Advanced Testing for Cardiovascular Risk

The New Gold Standard for Lipoprotein Analysis
Cardiovascular Disease Mortality Trends for Males and Females

United States: 1979-2002

Source: CDC/NCHS.
Evolution of Lipoprotein Testing

"The Lipid Panel"

Total Cholesterol = VLDL + LDL + HDL

Friedewald Equation: VLDL = TG/5

Calculated LDL = TC – (HDL + TG/5)

Direct LDL is Better

Consensus Statements: American Diabetes Association & American College of Cardiology (2008) and now the National Lipid Association (2011)

Lipoprotein Particles or Apo B-100 Predict Risk Better than Cholesterol

Lipoprotein Particle Numbers by Subgroup is Best
NCEP Guidelines for Cardiovascular Disease

NCEP - ATP III

- 50% of at Risk Individuals are **Not Identified**

- 50% of Heart Attack Victims have **Normal Cholesterol**

- NCEP Identified a Number of **New Lipoprotein Risk Factors** – To Help Assess Those at Risk

**ATP IV to come out soon**
NCEP New Lipoprotein Risk Factors

RLP (Remnant Lipoprotein)
- High in 25% of population
- One of the Most Atherogenic Lipoproteins
- Skips Oxidation Step in Forming Plaque

Small Dense LDL – High in 25% of population
- 3-fold Greater Risk of CVD than Buoyant LDL
- Penetrates Arterial Endothelial Lining Easily
- Less Recognized by LDL Receptors therefore Increases

Lp(a) - High in 25% of population
- Small Particles that are Easily Oxidized
- Competes with Plasminogen, Prevents Fibrinolysis

HDL 2b & 3 – Low in 25% of population
- HDL 3 Picks Up Excess Cholesterol and Becomes HDL 2b in Reverse Cholesterol Transport
Lp(a) Competes with Plasminogen and Prevents Fibrinolysis

Many of the over 40 genetic variations of Lp(a) mimic plasminogen

Fibrinogen → Fibrin → Blood Clot (MI, Stroke or DVT)

Plasminogen → Plasmin → Lp(a) → Fibrin → Fibrinolysis

Possible Antithrombotic Therapy Indicated
To Determine the NCEP New Risk Factors Lipoprotein Subgroup Information is Needed

What are Lipoproteins and their Subgroups?

How do they Cause Cardiovascular Disease?
Lipoprotein Particles

- Apolipoprotein A-1 (HDL) or B-100 (LDL)
- Cholesterol Ester
- Triglyceride
- Unesterified Cholesterol
- Phospholipid
Atherogenic Particles

<table>
<thead>
<tr>
<th>Size (nm)</th>
<th>Density (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>50 1.004</td>
</tr>
<tr>
<td>RLP</td>
<td>25 1.004</td>
</tr>
<tr>
<td>Buoyant LDL</td>
<td>22 1.023</td>
</tr>
<tr>
<td>Dense LDL</td>
<td>19 1.044</td>
</tr>
</tbody>
</table>

TG-rich Lipoproteins

Mean Endothelial Pore Size
CETP in Cholesterol Metabolism

VLDL $\rightarrow$ LPL $\rightarrow$ IDL $\rightarrow$ HDL Enters Cells & Arterial Intima $\rightarrow$ LDH 1-2 $\rightarrow$ CETP $\rightarrow$ TG's $\rightarrow$ CE $\rightarrow$ HDL Enter Cells & Arterial Intima $\rightarrow$ LDL 3-4 $\rightarrow$ LCAT $\rightarrow$ Apo A-1 $\rightarrow$ Nascent HDL

Apo B-100 Liver Reverse Cholesterol Transport Apo A-1

HDL2b LCAT HDL3 LCAT

Atherosclerotic Plaque Formation

- **HDL REMOVES EXCESS LIPIDS**
- **ARTERIAL INTIMA**
- **MACROPHAGE CELL**
- **FOAM CELLS BUILD PLAQUE**
- **INFLAMMATION RUPTURES PLAQUE**

- **ARTERIAL LUMEN**
- **RLP**
- **LDL**
- **Lp(a)**
- **MONOCYTE CELL**
- **DENSE LDL**
- **OXIDIZED LDL**
- **MACROPHAGE CELL**
- **DAMAGED ENDOTHELium CELLS**

Atherosclerotic Plaque Formation involves the accumulation of lipids and other substances in the arterial intima, leading to the formation of foam cells, which eventually rupture and lead to further plaque buildup.
Why Have the LPP Test?

Provides a More Complete Risk Answer than the Standard Cholesterol Test

LPP Has Three Risk Identifying Components

1. NCEP’s New Lipoprotein Risk Factors
2. Lipoprotein Particle Numbers vs. Cholesterol Content
3. Non-Lipid Risk Factors: CRP, Insulin and Homocysteine

These Components Identify Many at Risk Individuals that are Missed
Lipoprotein Particle Profile (LPP™) Process

- Separates by Density using Analytical Ultracentrifugation

the CDC’s Gold Standard

- The New Gold Standard - Enhancements

- Continuous Gradient

- Clinically Relevant Lipoprotein Particles are Detected Not the Surrogate Marker Cholesterol
Lipoprotein Particle Measurement

Fluorescent Dye – a Phospholipid Analog

Fluorescent - Hydrophilic End  Hydrophobic End

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

The Fluorescence is a Direct Measurement of Particle Number
Separation by Density

Centrifuge Tube with Mixture of Serum, Gradient and Dye

Intense Gravitational Force
600,000 G’s

Separated Lipoproteins

Density (g/ml)
1.000
1.006
1.030
1.063
1.100
1.300

VLDL
LDL
HDL
Proteins
LPP Test - Lipoprotein Groups and Subgroups

- **VLDL**
- **Buoyant - Large**
- **Dense - Small**

**HDL2b**, **HDL3**

**Particle Measurement**

**Accurate Densities**

- **Risk Factor**
- **Borderline**
- **Normal**
LPP showing NCEP’s New Lipoprotein Risk Factors

Healthy Profile

Atherogenic Profile

**Lipoprotein Particle Profile**

- Low RLP
- Buoyant LDL
- High HDL 2b

- High RLP
- Dense LDL
- Low HDL 2b

**Lipoprotein Particle Numbers (nmol/L)**

<table>
<thead>
<tr>
<th>Lipoprotein Particle</th>
<th>Value</th>
<th>Reference Value</th>
<th>Alert</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL Particles</td>
<td>24</td>
<td>&lt;85</td>
<td></td>
</tr>
<tr>
<td>Total LDL Particles</td>
<td>676</td>
<td>&lt;900</td>
<td></td>
</tr>
<tr>
<td>Non - HDL Particles</td>
<td>888</td>
<td>&lt;1000</td>
<td>Optimal</td>
</tr>
<tr>
<td>RLP (Remnant Lipoprotein)</td>
<td>75</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Small - Dense LDL I</td>
<td>335</td>
<td>&lt;300</td>
<td></td>
</tr>
<tr>
<td>Small - Dense LDL II</td>
<td>82</td>
<td>&lt;150</td>
<td></td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>8274</td>
<td>&gt;7000</td>
<td></td>
</tr>
<tr>
<td>Large - Buoyant HDL 2b</td>
<td>2937</td>
<td>&gt;1500</td>
<td></td>
</tr>
</tbody>
</table>

**Biomarkers and Risk Factors**

- Apo B-100 (mg/dL): 67
- Lip(a) (mg/dL): 6.3
- Metabolic Syndrome Traits: 0
- C-Reactive Protein-β (mg/L): 0.2
- Insulin (uU/mL): 0.0
- Homocysteine (μmol/L): 18.2

**Lipid Panel (mg/dL)**

- Total Cholesterol: 145
- LDL - Cholesterol: 27
- HDL - Cholesterol: 61
- Triglycerides: 47
- Non - HDL - Chol (calc): 84

- Total Cholesterol: 204
- LDL - Cholesterol: 95
- HDL - Cholesterol: 79
- Triglycerides: 340
- Non - HDL - Chol (calc): 120
Correlation of Apo B and LPP non-HDL Particle Numbers is Excellent

One Apo B Molecule per non-HDL Particle

ADA and ACC Consensus: Apo B More Predictive of CVD Risk than Cholesterol*

Correlation of Apo B and LPP non-HDL Particle Numbers is Excellent

r = 0.93
n = 2990

* Diabetes Care, Volume 31, Number 4, April 2008
Lipoprotein Particle Numbers

All of These Patients have the **Same LDL** “Cholesterol”

A
125 mg/dL

B
125 mg/dL

C
125 mg/dL

D
125 mg/dL

**LDL Particles with Varying Cholesterol Content**

- Large - Buoyant
  - TG’s & CETP
  - Large - Buoyant
  - Depleted

- Small - Dense
  - TG’s & CETP
  - Small - Dense
  - Enriched

- Small - Dense
  - TG’s & CETP
  - Small - Dense
  - Depleted

Particle Numbers or Apo B-100

- Low
  - Increased

- Increased

- High

Risk

Relevance of Lipoprotein Particle Numbers

- A Disconnect Exists between Lipoprotein Particle Number and Cholesterol Number

- 30% of the Population is Cholesterol Depleted
  20% of the Population is Cholesterol Enriched

- The LPP Test is Like Having Apo B-100 Broken Down into Subgroups for a Better Therapeutic Approach
The Magnitude of the Result is Easy to Judge with Respect to the Reference Value.

Color Highlighted Numerical Results

Reference Value

Green is Normal

Yellow is Borderline

Red is High
Primary Risk Assessment & Therapeutic Approach

Risk Modification

Includes Apo B-100, Lp(a), Metabolic Syndrome

LPP+ C-Reactive Protein, Insulin and Homocysteine

Lipid Panel

Graph – Quick Assessment

Direct Measurement of Lipoprotein Particle Numbers
Interrelation Between Atherosclerosis and Metabolic Syndrome - Insulin Resistance

Six Possible Traits
- Hypertension
- Obesity
- High Glucose
- High Triglycerides
- Small, Dense LDL
- Low HDL

Metabolic Syndrome
- Insulin Resistance

Atherosclerosis
- Risk Increases
The Role of CETP and TG’s in Metabolic Syndrome

Atherogenic Profile with High TG’s, Low HDL and Dense LDL. Patient has **Metabolic Syndrome**

Three Lipid Traits is a Probable Diagnosis of Metabolic Syndrome

Most Likely the Patient has One or More non-Lipid Traits:
1. Hypertension
2. Obesity
3. High glucose

Risk Level Increases

Check for **Insulin Resistance**
LPP Treatment Strategy

1. **Primary Risk Assessment using Non-HDL Particle Numbers**

2. **Modify Risk Level Using Metabolic Syndrome Traits Lp(a), CRP and Insulin**

3. **Direct Therapeutic Approach Based on LDL and HDL Subgroup Distributions and Lp(a)**
Important Benefits of the LPP™ Test

- Identification of the NCEP New Risk Factors
- Measurement of Lipoprotein Particles Numbers with Lipid Panel in Cholesterol Equivalents
- Identification of Lipid Metabolic Syndrome Traits
- LPP+ Adds Additional Risk Factors hs-CRP plus Insulin and Homocysteine
Male Patient on 4 gm EPA & DHA Omega-3’s, adding 10 mg Rosuvastatin and then 145 mg Tricor

10 mg Rosuvastatin
LDL decreased (48%)

145mg Tricor
Dense LDL decreased 29%

TG’s decreased 57%

Metabolic Syndrome Traits from 2 to 0
62 year old female MD with a healthy profile like shown found that her calcium score was in the 90th percentile.

Examples of the Atherogenic Apo C-1 Enriched HDL and Low Birth Weight Babies (~6 lb vs ~7 lb)*

Apo C-1 HDL Characteristics Observed in 1-2% of Specimens
Proinflammatory HDL Associated with Endothelial Dysfunction

High Very Buoyant HDL – Apo C-1 or Lp(a)?

Profile Most Likely has Apo C-1 Enriched HDL Since Lp(a) is Low

If Patient History Indicates a Low Birth Weight the Probability Would Increase

Confirmation with an Apo C-1 assay is Currently Only Done as Research

Mass Spectrometry shows two isoforms of Apo C-1, one appears to always be associated with CVD*.

## Lipoprotein Particle Numbers Therapeutic Guidelines

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Therapeutic</th>
<th>Statins</th>
<th>Niacin</th>
<th>Fibrates</th>
<th>Estrogens</th>
<th>Resins</th>
<th>Absorption Inhibitors</th>
<th>Omega-3’s EPA &amp; DHA</th>
<th>Alcohol (moderate)</th>
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<tbody>
<tr>
<td>VLDL (Triglycerides)</td>
<td>♥</td>
<td>♥</td>
<td>♥♥</td>
<td>♥♥</td>
<td>X</td>
<td>X</td>
<td>♥</td>
<td></td>
<td>■</td>
</tr>
<tr>
<td>RLP (IDL)</td>
<td>♥</td>
<td>♥</td>
<td>♥</td>
<td>♥</td>
<td>X</td>
<td>X</td>
<td>♥</td>
<td>♥♥</td>
<td>■</td>
</tr>
<tr>
<td>LDL I &amp; II - buoyant</td>
<td>♥</td>
<td>♥</td>
<td>♥♥</td>
<td>♥♥</td>
<td></td>
<td></td>
<td>♥</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>LDL III - dense</td>
<td>♥</td>
<td>♥**</td>
<td>♥♥</td>
<td>♥</td>
<td>♥</td>
<td></td>
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<td>LDL IV or Lp(a)</td>
<td>■</td>
<td>■</td>
<td></td>
<td>♥</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td></td>
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<tr>
<td>HDL 2b - buoyant</td>
<td>♥♥</td>
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<tr>
<td>HDL 2a &amp; 3</td>
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<td></td>
<td>■</td>
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<td>■</td>
</tr>
</tbody>
</table>

- **Therapeutic**: ♥♥
- **Beneficial**: ♥
- **Little or No Effect**: ■
- **Negative Result**: X

*These guidelines provide some of the treatment options available to modify lipoprotein particle numbers determined by the LPP™ test. Spectracell Laboratories observed response to treatment. The National Cholesterol Education Program (NCEP) guidelines provide dosage information on the treatment options.