I. Establishing causes of coronary artery disease (CAD)

II. Blood lipids, lipoproteins & CAD
   - Cholesterol
   - LDL cholesterol
   - Lp(a)
   - HDL cholesterol

III. Atherogenic Potential of Triglyceride and non-HDL cholesterol

IV. Future Directions in Related Clinical Laboratory Measurements

1. Strength
   “significant different” relative risk than
   the general population

2. Consistency
   evidence across studies in different
   settings and with different populations

3. Specificity
   exposure results in outcome

4. Dose - Response
   increased incidence or severity of
   “outcomes” occur with higher levels of
   “exposure”

5. Temporal Sequencing
   “exposure” occurs before the
   “outcome”

6. Biological Plausibility
   logical (theoretical or observed)
   empirically-based explanation that links
   the exposure with the outcome

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Establishing Causes of CAD

Age
Family History
Cigarette Smoking §
Dyslipidemia §
Hypertension §
Sedentary Lifestyle §
Obesity §
Pre-diabetes §

§ Major Alterable Risk Factors

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1904: Felix Marchand
Coined the term “atherosclerosis”

1908: A.I. Ignatowski
Described the relationship
between cholesterol-rich food
and experimental atherosclerosis

1910: Adolf Windaus
Showed atherosclerotic lesions
contained 6 to 20 times more
cholesterol than normal arterial wall

1913: Nikolai Anichkov
Showed cholesterol alone caused
atherosclerotic changes in the
vascular wall
Atherosclerotic lesions in coronary arteries are largely composed of cholesterol.

6/23/2014

The relationship between blood cholesterol and coronary artery disease is strong, graded and independent.

A 1 mmol/L (38.6 mg/dL) increase in blood cholesterol is associated with a 20 to 25% increase in CAD.
Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths


Figure 1A: Mortality related to serum total cholesterol concentration

Figure 1B: Mortality related to serum total cholesterol concentration

Figure 1C: Serum cholesterol distribution and mean levels

Benefits of LDL Cholesterol Reduction
Cholesterol Treatment Trialists Collaboration

- 12% all-cause mortality
- 19% coronary mortality
- 21% major cardiovascular events
- 17% fatal and non-fatal stroke

In those with CVD, more intensive LDL cholesterol lowering resulted in further reduction in major vascular events, even when LDL cholesterol was already lower than 2 mmol/L.

Baigent et al., Lancet 366: 1267 – 1278, 2005

Cholesterol Treatment Trialists’ Collaborators, Lancet 2012

Benefits of LDL Cholesterol Reduction

Lipoprotein (a)

Lp(a) concentrations are genetically determined.
The function of Lp(a) is unknown.
Lp(a) relationship to CVD is graded and independent.
Lp(a) may exert a proatherothromogenic influence only in specific subgroups (e.g., in those with high LDL-cholesterol)
Lp(a) is relatively refractory to lifestyle and pharmacological interventions.

Tziomalos et al., Curr Opin Cardiol 24: 351 – 357, 2009
Bruckert et al., Atherosclerosis 210: 353 – 361, 2010
NCEP: ATP III Classification of Blood Lipids

<table>
<thead>
<tr>
<th>Total Cholesterol</th>
<th>Classification</th>
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<tbody>
<tr>
<td>&lt; 200</td>
<td>Desirable</td>
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<td>200 - 239</td>
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<tr>
<td>≥ 240</td>
<td>High</td>
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<table>
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<tr>
<th>LDL-Cholesterol</th>
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<tr>
<td>&gt; 190</td>
<td>Very High</td>
</tr>
<tr>
<td>160 - 189</td>
<td>High</td>
</tr>
<tr>
<td>130 - 159</td>
<td>Lower Risk</td>
</tr>
<tr>
<td>100 - 129</td>
<td>Moderate High Risk</td>
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<tr>
<td>&lt; 100 (optional &lt;70)</td>
<td>Very High Risk / High Risk</td>
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Calculated LDL-cholesterol

Most standard lipid panels include LDL-C estimates calculated from the Friedewald Equation: 
LDLC = TC – HDLC – (TG/5)

1. Multiple fasting samples are required prior to initiating treatment
2. Poor estimates when TG > 200 mg/dL
3. Inaccurate at low LDL-C (e.g., error is 15% when LDL-C is 100 mg/dL)

Automated Measurements of LDL-cholesterol

Several direct homogenous assays using multiple detergents to achieve specificity for LDL have been certified by the Cholesterol Reference Methods Lab of the CDC/P.

Designed to provide accurate LDL-C quantification when specimen TG >400 mg/dL, but have proven to be unsuitable for use in dyslipidemia

Unable to meet the NCEP goal of <12% total error for LDL-C
The inverse relationship between HDL cholesterol and coronary artery disease incidence and mortality is strong, graded and independent.

A 0.5 mmol/L (20 mg/dL) increment in HDL cholesterol is associated with a ~26% reduction in CAD risk.
Clinical benefits of increasing HDL cholesterol remains elusive (ILUMINATE; AIM HIGH; HPS2-THRIVE)

Clark et al., ATVB 24: 490 – 497, 2004

NCEP: ATP III Classification of Blood Lipids

<table>
<thead>
<tr>
<th>Total Cholesterol</th>
<th>Desirable</th>
<th>Borderline High</th>
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<th>Very High</th>
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<td>150 - 199</td>
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<tr>
<td>&gt; 500</td>
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</table>

“Ooo, I just felt the good cholesterol kick the bad cholesterol.”
Postprandial Lipemia Defined

Genetic
- Familial Hypertriglyceridemia
- Apolipoprotein CII Deficiency
- Apo A-V Deficiency
- GPIHBP1 Deficiency
- Anderson Disease

Acquired Metabolic Disorders
- Hypothyroidism
- Diabetes
- Pregnancy

Drugs
- α-interferon
- β-blockers
- Estrogens
- Steroids
- Thiazides
- Antipsychotics
- Bile Acid Resins
- Protease Inhibitors
- Tamoxifen

Diet
- Saturated Fats
- Alcohol
- Sugar-sweetened foods

Plasma Triglyceride (mg/dL)

- Normal
- Elevated
- Lipemic

0 20 50 100 150 200 250 300

2 hr 4 hr 6 hr 8 hr
Developing Hypertriglyceridemia & Postprandial Lipemia

Macronutrient Composition

U.S. Adult Men
2520 kcal/day

- PRO 18%
- CHO 48%
- FAT 34%

U.S. Adult Women
1775 kcal/day

- PRO 15%
- CHO 51%
- FAT 34%


Dietary Trends

- 53% consume 1 to 3 meals per week in restaurants
- 23% consume > 4 meals per week in restaurants

More consumption of commercially-prepared meals, fast foods and energy-dense foods with portion sizes that have increased > 33% over the last 3 decades.

- 50 to 67% consume > 10% of our calories from saturated fats
- 20 to 25% consume > 2 servings of fish and 4 to 15% get > 250 mg/day of EPA/DHA
- 20 to 29% get > 5 servings/day of fruits and veggies
- 50 to 76% consume > 36 oz of sugar-sweetened beverages per week
- 50 to 66% consume > 2.5 servings of sweets and bakery goods/day
- 2 to 11% consume > 28 g/day dietary fiber/day

Developing Hypertriglyceridemia & Postprandial Lipemia

Dietary Trends

> 200 kcal/day increase in energy intake over the last 3 decades

Mostly due to increased consumption of sugar-sweetened beverages and snacks

21% of all calories from sweetened beverages, fruit juices, alcohol, and soda/cola
We spent a considerable amount of time in the non-fasting, postprandial state.

Most of us consume three or more meals/day – each containing 20 to 70 g of fat.

Each of these meals is often consumed before plasma lipids have returned to levels that existed prior to the previous meal.

**Metabolic Syndrome**

- Obesity
- Dyslipidemia
- HTN
- Insulin Resistance
- Vascular Inflammation
- Elevated Blood Glucose

**Measuring Postprandial Lipid Responses**

- Blood Sample
- Test Meal
Non-Fasting Lipids & Lipoproteins

**Triglyceride**: triglyceride in all lipoprotein fractions

**Remnant Cholesterol (calculated)**: cholesterol in all lipoproteins larger than LDL

**Remnant-Like Particle Cholesterol**: immunoseparation to determine cholesterol in chylomicron, VLDL and IDL remnants

**Lipoprotein-Specific Markers**: apo B-48; apo B-100; apo Al apolipoproteins

**Stable Isotopes & Mass Spectrometry**: (leucine and glycine) kinetics of apo-B48, apo-B100, apo Al; (albumin-bound FA – orally and infused) compare the extraction of TG in specific tissues

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Figure 1: Atherogenic vs. anti-atherogenic lipoproteins. The diagram shows that there is a single apolipoprotein B (apoB) molecule in each large buoyant or small, dense particle of very low-density (VLDL) and intermediate density (IDL) lipoproteins. Transfers: small (apoB) represents the total number of potentially atherogenic particles. ApoB in HDL particle (apoB) is the predominant apoB-containing lipoprotein (apoB48) responsible for binding transfers cholesterol transport. The extent of LDL transfer between specific lipoproteins (apoB) is essential for the regulation of cholesterol transport.

Abbreviations: TG, triglycerides; C, cholesterol; apoB, apoB48; HDL, high density lipoprotein.

Millan et al., Vasc Health Risk Mgmt. 5: 757-765, 2009

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Non-HDL cholesterol & CAD Risk

Non-HDL = serum TC – HDL-C

NCEP cut points are arbitrarily set at 30 mg/dL above LDL-C and assume a triglyceride of ≤ 150 mg/dL (a calculated VLDL-C of 30 mg/dL.)

Non-HDL-C can be calculated in non-fasting specimens and avoids the problem of calculating LDL-C in hypertriglyceridemia

Recognized by NCEP ATP III (2001) as a secondary target for those with hypertriglyceridemia

Provides an estimate of cholesterol in atherogenic particle spectrum:
(i.e., VLDL, IDL, LDL, Lp(a))

2008 National Lipid Association Task Force recognized the superiority of non-HDL-C to LDL-C as a measure of vascular event risk and equivalent with apo B & LDL particle number in some clinical trials.

Atherogenic Effects of Lipoproteins

Altered Endothelial Adhesion Characteristics
- Oxidative Stress (reduces NO availability)
- Cholesterol Deposition
- Inflammation
Apolipoprotein B & CAD Risk

Immunophelometric and immunoturbidometric techniques are accurate (CVs 3 – 7%) supported by international reference materials - International Federation of Clinical Chemistry

Commercially available in a variety of automated platforms

2008 ADA / ACC Consensus Statement recommends that apolipoprotein B be included in a lipid profile with non-HDL-C and LDL-C in high-risk patients

\[ \text{LDL-C} < 70 \text{ mg/dL}; \quad \text{non-HDL-C} < 100 \text{ mg/dL}; \quad \text{apo B} < 80 \text{ mg/dL} \]

Treatment goals for apo B supported by several large prospective studies:

- CVD prevention: AMORIS INTERHEART
- Assessing residual risk in patients receiving lipid lowering therapy: AFCAPS / TexCAPS TNT IDEAL

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Larger Average LDL Size
Smaller Average LDL Size

Fewer Particles
More Particles

Mora, Circulation 119: 2396 – 2404, 2009

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Figure 5. Higher concentrations of either large or small LDL were both positively associated with carotid intima-media thickness (IMT; \( y \)-axis) in a multietnic study of asymptomatic individuals (n=5638), the MESA Study. Reprinted from Mora et al\(^7\), copyright © 2007, with permission from Elsevier.
Gradient Gel Electrophoresis (Berkley Heart Lab)
Proprietary segmented polyacrylamide gradient gels provide information on LDL size phenotypes. Apo B can be measured separately.

Density Gradient Ultracentrifugation (Atherotech, Spectracell)
Provides information on lipoprotein particle size distribution; cholesterol content of lipoprotein classes; no information on particle number; estimated apo B

Nuclear Magnetic Resonance Spectroscopy (Liposcience)
Particle concentrations of lipoprotein sub-fractions are determined from the measured amplitudes of their lipid methyl group NMR signals.
Particle size distribution; particle number

Ion-Mobility Analysis (Quest)
Gas-phase differential electric mobility provides information on particle concentrations and sizes in subclasses

Table. Summary of Current Limitations to the Clinical Utility of Advanced Lipoprotein Tests

- Lack of standardization and comparability of information provided by various tests
- Information received can be minimized by focusing on several key lipoprotein measures
- Lack of comparability
- Lack of demonstration that tests alter clinical management and outcomes of patients, such as by improving risk classification or targeting of therapy
- Subgroups of individuals have not been identified who may particularly benefit from testing (e.g., those with cardiometabolic risk factors)
- Favorable cost-benefit ratios have not been demonstrated


High Density Lipoprotein

Promotes cholesterol efflux

- Anti-oxidant
- Anti-Inflammatory
- Anti-fibrotic
- Anti-thromboid
- Increases endothelial NO production
- Prevents vascular endothelial apoptosis
- Vasoprotective

- Downregulates endothelial VCAM-1 and ICAM-1
- Prevents endothelial IL-8 and MCP-1 expression
- PON-1
- Downregulates macrophage TNFα
- Protects against endotoxins