Lipoprotein (a) 2010

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Lipoprotein little a (small case a as opposed to a capital A) has been confusing lipidologists for a long, long time since it was first discovered in 1963. It seems to be a risk factor in some people and not in others and race is involved. Over time the data associating Lp(a) with CV risk has gotten stronger and stronger and current opinion is until proven otherwise that Lp(a) is an independent risk factor for CHD.

Lipoprotein (a), pronounced lipoprotein "little a" is simply an LDL (a collection of core cholesteryl ester or CE and triglyceride or TG in a 4:1 ratio) with a phospholipid and free cholesterol surface enwrapped with a single molecule of apolipoprotein B 100 that has attached to it via a covalent disulfide bond a glycoprotein called apoprotein (a) which is a plasminogen-like glycoprotein. Once apoprotein (a) is attached to an apoB particle the particle is called lipoprotein (a) which is abbreviated as Lp(a). Apoprotein (a) abbreviated as apo(a) should not be confused with apoprotein A (upper case A) which is a family of very different apoproteins (apoA-I through apoA-V). In reality apo(a) can attached to any apoB particle, but of course, because of its long half life, the vast majority of circulating apoB particles are LDLs. Apo(a) can also be attached to TG-rich apoB 100 particles as they exit the liver. The Fractional Catabolic Rate of apo(a) is approximately half that of Lp(a) B-100. This newly formed Lp(a) particle releases apo(a) as the triglyceride-rich lipoprotein portion is catabolized via receptor-mediated clearance. It is not known which receptor clears Lp(a) but it is not the LDL receptor. The free apo(a) then recombines with another apoB-100 particle, most likely of triglyceride-rich lipoprotein origin. Because about 50% of triglyceride-rich lipoprotein is converted to LDL in the fed state, the second Lp(a) particle may survive catabolism [please see: The metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein (a) in human beings. Jennifer L. Jenner et al. Metabolism Clinical and Experimental 2005;54:361–369]

NCEP ATP-III gave little impact or discussion to elevated Lp(a) levels in the final report published in 2002. They stated it may be a major risk factor but the studies at that time were inconclusive. They did note African American's can have high levels without risk and they pointed out the lab assays were far from properly developed. They state: *"the quantitative contribution of elevated Lp(a) to CHD risk beyond the major risk factors is uncertain."* They correctly pointed out back then (and it remains true today) that there is no outcome evidence related to lowering Lp(a) with drugs. They concluded *"some authorities believe that Lp(a) measurement is a useful addition to the major risk factors for identifying persons at still higher risk than revealed by those factors. According to advocates for Lp(a), the option of measurement is best reserved for persons with a strong family history of premature CHD or those with genetic causes of hypercholesterolemia, such as familial hypercholesterolemia. An elevated Lp(a) thus presents the option to raise a person's risk to a higher level. ATP III did not find strong evidence to support this approach, but accepts it as an option for selected persons."* NCEP ATP-III did not discuss measuring Lp(a)-C in 2001.

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Apoprotein (a) protein is made up of multiple repeated amino acid loop-like domains or motifs resembling a German or Scandinavian pretzel called kringles (K).These protein motifs or "kringles" (K) on apo(a) resemble the protein motifs on kringles IV and V that are present on the plasminogen molecule. Hence apo(a) and plasminogen have significant structural homology. Plasminogen has five different kringle domains [KI through KV or K1 through K5]. Henceforth I will use the Roman Numerals. Two of these domains are present in apoprotein (a): KIV and KV. However the KIV domain is where things become complicated. Its genetic sequence coding which controls the KIV structure is very variable among individuals. Altogether, the apo(a) gene has 10 different types of KIV domains, referred to as KIV types 1 through 10. KIV types 1 and 3 through 10 are present as single copies, whereas KIV type 2 is present as multiple copies, varying in number from 3 to more than 40 copies. Those with multiple copies have the high molecular weight isoform and those with few copies will have the lower molecular weight isoform of apo(a). Apolipoprotein(a) genotype, which determines both the synthetic rate and size of the apolipoprotein(a) moiety of Lp(a), alone accounts for 90% of plasma concentrations of Lp(a). As hepatic secretion rates are lower for large apolipoprotein(a) isoforms, and as most individuals are heterozygous for two different isoforms, the smallest isoform typically but not always predominates in plasma. It is for this reason Lp(a)-P [or Lp(a),particle count] does not always correlate with Lp(a) mass measurements. The heterogeneity of Lp(a), particularly with respect to apo(a) isoform size, has posed significant challenges for measurement of Lp(a) in clinical samples. Virtually all of the commercially available Lp(a) assays display an isoform size–dependent bias. Assays insensitive to isoform size are not yet widely available.

So let's closely examine the KIV domain on apo(a): KIV consists of distinct kringle types called 1 to 10 (KIV-1 through KIV-10). Kringle IV types 1-3 and 5-10 (KIV 1-3 and KIV 5-10) only exist in one copy. No inter-individual differences exist. KIV-2 type 2 (kringle IV type 2) has a genetically determined variable structure. KIV-2 can exist from 2 to > 40 copies of 5.6 kb repeats which results in the large number of different sized isoforms of apolipoprotein(a). Obviously a person having KIV-2 containing 2 or 3 repeats will have a smaller, lower molecular weight apo(a) than a person having a larger, higher molecular weight KIV-2 with 40 or more repeats. In other words, some folks have just a few copies of the KIV-2 and the next patient might have several. Thus with respect to apo(a) makeup, molecular weight and size, the most clinically important of the apo(a) polymorphisms is the KIV-2 size polymorphism. Plasma levels of lipoprotein(a) vary greatly among individuals and are determined by the KIV polymorphisms that are present.

Simply put, apoprotein (a) has a variable number of genetically determined repeats of a protein domain, kringle IV (specifically KIV-2). There are different apo(a) isoforms that account for a range of Lp(a) molecular weights (from 280 to 800 kDa). The MW of the apo(a) isoform rises in proportion to the KIV-2 repeats. Thus the KIV-2 size (how many repeats or the copy number variability) polymorphism is the reason why there are different apo(a) or Lp(a) isoforms (small and large). Patients with the smaller isoforms have less KIV-2 repeats (< 22) than the larger isoforms (> 22). The number of KIV-2 repeats correlates inversely with plasma levels of lipoprotein(a); small apolipoprotein(a)

isoforms associate with high lipoprotein(a) plasma concentrations, and vice versa. Why is that? It seems the liver is much better at secreting the smaller apo(a) isoforms than the larger ones. The molecular weight of the apolipoprotein (a) molecule depends on how many kringle repeats are present. The less the number of KIV-2 repeats, the lower the MW and the higher the hepatic secretion rate. So paradoxically, even though small isoforms have a lower molecular weight than the larger isoforms (which have more KIV-2 repeats), serum levels of apo(a) mass of Lp(a) will usually be higher patients with the smaller, lower molecular weight isoforms compared to the larger and higher molecular weight isoforms. The small, low molecular weight isoforms of apo(a) or Lp(a) are in epidemiological trials associated with more CV risk and considered more atherogenic than the larger high molecular weight isoforms. If a patient does secrete too many larger higher MW isoforms, Lp(a) mass will be high but CV risk may be lower than that suggested by the apo(a) mass measurement. That is the shortcoming of Lp(a) mass concentration testing.

The literature until recently has been quite conflicting in large part because there has been no standard assay and every study used something different. In most of the epidemiological studies the risk of elevated apo(a) depends in a linear fashion on the LDL-C concentration. Indeed, data from the large Physicians Health Study, revealed that Lp(a) conveyed no risk if the LDL-C was less than 160 mg/dL. In the Women's Health Study Lp (a) conveyed no risk unless it was extremely elevated (>90th percentile) and the LDL-C (apoB) was also elevated. Thus elevated Lp(a) in the face of normal LDL-C is not a risk factor.

1) Men: High Lp(a) predicts risk of angina, and the risk is substantially increased with high concomitant LDL-cholesterol (reported as > 160 mg/dL). The study found that Lp(a) concentration strongly contributed to CHD risk when LDL-C was concomitantly increased, consistent with several other studies. In other words the risk of Lp(a) is not there if LDL-C is OK (< 160 mg/dL). The men with the highest risk had Lp(a) concentrations > than the 80th percentile and LDL-C > 160 mg/dL. The reference is: Apolipoprotein(a) Size and Lipoprotein(a) Concentration and Future Risk of Angina Pectoris with Evidence of Severe Coronary Atherosclerosis in Men: The Physicians' Health Study Nader Rifai et al. Clinical Chemistry 2004;50:1364–1371.

2) Women: In this cohort of initially healthy women, extremely high levels of lipoprotein(a) (90th percentile), measured with an assay independent of apolipoprotein(a) isoform size, were associated with increased cardiovascular risk, particularly in women with high levels of LDL-C. However, the threshold and interaction effects observed do not support routine measurement of lipoprotein(a) for cardiovascular stratification in women. Lipoprotein(a), Measured With an Assay Independent of Apolipoprotein(a) Isoform Size, and Risk of Future Cardiovascular Events Among Initially Healthy Women Jacqueline Suk Danik, et al. JAMA. 2006;296:1363-1370

In 2003 the NHLBI issued a recommendation that Lp(a) concentrations be reported not in mg/dL but in molar concentrations, yet there are no real world labs who have such an assay. Any lab now reporting Lp(a) in molar units is simply takes mg/dL value and uses a

molecular weight (MW) formula to convert it. Unfortunately because the apoprotein (a) isoforms can vary so significantly in MW due to the variable KIV-2 domains one cannot use such conversion formulas without knowing what isoform is present (isoform testing is not available to real world clinicians). If one uses molar apo (a) mass measurements (readily available) errors can be made (as discussed above) depending if the patient has the more atherogenic isoforms (small) vs. the less atherogenic larger isoforms. More on this later. New data looking at apo(a) SNPs (single nucleotide polymorphisms) might one day help us better understand the all of these relationships.

In the Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease: Recent Advances and Future Directions authored by Santica M. Marcovina et al. Clinical Chemistry 49:11 1785–1796 (2003). The authors stated: *"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."* They go on to state: *"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."* The committee had several recommendations including*: "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. 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."*

However in 2010 (7 years after the above article) Marcovina in the J Clin Lip reference cited above states: *"The conversion factor from mg/dL to nmol/L varies from 2.85 for a small Lp(a) size to 1.85 for a large one. Therefore, a factor of 3.5 is too high, and we suggest a mean conversion factor of 2.4, even though the conversion can be more or less imprecise depending on the apo(a) size. However,* ***the major problem of Lp(a) values*** *is not the units used to report the results but is related to the inaccuracy of the methods that are affected by apo(a) size heterogeneity. These methods overestimate the levels of Lp(a) in individuals with large Lp(a) molecules and consequently underestimate the levels in individuals with small Lp(a) molecules.*

One can now order a reliable Lp(a)-C which is the amount of cholesterol carried by all of the Lp(a) particles that exist per deciliter of plasma (mg/dL). As discussed below, neither Lp(a) mass levels or Lp(a)-C by themselves can help us discern risk with the highest degree of accuracy, but when used together we have a real world tool on more accurately guessing isoform size. Health diagnostic labs in Richmond VA (www.myhdl.com) offers Lp(a) mass and Lp(a)-C testing (developed by Joe McConnell at the Mayo clinic). As mentioned, Lp(a)-C is simply the amount of cholesterol trafficked within all of the Lp(a) particles that exist in a dL of plasma. Let's look at two patients with high Lp(a) mass

levels, but one has the large, high molecular weight isoform and is thus likely not at CV risk and one has the smaller, low molecular weight isoform and likely is at risk. Because there are so many more Lp(a) particles [Lp(a)-P)] in the person with the small isoforms (due to its high hepatic secretion rate) compared to the patient with larger isoforms (liver has a hard time secreting such a large molecule), the former will have a high Lp(a)-C and the latter will not. So when I now order Lp(a) mass I always get Lp(a)-C. In the above instance two patients both have high Lp(a) mass, but only the higher risk person with the smaller isoform will have the higher Lp(a)-C. So the easiest way for me to understand risk related to high Lp(a) mass is to always look at both Lp(a) mass and Lp(a)-C: if both are up the isoform is small and risk is present. If mass is up and Lp(a)-C is normal, the isoform is large and no risk exists. The 75th percentile population cut point (high risk) for Lp(a) is 30 mg/dL or if one does the molar conversion it is 70 nmol/L. But here is where one gets into trouble. If one has large isoform apo(a), a level of 30 mg/dL is only the 50th percentile cutpoint (not a high risk). I hope you see how Lp(a)-C can help us very much in these scenarios. Conclusion: Lp(a) mass or Lp(a)-C by themselves are not really that helpful in adjudicating CV risk. It is hoped that one day soon we will have Lp(a)-B measurements. Remember apo(a) traffics on an apoB lipoprotein: LDL is a apoB containing particle. Would not it be nice to simply count Lp(a) particles. Maybe that is all we will need. Or else we can use it with in addition to Lp(a) mass and Lp(a)-C. That day is not that far away.

Exactly, why Lp(a) may be a risk factor is still debated but the evidence is now pointing to not only the acting as a faulty plasminogen (inhibiting fibrinolysis) and thrombotic risk but to the fact it is an inflammatory marker and that the apo(a) (especially the small isoforms) traffics oxidized phospholipids, many of which are generated as a result of lipoprotein phospholipase A2 (Lp-PLA2). Of course ox-PL are very good at causing endothelial dysfunction and aggravating the maladaptive inflammatory process that occurs when apoB particles enter the arterial wall and get ingested by macrophages.

No randomized clinical trial of the effect of lowering lipoprotein(a) levels on CHD prevention has ever been conducted. The well illustrated editorial in JACC cited above (2010;55:2168-2170) entitled The Mysteries of Lipoprotein(a) and Cardiovascular Disease Revisited by Kiechl and Willeit concludes *"the puzzling pieces of knowledge are being assembled to a promising whole. - We are on the verge of understanding the physiologic role and pathologic properties of Lp(a) particles and await the development of specific Lp(a)-lowering therapies. - But alas for those requiring level 1 evidence: we are not close."* Are there any official guideline treatment recommendations regarding treating at risk patients with high small isoform Lp(a)? No other than to lower LDL-C. However, expert opinion (see the J Clin Lip reference cited above) suggests lowering LDL-C and apoB (LDL-P) with a statin and then adding Niaspan for multiple lipoprotein benefits (including additional LDL-P and HDL-P benefits) and whatever apo(a) lowering one might get. This will never be solved without trial data - maybe AIM-HIGH will offer some data.

It is well known statins do not seem to lower Lp (a) and in some studies have actually increased Lp (a) levels. Structurally, lipoprotein (a) is an LDL particle with an

apoprotein (a) attached via a disulfide bond to the apoprotein B (apoB) on the surface of the LDL particle. In effect the apo(a) covers or camouflages the LDL-receptor binding site on the apoB molecule from hepatic LDL receptors (LDLr). Thus the liver is not likely to clear Lp(a) particles, even if one is on a statin which causes upregulation of LDLr. Fortunately, patients with elevated Lp(a) have the apo(a) attached to some but not all of their LDL particles. So the upregulated LDLr can clear LDLs which do not carry apo(a). So statins lower LDL-C and LDL-P but have no effect on Lp(a). Thus the statin will likely cause event reduction based on LDL-P lowering even though they do nothing to Lp(a) levels.

Many labs perform apolipoprotein (a) mass concentrations: however this assay is very tricky and by no means standardized. The Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein(a) and Cardiovascular Disease: Recent Advances and Future Directions Santica M. Marcovina, et al. Clinical Chemistry 2003;49:11:1785–1796) advocate measuring apo(a) in molar concentration, because 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. They also state, 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. LipoScience reports Lp(a) in molar concentrations but no other lab does. The problem is LipoScience uses a calculation to convert mg/dL to nmol/L but since LipoScience is not identifying which isoform is present (high or low molecular weight), one cannot convert mg/dL to molar units.

VAP does not report Lp(a) mass but rather reports how much cholesterol is within the Lp(a) particles and thus provides Lp(a)-C concentrations. Since the risk of apo(a) seems to be related to LDL-C (see below) some find the Lp(a)-C measurement helpful. In reality the best way in 2010 to know if Lp(a)-P is elevated, one needs Lp(a) mass and Lp(a)-C. You need to do Lp(a) mass and Lp(a)-C on the same specimen. If both are elevated the person likely has a high Lp(a)-P of the low molecular weight isoform and is at risk. The problem is the risk depends on LDL-P and apoB cannot be used to count particles in persons with elevated Lp(a) as there apo(a) can interfere with the apoB test. So you can only judge risk by looking at LDL-C or better yet LDL-P or best of all Lp(a)-P (not yet readily available). Since Lp(a)-P or Lp(a) mass in molar concentration is not available, Lp(a)-C serves as the Lp(a)-P test at present. Thus high Lp(a) mass + high Lp(a)-C means high Lp(a)-P and risk where high Lp(a) mass but normal or absent Lp(a)-C means low Lp(a)-P and less risk. Be sure you lab provides both Lp(a) mass and Lp(a)-C. So in the patient at hand the Lp(a) situation adds to his risk.

Be sure you know how your lab reports the data and insist on molar concentrations. I believe looking at apo(a) molar concentrations with respect to LDL-P or apoB is a far better way to understand the risk.

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Statins deplete the LDL cholesterol, apoB and LDL-P levels by inhibiting hepatic HMG CoA reductase mediated production of cholesterol. The liver in an attempt to restore hepatic cholesterol levels (necessary for bile acid synthesis) upregulates LDL receptors. The apoB on the LDL (or other betalipoprotein surface) is a major ligand for LDL receptors.

Of course the best proven way to reduce atherosclerosis is to reduce apoB or LDL-P (or their lipid surrogates like LDL-C and Non HDL-C). Any LDL particle with an apoprotein (a) attached in effect has a camouflaged apoB on its surface. Such particles are far less susceptible to removal via the LDL receptor LDLr) mechanism (upregulated by statins and ezetimibe), as the apoB is not visible to the LDL receptor. Of course LDL particles that do not have the apoprotein (a) attached to their apoB are cleared normally by statin or statin/ezetimibe upregulated LDLr. This scenario of removal of normal LDL particles, but no removal of Lp(a) particles would result in little change of Lp (a) levels with statin therapy even though LDL-C, apoB and LDL-P and clinical risk would be reduced. Thus the most potent therapy to most significantly upregulate LDL receptors and lower LDL-P is combination therapy with a statin and ezetimibe or bile acid sequestrant.
Thus one can normalize LDL-C, LDL-P, apoB and lower CV risk without doing anything to apo(a) levels.

The drugs that can reduce apo(a) mass like niacin (the most efficacious), fenofibrate (in some studies elevated it), estrogen, and raloxifene will cause less apoprotein (a) to be attached to LDL particles. These drugs reduce Lp(a) levels but none as monotherapy are particularly efficacious as statins in reducing LDL-P or LDL-C. There are no outcome studies relating clinical event reduction to what a drug does to Lp(a) levels. There are all sorts of studies showing lowering LDL-C or LDL-P saves lives and that is why those surrogates are what NCEP strongly suggests clinician's direct therapy at. Most advocate statin use first followed by the addition of niacin to lower apo (a) levels, yet there is no clinical trial evidence whatsoever that clinical events would be affected. Many people have extremely high Lp(a) levels. Niacin can only lower it 25-30% which would never get the Lp(a) concentration close to a normal level. Since atherosclerosis risk depends on apoB or LDL-P, if we simply normalize those measures or their lipid surrogates (LDL-C and Non HDL-C) there will likely be few events.

Dr Greg Brown has reported: "In an analysis by Maher et al. of the Lp(a) data in the FATS trial, lowering LDL levels in those with high LDL and high Lp(a) levels dramatically reduced risk. Without treatment, these patients had a 42% risk of a major clinical event, including MI, the need for revascularization, or CV death over the 2.5 year study. When LDL levels were lowered aggressively, even though the Lp(a) levels remained high, the risk of this group was reduced to less than 10%, for a roughly 75% reduction in the risk of a major cardiovascular event. While Lp(a) (and probably risk) may be modestly lowered with niacin therapy, and with estrogens in women, aggressive lowering of LDL levels appears to be the most reliable way to treat patients at high risk due to elevated Lp(a)."

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