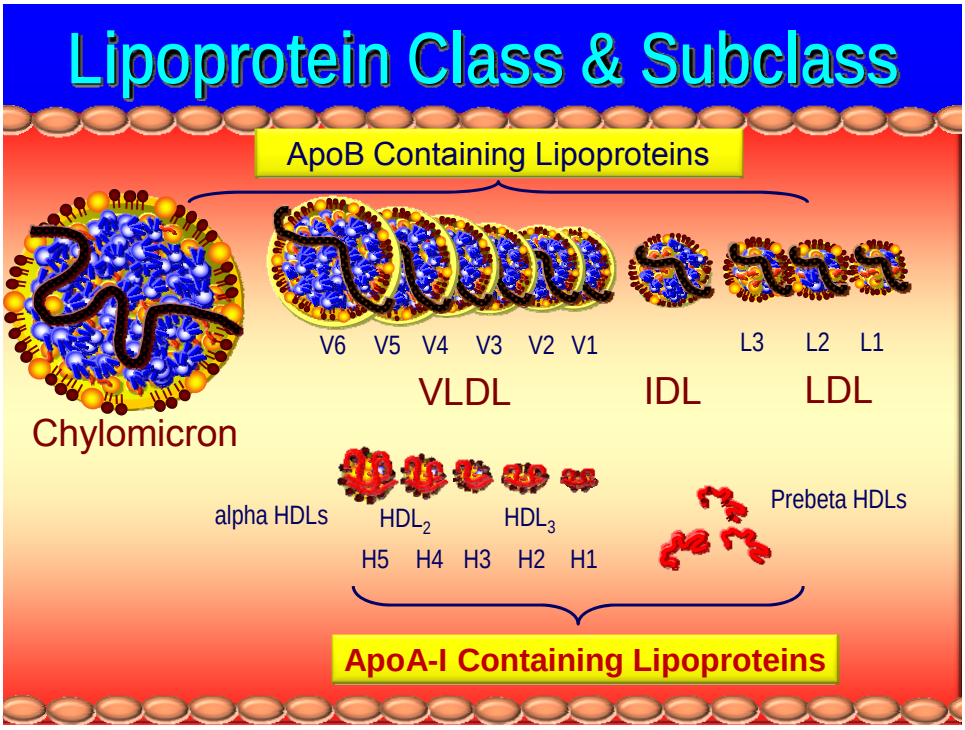
ADVANCED LIPOPROTEIN TESTING
Thomas Dayspring MD, FACP

To my friends: I have been getting more and more inquires as to what is the preferred type of advanced lipoprotein testing. Seems like more and more physicians realize this is the only way to truly know what you are doing with risk assessment and treatment. Due to all sorts of crazy marketing and "education" by those with little knowledge of the subject or other interests at heart (the bottom line), healthcare professionals are getting all sorts of misinformation. So let me help you understand what you get when you order certain tests.

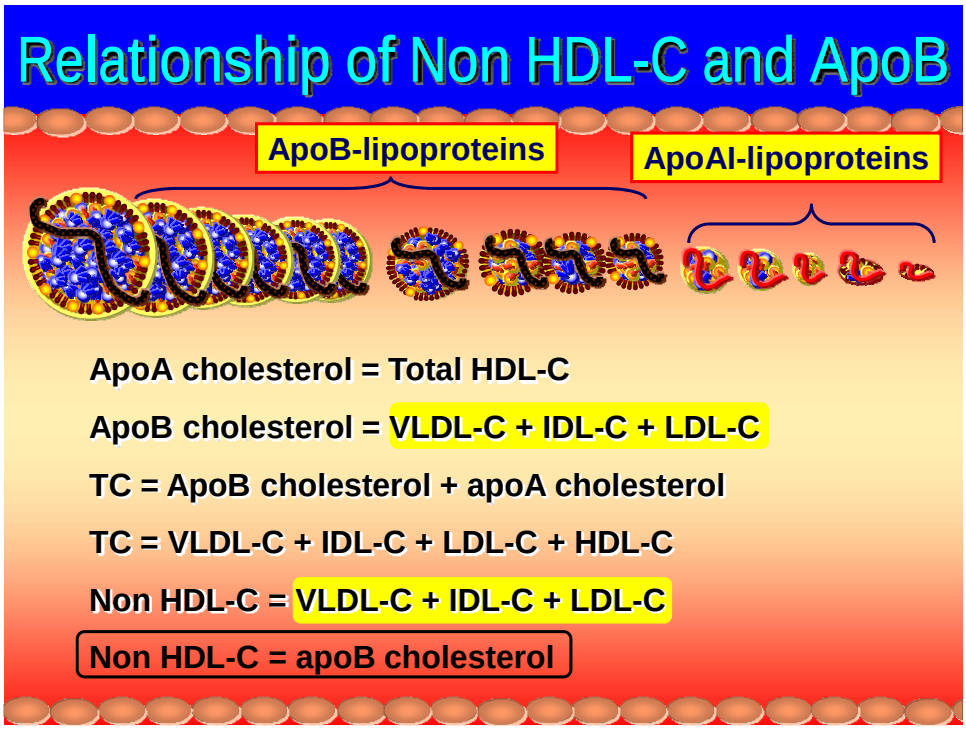
Atherosclerotic plaque begins when lipoproteins invade the arterial wall (intima) and "dump" their sterol (both cholesterol and other non-cholesterol sterol) contents. All lipoproteins less than 70 nm in diameter have the ability to pass through the endothelium and invade the arterial wall. The "beta" lipoproteins, characterized by having apolipoprotein B or "apo" B (there is one molecule of apo B on every beta-lipoprotein) on their surface are the "atherogenic" lipoproteins, because apoB adheres the particle to intimal proteoglycans. Once trapped these particles are prone to oxidative forces and surface phospholipids are hydrolyzed by lipoprotein associated phospholipase A2 (Lp-PLA2 available commercially as the PLAC test): these modified beta-lipoproteins are then ingested by macrophages which then become foam cells (the hallmark of atherosclerosis). Alpha-lipoproteins (HDL) enter the intima but do not adhere to the intimal tissue and not ingested by macrophages and thus their sterol content is considered "non-atherogenic). The major surface apolipoprotein on HDLs are two to four molecules of apoA-I. ApoB and apoA-I levels are thus lab measures which can be used to quantify the beta and alpha lipoproteins. In the large, AFCAPS-TexCAPS as well as the AMORIS trial increased apoB and decreased apoA-I levels were the best predictors of baseline and in AFCAPS, on-treatment residual risk.



The beta-lipoproteins consist of chylomicrons, chylomicron remnants, VLDL, VLDL remnants, IDL (intermediate density lipoprotein), LDL and lipoprotein (a). All of the beta-lipoproteins can be atherogenic (if they enter the arterial wall), but their respective concentrations do vary. 90%

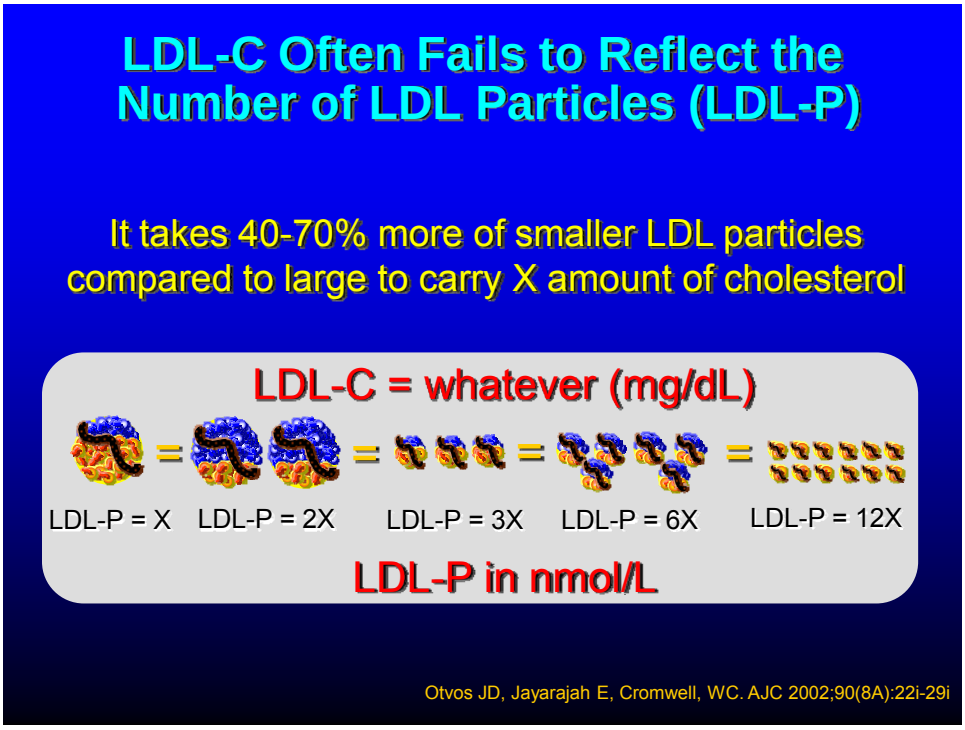
or more of an Apo B measurement are LDL particles as most of the above beta-lipoproteins have very short half lives (hours) with the exception of the LDL which lasts for 2-3 days (longer if the LDL is small).

The cholesterol or cholesteryl ester (CE) inside of the beta-lipoproteins is called non-HDL-C and is determined by subtracting HDL-C from the total cholesterol level (or adding LDL-C to VLDL-C). Thus, non HDL-C is a lipid concentration estimation of beta-lipoprotein concentration or apoB. If non HDL-C is elevated the patient probably has too many beta-lipoproteins, most of which will be LDLs. One could look at non-HDL-C as the "poor man's LDL-P."



ALL of the above lipoproteins come in varying sizes (small to big, based on diameter) and all have different lipid compositions (amount of TG and CE within the particle). Each group of lipoproteins (chylomicrons, VLDL, LDL, and HDL) is a heterogeneous mixture of large, intermediate and small sizes. All people do have "predominant" sizes of LDL particles with Pattern A being normal or large and Pattern B being small). Some labs report LDL size in nanometers (nm) and others in Angstroms (A°). Both LDL particle concentration (LDL-P) and LDL size are related in determining the particle's atherogenicity but particle number is the only lipoprotein parameter that is statistically significantly associated with CV risk. Too many LDL particles (be they large or small) are a major CHD risk factor. LDL-C, especially when less than 140 mg/dL does not always correlate with apoB or LDL-P because the LDL particles can have variable amounts of cholesterol per particle depending on either size and/or TG/CE composition. Because the volume of a sphere is related to the third power of the radius ($4/3\pi r^3$), it will take many more small rather than large LDLs to traffic a given amount of cholesterol. Likewise a TG-rich and thus CE-poor LDL is a cholesterol depleted particle and more particles will be required to traffic a given LDL-C. Thus LDL-C may be normal but LDL-P or apoB will be high and as

mentioned risk is more directly related to particle number than particle size or cholesterol content.



With respect to VLDL particles, increased numbers of large VLDL are related to risk in population studies: Large VLDL, although usually too large (> 70 nm) to penetrate the endothelium, after lipolysis (i.e. TG hydrolysis or removal via cholesterol ester transfer protein or CETP) become smaller, highly atherogenic, cholesterol laden, VLDL remnants (↑ VLDL-C). The CETP mediated shift of TG to LDLs and HDLs causes the formation of TG-rich, CE-poor LDLs and HDLs and after their subsequent hydrolysis by hepatic lipase, small sized LDLs and HDLs are created. Thus large VLDL is often associated with increased production of cholesterol-enriched VLDL remnants (smaller VLDL or chylomicron particles that have acquired CE from HDL via CETP) and small LDL and HDL particles (and also less large LDLs and HDLs). In other words elevated T are often associated with increased numbers of large VLDLs, small LDLs and HDLs (↑ large VLDL-P, total and small LDL-P and ↓ total and large HDL-P). The TG-depleted, CE–rich remnant lipoproteins are less than 70 nm in diameter and can enter the arterial wall but both they and large VLDLs are also associated with abnormal coagulation factors, increased blood viscosity, inflammatory markers and endothelial dysfunction. Also there are always tremendously more LDLs than VLDLs because of the half-life differences (i.e. the LDL-P elevation is much higher than the VLDL-P).

As noted in AFCAPS, AMORIS, Framingham Offspring trial and many others, persons who are at high risk for CHD are those that have too many apoB-containing or beta-lipoproteins (especially LDL-P) or too few apoA-I-containing lipoproteins (alpha). The goal of therapy in treating lipoprotein disorders is to prevent the beta-lipoproteins from invading the arterial wall (reduce apoB) and by increasing the concentration and functionality of alpha-lipoproteins concentration: however the functionality of the HDL particles cannot be determined by concentration and size measurements.

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So how do you determine if there are too many beta-lipoproteins and too few alpha-lipoproteins other than measuring apoB and apoA-I? You certainly cannot rely on the LDL-C level as a lipid surrogate of LDL-P unless the LDL-C is very high (> 140 mg/dL). Non-HDL-C (LDL-C plus VLDL-C) would be a more accurate estimate of apoB than is LDL-C and it correlates well with apoB but is only moderately concordant. Treatment errors are often made by looking solely at LDL-C by itself (especially in patients with TG/HDL axis disorders). Normal or slightly elevated LDL-C levels (rarely treated) are very frequently associated with elevated LDL-P concentrations (increased risk). In both the Heart Protection and PROVE-IT trials there was still significant residual risk in some patients with mean LDL-C levels of 70 and 62 respectively. If one has 62 mg of cholesterol in all of the LDL particles in a deciliter (dL) of plasma, it could take up to 70 % more very small LDL particles or TG-rich, CE-poor LDLs to transport that amount of cholesterol compared to how many large, CE-rich particles would ne required. In both patients with an LDL-C of 62, the one with large LDL particles will have an normal (physiologic) LDL-P and the one with very small particles or TG-rich LDLs will have an LDL-P that is considerably higher (explaining much the residual risk seen in clinical trials when LDL-C is satisfactory).

NCEP ATP-III in 2001 wanted you to calculate the non-HDL-C (TC minus HDL-C) and use that value as a secondary goal of therapy in patients with elevated triglycerides (> 200 mg/dL). (NCEP's goal is under 160 mg/dL in primary prevention and under 130 in secondary prevention and under 100 in the very high risk patient). Fasting is not required when calculating non-HDL-C. As mentioned above, non-HDL-C is certainly a better predictor of risk than that predicted by LDL-C. However, like all of these calculations or ratios, they pick up the patients in the middle of the proverbial bell shaped curve and miss those on the outside or fringes. NCEP suggests that you calculate non-HDL-C in all patients with TG above 200 mg/dl because those patients with elevated TG are apt to be the patients with small LDL particles and increased LDL-P and decreased LDL-C and HDL-C; however newer data from the Framingham Heart Study shows the TG levels are irrelevant: i.e. whether the TG are greater or less than 200 mg/dL, non-HDL-C is a better predictor of risk than is LDL-C. The non-HDL-C values, if abnormal, can alert the clinician, especially when LDL-C is unremarkable, that a person might still be at risk despite the "normal" LDL-C level.

The data on particle concentration superiority over standard lipid measurements is spo strong that in April of 2008, a joint consensus statement from the American Diabetes Association and the American College of Cardiology have now stated that when assessing or treating those with cardiometabolic risk, all pharmacological decisions must be based on a measured apoB or LDL-P). Those of us interested in making the most accurate risk assessments and in making sure our therapies maximally reduce risk (reduce the numbers of circulating atherogenic particles) have to rely on the available types of advanced lipoprotein testing that quantifies particle concentrations and not the non-HDL-C level. Indeed a recent consensus statement from the ACC and ADA states that apoB or LDL-P is required to make therapeutic judgments. The only currently available tests that quantify atherogenic lipoproteins I(and have published data from large clinical trials) are apolipoprotein B (apoB) measurements (widely available and now standardized) and nuclear magnetic resonance spectroscopy (NMR LipoProfile from LipoScience) determination of LDL-P.

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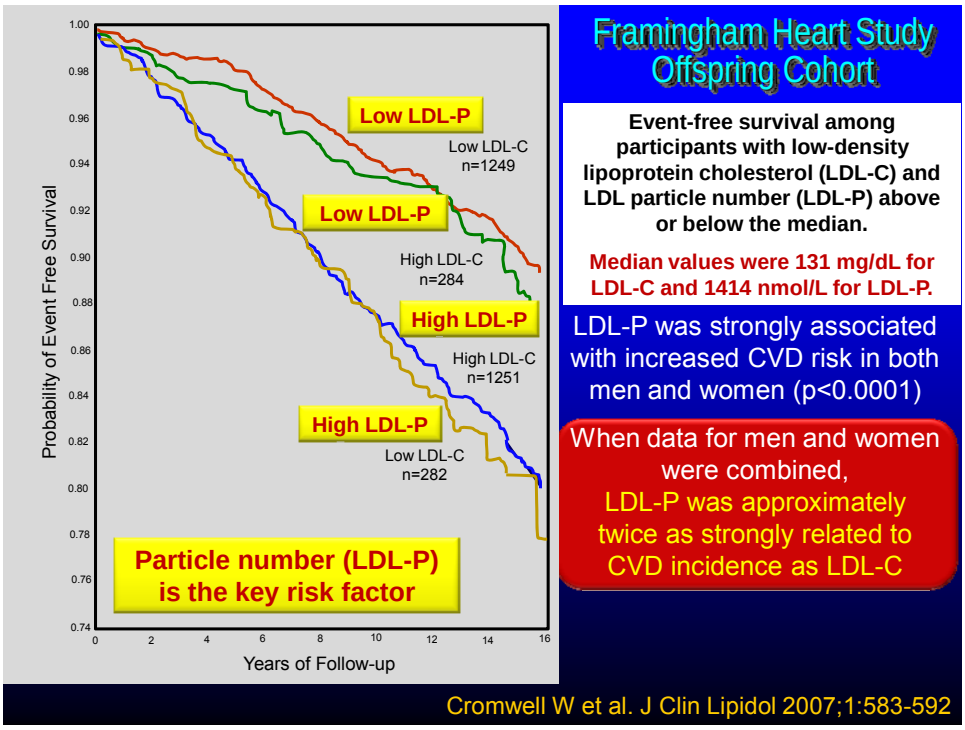

ADVANCED TESTING TECHNIQUES

1) Gradient Gel fractionation tests: such as Berkeley labs, etc. By subjecting lipoproteins lying in gels with diminishing pore sizes, to an electric current one can separate lipoproteins by sizes (a very complicated procedure which can be subject to error: Berkeley is the best lab at performing gradient gel fractionation). This technique sizes LDL and HDL particles into numerous subclasses based on size. You get nothing about VLDL in the Berkeley report, other than a graphic curve without reported numbers. HDL size separation adds considerable cost to the test. Berkeley does provide apoB, using standard immunoassays as their way of collectively quantitating all beta-lipoproteins. They also can report ultra-apoB which is the apoB on LDL particles.

2) Atherotec's VAP (vertical auto-profile): VAP is really a cholesterol assay and not a lipoprotein quantification test. It provides cholesterol concentration within the various particles without providing any lipoprotein concentrations. The lipoproteins are separated and sized by ultracentrifugation and then a migration technique (prone to error). The cholesterol concentration (not the particle concentration) is then determined for each of the particles. Note that this technique does not actually report the size of the LDL particles, but rather the phenotype as Pattern A, Pattern B or AB. Does the cholesterol content of lipoprotein particles of any value whatsoever without knowing the concentration of the particle? There is data from the Quebec Cardiovascular trial that small LDL particles which contain increased cholesterol are more atherogenic than small LDL particles carrying less cholesterol (however since no LDL-P measurements were made in this study the small LDL-C could not be adjusted for this parameter). Thus subparticle cholesterol content is nice to know, but clinically fairly meaningless without particle concentration data. The 4S trial with simvastatin (Zocor) and the AFCAPS

TexCAPS trial with lovastatin (Mevacor) both revealed that CV event reductions was more correlated with LDL particle concentration (as measured by apoB) than any cholesterol measurement. Recently VAP has been reporting a calculated apoB concentration, based on non-HDL-C and LDL size. The formula used has not been published in any peer reviewed scientific study and has not been validated in any large clinical trial (as measured apoB and LDL-P via NMR have). Why calculate apoB (based on lipids) when it is so easily measured in the any chemistry lab?

LDL size or LDL particle cholesterol concentration is not a statistically significant risk factor in persons with normal LDL particle concentrations. Patients with normal numbers of small or large LDL particles are not at risk for CHD! Yet many physicians subject the patient to therapy based solely on small LDL size. That is not evidenced-based medicine and is gross over treatment! Unfortunately the VAP profile does not give you LDL particle concentrations: you would have to order a measured apoB which adds expense.

Many do not realize it but LDL-C as reported by virtually all labs is actually the sum of the cholesterol within all of the IDL, LDL and Lp(a) particles in plasma. Real LDL as reported by VAP is a direct LDL-C measurement where they have excluded IDL-C and Lp(a)-C. Atherotech separates LDL particles by ultracentrifugation and other labs who report direct LDL-C use antibody tests to remove apoA1 (HDL) and apoE particles (VLDL & IDL) and then measure cholesterol in the remaining LDL particles. Of course it is not very important to know what the calculated or measured LDL-C is when one has lipoprotein particle concentrations (apoB or LDL-P) as it is particle number, not particle cholesterol that most closely correlates with risk. Neither a directly measured or calculated LDL-C correlates very well with LDL-P, when the LDL-C is < 140 mg/dL or when TG levels are elevated.

VAP does not report apo(a) or Lp(a) concentrations, but rather the cholesterol content of Lp(a) of various sized LDL particles. Some find this convenient as the risk of apo(a) is related to LDL-C.

3) Nuclear Magnetic Resonance (NMR) Spectroscopy: by LipoScience. By subjecting plasma (which needs no alteration by any reagents: minimizing chance for errors) to magnetic waves, lipoproteins can be sized and enumerated (concentrations can be determined). It is the only advanced lipoprotein test that does both (quantity and quality) and of course you get the all important beta and alpha particle concentrations. You get both VLDL concentrations and various HDL subparticle concentrations (too get a total HDL-P, one has to sum small, medium and large HDL-P). No other technique offers you that. HDL-P is also emerging as a very important clinical tool, unlike HDL-C. NMR technology cannot quantitate the very small pre-beta HDLs, and they typically represent ~ 5% of circulating HDLs.

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