Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease: Recent Advances and Future Directions

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It has been estimated that ~37% of the US population judged to be at high risk for developing coronary artery disease (CAD), based on the National Cholesterol Education Program guidelines, have increased plasma lipoprotein(a) [Lp(a)], whereas Lp(a) is increased in only 14% of those judged to be at low risk. Therefore, the importance of establishing a better understanding of the relative contribution of Lp(a) to the risk burden for CAD and other forms of vascular disease, as well as the underlying mechanisms, is clearly evident. However, the structural complexity and size heterogeneity of Lp(a) have hindered the development of immunoassays to accurately measure Lp(a) concentrations in plasma. The large intermethod variation in Lp(a) values has made it difficult to compare data from different clinical studies and to achieve a uniform interpretation of clinical data. A workshop was recently convened by the National Heart, Lung, and Blood Institute (NHLBI) to evaluate our current understanding of Lp(a) as a risk factor for atherosclerotic disorders; to determine how future studies could be designed to more clearly define the extent to which, and mechanisms by which, Lp(a) participates in these processes; and to present the results of the NHLBI-supported program for the evaluation and standardization of Lp(a) immunoassays. This report includes the most recent data presented by the workshop participants and the resulting practical and research recommendations.
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Lipoprotein(a) [Lp(a)],[4] first discovered by Kåre Berg in 1963 (1), is a specific class of lipoprotein particles found in human plasma. The protein moiety comprises two components, a single copy of apolipoprotein B-100 linked to a single copy of a protein referred to as apolipoprotein(a) [apo(a)]. Treatment of Lp(a) with reducing agents yields apo(a) and a lipoprotein particle that is essentially indistinguishable from LDL, both in structure and in its physical and chemical properties. Because Lp(a) and LDL are metabolically distinct, it is evident that the special characteristics of Lp(a), including its size and density heterogeneity, are almost entirely attributable to apo(a). apo(a) is a carbohydrate-rich, highly hydrophilic protein characterized by a marked size heterogeneity that is primarily attributable to a genetic size polymorphism of the polypeptide chain (2). Numerous studies have documented that high plasma Lp(a) concentrations are associated with a variety of cardiovascular disorders, including peripheral vascular disease, cerebrovascular disease, and premature coronary disease [for a review, see Ref. (3)]. However, despite intense scientific efforts, Lp(a) continues to be an enigmatic lipoprotein particle that has defied the ability of scientists to elucidate its physiologic role and

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[4] Nonstandard abbreviations: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); apo B, apolipoprotein B; NHLBI, National Heart, Lung, and Blood Institute; CVD, cardiovascular disease; WHHL, Watanabe Heritable Hyperlipidemia; MAb, monoclonal antibody; and CHD, coronary heart disease.

its pathologic mechanisms of action, thus making it difficult for clinicians to develop effective interventions in clinical practice. Although the mechanism of Lp(a) pathogenicity in the process of atherosclerosis remains unclear, it has been well established that Lp(a) is deposited at sites of vascular injury (4–6). As such, it is reasonable to postulate that the contribution of Lp(a) to atherosclerosis is facilitated by interactions of apo(a) with plaque elements.

Analysis of an apo(a) cDNA from a human liver library in 1987 revealed that apo(a) bears striking homology with the fibrinolytic proenzyme plasminogen and is a member of the plasminogen gene superfamily (7). apo(a) contains domains that correspond to single copies of plasminogen kringle 5 and the serine protease domain, as well as multiple copies of a sequence that closely resembles plasminogen kringle 4. On the basis of amino acid sequencing, apo(a) contains 10 types of plasminogen kringle 4-like motifs (designated K4 types 1–10). Each K4 motif is present in a single copy, except for apo(a) K4 type 2, which is present in a variable number of identically repeated copies (3 to >40) (8, 9). This forms the molecular basis of the Lp(a) isoform size heterogeneity that is a hallmark of this unique lipoprotein. It is noteworthy that despite a high degree of sequence similarity, the serine protease-like domain of apo(a) is catalytically inactive (10), which has led to speculation that Lp(a) can interfere with the physiologic functions of plasminogen in the fibrinolytic cascade. This could provide a direct link between the processes of atherosclerosis and thrombosis.

Sites for N-linked glycosylation are present within the core of each apo(a) K4 motif (7, 11), whereas a minimum of six O-linked glycosylation sites are present within the linker sequences that join individual kringles (12). The hydrophilic O-linked glycans are primarily (~80%) composed of monosialylated core structures (13), which may have implications for Lp(a) catabolism. apo(a), and thus Lp(a), is heterogeneous in its glycosylation, although the significance of the glycosylation modification with respect to the in vivo properties of Lp(a) is still unclear.

Discussion Highlights

ASSEMBLY AND CATABOLISM OF Lp(a): ROLE IN DETERMINATION OF PLASMA Lp(a) CONCENTRATIONS

Lp(a) concentrations vary greatly in the population, ranging from 0.1 to >250 nmol/L (14). The primary site of apo(a) synthesis is the liver (15); the site of assembly of apo(a) and apolipoprotein B (apo B)-100 to form Lp(a) particles is not clear at present, but may occur on the surface of hepatocytes (16). Assembly of Lp(a) particles on the cell surface is not inconsistent with in vivo kinetic data, which suggest that a separate pool of apo B is associated with Lp(a) secretion (17). Kinetic data from Su et al. (18) support the view that newly synthesized apo B rather than circulating LDL-apo B is used in the formation of Lp(a) particles.

There is evidence to suggest that the rate of formation of Lp(a), rather than its catabolism, is the primary determinant of plasma Lp(a) concentrations (19). This is supported by data demonstrating that the fractional catabolic rates of ^{125}I-labeled Lp(a) of differing isoform size composition are not significantly different (20), despite the general inverse correlation that has been reported between apo(a) isoform size and plasma Lp(a) concentrations (2). This inverse correlation has been attributed at least in part to the increased endoplasmic reticulum retention time that has been reported for larger apo(a) species, which leads to a greater extent of intracellular degradation in the hepatocytes (21).

In Lp(a) particles, apo(a) is covalently linked to apo B-100 by a single disulfide bond (22, 23); the stoichiometry of apo(a) and apo B-100 in the Lp(a) particle is 1:1 (24). Considerable evidence suggests that Lp(a) assembly occurs as a two-step process in which initial noncovalent interactions between apo B and apo(a) precede disulfide bond formation; this initial interaction can be effectively inhibited by lysine and lysine analogs (25–27). Experiments using site-directed mutagenesis of recombinant apo(a) suggest that the weak lysine binding sites present within apo(a) K4 types 7 and 8 play a major role in noncovalent association with apo B-100 [M.L. Koschinsky and L. Becker, National Heart, Lung, and Blood Institute (NHLBI) conference presentation]; other work from this group has identified key lysine residues within the NH$_2$-terminal 18% of apo B, which bind to the weak lysine binding sites in apo(a) (28). A peptide spanning amino acid residues 680–704 in the NH$_2$ terminus of apo B can reduce Lp(a) formation in vitro (28). Additional sites of noncovalent interaction within the COOH terminus of apo B have also been identified (29); Sharp et al. (30) have recently shown that a peptide spanning amino acid residues 4372–4392 in apo B-100 can completely inhibit the apo(a)–apo B binding interaction. The use of peptide inhibitors may represent an interesting strategy for inhibiting Lp(a) assembly. The ability of the lysine analog tranexamic acid to reduce plasma Lp(a) concentrations in humans has also been reported (31), again underscoring the potential utility of inhibiting Lp(a) assembly to lower plasma Lp(a) concentrations. Most recently, Becker et al. (32) have reported that a conformational change in apo(a), which can be elicited in vitro by lysine analogs, is accompanied by a significant increase in the rate of the covalent step of Lp(a) assembly; this may provide the rationale for the development of a new generation of compounds aimed at lowering plasma Lp(a) concentrations by inhibiting the process of covalent Lp(a) assembly.

The pathway(s) of Lp(a) catabolism in vivo remain(s) unclear. A study using primed constant infusion of deuterated leucine in 23 individuals revealed that the residence time of apo(a) in plasma was 4.8 days, whereas the residence time of apo B-100 associated with Lp(a) particles was only 2.5 days (E. Schaefer, NHLBI conference presentation). These data were taken to suggest that apo(a) recirculates twice on an apo B-100 particle in

plasma before catabolism. However, the mechanism that would allow for cellular recycling of apo(a) remains unclear at present. Additionally, this finding is not in agreement with other kinetic studies showing similar plasma residence times of apo(a) and apo B-100 in Lp(a) *(18)*.

The major site of catabolism of Lp(a) particles in humans remains unidentified. Although fragments of apo(a) have been identified in urine, this pathway likely does not account for more than 1% of the fractional catabolic rate of Lp(a) *(33)*. Several studies have identified receptors that can bind to and internalize Lp(a) [for a review, see Ref. *(34)*]. Kostner and colleagues have recently identified the mammalian hepatic asialoglycoprotein receptor, an endocytotic recycling receptor that mediates the binding and internalization of desialylated glycoproteins as a selective receptor and may be responsible for Lp(a) catabolism in vivo (G.M. Kostner, NHLBI conference presentation). Further studies will be required to assess the relative contributions of receptor- and non-receptor-mediated catabolism of Lp(a).

Studies by Gaubatz et al. *(35)* have demonstrated the existence of Lp(a) noncovalently associated with triglyceride-rich lipoproteins in patients with hyperlipidemia; they also reported that Lp(a) containing higher molecular weight apo(a) isoforms preferentially bound to the triglyceride-rich lipoprotein fraction. The catabolism of the Lp(a)–triglyceride-rich lipoprotein complex, which is more abundant in patients with hypertriglyceridemia, may be different from that of conventional Lp(a) particles and therefore warrants further study.

contribution of genetics to determination of plasma Lp(a) concentrations
Discovery of the genetic size polymorphism of apo(a) by Utermann et al. *(2)* has provided major insights into the genetic control of plasma Lp(a) concentrations. Results of early studies demonstrated that all apo(a) isoforms are codominantly expressed at a single locus by multiple autosomal alleles (differing in the number of sequences encoding K4 type 2 motifs) *(8, 36)*. Additionally, it was demonstrated that the sizes of apo(a) isoforms are, in general, inversely correlated with plasma Lp(a) concentrations *(2)*.

Examination of Lp(a) concentrations and apo(a) genotypes in 48 Caucasian families demonstrated that the apo(a) gene *LPA* accounts for >90% of the genetic variation in plasma Lp(a) concentrations and that the number of K4 repeats in *LPA* accounts for ~70% of this variation *(37)*. However, the extent to which variation in the size of *LPA* accounts for the variability in plasma Lp(a) concentrations is highly dependent on the ethnic composition of the study group. For example, although *LPA* itself is also the major determinant of plasma Lp(a) concentrations in the black American population, it has been reported to account for only one-half of the variance in the African population *(38)*. In the latter population, other genetic

factors (in addition to *LPA*) as well as nongenetic factors may contribute to the determination of Lp(a) concentrations.

Sequence variations in the coding and noncoding regions of *LPA* have been reported to contribute to variation in plasma Lp(a) concentrations. For example, a repeat polymorphism of the pentanucleotide sequence TTTTA present at −1371 upstream of the apo(a) translational start site in *LPA* may account for 10–14% of the interindividual variations in plasma Lp(a) concentrations in Caucasians *(39)*. Although an inverse relationship between the number of pentanucleotide repeats and Lp(a) concentrations in Caucasians has been reported, no such correlation was observed in African individuals *(39)*. Interestingly, a +93 C/T substitution in *LPA* has been reported to decrease translational efficiency in vitro and influences Lp(a) concentrations in African but not Caucasian populations *(40)*.

In a recent study, the size of apo(a) alleles together with the pentanucleotide repeat polymorphism and single-nucleotide polymorphisms in the 5′ and 3′ regions of *LPA* were analyzed in >2000 unrelated individuals from 17 populations of African, Arctic, Asian, European, and Pacific origins (G. Utermann, NHLBI conference presentation). Core haplotype frequencies for the single-nucleotide polymorphisms were found to be different among populations of different geographic origin. Significant linkage disequilibria exist between the polymorphisms separated by the K4 type 2 repeats, suggesting that the size polymorphism in the gene encoding apo(a) has arisen from gene conversion rather than by recombination. Additionally, this study confirmed that the relationship between *LPA* variation and Lp(a) concentrations is heterogeneous across ethnic groups. Although these studies suggest a causal relationship between polymorphisms in the gene encoding apo(a) and variation in plasma concentrations of Lp(a), the exact identity of the causal polymorphisms and the mechanisms underlying their effects on Lp(a) concentrations remain to be pinpointed.

On the basis of the studies described above, the strong ethnic dependence of the relationship between apo(a) size and Lp(a) concentration needs to be taken into account in the design of prospective studies aimed at evaluating the contribution of Lp(a) to future cardiovascular events. This is because there are indications that small apo(a) isoforms, in addition to being associated with higher plasma Lp(a) concentrations, may in and of themselves be more atherogenic *(41)*. As such, the relationship between apo(a) isoform size and risk for cardiovascular disease (CVD) needs to be examined in various ethnic groups.

use of genomics to study mechanisms of apo(a) gene regulation and evolution
The orthologous distribution of the apo(a) gene is limited to Old World monkeys and humans *(42)*, which prevents the use of a standard comparative genomics approach to identify key regulatory sequences in this gene. Recently, the technique of phylogenetic shadowing has been ap-

plied by Boffelli et al. (43) to uncover primate-specific regulatory elements in the apo(a) gene. Sequence analysis of the apo(a) gene locus in humans, chimpanzees, and baboons revealed a region (~1.6 kb) of extreme conservation adjacent to the transcription start site; this region was analyzed in 18 Old World monkeys and hominoids. Phylogenetic shadowing revealed that regions with the lowest variation corresponded to sequences containing the first exon, the TATA box, and a hepatocyte nuclear transcription factor-1α binding site; the last two have been functionally identified in a previous study (44). Eight additional regions (40–70 bp in length) showed a high degree of conservation, which suggests that they are also potentially important in regulation of the apo(a) gene. The 10 most conserved regions of sequence were found to interact with DNA binding proteins and had a larger functional impact on expression based on their ability to drive expression of a reporter gene in vitro, compared with nonconserved regions.

Extensive genomic sequence comparisons of species both with (e.g., hedgehog, baboon, and human) and without (e.g., mouse and lemur) the apo(a) gene indicate that twice during evolution, duplication of the plasminogen gene has occurred, giving rise to the apo(a) gene. In both cases, this has led to the generation of a molecule that can bind to both LDL and fibrin, thereby providing a compelling example of convergent evolution. Taken together, the studies presented by Boffelli et al. (43) underscore the usefulness of genome analysis in understanding the regulation of the apo(a) gene, as well as its evolutionary history.

MECHANISM OF Lp(a) ACTION: INSIGHTS FROM ANIMAL MODELS

Studies of Lp(a) biology using animal models have been complicated by the unusual species distribution of this lipoprotein. Lp(a) is present only in humans, Old World monkeys, and the hedgehog (45). The principal animal models for Lp(a) have been transgenic mice and rabbits. Both species have their advantages and disadvantages, and both models have provided fundamental insights into biochemical and pathophysiologic aspects of Lp(a). The most powerful application for transgenic apo(a) animals is assessment of the role of apo(a) or Lp(a) in the development of atherosclerosis and analysis of the mechanism(s) by which this may occur.

The earliest data suggesting that apo(a) is atherogenic came from transgenic mouse experiments. It was reported that apo(a) transgenic mice fed an atherogenic diet had significantly larger aortic lesion areas than their nontransgenic littermates fed the same diet, and apo(a) was found colocalized with (mouse) apo B in the lesions (46). Subsequent studies in mice transgenic for both human apo(a) and human apo B-100 confirmed that animals with the apo(a) transgene had increased aortic fatty streaks, whereas animals with both transgenes had lesions 2.5 times larger than those expressing the apo(a) transgene

alone (47). In contrast, Mancini et al. (48) found that expression of apo(a) alone did not promote atherosclerosis in the proximal aorta of fat-fed mice. A subsequent study comparing human apo B-100 with apo(a)/human apo B-100 transgenic mice in LDL-receptor knockout mice also found that expression of apo(a) did not potentiate aortic lesion development (49). However, Berg et al. (50), studying relatively aged apo(a) transgenic mice that possessed the LDL receptor and had been fed normal chow, found that these mice had more extensive atherosclerotic lesions than nontransgenic control mice.

In sharp contrast to the inconsistent atherogenic effects of apo(a) in transgenic mouse models is the striking atherogenicity of apo(a) when expressed from a transgene in rabbits. Studies by Fan et al. (51) evaluating diet-induced atherosclerosis in transgenic rabbits revealed that expression of apo(a) led to an approximately two- to fivefold increase in lesion area. Unlike transgenic apo(a) mice, in which atherosclerosis was limited to the proximal aorta, in the transgenic rabbits lesions were observed throughout the aorta and in the carotid, iliac, and coronary arteries. As reported recently, considerably more dramatic results were seen when apo(a) was expressed from a transgene in Watanabe Heritable Hyperlipidemia (WHHL) rabbits (52). The WHHL rabbit is an excellent model for atherosclerosis because the animals spontaneously develop severe atherosclerosis that is characterized by complex lesions closely resembling those found in advanced human disease (53). The morphologic features of the lesions differed markedly between nontransgenic and transgenic animals. The lesions in the nontransgenic WHHL rabbits resembled fatty streaks, whereas the apo(a)-expressing animals had much more advanced lesions that contained atheroma, fibroatheroma, and calcification. These lesions were covered by a fibrous cap containing smooth muscle cells and had a necrotic core containing macrophage-derived foam cells (52). These findings are particularly noteworthy because it is this type of lesion morphology that has been implicated in the "vulnerable plaque" in human disease that is prone to rupture, from which the catastrophic thrombotic sequelae of atherosclerosis ensue.

The animal model studies conducted to date provide prima facia evidence that apo(a) is an atherogenic molecule. It is clear that both the mouse and rabbit models have their advantages and disadvantages. The models should be viewed as complementary and should, coupled with continued in vitro studies of the biochemical properties of apo(a) and Lp(a), provide critical insights into the pathogenic mechanisms of Lp(a). In addition, the animal models can be used to investigate whether smaller apo(a) isoforms are more atherogenic and whether there are gender differences in risk attributable to Lp(a), as well as to discover and apply therapeutic strategies aimed at lowering plasma Lp(a) concentrations.

MEASUREMENT OF Lp(a) CONCENTRATIONS IN PLASMA

The high degree of size heterogeneity of apo(a), derived from the variable number of K4 type 2 repeats, provides a variable number of identical epitopes to interact with the antibodies used in immunoassays to measure the concentration of Lp(a) in plasma. Considering that the main prerequisite for the development of an accurate immunoassay is that the antibodies possess the same immunoreactivity per particle for the assay calibrator and for the samples being analyzed, it is evident that the size variation of apo(a) affects the development of immunoassays on two fronts: (*a*) the choice of apo(a) size in the calibrator is arbitrary, and independent of the choice, the calibrator would not be representative of the size of apo(a) in most of the samples; and (*b*) the immunoreactivity of the antibodies directed to the identically repeated epitopes in K4 type 2 will vary depending on the size of apo(a). As a consequence, the immunoassays will tend to underestimate Lp(a) in samples with apo(a) sizes smaller than the apo(a) size of the assay calibrator and, conversely, overestimate Lp(a) in samples with larger apo(a) isoforms.

To document the impact of apo(a) size on the accuracy of Lp(a) measurements, Marcovina et al. (*54*) generated and characterized a variety of monoclonal antibodies (MAbs) directed to different apo(a) epitopes. A MAb (a5) directed to an epitope present in K4 type 2, whose immunoreactivity per particle would therefore vary depending on apo(a) size, and another MAb (a40) specific for a unique epitope located in K4 type 9 were used to develop two enzyme immunoassay (ELISA) methods to measure Lp(a) in a large number of samples. Both assays were calibrated with the same serum containing an apo(a) with 21 K4 motifs. The Lp(a) protein value was assigned to the assay calibrator by use of a primary preparation, and the values were expressed in nmol/L to reflect the number of Lp(a) particles in plasma. As expected, practically identical values were obtained by the two ELISA methods in the samples with the same apo(a) size as in the assay calibrator, whereas the Lp(a) values measured by MAb a5 were under- or overestimated in samples with apo(a) containing <21 or >21 K4 repeats, respectively, compared with the values obtained by MAb a40. This MAb a40-based ELISA has been carefully optimized, extensively validated in a large number of individuals (*14*), and used as a reference method in the NHLBI-sponsored contract for the standardization of Lp(a) measurement.

The availability of a primary reference material is the first main step for the accuracy of immunoassays. As part of the NHLBI contract, Celina Edelstein, in collaboration with Dr. Angelo Scanu, developed a suitable method to prevent the marked physical, chemical, and immunologic changes that Lp(a) undergoes when isolated from human plasma (*55*). When isolated Lp(a) was lyophilized in the presence of suitable cryopreservatives and then reconstituted, it was found to be indistinguishable from the starting material in terms of composition and physical, chemical, and biological properties. Moreover, the reconstituted products exhibited unchanged immunochemical properties and appeared to have all of the requisites to be used as a primary reference material (C. Edelstein, NHLBI conference presentation).

The second step in the standardization process is the selection of a secondary reference material to be used to achieve comparability of values among different immunoassays. On the basis of extensive evaluations performed by the IFCC Working Group on Lp(a), a lyophilized serum pool preparation was selected as secondary reference material for Lp(a). As a collaboration between the recipients of the NHLBI contract and the IFCC Working Group on Lp(a), the MAb a40-based ELISA calibrated with a purified Lp(a) primary preparation was used to assign a target value to the IFCC secondary reference material. A target value of 107 nmol/L of Lp(a) protein was assigned to the IFCC preparation based on 144 replicate analyses. To evaluate the performance of commercially available methods, the IFCC reference preparation was then used to transfer an accuracy-based value to the various immunoassay calibrators. After uniformity of calibration was demonstrated in the 22 evaluated systems, Lp(a) was measured in 30 fresh-frozen samples covering a wide range of Lp(a) concentrations and apo(a) sizes with values assigned by the reference method. The CV among the methods on the IFCC preparation was 2.8%, clearly indicating the lack of matrix effect of this material. However, the CV among laboratories on the 30 samples ranged from 6% to 31%. Additionally, significant apo(a) size-dependent biases were observed among the values obtained by the majority of the evaluated systems. Only a turbidimetric assay was found to be insensitive to apo(a) isoform size. With this method, there was a nearly perfect correlation and a negligible bias between the obtained and the expected values, indicating the suitability of the IFCC reference material (*56*).

Despite the use of a common reference preparation, no harmonization in Lp(a) values among the different methods was achieved. This indicates that the impact of apo(a) isoform size on Lp(a) concentrations varies among the different methods as a function of the apo(a) size of the assay calibrators. In addition, other factors, such as differences in antibody properties; assay precision and robustness; and sensitivity of the assays to sample handling, storage conditions, and length of storage, play a role in and contribute to the lack of comparability of data obtained by different methods. All of these factors need to be taken into consideration in the standardization and validation of Lp(a) immunoassays. From these observations, it is obvious that no reference material, either primary or secondary, would be able to eliminate the biases in Lp(a) values obtained by methods that are affected by apo(a) size heterogeneity and/or are not properly optimized. However, the IFCC preparation has been shown to have excellent stability and commutability properties, and it has been proposed to the WHO to be accepted as the

WHO-IFCC Reference Material for Lp(a) (G.M. Kostner, NHLBI conference presentation). Its availability could play an important role by providing an accuracy-based calibration of validated assays.

To reduce the apo(a) size-dependent differences in results obtained with the immunoassays, a different approach to assay calibration was investigated. Instead of using serial dilutions of a single calibrator, five fresh-frozen samples with different apo(a) sizes from small to large and suitable Lp(a) concentrations were used to calibrate a turbidimetric assay affected by apo(a) size variation. This assay was selected because it had good robustness and precision characteristics, and no significant differences were observed in Lp(a) values between fresh samples and samples stored at −70 °C over a period of 15 months. Analyses were performed in parallel using the original assay calibrator and the five-sample calibrator on a large number of samples. Consistent apo(a) size-dependent biases were observed with the original assay calibrator. In contrast, comparability between the observed values and the values obtained by the reference method was good when the five independent samples were used to calibrate the assay (J.J. Albers, NHLBI conference presentation). Although this approach appears promising for reducing assay inaccuracy, it may not be equally effective in all of the methods or in all of the samples. Therefore, its potential use for assay standardization will require further evaluation using multiple methods and a large number of samples with a good representation of single and double apo(a) isoforms.

IMPACT OF Lp(a) METHOD INACCURACY ON THE INTERPRETATION OF Lp(a) VALUES

In the majority of clinical studies, Lp(a) concentrations have been determined by methods affected by apo(a) size heterogeneity. Therefore, for the conclusions of these studies to be valid, we must rely on the assumption that the distribution of apo(a) isoforms is similar between cases and controls, thus minimizing the potential that method-dependent over- or underestimation of Lp(a) values may contribute to the observed difference or lack thereof between cases and controls. Despite the importance of this topic, no studies have been performed to evaluate the impact of method inaccuracy on the interpretation of clinical data. To evaluate to what extent method inaccuracy affects the correct stratification of individuals for coronary artery disease risk, based on their Lp(a) values, Lp(a) concentrations were determined by the reference method in 2940 samples from the participants in the Framingham Study collected during the fifth cycle and apo(a) isoforms were determined as described previously (57). During the same cycle, Lp(a) values were also determined in other laboratories for 2556 of the samples by a turbidimetric method and for 2662 of the samples by a commercially available ELISA. A Lp(a) value of 75 nmol/L, which approximates the 75th percentile of the Framingham population as determined by the reference method, was arbitrarily selected as the clinical decision point.

On the basis of the Lp(a) values obtained by the turbidimetric assay, 136 individuals were misclassified as being at increased risk (false positive) and 23 were misclassified as being not at risk (false negative). On the basis of the Lp(a) values obtained by the commercial ELISA, 329 individuals were misclassified as being at increased risk (false positive) and 25 were false negative. The vast majority of the misclassifications observed with the turbidimetric method were explained by the over- or underestimation of Lp(a) values based on the apo(a) size in the samples. The larger number of false positives compared with the false negatives obtained by this assay is explained by the small apo(a) size in the assay calibrator and by the high frequency of samples in the general population with apo(a) sizes larger than that in the calibrator. This observation is consistent with previously reported data (56). However, the large number of false-positive values generated by the ELISA method was not explained by the apo(a) sizes in these samples. In fact, a high degree of variability in Lp(a) values was observed with this ELISA method. In addition, the values obtained from frozen samples were generally higher than those from fresh samples, but the magnitude of the increase was sample dependent. These findings clearly indicate that assay standardization can be achieved only if each assay is properly optimized in addition to being evaluated for its sensitivity to apo(a) size polymorphism (S.M. Marcovina, NHLBI conference presentation).

To evaluate method differences in the interpretation of clinical data, a study was performed to directly compare the ability of the ELISA reference method (54) and a commercially available latex-based nephelometric method to predict future angina pectoris in men participating in the Physicians' Health Study. apo(a) isoform size was determined and plasma Lp(a) concentration was simultaneously measured by the ELISA reference method and in a different laboratory by the nephelometric method. Analyses were performed in samples from 195 study participants who subsequently developed angina and from paired controls, matched for age and smoking, who remained free of reported vascular disease. The median baseline Lp(a) value in cases, as determined by ELISA, was ~35% higher ($P = 0.02$) than that in controls. Additionally, Lp(a) was associated with increased relative risk for angina, and this association was strengthened after controlling for lipid risk factors. However, the median Lp(a) value determined by the commercial method was not statistically different between cases and controls, and the association with angina was also not significant. On the other hand, very high Lp(a) values obtained by the nephelometric assay (>95th percentile of controls) significantly predicted angina, although the relative risks were not as strong than those with the reference method.

The results of these two studies clearly indicate the importance of using suitable and standardized methods

for risk assessment and for the interpretation of clinical outcomes (S.M. Marcovina, NHLBI conference presentation).

Lp(a) as an emerging risk factor for vascular disease: clinical perspectives

On the basis of the Adult Treatment Panel (ATP) III guidelines, Lp(a) is currently classified as an "emerging" lipid risk factor for CVD (58). For increased Lp(a) to be considered a "major" risk factor (i.e., in the same category as cigarette smoking and increased LDL-cholesterol) it has to meet the following criteria: (a) robust predictive power for CVD; (b) high prevalence of Lp(a) concentrations above an arbitrary risk threshold in the general population; (c) ready availability of clinical samples and a widely available, standardized, inexpensive means to measure Lp(a); and (d) evidence for a benefit of lowering Lp(a) concentrations (S. Grundy, NHLBI conference presentation).

For these reasons, it is essential to define stringent criteria for assay standardization. It is evident that a robust predictive power of Lp(a) can be fully explored only if measurements are made with methods that have been evaluated for their suitability to produce accurate Lp(a) values. On the basis of his long-standing experience with the Framingham Study, Dr. Kannel (NHLBI conference presentation) provided strong rationales for the use of highly standardized laboratory procedures for the measurement of lipids and lipoproteins. Standardized methods are essential for (a) promulgating guideline recommendations for establishing and implementing goals for therapy; (b) defining with confidence the existence, strength, and nature of the relationship of lipid and lipoproteins to disease; (c) estimating the prevalence and determinants of the abnormality within and among population subgroups; (d) comparing trends by age, sex, and populations; and (e) combining results from different studies to define critical values in relation to the disease outcome. It is clear that the above-mentioned goals need to be reached for defining the role of Lp(a) in clinical practice and that these can be accomplished only with the use of accuracy-based standardized methods.

Metaanalysis of 27 prospective studies provided information on 5436 coronary heart disease (CHD) cases observed during a mean follow-up time of 10 years (59). Despite differences in methods to measure Lp(a), this analysis indicates that individuals in the general population with Lp(a) concentrations in the top one-third of baseline measurements are at ~70% increased risk of CHD compared with individuals in the bottom one-third.

The predictive strength of Lp(a) for CHD was evaluated in the Atherosclerosis Risk in Communities (ARIC) Study, with 10-year follow-up and 725 CHD events (60). Lp(a) was found associated with modest risk ratios. In terms of population differences, the ARIC results suggest that Lp(a) confers less risk in blacks than in whites. A recent prospective study (61) examined a cohort of 1216 patients with a mean follow-up time of 6.7 years with total mortality and mortality attributable to CVD used as outcome variables. In this study, Lp(a) concentrations in excess of 300 mg/L were present in 30% of the study population and were found to be an independent predictor of death. On this basis, the authors suggested that Lp(a) values in excess of 300 mg/L are suggestive of a poor prognosis and may serve to identify patients who would benefit from aggressive secondary prevention programs.

Although there is little or no correlation between plasma Lp(a) concentrations and other vascular risk factors, evidence has been provided from several studies to suggest that the risk attributable to Lp(a) is dependent on the concomitant presence of other such risk factors. In the Familial Atherosclerosis Treatment Study (FATS), Lp(a) was a strong predictor of events at baseline, but lost its predictive value when LDL-cholesterol was reduced to <1000 mg/L in the treatment group (62). More recently, in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) (63), Lp(a) was investigated as a CHD risk factor in a prospective cohort of 9133 French and Northern Irish men, 50–59 years of age, without a history of CHD. Increased Lp(a) increased the risk for myocardial infarction and angina pectoris, and the effect was most pronounced in men with high LDL-cholesterol. The results of the Quebec Cardiovascular Study also suggest that Lp(a) is not an independent risk factor for ischemic heart disease in men, but increases the risk associated with increased apo B and total cholesterol and appears to attenuate the beneficial effects of increased HDL (64). The same interactions of increased Lp(a) with other risk factors were found in the PROCAM study. As a consequence, a high Lp(a) concentration further increased the risk of myocardial infarction in men with high or moderately increased estimated global risk (i.e., risk of a coronary event >10% in 10 years) but not in men with a low estimated global risk (65).

In addition to the potential synergy of Lp(a) risk with other markers of dyslipidemia, the interaction of Lp(a) with other thrombotic risk factors for stroke in children has been reported by Nowak-Gottl et al. (66). Increased plasma Lp(a) concentrations in combination with resistance to activated protein C, attributable to the presence of the factor V Leiden mutation, was associated with an odds ratio of 30 for stroke in children. In other studies, this group has also reported that increased Lp(a) concentration, familial protein C deficiency, and ischemic stroke of vascular origin had significant and independent associations with recurrent ischemic stroke in children (67). Clinical evidence suggests that Lp(a) may also be an independent risk factor for venous thromboembolism (68, 69). Recently, the role of Lp(a) as a risk factor for secondary thromboembolic events was investigated prospectively in a cohort of 301 children. The results suggest that the combination of increased Lp(a) with any thrombophilic risk factor increased the risk of a thromboembolic

event by a factor of 2.6; this risk ratio increased to 6.2 when increased Lp(a) occurred together with the factor V Leiden defect (70). Taken together, these studies clearly indicate that the risk contributed by Lp(a) to venous thrombosis is exacerbated by the presence of other vascular risk factors.

An area of clinical investigation that is receiving increased attention is the predictive value of apo(a) isoform size. The results of the Bruneck study demonstrated that small (<22 K4 repeats) apo(a) isoforms predicted risk for advanced atherosclerosis independently of Lp(a) concentration (41). Prospective data from the Physicians' Health Study examining apo(a) isoform size and Lp(a) concentrations [measured using an apo(a) size-independent method] and the risk of angina pectoris in men demonstrated that both Lp(a) concentrations and apo(a) isoform size were predictive for risk of future angina (F.M. Sacks, NHLBI conference presentation). On the basis of the independent contributions of apo(a) size and Lp(a) concentrations in a multivariate model, the authors of this study found that in men with LDL concentrations >1600 mg/L, smaller apo(a) isoform size was associated with a greater magnitude of risk and remained an independent risk factor after adjustment for the contribution of other variables.

MODULATION OF PLASMA Lp(a) CONCENTRATIONS
Compared with plasma LDL, Lp(a) concentrations are relatively resistant to alteration by traditional pharmacologic and nonpharmacologic approaches. However, there are reports of several interventions as well as disease conditions, such as renal disease and poorly controlled diabetes mellitus, that may significantly increase plasma Lp(a) concentrations [for a review, see Ref. (71)]. The most effective method to decrease Lp(a) concentrations by 50% or more is by LDL or Lp(a) apheresis procedures (72, 73). However, these procedures are costly and generally reserved for patients with extreme forms of familial hypercholesterolemia. Several agents have been reported to reduce Lp(a) concentrations, including niacin in high doses (74), L-carnitine at a dose of 2 g/day (75), and ascorbic acid (3 g/day) together with L-lysine monohydrochloride (3 g/day) (76). Various hormones have also been shown to modulate plasma Lp(a) concentrations, and both tamoxifen and estrogen have been shown to lower Lp(a) significantly in postmenopausal women (77).

Contradictory findings have been reported concerning the effect of statins on Lp(a) concentrations. A modest increase in Lp(a) concentrations was found in patients with increased cholesterol receiving simvastatin (78), and a 36% increase in plasma Lp(a) concentrations was reported in patients receiving atorvastatin (79). Use of pravastatin did not influence Lp(a) values (80), whereas fluvastatin was found to significantly reduce Lp(a) concentrations (81). In a recent study of a large patient cohort, both atorvastatin and simvastatin therapy for 6 weeks produced a modest but significant reduction in Lp(a)

values (82). Clearly the effect of statins on Lp(a) concentrations requires further analysis in large cohorts to define the roles of baseline Lp(a) concentration as well as apo(a) isoform size on the magnitude of the statin effect. Additionally, mechanistic studies need to be performed to identify the molecular basis for the potential modulation of Lp(a) by statin therapy.

Aspirin therapy (81 mg/day) was reported to lower serum Lp(a) concentrations in a recent study of 70 patients with atherosclerotic disease (83). Interestingly, the magnitude of the decrease in Lp(a) was larger in patients with high concentrations, irrespective of apo(a) isoform size. This has been speculated to result from a greater reduction by aspirin of apo(a) gene transcription in patients with high baseline transcriptional activity of the gene. Clearly these intriguing results need to be confirmed with a larger patient population, and the mechanism underlying the effect of aspirin on apo(a) gene transcription requires further analysis.

In contrast to plasma LDL concentrations, Lp(a) concentrations are thought to be relatively resistant to diet and exercise. However, in a study of obese women with high baseline Lp(a), a low-calorie diet with concomitant weight loss produced a significant reduction in Lp(a) values (84). In terms of specific dietary effects, the authors of a recent randomized cross-over study reported that almonds, rich in monounsaturated fat, significantly reduced Lp(a) concentrations (85). Ginsberg et al. (86) reported a significant increase in Lp(a) values in individuals who reduced their intake in saturated fat. Therefore, the results of these two studies support the notion that fat intake may lower Lp(a).

Systematic lowering of plasma Lp(a) concentrations can provide us with a study design in which the consequences of Lp(a) reduction can be evaluated prospectively. This could allow a direct assessment of the contribution of Lp(a) concentration to atherosclerotic risk.

Recommendations
To establish guidelines for the use of Lp(a) in clinical practice and to define its importance in relation to other risk factors for coronary and peripheral artery disease, we make the following recommendations:

- To compare data among different studies and populations, it is essential that assays for measuring Lp(a) in clinical and epidemiologic studies be validated for their ability to provide accurate values independently of the size of apo(a) in the samples.
- Manufacturers of Lp(a) assays should direct major efforts toward minimizing the impact of apo(a) size variability, as well as assay imprecision and batch-to-batch antibody variability.
- Because of the impact on the measurement, stringent protocols for blood collection and sample storage should be developed and followed. Most clinical and epidemiologic studies are performed on frozen sam-

ples. Therefore, the impact of freezing and storage conditions on individual assays should be evaluated; assay limitations need to be clearly indicated.

- The expression of Lp(a) values in terms of total Lp(a) mass should be abandoned because what is measured is the protein component of Lp(a) and not its lipid and carbohydrate content. In addition, to correctly reflect the number of Lp(a) particles and to compare data from different studies, the values should be expressed in terms of nmol/L of Lp(a) protein.
- The IFCC reference material with the assigned value of 107 nmol/L should be used as a point of reference for assay calibration.
- Screening for increases in Lp(a) in the general population is not recommended at this time. However, measurement of Lp(a) is recommended in individuals with an increased risk of CVD, particularly in those with borderline LDL-cholesterol or high apo B.
- On the basis of currently available data, individuals with Lp(a) values exceeding the 75th percentile are at increased risk for CVD. For Caucasians, based on the Framingham data, this percentile corresponds to an Lp(a) value of ~75 nmol/L. More studies performed with standardized methods are required to confirm the clinical validity of this cut-point value and to establish cut-points for other populations, particularly those of African origin.
- If methods sensitive to apo(a) isoform size are used for risk assessment, samples with values >50 nmol/L should be remeasured by referral laboratories using validated methods. The value of 50 nmol/L has been selected to minimize the possibility of misclassification attributable to method inaccuracy.
- Protocols for Lp(a) clinical studies supported by federal grants should be rigorously evaluated. A large sample size is required to take into consideration the large range of Lp(a) values. Because of race-related differences in Lp(a) values, results should be presented separately for each racial group. The sample size of each racial group should be large enough to achieve a satisfactory statistical power. To better define the role of apo(a) size, determination of apo(a) isoforms should also be performed. The suitability of assays for measuring Lp(a) and for determining apo(a) isoform size should be well-documented.

To gain a full understanding of the mechanisms by which Lp(a) may contribute to the risk for CVD, which may provide the rationale for the development of therapeutic strategies aimed at inhibiting the harmful effects of this lipoprotein, we make the following recommendations:

- Emphasis should be placed on studies of agents that specifically lower plasma Lp(a); this will allow randomized clinical trials to be conducted in which effects of systematic Lp(a) lowering can be examined prospectively.

- Efforts to identify the mechanism(s) that underlie the pathogenic role of Lp(a) in vascular disease should be continued. These types of studies will require the combined analysis of transgenic animals expressing a variety of apo(a) variants [including different apo(a) isoform sizes], as well as in vitro analyses.

Appendix

We thank the participants in the workshop, who all contributed to the concepts explored in this report. The following is the roster of participants.

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References

1. Berg K. A new serum type system in man: the Lp system. Acta Pathol Microbiol Scand 1963;59:362–82.
2. Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp(a)-

lipoprotein concentrations in plasma. J Clin Invest 1987;80:458–65.

3. Marcovina SM, Koschinsky ML. A critical evaluation of the role of Lp(a) in cardiovascular disease: can Lp(a) be useful in risk assessment? Semin Vasc Med 2002;2:335–44.

4. Rath M, Niendorf A, Reblin T, Dietel M, Krebber HJ, Beisiegel U. Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. Arteriosclerosis 1989;9:579–92.

5. Cushing GL, Gaubatz JW, Nava ML, Burdick BJ, Bocan TM, Guyton JR, et al. Quantitation and localization of apolipoproteins [a] and B in coronary artery bypass vein grafts resected at re-operation. Arteriosclerosis 1989;9:593–603.

6. Papagrigorakis E, Iliopoulos D, Asimacopoulos PJ, Safi HJ, Weilbaecher DJ, Ghazzaly KG, et al. Lipoprotein(a) in plasma, arterial wall, and thrombus from patients with aortic aneurysm. Clin Genet 1997;52:262–71.

7. McLean JW, Tomlinson JE, Kuang W-J, Eaton DL, Chen EY, Fless GM, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 1987;330:132–7.

8. Lackner C, Cohen JC, Hobbs HH. Molecular definition of the extreme size polymorphism in apolipoprotein(a). Hum Mol Genet 1993;2:933–40.

9. van der Hoek YY, Wittekoek ME, Beisiegel U, Kastelein JJ, Koschinsky ML. The apolipoprotein(a) kringle IV repeats which differ from the major repeat kringle are present in variably sized isoforms. Hum Mol Genet 1993;2:361–6.

10. Gabel BR, Koschinsky ML. Sequences within apolipoprotein(a) kringle IV types 6–8 bind directly to low-density lipoprotein and mediate noncovalent association of apolipoprotein(a) with apolipoprotein B-100. Biochemistry 1998;37:7892–8.

11. Sangrar W, Marcovina SM, Koschinsky ML. Expression and characterization of apolipoprotein(a) kringle IV types 1, 2 and 10 in mammalian cells. Protein Eng 1994;7:723–31.

12. Kratzin H, Armstrong VW, Niehaus M, Hilschmann N, Seidel D. Structural relationship of an apolipoprotein (a) phenotype (570 kDa) to plasminogen: homologous kringle domains are linked by carbohydrate-rich regions. Biol Chem Hoppe Seyler 1987;368:1533–44.

13. Garner B, Merry AH, Royle L, Harvey DJ, Rudd PM, Thillet J. Structural elucidation of the N- and O-glycans of human apolipoprotein(a): role of o-glycans in conferring protease resistance. J Biol Chem 2001;276:22200–8.

14. Marcovina SM, Albers JJ, Wijsman E, Zhang ZH, Chapman NH, Kennedy H. Differences in Lp(a) concentrations and apo(a) polymorphs between black and white Americans. J Lipid Res 1996;37:2569–85.

15. Kraft HG, Menzel HJ, Hoppichler F, Vogel W, Utermann G. Changes of genetic apolipoprotein phenotypes caused by liver transplantation. Implications for apolipoprotein synthesis. J Clin Invest 1989;83:137–42.

16. White AL, Lanford RE. Cell surface assembly of lipoprotein(a) in primary cultures of baboon hepatocytes. J Biol Chem 1994;269:28716–23.

17. Morrisett JD, Gaubatz JW, Nava MN, Guyton JR, Hoffmann AS, Opekun AR, et al. Metabolism of apo(a) and apo B-100 in human lipoprotein(a). In: Catapano AL, Gotto AM Jr, Smith LC, Paoletti R, eds. Drugs affecting lipid metabolism. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1993:161–7.

18. Su W, Campos H, Judge H, Walsh BW, Sacks FM. Metabolism of Apo(a) and ApoB100 of lipoprotein(a) in women: effect of postmenopausal estrogen replacement. J Clin Endocrinol Metab 1998;83:3267–76.

19. Krempler F, Kostner GM, Bolzano K, Sandhofer F. Turnover of lipoprotein (a) in man. J Clin Invest 1980;65:1483–90.

20. Rader DJ, Cain W, Ikewaki K, Talley G, Zech LA, Usher D, et al. The inverse association of plasma lipoprotein(a) concentrations with apolipoprotein(a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. J Clin Invest 1994;93:2758–63.

21. White AL, Hixson JE, Rainwater DL, Lanford RE. Molecular basis for "null" lipoprotein(a) phenotypes and the influence of apolipoprotein(a) size on plasma lipoprotein(a) level in the baboon. J Biol Chem 1994;269:9060–6.

22. Brunner C, Kraft HG, Utermann G, Muller HJ. Cys4057 of apolipoprotein(a) is essential for lipoprotein(a) assembly. Proc Natl Acad Sci U S A 1993;90:11643–7.

23. Koschinsky ML, Cote GP, Gabel B, van der Hoek YY. Identification of the cysteine residue in apolipoprotein(a) that mediates extracellular coupling with apolipoprotein B-100. J Biol Chem 1993;268:19819–25.

24. Albers JJ, Kennedy H, Marcovina SM. Evidence that Lp(a) contains one molecule of apo(a) and one molecule of apo B: evaluation of amino acid analysis data. J Lipid Res 1996;37:192–6.

25. Ernst A, Helmhold M, Brunner C, Petho-Schramm A, Armstrong VW, Muller HJ. Identification of two functionally distinct lysine-binding sites in kringle 37 and in kringles 32–36 of human apolipoprotein(a). J Biol Chem 1995;270:6227–34.

26. Trieu VN, McConathy WJ. A two-step model for lipoprotein(a) formation. J Biol Chem 1995;270:15471–4.

27. Gabel BR, May LF, Marcovina SM, Koschinsky ML. Lipoprotein(a) assembly. Quantitative assessment of the role of apo(a) kringle IV types 2–10 in particle formation. Arterioscler Thromb Vasc Biol 1996;16:1559–67.

28. Becker L, McLeod RS, Marcovina SM, Yao Z, Koschinsky ML. Identification of a critical lysine residue in apolipoprotein B-100 that mediates noncovalent interaction with apolipoprotein(a). J Biol Chem 2001;276:36155–62.

29. Cheesman EJ, Sharp RJ, Zlot CH, Liu CY, Taylor S, Marcovina SM, et al. An analysis of the interaction between mouse apolipoprotein B100 and apolipoprotein(a). J Biol Chem 2000;275:28195–200.

30. Sharp RJ, Perugini MA, Marcovina SM, McCormick SP. A synthetic peptide that inhibits lipoprotein(a) assembly. Arterioscler Thromb Vasc Biol 2003;23:502–7.

31. Frank S, Durovic S, Kostner K, Kostner GM. Inhibitors for the in vitro assembly of Lp(a). Arterioscler Thromb Vasc Biol 1995;15:1774–80.

32. Becker L, Webb BA, Chitayat S, Nesheim ME, Koschinsky ML. A ligand-induced conformational change in apolipoprotein(a) enhances covalent Lp(a) formation. J Biol Chem 2003;278:14074–81.

33. Kostner KM, Maurer G, Huber K, Stefenelli T, Dieplinger H, Steyrer E, et al. Urinary excretion of apo(a) fragments. Role in apo(a) catabolism. Arterioscler Thromb Vasc Biol 1996;16:905–11.

34. Koschinsky ML, Marcovina SM. Lipoprotein(a): structural implications for pathophysiology. Int J Clin Lab Res 1997;27:14–23.

35. Gaubatz JW, Hoogeveen RC, Hoffman AS, Ghazzaly KG, Pownall HJ, Guevara J Jr, et al. Isolation, quantitation, and characterization of a stable complex formed by Lp[a] binding to triglyceride-rich lipoproteins. J Lipid Res 2001;42:2058–68.

36. Utermann G, Duba C, Menzel HJ. Genetics of the quantitative Lp(a) lipoprotein trait. II. Inheritance of Lp(a) glycoprotein phenotypes. Hum Genet 1988;78:47–50.

37. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J Clin Invest 1992;90:52–60.

38. Scholz M, Kraft HG, Lingenhel A, Delport R, Vorster EH, Bickeboller H, et al. Genetic control of lipoprotein(a) concentrations is different in Africans and Caucasians. Eur J Hum Genet 1999;7:169–78.

39. Trommsdorff M, Kochl S, Lingenhel A, Kronenberg F, Delport R, Vermaak H, et al. A pentanucleotide repeat polymorphism in the 5′ control region of the apolipoprotein(a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. J Clin Invest 1995;96:150–7.

40. Kraft HG, Windegger M, Menzel HJ, Utermann G. Significant impact of the +93 C/T polymorphism in the apolipoprotein(a) gene on Lp(a) concentrations in Africans but not in Caucasians: confounding effect of linkage disequilibrium. Hum Mol Genet 1998;7:257–64.

41. Kronenberg F, Kronenberg MF, Kiechl S, Trenkwalder E, Santer P, Oberhollenzer F, et al. Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis. Prospective results from the Bruneck study. Circulation 1999;100:1154–60.

42. Lawn RM, Boonmark NW, Schwartz K, Lindahl GE, Wade DP, Byrne CD, et al. The recurring evolution of lipoprotein(a). Insights from cloning of hedgehog apolipoprotein(a). J Biol Chem 1995;270:24004–9.

43. Boffelli D, McAuliffe J, Ovcharenko D, Lewis KD, Ovcharenko I, Pachter L, et al. Phylogenetic shadowing of primate sequences to find functional regions of the human genome. Science 2003;299:1391–4.

44. Wade DP, Lindahl GE, Lawn RM. Apolipoprotein(a) gene transcription is regulated by liver-enriched trans-acting factor hepatocyte nuclear factor 1α. J Biol Chem 1994;269:19757–65.

45. Lawn RM. How often has Lp(a) evolved? Clin Genet 1996;49:167–74.

46. Lawn RM, Wade DP, Hammer RE, Chiesa G, Verstuyft JG, Rubin EM. Atherogenesis in transgenic mice expressing human apolipoprotein(a). Nature 1992;360:670–2.

47. Callow MJ, Verstuyft J, Tangirala R, Palinski W, Rubin EM. Atherogenesis in transgenic mice with human apolipoprotein B and lipoprotein (a). J Clin Invest 1995;96:1639–46.

48. Mancini FP, Newland DL, Mooser V, Murata J, Marcovina S, Young SG, et al. Relative contributions of apolipoprotein(a) and apolipoprotein-B to the development of fatty lesions in the proximal aorta of mice. Arterioscler Thromb Vasc Biol 1995;15:1911–6.

49. Sanan DA, Newland DL, Tao R, Marcovina S, Wang J, Mooser V, et al. Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). Proc Natl Acad Sci U S A 1998;95:4544–9.

50. Berg K, Svindland A, Smith AJ, Lawn RM, Djurovic S, Alestrom A, et al. Spontaneous atherosclerosis in the proximal aorta of *LPA* transgenic mice on a normal diet. Atherosclerosis 2002;163:99–104.

51. Fan J, Shimoyamada H, Sun H, Marcovina S, Honda K, Watanabe T. Transgenic rabbits expressing human apolipoprotein(a) develop more extensive atherosclerotic lesions in response to a cholesterol-rich diet. Arterioscler Thromb Vasc Biol 2001;21:88–94.

52. Sun H, Unoki H, Wang X, Liang J, Ichikawa T, Arai Y, et al. Lipoprotein(a) enhances advanced atherosclerosis and vascular calcification in WHHL transgenic rabbits expressing human apolipoprotein(a). J Biol Chem 2002;277:47486–92.

53. Watanabe Y. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). Atherosclerosis 1980;36:261–8.

54. Marcovina SM, Albers JJ, Gabel B, Koschinsky ML, Gaur VP. Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). Clin Chem 1995;41:246–55.

55. Edelstein C, Hinman J, Marcovina S, Scanu AM. Properties of human free apolipoprotein(a) and lipoprotein(a) after either freezing or lyophilization in the presence and absence of cryopreservatives. Anal Biochem 2001;288:201–8.

56. Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). Clin Chem 2000;46:1956–67.

57. Marcovina SM, Zhang ZH, Gaur VP, Albers JJ. Identification of 34 apolipoprotein(a) isoforms: differential expression of apolipoprotein(a) alleles between American blacks and whites. Biochem Biophys Res Commun 1993;191:1192–6.

58. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.

59. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 2000;102:1082–5.

60. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2001;104:1108–13.

61. Glader CA, Birgander LS, Stenlund H, Dahlen GH. Is lipoprotein(a) a predictor for survival in patients with established coronary artery disease? Results from a prospective patient cohort study in northern Sweden. J Intern Med 2002;252:27–35.

62. Maher VM, Brown BG, Marcovina SM, Hillger LA, Zhao XQ, Albers JJ. Effects of lowering elevated LDL cholesterol on the cardiovascular risk of lipoprotein(a). JAMA 1995;274:1771–4.

63. Luc G, Bard JM, Arveiler D, Ferrieres J, Evans A, Amouyel P, et al. Lipoprotein (a) as a predictor of coronary heart disease: the PRIME Study. Atherosclerosis 2002;163:377–84.

64. Cantin B, Gagnon F, Moorjani S, Despres JP, Lamarche B, Lupien PJ, et al. Is lipoprotein(a) an independent risk factor for ischemic heart disease in men? The Quebec Cardiovascular Study. J Am Coll Cardiol 1998;31:519–25.

65. von Eckardstein A, Schulte H, Cullen P, Assmann G. Lipoprotein(a) further increases the risk of coronary events in men with high global cardiovascular risk. J Am Coll Cardiol 2001;37:434–9.

66. Nowak-Gottl U, Strater R, Heinecke A, Junker R, Koch HG, Schuierer G, et al. Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. Blood 1999;94:3678–82.

67. Strater R, Becker S, von Eckardstein A, Heinecke A, Gutsche S, Junker R, et al. Prospective assessment of risk factors for recurrent stroke during childhood—a 5-year follow-up study. Lancet 2002;360:1540–5.

68. Nowak-Gottl U, Schobess R, Kurnik K, Schwabe D, Fleischhack G, Junker R. Elevated lipoprotein(a) concentration is an independent risk factor of venous thromboembolism. Blood 2002;99:3476–7,author reply 3477–8.

69. von Depka M, Nowak-Gottl U, Eisert R, Dieterich C, Barthels M, Scharrer I, et al. Increased lipoprotein (a) levels as an independent risk factor for venous thromboembolism. Blood 2000;96:3364–8.

70. Nowak-Gottl U, Junker R, Kreuz W, von Eckardstein A, Kosch A, Nohe N, et al. Risk of recurrent venous thrombosis in children with combined prothrombotic risk factors. Blood 2001;97:858–62.

71. Marcovina SM, Koschinsky ML. Lipoprotein(a): structure, measurement and clinical significance. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing, 2nd ed. Washington, DC: AACC Press, 2000:345–85.

72. Armstrong VW, Schleef J, Thiery J, Muche R, Schuff-Werner P, Eisenhauer T, et al. Effect of HELP-LDL-apheresis on serum concentrations of human lipoprotein(a): kinetic analysis of the

post-treatment return to baseline levels. Eur J Clin Invest 1989;19:235–40.

73. Pokrovsky SN, Adamova I, Afanasieva OY, Benevolenskaya GF. Immunosorbent for selective removal of lipoprotein(a) from human plasma: in vitro study. Artif Organs 1991;15:136–40.

74. Crouse JR 3rd. New developments in the use of niacin for treatment of hyperlipidemia: new considerations in the use of an old drug. Coron Artery Dis 1996;7:321–6.

75. Sirtori CR, Calabresi L, Ferrara S, Pazzucconi F, Bondioli A, Baldassarre D, et al. L-Carnitine reduces plasma lipoprotein(a) levels in patients with hyper Lp(a). Nutr Metab Cardiovasc Dis 2000;10:247–51.

76. Dalessandri KM. Reduction of lipoprotein(a) in postmenopausal women. Arch Intern Med 2001;161:772–3.

77. Shewmon DA, Stock JL, Rosen CJ, Heiniluoma KM, Hogue MM, Morrison A, et al. Tamoxifen and estrogen lower circulating lipoprotein(a) concentrations in healthy postmenopausal women. Arterioscler Thromb 1994;14:1586–93.

78. Plenge JK, Hernandez TL, Weil KM, Poirier P, Grunwald GK, Marcovina SM, et al. Simvastatin lowers C-reactive protein within 14 days: an effect independent of low-density lipoprotein cholesterol reduction. Circulation 2002;106:1447–52.

79. Dujovne CA, Harris WS, Altman R, Overhiser RW, Black DM. Effect of atorvastatin on hemorheologic-hemostatic parameters and serum fibrinogen levels in hyperlipidemic patients. Am J Cardiol 2000;85:350–3.

80. Cobbaert C, Jukema JW, Zwinderman AH, Withagen AJ, Lindemans J, Bruschke AV. Modulation of lipoprotein(a) atherogenicity by high density lipoprotein cholesterol levels in middle-aged men with symptomatic coronary artery disease and normal to moderately elevated serum cholesterol. Regression Growth Evaluation Statin Study (REGRESS) Study Group. J Am Coll Cardiol 1997;30:1491–9.

81. Duriez P, Dallongeville J, Fruchart JC. Lipoprotein(a) as a marker for coronary heart disease. Br J Clin Pract 1996;77A:54–61.

82. Gonbert S, Malinsky S, Sposito AC, Laouenan H, Doucet C, Chapman MJ, et al. Atorvastatin lowers lipoprotein(a) but not apolipoprotein(a) fragment levels in hypercholesterolemic subjects at high cardiovascular risk. Atherosclerosis 2002;164:305–11.

83. Akaike M, Azuma H, Kagawa A, Matsumoto K, Hayashi I, Tamura K, et al. Effect of aspirin treatment on serum concentrations of lipoprotein(a) in patients with atherosclerotic diseases. Clin Chem 2002;48:1454–9.

84. Kiortsis DN, Tzotzas T, Giral P, Bruckert E, Beucler I, Valsamides S, et al. Changes in lipoprotein(a) levels and hormonal correlations during a weight reduction program. Nutr Metab Cardiovasc Dis 2001;11:153–7.

85. Jenkins DJ, Kendall CW, Marchie A, Parker TL, Connelly PW, Qian W, et al. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. Circulation 2002;106:1327–32.

86. Ginsberg HN, Kris-Etherton P, Dennis B, Elmer PJ, Ershow A, Lefevre M, et al. Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the DELTA Study, protocol 1. Arterioscler Thromb Vasc Biol 1998;18:441–9.