LIPID CASE 260 Lipoprotein Core Composition

Over the last many years using my Lipidaholic's Anonymous case discussions I have demonstrated time and again the significant limitations of lipid concentrations in determining CV risk and in serving as goals of therapy. We really need to understand why that is so and comprehend the concepts of concordance and discordance. This means although two tests have ability to indicate something, are they always equally predictive? If so they are concordant. If they are not always equally predictive, then in those instances they are discordant. When two tests are discordant, only clinical trials can tell us which one is more accurate and obviously we should then use the superior (more accurate) test.

It is possible for two usually correlating CV risk factors to be concordant in many patients but then discordant in others. This definitely applies to lipid and lipoprotein testing - Several studies have shown that standard lipid concentrations or ratios (TC, HDL-C, LDL-C, VLDL-C, LDL-C, non-HDL-C, TC/HDL-C and TG/HDL-C ratios) correlate with CV risk. Of course, directly measuring atherogenic lipoprotein concentrations apoB and LDL-P or apoA-I and HDL-P also correlate with CV risk. When lipids and lipoprotein assays both indicate risk equally, they are concordant and we can trust either. When one is normal and the other is abnormal - they are said to be discordant. Well all my readers certainly know that there are many instances when LDL-C and non-HDL-C are fine but apoB and LDL-P are ugly. Thus discordance can exist. We have plenty of trials that show when discordance is present; risk always follows lipoprotein concentrations - not lipid concentrations.

Unfortunately I know lots of lipid guys and gals, even certified lipidologists, who think they can look at the various lipid concentrations or ratios and very accurately guess whether atherogenic lipoprotein assays (apoB or LDL-P), would be normal or abnormal. If I can be blunt: anyone believing that is delusional. I used to be one of them. However, I gave up on these guesstimates years ago. It simply cannot be done, no matter how sophisticated your lipid/lipoprotein knowledge is. I realized this after I went to a case discussion seminar at a NELA meeting several years ago by Jamie Underberg of NYC (Bellevue Lipid Clinic). He showed cases and provided medical history and lipid panels. The audience, including me would shout out what we perceived the risk was and if apoB or LDL-P was an issue. To my shock, I would have done better by flipping a coin. I was embarrassed how wrong I was in so many cases. Over the last 20 years a large part of my professional life has been studying lipidology. Well if I cannot accurately estimate atherogenic lipoproteins by looking at a lipid profile, then I doubt anyone else can do any better. That is why all of my patients get NMR particle concentrations.

Recent data from NHANES shows that the single biggest cause of myocardial infarction in the US is insulin resistance. In young adults, preventing insulin resistance is predicted to prevent 42% of myocardial infarctions. IR is associated with such a high incidence of atherosclerosis because of: systolic hypertension, elevated TG, low HDL-C, elevated glucose and especially **elevated apolipoprotein B** (Diabetes Care 32:361–366, 2009). IR is of course related to lifestyle, obesity (although 20% of metabolic syndromes are lean), genes and especially age. Notice that there is now no mention of LDL-C as having any relationship to IR. It is absolutely crucial that clinicians understand why patients with cardiometabolic risk have such a high incidence of atherosclerosis and that risk is **not related to LDL-C** but rather to apoB – i.e. LDL-C and particle measurements are often very discordant in this cohort.

In relatively new data (Amit Sachdeva, et al Am Heart J 2009;157:111-7.e2): data from the Get with The Guidelines Study looked at lipid levels in patients hospitalized with coronary artery disease: In this large cohort of patients hospitalized with CAD, almost half have admission LDL-C levels <100 mg/dL. More than half the patients have admission HDL levels < 40 mg/dL (but 45% did not), and the vast majority had TG values between 80 and 200 mg/dL (keep in mind a physiologic TG is typically 10-70 with a mean in the 30s). More NHANES analysis shows that over the last 30 years, LDL-C levels have been dropping but alas so has the number of low risk

people been dropping. Interestingly over that time period triglycerides (TG) have doubled in the adult population. HDL-C has also been dropping. What is the diagnosis when LDL-C falls at the expense of rising TG and dropping HDL-C ------ ????? ------- Of course the dyslipoproteinemia of IR, Metabolic Syndrome, T2DM.

To truly understand atherogenesis (the accumulation of sterols in the arterial wall macrophages) it is crucial that one realizes that hydrophobic lipids go nowhere in human plasma unless they are passengers in protein wrapped vehicles conveniently termed called lipoproteins. To be technically correct fatty acids (FA, a lipid,) can also traffic in plasma attached to albumen, phospholipids (PL) to phospholipid transfer protein (PLTP) and both TG and cholesteryl ester (CE) to cholesteryl ester transfer protein (CETP). Of course the vast majority of plasma lipids are in lipoproteins and lipid concentrations are measureable. Since it is the lipoproteins which are the vehicles trafficking sterols and unfortunately in some patients they enter the arterial wall, get oxidized and are then internalized by monocytes turned into macrophages. You must understand that although caused by oxysterols, atherogenesis is in effect mediated by pathological lipoproteins. There is no way for sterols to get into the artery other than being a passenger within a lipoprotein. One must understand which of the sterols (cholesterol, cholesteryl ester and noncholesterol sterol) containing lipoproteins have the ability to enter the arterial wall to set off the process. "Which lipoproteins are the illegal dumpers?" So here is my tutorial on lipoprotein “anatomy”.

Discovered in the 1940s using the ultracentrifuge, lipoproteins were first separated by their buoyancy (flotation). Those with large amounts of lipids attached to surface proteins are quite buoyant and float and those with few lipids (denser) sink lower in the centrifuge tube. Thus ranked by buoyancy or size (from largest to smallest) are chylomicrons, very low density (VLDL), intermediate density (IDL), low density (LDL) and high density lipoproteins (HDL). Paper electrophoresis soon followed and the following terms appeared: Beta-lipoproteins for LDLs, alpha lipoproteins for HDLs and prebeta lipoproteins for VLDLs. Thus the term beta-quantification used to be used when assaying LDL particles. Later on the term betalipoproteins has been applied generically and collectively to VLDLs, IDLs and LDLs and alphalipoproteins to HDLs.

Providing structure, stability and solubility to lipoproteins are structural proteins called apoproteins and once they attached to lipids, apolipoproteins. The major structural apoproteins are hepatic or intestinally produced apolipoprotein A-I (for HDL or alpha-lipoproteins), hepatic produced apolipoprotein B100 (for VLDLs and their byproducts IDLs and LDLs) and intestinally produced apolipoprotein B48 (for chylomicrons): apoB 48 is simply a truncated apoB molecule having 48% of the molecular weight of hepatic produced apoB100.

So now let's dissect a lipoprotein: other than the very tiny prebeta-HDL species, lipoproteins are circular collections of lipids and proteins and some other molecules that may hang along for the ride such as (fat soluble vitamins). Lipoproteins have the following structure:

1) **LIPOPROTEIN SURFACE**: A surface which is composed of a single layer of PL and free or unesterified cholesterol (FC). Remember the surface of the lipoprotein floats in aqueous plasma and surface molecules interface with water. Both FC and PL are amphipathic molecules meaning they have both a hydrophilic end and a lipophilic end enabling the hydrophilic ends (phosphorous end of phospholipids, and hydroxy or -OH moiety of FC) of these lipids to be aligned on the plasma surface of the particle. The hydrophobic (lipophilic) FA tails of PL and the terminal aliphatic ethyl-methyl groups of FC extend into the inside or core of the lipoprotein where they are not exposed to plasma.

2) **LIPOPROTEIN CORE**: This refers to the internal hydrophobic lipids, namely triacylglycerol or TG and cholesteryl ester (CE) which is a cholesterol molecule on which a long chain fatty acid has replaced the 3-hydroxy group of FC, thereby making both ends of the cholesteryl ester molecule hydrophobic. **All** lipoprotein cores carry variable degrees of TG and CE.

Normal core compositions
VLDL 5/1 (5 times more TG than CE)
LDL 4/1 (4 times more CE than TG)
HDL 90-95% of core is cholesteryl ester – very little TG

The Friedewald formula calculates VLDL-C by dividing TG by 5. This presumes all TG are in VLDLs and the VLDLs have normal core compositions of 5 times more TG than cholesterol.

3) **SOLUBILIZING PROTEINS**: include the structural and nonstructural apoproteins: We have discussed apoB 100 and apoB 48: there is one apoB molecule per VLDL, IDL, LDL and chylomicron. ApoB is the only nontransferable apolipoprotein meaning it is with the particle from its origin to its final destruction: it cannot be shared. Measuring apoB, using standard and readily available protein immunoassays, quantifies the number of apoB-containing lipoproteins per deciliter (dL) of plasma. ApoA-I is the main apoprotein on HDLs (although it can also be found on chylomicrons) and there can be from 2 to 4 molecules per HDL particle. Numerous other apoproteins exist and they perform numerous other functions many of which are related the lipolytic (catabolic) fate of the various lipoproteins. The only lipoproteins that carry a single apolipoprotein are LDLs which under normal circumstances only have apoB100. Every other lipoprotein has numerous apolipoproteins on its surface.

It is also **CRUCIALLY** important to recognize that lipoproteins are dynamic particles in a constant state of lipidation (acquiring lipids) and delipidation (shedding lipids). This constant process or remodeling will affect particle surface, particle core lipid content, particle apoprotein content as well as particle size, density and buoyancy. Obviously this process will ultimately also impact particle concentrations.

Why did lipoproteins evolve: clearly they are in reality lipid transportation vehicles. The vast majority of tissues in humans can for the most part denovo synthesize all of their cholesterol needs. However, most tissues do not synthesize enough PL for their cell membranes and muscles certainly require a lot of energy which they get by oxidizing FA. Of course trios of FA are trafficked as little energy bundles called triacylglycerols (TG). Thus the apoB-containing lipoproteins evolved to traffic energy (TG). Any FC or CE they carry is along for the ride as it is not an apoB particle's mission to deliver cholesterol. The TG-rich lipoproteins are the very large chylomicrons (bringing in exogenous energy from the gut) and the large VLDLs (trafficking endogenously or hepatic produced energy). Once VLDLs and chylos delipidate (give up their TG) upon the action of lipoprotein lipase (mostly in myocyte or adipocyte beds) they become smaller apoB particles often called remnants or IDLs (carrying far less TG but still carrying their CE load). Under normal circumstances these remnants and IDLs are rapidly cleared by hepatic LDL receptors (LDLr) or LDL receptor related proteins (LRP). LDLr binds to particle apoB100 or apoE (of which chylos & VLDLs have multiple copies of). LRP looks for apoE. ApoB48 does not bind to any receptors. In reality the main function of apoB particles is to bring energy from the gut or liver in the form of TG to tissues for use or adipocytes for storage. The TG-poor remnants then return FC and CE to the liver in a process called indirect reverse cholesterol transport (indirect RTC). At the liver some of the small remnants or IDLs undergo further lipolysis via hepatic lipase and become LDLs. Ultimately after their 1.5-3 day half life LDLs are cleared. The function of apoB particles is not to deliver cholesterol per se but rather deliver energy! The majority of their cholesterol is returned to the liver in the indirect RCT process. VLDLs and chylomicrons deliver TG and remnants and LDLs return cholesterol to the liver.

Again: let's review the core composition of normal lipoproteins:

Chylomicrons and VLDLs are TG-rich with a core of mostly TG and some CE (a 5/1 ratio). Since chylomicrons are normally very transient postprandial particles, the vast majority of TG are carried in VLDLs. Using the Friedewald formula VLDL-C = TG/5.

IDLs: very transient particles which are simply remnants of VLDLs that have lost a lot of their TG in the lipolytic process (lipolysis refers to loss or hydrolysis of lipids). IDLs are rapidly cleared by LDLr. IDLs are CE rich particles. Consider them as very large LDLs.

LDLs: Some very small VLDLs and some IDLs undergo further lipolysis at the liver (via hepatic lipase) and thus loose additional surface PL and core TG, and become LDLs. Normally, the core of an LDL is 4 times more CE than TG.

HDLs contain mostly CE and very little TG and their normal core is > 90% CE and < 10% TG.

LAB ANALYSIS OF LIPIDS:

TC = cholesterol in all of the lipoproteins: Chylomicron-C + VLDL-C + IDL-C + LDL-C
VLDL-C = the cholesterol within all of the VLDLs and chylos if present in a dL (100 cc) of plasma
LDL-C = the cholesterol within all IDLs and LDLs regardless of size: IDL-C + LDL-C
HDL-C = all of the cholesterol trafficked within all of the HDLs per dL
Non-HDL-C = the cholesterol within all of the apoB particles. Since 90% of apoB particles are LDLs, non-HDL-C is in essence a surrogate of LDL-P.

LAB ANALSIS OF LIPOPROTEINS

ApoB (which should as per ADA/ACC consensus statement be measured using standard assay - not calculated) is the number of apoB particles per dL. Because of its long half life compared to that of VLDLs and IDLs, well over 90% of apoB particles are LDL particles. Unless Type III Hyperlipidemia is present, when you get an apoB level you are really looking at LDL-P.

Total LDL-P the number of LDL particles of all sizes per L of plasma (measured in nanomoles per liter)
Small LDL-P: the number of smaller LDLs (size <20.6 nm) per liter
VLDL-P - total number of VLDLs of all sizes in nmol/L
Large VLDL-P the number of large VLDL particles per liter (these are large, very TG-rich VLDLs)
Total HDL-P the number of HDLs of all sizes per L (does not measure unlipidated apoA-I or prebeta
HDLs)

A reality not understood by many is that in all but Type III patients, apoB is not a measure of VLDL and IDL particles (because there are so few of them) To put this into perspective: a normal person would have the following particle concentrations at the 20th percentile population cutpoint):

The 20th percentile particle cut points of the apoB particles using MESA data are
LDL-P = 1000 nmol/L
VLDL-P = 40 nmol/L
IDL-P = 0 nmol/L (very transient postprandial particles)

So although apoB measures LDL-P + IDL-P + VLDL-P, for every 1000 nmol of LD- P there are 40 nmols of VLDL-P. VLDLs make up 4 % of apoB particles under normal circumstances. Under IR conditions and elevated TG where LDL-P becomes extremely high, the % of particles that are VLDLs (compared to LDLs) are even less. So even though there is increased VLDL-P in IR, high TG states, there are even vastly more LDLs (1600-2000 nmol/L). Do not get me wrong: VLDLs and especially their remnants must be considered for a variety of reasons as very atherogenic particles but their atherogenicity is not related to their contribution to apoB.

We now know that atherogenesis occurs if there are increased numbers atherogenic apoB particles (almost all of which are LDLs) in plasma which explains why apoB or LDL-P are the best predictors of CV risk and best goals of therapy. The way people, especially IR people get plaque, no matter what their LDL-C is (including perfect or desirable levels), is that they have high apoB and LDL-P concentrations. In other words in many patients there is a disconnect (discordance) between cholesterol measurements (LDL-C, non-HDL-C) and apoB or LDL-P. The big question is what explains this disconnect?

THIS PARAGRAPH IS CRUCIAL: LDL-P is related to several things: The size of the particle and the core composition of the particle as well as particle synthesis and particle clearance. A better way to examine the core composition is to ask, how many molecules of cholesterol are carried in the typical LDL particle. Any LDL that is carrying (trafficking) lesser numbers of cholesterol molecules will henceforth be referred to as cholesterol-depleted. It will clearly take many more cholesterol-depleted LDLs to traffic a given plasma level of LDL-C than cholesterol-rich LDLs.

a) Particle volume or size: Since the volume of a sphere is 4/3 x pi x the radius cubed, it is obvious that even subtle changes in LDL size will drastically affect how many lipid molecules (CE and TG) an LDL can carry in its core. Amazingly, because of cubing of the radius in the volume formula, it takes 16 marbles to equal the volume of one ping pong ball. With respect to LDLs, presuming a normal core composition of CE/TG of 4:1 it takes 40-70% more (depending on their actual size) small LDLs to traffic the same amount of cholesterol than large LDLs.

b) Particle composition: What about core composition: if it is abnormal meaning the LDL is carrying larger amounts of TG than usual, then the LDLs will have less CE per particle. So anyone with TG-rich LDLs will have CE-poor (depleted) LDL particles. It will obviously take a lot more CE-depleted LDLs than CE-rich LDLs to traffic a given LDL-C. How do LDLs become TG-rich and CE-poor. Increased cholesteryl ester transfer protein (CETP) activity which exchanges core TG for core CE between lipoproteins. TGs go from TG-rich particles (typically chylos postprandially and VLDLs postprandially and all day long to CE-rich particles like LDLs and HDLs. As CETP activity goes up, LDLs and HDLs receive TG in exchange for their CE which winds up in VLDLs and chylo remnants.

From paragraphs a and b above, does everyone see that one could have the following scenarios all of which lead to discordance:

[l] Large LDLs that are normally composed (4:1 CE to TG) likely has elevated LDL-P and LDL-C. Yet if the LDLs are very large, LDL-C may be high and LDL-P is normal: a type, of discordance that is often missed

[2] Large LDLs that are TG-rich and CE-poor. LDL-C is normal or even low, but LDL-P is high. This is a situation where LDL-TG would be high if we could measure it. It is also a situation where one has normal LDL-C, high LDL-P with large LDL particles. This is not uncommon and might indicate hepatic lipase deficiency.

3] Small LDLs that are CE rich. LDL-C may or may not be elevated, but LDL-P is high as it always takes more small LDLs than large to carry a given LDL-C.

4] Small LDLs that are CE-poor and TG-rich: These folks have extremely high LDL-P levels because their LDLs are extremely CE-depleted due to their small size and their increased core TG.

c) Particle synthesis: Hepatic VLDL (apoB) production is dependent on hepatic lipid stores. Livers produce way more apoB than anyone ever uses (most is rapidly degraded). The more lipids (CE or TG in the liver, then more apoB lipidation occurs and more VLDLs and large VLDLs are produced. VLDLs are in effect the grandfathers of one's LDLs. So in IR patients TG can drive

apoB particle production and levels. The larger TG-rich VLDL will also set off CETP activity, affecting LDL core composition (making the LDL TG-rich and CE-poor).

d) LDL particle clearance: this is mediated by LDL receptors (LDLr): it is also well known that LDLr are more efficient at recognizing and clearing normal sized LDLs than small or very large LDLs. Thus IR folks who have small LDLs (because of TG, CETP and hepatic lipase) all have high small and total LDL-P because the small LDLs are not rapidly cleared and thus they accumulate driving up LDL-P. Thus the real danger to small LDLs is not their size but rather that they are present in very large numbers which drives arterial wall entry.

The truth is our patients typically have heterogeneous collections of big and small LDLs that are in a complex dynamic state of flux as they are constantly swapping core CE and TG and constantly being acted up by TG and phospholipid hydrolyzing enzymes. So there is absolutely no way for any practitioner to know with any certainty in any patients how particle synthesis, dynamic remodeling, core composition and clearance is at play and that is why it is an exercise in futility to estimate LDL-P using lipid concentrations. The sooner we all start checking LDL-P and run away from cholesterol measurements we can say goodbye to discordance and be more accurate at risk prediction and getting to goal.