HIGH DENSITY LIPOPROTEINS Classification

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High density lipoprotein is the smallest of the lipoproteins that transport lipids in the plasma. We are beginning to realize that HDL particles perform numerous functions including lipid transport (to and from the periphery) as well as innate immune functions. The particles are in a constant state of synthesis, remodeling (lipidation and delipidation) and excretion and they also traffic both phospholipids as well as numerous proteins on their surface.

The core of the HDL particle is cholesteryl ester and TG. The surface is phospholipids and some free cholesterol and proteins (apoproteins). The main surface apoprotein is apoA-I , but there are also several other types: A-I, A-II, A-IV, A-V. Apolipoprotein A-I is made in the liver and proximal intestine and the other apoAs in the liver. HDLs at some point in their lifespan also temporarily transport many other apolipoproteins (D, C, E, J, L and M) as well as other lipid transfer proteins (LCAT, CETP, PLTP). Numerous other proteins, capable of many other actions, are also trafficked by HDLs.

HDL particles have several classifications depending on the methodology used, some of which can be very confusing to master:

1) By apolipoprotein content

LPA-I is an HDL with only ApoAI on its surface
LP A-I:A-II is an HDL with apoA-I and A-II on its surface

2) Size:

By size using gel fractionation

HDL_3 (small) subfractions a,b,c (a is larger than c)
HDL_2 (large) subfractions a,b (b is larger than a)

By using NMR (nuclear magnetic resonance spectroscopy)

H1, H2, H3, H4, H5 (smallest to largest)

3) By surface charge

There are three ApoA-I subpopulations separated by charge on agarose gel based on whether the motilities are slower, the same as or faster migrating than albumin: pre-beta, alpha and pre-alpha. The smallest are the pre-beta and these are lipid poor LPA-I or discoidal particles of one or two molecules of apoAI complexed with phospholipids and perhaps a tiny amount of free cholesterol (these were previously referred to as nascent HDL). The alpha particles are spherical and larger particles and account for the majority of HDL particles in the plasma. They can be HDL_3 or HDL_2 as well as LPA-I and LPA-I:A-II. ApoA-II is present only in α_2 and α_3 subpopulations. There is a strong correlation with total HDL-C and α_1 subpopulation as it is the largest HDL particle. Persons with HDL-C < 40 usually have very few, large mature HDL species.

There are 12 apoAI subpopulations on nondenaturing gel electrophoresis:

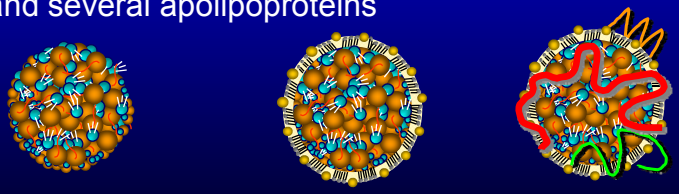
Prebeta 1,2,3, (small)
Pre-alpha 1,2,3
Alpha 1,2,3 (large) (1>2>3 in size)

Note: All of the HDL particles reported by LipoScience (NMR) or Berkeley Heart Lab are pre-alpha or alpha. Prebeta HDLs are not reported by those methodologies.

4) By ultracentrifugation: separated by density which is proportional to the protein/lipid makeup: the more protein, less lipid, the denser the particle.

High Density Lipoproteins

- The smallest lipoproteins (7-12 nm in diameter) as well as the densest (1.063-1.25 g/ml)
- Hydrophobic core of cholesterol esters plus a small amount of TG
  - Surrounded by a surface monolayer of phospholipids, free cholesterol
  - and several apolipoproteins

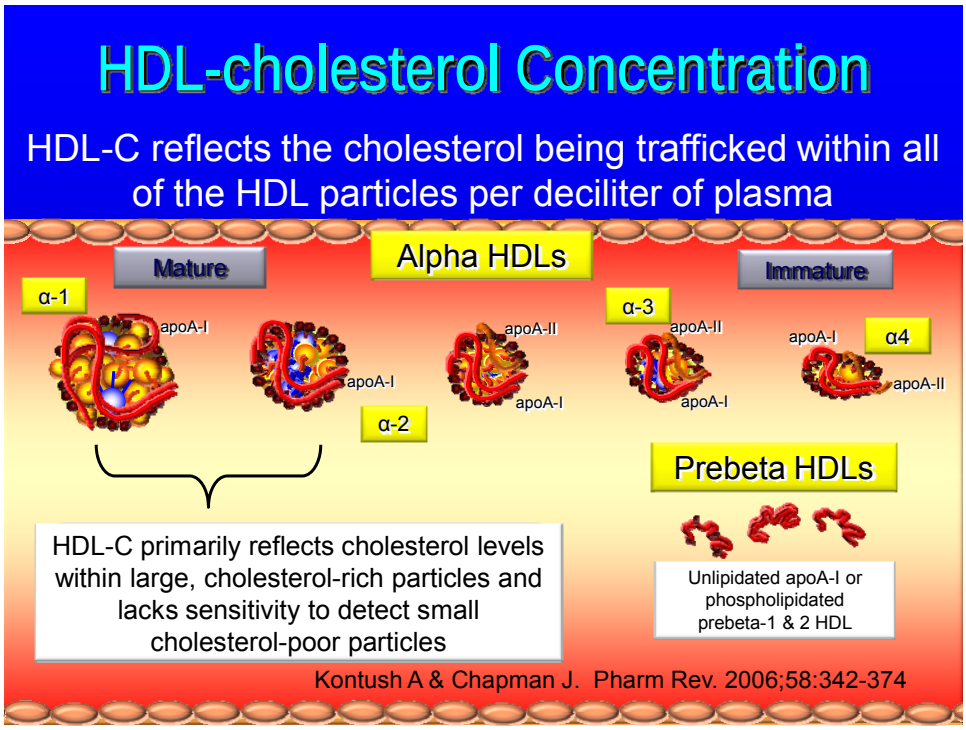


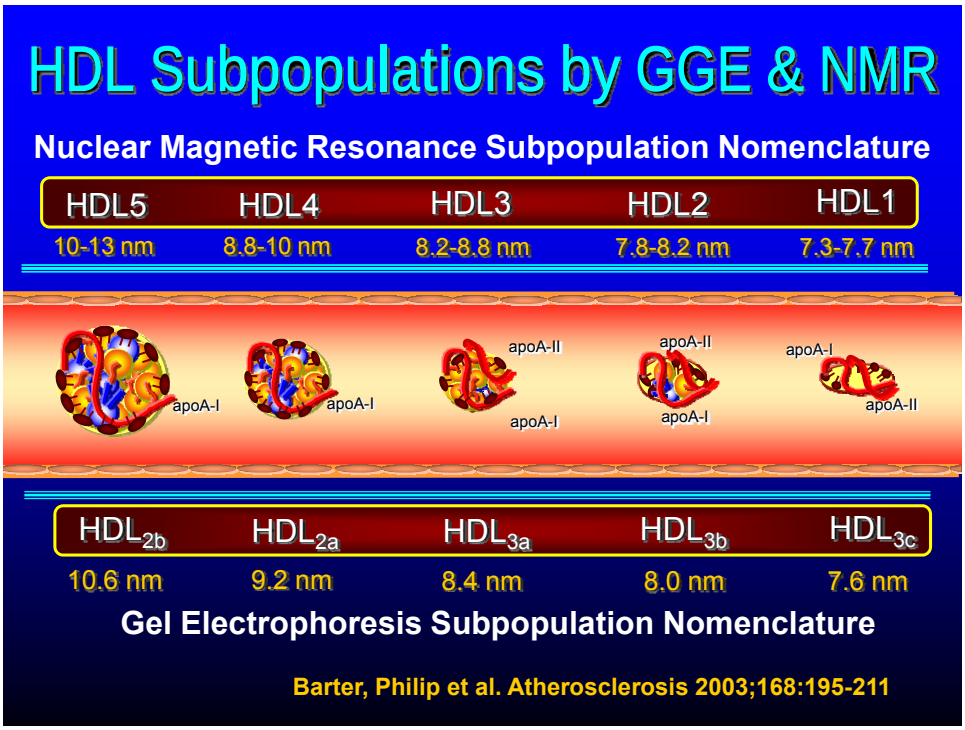
Barter, Philip et al. Atherosclerosis 2003;168:195-211

High Density Lipoproteins
Density by Ultracentrifugation

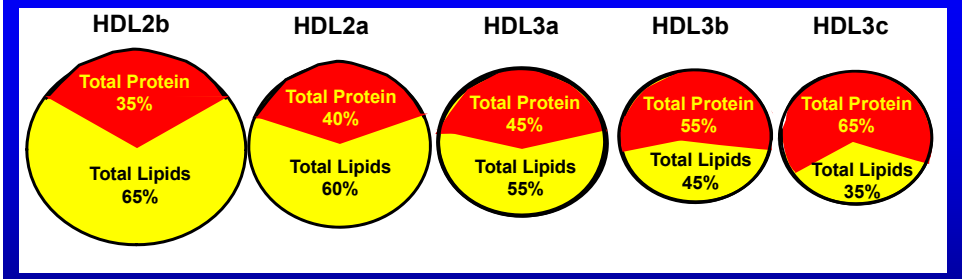
- Two major subfractions
  - HDL_2 (1.063 < density < 1.125 g/ml)
  - HDL_3 (1.125 < density < 1.21 g/ml)
  - Non-denaturing gel electrophoresis separates the HDLs into 5 distinct subpopulations
    - HDL_{2b}, HDL_{2a} HDL_{3a}, HDL_{3b}, HDL_{3c}

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


HDL-cholesterol Concentration
HDL-C reflects the cholesterol being trafficked within all of the HDL particles per deciliter of plasma
Mature
Alpha HDLs
Immature
α-1
α-2
α-3
α4
apoA-I
apoA-II
Prebeta HDLs
HDL-C primarily reflects cholesterol levels within large, cholesterol-rich particles and lacks sensitivity to detect small cholesterol-poor particles
Unlipidated apoA-I or phospholipidated prebeta-1 & 2 HDL
Kontush A & Chapman J. Pharm Rev. 2006;58:342-374


HDL Subpopulations by GGE & NMR
Nuclear Magnetic Resonance Subpopulation Nomenclature
HDL5 HDL4 HDL3 HDL2 HDL1
10-13 nm 8.8-10 nm 8.2-8.8 nm 7.8-8.2 nm 7.3-7.7 nm
apoA-I
apoA-II
HDL2b HDL2a HDL3a HDL3b HDL3c
10.6 nm 9.2 nm 8.4 nm 8.0 nm 7.6 nm
Gel Electrophoresis Subpopulation Nomenclature
Barter, Philip et al. Atherosclerosis 2003;168:195-211

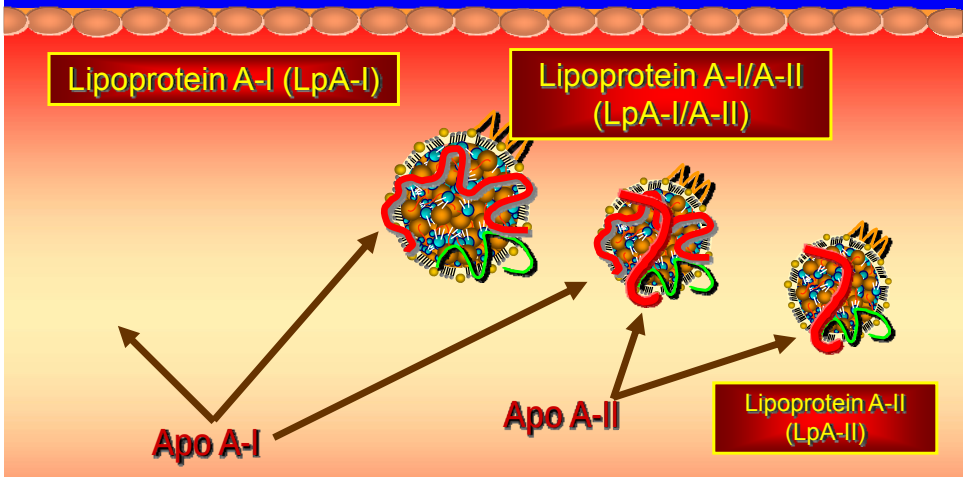
Alpha HDL Buoyancy



Functional plasma HDL are spherical or discoidal particles of high hydrated density (1.063–1.21 g/ml) due to elevated protein content (30% by weight) compared with other lipoproteins

Kontush A & Chapman MJ Pharmacol Rev 58:342–374, 2006

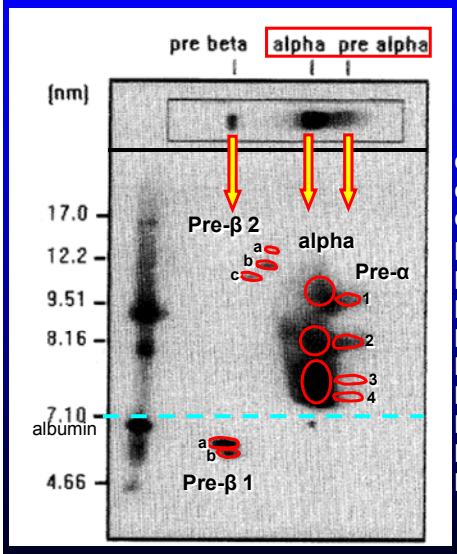
HDL by Apolipoprotein A-I A-II



Most of the LpA-I/A-II are found in the small HDL_3 density range, while LpA-I are prominent components of both HDL_2 and HDL_3

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

HDL Subpopulations by Surface Charge



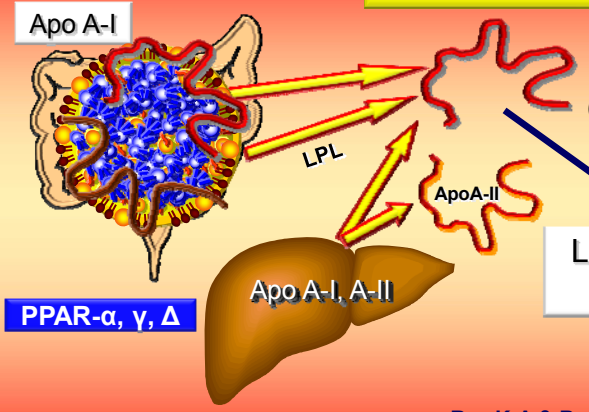
12 ApoA-I HDL Subpopulations

	First Dimension (median R_f)	Second Dimension modal diameter (nm)	% Distribution
α_1	0.99±.03	10.97±.10	18.2%
α_2	0.93±.04	9.20±0.18	29.0%
α_3	1.01±.03	7.62±0.25	20.2%
pre-α_1	1.39±.03	11.05±0.31	6.5%
pre-α_2	1.39±.03	9.42±0.21	7.8%
pre-α_3	1.39±.03	8.40±0.27	3.2%
pre-α_4	1.39±.03	7.67±.0.15	2.5%
pre-β_{1a}	0.38±.04	5.79±0.18	3.5%
pre-β_{1b}	0.42±.09	5.38±0.21	3.1%
pre-β_{2a}	0.76±.02	13.74±0.27	1.8%
pre-β_{2b}	0.72±.02	12.85±0.14	2.2%
pre-β_{2c}	0.64±.02	12.17±0.20	1.6%

Asztalos BF Biochim Biophys Acta 1992;1169:291-300

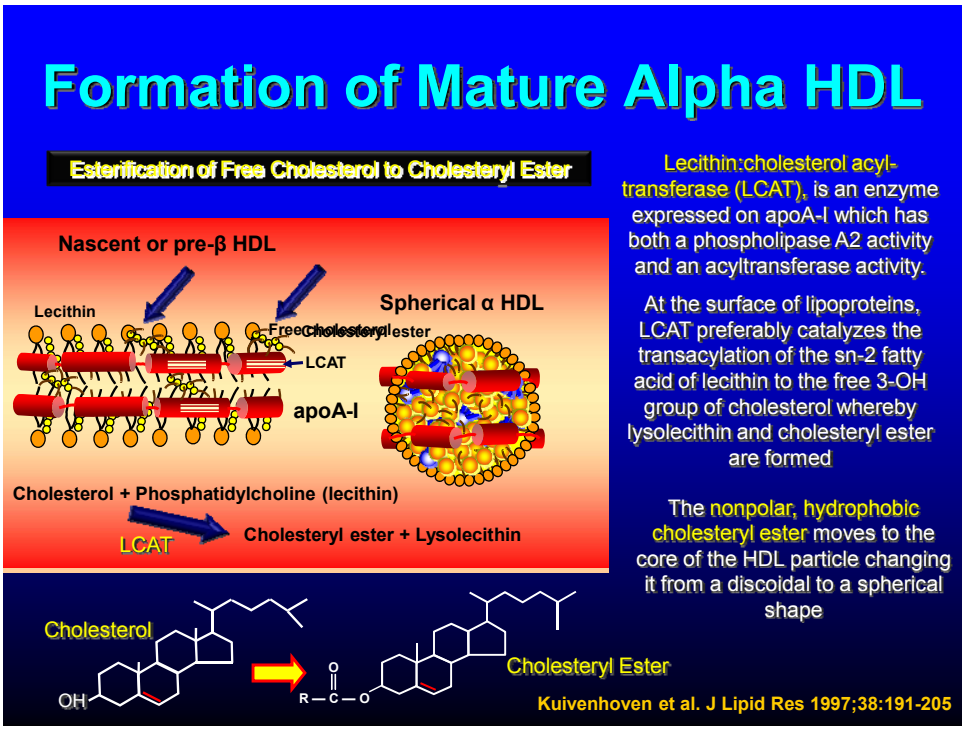
Apolipoprotein A-I Production

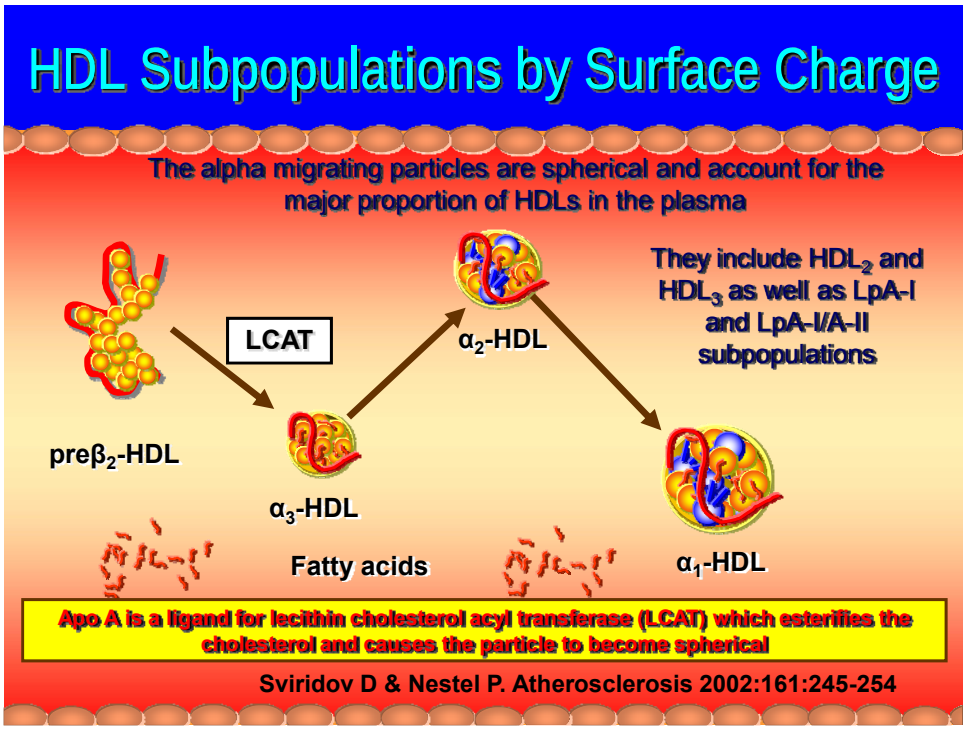
ApoA-I (prebeta$_1$ HDL) is a α-helical protein which serves as a cholesterol acceptor



Lipidation of apoA-I can now occur

Rye K-A & Barter PJ. ATVB 2003;24:1-8
Sviridov D & Nestel P. Atherosclerosis 2002:161:245-254


Formation of Mature Alpha HDL
Esterification of Free Cholesterol to Cholesteryl Ester
Nascent or pre-β HDL
Lecithin
Free Cholesterol
Cholesteryl ester
LCAT
apoA-I
Spherical α HDL
Cholesterol + Phosphatidylcholine (lecithin)
LCAT
Cholesteryl ester + Lysolecithin
Lecithin:cholesterol acyl-transferase (LCAT), is an enzyme expressed on apoA-I which has both a phospholipase A2 activity and an acyltransferase activity.
At the surface of lipoproteins, LCAT preferably catalyzes the transacylation of the sn-2 fatty acid of lecithin to the free 3-OH group of cholesterol whereby lysolecithin and cholesteryl ester are formed
The nonpolar, hydrophobic cholesteryl ester moves to the core of the HDL particle changing it from a discoidal to a spherical shape
Cholesterol
OH
R—C—O
Cholesteryl Ester
Kuivenhoven et al. J Lipid Res 1997;38:191-205


HDL Subpopulations by Surface Charge
The alpha migrating particles are spherical and account for the major proportion of HDLs in the plasma
LCAT
preβ2-HDL
α3-HDL
α2-HDL
α1-HDL
Fatty acids
They include HDL2 and HDL3 as well as LpA-I and LpA-I/A-II subpopulations
Apo A is a ligand for lecithin cholesterol acyl transferase (LCAT) which esterifies the cholesterol and causes the particle to become spherical
Sviridov D & Nestel P. Atherosclerosis 2002:161:245-254