All About Lipid and Lipoprotein Testing

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North Jersey Institute of Menopausal Lipidology
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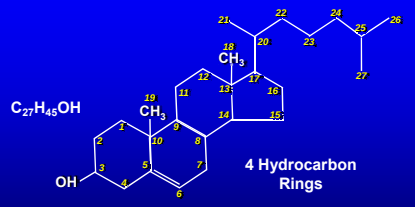
A sterol with 27 carbon molecules with an -OH group at the # 3 position

Hydrocarbon tail



Cholesterol, which can be synthesized de novo or absorbed intestinally, is required by humans for cell membrane integrity and function, as well as bile acid, steroid and vitamin D production.

Linking Cholesterol to Atherosclerosis

Nikolai N. Anitschkow
ca.1904, at the time a student at the Military Medical Academy in St. Petersburg.

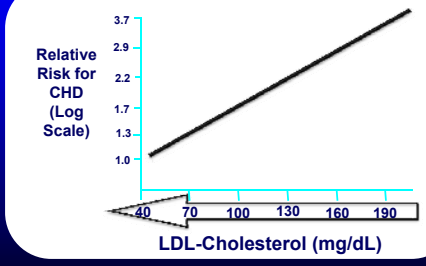
His drawing of a typical foam cell-rich lesion in a rabbit

Anitschkow, N. 1913. Ueber die Veranderungen der Kaninchenaorta bei experimenteller Cholesterinsteatose. Beitr. Pathol. Anat.56:379–404.

National Cholesterol Education Program

Adult Treatment Panel III NCEP-ATP III

2004 Addendum



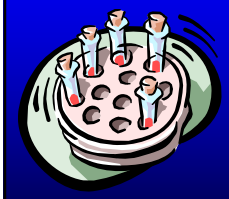
Circulation 2004;110:227-239

Lab Measurement of Lipids and Lipoproteins

What are the available analytic measures of cholesterol which accurately evaluate its association with CVD

Classic Lipoprotein Testing

Separation by Ultracentrifuge **1949**



The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease

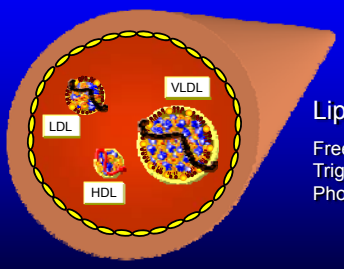
by John W. Gofman, Oliver Delalla, Frank Glazier, Norman K. Freeman, Frank T. Lindgren, Alex V. Nichols, Beverly Strisower, Arthur R. Tamplin

From the Donner Laboratory of Medical Physics, Division of Medical Physics, Department of Physics and the Radiation Laboratory, University of California, Berkeley, California USA

Plasma 1955;2:413-484

Reproduced in: Journal of Clinical Lipidology (2007) 1, 104–141

Normal Lipid Transportation



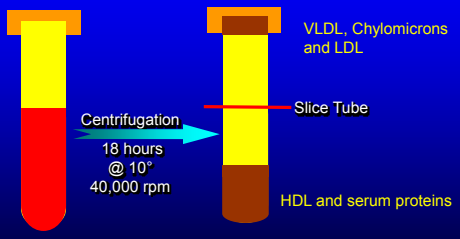
Lipids

Free & esterified sterols
Triglycerides
Phospholipids

"Lipid" movement is **lipoprotein** driven

Ultracentrifugation

- Lipoproteins can be separated on the basis of their differing hydrated densities using ultracentrifugation.



Ultracentrifugation at 1.063 kg/L

Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

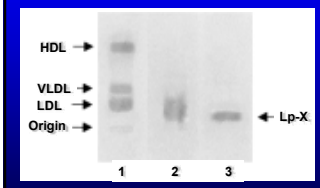
Ultracentrifugation

- The proportion of lipid, especially TG adds to the buoyancy of the total complex allowing the major classes to be separated by equilibrium or weight methods.
  - VLDL < 1.006 kg/L
  - LDL range in density from 1.006 to 1.0063 kg/L
  - HDL from 1.063 – 1.210 kg/L
  - These classes are comparable to electrophoretic fractions designated pre-beta, beta and alpha respectively.
- Ultracentrifugation cannot meet the stringent requirements of a reference method. Achieving complete and reproducible recovery is very difficult

Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

Classic Lipoprotein Testing

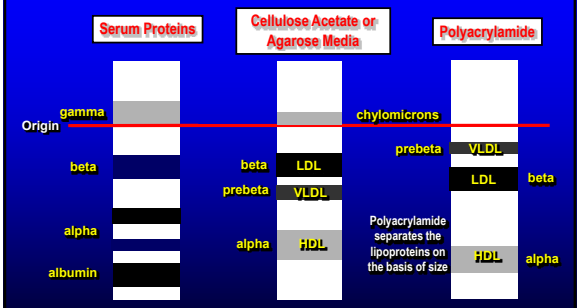
Separation by Electrophoresis



Fredrickson DS. Levy RI. Lees RS. Fat transport in lipoproteins: an integrated approach to mechanisms and disorders.

N Engl J Med. 1967; 276:148 –156; 215–225; 273–281.

Electrophoresis

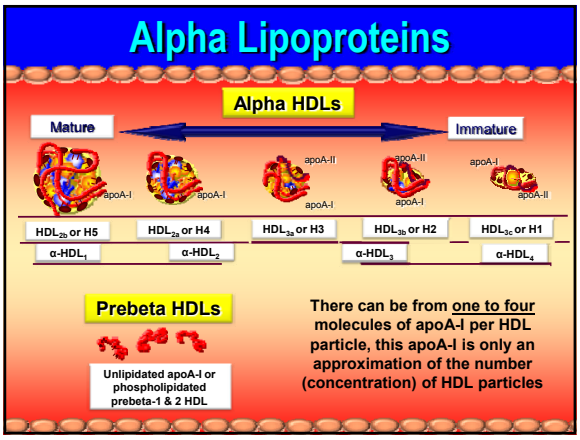


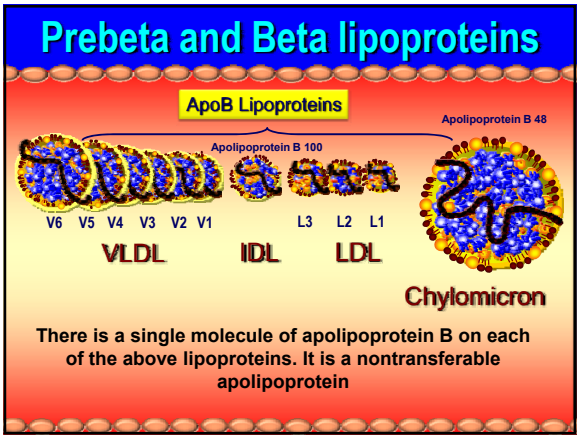
Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

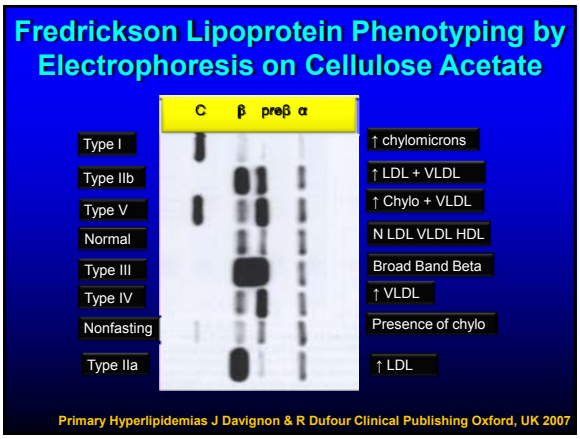
Electrophoresis

- Lipoproteins can be isolated using electrophoretic techniques and the lipoproteins visualized with lipophilic dyes (virtually all of the lipids are in lipoproteins: not other proteins)
- The lipoproteins are separated by their charge and size
- They are named on the basis of mobility by comparison to mobilities of common serum proteins
  - Alpha
  - Pre-beta
  - Beta
- Electrophoresis is used for qualitative analysis and is not appropriate for quantification

Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000


Alpha Lipoproteins
Alpha HDLs
Mature
Immature
apoA-I
apoA-II
HDL2b or H5
HDL2a or H4
HDL3a or H3
HDL3b or H2
HDL3c or H1
α-HDL1
α-HDL2
α-HDL3
α-HDL4
Prebeta HDLs
Unlipidated apoA-I or phospholipidated prebeta-1 & 2 HDL
There can be from one to four molecules of apoA-I per HDL particle, this apoA-I is only an approximation of the number (concentration) of HDL particles


Prebeta and Beta lipoproteins
ApoB Lipoproteins
Apolipoprotein B 48
Apolipoprotein B 100
V6 V5 V4 V3 V2 V1
L3 L2 L1
VLDL
IDL
LDL
Chylomicron
There is a single molecule of apolipoprotein B on each of the above lipoproteins. It is a nontransferable apolipoprotein


Fredrickson Lipoprotein Phenotyping by Electrophoresis on Cellulose Acetate
C β preβ α
Type I
Type IIb
Type V
Normal
Type III
Type IV
Nonfasting
Type IIa
↑ chylomicrons
↑ LDL + VLDL
↑ Chylo + VLDL
N LDL VLDL HDL
Broad Band Beta
↑ VLDL
Presence of chylo
↑ LDL
Primary Hyperlipidemias J Davignon & R Dufour Clinical Publishing Oxford, UK 2007

Diseases of The Heart and Circulation
Paul Wood MD FRCP
1958

The total serum cholesterol, which is normally around ***150-300*** mg% is certainly related to atherosclerosis, but has found to be only a crude measure of blood lipid disturbance.

Cholesterol is insoluble in water and is carried in combination with lipoproteins which are microscopically invisible macromolecules of various sizes and densities.

In atherosclerosis there is a relative and absolute increase in the **ß lipoproteins,** even when the blood cholesterol is normal.

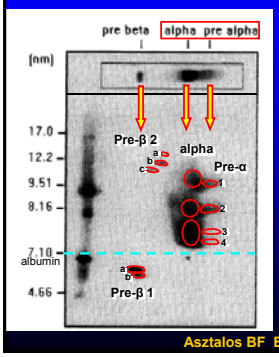
J B Lippincott Co Philadelphia, PA

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 Two Dimension al Electrophoresis and Surface Charge



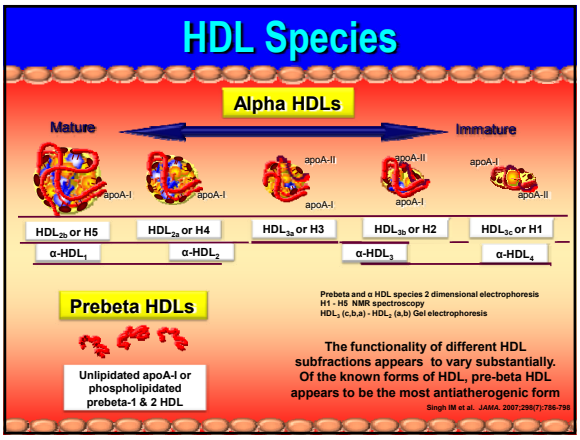
First Dimension

Prebeta & alpha (α) migrating apoA-I on agarose gel electrophoresis

Second Dimension

Separation by size: Nondenaturing concave polyacrylamide gel electrophoresis & immunolocalization

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



High Density Lipoprotein Cholesterol: The Editor's Roundtable

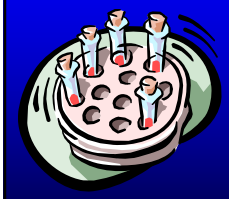
H Bryan Brewer

- We do not have sufficient information bout the clinical utility of HDL subfractions to warrant their measurement in the clinical setting
  - Dr Rader: There are many misconceptions about HDL subfractions
- The concept of "normal" level is changing to "ideal," which in the individual patient depends on that person's other risk factors and risk benefits.
  - Unfortunately we have little data about the "ideal" HDL-C level, because we lack data on its (*HDL particle*) functionality
- We do not have the data to know with certainty whether in individual patients, elevated HDL-C reduces the risk for CVD
  - There are many people with high HDL-C and CAD

Vincent Friedewald, H Bryan Brewer, Scott Grundy, Daniel Rader and William Roberts. Amer J Cardiol 2007;99:1698-1705

Advanced Lipoprotein Testing

Measurement of apoproteins



Apolipoprotein B
Apolipoprotein A-I
Apolipoprotein E genotype
Lipoprotein (a)

Apoprotein-related MOrtality RISk AMORIS Study

- 175,553 patients from screening programs
  - 98,722 men and 76,831 women
- Examined relationship of apoproteins and lipids and prediction of fatal MI
- Mean Follow up 66-68 months

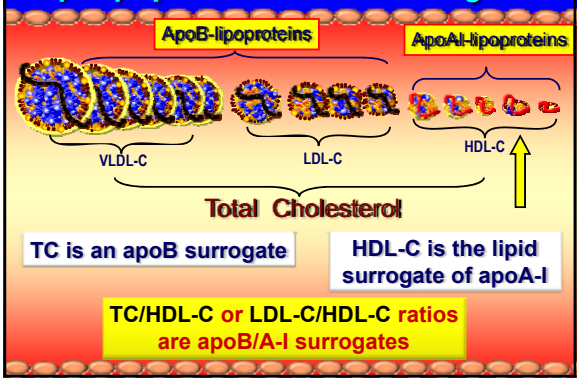
Wallidius G et al Lancet 2001;358:2026-2033

Apoprotein-related MOrtality RISk AMORIS Study

- In multivariate analyses adjusted for age, TC and TG
- **Apolipoprotein B** was a stronger predictor of risk than LDL-C in both sexes
- **Apolipoprotein A-I** was protective
  - The values for **Apo B** and the **ApoB/ApoA-I** ratio were strongly and positively related to risk of fatal MI in men and women

Wallidius G et al Lancet 2001;358:2026-2033

Apolipoprotein B & A-I Surrogates



International Position Paper

All of the national and transnational screening and therapeutic guidelines are based on total or LDL cholesterol. This presumes that cholesterol is the most important lipoprotein-related proatherogenic risk variable.

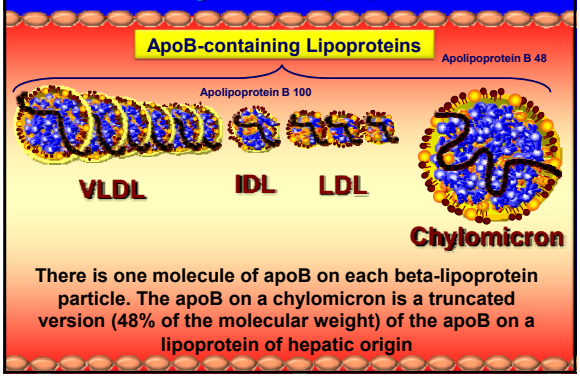
On the contrary, risk appears to be more directly related to the number of circulating atherogenic particles that contact and enter the arterial wall than to the measured concentration of cholesterol in these lipoprotein fractions.

Each of the atherogenic lipoprotein particles contains a single molecule of apolipoprotein (apo) B and therefore the concentration of apo B provides a direct measure of the number of circulating atherogenic lipoproteins.

Evidence from fundamental, epidemiological and clinical trial studies indicates that apo B is superior to any of the cholesterol indices to recognize those at increased risk of vascular disease and to judge the adequacy of lipid-lowering therapy.

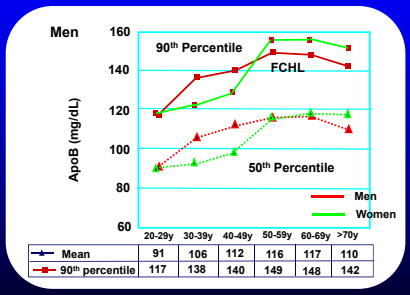
Barter PJ et al. J Intern Med 2006;249:247-258

Atherogenic Lipoproteins



There is one molecule of apoB on each beta-lipoprotein particle. The apoB on a chylomicron is a truncated version (48% of the molecular weight) of the apoB on a lipoprotein of hepatic origin

National Health And Nutrition Examination Survey III (NHANES): Apolipoprotein B Levels by Age 50th and 90th Percentile



	20-29y	30-39y	40-49y	50-59y	60-69y	>70y
Mean	91	106	112	116	117	110
90th percentile	117	138	140	149	148	142

Carr M & Brunzell J J Clin Endo & Metab 2004;89:2601-2607

Apolipoprotein Testing

- Currently, most commercial methods are based on the use of specific antibodies to precipitate apo A-I and apo B in liquid phase.
  - The immunocomplexes that form are then quantitated using turbidimetric or nephelometric approaches on highly automated instruments.
- As part of a standardization project of the International Federation of Clinical Chemistry (IFCC), based on extensive studies (NHANES, Sweden), the World Health Organization (WHO) accepted these materials as WHO-IFCC International Reference Material for apo A-I and apo B and designated the CDC as the depository of the preparations.
- Apo A-I and B values in individuals who fasted versus those who did not were not significantly different

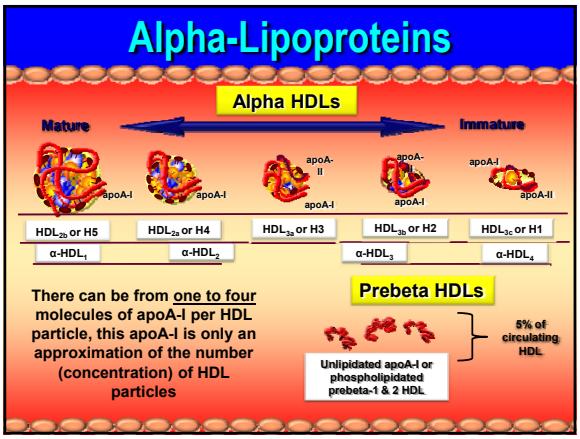
Sniderman, AD & Marcovina, SM Clin Lab Med 26 (2006) 733–750

Canadian Medical Association Recommendations for Management of Dyslipidemia

- Apo B has been standardized and most labs have the equipment to measure it
- Population levels (Canadian)
  - 90 mg/dL 20th percentile
  - 105 mg/dL 50th percentile
  - 120 mg/dL 75th percentile

Genest J et al. CMAJ 2003;168:921-924

Alpha-Lipoproteins

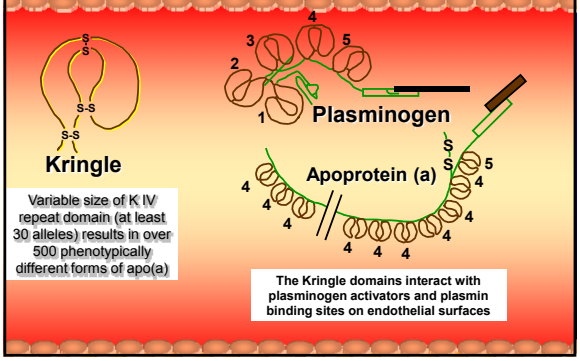


Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein (a)

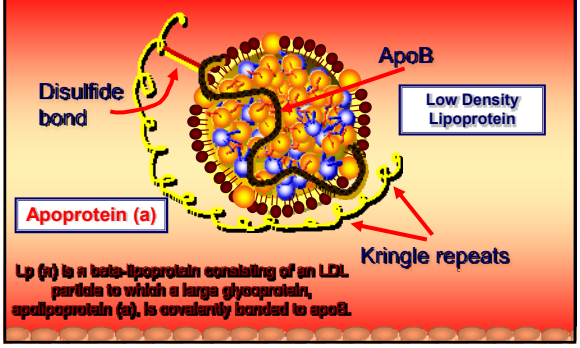
- In Lp(a) particles, apo (a) is covalently linked to apo B-100 by a single disulfide bond *(22, 23)*; the stoichiometry of apo (a) and apo B-100 in the Lp (a) particle is 1:1

Santica M. Marcovina, et al. Clinical Chemistry 2003;49:111785–1796

Apoprotein (a)



Lipoprotein (a)



Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein (a)

- The expression of Lp (a) values in terms of total Lp (a) mass (mg/dL) **should be abandoned** because what is measured is the protein component of Lp (a) and not its lipid and carbohydrate content.
- In addition, to correctly reflect the number of Lp (a) particles and to compare data from different studies, the values should be expressed in terms of nmol/L of Lp (a) protein.
- On the basis of currently available data, individuals with Lp (a) values exceeding the 75th percentile are at increased risk for CVD. For Caucasians, based on the Framingham data, this percentile corresponds to an Lp(a) value of 75 nmol/L.

Santica M. Marcovina, et al. Clinical Chemistry 2003;49:111785–1796

Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein (a)

- Screening for increases in Lp (a) in the general population is not recommended at this time.
- However, measurement of Lp (a) is recommended in individuals with an increased risk of CVD, particularly in those with borderline LDL-cholesterol or high apo B.

Santica M. Marcovina, et al. Clinical Chemistry 2003;49:111785–1796

Apolipoprotein E

- The plasma concentrations of lipoproteins and their metabolic fates are modulated by apolipoproteins on the surface of these lipid-rich particles.
  - It is thought that the genetic variation in apolipoproteins is a major determinant of the interindividual variation ion susceptability to atherosclerosis, specifically CAD[1]
- Apolipoprotein E is a multifunctional protein that plays a key role in the metabolism of cholesterol and triglycerides by binding to receptors on the liver to help mediate clearance of chylomicrons and very low-density lipoproteins from the bloodstream.[2]

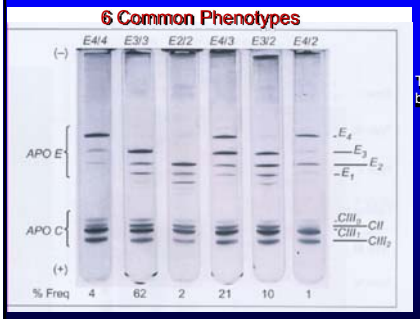
1 Davignon J et al. Arterioslcerosis 1998;8:1-21.
2 Bennet AM et al. JAMA. 2007;298(11):1300-1311

Three Common Human apoE Alleles

		Position 112	158
Parent Form	ApoE3	Cys	Arg
Variant	ApoE4	Arg	Arg
Variant	ApoE2	Cys	Cys

Six phenotypes are possible with their ranking from most to least common being E3/3, E4/3, E3/2, E4/4, E4/2 and E2/2

Electrophoresis of Human apoE Alleles

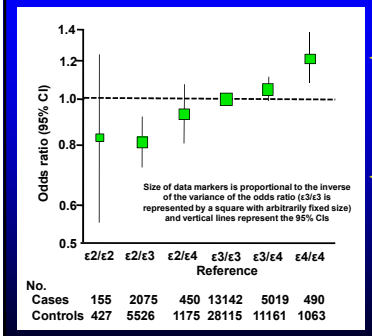


The separation is driven by the relative charge of each isoform

Each arginine residue confers an added positive charge, hence apo E4 which is the most basic of the 3 isoforms has the strongest positive charge

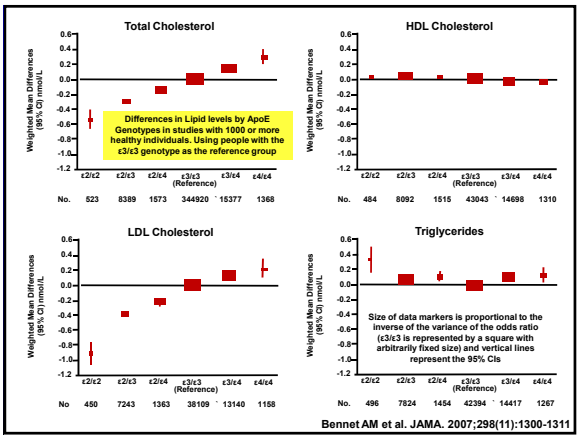
Primary Hyperlipidemias J Davignon & R Dufour Clinical Publishing Oxford, UK 2007

Apolipoprotein E Genotype and CAD Risk



- Odds ratio for CAD with apoE genotypes using individuals with ε3/ε3 genotype as the reference group
- **Data from 21,331 cases and 47,467 controls in studies with 500 or more cases**

Bennet AM et al. JAMA. 2007;298(11):1300-1311



Apolipoprotein E Genotypes, Lipids and CHD Risk

Conclusions

- There are approximately linear relationships of apoE genotypes with both LDL-C levels and coronary risk.
- Compared with individuals with the ε3/ε3 genotype, ε2 carriers have a 20% lower risk of coronary heart disease and ε4 carriers have a slightly higher risk.

Bennet AM et al. JAMA. 2007;298(11):1300-1311

Apolipoprotein E Genotypes, Lipids and CHD Risk

Screening

- Given that the prevalence of the ε2 allele is only about 7% in Western populations, even if the 20% lower coronary risk associated with it were to be entirely causal, it would still explain only a few percent of coronary disease cases in Western populations.
- Although the magnitude of this relative risk is insufficiently strong to justify population-wide screening for apoE genotypes, it has been proposed that the effects of apoE genotypes may be particularly strong in certain subgroups

Bennet AM et al. JAMA. 2007;298(11):1300-1311

Real World Lipoprotein Testing

Lipid Concentrations

Lipid concentrations determined by direct measurement or by calculation serve as surrogates of lipoprotein concentrations

The Lipid Profile

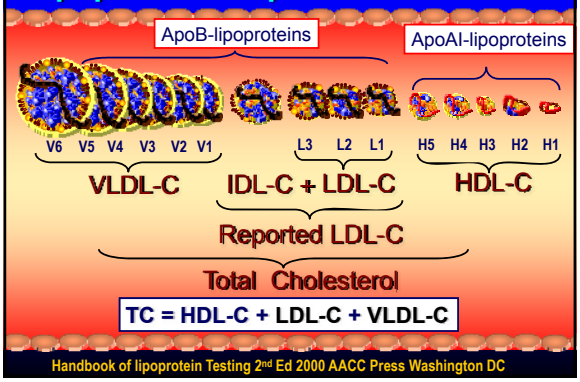
Using Lipid Concentrations as surrogates of Lipoprotein Characteristics

Lipid Concentrations

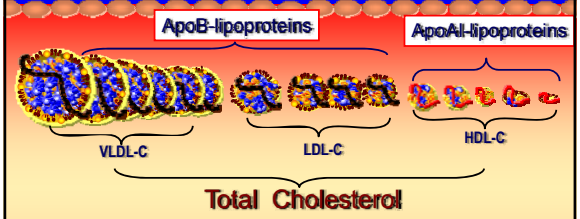
Total cholesterol (TC) is the cholesterol trafficked within all of the lipoproteins in a deciliter of plasma

TC is determined analytically and does not require fasting

Lipoprotein & Lipid Concentrations



Apolipoprotein B & A-I Surrogates



TC > 200 mg/dL is a surrogate of ↑ apoB

TC > 135 mg/dL is a surrogate ↑ apoB in T2DM

Lipid Concentrations

LDL cholesterol (LDL-C) is the cholesterol trafficked within all of the low and intermediate density lipoproteins in a deciliter of plasma

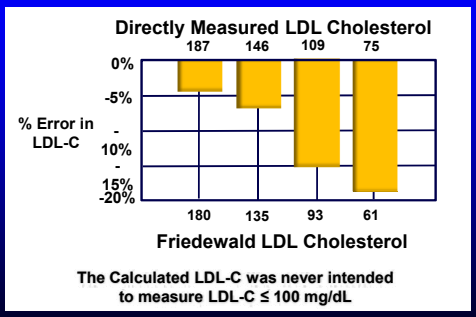
LDL-C can be determined analytically (directly) and does not require fasting

LDL-C is most commonly estimated using the Friedewald formula (fasting required)

✦Friedewald formula

LDL-C = Total Cholesterol - ([HDL-C] + [VLDL-C])

Friedewald Calculated LDL-C

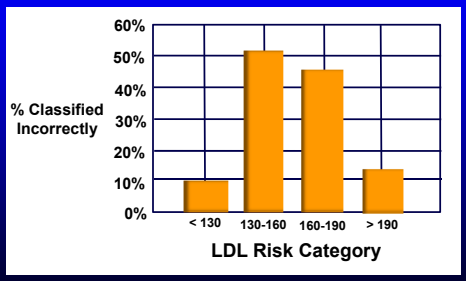


The Calculated LDL-C was never intended to measure LDL-C ≤ 100 mg/dL

Scharnagl H et al. Clin Chem Lab Med 2001;39(5):426-31

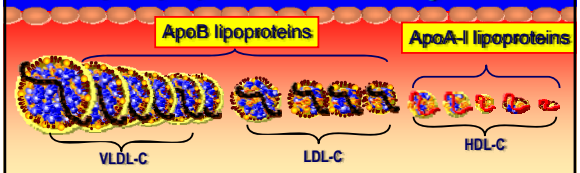
Friedewald Calculated LDL-C

Calculated LDL-C misclassifies patient risk when TG > 177 mg/dL



Marniemi J et al. Clin Biochem 1995;28:285-289

Apolipoprotein B Surrogates

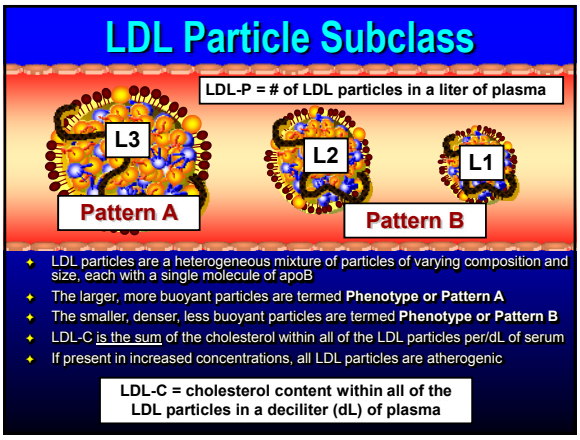


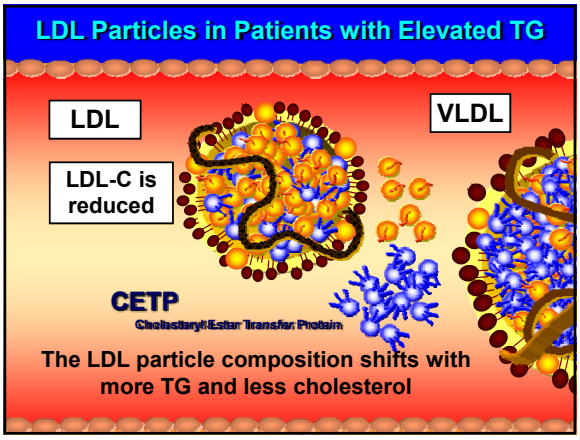
Since LDL-P makes up more than 90% of apoB particles, LDL-C is an important apoB surrogate unless the predominant LDL species are small or TG-rich

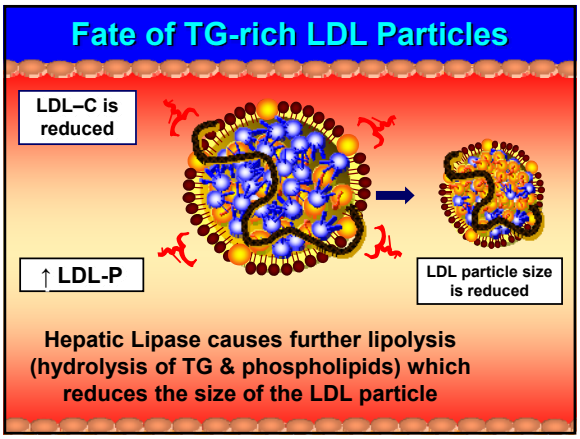
Evidence Supporting Apo B over LDL-C: Prospective Epidemiologic Studies & Placebo Wing of Major Statin Trials

QCVS-13:	Quebec Cardiovascular Study 13 year follow up
THROMBO MS	Thrombogenic Factors & Recurrent Coronary Events Metabolic Synd
LIPID	Long-term Intervention with Pravastatin in Ischemic Disease
AFCAPS/TexCAPS	Air Force Texas Coronary Atherosclerosis Prevention Study
4S	Scandinavian Simvastatin Survival Study
Womens HS	Women's Heart Study
THROMBO	Thrombogenic Factors & Recurrent Coronary Events
NPHS	Northwick Park Heart Study
AMORIS	Apolipoprotein-related Mortality Risk
QCVS-5	Quebec Cardiovascular Study 5 year follow up

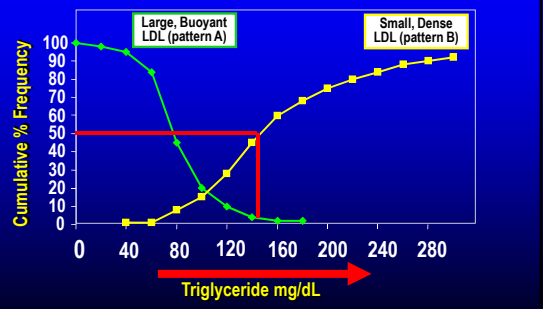
Sniderman A D& Marcovina SM. Clin Lab Med 26 (2006) 733–750


LDL Particle Subclass
LDL-P = # of LDL particles in a liter of plasma
L3
L2
L1
Pattern A
Pattern B
LDL particles are a heterogeneous mixture of particles of varying composition and size, each with a single molecule of apoB
The larger, more buoyant particles are termed Phenotype or Pattern A
The smaller, denser, less buoyant particles are termed Phenotype or Pattern B
LDL-C is the sum of the cholesterol within all of the LDL particles per/dL of serum
If present in increased concentrations, all LDL particles are atherogenic
LDL-C = cholesterol content within all of the LDL particles in a deciliter (dL) of plasma


LDL Particles in Patients with Elevated TG
LDL
VLDL
LDL-C is reduced
CETP
Cholesteryl Ester Transfer Protein
The LDL particle composition shifts with more TG and less cholesterol

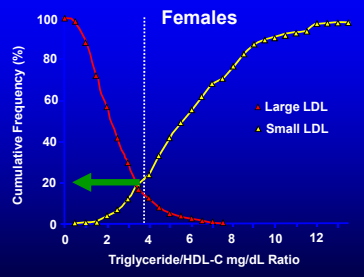

Fate of TG-rich LDL Particles
LDL–C is reduced
↑ LDL-P
LDL particle size is reduced
Hepatic Lipase causes further lipolysis (hydrolysis of TG & phospholipids) which reduces the size of the LDL particle

Relationship of Triglycerides and LDL Particle Size



Austin M, et al. Circulation. 1990;82:495-506.

Relationship of Small LDL to Triglyceride/HDL-C Ratio



At a ratio ≥ 3.8, 80% of patients will have small LDL phenotype

NMR Spectroscopy

Hanak, V. et al. Am J Cardiol 2004;94:219–222

European Prospective Investigation into Cancer and Nutrition- Norfolk Study (EPIC-Norfolk)

- Whereas LDL size was related to CAD risk, this relationship was abolished after adjusting for LDL-P. Both LDL-P and non–HDL-C had incremental value on top of the Framingham Risk Scoring in multivariate analyses.
- Recognition that patients with low HDL-C and/or high triglycerides often have elevated numbers of LDL particles without having elevated LDL-C may enable their LDL-related CAD risk to be managed more effectively.

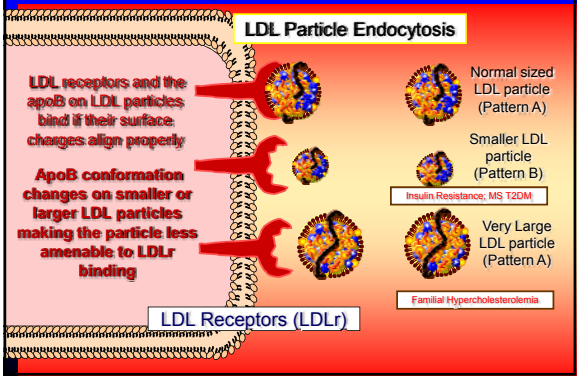
Karim El Harchaoui, et al. J. Am. Coll. Cardiol. 2007;49;547-553

Multi-Ethnic Study of Atherosclerosis (MESA)

- Contrary to current opinion, both small and large LDL were significantly associated with subclinical atherosclerosis independent of each other, traditional lipids, and established risk factors, with **no association between LDL size and atherosclerosis** after accounting for the concentrations of the two subclasses.

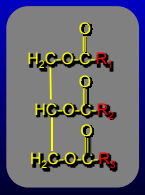
Mora S, Szklo S, Otvos JD et al. Atherosclerosis 2007;192:211-217

LDL Receptor & LDL Particles



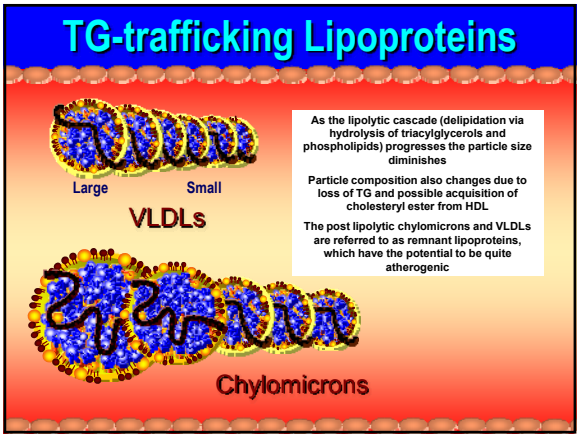
Lipid Concentrations

Triglycerides: is the triacylglycerol (TG) concentration trafficked in all of the lipoproteins found in a deciliter of plasma



R = Fatty acid chain

TG-trafficking Lipoproteins



As the lipolytic cascade (delipidation via hydrolysis of triacylglycerols and phospholipids) progresses the particle size diminishes

Particle composition also changes due to loss of TG and possible acquisition of cholesteryl ester from HDL

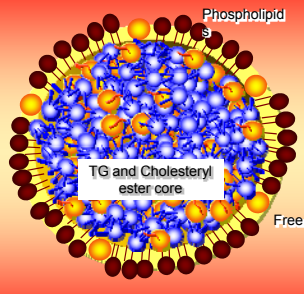
The post lipolytic chylomicrons and VLDLs are referred to as remnant lipoproteins, which have the potential to be quite atherogenic

Lipid Concentrations

VLDL cholesterol (VLDL-C) is the cholesterol trafficked within all of the very low density lipoproteins (and chylomicrons if present) in a deciliter of plasma

VLDL-C is determined using the Friedewald formula, by dividing TG by 5

Very Low Density Lipoprotein (VLDL)



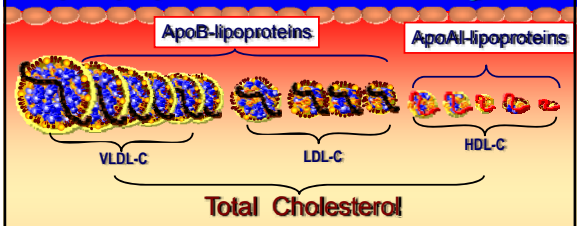
- The primary TG transporting lipoprotein
- Size, depending on TG content (varies from 300 Å (30 nm) to 700 Å (70 nm)
- Normal particle composition is 80% TG and 20% cholesterol (or a 3 to 1 ratio)
- VLDL-C = TG/5

Friedewald Equation

- Using the Friedewald equation makes three assumptions
  - All TG-rich lipoproteins are VLDL particles (no chylomicrons are present)
  - All serum TG are in VLDL particles, with none in any other lipoproteins
  - The relative proportion of cholesterol in VLDL is constant at 20% of VLDL mass
- These assumptions which are only partially true are increasingly unreliable when TG levels > 250 and completely unreliable at TG > 400 and in individuals with Type III hyperlipidemia

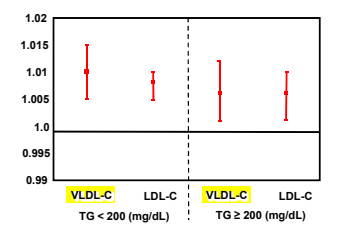
Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

Apolipoprotein B & A-I Surrogates



VLDL-C > 30 mg/dL is a surrogate of ↑VLDL-P or apoB

Framingham Heart Study: Non HDL-C and VLDL-C and Their Risk Predictive Values in Coronary Heart Disease

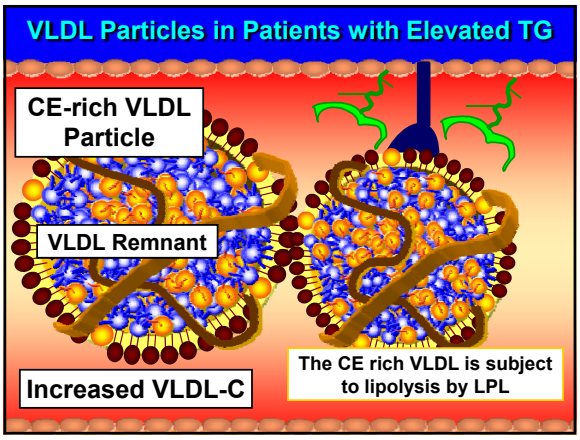


If LDL cholesterol and VLDL cholesterol were added into the model simultaneously, the RR estimates of CHD risk for LDL-C and VLDL-C remained approximately the same

Risk of CHD incidence for VLDL-C and LDL-C as continuous variables, by TG levels, adjusted form age, gender , study, smoking status, systolic BP and prevalent diabetes at baseline

Jiian Liu -- Scott Grundy et al. Am J Cardiol 2006;98:1363-1368

VLDL Particles in Patients with Elevated TG



National Cholesterol Education Program Adult Treatment Panel III NCEP-ATP III Risk of Triglycerides

When **triglyceride levels are ≥200 mg/dL**, the presence of increased quantities of atherogenic remnant lipoproteins can heighten CHD risk **substantially** beyond that predicted by LDL cholesterol alone.

NCEP JAMA 2001;285:2486 Final Report Circulation 2002;106:3143-3421

European Prospective Investigation into Cancer and Nutrition- Norfolk Study (EPIC-Norfolk)

- Recognition that patients with low HDL-C and/or high triglycerides often have elevated numbers of LDL particles without having elevated LDL-C may enable their LDL-related CAD risk to be managed more effectively.

Karim El Harchaoui, et al. J. Am. Coll. Cardiol. 2007;49;547-553

Lipid Concentrations

HDL cholesterol (HDL-C) is the cholesterol trafficked within all of the high density lipoproteins in a deciliter of plasma

HDL-C is determined analytically and does not require fasting

High Density Lipoprotein Cholesterol: The Editor's Roundtable

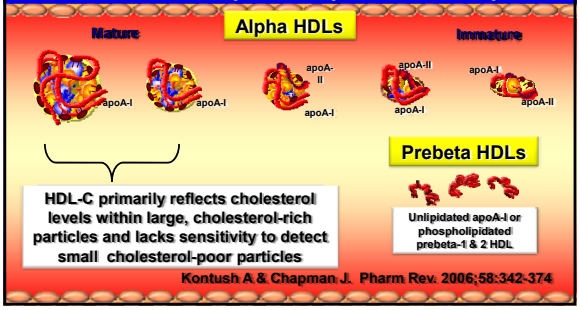
Dan Rader

- Plasma HDL-C is the least accurate of standard lipid measurements
- Performed correctly, which is true with large labs, HDL-C accuracy is ± 10%
- You do not make a treatment recommendation based on a single measurement of HDL-C. A low HDL-C or one that falls unexpectedly should be confirmed with at least one repeat measurement
- Dr William Roberts: An accuracy or ± 10% could give errors of up to 4 mg/dL

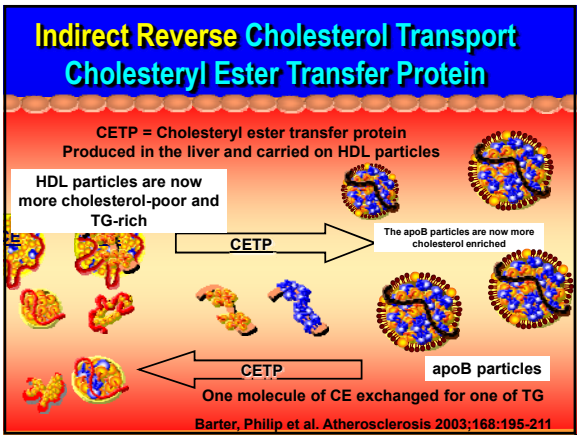
Vincent Friedewald, H Bryan Brewer, Scott Grundy, Daniel Rader and William Roberts. Amer J Cardiol 2007;99:1698-1705

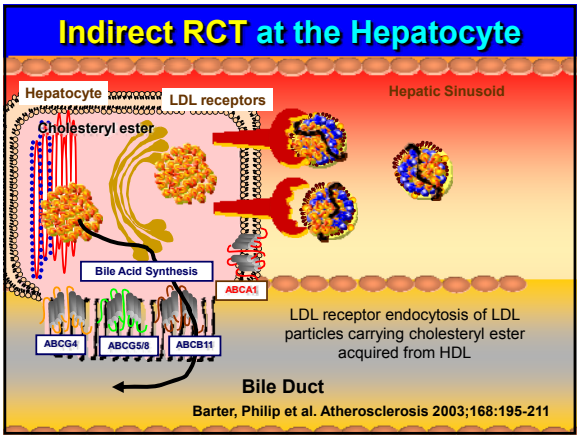
HDL-cholesterol Concentration

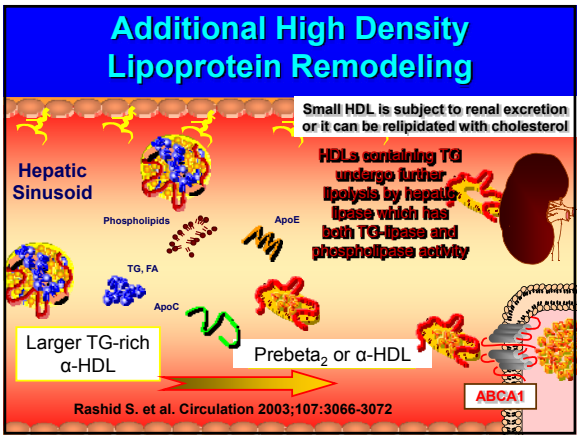
HDL-C reflects the cholesterol being trafficked within all of the HDL particles per deciliter of plasma

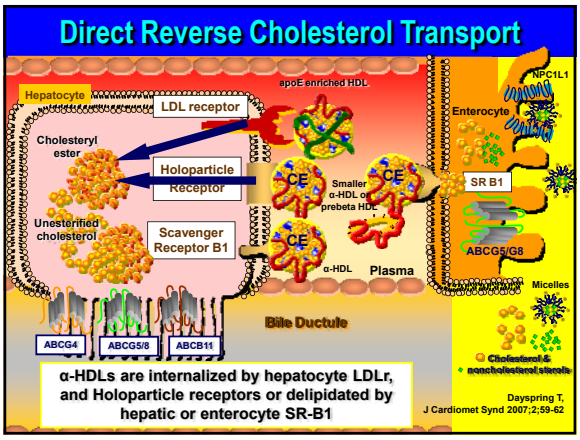


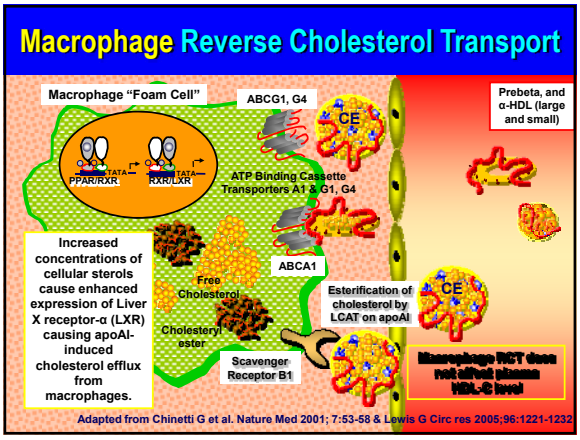
Kontush A & Chapman J. Pharm Rev. 2006;58:342-374

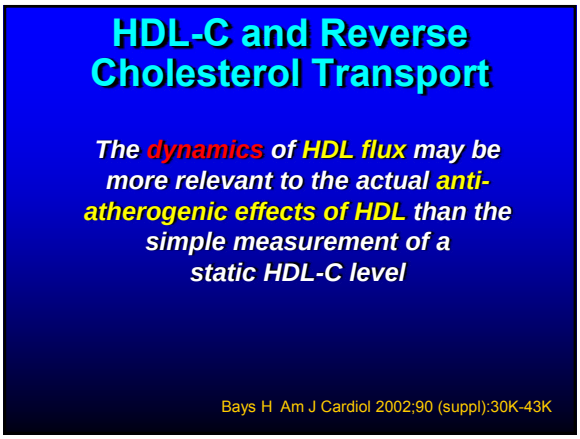

Indirect Reverse Cholesterol Transport
Cholesteryl Ester Transfer Protein
CETP = Cholesteryl ester transfer protein
Produced in the liver and carried on HDL particles
HDL particles are now more cholesterol-poor and TG-rich
CETP
The apoB particles are now more cholesterol enriched
CETP
apoB particles
One molecule of CE exchanged for one of TG
Barter, Philip et al. Atherosclerosis 2003;168:195-211


Indirect RCT at the Hepatocyte
Hepatocyte
LDL receptors
Hepatic Sinusoid
Cholesteryl ester
Bile Acid Synthesis
ABCA1
ABCG4
ABCG5/8
ABCB11
LDL receptor endocytosis of LDL particles carrying cholesteryl ester acquired from HDL
Bile Duct
Barter, Philip et al. Atherosclerosis 2003;168:195-211


Additional High Density Lipoprotein Remodeling
Small HDL is subject to renal excretion or it can be relipidated with cholesterol
Hepatic Sinusoid
HDLs containing TG undergo further lipolysis by hepatic lipase which has both TG-lipase and phospholipase activity
Phospholipids
ApoE
TG, FA
ApoC
Larger TG-rich α-HDL
Prebeta2 or α-HDL
ABCA1
Rashid S. et al. Circulation 2003;107:3066-3072


Direct Reverse Cholesterol Transport
Hepatocyte
LDL receptor
apoE enriched HDL
NPC1L1
Enterocyte
Cholesteryl ester
Holoparticle Receptor
CE
Smaller α-HDL or prebeta HDL
SR B1
Unesterified cholesterol
Scavenger Receptor B1
ABCG5/G8
α-HDL
Plasma
Micelles
Bile Ductule
ABCG4
ABCG5/8
ABCB11
Cholesterol & noncholesterol sterols
α-HDLs are internalized by hepatocyte LDLr, and Holoparticle receptors or delipidated by hepatic or enterocyte SR-B1
Dayspring T, J Cardiomet Synd 2007;2:59-62


Macrophage Reverse Cholesterol Transport
Macrophage "Foam Cell"
ABCG1, G4
CE
Prebeta, and α-HDL (large and small)
PPAR/RXR
RXR/LXR
ATP Binding Cassette Transporters A1 & G1, G4
Increased concentrations of cellular sterols cause enhanced expression of Liver X receptor-α (LXR) causing apoAI-induced cholesterol efflux from macrophages.
ABCA1
Free Cholesterol
Esterification of cholesterol by LCAT on apoAI
Cholesteryl ester
Scavenger Receptor B1
Macrophage RCT does not affect plasma HDL-C level
Adapted from Chinetti G et al. Nature Med 2001; 7:53-58 & Lewis G Circ res 2005;96:1221-1232

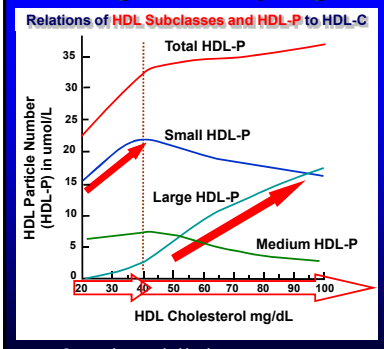

HDL-C and Reverse Cholesterol Transport
The dynamics of HDL flux may be more relevant to the actual anti-atherogenic effects of HDL than the simple measurement of a static HDL-C level
Bays H Am J Cardiol 2002;90 (suppl):30K-43K

Macrophage Reverse Cholesterol Transport

When we speak of reverse cholesterol transport, *in terms of cardiovascular benefit*, we are really speaking of MACROPHAGE RCT

Which does not affect Total HDL-C

Framingham Offspring Study (n = 3,467)



Lowess plot: smoothed levels

For HDL-C < 40 mg/dL, increasing HDL-C is accounted for by increases in levels of all three HDL subclasses, but primarily small HDL.

Increases in HDL-C beyond 40 mg/dL are due primarily to changes in HDL particle composition, with large HDL increasing steadily at the expense of decreasing numbers of the smaller HDL subclasses.

This is probably due to a product-precursor relationship between these species.

Cromwell, WC. Otvos JD. In Davidson MH, Toth PP, Maki K. eds Therapeutic Lipidology. Totowa, NJ, Human Press

HDL Particles & Cardioprotection

Collectively, the data leads to the conclusion that both large and small HDL subclasses are cardioprotective.

Determining whether one subclass is more cardioprotective than the other and whether therapies that primarily affect levels of one or the other subclass are more or less beneficial are questions that await further investigation.

Cromwell WC. Journal of Clinical Lipidology (2007) 1, 57–64

Plasma HDL-Cholesterol (HDL-C)

- The cholesterol content of HDL particles (HDL-C) depends on:
  - apoA-I production
  - apoA-I lipidation
    - Hepatic and peripheral cell ABCA1, A7, G1, G4
  - apoA-I delipidation
    - Steroid gland & adipocyte SRB1
    - Hepatic and Enterocyte SR-B1,
    - CETP activity (lipoproteins and adipocytes)
  - apoA-I removal
    - Holoparticle receptor endocytosis
    - LDL-receptor endocytosis: HDL-apoE
    - apoA-I excretion

HDL Cholesterol Trafficking

Plasma steady state **HDL-C levels are not an assay of the rate of RCT**, which is a dynamic process that can only be assessed through kinetic measures of cholesterol flux.

Duffy D & Rader D. Circulation 2006;113:1140-1150

HDL-C and Reverse Cholesterol Transport

The dynamics of HDL flux may be more relevant to the actual anti-atherogenic effects of HDL than the simple measurement of a static HDL-C level

Bays H Am J Cardiol 2002;90 (suppl):30K-43K

High Density Lipoproteins

- The functionality of different HDL subfractions appears to vary substantially.
- Of the known forms of HDL-C (pre-β HDL, HDL2, HDL3) pre-β HDL appears to be the most antiatherogenic form.
- Therefore, therapies that increase the most atheroprotective subfraction(s) of functioning HDL may be most promising.
- Additionally, functional testing of HDL may provide insight as to the therapeutic promise of investigational compounds.

Singh, Shishehbor, & Ansell,. JAMA. 2007;298(7):786-798

Lipid Concentrations

Non HDL cholesterol (Non HDL-C) is the cholesterol trafficked within all of the apoB-containing (potentially atherogenic) lipoproteins in a deciliter of plasma

Non HDL-C is a calculation and does not require fasting

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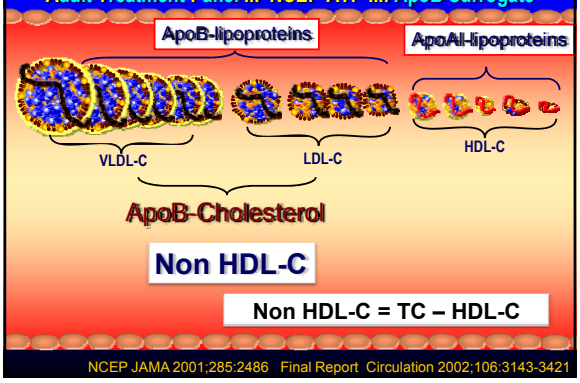
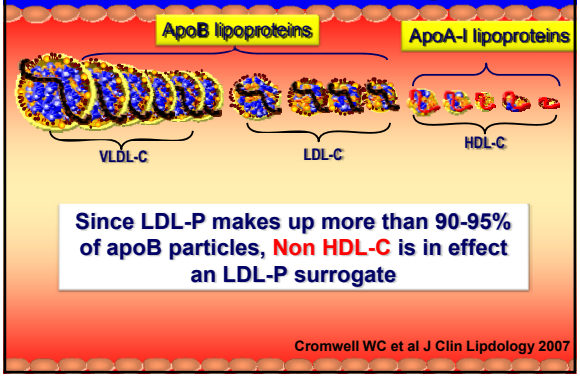
National Cholesterol Education Program

Adult Treatment Panel III NCEP-ATP III: ApoB Surrogate



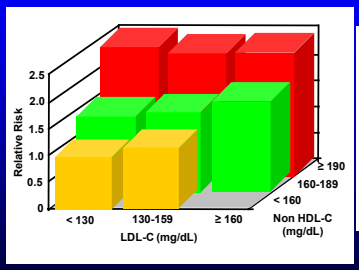
NCEP JAMA 2001;285:2486 Final Report Circulation 2002;106:3143-3421

Apolipoprotein B Surrogates



Cromwell WC et al J Clin Lipdology 2007

Framingham Heart Study: Non HDL-C and VLDL-C and Their Risk Predictive Values in Coronary Heart Disease



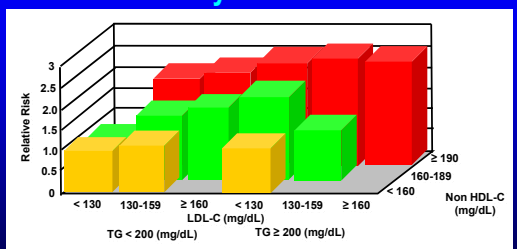
Within non HDL cholesterol levels, no association was found between LDL-C and the risk for incident CHD.

In contrast, a strong positive and graded association between non-HDL-C and risk for CHD incidence occurred within every level of LDL-C

That is, non HDL-C appears to be a better predictor of CHD incidence.

Jian Liu, -- Scott Grundy et al. Am J Cardiol 2006;98:1363-1368

Framingham Heart Study: Non HDL-C and VLDL-C and Their Risk Predictive Values in Coronary Heart Disease



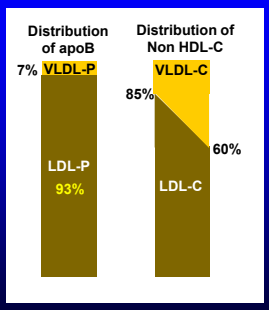
The association with CHD incidence was stronger for non-HDL cholesterol within every level of LDL cholesterol than that for LDL cholesterol within each level of non-HDL cholesterol, regardless of TG levels.

Jian Liu, -- Scott Grundy et al. Am J Cardiol 2006;98:1363-1368

Non HDL-C Concentrations

- Non-HDL-C (VLDL + LDL cholesterol) and total serum apoB (LDL + VLDL apoB) are different quantitative measures of the atherogenic lipoproteins in blood.
- Non-HDL-C measures the cholesterol contained within these lipoproteins and apoB measures the number of lipoprotein particles that carry this cholesterol.
- Due to wide variability in the cholesterol content of both VLDL and LDL particles, these 2 measures are not equivalent clinically
  - apoB and NMR measures of particle number are related more strongly to CHD risk than non-HDL-C
  - analytically (apoB and non-HDL-C are significantly discordant in many patients), despite being highly correlated (r ~ 0.9) in the overall population.

Non HDL-C and VLDL-C



The variable contributions of VLDL and LDL cholesterol to non-HDL cholesterol.

Because the amount of cholesterol in VLDL particles is variable, the proportion that VLDL cholesterol contributes to non-HDL cholesterol varies.

By contrast, the proportion of VLDL apoB to LDL apoB varies little.

Sniderman A D& Marcovina SM. Clin Lab Med 26 (2006) 733–750

Evidence Supporting Apo B over Non HDL-C: Prospective Epidemiologic Studies & Placebo Wing of Major Statin Trials

ApoB Superior as a Predictor

Carotid IMT	Carotid Intimomedial Thickness Studies (4)
CMS	Casale Monferrator Study
HHMS	Harvard healthy Men Study
AMORIS	Apolipoprotein-related Mortality Risk

Equal Predictors of Risk

Womens HS	Women's Heart Study
J-DM	Jiang Diabetes Mellitus

Sniderman A D& Marcovina SM. Clin Lab Med 26 (2006) 733–750

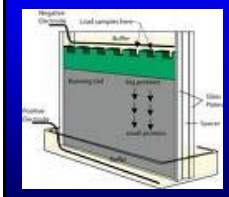
Apolipoprotein Testing

- Currently, most commercial methods are based on the use of specific antibodies to precipitate apo A-I and apo B in liquid phase.
  - The immunocomplexes that form are then quantitated using turbidimetric or nephelometric approaches on highly automated instruments.
- As part of a standardization project of the International Federation of Clinical Chemistry (IFCC), based on extensive studies (NHANES, Sweden), the World Health Organization (WHO) accepted these materials as WHO-IFCC International Reference Material for apo A-I and apo B and designated the CDC as the depository of the preparations.
- Apo A-I and B values in individuals who fasted versus those who did not were not significantly different

Sniderman, AD & Marcovina, SM Clin Lab Med 26 (2006) 733–750

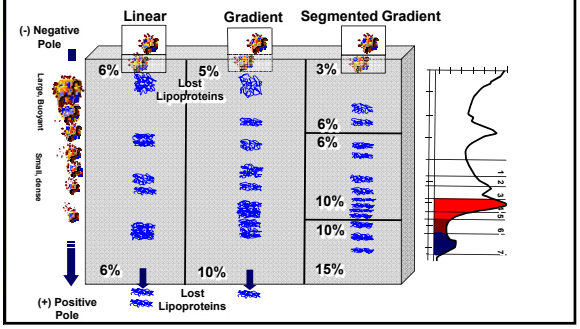
Advanced Lipoprotein Testing

Separation by size

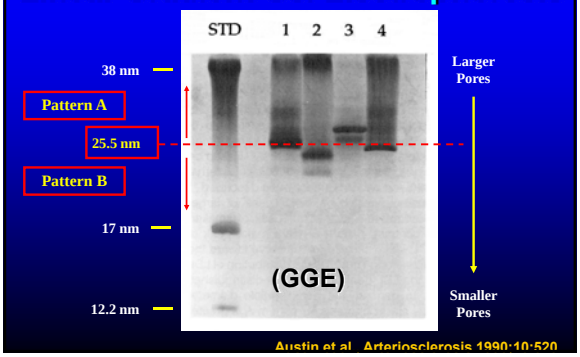


Gradient & Segmented Gel Electrophoresis (S-GGE)
Berkeley HeartLab, Quest
Tube Gel Electrophoresis
Lipoprint

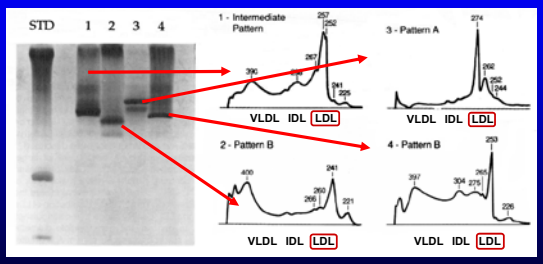
Increased Resolution with Gradient & Segmented-Gradient (S-GGE) Gel Electrophoresis



LDL Sizing by 3 Segmented Non-Linear Gradient Gel Electrophoresis



LDL Sizing by 3 Segmented Non-Linear Gradient Gel Electrophoresis

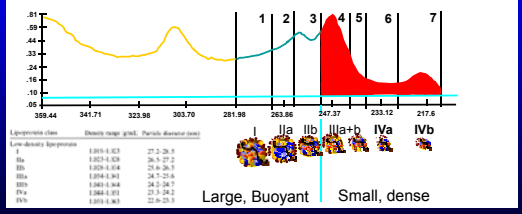


Densitometry scans give graphical output showing major LDL peak particle diameter (LDL-PPD)

LDL Sizing by 3 Segmented Non-Linear Gradient Gel Electrophoresis

LDL-S_3GGE™

A pattern with a high concentration of small LDL particles (ie, LDL-III or LDL-IV) and a peak particle diameter less than or equal to 25.5 nm has been designated a type B LDL phenotype, whereas a predominance of large LDL particles characterizes a type A phenotype

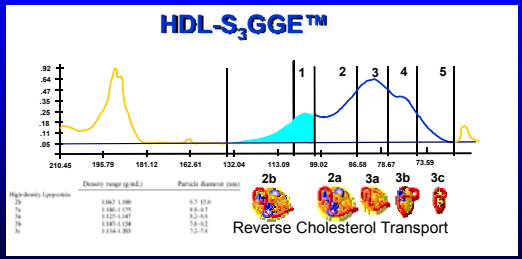


The LDL subfractions are reported as percentages based on the area under the curve for each of the seven subfractions.

Warnick GR et al. Clin Lab Med: 2006:803-846

HDL Sizing by 3 Segmented Non-Linear Gradient Gel Electrophoresis

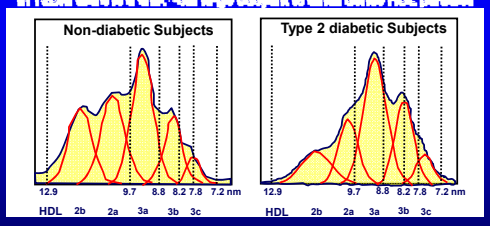
HDL-S_3GGE™



Segmented HDL Subclass Determination

GGE HDL Populations in T2DM

In T2DM there is a shift from large buoyant to small dense HDL particles



The thick bell-shaped lines represent Gaussian fitting of the scanline. The continuous line at the top of each panel is the original scan line. Vertical dotted lines are the fixed cut-points of HDLs according to size (nm)

J Lipid Res 1995;36:573-582. Taskinen MR. Diabetologia (2003) 46:733–749

Clinical Test Summary

	Test Result	Alert Value	Reference Range ‡	BHL Goal †
Total Cholesterol (mg/dL)	187		146-257	<200 *
LDL-C(mg/dL)	117		69-177	<100 *
HDL-C(mg/dL)	42		35-76	> 40*
Triglycerides(mg/dL)	142		61 - 269	< 150 *
Apo B particle #(mg/dL)	95		57 - 118	< 60*
Apo E Genotype	3/3		†	

*ATP III Goal

1 Near Optimal / Above Optimal LDL Cholesterol
2 Normal Triglycerides
3 Apo E 3/3
4 High LDL IIa + IIb with LDL Pattern Intermediate
5 Low HDL2b
(see below for specific details of clinical implications)

LDL-S_3GGE ™ Subclass Distribution

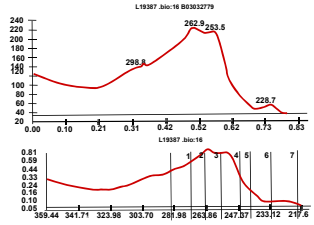
	Test Result	Alert Value	Population (10-90)*%	BHL Goal †
IIa + IIb (%) ▶	28.6	> 20	13.7 – 41.3	< 15
IVb (%)	1.7		1.5 – 9.5	< 5

1 = LDL I (%)	20.2
2 = LDL IIa (%)	18.6
3 = LDL IIb (%)	28.1
4 = LDL IIIa (%)	23.7
5 = LDL IIIb (%)	4.9
6 = LDL IVa (%)	2.7
7 = LDL IVb (%)	1.7

LDL Particle sizes are measured in angstroms (A˚)

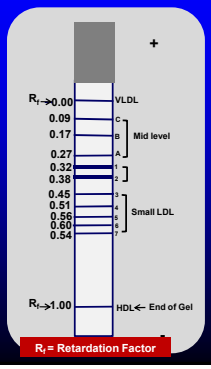
LDL Peak 1 (A˚)	263
LDL Peak 2 (A˚)	254

LDL Pattern Interpretation	Peak Diameter (A˚)
Pattern A (Large LDL)	263.5 - 285
Pattern Int (Intermediate LDL)	257.5 – 263.4
Pattern B (Small LDL)	220 – 257.4



Berkeley HeartLab, Inc.
advanced cardiovascular informatics

Tube Gel Electrophoresis: Lipoprint



The Lipoprint System is a device for the separation and measurement of LDL subfraction cholesterol.

After the completion of the electrophoresis, the gel tubes are scanned and the data is then exported to a computer and graphically analyzed.

The program provides a graphical representation of the lipoproteins and the subfractions present in each sample.

The test provides detailed results within the subfractions of low density lipoproteins (LDL).

DSI Laboratories www.dsilabs.com
http://www.4qc.com/products/electrophoresis

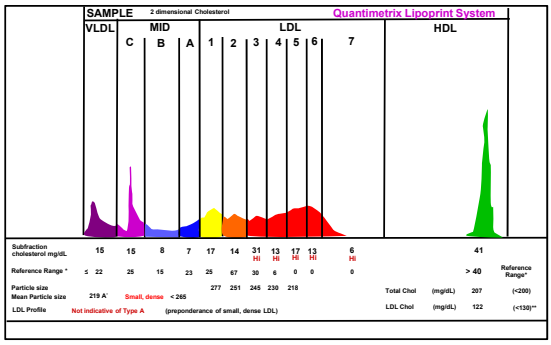
Tube Gel Electrophoresis: Lipoprint

This technology does not measure LDL particle size directly, but estimates LDL particle size by comparing particle electrophoretic mobility with the electrophoretic mobilities of particles of know sizes.

The total cholesterol of the sample must be measured independently of the Lipoprint system. The report contains the patient's scan and the cholesterol within each fraction based on retardation factor (Rf) which correspond to each of the LDL subfractions. The scan contains 7 possible LDL subfractions

Ensign, Hill and Heward. Clin Chem 2006;52:1722-1727

Tube Gel Electrophoresis: Lipoprint Report



http://www.4qc.com/products/electrophoresis/lipoprintLDLandHDL.html

Tube Gel Electrophoresis: Lipoprint

LDL Particle diameter: >268 Å (Indicative of Type A)

Total LDL cholesterol <100 mg/dL (<70 optional)
VLDL cholesterol 0-22 mg/dL
Midband C 0-13 mg/dL
Midband B 0-15 mg/dL
Midband A 0-25 mg/dL

LDL 1 Fraction 0-57 mg/dL
LDL 2 Fraction 0-37 mg/dL
LDL 3 Fraction 0-6 mg/dL
LDL 4-7 Fractions None detected.

Total cholesterol: <200 mg/dL, Desirable; 200-239 mg/dL, Borderline High; >239 mg/dL, High

DSI Laboratories www.dsilabs.com http://www.4qc.com/products/electrophoresis

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

- Single vertical spin density-gradient ultracentrifugation
- Direct measurement of lipoprotein cholesterol content, not particle concentration
- Subcurves empirically defined with "optimal fit" mathematical model
- Reports relative flotation index of particles not angstroms of diameters

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Advanced Lipoprotein Testing

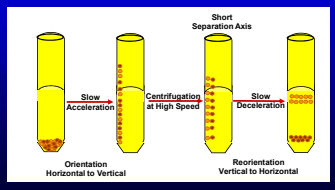
Separation by Density

Equilibrium Density Gradient Ultracentrifugation (Atherotech)

Vertical Auto Profile (VAP) Technology

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

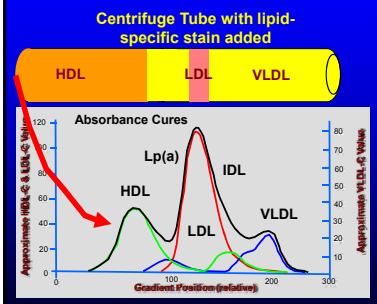
- Single vertical spin density-gradient ultracentrifugation (45 minutes)
  - Two distinct layers (KBr at 1.210 and saline at 1.006) blend to form continuous density gradient



- VLDL floats to the top
- LDL migrates to the middle
- HDL remains at the bottom

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology



Tube is placed in a fractionator apparatus and drained from the bottom into a Technicon AutoAnalyzer, where tube eluent is mixed with an enzymatic cholesterol reagent: a spectrophotometric tracing is obtained

Cholesterol profile is decomposed mathematically into individual lipoprotein species by computer

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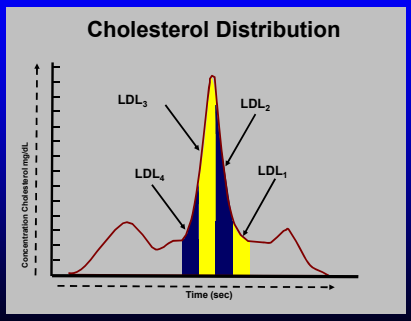
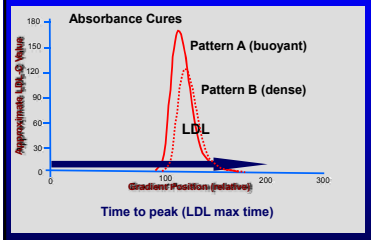
Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology



Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology



Because LDL max time decreases as the density of LDL peak increase (ie, dense LDL elutes from the tube before buoyant LDL), patient who have predominantly small and dense LDL (LDL pattern B) have lower LDL max times (≤115 seconds) compared with those who have predominantly large and buoyant LDL particles (≥118 seconds; LDL pattern A).

All patients who have LDL max times between 115 and 118 seconds are classified as having intermediate LDL pattern (LDL pattern A/B).

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

- Individual lipoproteins and their subclasses are then quantified using another software, also developed in-house, which deconvolutes the main absorbance curve into its component lipoprotein classes and subclasses.
- The deconvolution software is based on knowledge of the position and shape of individual lipoprotein peaks determined through VAP analysis of isolated lipoprotein classes
  - LDLr-C into 4 density subfractions
  - IDL-C
  - HDL-C into 5 subfractions and reported as HDL_2-C and HDL_3-C
  - VLDL-C into multiple subfractions and reported as $VLDL_3$ subfraction

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

- In contrast to other Lp(a) methods, the VAP method measures cholesterol concentration of Lp(a) particles instead of apolipoprotein (a) or Lp(a) particle concentration.
- Measuring cholesterol concentration enables VAP testing of Lp(a) without influence of apo(a) size, which is known to vary among patients.
  - Varying apo(a) size has been a major problem in the accuracy of almost all immunoassay-based methods

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

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Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

Atherotech has recently begun reporting to physicians apoB values that are not measured, but instead calculated from cholesterol information supplied by its VAP test, specifically non-HDL-C and LDL size pattern.

The LDL size information is purported to make the calculated VAP apoB, obtained from measured non-HDL-C values simply by applying a constant conversion factor, more "accurate".

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

- apoB and non-HDL-C were measured on 517 patient serum specimens and found to be highly correlated (r = 0.956, somewhat higher than correlations reported in other studies).
- By linear regression, an equation was derived relating these non-HDL-C and apoB values
  - **(apoB = 0.559 [non-HDL-C] + 19.8).**
- Non-HDL-C was measured on another 400 specimens and apoB values were calculated using the equation. This transformation does not produce particle number (apoB) information – it simply converts the cholesterol information into apoB units.
- The correlation of calculated and measured apoB values for these 400 specimens was r = 0.950.

Kulkarni KR, French KW. Clin Chem 2007;53(S6):A41.

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

Cholesterol content within major particles

Direct Measured Lipid Panel	Actual	Desirable	Risk
Total LDL-Cholesterol - Direct (CAD, Diabetes, or its equivalent – desirable range < 100 mg/dL)	166 mg/dL	<130 mg/dL	X
Total HDL-Cholesterol - Direct	57 mg/dL	≥ 40 mg/dL	
Total VLDL-Cholesterol - Direct	22 mg/dL	< 30 mg/dL	
Sum Total Cholesterol	245 mg/dL	< 200 mg/dL	X
Triglyceride - Direct	148 mg/dL	< 150 mg/dL	
Total Non HDL Cholesterol (LDL + VLDL)	188 mg/dL	< 160 mg/dL	X
Total Apo B100 (calc)	123 mg/dL	< 109 mg/dL	X

Calculated, not measured apolipoprotein B

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

Cholesterol content within Lp (a), IDL LDL particles

For Clinical Judgment in Setting LDL-C Goal	Actual	Desirable	Risk
Lp (a) Cholesterol	35 mg/dL	< 10 mg/dL	x
IDL Cholesterol	26 mg/dL	< 20 mg/dL	x
Real LDL Cholesterol	60 mg/dL	< 100 mg/dL	
Sum Total LDL-C	121 mg/dL	< 130 mg/dL	
Real LDL-C Size Pattern (Pattern B / Pattern A/B / Pattern A)	B	A	x
Remnant Lipoproteins (IDL + VLDL3)	38mg/dL	< 30 mg/dL	x

▶▶▶▶▶▶ Due to the presence of additional risk factors, consider lowering LDL-C goal

No particle size measurement, only phenotype reported

Density Gradient Ultracentrifugation Vertical Auto Profile (VAP) Technology

Subclass Information	Actual	Desirable	Risk
HDL-2 (Large, Buoyant; most protective)	6 mg/dL	> 10 mg/dL	x
HDL-3 (Small, Dense; least protective)	29 mg/dL	> 30 mg/dL	x
VLDL-3 (Small Remnant)	12 mg/d L	< 10 mg/dL	x

For Lab Use Only: Subspecies Real LDL (Cholesterol concentrations in mg/dL)

LDL_4 18 — LDL_3 2.5 (Pattern B) — LDL_2 5 — LDL_1 12 (Pattern A)

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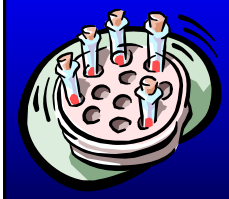
- Measuring cholesterol concentration enables VAP testing of Lp(a) without influence of apo(a) size, which is known to vary among patients.
  - Varying apo(a) size has been a major problem in the accuracy of almost all immunoassay-based methods

VAP provides Lp(a)-C concentrations not Lp(a) values

Kulkarni, KR Clin Lab Med 26 (2006) 787–802

Advanced Lipoprotein Testing

Separation by Staining

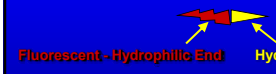


Density Gradient Ultracentrifugation & Particle Staining

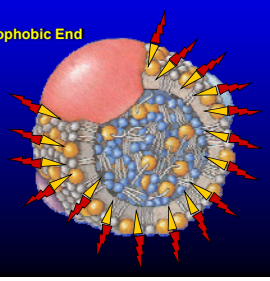
Lipoprotein Particle Profile (LPP) Technology: Spectracell Labs

Lipoprotein Particle Measurement via Ultracentrifugation & Particle Staining

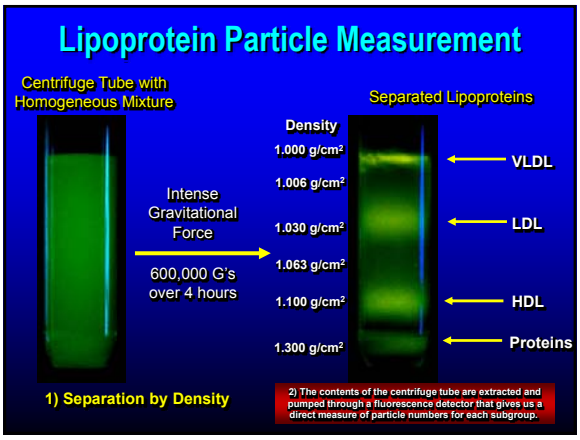
Fluorescent Dye – a Phospholipid Analog

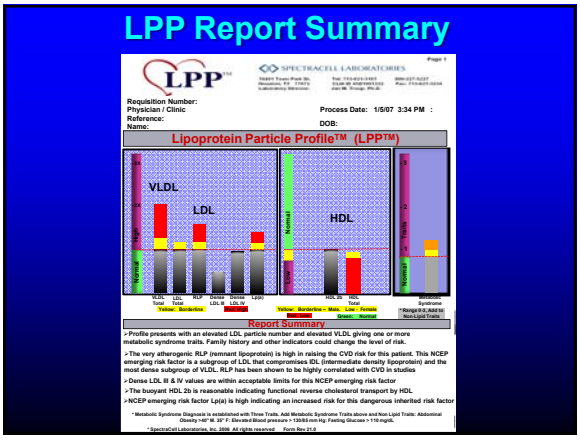


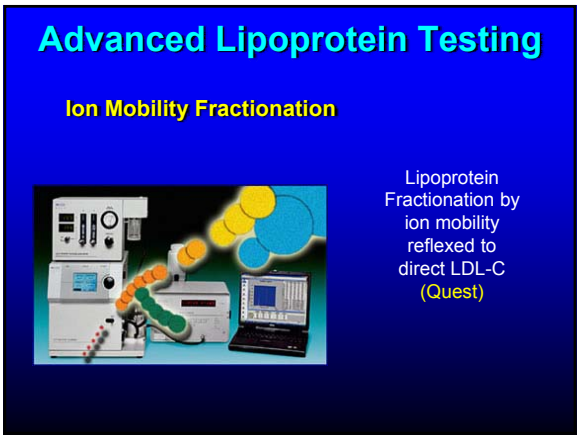
When Hydrophobic End Imbeds into the Phospholipid Shell of the Lipoprotein the Hydrophilic End Fluoresces



The Fluorescence is a Direct Measurement of Particle Number

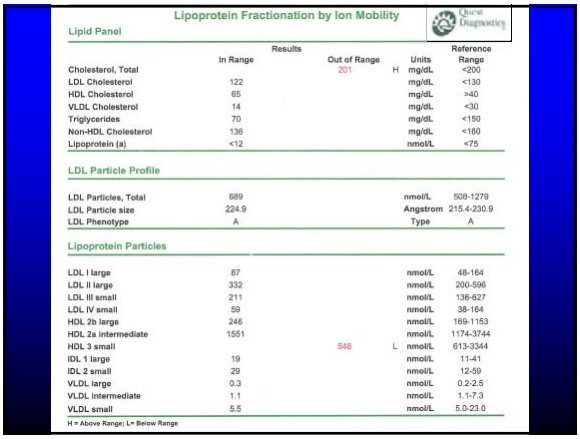

Lipoprotein Particle Measurement
Centrifuge Tube with Homogeneous Mixture
Separated Lipoproteins
Density
1.000 g/cm²
1.006 g/cm²
1.030 g/cm²
1.063 g/cm²
1.100 g/cm²
1.300 g/cm²
Intense Gravitational Force
600,000 G's over 4 hours
VLDL
LDL
HDL
Proteins
1) Separation by Density
2) The contents of the centrifuge tube are extracted and pumped through a fluorescence detector that gives us a direct measure of particle numbers for each subgroup.


LPP Report Summary
LPP
SPECTRACELL LABORATORIES
Requisition Number:
Physician / Clinic
Reference:
Name:
Process Date: 1/5/07 3:34 PM
DOB:
Lipoprotein Particle Profile™ (LPP™)
VLDL
LDL
HDL
Report Summary
Profile presents with an elevated LDL particle number and elevated VLDL giving one or more metabolic syndrome traits. Family history and other indicators could change the level of risk.
The very atherogenic RLP (remnant lipoprotein) is high in raising the CVD risk for this patient. This NCEP emerging risk factor is a subgroup of LDL that compromises IDL (intermediate density lipoprotein) and the most dense subgroup of VLDL. RLP has been shown to be highly correlated with CVD in studies
Dense LDL III & IV values are within acceptable limits for this NCEP emerging risk factor
The buoyant HDL 2b is reasonable indicating functional reverse cholesterol transport by HDL
NCEP emerging risk factor Lp(a) is high indicating an increased risk for this dangerous inherited risk factor


Advanced Lipoprotein Testing
Ion Mobility Fractionation
Lipoprotein Fractionation by ion mobility reflexed to direct LDL-C (Quest)

Ion Mobility Fractionation

- Ion mobility analysis measures the size distribution and count the number of individual particles in all classes of lipoproteins in a single analytical step.
- The technology measures the drift of charged, aerosolized lipoproteins as they are dragged through air by the force of an electric field.
- Charge and drift velocity separate the particles by weight and size. The sorted particles travel to a detector for counting.



Lipoprotein Fractionation by Ion Mobility

Lipid Panel

	Results			Units	Reference Range
	In Range	Out of Range			
Cholesterol, Total		201	H	mg/dL	<200
LDL Cholesterol	122			mg/dL	<130
HDL Cholesterol	65			mg/dL	>40
VLDL Cholesterol	14			mg/dL	<30
Triglycerides	70			mg/dL	<150
Non-HDL Cholesterol	136			mg/dL	<160
Lipoprotein (a)	<12			nmol/L	<75
LDL Particle Profile					
LDL Particles, Total	689			nmol/L	508-1279
LDL Particle size	224.9			Angstrom	215.4-230.9
LDL Phenotype	A			Type	A
Lipoprotein Particles					
LDL I large	87			nmol/L	48-164
LDL II large	332			nmol/L	200-596
LDL III small	211			nmol/L	136-627
LDL IV small	59			nmol/L	38-164
HDL 2b large	246			nmol/L	169-1153
HDL 2a intermediate	1551			nmol/L	1174-3744
HDL 3 small		548	L	nmol/L	613-3344
IDL 1 large	19			nmol/L	11-41
IDL 2 small	29			nmol/L	12-59
VLDL large	0.3			nmol/L	0.2-2.5
VLDL intermediate	1.1			nmol/L	1.1-7.3
VLDL small	5.5			nmol/L	5.0-23.0

H = Above Range; L= Below Range

Advanced Lipoprotein Testing

Nuclear Magnetic resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy
(LipoScience)

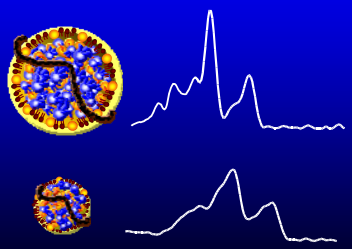
NMR LipoProfile

NMR Lipoprotein Analysis

- **NMR spectroscopic** analysis does not require physical fractionation of lipoproteins
- NMR provides access to lipoprotein quantification data, not based on apolipoproteins or cholesterol measurements

NMR Lipoprotein Analysis

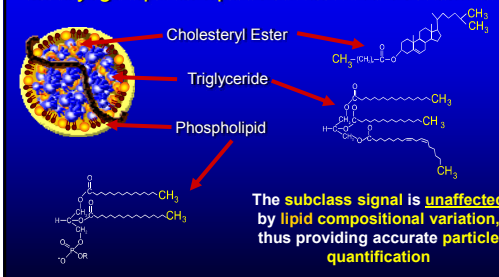
Lipoprotein subclasses of different size broadcast lipid NMR signals that **are naturally distinguishable**. The measured amplitudes of these signals provide subclass quantification.



Otvos JD. Handbook of Lipoprotein Testing, AACC Press 2000

NMR Lipoprotein Analysis

Schematic of lipoprotein lipid structure showing the methyl groups that produce the subclass NMR signal



The subclass signal is unaffected by lipid compositional variation, thus providing accurate particle quantification

Otvos JD. Handbook of Lipoprotein Testing, AACC Press 2000

NMR Lipoprotein Analysis

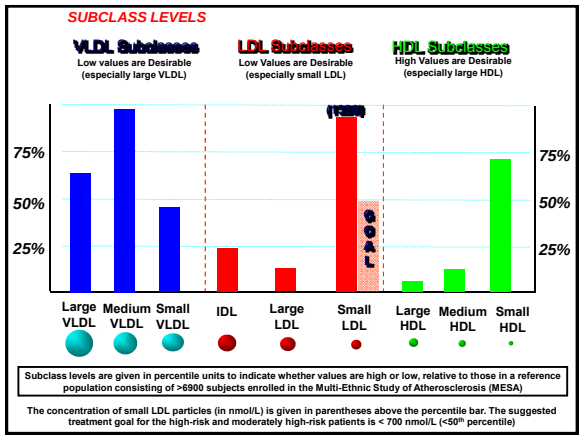
- The subclass signal is contributed by the aggregate number of terminal methyl groups on lipids within the particle
- The number of methyl groups depends only on the particle diameter and is not affected by lipid composition of the particle
- The methyl NMR signal emitted by each subclass serves as a direct measurement of that subclass

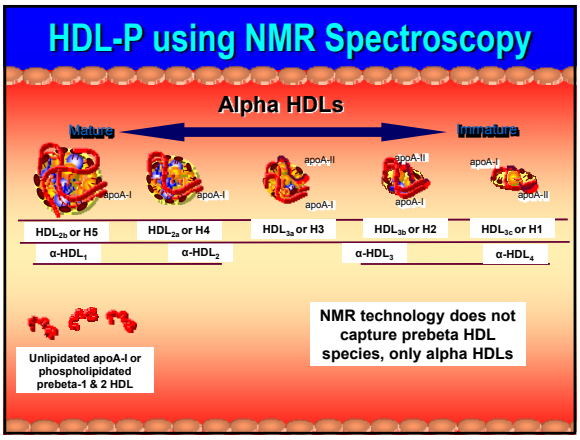
Otvos J. J Lab Med 2002;26:544-550

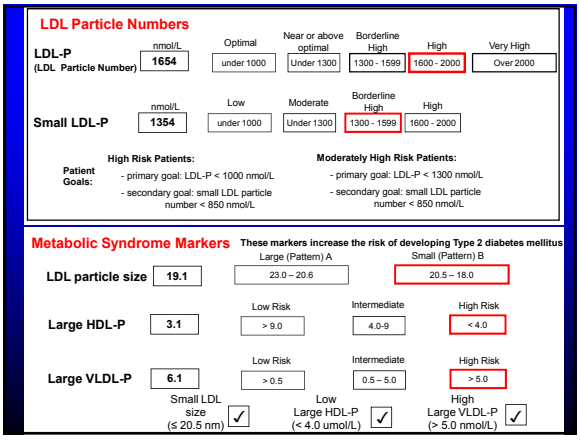
NMR Lipoprotein Analysis

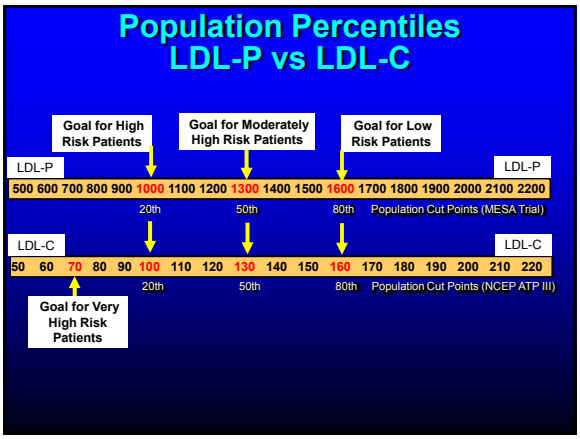
- Low-density lipoprotein subclass distributions determined by NMR and gradient gel electrophoresis are highly correlated.
- Low-density lipoprotein subclass diameters, which are consistent with electron microscopy data, are uniformly 5 nm smaller than those estimated by gradient gel electrophoresis.

Blake GJ, et al. Circulation 2002;106:1930 –7.
Rumsey SC, et al. J Lipid Res 1992;33:1551– 61.

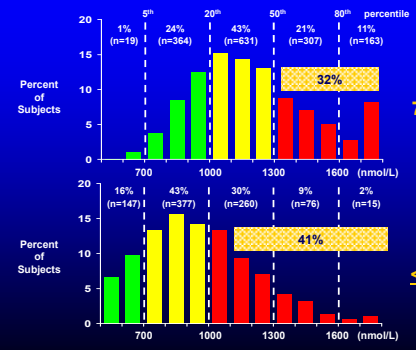



HDL-P using NMR Spectroscopy
Alpha HDLs
Mature
Immature
apoA-I
apoA-II
HDL2b or H5
HDL2a or H4
HDL3a or H3
HDL3b or H2
HDL3c or H1
α-HDL1
α-HDL2
α-HDL3
α-HDL4
Unlipidated apoA-I or phospholipidated prebeta-1 & 2 HDL
NMR technology does not capture prebeta HDL species, only alpha HDLs


LDL Particle Numbers
LDL-P
(LDL Particle Number)
nmol/L
1654
Optimal
under 1000
Near or above optimal
Under 1300
Borderline High
1300 - 1599
High
1600 - 2000
Very High
Over 2000
Small LDL-P
nmol/L
1354
Low
under 1000
Moderate
Under 1300
Borderline High
1300 - 1599
High
1600 - 2000
Patient Goals:
High Risk Patients:
- primary goal: LDL-P < 1000 nmol/L
- secondary goal: small LDL particle number < 850 nmol/L
Moderately High Risk Patients:
- primary goal: LDL-P < 1300 nmol/L
- secondary goal: small LDL particle number < 850 nmol/L
Metabolic Syndrome Markers
These markers increase the risk of developing Type 2 diabetes mellitus
Large (Pattern) A
Small (Pattern) B
LDL particle size
19.1
23.0 – 20.6
20.5 – 18.0
Large HDL-P
3.1
Low Risk
> 9.0
Intermediate
4.0-9
High Risk
< 4.0
Large VLDL-P
6.1
Low Risk
> 0.5
Intermediate
0.5 – 5.0
High Risk
> 5.0
Small LDL size (≤ 20.5 nm)
Low Large HDL-P (< 4.0 umol/L)
High Large VLDL-P (> 5.0 nmol/L)


Population Percentiles
LDL-P vs LDL-C
Goal for High Risk Patients
Goal for Moderately High Risk Patients
Goal for Low Risk Patients
LDL-P
LDL-P
500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200
20th
50th
80th
Population Cut Points (MESA Trial)
LDL-C
LDL-C
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
20th
50th
80th
Population Cut Points (NCEP ATP III)
Goal for Very High Risk Patients

LDL Particle Number Distribution in T2DM Subjects



Cromwell W & Otvos J (Am J Cardiol 2006;98:1599–1602

Framingham Heart Study: Offspring Cohort

►In multivariable models adjusting for non-lipid CVD risk factors, LDL-P was related more strongly to future CVD in both sexes than LDL-C or non- HDL-C in 3066 patients.

► Subjects with a low level of LDL-P (<25th percentile) had a lower CVD event rate (59 events per 1000 person-years) than those with an equivalently low level of LDL-C or non-HDLC (81 and 74 events per 1000 person-years, respectively).

►LDL particles are more cholesterol-depleted when LDL concentrations are lower, independent of triglycerides or LDL particle size, helps to explain why patients with low LDL-C often have disproportionately higher numbers of LDL particles

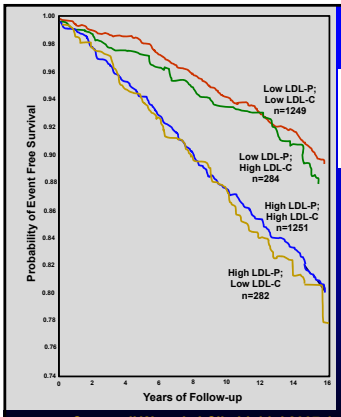
Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►Low LDL particle number was a better index of low CVD risk than low LDL-C.

►Non-HDL-C provided risk prediction intermediate between LDL particle number and LDL-C, with evidence suggesting that the better prediction relative to LDL-C was due les to non-HDL-C including atherogenic triglyceride-rich particles (VLDL and remnants) and more to its strong correlation with LDL particle number.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress



Framingham Heart Study Offspring Cohort

Event-free survival among participants with low-density lipoprotein cholesterol (LDL-C) and LDL particle number (LDL-P) above or below the median. Median values were 131 mg/dL for LDL-C and 1414 nmol/L for LDL-P.

When data for men and women were combined, LDL-P was approximately twice as strongly related to CVD incidence as LDL-C

LDL-P was strongly associated with increased CVD risk in both men and women (p<0.0001)

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Framingham Heart Study: Offspring Cohort

►The novel finding is that LDL particle are more cholesterol-depleted when LDL concentrations are lower, independent of triglyceride or LDL particle size.

►This helps to explain why patients with low LDL-C often have disproportionately higher numbers of LDL particles

►Our data show that persons with this LDL disconnect have higher CVD risk. It is therefore reasonable to anticipate that such discordant individuals would derive clinical benefit from more intensive LDL lowering than would have been indicated by their LDL-C level.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►Estimates of the cholesterol content of the LDL particles of individual subjects were obtained by dividing LDL-C (in mmol/L units, obtained by multiplying the mg/dL mass concentrations by 0.0259) by LDL-P (nmol/L).

►This ratio provides the approximate number of cholesterol molecules per LDL particle.

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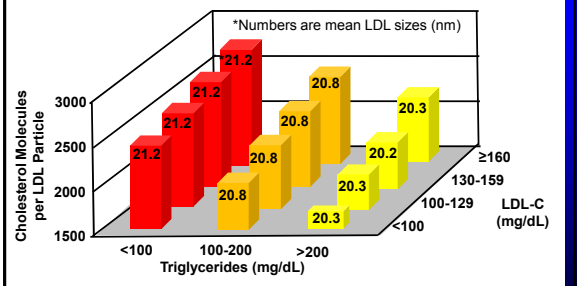
Framingham Heart Study: Offspring Cohort

►The amount of cholesterol per LDL particle varied substantially in the study population not only as a function of triglyceride level, but also as a function of LDL concentration. Within each triglyceride subgroup, the lower the LDL level, the lower was the amount of cholesterol per particle.

►This progressive cholesterol compositional depletion of LDL particles at lower LDL concentrations was not associated with smaller LDL particle sizes.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort



Cholesterol content of low-density lipoprotein (LDL) particles in subgroups defined by levels of LDL cholesterol (LDL-C) and triglycerides

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►LDL-P was strongly associated with increased CVD risk in both men and women ($p<0.0001$), though less strongly in men. LDL-C was not associated with CVD in men ($p=0.33$) and LDL-C was only modestly associated with CVD risk in women ($p=0.03$). When data for men and women were combined, LDL-P was approximately twice as strongly related to CVD incidence as LDL-C (□-coefficient 0.24 for LDL-P vs 0.11 for LDL-C).

►Non-HDL-C, which includes contributions from the cholesterol in VLDL as well as LDL, was more strongly associated with CVD than LDL-C in both men and women, but was less predictive of CVD events than LDL-P.

►Adding VLDL-P to LDL-P only very marginally strengthened CVD associations compared to LDL-P alone.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►LDL cholesterol levels under-represent the number of LDL particles in persons with relatively cholesterol-poor particles

► As expected, because the particles are smaller, we found cholesterol-poor LDL among individuals with elevated triglycerides.

►But irrespective of triglyceride level and LDL size, individuals with low LDL concentration also have cholesterol-poor particles.

►This interesting finding suggests that simply having low LDL levels, either naturally or as a result of LDL-lowering therapy, can create a discrepancy between LDL-C and LDL particle number and contribute to the underestimation of both LDL and CVD risk by measured levels of LDL-C.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►Among individuals with low LDL-C (quartile 1), most had concordantly low LDL-P (quartile 1) and a low CVD risk.

►However, a substantial subset (21%) had higher LDL-P and these discordant individuals had a higher CVD event rate.

►The results suggest that low LDL particle numbers may be a better indicator of low risk than equivalently low LDL cholesterol values.

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Framingham Heart Study: Offspring Cohort

►The data also indicate that LDL particles become progressively cholesterol depleted as LDL concentrations decrease.

►This relationship is independent of triglyceride level and is not associated with any change in LDL size.

►We speculate that the cause of this particle size-independent cholesterol compositional change is the lipid exchange reaction mediated by cholesterol ester transfer protein (CETP)

►Even with serum triglyceride (VLDL) levels that are not elevated, LDL particles can become cholesterol-depleted and triglyceride-enriched if LDL concentrations are low

Cromwell W et al. J Clin Lipidol 2007 http://www.lipidjournal.com/inpress

Clinical Chemistry 52:9
1722–1727 (2006)

Lipids, Lipoproteins, and Cardiovascular Risk Factors

Disparate LDL Phenotypic Classification among 4 Different Methods Assessing LDL Particle Characteristics

Wayne Ensign,[1] Nicole Hill,[2] and Christopher B. Heward[2]

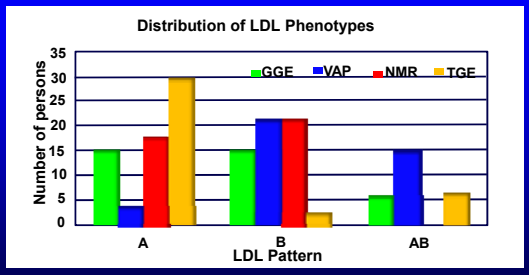
- Study seeking to clarify the differences in analytical results of 4 leading technologies used for analysis of LDL subfractions:
  - Gradient Gel Electrophoresis (GGE)
  - Ultracentrifugation – vertical auto profile (VAP)
  - Nuclear magnetic resonance (NMR)
  - Tube Gel Electrophoresis (TGE)
- 4 samples from 40 persons were analyzed

Distribution of LDL Phenotypes

- There was substantial heterogeneity of results and interpretations among 4 methods.
- Complete agreement among methods with respect to LDL subclass phenotyping occurred in only 8% (n=3) of the persons studied.
- NMR and GGE agreed most frequently at 70% (n = 28), whereas VAP matched least often.
- As measurement of LDL subclasses becomes increasingly important, standardization of methods is needed.
- Variation among currently available methods renders them unreliable and limits their clinical usefulness.

Ensign W, Hill N & Heward CB. Clin Chem 2006;59:1722-1727

Distribution of LDL Phenotypes



Histogram showing the number of persons categorized into each of the three LDL pattern types (A,B or AB) by each of the four measurement methods

Ensign W, Hill N & Heward CB. Clin Chem 2006;59:1722-1727

LDL-P vs Apo B & CVD Risk

Four studies have included measures of plasma apo B (highly correlated with LDL apo B) and NMR LDL-P, allowing comparison of the strengths of their disease associations. In all four studies, apo B was related less strongly to CVD than to LDL-P.

VA-HIT: Circulation 2006;113:1556–63.

Women's Health Study: Circulation 2002;106:1930–7.

John Hopkins' Sibling Study: JACC 2002;39:274A

VTE Study in men: Circulation 2005;112:893–9.

Jeyarajah EJ et al. Clin Lab Med 26 (2006) 847–870

LDL-P vs Apo B and CVD Risk

The reasons why apo B has so far exhibited a weaker relationship with CVD outcomes compared with LDL-P are not understood and deserve further investigation.

Speculation has centered on the apparently better measurement precision of the NMR assay and the fact that plasma apo B is only a surrogate for LDL particle number because of the inclusion of variable numbers of VLDL particles.

apo B, relative to LDL-P, "undervalues" small LDL particles compared with large LDL particles. A possible reason is that apo B adopts a substantially different conformation on small LDL than it does on large LDL, potentially causing differential exposure of the epitopes and differential antibody binding.

Jeyarajah EJ et al. Clin Lab Med 26 (2006) 847–870