Cardiovascular Disease Risk Assessment Factors

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Learning objectives

• Understand the biochemistry of lipids and lipoproteins
• Know the methodologies for measurement of lipids and lipoproteins
• Understand how results of traditional markers of cardiovascular disease risk are used in clinical practice
• Understand novel lipids and non-lipid-based biomarkers
Case report: 40 yoF with uncontrolled DM

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Notes</th>
<th>High Risk</th>
<th>Intermediate Risk</th>
<th>Optimal</th>
<th>High Risk Range</th>
<th>Intermediate Risk Range</th>
<th>Optimal Range</th>
<th>Previous Results</th>
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<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>223</td>
<td>≥ 240</td>
<td>200 - 239</td>
<td>&lt; 200</td>
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<tr>
<td>LDL-C Direct (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>109</td>
<td>≥ 130</td>
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<tr>
<td>HDL-C (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>≤ 40</td>
<td></td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>299</td>
<td>≥ 200</td>
<td>150 - 199</td>
<td>≤ 150</td>
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<tr>
<td>Non-HDL-C (mg/dL) (calculated)</td>
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<td></td>
<td></td>
<td>178</td>
<td>≥ 160</td>
<td>130 - 159</td>
<td>≤ 130</td>
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<td><strong>In Particles and lipoproteins</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Apo B (mg/dL)</td>
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<td></td>
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<td></td>
<td>≥ 80</td>
<td>60 - 79</td>
<td>&lt; 60</td>
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<td>LDL-P (nmol/L)</td>
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<td></td>
<td></td>
<td>2498</td>
<td>≥ 1300</td>
<td>1000 - 1299</td>
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<td>sdLDL (mg/dL)^a</td>
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<td>≥ 31</td>
<td>21 - 30</td>
<td>≤ 20</td>
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<td>% sdLDL (calculated)</td>
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<td></td>
<td>≥ 30</td>
<td>26 - 30</td>
<td>≤ 26</td>
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<td>Apo A-I (mg/dL)</td>
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<td>HDL-P (μmol/L)</td>
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<td></td>
<td></td>
<td>34.1</td>
<td>&lt; 28.0</td>
<td>28.0 - 34.0</td>
<td>≥ 35.0</td>
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</table>

Therapeutic recommendations to be presented at the end of lecture.
Cardiovascular disease
Cardiovascular disease overview
Go et al. Circ 2013;127:e6-245

Risk factor incidence

- Current Smoking: 0.0%
- Body Mass Index: 14.8%, 18.6%
- Physical Activity: 8.0%
- Healthy Diet Score*: 91.5%
- Total Cholesterol: 8.0%
- Blood Pressure: 12.4%
- Fasting Plasma Glucose: 11.7%
Cardiovascular disease overview
Go et al. Circ 2013;127:e6-245

Death rates
Cardiovascular disease overview
Go et al. Circ 2013;127:e6-245

Obesity

Percent of Population

Men

Women


10.7 12.2 12.8 20.6 34.4 36.1

15.7 16.8 17.1 26.0 34.0 36.1
Traditional lipids and lipoproteins

Biochemistry
Lipids and lipoproteins

- Compounds that combine with or produce fatty acids
- Cholesterol and triglyceride are the major lipids in blood
- Water insoluble so must be solubilized for absorption
- Bile acids and lipase facilitate absorption in the intestine
- Packaged into lipoprotein particles for transport
- Apolipoproteins are protein part of lipoprotein, help solubilize lipoproteins, and serve as ligands for cellular receptors and enzymes
- Composition varies among lipoproteins
Lipoproteins

Core containing triacylglycerols and cholesteryl esters

Phospholipids

Cholesterol

Apolipoproteins
## Characteristics of Lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Chylo-</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDH</th>
<th>HDL</th>
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</thead>
<tbody>
<tr>
<td><strong>Density</strong></td>
<td>&lt; 1.006</td>
<td>&lt; 1.006</td>
<td>1.006–1.019</td>
<td>1.019–1.063</td>
<td>1.063–1.21</td>
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<tr>
<td><strong>Diameter (nm)</strong></td>
<td>80–500</td>
<td>40–80</td>
<td>24.5</td>
<td>20</td>
<td>7.5–12</td>
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<tr>
<td><strong>Cholesterol (%)</strong></td>
<td>9</td>
<td>22</td>
<td>35</td>
<td>47</td>
<td>19</td>
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<tr>
<td><strong>Triglyceride (%)</strong></td>
<td>82</td>
<td>52</td>
<td>20</td>
<td>9</td>
<td>3</td>
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<tr>
<td><strong>Major Apolipoprotein</strong></td>
<td>B-48</td>
<td>B-100</td>
<td>B-100</td>
<td>B-100</td>
<td>A-I</td>
</tr>
<tr>
<td></td>
<td>C-I,II, III</td>
<td>C-I, II, III</td>
<td>E</td>
<td>A-II</td>
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</table>
Exogenous pathway
Endogenous pathway
Clinical interpretation
Total cholest distribution: CHD vs. non-CHD

Framingham Heart Study—26-Year Follow-up

35% of CHD Occurs in People With TC <200 mg/dL

No CHD

CHD

150  200  250  300

Total Cholesterol (mg/dL)
### Fredrickson classification of hyperlipoproteinemias

<table>
<thead>
<tr>
<th>Type</th>
<th>Elevated lipoprotein</th>
<th>Electrophoresis</th>
<th>Infranate</th>
<th>Supernatant</th>
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<tbody>
<tr>
<td>I</td>
<td>Chylomicrons</td>
<td>Origin</td>
<td>Clear</td>
<td>Cloudy</td>
</tr>
<tr>
<td>IIA</td>
<td>LDL</td>
<td>( \beta )-LDL</td>
<td>Clear</td>
<td>None</td>
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<tr>
<td>IIB</td>
<td>LDL, VLDL</td>
<td>( \beta )-LDL, pre-( \beta ) VLDL</td>
<td>Turbid</td>
<td>None</td>
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<tr>
<td>III</td>
<td>IDL and remnant lipoproteins</td>
<td>X-broad band</td>
<td>Turbid</td>
<td>None</td>
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<tr>
<td>IV</td>
<td>VLDL</td>
<td>Pre-( \beta )</td>
<td>Turbid</td>
<td>None</td>
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<tr>
<td>V</td>
<td>Chylomicrons, VLDL</td>
<td>Origin, pre-( \beta ) VLDL</td>
<td>Turbid</td>
<td>Cloudy</td>
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## NCEP Guidelines Step 1: determine levels

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<tr>
<th>Component</th>
<th>Optimal</th>
<th>Desirable</th>
<th>Borderline</th>
<th>Undesirable</th>
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<tbody>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total cholesterol</td>
<td>----</td>
<td>&lt;200</td>
<td>200-239</td>
<td>≥240</td>
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<tr>
<td>Triglycerides</td>
<td>----</td>
<td>&lt;150</td>
<td>150-199</td>
<td>≥200</td>
</tr>
<tr>
<td>HDL-C</td>
<td>≥60</td>
<td>≥40</td>
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<td>&lt;40</td>
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<tr>
<td>LDL-C</td>
<td>&lt;100</td>
<td>&lt;130</td>
<td>130-159</td>
<td>≥160</td>
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<tr>
<td><strong>Children</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>&lt;170</td>
<td>170-199</td>
<td>≥200</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;125</td>
<td>----</td>
<td>≥125</td>
<td></td>
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<tr>
<td>HDL-C</td>
<td>&gt;45</td>
<td>35-45</td>
<td>&lt;35</td>
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<tr>
<td>LDL-C</td>
<td>&lt;110</td>
<td>110-129</td>
<td>≥130</td>
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</tbody>
</table>
NCEP Steps 2, 3: identify CVD risk factors

Step 2: Clinical atherosclerotic diseases:
clinical CHD, peripheral artery disease, abdominal aortic aneurysms, diabetes

Step 3. Major CVD Risk factors
• Cigarette smoking
• Hypertension (≥140/90 mm Hg)
• Low HDL cholesterol (<40 mg/dL)
• Family history of premature CHD in 1st degree relative
• Age (male >45; female >55)
• HDL ≥60 mg/dL negative risk factors
Step 4: Clinical atherosclerotic diseases:
2+ risk factors (other than LDL) present without CHD, assess 10-y CHD risk

Step 5. Determine risk category
- Establish LDL goal of therapy
- Determine need for therapeutic lifestyle changes (TLC)
- Determine level for statin drug consideration

Diet: Saturated fat <7% of calories, cholesterol <200 mg/day and consider increased viscous (soluble) fiber (10-25 g/day) and plant stanols/sterols (2g/day). Weight management and increased physical activity
<table>
<thead>
<tr>
<th>Risk Category</th>
<th>LDL Goal</th>
<th>LDL Level at which to initiate therapeutic lifestyle changes</th>
<th>LDL Level at which to consider drug therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD or CHD risk equivalents (10-y risk &gt;20%)</td>
<td>&lt;100 (&lt;70)</td>
<td>≥ 100 mg/dL</td>
<td>≥130 mg/dL (100-129 mg/dL: drug optional)</td>
</tr>
<tr>
<td>2+ risk factors (10-year risk &lt; 20%)</td>
<td>&lt;130</td>
<td>≥ 130 mg/dL</td>
<td>10-y risk 10-20%: ≥130 mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10-y risk &lt;10%: ≥ 160 mg/dL</td>
</tr>
<tr>
<td>0-1 risk factor</td>
<td>&lt;160</td>
<td>≥ 160 mg/dL</td>
<td>≥ 190 mg/dL (160-189 mg/dL: LDL-lowering drug optional)</td>
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</tbody>
</table>
Analytical methods
Lipoprotein electrophoresis
Cholesterol (non-fasting)

- **CDC reference method (Abell-Kendall)**
  hydrolyze, hexane extraction, Liebermann-Burchard reagent; acetic acid, acetic anhydride and sulfuric acid

- **Routine methods (enzymatic)**
  - C. esterase
    cholesterol esters $\rightarrow$ cholesterol + fatty acids
  - C. oxidase
    cholesterol + $O_2$ $\rightarrow$ cholest-4-en-3-one + $H_2O_2$
  - Peroxidase
    $H_2O_2$ + dye $\rightarrow$ color indicator
Triglycerides (fasting)

- **CDC reference method**
  Serum lipids are extracted with CHCl₃, treated with silicic acid to remove phospholipids and free glycerol, saponified to release glycerol, oxidized to produce formaldehyde, chromotropic acid to produce a chromogen.

- **Routine methods (enzymatic)**
  - Lipase
    \[ \text{triglyceride} + H₂O \rightarrow \text{glycerol} + \text{FA} \]
  - Glycerol kinase
    \[ \text{glycerol} + \text{ATP} \rightarrow \text{glycerol-3-P} + \text{ADP} \]
  - Phosphokinase
    \[ \text{ADP} + \text{PEP} \rightarrow \text{ATP} + \text{pyruvate} \]
HDL cholesterol assays

• **CDC reference method**
  - ultracentrifugation (removes <1.006)
  - heparin/MnCl$_2$
  - measure HDL cholesterol in supernatant
  - comparison method uses dextran sulfate/MgCl$_2$

• **Routine methods**
  - precipitation with polyanion solutions
  - dextran sulfate-iron particles
  - homogenous methods: anti-apo B and/or apo C-III or agents that form insoluble complexes with non HDL
Direct LDL chol assays (IDL and Lp(a))

- **CDC reference method**
  - ultracentrifugation ($\beta$ quantitation)
  - measure cholesterol in $>1.006$ fraction and supernatant after precipitation
  - calculate LDL cholesterol (difference)

- **Routine methods (direct)**
  - selective precipitation
  - homogeneous methods use detergents and/or chemicals to block or solublize lipoproteins
  - antibody based
LDL chol = total chol – HDL chol – VLDL chol (triglyceride/5)

Assumes that 20% of triglycerides are in VLDL.

Inaccurate when triglyceride is >400 mg/dL (overestimates VLDL, underestimates LDL)
Total Cholesterol – HDL-C = Non-HDL-C

Can be accurately measured in nonfasting state

The “atherogenic cholesterol”
## Non-HDL-C goals

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>LDL Goal</th>
<th>Non-HDL Goal</th>
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<tbody>
<tr>
<td>CHD or CHD Risk Equivalent</td>
<td>&lt;100</td>
<td>&lt;130</td>
</tr>
<tr>
<td>(2+) Risk Factors and 10-year risk &lt; 20%</td>
<td>&lt;130</td>
<td>&lt;160</td>
</tr>
<tr>
<td>0-1 Risk Factor</td>
<td>&lt;160</td>
<td>&lt;190</td>
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## NCEP Acceptable assay performance

<table>
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<tr>
<th>Marker</th>
<th>Total Error</th>
<th>Bias</th>
<th>Precision</th>
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<tbody>
<tr>
<td>Cholesterol</td>
<td>8.9%</td>
<td>≤±3%</td>
<td>≤±3%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≤15%</td>
<td>≤±5%</td>
<td>≤±5%</td>
</tr>
<tr>
<td>HDL-C</td>
<td>≤13%</td>
<td>≤±5%</td>
<td>≤±4%</td>
</tr>
<tr>
<td>LDL-C</td>
<td>≤12%</td>
<td>≤±4%</td>
<td>≤±4%</td>
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</table>
HDL-C and CHD risk with TC < 200 mg/dl
Castelli et al. JAMA 986;256:2835-9.
Hypertriglyceridemia and CHD with low HDL-C


![Graph showing incidence per 1,000 (in 6 years) for LDL-C/HDL-C ratio.]

- **TG < 200 mg/dL**
  - Incidence: 24 per 1,000
  - LDL-C/HDL-C ratio ≤ 5.0
- **TG ≥ 200 mg/dL**
  - Incidence: 245 per 1,000
  - LDL-C/HDL-C ratio > 5.0

*Note: The incidence of CHD is significantly higher in the TG ≥ 200 mg/dL group compared to the TG < 200 mg/dL group.*
C-reactive protein
C-reactive protein

- Member of the pentraxin family
- 5 non-covalently associated protomers (205 aminoacids) arranged symmetrically around a central pore
- Molecular mass of 118 kDa
- Gene mapped to chromosome 1
- Twin studies show highly heritable component in baseline CRP
- High sensitivity assay needed for CVD risk.
  - <1 mg/L  Low risk
  - 1.0 - 3.0 mg/L  Average risk
  - > 3.0 mg/L  High risk
hs-cRP and CVD risk
Ridker et al. NEJM 1997;336:973–979.

![Graph showing relative risk of MI across quartiles of hs-CRP levels with statistical significance indicated.](image-url)
CRP as a risk marker for CHD

- MRFIT: Kuller 1996 CHD
- PHS: Ridker 1997 MI
- PHS: Ridker 1997 Stroke
- CHS/RHPP: Tracy 1997 CHD
- PHS: Ridker 1998 PVD
- WHS: Ridker 1998 CVD
- MONICA: Koenig 1999 CHD
- HELSINKI: Roivainen 2000 CHD

Relative Risk (upper vs lower quartile)
Comparison of risk markers

Lipoprotein (a)
Homocysteine
Total Cholesterol
Fibrinogen
tPA Antigen
TC/HDL
hs-CRP
hs-CRP + TC/HDL

Relative Risk for Future Myocardial Infarction
Jupiter trial
Ridker et al. NEM 2008;359:2195-207.
Jupiter trial
Ridker et al. NEM 2008;359:2195-207.
Jupiter trial
Ridker et al. NEM 2008;359:2195-207.

Relative risk for CV events based on achieved reductions in LDL and CRP

( LDL target < 70 mg/dL and CRP target < 2 mg/L)

- Placebo group
  - LDL not reduced, CRP not reduced
  - Only LDL reduced to target < 70 mg/dL
  - Only CRP reduced to target < 2 mg/L
  - Both LDL and CRP reduced

P < 0.001

Rosuvastatin Better

Rosuvastatin Worse
Lipoprotein (a)
Structure of Lp(a)

- Central LDL core
- Two proteins - single copy of apo B-100 linked to single copy of apo (a) (not found in any other lipoprotein class)
  - Apo (a) - CHO-rich, highly hydrophilic protein; marked size heterogeneity due to differences in length of polypeptide chain; high structural homology with plasminogen
  - Protease domain and Kringle 5 domain and multiple copies of Kringle 4 (10 distinct K4 types)
  - K4 types 1 and 3-10 are present as 1 copy/apo(a) particle, while K4 type 2 has variable number of copies (range 3->40).
  - The K4 type 2 variability responsible for multiple isoforms found in plasma (MW varies from 400-700 kDa)
- Levels genetically determined and not modified by traditional lipid lowering agents
Lp(a) assay

• Size variability of apo(a) is major problem
  – Immunoassays will underestimate Lp(a) levels in samples with apo(a) sizes smaller than calibrator, and overestimate the levels of larger apo(a) particles

• Need antibodies not directed to K4 type 2

• Gender and race differences
Prospective trials of Lp(a) and CVD
Danesh, et.al  Circ. 2000; 102: 1082-1085

<table>
<thead>
<tr>
<th>Type of cohort and source</th>
<th>No. of cases</th>
<th>Degree of adjustment</th>
<th>Risk ratio &amp; confidence limits (top third vs bottom third)</th>
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<tbody>
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<td>Population-based</td>
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<tr>
<td>Nguyen et al, 1997</td>
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<td>Cremer et al, 1994</td>
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<td>Schaefer et al, 1994</td>
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<td>Wald et al, 1994</td>
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<td>Althn et al, 1994</td>
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<td>Bostom et al, 1994</td>
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<td>Wild et al, 1997</td>
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<td>Jauhiainen et al, 1991</td>
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<td>Bostom et al, 1996</td>
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<td>Assman et al, 1996</td>
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<td>Rosengren et al, 1990</td>
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<tr>
<td>Coleman et al, 1992</td>
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<td>Dahlen, 1988</td>
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<tr>
<td><strong>Subtotal</strong></td>
<td><strong>4044</strong></td>
<td></td>
<td><strong>1.7 (95% CI 1.4 to 1.9)</strong></td>
</tr>
</tbody>
</table>
LDL small dense particles

- LDL exists in different particle sizes. Small dense particles contain less cholesterol.
- There is increased deposition of small, dense LDL into sub-endothelial space, thus it is more atherogenic.
- High levels are associated with high triglycerides.
- Small dense particles can be measured by LDL gradient gel electrophoresis.
LDL small dense particles
LDL particles
Underestimation of LDL-C w/ chol depletion

Non-HDL-C = Non-HDL-C
LDL particles

Case summary

Using the results of traditional lipids, the patient has moderate risk which may not justify use of lipid lowering drugs.

However, the results of LDL particles suggest that she is at high risk because of the high density particles. She is put on a statin regimen in addition to lifestyle change recommendations.
Cholesterol balance tests

• Lathosterol and desmosterol are intermediates to cholesterol biosynthesis.
• Increased levels indicate increased synthesis that can be reduced by statins.
• Sitosterol and campesterol are plant steroids that indicate increased cholesterol absorption.
• These patients can be treated with cholesterol absorption drugs such as ezetimide.
Self assessment questions

Calculate the LDL cholesterol concentration in mg/dL using the Friedewald equation:

- Total cholesterol: 245 mg/dL
- HDL cholesterol: 60 mg/dL
- Triglycerides: 180 mg/dL

Answer:

$$LDL-C + 245 - 60 - 180/5 = 149 \text{ mg/dL}$$
Self assessment questions

According to the NCEP, the result from the previous case represents a patient with:

A. Optimum level
B. Desirable level
C. Borderline level
D. Undesirable level
E. Cannot determine without other risk factors

Answer: C.
The previous patient is male, 35 years old, smokes, has blood pressure of 150/110 mmHg, no diabetes but mother suffered a heart attack at age 53 y. With the LDL and HDL cholesterol, how many risk factors does this subject have?

A. None  
B. One  
C. Two  
D. Three  
E. Four

Answer: C. He smokes, has high blood pressure and family history but also has low HDL-C.
Self assessment questions

• According to the NCEP, the patient’s LDL-C is in the intermediate range.
• However, the patient has a high triglycerides prompting further investigation.
• The high non-HDL-C and high LDL-P suggests that she is at high not borderline risk.
• Initiation of drug therapy now rather than later.