LIPOPROTEIN COMPOSITION REGULATES LDL-P
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We really need to comprehend the statistical concepts of concordance and discordance. This relates to the fact although two tests may have ability to indicate something, they may not always equally predictive? If they are equally predictive then they are said to be 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.

With respect to cardiovascular risk factors, it is possible for two lab assays that usually correlate to be concordant in many patients but then discordant in others. This definitely applies to lipid concentration and lipoprotein concentration 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 such as 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. Indeed there are many instances when LDL-C and non-HDL-C are fine but apoB and LDL-P are ugly, meaning indicative of risk. Thus discordance can exist. We have several trials that show when discordance is present; risk always follows lipoprotein concentrations - not lipid concentrations.

Unfortunately there are many practitioners, even certified lipidologists, who think they can look at various lipid concentrations or ratios and 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. That is why all of my patients get NMR particle concentrations, apolipoprotein B and A-I measurements or both.

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 (who have what is termed TG/HDL axis disorders).

 In relatively new data (Amit Sachdeva, et al Am Heart J 2009;157:111-7.e2) 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-C 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). Other 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. Interestingly over that time period triglycerides (TG) have doubled in the adult population. HDL-C has also been dropping over that time period. 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, the cohort where LDL-C/LDL-P discordance is so prominent.

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 albumin, 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 of course 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 ultimately caused by oxysterols, atherogenesis is in effect related to the delivery of sterols to the arterial wall is 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 sterol (cholesterol, cholesteryl ester and noncholesterol sterols) containing lipoproteins have the ability to enter the arterial wall to set off the process. "Which lipoproteins are the illegal dumpers or in scientific terms the potentially atherogenic particles?"

Discovered in the 1940s using the ultracentrifuge, lipoproteins were first separated by their buoyancy (flotation). Those particles 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 ultracentrifugation and the following terms appeared: Beta-lipoproteins for LDLs, alpha lipoproteins for HDLs and prebeta lipoproteins for VLDLs. Thus the term beta-quantification was 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 unique lipid-binding proteins called apoproteins and once they attach to lipids, apolipoproteins. The major <u>structural</u> 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. Let's cross-sectionally dissect a lipoprotein to better understand its structure: 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). <u>**All**</u> lipoproteins have the following structure:

LIPOPROTEIN SURFACE: A surface which is composed of a single layer of phospholipids (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 hydrophobic or lipophilic end. The hydrophilic ends of these lipids (phosphorous end of phospholipids, and the 3- hydroxy or 3-OH moiety of FC) are aligned on the plasma surface of the particle. The hydrophobic (lipophilic) FA tails of PL and the terminal aliphatic ethyl-methyl groups of FC or noncholesterol sterols extend into the inside or core of the lipoprotein where they are not exposed to aqueous plasma.

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. This hydrophobic quality of CE is crucial to changing the lipoprotein into a sphere which gives the particle a tremendous increase in volume (to traffic lipids) compared to discoid particles. The volume of a sphere is related to the third power of the radius. It may well be the primary teleological reason CE is in the core of beta-lipoproteins whose main mission is to deliver energy on the form of TG.

<u>**All**</u> lipoprotein cores carry variable degrees of TG and CE (and sometimes noncholesterol sterols).

Typical 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. So if serum TG is 150 mg/dL, the VLDL-C is 150/5 or 30 mg/dL. Of course, using the Friedewald formula, VLDL-C is used to calculate LDL-C.

STRUCTURAL (SOLUBILIZING) SURFACE PROTEINS: include the structural 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 with other lipoproteins. 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 remodeling process 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 hydrophobic lipid transportation vehicles capable of trafficking FC, CE, TG and PL. 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 from TG. Of course trios of FA are trafficked as little energy bundles called triacylglycerols (TG). 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 and plays no role in particle clearance. In reality the main function of apoB particles is to bring energy from the gut or liver in the form of TG to tissues (myocytes) 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 smaller remnants or IDLs undergo further lipolysis via hepatic lipase and become LDLs. Ultimately after their 1.5-3 day half life LDLs are cleared by LDLr. The function of apoB particles is not to deliver cholesterol to tissues per se but rather deliver energy! The majority of apoB-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. The CE is needed to keep beta-lipoproteins spherical. Although other cells can upregulate LDLr and acquire FC and CE, that is not the primary function of apoB particles including LDLs. The fact that persons with complex hypobetalipoproteinemia live long lives with LDL-C in the 5-20

mg/dL range demonstrates that apoB particles are not needed to deliver cholesterol. HDLs deliver cholesterol to steroidogenic tissues.

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. Most of their cholesterol is hepatic and enterocyte obtained, trafficked to steroidogenic and adipocyte tissues and ultimately returned to the liver or intestine directly (direct RCT) or indirectly by transfer of their core CE to apoB particles (see below).

LAB ANALYSIS OF LIPIDS:

TC = cholesterol in all of the lipoproteins: Chylomicron-C + VLDL-C + IDL-C + LDL-C + Lp(a)-C
VLDL-C = the cholesterol within all of the VLDLs and chylomicrons 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 + Lp(a)-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% or more of apoB particles are LDLs, non-HDL-C is in essence a surrogate of apoB or 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 of plasma. 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 and in the vast majority of patients apoB must simply be looked at as an LDL-P measurement.

Total LDL-P is the number of LDL particles of all sizes per L of plasma (measured in nanomoles per liter). It can be determined using NMR spectroscopy, ultracentrifugation and staining or ion mobility transfer techniques.
Small LDL-P: the number of smaller LDLs (size <20.6 nm using NMR criteria) 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). HDL are reported in micromols/L.

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 NMR derived 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 often 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 ultimately 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 think about the core composition of lipoproteins 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 π r^3 (radius cubed), it is obvious that even subtle changes in LDL (or any lipoprotein) size will drastically affect volume and 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 a lot more small particles compared to large to traffic a given amount of lipid molecules. For example, it takes the volume of 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 particle core composition: if it is abnormal meaning the LDL is carrying larger amounts of TG than usual, and 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. It is due to 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 chylomicron remnants.

From paragraphs a and b above, it is clear that one could have the following scenarios all of which lead to discordance between LDL-C and LDL-P:

   [I] Large LDLs that are normally composed, traffic more CE than TG (4:1 CE to TG). Such persons can have normal or high LDL-C and/or LDL-P. If LDL-C is very high the patient likely has elevated LDL-P (typical of FH to many large LDL particles). In such a case the values are concordant and equally predictive. 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. It simply does not take a lot of very large CE-rich LDLs to traffic a lot of CE (high LDL-C). This is often seen in women with very high TC, LDL-C and HDL-C levels. LDL-C looks high risk but normal apoB and LDL-P and high HDL-C indicate no risk. The lab values are discordant. The LDL-P (apoB) needs to be followed and the LDL-C ignored.

[2] Large LDLs that are TG-rich and CE-poor. LDL-C is normal or even low, but LDL-P is high as it takes a lot of CE-depleted LDLs to traffic a given level of LDL-C compared to CE-rich LDLs. This is a situation where LDL-TG would be high (if we could measure it). This is also a situation where there is apt to be serious discordance between LDL-C and LDL-P. Recognize that the elevated LDL-P is due to abnormal core composition and not the size of particles. The LDLs are large because they are full of TG, not CE. This situation 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. Again LDL-C and LDL-P are discordant and risk follows the LDL-P. In this case particle size is the culprit.

[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 both their small size and their increased core TG. The discordance between LDL-C and LDL-P is extreme. It is not uncommon to see LDL-C below the 20th percentile population cutpoints with LDL-P values greater than the 90th percentile population cutpoint.

c) Particle synthesis: Hepatic VLDL (apoB) production is dependent on hepatic lipid stores. Livers produce way more apoB molecules 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, which tend to have delayed catabolism, will also set off CETP activity, obviously 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 increased 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 so often present in very large numbers which drives arterial wall entry.

Since 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 the following: particle synthesis, dynamic remodeling, core composition and clearance. That is why it is an exercise in futility to accurately estimate apoB or LDL-P using lipid concentrations. The sooner we all start checking LDL-P and run away from cholesterol measurements we will not have to deal with discordance and we will be more accurate at risk prediction and getting to goal.