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Review Article

Lipoprotein(a) mass: A massively misunderstood metric

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Abstract: The importance of lipoprotein (a)—Lp(a)—as a cardiovascular (CV) risk marker has been underscored by recent findings that CV risk is directly related to baseline Lp(a) levels, even in well-treated patients. Although there is currently little that can be done pharmacologically to lower Lp(a) levels, knowledge of its serum concentration is important in overall risk assessment. This review focuses on 1 aspect of Lp(a) that is rarely discussed directly: how to express its levels in serum. There is considerable confusion on this point, and a fuller understanding of what the concentration units mean will help improve study-to-study comparisons and thereby advance our understanding of the pathobiology of this lipoprotein particle. As discussed here, the term Lp(a) mass refers to the entire mass of the particle: lipids, proteins, and carbohydrates combined. At present, there are no commercially available assays that are completely insensitive to the variability in particle mass, which arises not only from differences in apo(a) isoform mass but also from variations in lipid mass. Because lipoprotein "particle number" (molar concentration) has been found to be superior to component-based metrics (ie, low-density lipoprotein particle vs cholesterol concentrations) for CV disease risk prediction, the development of a mass-insensitive Lp(a) assay should be a high priority.

Introduction

Baseline lipoprotein (a)—Lp(a)—levels were recently found to be a significant predictor of cardiovascular disease (CVD) risk in both the placebo and treatment groups of the AIM-HIGH study, whereas apoB and the apoB/apoA-I ratio were only predictive in the placebo group.[1] This suggests that Lp(a) makes a unique contribution to residual CVD risk, even in statin/niacin-treated patients. Findings such as these have generated renewed interest in this most enigmatic of all lipoprotein particles, and understanding both its biology and predictive power has taken on a new urgency.

The purpose of this short review is to focus on only one aspect of this lipoprotein: its units of measurement. In an otherwise excellent recent review on Lp(a),[2] Lp(a) *cholesterol* was mistaken for Lp(a) *mass*. This illustrates that there is considerable confusion regarding the units for this lipoprotein particle, even among expert lipidologists.

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This confusion only adds to the challenges of knowing how to use information about Lp(a) in the clinic.

Lp(a) units

Because all lipoproteins are composed of lipids and proteins, it is possible to refer to any 1 chemical species (eg, cholesterol, triglyceride, protein), to several, or to all components combined using the term "mass." (Fig. 1)

In 1974, Albers et al. defined Lp(a) mass as that of the entire particle.[5] An Lp(a) mass of >30 mg/dL has been considered elevated,[6] being at about the 75th percentile for most populations.[2] However, because of marked heterogeneity of particle chemical components (as described in the following section), the use of total mass was discouraged.[7] Nakajima et al. referred to Lp(a) "protein" levels >7 mg/dL as being at the 90th percentile,[8] and Rubin et al. reported median Lp(a) "levels" in whites as 25 nmol/L and in blacks as between 103 and 125 nmol/L.[9] In another article, Lp(a) "mass" and "protein mass" were used interchangeably.[10] Lp(a) status can also be expressed as Lp(a) cholesterol, for which 10 mg/dL represented the 75th percentile in Framingham participants.[11] Thus, serum levels of this one lipoprotein particle are reported in many different ways, engendering considerable confusion.[12]

While developing a new electrophoresis-based assay for Lp(a) (lipoprotein immunofixation electrophoresis, or Lipo-IFE, which uses a novel approach to directly measure particle concentrations),[13] we had to address the varying

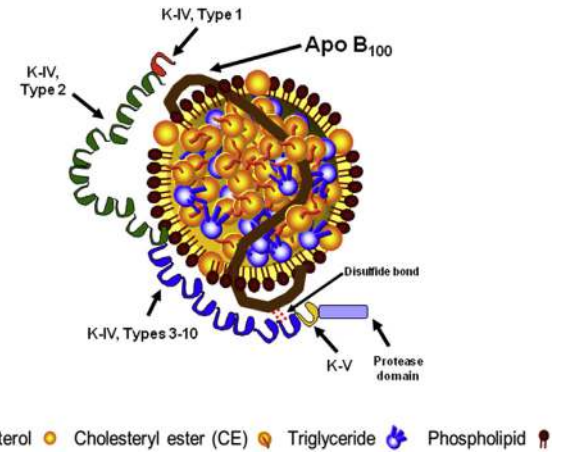

Figure 1 Lp(a) particle chemical composition. Lp(a) comprises an LDL particle, the apoB-100 moiety of which is attached to a protein called apolipoprotein(a)—apo(a)—via a disulfide bond. Apo(a) comprises a protease domain and a series of peptide subunits called "kringles" (K; because of their resemblance to a Danish pastry of the same name). Two K versions are present: 4 and 5 (KIV and KV). The former is subcategorized into 10 subtypes (types 1-10). KIV type 1 (KIV-1) and types 3-10, and KV are identical in all apo(a) isoforms. Variability in apo(a) isoform size (molecular mass) is due only to differences in the number of KIV-2 repeats. Apo(a) with 12 KIV-2 repeats is shown here; 3 to 43 repeats have been reported[3] with resulting apo(a) masses varying from 200 to 800 KDa.[4]

modes of expression for this particle's concentrations in serum. Lp(a) cholesterol content has been measured by at least 2 methods,[10,14] but the meaning of this metric is unambiguous because similar terminology has been used for decades in expressing the cholesterol content of low-density lipoproteins (LDL) and high-density lipoproteins (HDL) as surrogates for particle concentrations. However, the meaning of Lp(a) mass is not as clear: to what, exactly, does mass refer? As noted previously, it could be the entire particle's mass, the mass of just the proteins—apolipoproteins [apo] B + apo(a)—or the mass of just apo(a) (Fig. 2).

One approach to determining the "mass" to which this Lp(a) mass refers is to apply a conversion factor that has been recommended to translate mass-based concentrations (mg/dL) of Lp(a) to molar concentrations (nmol/L): 1 mg/dL equals 2.4 nmol/L.[15] Subjecting this factor to basic dimensional analysis (see the following section), one can calculate what the molecular mass of Lp(a) is, and with that information, determine to which component(s) of the particle this mass applies.

To convert mg/dL to nmol/L, one first converts the former unit to "per L:" 1 mg/dL = 10 mg/L. Thus, 2.4 nmol/L = 10 mg/L, which means that 2.4 nmol = 10 mg. Converting mg to ng gives 10×10^6 ng, or more simply, 10^7 ng. From this equation, the molecular mass (ng/nmol) can easily be calculated: $1 \times 10^7/2.4 = 0.4167 \times 10^7$ ng/nmol. To convert this to the more familiar kilodaltons (kDa, which is 1000 g/mol, used for very large molecular species), one arrives at 4167 kDa for the molecular mass of Lp(a).

To which component(s) of Lp(a) does this mass apply? Lp(a) is composed of 2 primary proteins: apoB (molecular mass ~530 kDa) and apo(a) (whose mass ranges from about 200 to 800 kDa depending on the number of KIV-2 repeats it contains Figures 1 and 2); for the present purposes, we assume an average mass of about 500 kDa.[4] So the total *protein* of Lp(a) would have a mass of approximately 1030 kDa. This is only 25% of the calculated mass of Lp(a) (assuming a 2.4 conversion factor), hence we must look beyond the proteins for more mass. The non-protein components of Lp(a) include lipids (primarily free and esterified cholesterol, triglycerides, and phospholipids) and carbohydrates (estimated to be between 4% and 10% of apoB mass).[16] Assuming that the non-apoprotein(a) components of Lp(a) are similar to those of LDL, then using the LDL chemical compositional data from Shen et al.[17] and the approximate molecular masses of the non-protein components, one arrives at a non-protein molecular mass of about 1971 kDa per particle (Table 1).

Summing all of the chemical components of Lp(a), one arrives at a *total particle* molecular mass of about 3002 kDa. This is clearly closer to the 4167 kDa than is any one component of the particle, but still accounts for only 72% of the calculated particle mass, again assuming the 2.4 conversion factor is correct. Another approach is to begin by assuming that the molecular masses in Table 1 are all approximately

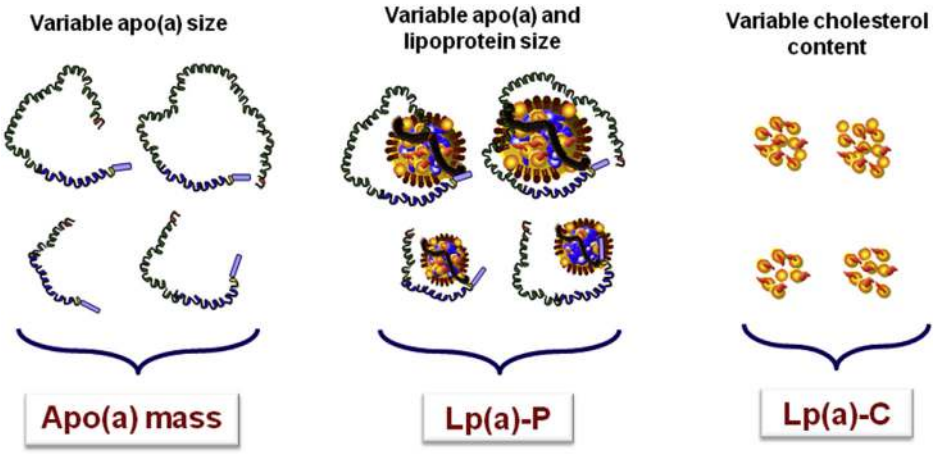

Figure 2 Various approaches to quantifying Lp(a). Left: Apo(a) mass is the amount of apolipoprotein(a) present expressed as mg/dL. Middle: Lp(a)-P is the concentration (directly proportional to particle number) of Lp(a) particles, regardless of apo(a) size or amount of lipid present, and is expressed as nmol/L. Right: Lp(a)-cholesterol (C) is the amount of C trafficked by Lp(a) particles and is expressed as mg/dL. Note that variability in lipoprotein size and in apo(a) molecular mass can both contribute to variability in Lp(a) total particle mass. In the middle panel, the Lp(a) particle that is considered most atherogenic is in the lower left, with the smallest lipid core and the smallest apo(a) isoform.

correct, and then to back calculate to the conversion factor. Doing this, one arrives at a factor of 1 mg/dL ≈ 3.3 nmol/L. (This value is close to that experimentally determined comparing Lp(a) mass [by immunoturbidimetry, Denka Seiken] to Lp(a)-P by Lipo-IFE.)[13]

Mass-based concentrations cannot be converted into particle concentrations

Regardless of whether the factor is 2.4 or 3.3, it appears that the usual "mass" value for Lp(a) refers to the entire lipoprotein particle, not any one of its component parts. More to the point, it is obvious that no single conversion factor can be accurately applied to convert mass units to molar units because the complete chemical composition of Lp(a) is never determined in the clinical laboratory.

Table 1 Chemical composition of lipoprotein(a)

	Molecules per LDL Particle	Approximate molecular mass (Da)	Mass per particle (kDa)	
Amino acids	4830	110	531	
Carbohydrate*			37	
PL	653	645	421	1971
FC	475	387	184	
CE	1310	813	1065	
TG	298	886	264	
Total LDL			2502	
Apo(a) (approximate)			500	
Total Lp(a)			3002	

CE, cholesteryl ester; FC, free cholesterol; LDL, low-density lipoprotein; PL, phospholipid; TG, triglyceride.

LDL particle composition taken from Shen et al.[17]

*Between 4% and 10% of apoB mass per Yang et al.[16]

Variability in Lp(a) total particle mass arises not only from variations in apo(a) isoform mass, but also from differences in the small amounts of other apolipoproteins present as well as potentially major variations in the lipid cargo of the LDL particle (Figure 2). In addition to different amounts of individual lipid class components, there is also interindividual variability in the fatty acid species esterified within each of these lipid classes. Small amounts of fat-soluble vitamins are also likely to be present in the LDL core of Lp(a). All of these factors determine the true mass for the Lp(a) particle for a particular individual, and thus any attempt to convert measured Lp(a) "mass" to molar (ie, particle) concentration would produce only a rough approximation.

Some clinical laboratory assays of Lp(a) mass use polyclonal antibodies that target, at least in part, the KIV-2 region on apo(a). These will naturally overestimate the mass of apo(a) for molecules with many KIV-2 repeats and will underestimate the mass of those with few repeats. Other assays use antibodies that target nonvariable regions of apo(a),[18] but these could still be influenced by overall differences in particle size because turbidimetric/nephelometric assays measure the absorbed/scattered light from the entire particle-antibody complex. In other words, isoform-insensitive *antibodies* do not necessarily result in isoform-insensitive *assays* when measuring Lp(a) mass. A true assessment of Lp(a) status will require a method that is completely insensitive to variations in total particle [not just apo(a)] mass. Evidence to date suggests that Lipo-IFE accomplishes this, and it does so using a modern lipoprotein electrophoretic approach that bears little resemblance to the qualitative paper electrophoresis method used in the 1990s.[13,19]

Finally, the use of the term "P" as a common abbreviation for "particle number" (eg, Lp(a)-P, LDL-P, HDL-P)

is not strictly correct. A serum level of 100 nmol/L of Lp(a)-P does not mean that there are 100 Lp(a) particles per liter. Applying Avogadro's number to convert nmoles to actual particle numbers, one arrives at a true concentration of 6×10^{16}, or 60 quadrillion Lp(a) particles/L. Because total plasma volume is approximately 2.8 L, the total number of circulating Lp(a) particles would be about 170 quadrillion. A similar calculation for a normal LDL-P concentration of 1000 nmol/L would equate to about 1.7 quintillion particles circulating at any one time. Particle-based (but not mass-based) concentrations are directly proportional to the actual number of particles per unit volume, but "particle number" is incorrect terminology.

Lp(a)-P and CVD

An important question that can only be answered in future clinical trials is, "will Lp(a)-P predict CVD risk better than current Lp(a) mass methods?" Several studies have shown improved risk prediction for LDL-P over LDL-cholesterol[20,21] and for HDL-P over HDL-cholesterol,[22] and thus one can hypothesize that the same will be true for Lp(a)-P. Because smaller apo(a) isoforms predominate in plasma (being more readily secreted from the liver[23,24]), there will naturally be a correlation between Lp(a) particle concentrations and apo(a) isoform size. In addition, because the smaller isoforms are more atherogenic than larger isoforms,[25,26] particle concentrations should reflect the increased risk associated with smaller isoform predominance. But until new studies are undertaken to examine these hypotheses, one can only speculate.

Conclusion

With the increasing recognition of the importance of Lp(a) as a risk marker for CVD, understanding the basics of the metric, and using consistent and accurate units of measurement, will become essential. Assays that reliably report Lp(a) particle concentrations are likely to be, as is the case for LDL,[20,21] the preferred approach to assessing Lp(a) status.

References

1. Albers JJ, Slee A, O'Brien KD, et al. Relationship of apolipoproteins A-1 and B, and lipoprotein(a) to cardiovascular outcomes: The AIM-HIGH Trial (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglyceride and Impact on Global Health Outcomes). *J Am Coll Cardiol*. 2013;62(17):1575–1579.
2. Jacobson TA. Lipoprotein(a), cardiovascular disease, and contemporary management. *Mayo Clin Proc*. 2013;88(11):1294–1311.
3. Lackner C, Cohen JC, Hobbs HH. Molecular definition of the extreme size polymorphism in apolipoprotein(a). *Hum Mol Genet*. 1993;2(7):933–940.
4. Utermann G. The mysteries of lipoprotein(a) [Review]. Science. 1989;246:904–910.
5. Albers JJ, Hazzard WR. Immunochemical quantification of human plasma Lp(a) lipoprotein. *Lipids*. 1974;9(1):15–26.
6. Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Qunici GB. Lipoprotein Lp(a) and the risk for myocardial infarction. *Atherosclerosis*. 1981;38(1-2):51–61.
7. Albers JJ, Marcovina SM, Lodge MS. The unique lipoprotein(a): properties and immunochemical measurement. *Clin Chem*. 1990;36(12):2019–2026.
8. Nakajima K, Hinman J, Pfaffinger D, Edelstein C, Scanu AM. Changes in plasma triglyceride levels shift lipoprotein(a) density in parallel with that of LDL independently of apolipoprotein(a) size. *Arterioscler Thromb Vasc Biol*. 2001;21(7):1238–1243.
9. Rubin J, Paultre F, Tuck CH, et al. Apolipoprotein [a] genotype influences isoform dominance pattern differently in African Americans and Caucasians. *J Lipid Res*. 2002;43(2):234–244.
10. Baudhuin LM, Hartman SJ, O'Brien JF, et al. Electrophoretic measurement of lipoprotein(a) cholesterol in plasma with and without ultracentrifugation: comparison with an immunoturbidimetric lipoprotein(a) method. *Clin Biochem*. 2004;37(6):481–488.
11. Seman LJ, DeLuca C, Jenner JL, et al. Lipoprotein(a)-cholesterol and coronary heart disease in the Framingham Heart Study. *Clin Chem*. 1999;45(7):1039–1046.
12. Marcovina SM, Koschinsky ML. Lipoprotein(a): Structure, Measurement, and Clinical Significance Edtion ed. . In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein Testing. Washington, DC: AACC Press, 2000. p. 345–385.
13. Guadagno PA, Summers E, Stanovick B, et al. Validation of Lp(a) particle number analysis by quantitative Lipo-IFE. *J Clin Lipidol*. 2013;7(3):240–241.
14. Seman LJ, Jenner JL, McNamara JR, Schaefer EJ. Quantification of lipoprotein(a) in plasma by assaying cholesterol in lectin-bound plasma fraction. *Clin Chem*. 1994;40(3):400–403.
15. Brown WV, Ballantyne CM, Jones PH, Marcovina S. Management of Lp(a). *J Clin Lipidol*. 2010;4(4):240–247.
16. Yang CY, Lee FS, Chan L, Sparrow DA, Sparrow JT, Gotto AM Jr. Determination of the molecular mass of apolipoprotein B-100. A chemical approach. *Biochem J*. 1986;239(3):777–780.
17. Shen BW, Scanu AM, Kezdy FJ. Structure of human serum lipoproteins inferred from compositional analysis. *Proc Natl Acad Sci U S A*. 1977;74(3):837–841.
18. 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(2):246–255.
19. Ellefson RD, Jimenez BJ, Smith RC. Pre-beta (or alpha 2) lipoprotein of high density in human blood. *Mayo Clin Proc*. 1971;46(5):328–332.
20. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med*. 2006;26:847–870.
21. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. *J Clin Lipidol*. 2007;1(6):583–592.
22. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation*. 2013;128(11):1189–1197.
23. Rader DJ, Cain W, Ikewaki K, 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(6):2758–2763.
24. Dieplinger H, Utermann G. The seventh myth of lipoprotein(a): where and how is it assembled? *Curr Opin Lipidol*. 1999;10(3):275–283.
25. Clarke R, Peden JF, Hopewell JC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med*. 2009;361(26):2518–2528.
26. Erqou S, Thompson A, Di Angelantonio E, et al. Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. *J Am Coll Cardiol*. 2010;55(19):2160–2167.