**HDL Terminology:**

HDL literally means High Density Lipoprotein and nothing else. HDL is not a biologic marker or concentration of anything, it is a noun used to identify one class of lipoproteins. HDL particles have several functions related to trafficking cholesterol and proteins. If HDL particles perform these biologic tasks they are termed functional. If they do not they are dysfunctional. With respect to atherosclerosis, if an HDL does not perform antiatherogenic functions, it is called a “proatherogenic” HDL. Laboratory analysis (that are readily available to clinicians) of HDLs include HDL-C, HDL-P, apoA-I, HDL sizes. Thus one does not say a patient has an HDL of 42 – but rather to be accurate one should state the patient has an HDL-C or 42.

1) HDL particles exist as a group of heterogeneous, apoA-I enwrapped, rapidly and constantly remodeling particles that are in a constant state of flux. Most humans have far more HDL particles than they do betalipoproteins (LDL and VLDL and IDL): HDLs are measured in micromolar concentrations and apoB particles in nanomolar concentrations. Yet the betalipoproteins carry the majority of cholesterol in the plasma: Even though there are far fewer apoB particles (than A-I particles), the apoB species are so much larger, they carry the majority (>70%) of the cholesterol in plasma.

2) HDL particles can be separated by several lab techniques: ultracentrifugation, nuclear magnetic resonance spectroscopy, and electrophoresis just to name a few. In clinical practice we do not measure the flux or remodeling of the particles. The two major categories of HDL particles separated by electrophoresis are pre-beta and alpha. To few HDL particles associated with very low HDL-C, is termed hypoalphalipoproteinemia and very high numbers of HDLs (very elevated HDL-C) is called hyperalphalipoproteinemia.

A) ApoA-I is the HDL precursor protein manufactured by and secreted by hepatocytes or enterocytes. ApoA-I is an unlipidated cholesterol seeking protein, with amphipathic properties. Phospholipidated ApoA-I is sometimes referred to as prebeta-1 HDL. ApoA-I attaches to cellular cholesterol efflux pumps (ATP Binding Cassette Transporters A1 or ABCA1) and is lipidated with free cholesterol and phospholipids: it is now referred to as prebeta-1 or ultimately prebeta-2 HDL. This is the critical step in the formation of HDL particles. Without this lipidation step there will be no development of mature or HDL. One can easily understand the importance of the ABCA1 transporter (the major HDL lipidation pump). No laboratory test available to clinicians in practice can measure (quantitate) prebeta HDL (too small and too transient). Prebeta HDL is not reported by NMR, Berkeley or VAP. There are two apoA-I molecules on each prebeta HDL and two or more apoA-I molecules are on most other HDLs, so even apoA-I cannot help you in determining prebeta HDL particle number. The two cells which typically have large cholesterol loads and thus upregulate ABCA1 are hepatocytes and enterocytes. Those two cells serve as the origins of the vast majority of the cholesterol carried in HDL.

a) ApoA-I is also found on large TG-rich lipoproteins like chylomicrons. As these particles undergo lipolysis (hydrolysis of TG), they loose surface phospholipids and apolipoproteins like A-I. These A-I molecules are gathered up by Phospholipid Transfer Protein (PLTP) where they can be reassembled into prebeta-2 HDL.

B) ApoA-I carries an enzyme called lecithin acyl cholesterol acyl transferase (LCAT) which rapidly esterifies (attaches a fatty acid to the OH group at the # 3 position of the cholesterol molecule, forming cholesteryl ester abbreviated as CE). The majority of cholesterol is transported as CE in lipoproteins including HDL. As the cholesterol esterifies (becomes more hydrophobic) it seeks the innermost part of the forming HDL particles which cause the particle to go from discoidal to spherical. As the HDL particle matures it is no longer called prebeta, but rather alpha 1, 2 or 3 (with 1 being the largest and 3 the smallest). Alpha HDL is what is commonly measured in clinical labs: with electrophoresis the smaller alpha-3 HDL are called HDL3, and the larger alpha-1 HDL are HDL2. With NMR H1 & 2 are the smaller and H4 & H5 the larger alpha HDLs.
C) If you want to be more confused and look at apolipoproteins, prebeta HDL is purely an apoA-I particle (one or 2 apoA-I proteins). Alpha-3 HDL (small HDL₃) most often contains apoA-I and apoA-II. Large alpha-1 HDL (HDL₂) most often contain 3-4 molecules of apoA-I. There is significant overlap with apoA-I and A-II and no one in the clinical world uses apoA-I & A-II to classify HDL particles.
Understanding HDL Complexities
Thomas Dayspring MD, FACP

3) HDL-C is the cholesterol content within all of the alpha HDL particles that exist in a deciliter (100 cc) of plasma. That’s it: nothing more can be garnered from an HDL-C level. It makes no definitive statement about HDL-P, apoA-I or HDL size. HDL-C also has no major relationship with how the HDL particles are being dynamically remodeled or HDL functionality. It refers to how much cholesterol the particles present in 100 cc of plasma are transporting. HDL-C is a function of both the number and the size of the HDL particles. HDL-C level is a function of the number of HDLs, their size and their cholesterol content.

4) HDL-P, determined by nuclear magnetic resonance spectroscopy (NMR LipoProfile) refers to the number of HDL particles that exist in a liter of plasma.

5) ApoA-I is a measure of how much apoA-I exists in a deciliter of plasma, including prebeta species. In general, it is a gross estimation of HDL-P, because there is no linear relationship to total HDL-P as each HDL particle can have more than two or more apoA-I proteins on it.

6) The function of HDL particles is too complex for this paper. Basically the particles carry cholesterol to and from various tissues in “forward and reverse” directions (they are in effect vehicles trafficking cholesterol, triglycerides, phospholipids and multiple proteins). However HDLs perform dozens of other functions including several immunological activities (including fighting viruses and parasites). They carry a multitude of surface proteins that have anti-inflammatory, antioxidant, anticoagulant, and profibrinolytic functions. They promote endothelial function through nitric oxide upregulation and downregulate several inflammatory proteins. HDLs, in a process termed macrophage RCT, also can help delipidate macrophages of atherogenic sterols (termed macrophage reverse cholesterol transport). These, potentially lifesaving antiatherogenic activities have no effect on HDL-C or HDL-P or apoA-I levels and thus HDL functionality cannot be estimated or monitored by clinicians.

7) After alpha HDLs mature (remember the majority of their cholesterol was obtained from hepatic and enterocyte ABCA1 transporters), they deliver the cholesterol to steroidogenic tissues (mostly adrenal) and adipocytes (for cell membrane use). After delipidation (by scavenger receptor B1 or SR-B1), the now smaller alpha or pre-beta HDLs return to tissue ABCA1 for relipidation. Large HDLs that remain after the adrenal has satisfied its need for cholesterol have several options of what to do with their cholesterol content and the remaining transport options are collectively termed Reverse Cholesterol Transport (RCT) as opposed to the forward cholesterol transport option I have already discussed.
A) Large alpha-1 or -2 HDLs (HDL2) can proceed directed to the liver for:

   a) Particle Delipidation by hepatic or enterocyte Scavenger Receptors B1 (SR-B1): As the mature HDL looses cholesteryl ester it reforms into small alpha-3 (HDL3) or pre-beta-2 HDLs which are available for relipidation or for renal excretion. SR-B1 is regulated by PPAR alpha.

   b) Particle Endocytosis by what are termed holoparticle or catabolism receptors (apoA-I beta chain synthase): once endocytosis occurs the entire HDL is catabolized: it is no longer available.

   c) Additional Particle Endocytosis: If the HDL is apoE enriched, LDL receptors (using apoE as a ligand) can endocytose the entire HDL particle.

Avenues a, b and c described above are collectively referred to as **Direct RCT**.
C) Under conditions of elevated triglycerides (>100 mg/dL) a different RCT option occurs. The apoB particles (VLDL and LDL) will be TG-rich. A lipid transfer protein called cholesteryl ester transfer protein (CETP) traffics with HDL particles; it exchanges one molecule of CE for one molecule of TG. This neutral transfer of lipids between HDL and apoB particles results in cholesterol-poor HDLs and more cholesterol enriched apoB particles. The cholesteryl ester that was in the HDLs is now in the apoB particles. Liver LDL receptors (LDLr) attach to and endocytose the LDL (or VLDL) particles (which are carrying the CE acquired from HDL). This RCT pathway where HDLs transfer CE to LDLs for return to the liver is termed **Indirect RCT**.

In most patients there are varying degrees of both direct and indirect RCT at play. Clearly as TG rise, CETP activity increases and indirect RCT increases and direct diminishes. Of course a serum HDL-C would tell a clinician nothing about this complex dynamic HDL remodeling process (lipidation and delipidation) that is ongoing.
Understanding HDL Complexities
Thomas Dayspring MD, FACP

**Indirect Reverse Cholesterol Transport**

**Cholesteryl Ester Transfer Protein**

CETP = Cholesteryl ester transfer protein
Produced in the liver and carried on HDL particles

HDL particles are now more cholesterol-poor and TG-rich

One molecule of CE exchanged for one of TG

The apoB particles are now more cholesterol-enriched

Barter, Philip et al. Atherosclerosis 2003;168:195-211

**Indirect RCT at the Hepatocyte**

Hepatocyte
LDL receptors
Hepatic Sinusoid

Cholesteryl ester

ABCG4 ABCG5/8 ABCB11

LDL receptor endocytosis of LDL particles carrying cholesteryl ester

Bile Duct
Barter, Philip et al. Atherosclerosis 2003;168:195-211
SUMMARY OF HDL CHOLESTEROL TRANSPORT:

Hepatocytes and enterocytes synthesize and secrete unlipidated apoA-I (prebeta HDL-1). Lipidation can occur in any cell with extra free cholesterol (in reality the majority of lipidation occurs in enterocytes and hepatocytes) via ABCA1 transporters resulting in prebeta-2 HDL. Polymorphisms of ABCA1 greatly affect HDL-C and are major determinants of a person’s HDL-C level. The enzyme LCAT esterifies the cholesterol causing formation of larger, mature alpha HDLs (3,2,1). The alpha HDLs perform forward cholesterol transport. Any remaining mature alpha HDLs then participate in direct (several avenues) and indirect RCT. HDLs also perform macrophage RCT, a specialized avenue of the potential HDL traffic routes. The main determinants of HDL-C levels are the ABCA1 transporters, LCAT, CETP and SR-B1. Since different drugs affect all of these proteins in very different ways, drugs will vary widely in their ability to affect HDL-C. See my paper on Drugs and HDL modeling)

UNDERSTANDING DISORDERS OF HDL PARTICLES (briefly)

Low HDL-C or Hypoalphalipoproteinemia:

1) Decreased production of apoA-I Rare disorder
2) Lack of ABCA1 transporters
   a) Homozygous: Tangier's Disease HDL-C is extremely low (<10)
   b) Heterozygous: HDL-C 20-30 (often not associated with CHD)
3) LCAT Deficiency (homo or heterozygous): the latter is termed Fish Eye Disease
   HDL-C very low even zero Lipoprotein X often found in plasma
4) ApoA-I Milano (and other variants): A mutant type of apolipoprotein A-I that is extremely functional. Associated with very low HDL-C levels (10-20) and longevity
4) Drug induced CETP elevation: Probucol, succinylbuccol
5) TG-induced: Under conditions of insulin resistance and elevated TG, CETP results in TG-rich, CE-poor HDL particles that are subject to lipolysis (hydrolysis of TG) resulting in very small HDLs many of which are excreted by the kidneys.
5) Conditions of overexpression of endothelial lipase: (inflammation and infection)

High HDL-C or hyperalphalipoproteinemia (characterized by large HDL particles)

1) Hepatic lipase deficiency’ (larger HDLs which cannot be remodeled by HL). TG will also be elevated.
2) CETP deficiency: CE remains in the HDLs creating very large HDLs. Some of these may be dysfunctional and associated with CHD.
3) Unclassified

In data from IDEAL and EPIC-Norfolk studies there were patients with CHD and very high HDL-C. High HDL-C can be explained by having very large HDL particles without a high HDL-P, or having lots of smaller HDL particles. The CV risk was seen in those when high HDL-C was not accompanied by high HDL-P or apoA-I. Thus, like LDLs the most important parameter to watch may be HDL-P and not HDL-C.