High Density Lipoprotein (HDL) Classification

Thomas Dayspring, MD, FACP

Clinical Assistant Professor of Medicine

University of Medicine and Dentistry of New Jersey

Attending in Medicine: St Joseph's Hospital, Paterson, NJ

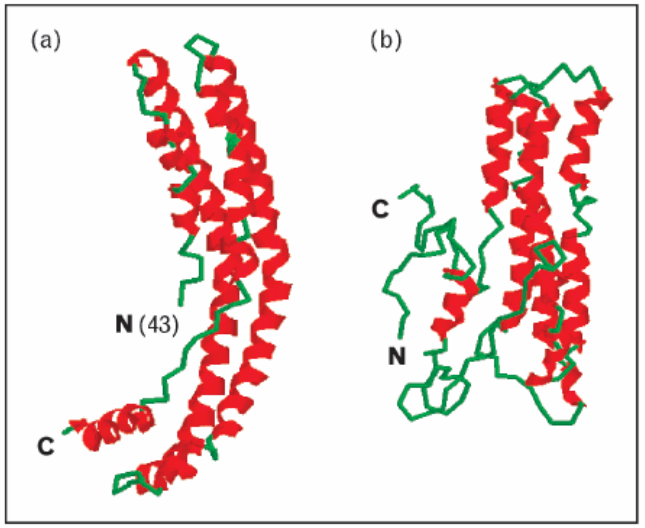
Certified Menopause Clinician: North American Menopause Society

North Jersey Institute of Menopausal Lipidology

Wayne, New Jersey

Apolipoprotein A-I

- ApoA-I is an exchangeable helical, apoprotein that is the major constituent of HDL
- In lipid-free and lipid-bound states, apolipoproteins span multiple conformations, some of which are required for apolipoprotein functions such as:
  - Binding to lipid surfaces
  - Interactions with cellular receptors, lipid transporters, charged ligands
  - Enzyme activation

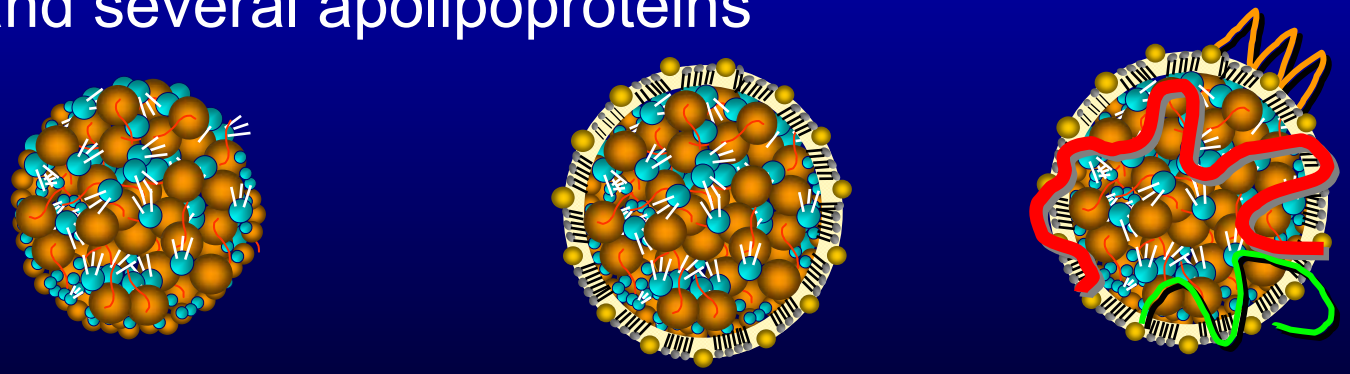


Atomic models of monomeric lipid-free apoA-I

Olga Gursky. Curr Opin Lipidol 2005;16:287–294.
Davidson WF & Silva G. Curr Opin Lipidol 2005;16;295-300.

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 Lipoprotein

- HDL includes a complex family of lipoprotein particles that exist in a constant state of dynamic flux as they interact with other HDL particles and beta-lipoproteins.
- HDL has the highest proportion of protein (>50%) relative to lipid compared to other lipoproteins
- Lipid Composition
  - Phospholipids 50% Cholesteryl Ester 30% Free Cholesterol 10% Triglycerides 10%

Nauck et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

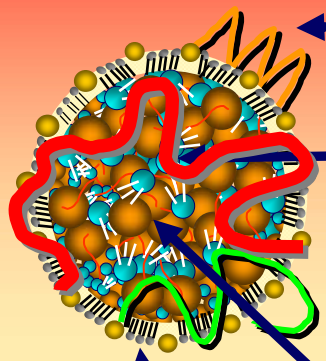
High Density Lipoprotein

- Historically HDLs have been subfractionated into 2 or 3 subclasses based on the density of particles and at least 5 subclasses based on particle size using nondenaturing gel electrophoresis
- Immunoaffinity chromatography has been used to separate HDL by apolipoprotein content into LpA-I and LpA-I/A-II
- Agarose gel electrophoresis is used to subfractionate HDL into lipid-containing spherical particles (α HDL) and lipid-free or lipid-poor apoA-I (preβ HDL) based on electrophoretic mobility

Lee, J et al J Lipid Res 2004;45:716-728

High Density Lipoprotein (HDL)

Apo A-I is a surrogate of HDL particle concentration



Apo A Family
(A-I, A-II, A-IV, A-V)

Apo E

Triglycerides

Apo C
(C-I,C-II,C-III)

Cholesterol ester

HDL-C

Phospholipids

HDLs also serve as a transport vehicle for CETP, LCAT and PLTP

Apo J (clusterin) and D, F and L are also associated with HDL

Apo B / Apo A is an advanced TC / HDL-C or LDL-C / HDL-C ratio

High Density Lipoprotein Classification

- **The HDL is heterogeneous in terms of shape, size, density, composition and surface charge and is classified:**
  - **By density (ultracentrifugation)**
  - **By gradient gel electrophoresis (GGE)**
  - **By NMR-spectroscopy spectral signals**
  - **By surface charge (agarose gel electrophoresis)**
  - **By Apolipoprotein composition**

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}

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High Density Lipoproteins by NMR Nuclear Magnetic Resonance Spectroscopy

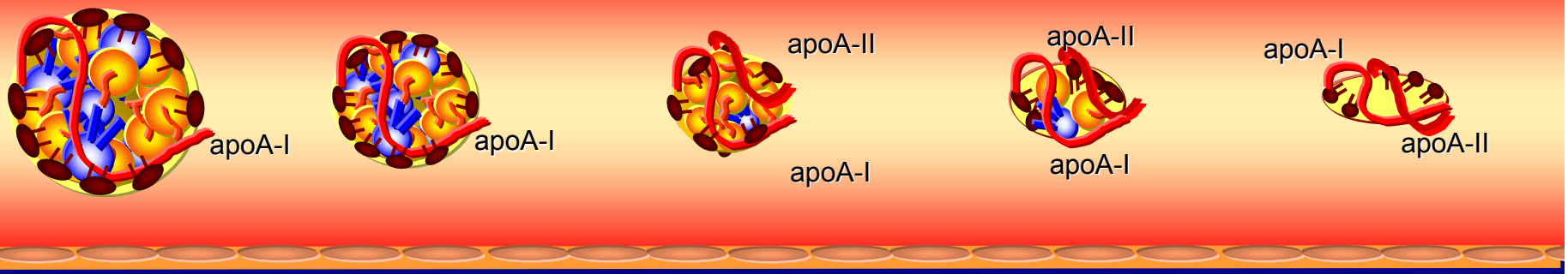
- **Quantification** is based on the detected amplitudes of spectral signals emitted by HDL subclasses of different size
- Each subclass signal emanates from the aggregate number of terminal methyl groups on the lipids within the particle
- This technique identifies 5 subclasses
  - H1 H2 H3 H4 H5

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

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



HDL_{2b}	HDL_{2a}	HDL_{3a}	HDL_{3b}	HDL_{3c}
10.6 nm	9.2 nm	8.4 nm	8.0 nm	7.6 nm

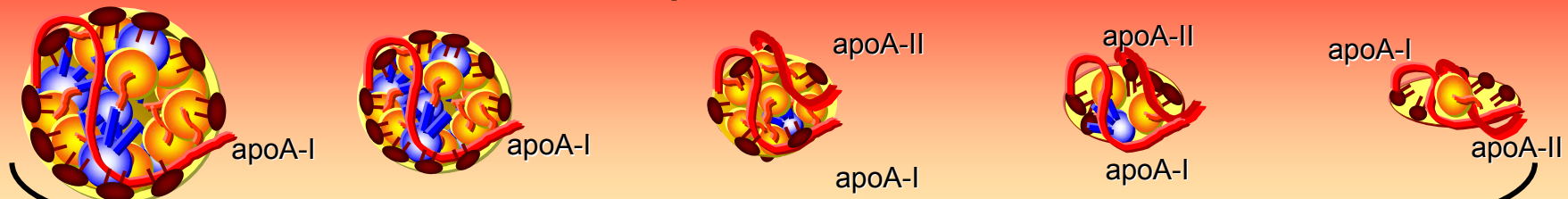
Gel Electrophoresis Subpopulation Nomenclature

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

HDL-cholesterol Concentration

HDL5	HDL4	HDL3	HDL2	HDL1
HDL_{2b}	HDL_{2a}	HDL_{3a}	HDL_{3b}	HDL_{3c}

Alpha HDLs



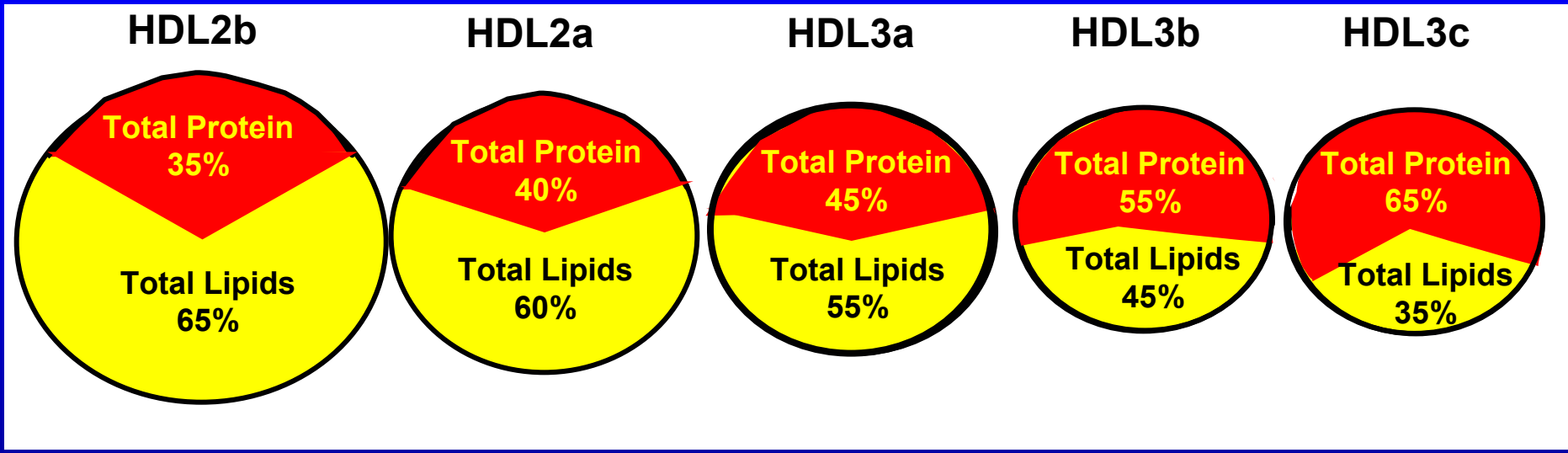
HDL-P reflects the number of α-HDL particles

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

Alpha HDL Buoyancy



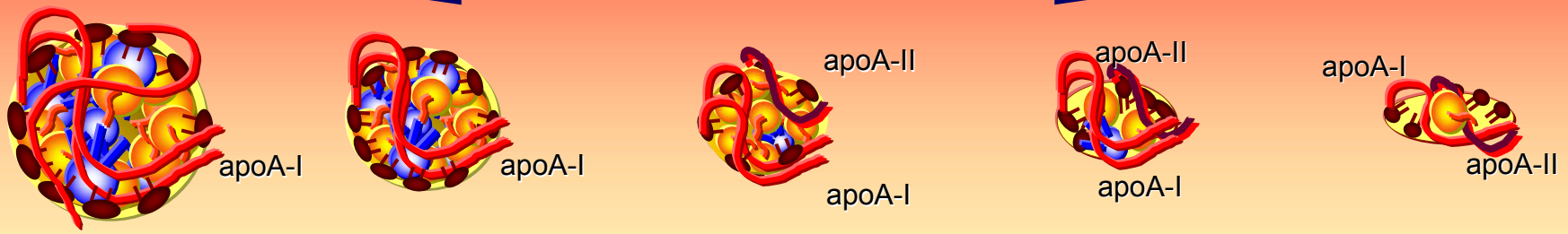
Functional plasma HDL are spherical or discoidal particles of high hydrated density (1.063–1.21 g/ml) due to elevated protein content (30% by weight) compared with other lipoproteins

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

Apolipoprotein A-I

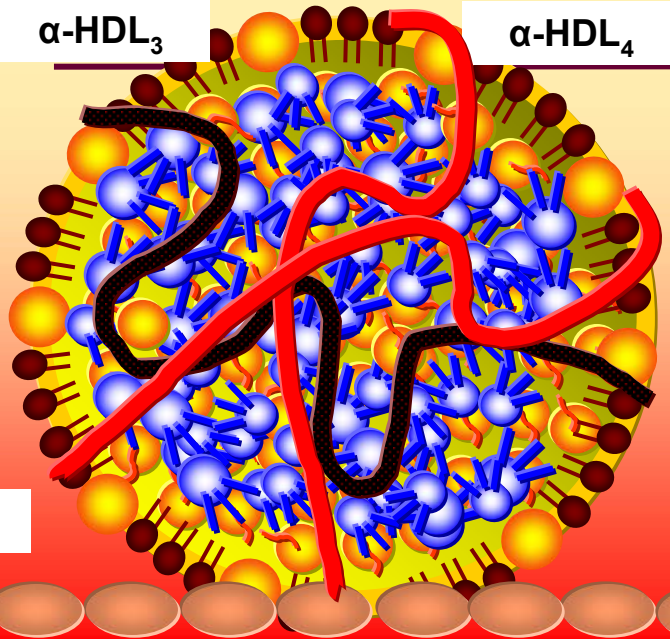
Alpha HDLs

Mature ⟷ Immature



HDL_{2b} or H5	HDL_{2a} or H4	HDL_{3a} or H3	HDL_{3b} or H2	HDL_{3c} or H1
α-HDL_1	α-HDL_2		α-HDL_3	α-HDL_4

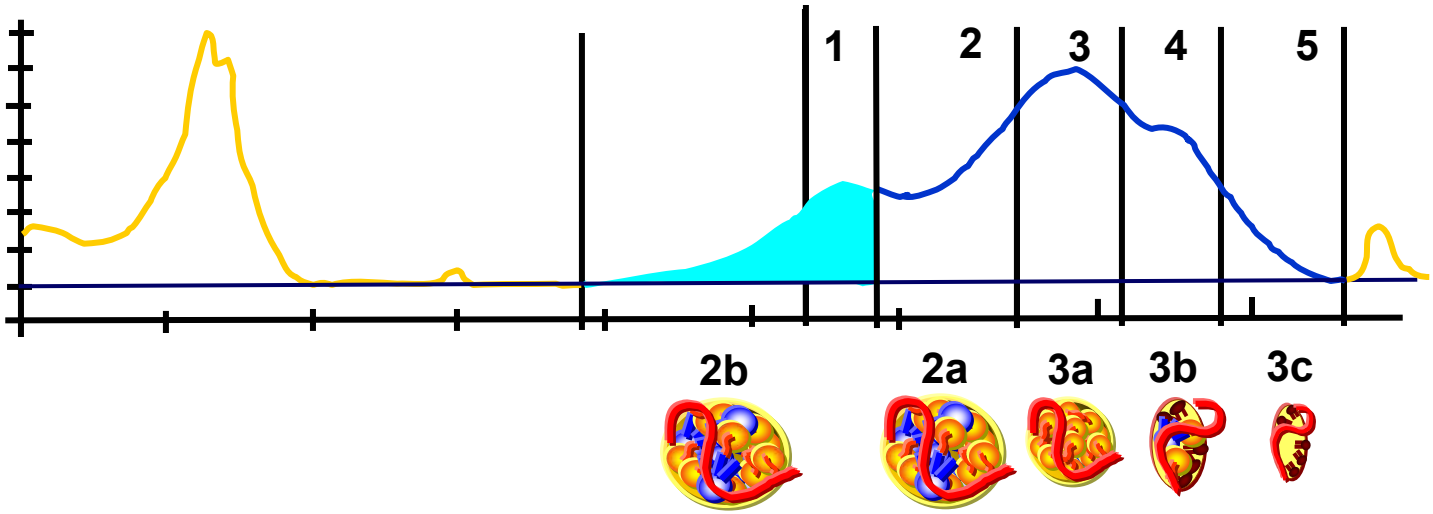
Unlipidated apoA-I or phospholipidated prebeta-1 & 2 HDL



Chylomicron

LDL Sizing by Gradient Gel Electrophoresis

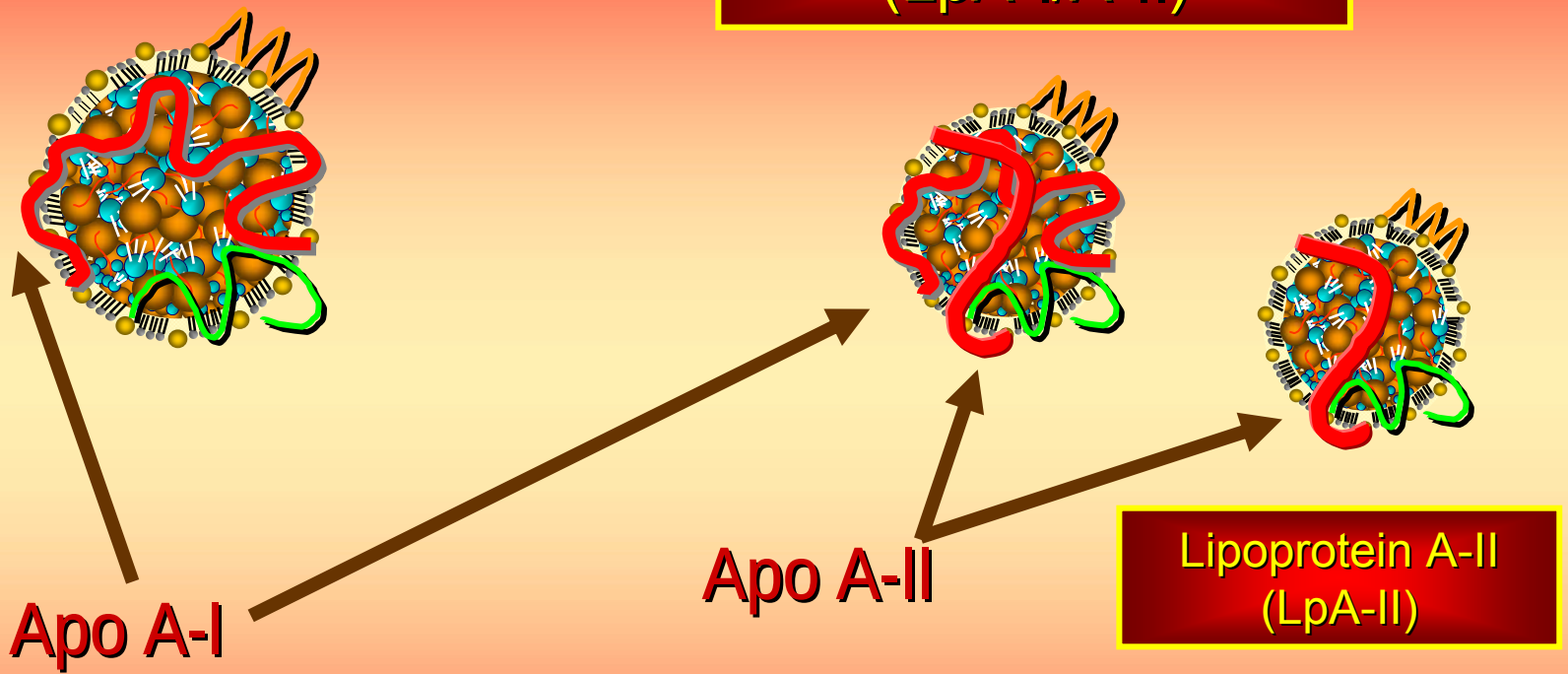
HDL-S_3GGE™



ApoA-I Cholesterol Transport

Segmented HDL Subclass Determination

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

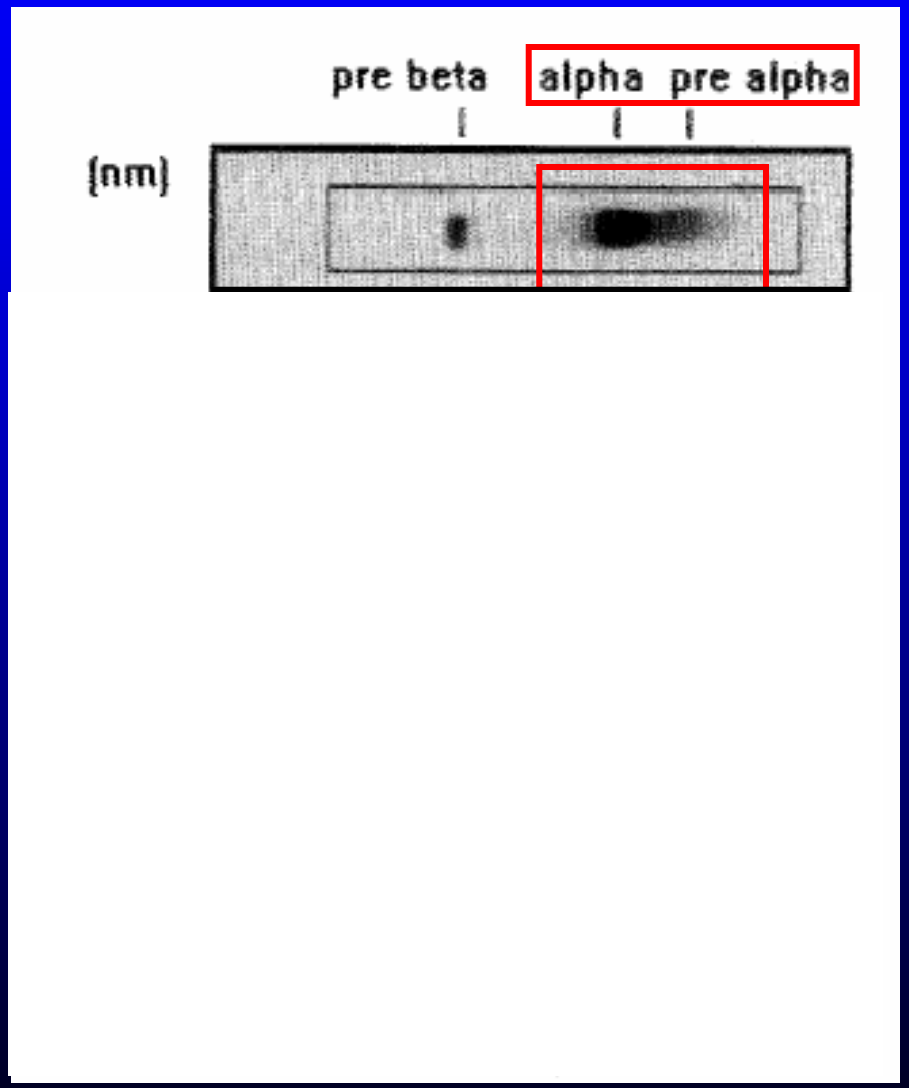
Barter, Philip et al.
Atherosclerosis
2003;168:195-211

HDL Subpopulations by Surface Charge

- In the first dimension **(mobility)**, there are three ApoA-I HDL subpopulations separated by charge on agarose gel (on the basis of electrophoretic mobilities relative to albumin)
  - Alpha: α (R_f = 1) mobility similar to albumin
  - Pre-alpha: Pre-α (R_f > 1) mobility faster than albumin
  - Pre-beta: Pre-β (R_f < 1) mobility slower than albumin
- In the second dimension **(size characterization)**, the particles (12) were differentiated on nondenaturing gel electrophoresis by modal diameters

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

HDL Subpopulations by Surface Charge



First Dimension

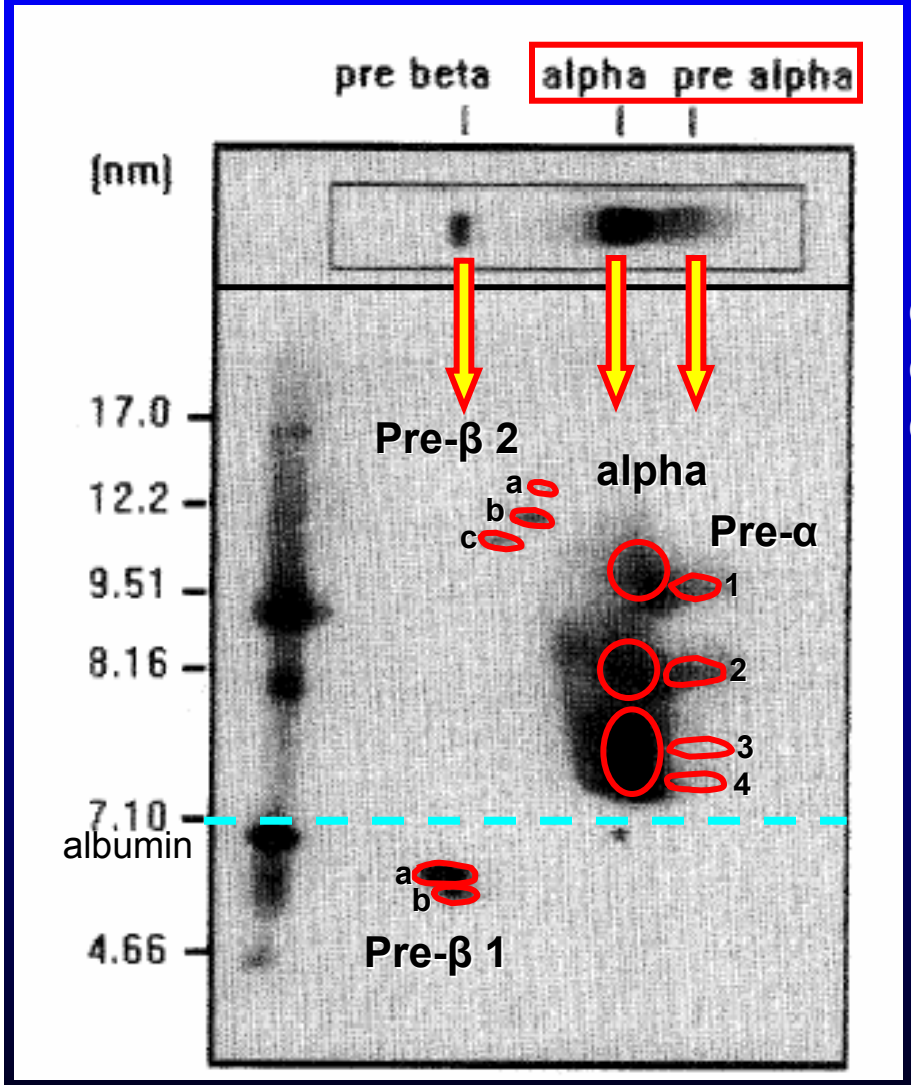
Alpha (α) migrating ApoA-I on agarose gel electrophoresis

Second Dimension

Nondenaturing concave polyacrylamide gel electrophoresis & immunolocalization

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

HDL Subpopulations by Surface Charge

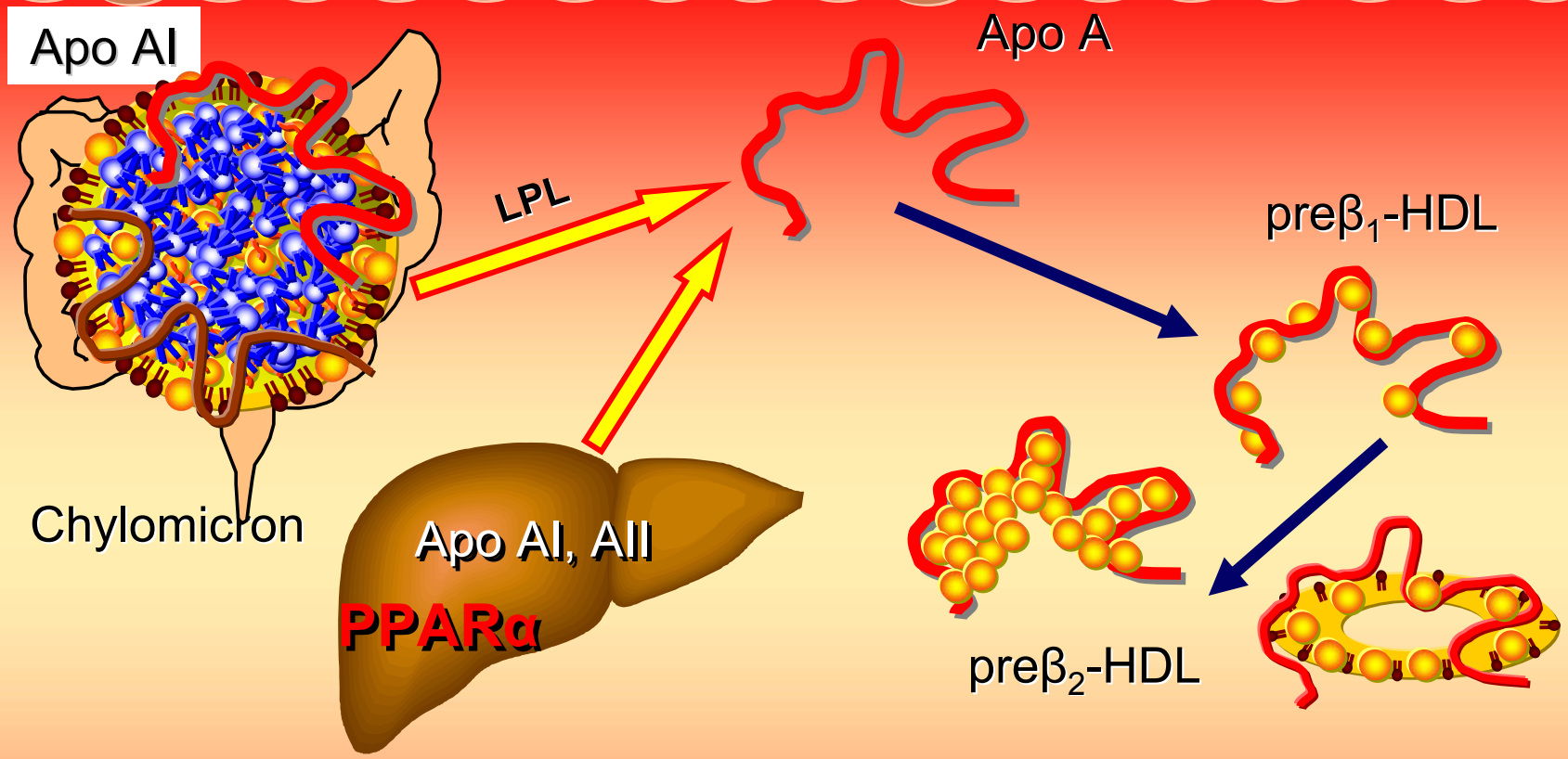


12 ApoA-I HDL Subpopulations

	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

HDL Subpopulations by Surface Charge

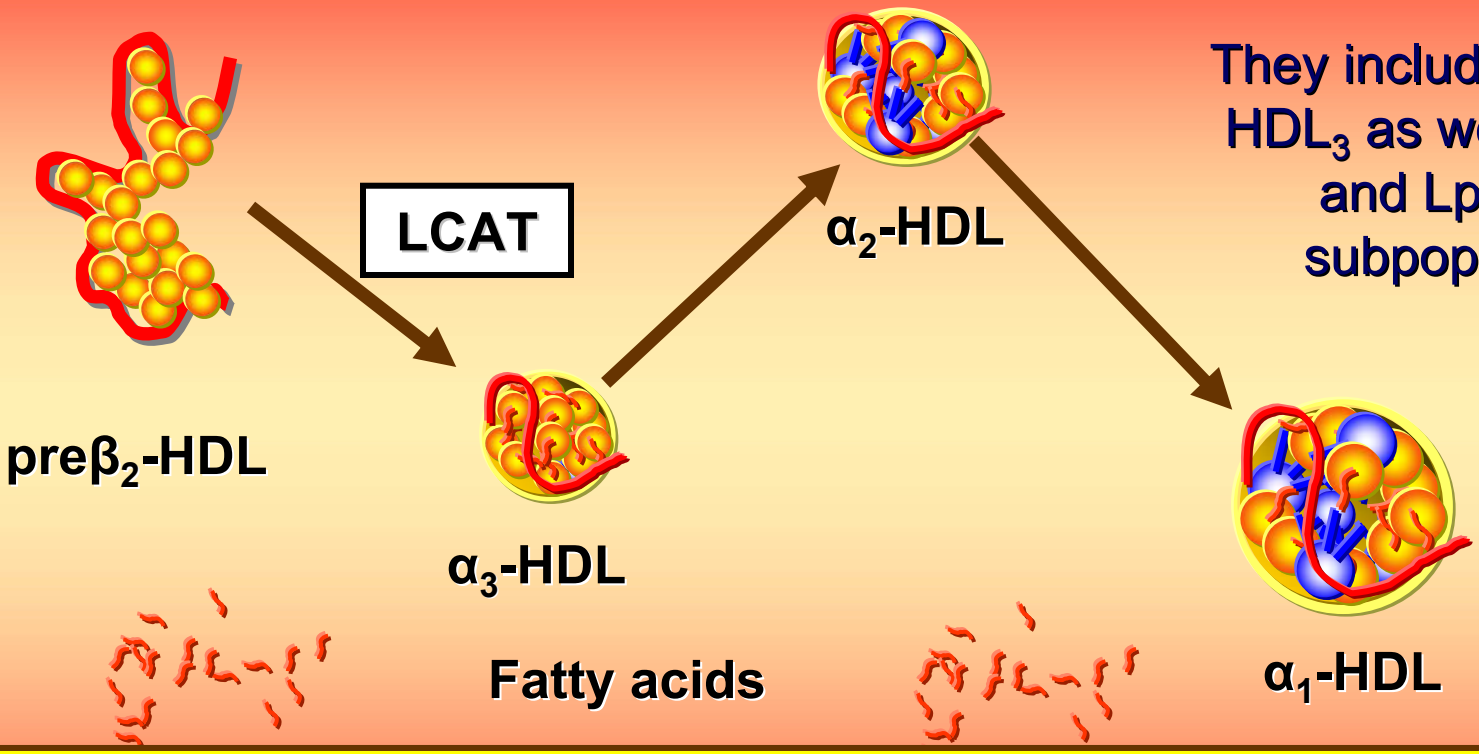


Pre-beta HDLs are either lipid poor Apo AI or discoidal particles consisting of one or two molecules of Apo AI complexed with phospholipids and possibly a small amount of unesterified cholesterol.

Sviridov D & Nestel P.
Atherosclerosis
2002:161:245-254

HDL Subpopulations by Surface Charge

The alpha migrating particles are spherical and account for the major proportion of HDLs in the plasma



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