Understanding the Entire Lipid profile

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There is only one absolute in atherosclerosis: sterols (predominantly cholesterol) entering the artery wall and being internalized by macrophages, creating foam cells (the histologic diagnostic marker of atherosclerosis) and the associated inflammatory process. If one can keep sterols out of the intimal layer there cannot be atherosclerosis. Sterols, other than cholesterol are termed noncholesterol sterols (sitosterol, campesterol, stigmasterol and many others). Some term these phytosterols but not all are of plant origin.

Since lipids (sterols, phospholipids and triacylglycerols or triglycerides) are oils (hydrophobic) they are transported in plasma in protein wrapped vehicles called lipoproteins. The surface proteins which convey solubility and structure to lipoproteins are called apoproteins or apolipoproteins. Thus, the only way sterols get into the artery wall is as passengers in the lipoprotein particles transporting or trafficking them. The particles are driven into the arterial intima by concentration gradients. Quantitating atherogenic lipoproteins is the best determinant of CV risk and thus particle concentrations are not only the best way to predict risk, but are also the best goals of therapy. Historically, for a variety of logistic reasons, few clinicians have performed lipoprotein testing. But this is changing and in April 2008, the ADA and ACC issued a consensus statement on LipoProtein Management mandating particle quantification in patients with moderate, high or very high risk; if you are basing CV risk and goals of therapy using lipid profiles, you are simply using lipid concentrations as surrogates or proxies of lipoprotein concentrations. You are guessing! Previously we have assumed that if LDL-C is elevated LDL-P (LDL particle concentration) is also elevated. Unfortunately, in an insulin resistant world, where small LDL particles predominate, that is not always true and very often the least useful surrogate of LDL-P is LDL-C! In these patients there is a major disconnect between LDL cholesterol and LDL particle concentrations. Lipid profiles report lipid concentrations: how much cholesterol is within all of the LDL, HDL or VLDL particles that exist in a deciliter of plasma. Never confuse LDL-C or HDL-C with LDL-OP or HDL-P.

Before we get to understanding the lipid profile, a few lipoprotein basics. Lipoproteins can be separated in the lab using several techniques such as:

1) Ultracentrifugation: (VAP)
2) Surface apolipoprotein content (apoB, apoA-I and others) using antibodies
   Beta-lipoproteins: apoB is on chylomicrons, VLDLs, IDLs, LDLs, remnants, Lp(a)
   Alpha-lipoproteins: apoA-I is on HDLs
3) Electrophoretic mobility: (beta, alpha) (Berkeley)
4) Surface charge (used predominantly for HDL: prebeta 1 &2) and alpha (1,2,3)
5) NMR or Nuclear magnetic resonance spectroscopy: (VLDLs, LDLs, IDLs and HDLs)

Depending on the technique used, the apoB (historically called beta) and apoA-I (historically called alpha) particles can be grouped into subparticles based on size or the presence of other apolipoproteins. The terminology can be complex and confusing, as there is no standard.
To keep it simple, the apoB-containing lipoproteins are the potentially atherogenic particles and apoA-I particles are the presumed non-atherogenic particles (HDLs). ApoB (a collective measurement of how many chylomicrons, VLDLs, IDLs, LDLs and Lp(a) particles are in plasma, measurement of apoB has long been recognized as a far superior predictor of CHD events than are any lipid concentrations including LDL-C. In several major studies elevations of the apoB/ApoA-I ratio has proved to be the best marker of CHD risk: such patients have too many atherogenic and too few non-atherogenic particles.

If an apoB particle enters the vessel wall, the apoB moiety attaches (fixes) to arterial wall proteoglycans and the surface phospholipids are subject to modification by reactive oxygen species, and the proteins by glycation from glucose, etc. The oxidized phospholipids are subject to further lipolysis by an enzyme called lipoprotein associated phospholipase A2 (Lp-PLA_2), the hydrolytic products of which (oxidized fatty acids and lysophosphatidyl choline) create endothelial dysfunction and recruitment of macrophages. The modified or oxidized particles are then

internalized by macrophage scavenger receptors and CD36, 40, etc. The factors that determine whether a lipoprotein will invade the intimal layer are 1) concentration, 2) size < 70 nm and endothelial integrity. LDL particles have a diameter in the range of 18-23 nm. Of these measurements (particle concentration and particle size), only particle concentration is statistically significantly related to atherosclerosis and clinical events. Particle size is important, but by itself has no statistical independency in any study (where both LDL-P and LDL-size were both measured) to predict events. Concentration of lipoproteins is referred to as quantity and lipoprotein size as quality. No one should be making diagnoses or risk assessment or judging therapy by looking only at lipoprotein sizes! In reality if you have concentration data, particle size is not that important. Put another way without concentration data, particle size cannot help you.

Real world clinicians have two ways of quantitating lipoproteins: 1) apoB and apoA-I or 2) the NMR LipoProfile (www.lipoprofile.com) offered by LipoScience which provides VLDL-P, IDL-P, LDL-P and HDL-P. Since there is one apoB protein on every beta-lipoprotein, apoB is a collective measurement all of the apoB particles in a deciliter (dL or 100 cc) of plasma. Because of their long half life, 85-90% of apoB represents LDL particles. ApoA-I is an estimate of HDL particle concentration. However, each HDL particle usually has 2 to 4 apoA-I proteins, so the relationship between apoA-I and HDL-P is not as exact as apoB is with beta-lipoprotein concentrations. The NMR LipoProfile reports individual concentrations of each of the apoB (VLDL, IDL and LDL) and apoA-I (HDL) subparticles in umol/L or nmol/L. The NMR LipoProfile does not report apoB or apoA-I levels, as there is no need for them with the NMR reporting individual particle concentrations. Note that the NMR HDL-P contains only alpha-HDL numbers (small and large or HDL_2 and HDL_3). Free (unlipidated) apoA-I or prebeta (nascent) HDLs (5% of total HDL-P) are not captured by NMR technology.

Most clinicians order lipid profiles which as mentioned report lipid, not lipoprotein concentrations. The educated provider then uses the various lipid concentrations as surrogates or proxies of apolipoprotein B and A-I (particle concentrations) and as proxies of lipoprotein particle sizes. In order to make everyone as expert as possible, here are tips to help you get the most value from a lipid profile.

First recognize that only Total Cholesterol (TC) and HDL-C are actual lab assays. VLDL-C and LDL-C are calculated using the TG level (that is why lipid profiles should be done fasting).

TC = HDL-C + LDL-C + VLDL-C + IDL-C + Chylomicron-C + Lp(a)-C + Remnant-C

Keep in mind: under normal circumstances, when fasting, there are no chylomicrons, remnants (smaller chylomicrons or VLDL particles) or very many if any IDL particles (these are postprandial lipoproteins):

TG is the triacylglycerol concentration within ALL of the TG-trafficking lipoproteins in 100 cc or a dL of plasma
TC is the cholesterol content of every lipoprotein in a deciliter of plasma
LDL-C is the cholesterol content of all the IDL and LDL particles in a dL of plasma
HDL-C is the cholesterol content of all of the HDL particles in a dL of plasma
VLDL-C is the cholesterol content of all of the VLDL particles in a dL of plasma
Remnant-C is the cholesterol content of all of the remnants in a dL of plasma
(Note NCEP recommends using VLDL-C as your surrogate of remnant-C)
Lp(a)-C is the cholesterol content of LDL particles that have apo(a) attached
Lp(a) concentration is the concentration of apo(a) in 100 cc of plasma

LDL-P is the number of LDL particles in a liter of plasma (expressed in nmol/L): this represents all sized LDL particles: large, intermediate or small
HDL-P is the number of HDL particles in a liter of plasma (umol/L)
VLDL-P is the number of VLDL particles in a liter of plasma (nmol/L)

Labs like LipoScience break particle concentrations down even further:

Total LDL-P is the sum of all of the LDLs in a liter of plasma
Small LDL-P is the number of small and intermediate LDL particles per liter of plasma

They also report particle concentrations for all of the IDL, HDL and VLDL subparticles with actual molar concentrations.

Of course when labs report calculated or direct LDL-C to you, it is really LDL-C + IDL-C, as labs cannot separate IDLs from LDLs (without significant expense). Remnants are smaller, cholesterol-enriched VLDLs and chylomicrons. In fasting states chylomicrons and IDLs and remnants should not even exist to any appreciable extent. Most people do not have significant quantities of Lp(a), so in effect in most patients:

TC = HDL-C + LDL-C + VLDL-C

HDL particles are apoA-I particles and LDL and VLDL (plus all of the others mentioned above) are apoB particles, so in reality TC is the sum of the cholesterol in all apoA-I and apoB particles. :

TC = apoA-I-C + apoB-C

It is conventional to refer to the cholesterol in the apoB particles as Non HDL-cholesterol or Non HDL-C. Simply put, apoB-C is the cholesterol that is not in the HDL particles. In fact since apoB particles are the potentially atherogenic particles, Non HDL-C is our best surrogate (in the lipid profile) of apoB or potentially atherogenic particles.

In almost all circumstances the majority of the apoB particles are LDLs. Chylomicrons, VLDLs, IDLs, remnants have half life's of minutes to 4-6 hours, LDL particles have half life's of 2-3 days. Thus under most circumstances, ~90% of an apoB level represents LDL particles.

NCEP uses TC and especially LDL-C as its apoB surrogate and Non HDL-C as its surrogate when hypertriglyceridemia is present (at a level > 200 mg/dL). The reason being is that when TG levels are elevated there is apt to be delayed catabolism (lipolysis) of TG-rich, apoB lipoproteins (VLDL, Chylomicrons, IDLs, remnants). My personal belief, supported in the literature, is that Non HDL-C is a better surrogate of apoB than is LDL-C at any TG > 70 mg/dL (for sure, when TG are > 100-130). By the time a TG is 200 mg/dL, most people are drowning in apoB particles.

In summary the apoB surrogates you should be using are

1) TC (unless HDL-C is very high)
2) LDL-C
3) Non HDL-C
4) TG or VLDL-C

How about apoA-I? This is easy: Your only surrogate of apoA-I in the lipid profile is the HDL-C concentration. However some caution is needed. On the apoB particles, there is one apoB per lipoprotein particle. HDL particles can contain several (typically 1-4) apoA-I proteins. Thus the relationship between apoB and apoB particles is more linear that is apoA-I to HDL-P relationship.

Please note that if TG are perfect (<70-100 mg/dL), for all intents and purposes Non HDL-C and LDL-C are one in the same as a surrogate of apoB and there is no major advantage of Non HDL-C calculations over LDL-C. However, in America, how many patients have TG < 70-100 mg/dL? Yet why not always use Non HDL-C, instead of LDL-C as the apoB surrogate (as long as the TG <500 mg/dL). At TG> 500 mg/dL there is no good surrogate of apoB other than TC: reason ---

when TG are grossly elevated, one may have few but very large VLDL or chylomicrons which of course grossly elevate the TG level, but not the apoB level.

How about guessing or estimating lipoprotein particle sizes? There are many clues in the lipid profile. But first a word about particle sizes.

The major driving force with respect to apoB particles entering the arterial intima is particle number (especially LDL-P) and of course endothelial function. A particle larger than 70 nm will not enter the artery wall (the very large VLDL subparticles and Chylomicrons). Smaller VLDLs and chylomicrons (remnants), IDLs and of course LDLs easily enter the intima if present in increased concentrations. Since LDLs are the most numerous apoB particle present in plasma (due to their half life), it is obvious that LDL particles (small and large) are in most patients, the most atherogenic particles that exist.

With respect to HDL particles, size does not really matter. HDL particles of any size are so small that they can easily enter an artery wall. Of course, HDL particles are in a constant state of dynamic flux (remodeling). Little particles lipidate (become large) and then delipidate (become small). We really have no way of determining HDL functionality (ability to perform lipidation or delipidation, peripheral cholesterol transport or numerous other non-lipid antiatherogenic functions). **Neither HDL-C, apoA-I or HDL-P or HDL size seem to be related to HDL particle functionality.** One needs HDL particles of all sizes and they must be capable of rapidly changing.

Let's look at the atherogenic apoB particles and see what in the lipid profile indicates LDL particles are likely small:

Small LDL particles:

1) Low HDL-C
2) TG > 130-150
3) TC/HDL-C ratio > 4.0
4) TG/HDL-C ratio > 3.8 in women or 4.0 in men
5) Unremarkable LDL-C, but elevated non HDL-C

Remnant lipoproteins (smaller cholesterol-laden VLDL and chylomicrons)

1) TG > 150- 200
2) Elevated VLDL-C > 30
3) Unremarkable LDL-C with an elevated Non HDL-C
4) Low HDL-C in insulin resistant patients
5) Elevated TC/HDL-C ratio and TG elevation (>150)

Large VLDL particles which convey risk through rheologic, multiple coagulation and inflammatory associations and their role in creating atherogenic remnants, small LDL and small HDL particles (which are then excreted in the urine, causing a reduced concentration of HDL particles)

1) TG > 150-200 and/or VLDL-C > 30
2) Postprandial hypertriglyceridemia (> 170-200)
3) Decreased HDL-C
4) Elevated TC/HDL-C ratio and TG elevation (>150)

If you were paying strict attention you have noticed that low HDL-C is almost always associated with abnormal apoB particles (especially if insulin resistance is present): small LDL, remnant particles and large VLDL. Indeed, low HDL-C may simply be an epi-phenomenon alerting you to all of those atherogenic apoB particles. This is a crucial point and it is why treatment in low HDL-C states should always be directed first at apoB (LDL-C, VLDL-C or non HDL-C), rather than

apoA-I or HDL-C. In summary, especially in insulin resistant patients, with unusual exceptions low HDL-C means elevated apoB.

SUMMARY: To read a lipid profile properly and effectively:

1) Look at the TG: If > 500 treatment is needed and the TG takes precedence over all other lipid concentrations. If TG are less than 500, go to step (2)
2) Look at the LDL-C, because if it is above 190 mg/dL, drug therapy is indicated no matter what else you find. At lesser levels it depends on the risk of the patient whether drug treatment is indicated.
3) Look at the HDL-C: concern if < 40 male or 50 woman
4) Calculate the TC/HDL-C ratio: Concern if > 4.0
Some advocate LDL-C/HDL-C ratio, but I do not since LDL-C is a calculated value
5) Calculate the non HDL-C (TC minus HDL-C). NCEP says to do this only if TG are > 200, but in reality this calculation is always equal to or better than LDL-C (less valuable if TG >500)
6) Calculate the TG/HDL-C ratio
7) If apoB/apoA-I ratio is so predictive (and it is): you can estimate it by looking at

TC/HDL-C or Non HDL-C/HDL-C

All should know that the NCEP goal for Non HDL-C is 30 mg/dL higher than whatever the LDL-C goal is for a given patient. Thus if your LDL-C goal is 70 (very high risk patient), the Non HDL-C goal would be 70 + 30 or 100 mg/dL. In a primary prevention setting where NCEP calls for an LDL-C of 130, the non HDL-C goal is 160. I agree with the AHA Women's Guidelines: all adults should have a non HDL-C < 130 mg/dL.

Interesting note: The Canadian Lipid Guidelines call for normalization of both LDL-C and then the TC/HDL-C ratio (make it < 4.0).

Example: If you ignore a very high risk person with an LDL-C of <70 but who has a TC/HDL-C ratio > 4.0 you have not achieved goals. Obviously there are still apoB particles present! Additional therapy is warranted.

NCEP uses the TC/HDL-C as a powerful risk predictor, but as a goal of therapy wants us to use LDL-C and then Non HDL-C.

Example: If you ignore a very high risk person with an LDL-C of <70 but who has a Non HDL-C > 100 mg/dL and the TG are still elevated (>200) you have not achieved goals. Additional therapy is warranted.

In closing, the lipid profile provides a significant amount of information on what lipoproteins might be present or absent. Tragically far too many clinicians are too focused on LDL-C and do not use the rest of the profile. That shortcoming is a big reason why patients are so under treated in the US. If you truly understand all I have written you will also rapidly appreciate how necessary combination lipid therapy is. Keep in mind that NCEP calls for combo therapy, when monotherapy fails to achieve either LDL-C or Non HDL-C goal.