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.
Understanding Lipid & Lipoprotein Testing

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 Strissower, 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

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

Thomas Dayspring MD, FACP
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.
- VLDL, Chylomicrons, and LDL
- HDL and serum proteins

Ultracentrifugation at 1.063 kg/L

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 1.065 – 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.

**Classic Lipoprotein Testing**

**Separation by Electrophoresis**


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**Electrophoresis**

![Electrophoresis Diagram](Image)


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**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

Understanding Lipid & Lipoprotein Testing

**Alpha Lipoproteins**

- Mature 
- Immature

<table>
<thead>
<tr>
<th>HDL, or H5</th>
<th>HDL, or H4</th>
<th>HDL, or H3</th>
<th>HDL, or H2</th>
<th>HDL, or H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HDL5</td>
<td>α-HDL4</td>
<td>α-HDL3</td>
<td>α-HDL2</td>
<td>α-HDL1</td>
</tr>
</tbody>
</table>

Prebeta HDLs

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

- 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**


- Type I: ↑ chylomicrons
- Type IIb: ↑ LDL + VLDL
- Type V: ↑ Chylo + VLDL
- Type III: Broad Band Beta
- Type IV: Presence of chylo
- Type IIa: ↑ Chylo + VLDL

- T Chylomicrons
- T LDL + VLDL
- T Chylo + VLDL
- N LDL, VLDL, HDL
- Broad Band Beta
- T VLDL
- Presence of chylo
- T LDL
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 β lipoproteins, even when the blood cholesterol is normal.

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 (Rf = 1) mobility similar to albumin
  - Pre-alpha: Pre-α (Rf > 1) mobility faster than albumin
  - Pre-beta: Pre-β (Rf < 1) mobility slower than albumin

- In the second dimension (size characterization), the particles (12) were differentiated on nondenaturing gel electrophoresis by modal diameters.

HDL Subpopulations by Two Dimensional Electrophoresis and Surface Charge

- Separation by size: Nondenaturing concave polyacrylamide gel electrophoresis & immunolocalization.

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

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

Mature

Immature

Prebeta HDLs

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

Alpha HDLs

HDL_{3b} or H5
HDL_{2b} or H4
HDL_{1a} or H3
HDL_{1b} or H2
HDL_{1c} or H1

Prebeta and α-HDL species 2 dimensional electrophoresis
HDL_{1a}, HDL_{1b}, HDL_{1c}, HDL_{2a}, HDL_{2b}, HDL_{2c}, HDL_{3a}, HDL_{3b}, HDL_{3c}

The functionality of different HDL subfractions appears to vary substantially.

Of the known forms of HDL, pre-beta HDL appears to be the most antiatherogenic form.

Singh IM et al. JAMA. 2007;298(7):786-798

Prebeta and alpha HDL species 2 dimensional electrophoresis
HDL_{1a}, HDL_{1b}, HDL_{1c}, HDL_{2a}, HDL_{2b}, HDL_{2c}, HDL_{3a}, HDL_{3b}, HDL_{3c}

High Density Lipoprotein Cholesterol: The Editor’s Roundtable

H Bryan Brewer

• We do not have sufficient information about the clinical utility of HDL subfractions to warrant their measurement in the clinical setting.
• 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.


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**

- TC is an apoB surrogate
- HDL-C is the lipid surrogate of apoA-I
- TC/HDL-C or LDL-C/HDL-C ratios are apoB/A-I surrogates
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.


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.

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

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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.


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

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.

Thomas Dayspring MD, FACP
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.

The Kringle domains interact with plasminogen activators and plasmin binding sites on endothelial surfaces.

Lp(a) is a beta-lipoprotein consisting of an LDL particle to which a large glycoprotein, apolipoprotein (a), is covalently bonded.
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.

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.

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 in susceptibility to atherosclerosis, specifically CAD. 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.
Three Common Human apoE Alleles

<table>
<thead>
<tr>
<th>Parent Form</th>
<th>ApoE3</th>
<th>Cys</th>
<th>Arg</th>
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<tr>
<td>Variant</td>
<td>ApoE4</td>
<td>Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Variant</td>
<td>ApoE2</td>
<td>Cys</td>
<td>Cys</td>
</tr>
</tbody>
</table>

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.

Apolipoprotein E Genotype and CAD Risk

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

Data from 21,331 cases and 47,467 controls in studies with 500 or more cases
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.

- 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.
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

Reported LDL-C

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

Handbook of Lipoprotein Testing 2nd Ed 2000 AACC Press Washington DC
Apolipoprotein B & A-I Surrogates

VLDL-C
HDL-C
LDL-C
Total Cholesterol

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.
Friedewald Calculated LDL-C

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


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

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCVS-13:</td>
<td>Quebec Cardiovascular Study 13 year follow up</td>
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<tr>
<td>THROMBO MS LIPID</td>
<td>Thrombogenic Factors &amp; Recurrent Coronary Events Metabolic Synd</td>
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<tr>
<td>AFCAPS/TexCAPS 4S</td>
<td>Long-term Intervention with Pravastatin in Ischemic Disease</td>
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<tr>
<td>Womans HS</td>
<td>Air Force Texas Coronary Atherosclerosis Prevention Study</td>
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<tr>
<td>THROMBO NPHS</td>
<td>Scandinavian Simvastatin Survival Study</td>
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<tr>
<td>AMORIS</td>
<td>Women’s Heart Study</td>
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<tr>
<td>QCVS-5</td>
<td>Thrombogenic Factors &amp; Recurrent Coronary Events</td>
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<tr>
<td></td>
<td>Apolipoprotein-related Mortality Risk</td>
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<tr>
<td></td>
<td>Northwick Park Heart Study</td>
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<tr>
<td></td>
<td>Apolipoprotein-related Mortality Risk</td>
</tr>
<tr>
<td></td>
<td>Quebec Cardiovascular Study 5 year follow up</td>
</tr>
</tbody>
</table>

Understanding Lipid & Lipoprotein Testing

**LDL Particle Subclass**

- 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-P** = # of LDL particles in a liter of plasma

**LDL Particles in Patients with Elevated TG**

- The LDL particle composition shifts with more TG and less cholesterol.
- CETP (Cholesteryl Ester Transfer Protein) reduces LDL-C.
- Hepatic Lipase causes further lipolysis (hydrolysis of TG & phospholipids) which reduces the size of the LDL particle.

**Fate of TG-rich LDL Particles**

- LDL-C is reduced.
- LDL particle size is reduced.
Relationship of Triglycerides and LDL Particle Size


Relationship of Small LDL to Triglyceride/HDL-C Ratio


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.

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 Receptors (LDLr):
- Smaller LDL particle (Pattern B)
- Very Large LDL particle (Pattern A)
- Normal sized LDL particle (Pattern A)

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
As the lipolytic cascade (degradation via lipase of triglycerides 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)**

- TG and Cholesteryl ester
- Phospholipid
- Free cholesterol
- TG and Cholesteryl ester
- Phospholipid
- Free cholesterol
- TG and Cholesteryl ester
- Phospholipid
- Free cholesterol
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


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

Jian Liu, Scott Grundy et al. Am J Cardiol 2006;98:1363-1368
Understanding Lipid & Lipoprotein Testing

VLDL Particles in Patients with Elevated TG

CE-rich VLDL Particle

VLDL Remnant

Increased VLDL-C

The CE rich VLDL is subject to lipolysis by LPL

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.

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.


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.
- Perform 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.

HDL-cholesterol Concentration

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

HDL-C primarily reflects cholesterol levels within large, cholesterol-rich particles and lacks sensitivity to detect small, cholesterol-poor particles.

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

The apoB particles are now more cholesterol-enriched

One molecule of CE exchanged for one of TG

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

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**Indirect RCT at the Hepatocyte**

Hepatocyte

LDL receptors

Cholesteryl ester

Bile Acid Synthesis

ABCA1

ABCG4

ABCG5/8

LDL receptor endocytosis of LDL particles carrying cholesteryl ester acquired from HDL

Bile Duct

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

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**Additional High Density Lipoprotein Remodeling**

Hepatic Sinusoid

Smaller HDL is subject to renal excretion or it can be relipidated with cholesterol

HDLs remaining TG undergo further lipolysis by hepatic lipase which has both TG-lipase and phospholipase activity

Larger TG-rich α-HDL

Prebeta2, or α-HDL


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Understanding Lipid & Lipoprotein Testing

Direct Reverse Cholesterol Transport

- e-HDLs are internalized by hepatocyte LDLr, and Holoparticle receptors or delipidated by hepatic or enterocyte SR-B1

Macrohage Reverse Cholesterol Transport

- Increased concentrations of cellular sterols cause enhanced expression of Liver X receptor-α (LXR) causing apoAI-induced cholesterol efflux from macrophages.

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)

For HDL-C < 40 mg/dL, increasing HDL-C is accompanied 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 appearing alongside the decrease in numbers of the smaller HDL subclasses. This is probably due to a product-precursor relationship between these species.

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, AT, 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.
Lipoprotein Testing

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.

Non HDL-C = TC – HDL-C

Cromwell WC et al J Clin Lipidology 2007

Since LDL-P makes up more than 90-95% of apoB particles, Non HDL-C is in effect an LDL-P surrogate.

Cromwell WC et al J Clin Lipidology 2007
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.

LDL-C (mg/dL) | Non HDL-C (mg/dL) | Risk Ratio
--- | --- | ---
< 130 | < 160 | 0
130-159 | < 160 | 0.5
160-189 | < 160 | 1.0
≥ 190 | < 160 | 1.5
TG ≥ 200 (mg/dL) | Non HDL-C (mg/dL) | Risk Ratio
--- | --- | ---
< 130 | < 160 | 0
130-159 | < 160 | 0.5
160-189 | < 160 | 1.0
≥ 190 | < 160 | 1.5
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.

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.

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

Carotid IMT Carotid Intimomedial Thickness Studies (4)
CMS Casale Montefiora Study
HHMS Harvard healthy Men Study
AMORIS Apolipoprotein-related Mortality Risk
Womens HS Women’s Heart Study
J-DM Jiang Diabetes Mellitus

ApoB Superior as a Predictor

Equal Predictors of Risk

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.
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

Austin et al. Arteriosclerosis 1990;10:520
Densitometry scans give graphical output showing major LDL peak particle diameter (LDL-PPD)


A pattern with a high concentration of small LDL particles (i.e., 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 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.


Reverse Cholesterol Transport

Segmented HDL Subclass Determination
GGE HDL Populations in T2DM

In T2DM there is a shift from 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).

12.9 9.7 8.8 8.2 7.8 7.2 nm
2b 2a 3a 3b 3c HDL 2b 2a 3a 3b 3c HDL

Non-diabetic Subjects
Type 2 diabetic Subjects


Clinical Test Summary

Test
Result
Alert
Value
Reference Population (10-90%)

BHL Goal †

Total Cholesterol (mg/dL) 147 160.0 180.0
LDL-C (mg/dL) 42 50.0 70.0
HDL-C (mg/dL) 66 50.0 70.0
Lipoprotein(a) (mg/dL) 124 40.0 140.0
Rf & percent (mg/dL) 96 61 - 114
Peak 1 (nm) 26.3
Peak 2 (nm) 26.2

LDL Particle sizes are measured in angstroms (Å)

LDL Peak 1 (Å) 263 254 257 263.4 263.5
LDL Peak 2 (Å) 253 254 257 263.4 263.5
Peak Diameter (Å) 263.5 285 257.4 263.4 257.5

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

L19387.bio:16
L19387.bio:17

IIIa + IIIb (%)
IVb (%) 1.7
28.6 > 20 13.7 – 41.3
1.5 – 9.5 < 5
< 15

Clinical Test Summary

Berkeley HeartLab, Inc.
advanced cardiovascular informatics

1 = LDL I (%) 20.2
18.8
28.1
23.7
4.9
2.7
1.7

2 = LDL IIa (%)
3 = LDL I1b (%)
4 = LDL IIIa (%)
5 = LDL IIIb (%)
6 = LDL IVa (%)
7 = LDL IVb (%)

LDL Particle sizes are measured in angstroms (Å)

LDL Pattern Interpretation

Pattern A (Large LDL)
Pattern Int (Intermediate LDL)
Pattern B (Small LDL)

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

Test
Result
Alert
Value
Reference Population (10-90%)

BHL Goal †

Total Cholesterol (mg/dL) 147 160.0 180.0
LDL-C (mg/dL) 42 50.0 70.0
HDL-C (mg/dL) 66 50.0 70.0
Triglycerides (mg/dL) 147 142 137
Apo B particle # (mg/dL) 187 146-257
Apo E Genotype 3/3

† <200 *<100 *> 40*< 150 *< 60*

1 Near Optimal / Above Optimal
2 Normal
3 Apo E 3/3
4 High LDL IIIa + IIIb with LDL Pattern Intermediate
5 Low HDL2b

損害for specific details of clinical implications

Clinical Test Summary

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).

Rf = Retardation Factor

Tube Gel Electrophoresis: Lipoprint

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

Rf = Retardation Factor
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 known 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.


Tube Gel Electrophoresis: Lipoprint Report

VLDL C D B A 1 2 3 4 5 6 7

Quantimetrix Lipoprint System

25 23 15 6 0 0 0

© Laboratories www.dsilube.com

Tube Gel Electrophoresis: Lipoprint

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

Total LDL cholesterol <100 mg/dL (<70 optional)

VLDL cholesterol 0-22 mg/dL

Midband C 0-15 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


Advanced Lipoprotein Testing

Separation by Density

Equilibrium Density Gradient Ultracentrifugation (Atherotech)

Vertical Auto Profile (VAP) Technology

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

Density Gradient Ultracentrifugation
Vertical Auto Profile (VAP) Technology

Cholesterol Distribution

Because LDL max time decreases as the density of LDL peak increases (ie, dense LDL elutes from the tube before buoyant LDL), patients who have predominantly small and dense 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).


Density Gradient Ultracentrifugation
Vertical Auto Profile (VAP) Technology

Time to peak (LDL max time)

Pattern A (buoyant)

Pattern B (dense)

Because LDL max time decreases as the density of LDL peak increases (ie, dense LDL elutes from the tube before buoyant LDL), patients who have predominantly small and dense 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).

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:
- LDL-C into 4 density subfractions
- IDL-C
- HDL-C into 5 subfractions and reported as HDL2-C and HDL3-C
- VLDL-C into multiple subfractions and reported as VLDL3 subfraction

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.

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
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

\[ \text{apoB} = 0.559 \times \text{non-HDL-C} + 19.8. \]

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.

### Density Gradient Ultracentrifugation

**Vertical Auto Profile (VAP) Technology**

#### Cholesterol content within Lp (a), IDL LDL particles

<table>
<thead>
<tr>
<th>For Clinical Judgment in Setting LDL-C Goal</th>
<th>Actual</th>
<th>Desirable</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp (a) Cholesterol</td>
<td>35 mg/dL</td>
<td>&lt; 16 mg/dL</td>
<td></td>
</tr>
<tr>
<td>IDL Cholesterol</td>
<td>26 mg/dL</td>
<td>&lt; 20 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Real LDL Cholesterol</td>
<td>60 mg/dL</td>
<td>&lt; 165 mg/dL</td>
<td></td>
</tr>
</tbody>
</table>

#### Sum Total LDL-C

- **Actual:** 121 mg/dL
- **Desirable:** < 130 mg/dL
- **Risk:**  
  - Pattern B
  - Pattern A

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

#### Density Gradient Ultracentrifugation

**Vertical Auto Profile (VAP) Technology**

#### LDL subclass Information

<table>
<thead>
<tr>
<th>Actual</th>
<th>Desirable</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-2 (Large, Buoyant; most protective)</td>
<td>6 mg/dL</td>
<td>&gt; 16 mg/dL</td>
</tr>
<tr>
<td>HDL-3 (Small, Dense; least protective)</td>
<td>23 mg/dL</td>
<td>&gt; 33 mg/dL</td>
</tr>
<tr>
<td>VLBD-3 (Small Remnant)</td>
<td>12 mg/dL</td>
<td>&lt; 16 mg/dL</td>
</tr>
</tbody>
</table>

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

- LDL2 18 mg/dL
- LDL3 2.5 mg/dL
- LDL4 5 mg/dL
- LDL5 12 mg/dL


---

#### 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
  
  \[ \text{apoB} = 0.559 \times \text{non-HDL-C} + 19.8 \]

- 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 $r = 0.950$.

---

**Thomas Dayspring MD, FACP**
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.

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


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

Fluorescent - Hydrophilic End

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

The Fluorescence is a Direct Measurement of Particle Number
Lipoprotein Particle Measurement

1) Separation by Density

- Intense Gravitational Force
- 600,000 G's over 4 hours

- Density
  - 1,000 g/cm²
  - 1,016 g/cm²
  - 1,063 g/cm²
  - 1,126 g/cm²
  - 1,300 g/cm²

- Separated Lipoproteins
  - VLDL
  - LDL
  - HDL
  - Proteins

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

- Lipoprotein Particle Profile (LPP)

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.

<table>
<thead>
<tr>
<th>Lipoprotein Fractionation by Ion Mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Panel</td>
</tr>
<tr>
<td>Cholesterol, Total</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
</tr>
<tr>
<td>Triglycerides</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
</tr>
</tbody>
</table>

Lp(a) Particle Profile

<table>
<thead>
<tr>
<th>Lipoprotein Profile</th>
<th>Range</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL particles, Total</td>
<td>mg/dL</td>
<td>mg/dL</td>
</tr>
<tr>
<td>LDL particles, small</td>
<td>mg/dL</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>mg/dL</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Advanced Lipoprotein Testing

Nuclear Magnetic resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy

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.

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

The subclass signal is unaffected by lipid compositional variation, thus providing accurate particle quantification.
Understanding Lipid & Lipoprotein Testing

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

Olmos 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.


The concentration of small LDL particles (in nmol/L) is given in parentheses above the percentile bar. The suggested treatment goal for the high-risk and moderately high-risk patients is < 700 nmol/L (<50th percentile)

Subclass levels are given in percentile units to indicate whether values are high or low, relative to those in a reference population consisting of MESA subjects enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA)

Thomas Dayspring MD, FACP
HDL-P using NMR Spectroscopy

**Alpha HDLs**

- α-HDL₂ or HDL₂
- α-HDL₁ or HDL₁

NMR technology does not capture prebeta HDL species, only alpha HDLs

**HDL-P using NMR Spectroscopy**

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

- **Mature** apoA-I
- **Immature** apoA-I

**LDL Particle Numbers**

- **Optimal:** LDL-P < 100 nmol/L
- **Borderline high:** 100 to 199 nmol/L
- **High:** 200 to 299 nmol/L
- **Very high:** 300 nmol/L or above

**Patient Goals:**

- **High Risk Patients:**
  - Primary goal: LDL-P < 100 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**

- **LDL particle size**
  - Large (Pattern) A
  - Small (Pattern) B

- **HDL-P**
  - < 4.0 umol/L

- **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**
- **Goal for Very High Risk Patients**

**Populations Cut Points (MESA Trial)**

- **50th**
- **80th**
- **90th**

**Populations Cut Points (NCEP ATP III)**

- **20th**

**Thomas Dayspring MD, FACP**
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-HDL-C (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.

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 less to non-HDL-C including atherogenic triglyceride-rich particles (VLDL and remnants) and more to its strong correlation with LDL particle number.
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).

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 1444 nmol/L for LDL-P.

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.

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.
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.

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.
Understanding Lipid & Lipoprotein Testing

**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.


**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.


**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.

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. 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.

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.

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


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.