Lp(a)] resembles low-density lipoprotein (LDL), with an LDL lipid core and apolipoprotein B (apoB), but contains a unique apolipoprotein, apo(a). Elevated Lp(a) is an independent risk factor for coronary and peripheral vascular diseases. The size and concentration of plasma Lp(a) is related to the synthetic rate, not the catabolic rate, and is highly variable with small isoforms associated with high concentrations and pathogenic risk. Apo(a) is synthesized in the liver, although assembly of apo(a) and LDL may occur in the hepatocytes or plasma. While the uptake and clearance site of Lp(a) is poorly delineated, the kidney is the site of apo(a) fragment excretion. The structure of apo(a) has high homology to plasminogen, the zymogen for plasmin and the primary clot lysis enzyme. Apo(a) interferes with plasminogen binding to C-terminal lysines of cell surface and extracellular matrix proteins. Lp(a) and apo(a) inhibit fibrinolysis and accumulate in the vascular wall in atherosclerotic lesions. The pathogenic role of Lp(a) is not known. Small isoforms and high concentrations of Lp(a) are found in healthy octogenarians that suggest Lp(a) may also have a physiological role. Studies of Lp(a) function have been limited since it is not found in commonly studied small mammals. An important aspect of Lp(a) metabolism is the modification of circulating Lp(a), which has the potential to alter the functions of Lp(a). There are no therapeutic drugs that selectively target elevated Lp(a), but a number of possible agents are being considered. Recently, new modifiers of apo(a) synthesis have been identified. This review reports the regulation of Lp(a) metabolism and potential sites for therapeutic targets.

### Keywords

plasminogen mimetic; expression; assembly; catabolism; modification

### 1. Introduction

Lipoprotein(a) [Lp(a)] was first described nearly 50 years ago by Kåre Berg as a unique lipoprotein. Lp(a) is similar to low density lipoprotein (LDL) with the lipid core and apolipoprotein B (apoB), but it contains a unique apolipoprotein, apo(a), tethered to apoB by a covalent disulfide bond (Figure 1). Early studies identified Lp(a) as a risk factor for

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#### Conflict of Interest

None.

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cardiovascular disease (CVD), independent of LDL. However, the results of a few studies that suggested there was little or no association of Lp(a) with CVD risk, may have been due to size dependent assays or storage deterioration. Several recent studies that included large numbers of subjects have provided convincing evidence that small isoforms of elevated Lp(a) are a risk factor for CVD. Considerable interest was expressed in the pathological and physiological function of apo(a) when the sequence of apo(a) revealed a high homology to plasminogen (Plg), the zymogen for plasmin, which is the primary enzyme for clot lysis. Lp(a) concentration is genetically determined by apo(a) size, with small isoforms at higher concentrations than large isoforms. Plasma elevated Lp(a) concentrations are generally resistant to lipid lowering drugs and physiological changes, but exceptions are noted (Table 1). Niacin, not selective for Lp(a), has long been considered a Lp(a) lowering drug, but some recent studies of niacin in combination with other lipid lowering drugs are contradictory. Sex hormones, especially estrogen reduces plasma Lp(a), but are not recommended for therapy. In kidney disease, Lp(a) is elevated, and in obstructive cholestasis Lp(a) is reduced. Kinetic studies suggest plasma Lp(a) concentrations are regulated by synthesis rather than catabolism. Sequence polymorphisms of apo(a) may also determine concentration and pathological responses. In addition, modifications of circulating Lp(a) may alter Lp(a)/apo(a) functions. Importantly, the role of apo(a) in CVD and peripheral vascular diseases and its physiological function are still unclear and there is no effective therapeutic treatment for lowering elevated Lp(a). This review focuses on the regulation of metabolism of Lp(a), highlighting areas that need clarification and potential targets for therapeutic intervention.

## 2. Synthesis of Apolipoprotein(a)

### 2.1. Species and Tissue Expression

The occurrence of Lp(a) is documented by genetic, biochemical and immunological evidence in humans and other primates, including Old World monkeys, (baboon, rhesus, cynomolgus, cebus, pig-tailed, and stump-tailed), New World monkeys (marmoset), and lesser and greater apes (chimpanzee, orangutan, gibbon). Lp(a) also occurs in hedgehogs, but is not expressed in other animal species commonly used in research studies, including rats, mice, guinea pigs, rabbits, and pigs. Lp(a) is synthesized primarily in the liver, but apo(a) expression has been reported for other sites such as testes in cynomolgus monkeys, and in human aorta and carotid arteries. The mRNA size, but not the levels, correlates with the concentration of apo(a) in the plasma in humans and non-human primates.

### 2.2. Transcriptional regulation of the LPA gene

Plasma Lp(a) was considered to be largely determined by the isoform size due primarily to the variation in the number kringle IV Type 2 (KIV-2) domains, which may vary from 3–43 copies (Figure 2). Additional polymorphisms have been identified including copy variation of the pentanucleotide, TTTTA, repeats (PNR) in the promoter region, 1.3 kb from the transcription start site, and the C/T polymorphism in the coding region (+93) that creates an additional start codon. Haplotype variation of these three regions varies between ethnic populations. The LPA gene is located on Chromosome 6 and several loci on other chromosomes have been identified that regulate apo(a) expression. An 8–13% increase in plasma Lp(a) in women after menopause and hormone replacement therapy reduces Lp(a). In the apo(a) yeast artificial chromosome (YAC) transgenic mice, plasma apo(a) decreased by 50% during the estrus cycle. Using human HepG2 cells, Bofelli et al. identified an estrogen apo(a) enhancer region 26 kb upstream of the apo(a) promoter region. The estrogen receptor binds to DNA via a transcription factor at the negative enhancer estrogen receptor element (ERE). Wade et al. found a negative enhancer region and a positive enhancer at a HNF1A-binding site in the promoter region, a –98 to +130n bp region relative to the mRNA start site. Negi et al. reported the regulation of the LPA gene by a tissue specific (–716 to

–616 bp) region and a negative enhancer (–1432 to –716 bp) region where the transcription factors HNF3A (FOXA1) and GATA4 were found to bind and repress apo(a) expression. Recently, Chennamsetty et al. reported that patients with biliary obstruction and in apo(a) transgenic mice with a Farnesoid X receptor (FXR) deficient background have reduced apo(a) synthesis. FXR binds to a negative DR-1 control element located at the –826 bp region of the LPA. FXR activation by bile acids reduced plasma concentrations and hepatic expression of human LPA, but did not fully explain the decrease in plasma apo(a). Bile acids and an FXR agonist lower plasma lipids and may be candidates for reducing Lp(a) as well. However, Chennamsetty et al. identified a pathway involving fibroblast growth factor 19 (FGF19) binding to the FGFR4 receptor on hepatocytes and induced the translocation of Elk-1 to the nucleus to mediate repression of LPA transcription in the promoter region at –1630/–1615bp. Apo(a) transgenic mice had a significant reduction in plasma apo(a) (30%) and liver mRNA when injected with FGF19.

Apo(a) expression is regulated by both positive (IL-6) and negative (TNF- $\alpha$ , TGF- $\beta$ ) factors in primary monkey hepatocytes. Recent studies in humans suggest that inhibition of IL-6 reduces plasma Lp(a) and a specific polymorphism of IL-6 was associated with elevated Lp(a). Whether or not pathophysiological variations of IL-6, an inflammatory cytokine, and TNF- $\alpha$  and TGF- $\beta$  play a role *in vivo* in the regulation of LPA expression is unclear. Regulation of apo(a) expression also occurs in the intergenic region between LPA and Plg genes. Within this intergenic region multiple enhancer regions of apo(a) expression were identified as well as an apo(a) gene enhancer within a Long Interspersed Element. A regulatory region in the 40 kb intergenic region between apo(a) and Plg was identified 20 kb upstream of the LPA gene, that significantly increases the minimal promoter activity of the human LPA gene related to basal apo(a) values. Deletion of this region in apo(a) transgenic mice caused a 30% decrease in plasma apo(a), but mice with the mutation still responded to sexual maturation. Reduction of plasma Lp(a) concentration may require interference with the regulation of apo(a) transcription. A number of transcription sites are predicted for the promoter and the intergenic region, ([http://www.sabiosciences.com/chipqpcrsearch.php?factor=Over+200+TF&species\\_id=0&ninfo=n&ngene=n&nfactor=y&gene=LPA](http://www.sabiosciences.com/chipqpcrsearch.php?factor=Over+200+TF&species_id=0&ninfo=n&ngene=n&nfactor=y&gene=LPA)) and several of these sites have been shown to regulate apo(a) expression (Figure 2).

### 2.3 Regulation of secretion

Several investigations of the cellular secretion of apo(a) have been carried out in isolated hepatocytes from humans, baboons, and mice, as well as human HepG2 cells and McARH7777 cells. In pre-secretory processing, apo(a) enters the ER lumen, is folded, and then is either released for secretion into the Golgi or proceeds to the proteasome degradation pathway (Figure 3). A number of factors were shown to affect secretion of apo(a). Inhibition of N-linked glycosylation reduced secretion while protease inhibitors increased secretion. Transport of apo(a) to the post-ER premedial Golgi was required for apo(a) degradation. Calnexin, an ER chaperone, binds to apo(a) and prevents degradation. Epsilon-aminocaproic acid (EACA), a C-terminal lysine mimic, circumvented the requirement of calnexin and calreticulin and increased apo(a) secretion by reducing the pre-secretion degradation. In oleate treated cells, inhibition of protease digestion increased secretion. Treating with the reagent dithiothreitol decreased retention and was apo(a) size dependent. In hepatoma cells, apo(a) synthesis and secretion were coupled to TG synthesis and secretion. Although the folding of apo(a) does not appear to be size dependent, a higher percentage of large isoforms are degraded relative to secretion compared to the small isoforms.

### 2.4. Assembly Lp(a)

Reports of the location of Lp(a) assembly remain controversial (Figure 3). Studies suggest assembly may be either intracellular or extracellular. Apo(a):LDL complexes have been

found in cell lysates from primary human hepatocytes and in hepatoma HepG2 cell microsomes. Extracellular assembly was reported in baboon hepatocytes and HepG2 cells. Apo(a):LDL complexes were found in apo(a) transgenic mice when infused with human LDL. *In vivo* studies investigating the site, either intracellular or extracellular, of Lp(a) assembly are difficult and the few studies reported are inconsistent. In a study by Demant et al. using stable isotopes, the synthesis rates for apo(a) and apoB in Lp(a) were similar, but different for LDL, which suggests separate pools of apoB for Lp(a) and LDL. In this model, two pools of LDL from Lp(a), one from direct secretion of Lp(a) from the liver and one from derived LDL circulating in the plasma, are consistent with the study of fed subjects where Lp(a) is disassembled and reassembled in the plasma. These data suggest that apo(a) does not remain covalently linked to apoB but re-associates at least once. If Lp(a) associates and then re-associates in plasma, this may explain the inconsistent reports regarding the site of assembly.

Mutagenesis studies have identified amino acids in apo(a) and apoB required for the noncovalent interaction and the covalent binding in KIV-9. Apo(a) at [apo(a)Cys4057] binds to apoB at (apoBCys4326) through a covalent disulfide bond. Evidence suggests that for efficient assembly, noncovalent binding occurs between apo(a) and apoB in the KIV-7 of apo(a) and apoBLys680/apoBLys690, and KIV-8 of apo(a) and apoBLys4372 prior to the formation of the covalent disulfide bond. Inhibition of Lp(a) assembly has been investigated as a strategy to reduce plasma Lp(a). Inhibition of assembly Lp(a) *in vitro* by small molecules is most effective with the same molecules that inhibit lysine binding, including tranexamic acid, delta aminovaleric, gamma amino butyric acid, and EACA. However, in transgenic apo(a) or Lp(a) mice treated with tranexamic or delta aminovaleric acid, the plasma levels of Lp(a) doubled. This suggested that these inhibitors caused release of cell bound Lp(a) since the intracellular apo(a) concentration remained the same. In patients treated with tranexamic acid, there was a decrease of 18% in the plasma Lp(a). ApoB peptides in the 4372-4392 region also inhibit Lp(a) assembly by reducing the noncovalent binding and are better inhibitors than the lysine binding site (LBS) inhibitors. One of the issues with inhibitors that reduce of Lp(a) assembly is that apo(a) may not be reduced in the plasma, and apo(a) and its peptides may induce pathogenic effects outside the Lp(a) complex. Antisense oligonucleotide directed to human apoB reduced plasma Lp(a) in transgenic mice, but not apo(a). Apo(a) antisense-RNA expression with adenovirus in McARH7777 cells and in apo(a) transgenic mice effectively reduced apo(a) synthesis, and an antisense oligonucleotide directed to the KIV-2 region reduced both plasma apo(a) and Lp(a) in plasma in apo(a) transgenic mice. Thus, targets of apo(a) transcription may be better therapeutic targets than inhibition of the Lp(a) assembly process.

### 3. Catabolism of Lp(a)

#### 3.1. Cellular uptake via LDL receptors

Soon after the identification of Lp(a), evidence indicated that Lp(a) is not a metabolic product of other lipoproteins, VLDL or LDL, nor is it metabolized to other lipoproteins. Importantly, further *in vivo* studies suggested that the variations in Lp(a) plasma concentration in individuals with different isoforms was due to the production rate of apo(a) rather than by its clearance rate. Su et al. reviewed the kinetic studies for Lp(a) catabolism and found that the reported parameters were similar among the studies with a mean fractional catabolic rate (FCR) of  $0.28 \pm .05$  (0.16-0.36) pool/d and a mean production rate of  $3.6 \pm 1.1$  (2.1-4.5) mg/kg/d. These studies relied on labeled Lp(a) to determine Lp(a) metabolism, but Parhofer et al. measured Lp(a) in the non-steady state after LDL apheresis and found values similar to those reported with labeled Lp(a) despite different methods and the fact that the number of subjects was low. The low-density lipoprotein receptor (LDLR) was considered to be a possible site for the uptake and degradation of Lp(a), however

reports were controversial (Figure 4) regarding the significance of the role of the LDLR in Lp(a) catabolism. Hoffman et al. reported that Lp(a) clearance was accelerated in transgenic LDLR mice, and in Watanabe rabbits, a familial hypercholesterolemia model, there was an accumulation of Lp(a) in the plasma. Kraft et al. reported that for Lp(a) with the same allele size the concentration was dependent on the gene dose of the LDLR in familial hypercholesterolemia and deficient subjects had higher values of plasma Lp(a) than heterozygous subjects. However, kinetic studies suggested that the clearance of Lp(a) was not entirely dependent on the LDLR. LDL from Lp(a) was cleared by the LDLR only after release of apo(a). Reblin et al. reported that LDLR or low-density lipoprotein receptor-related protein (LRP) deficient fibroblasts did not alter their uptake and degradation of Lp(a). Although Lp(a) may bind to LDLR and LRP, the binding does not seem to be important for its degradation.

Lp(a) binds to two other related receptors in the LDLR family, VLDLR and megalin/330gp, with higher affinity than LDLR or LRP. The VLDLR is expressed in heart, skeletal muscle, and adipose tissue, but not the liver. Lp(a) was internalized and degraded by mouse embryonic fibroblasts expressing the VLDL receptor, but not by fibroblasts deficient in the receptor, and clearance of plasma Lp(a) injected into mice deficient in the VLDLR was delayed. Megalin/gp330/LRP2 receptor is in the LDLR family and similar to the VLDLR. Megalin is an endocytotic receptor expressed on the plasma membrane of epithelial cells and most abundantly expressed in thyroid tissue, and to a much lower extent in the proximal tubule cells of the kidney, and in skeletal muscle. Lp(a) binds to megalin and is taken up and degraded in megalin expressing fibroblasts. Megalin binds both Plg and apoB, and Lp(a) and LDL inhibit uptake of the labeled Lp(a) into fibroblasts, suggesting that uptake of Lp(a) is mediated partially by apoB and partially by apo(a). Lp(a) is elevated in hypothyroidism, and treatment with thyroid hormone decreases Lp(a). Recently, a study showed treatment with eprotirome, a thyroid hormone analogue, lowers plasma Lp(a) by 30%. The thymimetics reduce LDL in the plasma and are thought to act by increasing hepatic clearance, but the relationship of eprotirome to clearance of Lp(a) by binding to megalin has not been examined. The significance of the uptake and degradation of Lp(a) by the VLDLR and megalin *in vivo* is unclear.

### 3.2. Cellular uptake of Lp(a) via Plg receptors

In addition to the lipoprotein receptors, Lp(a) binds to Plg receptors on cells and requires the LBS of apo(a). The Plg receptors are found ubiquitously on cells, and the majority of the Plg receptors have C-terminal lysines. Apo(a) binds to these receptors and can displace Plg. The apo(a) LBSs have been identified in several of the KIV kringle domains and in KV, but not in KIV-1 or KIV-2. The LBS of KIV-10 in apo(a) is similar to the LBS of Plg, but not identical. The secondary LBS sites, important for the initial noncovalent binding of apo(a) to apoB, in KIV-6-8 have a weaker LBS. The noncovalent binding of these kringle domains to lysine residues in apoB tethers the apo(a) to the apoB prior to formation of the covalent disulfide bond. Plasmin, the active enzyme of the zymogen Plg, is the primary enzyme for fibrin degradation. Plasmin also plays an important role in leukocyte migration in inflammation by degrading ECM, either directly or by the activation of metalloproteinases. Plasmin may also activate cytokine and induce cell signaling. Plg is activated on cell surface receptors abundant on many types of cells. Several PR have been identified including,  $\alpha$ -enolase, H2B, annexin2, p11, Plg-RKT, and  $\alpha_m\beta_2$  integrin. The function that receptor bound plasmin may play in leukocyte migration and stimulus induction may be cell and stimulus dependent. The binding of Lp(a) and interference with plasmin function has not been systematically evaluated. Whether the Plg receptors play a role in the degradation of apo(a) is not known. To date, unique receptors for Lp(a) (non-LDL and non-Plg) have not been identified.

## 4. Catabolism of apo(a)

### 4.1. Fragmentation of apo(a)

Oida et al. were the first to identify apo(a) immunoreactive fragments in urine. These fragments were primarily from the N-terminal region with KIV-1, -2, and -3. Mice were injected with apo(a) fragments that were rapidly cleared into the urine, suggesting the fragments were formed extrarenally and then excreted by the kidney. Kostner et al. identified 10 distinct apo(a) fragments of 30–160 kDa not attached to apoB from 30 individuals. Although the intensity of the apo(a) bands varied, the molecular weights were comparable despite variation of allele size suggesting specific sites of cleavage. The portion of total apo(a) found in urine was 1–3% of the plasma apo(a). The fragments in the plasma were larger than those in the urine, were not attached to apoB, and the concentration was directly related to plasma and urine Lp(a). The source of the fragments was investigated in subjects before and after cardiac surgery with cardiopulmonary bypass. Despite an inflammatory response and activation of neutrophils and an increase in neutrophil elastase (an apo(a) cleavage enzyme), apo(a) fragments in plasma remained unchanged. No fragments of apo(a) were formed when apo(a) was incubated with plasma *ex vivo* suggesting that the fragmentation occurs in tissues. Frank et al. investigated the role of various tissues in apo(a) fragmentation in mice treated with the N-terminal region of apo(a) and found fragments rapidly filtered into the urine. Incubation of perfused tissues with the N-terminal region of apo(a) indicated greater fragmentation in perfused skeletal muscle and kidney than in liver or spleen. When compared to the urinary excretion of other glycoproteins of a similar size, apo(a) fragments are excreted at higher rates, and Plg fragments are not detected. These results suggest that the kringle structure did not mediate the excretion of apo(a) and the kidney apo(a) fragmentation is not a rate-limiting step in the catabolism of apo(a) (Figure 4). Gonbert et al. suggested the pattern of fragments could be a marker of CVD risk, but this has not been assessed.

### 4.2. Localization of apo(a) fragmentation

The plasma concentration of Lp(a) increases in patients with renal disease but the urinary excretion of apo(a) fragments decreased suggesting that the kidney is required for the degradation of apo(a). Reblin et al. found that after apo(a) injection into rats 50–160 kDa apo(a) fragments were excreted in urine and apo(a) and the fragments accumulated intracellularly in the kidney. Frishmann et al. measured the catabolic and synthetic rate of Lp(a)-apo(a) and Lp(a)-apoB in hemodialysis patients and found that the FCR for both Lp(a)-apo(a) and Lp(a)-apoB was significantly reduced and that the residence time of Lp(a) was 2–3-fold higher in the hemodialysis patients than controls. The prolonged retention may account for the higher Lp(a) concentrations in patients with kidney disease. In hedgehogs and mice, the liver had a 3–4-fold higher uptake of Lp(a) than the kidney. A kinetic study injecting Lp(a) in LDLR deficient mice or apoE deficient mice suggested that the LDLR, apoE, or the asialoglycoprotein receptor, did not have a major role in Lp(a) clearance, but injected apo(a) inhibited Lp(a) clearance, suggesting apo(a) mediates the Lp(a) clearance. In this study, hepatic uptake was 35% of the injected dose versus only 1.3% for the kidney.

### 4.3. Proteolytic enzymes

Proteolytic enzymes responsible for the degradation of apo(a) were investigated *in vitro*, and neutrophil elastase was found to cleave apo(a) between KIV-4 and -5, and MMP-12 or macrophage elastase was also found to cleave apo(a) between KIV-4 and KIV-5, but at a different position. The degradation of apo(a), Lp(a), and the apo(a) N-terminal region was assessed in control, NE-/- mice, and MMP-12-/- mice. When apo(a) was injected, fragments were similar in mice from the control and NE-/- mice, but in MMP-12 -/- mice very little apo(a) was degraded, suggesting MMP-12 is important *in vivo* for the

degradation of apo(a). No difference was found between NE and MMP-12 deficient mice and control mice when the N-terminal fragment was injected. The N-terminal fragment was not degraded in plasma but fragments were found in the urine, suggesting different mechanisms for the catabolism of the C-terminal and N-terminal regions. In human carotid plaque, MMP-9 and MMP-2 were enhanced in regions where the abluminal lipid core and fibrous cap co-localized with the apo(a) C-terminal domain fragment.

#### 4.4. Role of vascular uptake of Lp(a) in degradation

Studies show that apo(a) and Lp(a) accumulate in the vascular wall in atherosclerotic lesions in human and non-human primates, and the amount is proportional to that found in the plasma. While the gene expression of LPA has been reported in the aorta and carotid arteries, the contribution of extrahepatic LPA gene expression to apo(a) accumulation is unknown. Hoff et al. characterized the apo(a) and Lp(a) that accumulated in human atherosclerotic lesions and found oxidized Lp(a), complexed larger particles, and small particles of degraded apo(a). In addition, Lp(a)/apo(a) and LDL were localized to different sites in the vessel wall. Nielsen et al. measured the accumulation of injected Lp(a) and LDL into rabbit aortas with and without atherosclerotic lesions. In nonlesioned aorta Lp(a) degradation was less than for LDL, and in atherosclerotic lesions, the Lp(a) degradation rate increased to match the degradation rate of LDL.

The mechanism of the differential Lp(a) accumulation in the vessel wall is unclear. Just as with Lp(a) binding to cells, Lp(a) binding to the ECM could be mediated by the LDL moiety or by apo(a). Lp(a) binds with higher affinity and greater concentration to the extracellular matrix (ECM) than LDL. Lp(a) also binds to ECM with higher affinity than Plg but with fewer binding sites than for Plg. Both Lp(a) and Plg binding to ECM is inhibited by lysine analogs, indicating binding was mediated by the LBS domain of Lp(a) and Plg. Lp(a) binds to a number of immobilized proteins including, fibrinogen, laminin, fibronectin and collagen IV, all components of the vascular wall. Lp(a) and apo(a) bind to fibrin and compete with Plg binding. The KV-protease domain of apo(a) was identified as the binding site of apo(a) for fibrin. Apo(a) binding to fibronectin was localized to the C-terminal region of apo(a). DANCE (fibulin-5) was identified as a ligand for Lp(a). Vascular smooth muscle cells secrete fibulin-5, and in deficient mice the elastic fiber lamina is defective and there are abnormal elastin aggregates in the aortic wall. Fibulin-5 is the only protein reported to bind to the N-terminal region of apo(a). In a perivascular cuff model, collagen was increased in the adventitia in apo(a) transgenic mice. Elevated Lp(a) alters the structure of the fibrin clot, which suggests apo(a) may also perturb the structure of other proteins within the vessel wall. In apo(a) transgenic mice, fatty streak lesion formation is increased and Hughes et al. found that vascular accumulation of adenovirus apo(a) delivered to apoB transgenic mice were diminished with LBS (KIV-10) defective forms of apo(a). Lp(a) binds to fibrinogen and in fibrin-/- mice with the apo(a) transgene, apo(a) accumulation was reduced in the fatty streak lesion of the vessel wall, suggesting that fibrinogen in the vessel wall is important for the retention of apo(a). Defensin, released from activated neutrophils, promotes the binding of Lp(a) to the vascular ECM. Lp(a) was found co-localized with laminin in atherosclerotic lesions and was inhibited by EACA. The role of Lp(a) in the development of vascular injury may be independent of LDL and Plg.

## 5. Modifications of Lp(a)

### 5.1. Oxidized Lp(a)

Oxidized Lp(a) was found in human atherosclerotic lesions and is more closely associated with carotid artery intima-media thickness than Lp(a). Umahara et al. found differential expression of oxidized/native Lp(a) and Plg in human carotid and cerebral artery plaques.

Elevated plasma ox-Lp(a) levels are associated with the presence and severity of acute coronary syndrome (ACS), and may be useful for identification of patients with ACS Lp(a). However, Bossola et al. did not find an association of oxidized phospholipids on apoB-100 particles, lipoprotein(a) or oxidized LDL with CVD in chronic hemodialysis patients. In addition, studies demonstrate an association of oxidized Lp(a) with modified functions. Malondialdehyde modification of Lp(a) and LDL induces uptake by the scavenger receptor in macrophages, and causes changes in the structure and biological properties of apo(a). Hypochlorite oxidation causes cross-linking of Lp(a). Although less susceptible than LDL, Lp(a) undergoes non-enzymatic glycation in hyperglycemia.

## 5.2.Regulation of Lp(a) LBS activity

In addition to vascular accumulation and increases in plasma of oxLp(a), changes in LBS activity by enzymatic and chemical modifications of Lp(a) could alter its cellular uptake and vascular accumulation. Using an LBS-Lp(a) immunoassay and a lysine-Sepharose bead assay, the LBS activity of Lp(a) could be quantified. LBS activity decreased with oxidation of Lp(a) by 2,2'-azobis(2-amidinopropane) dihydrochloride oxidation, but increased with copper sulfate oxidation. N-acetyl treatment decreased LBS activity, and homocysteine treatment had no effect on LBS activity. Modification of Lp(a) with N-acetyl-cysteine decreased elevated Lp(a) *in vivo*. Only minimal changes occurred in Lp(a) LBS activity after treatment with proteins found in the plasma, including lipoprotein lipase, sphingomyelinase, or phospholipase C. However, after phospholipase A<sub>2</sub> treatment, the LBS activity of Lp(a) increased by 80%, and enhanced Lp(a) binding to the subendothelial matrix. In addition, Lp(a) LBS activity varies in the population. In patients with coronary artery disease, Lp(a) concentration and small isoform size were higher, and the LBS activity was higher in the small isoforms compared to control subjects. Compared to LDL, Lp(a) contains an enhanced association of lipoprotein-associated phospholipase A<sub>2</sub>. Oxidized phosphatidylcholine linked to lysine residues was not derived from LDL. Higher concentrations of oxidized phosphatidylcholine were found in Lp(a) compared to LDL in transgenic mice. Thus, Lp(a) is highly susceptible to modification in the circulation and vasculature that alters its function.

## 6. Pathological and physiological roles for Lp(a)

Lp(a) metabolism may impact its physiological and pathogenic roles (Figure 5). With the recent studies of large numbers of subject there is convincing evidence that elevated Lp(a) is an independent risk factor for cardiovascular diseases, however, the molecular basis for this pathological risk is not clear. Lp(a) may have both prothrombotic and antithrombotic properties, as well as atherothrombotic properties. The complexity of Lp(a) and the limited animal models has complicated the mechanistic studies of Lp(a) function. Lp(a) may have properties of both LDL and apo(a). One hypothesis is that Lp(a) may inhibit and interfere with Plg activation, receptor binding, or proteolysis. Another hypothesis is that Lp(a) binds to Plg receptors or ECM and deposits LDL in the vessel wall at unique sites. These hypotheses have been tested *in vitro* and *in vivo*, but the distinctions have not always been made as to whether Lp(a) role is due to its LDL component or Plg-like component or whether there are unique properties of Lp(a). More than likely Lp(a) and apo(a) are multi-functional. Lp(a) size and plasma concentration, determined genetically; cleavage of Lp(a) to Lp(a):LDL and apo(a); fragmentation of apo(a); and modification of Lp(a), Lp(a):LDL, and apo(a) may contribute to the pathogenesis of Lp(a). Decreasing synthesis to reduce plasma concentration may be the most expeditious way to reduce Lp(a) CVD risk. Since many mammals do not carry the gene for apo(a), it is likely that there may be a positive physiological survival role for Lp(a) in primates. Possible candidates for this physiological role may be the regulation of neutrophils, and as a scavenger of oxidized phosphatidylcholine with high concentrations of lipoprotein-associated phospholipase A<sub>2</sub>.



There was no significant difference in Lp(a) in subjects <75 and >75 yrs. Lp(a) was associated with CVD but not in men>65 yrs. suggesting that elevated Lp(a) is compatible with longevity and may serve a physiological function. Genetic polymorphisms have been associated with particular CVDs. Whether there are specific polymorphisms associated with a survival or physiological function remains to be seen. The genetic variations that mediate the modifications of circulating Lp(a) that alter function and metabolism may also be important.

## 7. Conclusions and Clinical Implications

Elevated Lp(a) is an established risk factor for cardiovascular and peripheral vascular diseases. Studies of Lp(a) have been hampered by the lack of Lp(a) expression in small animal models and must rely on transgenic models. While many aspects of Lp(a) metabolism are fairly well understood, other aspects of Lp(a) metabolism are unclear. Circulating and vascular bound Lp(a) are subject to modifications that may influence its function and clearance from the blood and increasing the variability found in the population. The uptake and site of Lp(a) catabolism and fragmentation of apo(a) are poorly understood. Studies of Lp(a) assembly and catabolism were conducted over a decade ago and there may be new approaches that could be informative. A clearer understanding of Lp(a)/apo(a) pathological and physiological functions and its catabolism could lead to new strategies for intervention. There are currently no established effective therapeutic drugs for lowering elevated Lp(a) (Table 1). LDL apheresis has the largest decrease in plasma Lp(a), but is not suitable for all patients. Aspirin used for coronary artery disease and cerebral infarct treatment may also reduce Lp(a). Reducing elevated cholesterol and triglycerides has some benefit in reducing Lp(a) risk. Niacin, a lipid-lowering agent, has a modest effect on lowering Lp(a) and may be related to lowering LDL-C. Drugs, including the antisense oligonucleotide for apoB mRNA and the cholesterol esterase transfer protein inhibitor, are being tested in clinical trials with small numbers of subjects. Currently these studies report only modest reductions of plasma Lp(a). Drugs that reduce LPA transcription could be effective, such as the newly identified FGF19. Potentially there could be drugs already approved that could alter expression of LPA, including anti-TNF- $\alpha$  agents that reduce inflammation, TGF- $\beta$ , and PPAR modulators, but these have not been evaluated. Initially, fibrates, the lipid lowering drugs that activate PPAR $\alpha$ , were suggested as a Lp(a) lowering drug, but further studies were not consistent. Recently, other PPAR modulators have been developed, but not tested for lowering LPA. A new source of therapeutic drugs is emerging with targets for microRNA, small RNA sequences that regulate expression of genes. The microRNA regulation of LPA has not been investigated. Factors that alter LPA expression hold promise as therapeutic agents.

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## Abbreviations

LPA	apolipoprotein(a) gene
Lp(a)	lipoprotein(a)

<b>LDL</b>	low density lipoprotein
<b>apoB</b>	apolipoprotein B
<b>apo(a)</b>	apolipoprotein(a)
<b>Plg</b>	plasminogen
<b>C-terminal</b>	carboxy terminal end polypeptide chain
<b>N-terminal</b>	amino terminal end polypeptide chain
<b>CVD</b>	cardiovascular diseases
<b>mRNA</b>	<u>messenger ribonucleic acid</u>
<b>YAC</b>	yeast artificial chromosome
<b>KIV-2</b>	kringle IV Type 2
<b>kb</b>	kilobase
<b>%</b>	percent
<b>HNF3A(FOXA1)</b>	hepatocyte nuclear factor 3, alpha (forkhead box protein A1)
<b>HNF1A</b>	hepatocyte nuclear factor1 homebox A
<b>GATA4</b>	transcription factor
<b>Elk-1</b>	transcription factor
<b>NRE <math>\beta</math></b>	transcription factor binding motif
<b>Ets-1</b>	transcription factor binding motif
<b>DR-1</b>	transcription factor binding motif
<b>TF</b>	transcription factor
<b>FXR</b>	Farnesoid X receptor
<b>FGF19</b>	fibroblast growth factor 19
<b>FGF4</b>	fibroblast growth factor 4
<b>IL-6</b>	interleukin-6
<i>et al</i>	and others
<b>ER</b>	endoplasmic reticulum
<b>EACA</b>	epsilon-aminocaproic acid
<b>Cys</b>	cysteine
<b>LBS</b>	lysine binding site
<b>FCR</b>	fractional catabolic rate
<b>LDLR</b>	low density lipoprotein receptor
<b>LRP</b>	low density lipoprotein receptor-related protein
<b>VLDLR</b>	very low density lipoprotein receptor
<b>Gp330</b>	glycoprotein 330
<b>LRP2</b>	low density lipoprotein receptor-related protein 2
<b>apoE</b>	apolipoprotein E

<b>NE</b>	neutrophil elastase
<b>MMP-12</b>	metalloproteinase 12
<b>MMP-2</b>	metalloproteinase 2
<b>ECM</b>	extracellular matrix
<b>TNF-<math>\alpha</math></b>	tumor necrosis factor alpha
<b>TGF-<math>\beta</math></b>	transforming growth factor beta
<b>PPAR<math>\alpha</math></b>	peroxisome proliferator-activated receptor alpha
<b>BiP</b>	endoplasmic molecular chaperone
<b>PDI</b>	protein disulfide isomerase

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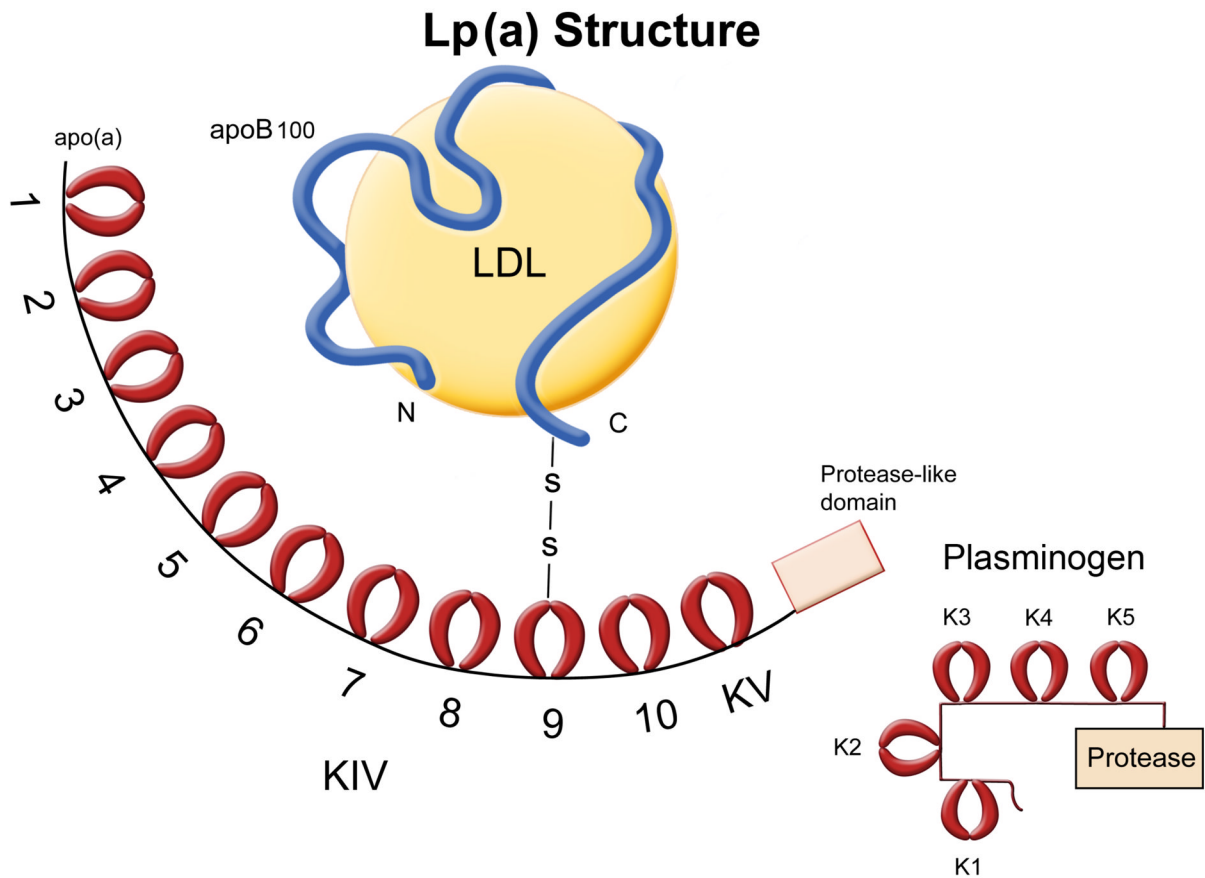
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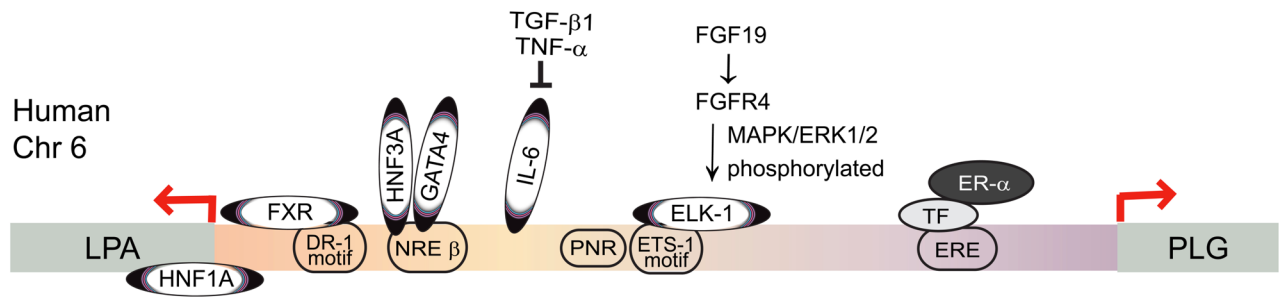


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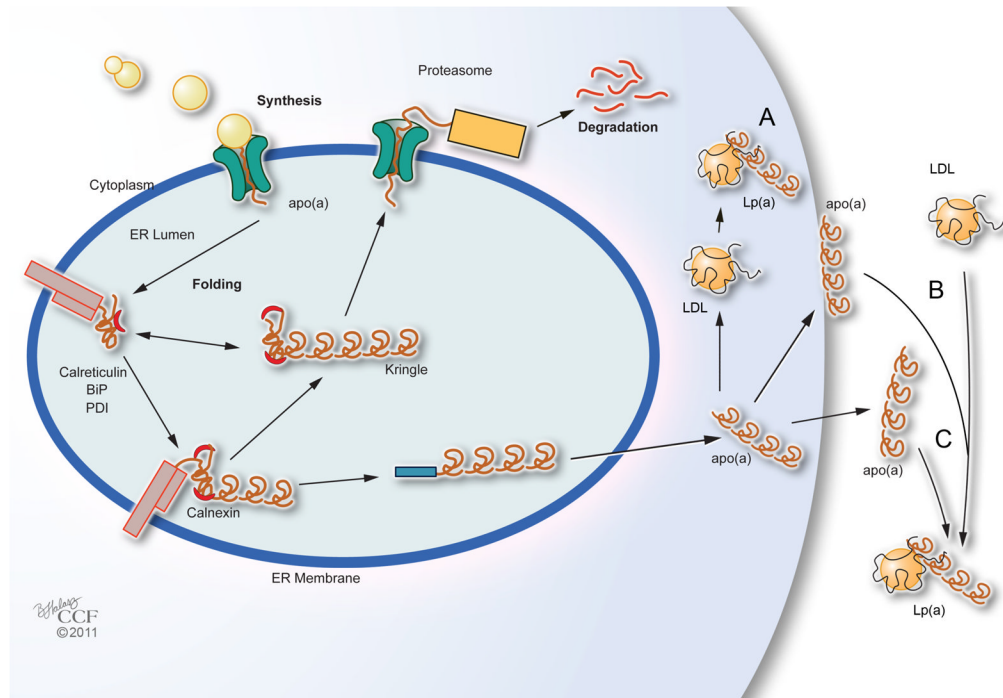
**Figure 1. Lp(a) Structure**

Lp(a) consists of a low-density lipoprotein lipid core with apolipoprotein B attached by a disulfide bond to apolipoprotein (a). Apo(a) contains a variable number of kringle domains (KIV-1-10) that have a high homology to K4 of plasminogen, one KV kringle similar to K5 of plasminogen, and a proteolytic-like domain. KIV-2 is present in 2-50 copies, imparting extreme heterogeneity to Lp(a). K4 and K5 domains of Plg bind to C-terminal lysines of cell surface proteins and ECM proteins. Apo(a) has a strong LBS in KIV-10 and weak LBSs in KIV-5-8.



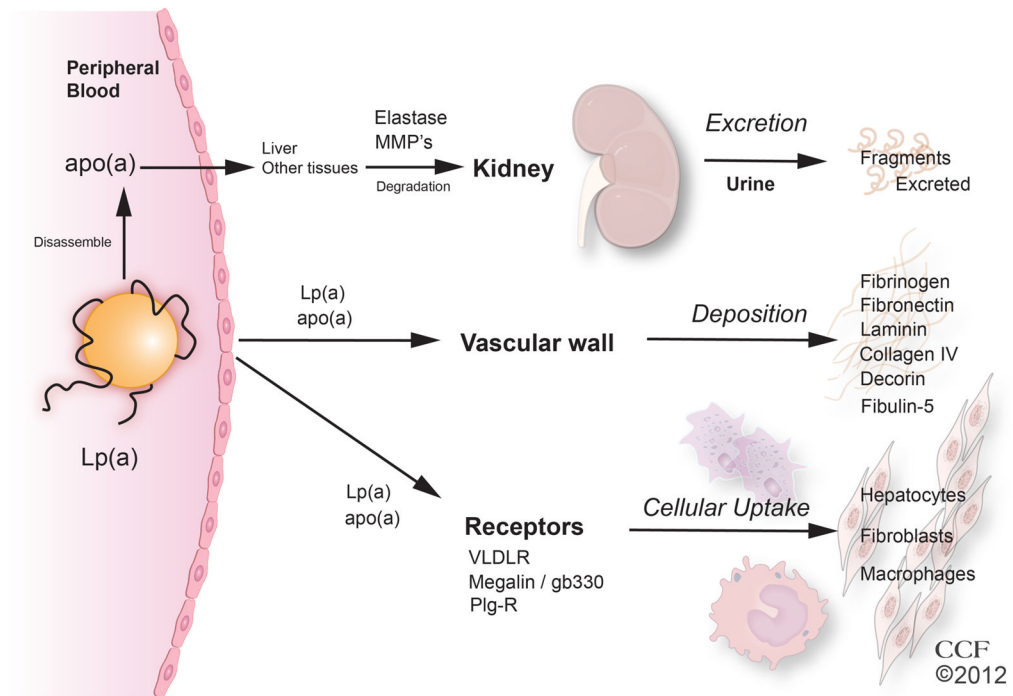
### Figure 2. Regulation of LPA Expression

Transcription factors bind to the 5' flanking sequence of LPA and regulate transcription activity. Shown are the sites that alter LPA expression. LPA and PLG genes are located on the same chromosome in humans. They are organized in a head to tail configuration and share the intergenic region in between. Trans-activation: HNF1A binds to 5' untranslated region and trans-activates the LPA promoter. IL-6 induces apo(a) and Lp(a) expression in acute response. It has several binding sites in the LPA promoter. Trans-repression: FXR binds to DR-1 site of LPA promoter and represses apo(a) expression in mice. HNF3A and GATA4 bind to NRE $\beta$  to represses gene transcription in HepG2 cells. Elk-1 binds to an Ets-1 motif in the human LPA promoter and mediates the expression of apo(a) repressed by FGF19.[10, 19, 23, 24, 25] In addition, a number of other transcription sites are predicted from the sequence of the promoter and the intergenic regions between the LPA gene and PLG gene.[36] **HNF1A** (+26/+42) GAGAGAATCATTAACT; **FXR** (-826/-814) GGGGGGCCAACGCGCACGG; **HNF3A** (-949/-939) ATTCTTGGCTC; **GATA4** (-973/-978) TGAGAG; **IL-6** (-1300/-800) CTGGGA; **Elk-1** (-1630/-1615) TTATCAGGATGTTTGC; **PNR** (-1432 to -1348); **ER- $\alpha$**  (-26kb): AGTTCAAGGATTGACCT



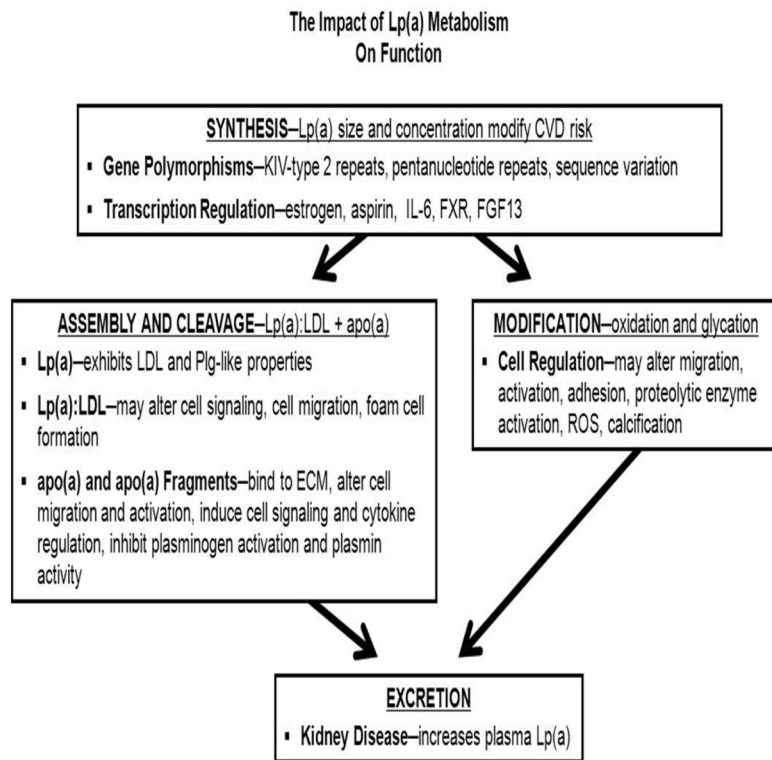
**Figure 3. Assembly of Lp(a)**

The site of Lp(a) assembly is unclear. Three possible sites have been proposed: A. intracellular; B. cell surface; and C. extracellular. BiP-endoplasmic molecular chaperone, PDI-protein disulfide isomerase.



**Figure 4. Catabolism of Lp(a)**

The site of Lp(a) and apo(a) degradation is not clear. Apo(a) may be degraded to fragments in the plasma, liver or other tissues with the fragments being excreted into the urine. Lp(a) and apo(a) and its fragments bind to ECM proteins of the vascular wall. Lp(a) may bind to VLDLR or megalin, and be taken up by hepatocytes, fibroblasts, or macrophages.



**Figure 5. Impact of Lp(a) metabolism on function**

The gene polymorphisms and transcription of the LPA gene have a striking influence on the Lp(a) plasma concentration and subsequently modify its risk. In addition, although LPA is located on Chromosome 6, loci on other chromosomes have been identified that alter plasma concentration and risk. Assembly and cleavage of Lp(a) may determine the portion of apo(a) and LDL that circulate in blood or deposited in the vessel wall. Modification of circulating or bound Lp(a) may have substantial role on its pathophysiology. Excretion of Lp(a) is poorly understood and may also impact the plasma concentration and pathophysiology.

**Table 1**

Therapeutic agents for decreasing elevated Lp(a).

Agent	Mechanism and Site of Intervention	Therapeutic Status	Ref.
<b><i>REDUCES Apo(a)</i></b>			
<b><i>SYNTHESIS</i></b>			
1. Estrogen	Hormone acts on responsive element in LPA promoter. In postmenopausal women (less estrogen) Lp(a) increases and with hormone replacement therapy Lp(a) decreases	NR*	
2. Anabolic Steroids	Hormone may act on gene expression	NR	
3. Tocilizumab	IL-6 receptor antagonist may inhibit gene expression	Approved <sup>^</sup>	
4. FGF19	Repression LPA gene expression	PreClinical	
5. FXR	Bile acid activated receptor that represses hepatic LPA gene expression	Preclinical	
6. Aspirin	Reduces LPA expression	Approved	
<b><i>REDUCES ApoB</i></b>			
<b><i>SYNTHESIS</i></b>			
7. Mipomersen	Antisense nucleotide decreases LDL synthesis	Phase III	
8. ApoB Peptides	Inhibit Lp(a) assembly	Preclinical	
<b><i>REDUCES LIPID</i></b>			
9. Apheresis	Removes LDL and Lp(a) from the circulation	Approved	
10. Niacin	Lowers diacylglycerol acyltransferase-2 (DGAT2) that decreases TG synthesis and VLDL assembly resulting in increased post-translational intrahepatic apoB degradation. Also reduces LDL- C and increases HDL	Approved	
11. Statins	Inhibits HMG-CoA reductase, the rate-limiting step in hepatic cholesterol synthesis. Decreases intracellular cholesterol and increases expression of hepatic LDL receptors resulting in increased hepatic clearance of circulating LDL	Approved	
12. Anacetrapib	Cholesterol ester transfer protein (CETP) inhibitor raises HDL and lowers LDL cholesterol	Phase III	
13. Eprotirone	Thyroid mimetic. Increases expression of hepatic LDLR and increases plasma clearance of LDL	Clinical trial discontinued due to side effects (2012)	
14. PCSK9 Inhibitor	PCSK9 promotes hepatic LDLR degradation reducing LDLR density and clearance of LDL particle. Inhibitor decreases plasma Lp(a)	Phase II	
15. PUFA	Long term consumption	Diet Supplement	
16. Carnitine	Important for transport of long chain fatty acids across mitochondrial membranes for mitochondrial $\beta$ -oxidation	Diet Supplement	

\* NR—not recommended;

<sup>^</sup> treatment of rheumatoid arthritis;

PCSK9-Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease; PUFA-polyunsaturated fatty acids.