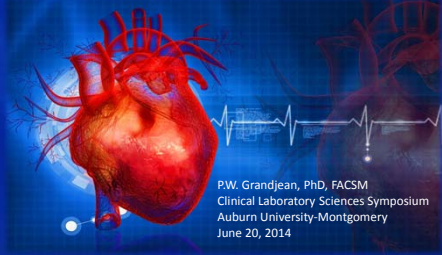


## Clinical Significance of Measuring Blood Lipids & Lipoproteins



P.W. Grandjean, PhD, FACS  
Clinical Laboratory Sciences Symposium  
Auburn University-Montgomery  
June 20, 2014

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## Outline

- 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

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## Atherosclerotic Disease



Black et al., *Angiology* 26: 759 – 783, 1975.  
Ragosta et al., *MSSE* 16:339 – 342, 1984.  
Davies et al., *Br Heart J* 53: 363 – 373, 1985.  
Libby, *Circulation*: 104(3): 365 – 372, 2001.

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## Establishing Causes of CAD

<b>1. Strength</b>	"significant different" relative risk than the general population
<b>2. Consistency</b>	evidence across studies in different settings and with different populations
<b>3. Specificity</b>	exposure results in outcome
<b>4. Dose - Response</b>	increased incidence or severity of "outcomes" occur with higher levels of "exposure"
<b>5. Temporal Sequencing</b>	"exposure" occurs before the "outcome"
<b>6. Biological Plausibility</b>	logical (theoretical or observed) empirically-based explanation that links the exposure with the outcome

Hill, Proc Royal Soc Med, 50: 295 - 300, 1965

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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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	<p><b>1904: Felix Marchand</b> Coined the term "atherosclerosis"</p> <p><b>1908: A.I. Ignatowski</b> Described the relationship between cholesterol-rich food and experimental atherosclerosis</p>	
	<p><b>1910: Adolf Windaus</b> Showed atherosclerotic lesions contained 6 to 20 times more cholesterol than normal arterial wall</p> <p><b>1913: Nikolai Anichkov</b> Showed cholesterol alone caused atherosclerotic changes in the vascular wall</p>	

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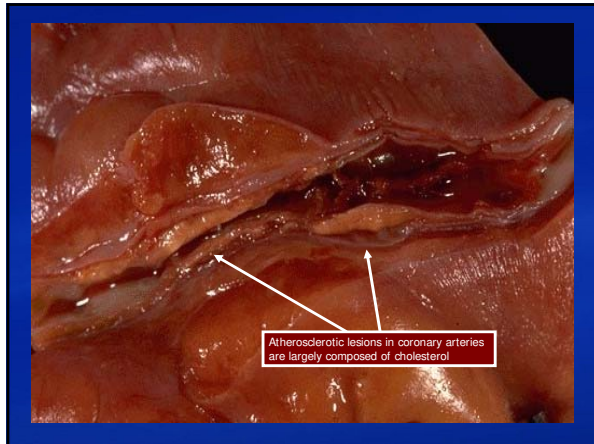
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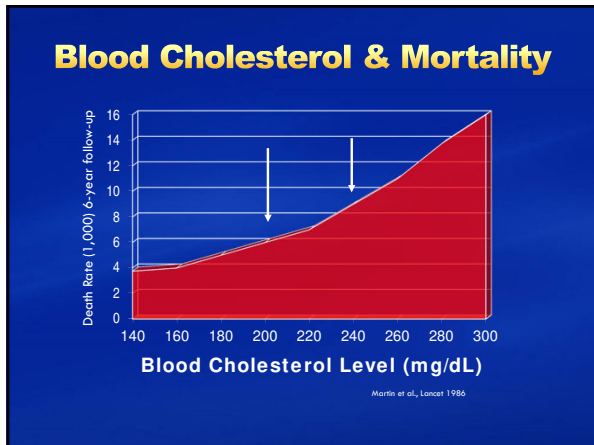
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### Blood Cholesterol & Mortality

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.

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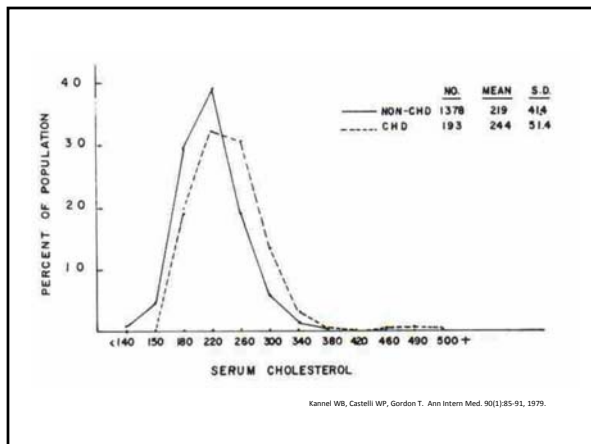
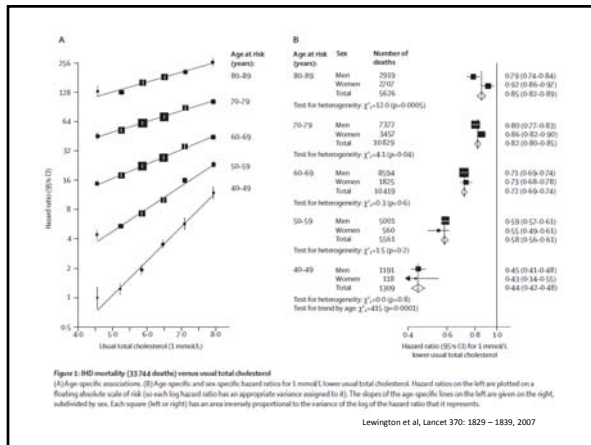
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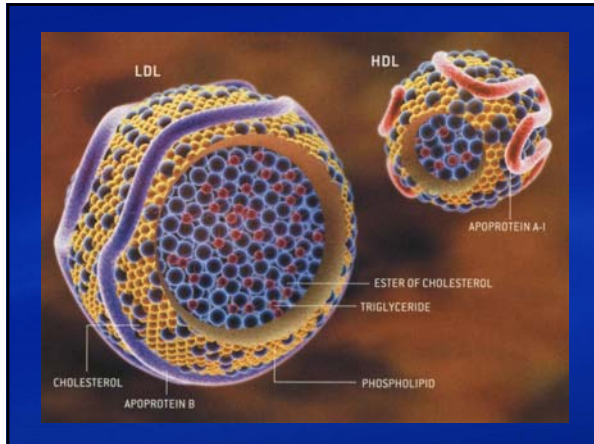
### Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths

Prospective Studies Collaboration\*

**Methods** Information was obtained from 61 prospective observational studies, mostly in western Europe or North America, consisting of almost 900 000 adults without previous disease and with baseline measurements of total cholesterol and blood pressure. During nearly 12 million person years at risk between the ages of 40 and 89 years, there were more than 55 000 vascular deaths (34 000 ischaemic heart disease [IHD], 12 000 stroke, 10 000 other). Information about HDL cholesterol was available for 150 000 participants, among whom there were 5000 vascular deaths (3000 IHD, 1000 stroke, 1000 other). Reported associations are with usual cholesterol levels (ie, corrected for the regression dilution bias).

Lancet 2007; 370: 1829-39






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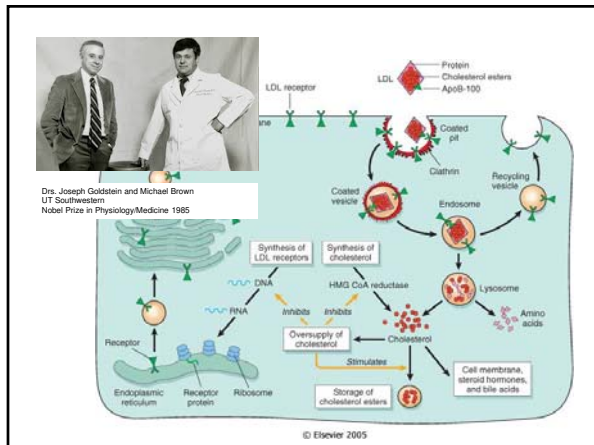
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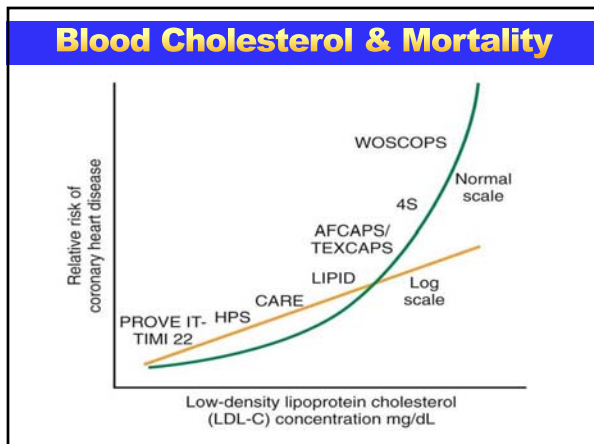
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### Benefits of LDL Cholesterol Reduction

*Cholesterol Treatment Trialists Collaboration*

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

1 mmol/L  
LDLC

Bolger et al., *Lancet* 366: 1267 – 1276, 2005

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.

Bolger et al., *Lancet* 376: 1670 - 1681, 2010

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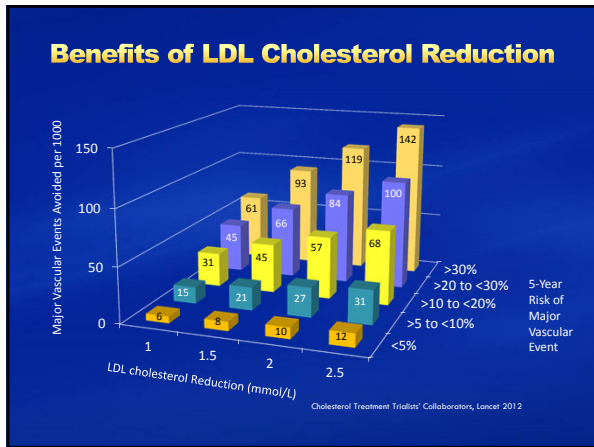
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### Lipoprotein (a)

Lp(a) concentrations are genetically determined.

The function of Lp(a) is unknown.

Apo B 100

LDL

Apo (a)

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.

impairs fibrinolysis  
promotes coagulation

Ruyy et al., *Athero Thromb* 11: 629 – 638, 1991  
Tsimikas et al., *Curr Opin Cardiol* 24: 351 – 357, 2009  
Bruckert et al., *Atherosclerosis* 210: 353 – 361, 2010

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**NCEP: ATP III Classification of Blood Lipids**

**Total Cholesterol**

< 200	Desirable
200 - 239	Borderline High
≥ 240	High

**LDL-Cholesterol**

< 100 (optional <70)	Very High Risk / High Risk
100 - 129	Moderate High Risk
130 - 159	Lower Risk
160 - 189	High
≥190	Very High




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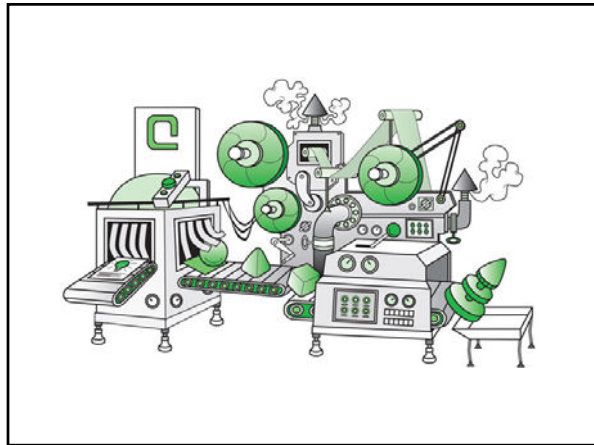
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**Calculated LDL-cholesterol**

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

- Multiple fasting samples are required prior to initiating treatment
- Poor estimates when TG > 200 mg/dL
- Inaccurate at low LDLC (e.g., error is 15% when LDLC 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 LDLC 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 LDLC

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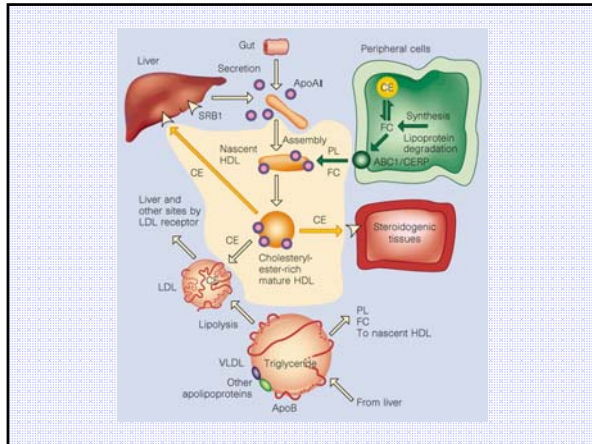
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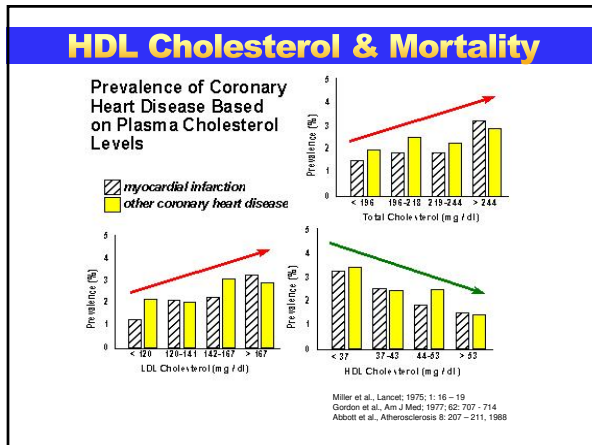
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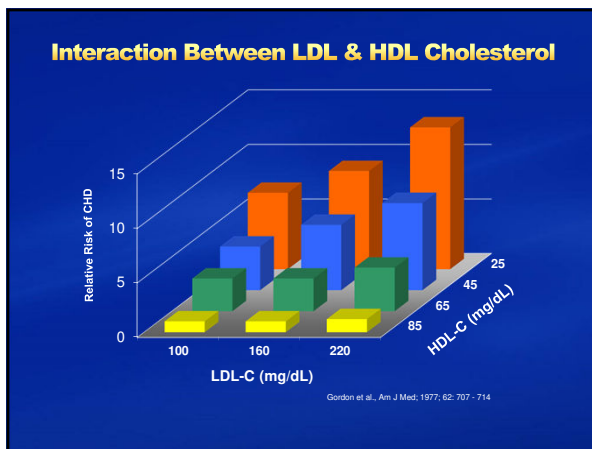
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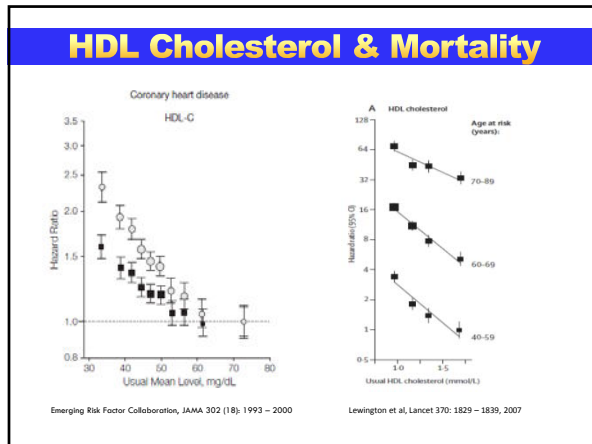
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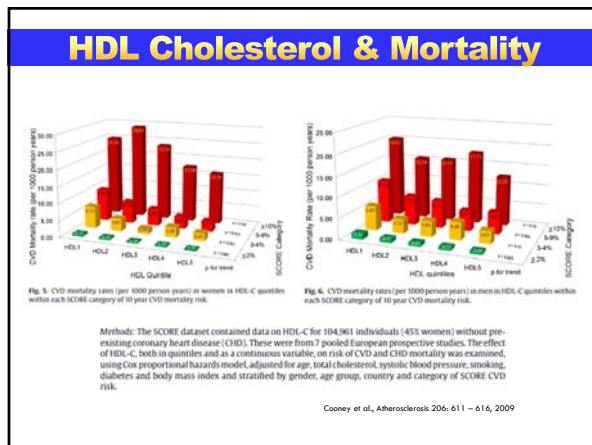
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### HDL Cholesterol & Mortality

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.

Gordon, Castelli et al., Am J Med. 1977; 62: 707 – 714  
Gordon, Kroke et al., Circulation; 1986; 74: 1217 - 1225

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## HDL Cholesterol & Mortality

Clinical benefits of increasing HDL cholesterol remains elusive (ILUMINATE; AIM HIGH; HPS2-THRIVE)

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

Barter et al., N Engl J Med. 357: 2109 – 2022, 2007

Boden et al., N Engl J Med. 365: 2255 – 2267, 2011

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## NCEP: ATP III Classification of Blood Lipids

<p><b>Total Cholesterol</b></p> <p>&lt; 200</p> <p>200 - 239</p> <p>≥ 240</p> <p><b>LDL-Cholesterol</b></p> <p>&lt; 100 (optional &lt;70)</p> <p>100 - 129</p> <p>130 - 159</p> <p>160 - 189</p> <p>≥ 190</p> <p><b>HDL-Cholesterol</b></p> <p>ⅴ &lt; 40 (males), ≤ 50 (females)</p> <p>ⅴ &gt; 60</p> <p><b>Triglycerides</b></p> <p>&lt; 150</p> <p>150 - 199</p> <p>200 - 499</p> <p>≥ 500</p>	<p>Desirable</p> <p>Borderline High</p> <p>High</p> <p>Optimal</p> <p>Near/Above Optimal</p> <p>Borderline High</p> <p>High</p> <p>Very High</p> <p>Low</p> <p>High</p> <p>Normal</p> <p>Borderline High</p> <p>High</p> <p>Very High</p>	
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"Ooo, I just felt the good cholesterol kick the bad cholesterol."

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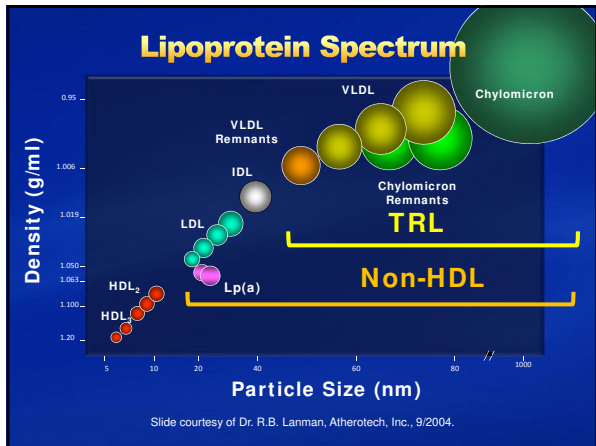
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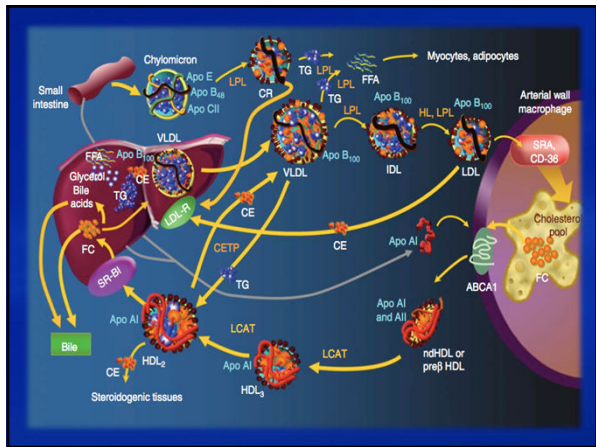
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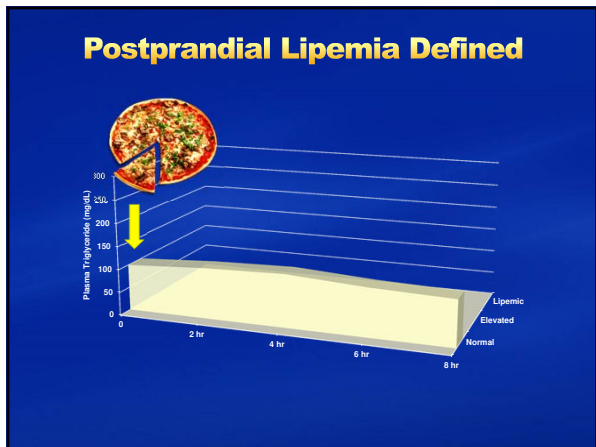
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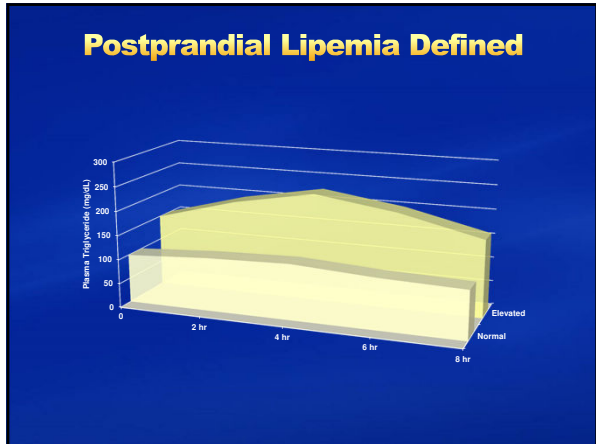
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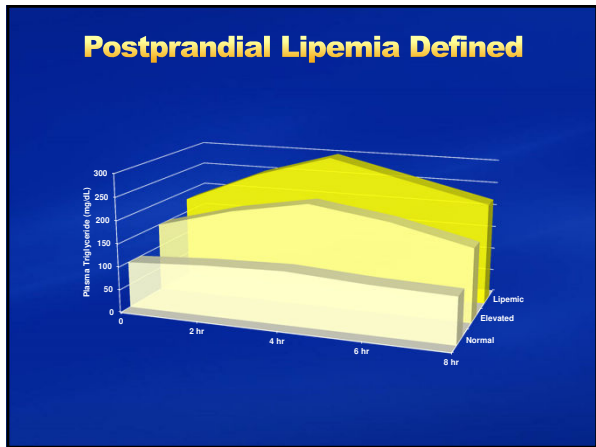
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Genetic	
Familial Hypertriglyceridemia	LPL deficiency
Apolipoprotein CII Deficiency	Apo A-V Deficiency
GPIIIBP1 Deficiency	Anderson Disease
Acquired Metabolic Disorders	
Hypothyroidism	Pregnancy
Diabetes	
Drugs	
$\alpha$ -interferon	Antipsychotics
$\beta$ -blockers	Bile Acid Resins
Estrogens	Protease Inhibitors
Steroids	Tamoxifen
Thiazides	
Diet	
Saturated Fats	Sugar-sweetened foods
Alcohol	

FIGURE 100-10 Postprandial Lipemia. From: Harrison's Principles of Internal Medicine, 19th ed. Copyright © 2011.

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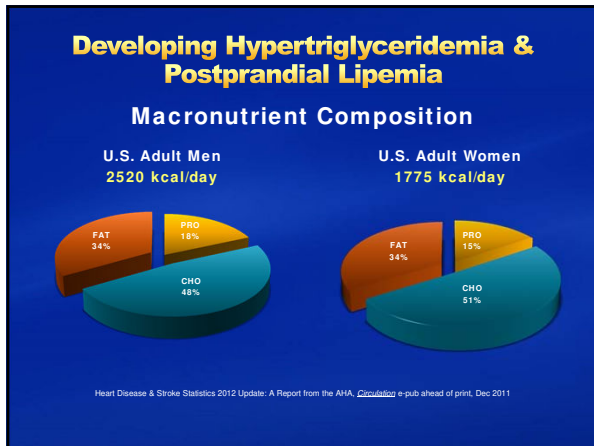
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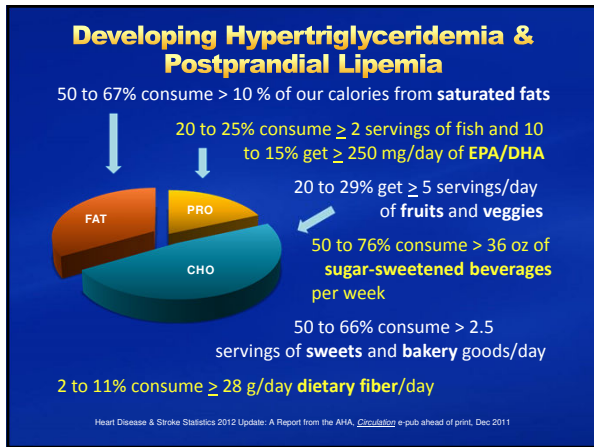
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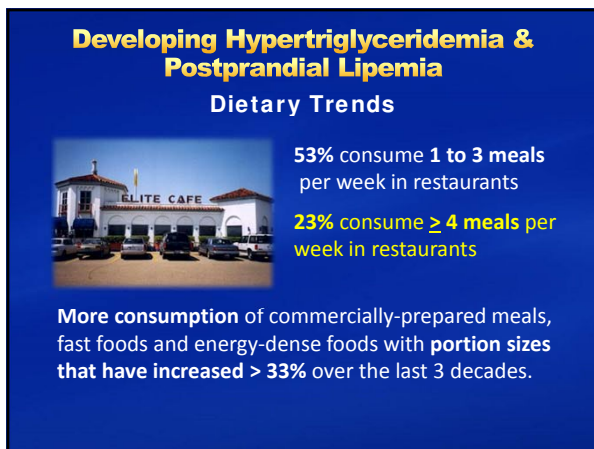
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### Developing Hypertriglyceridemia & Postprandial Lipemia

#### 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

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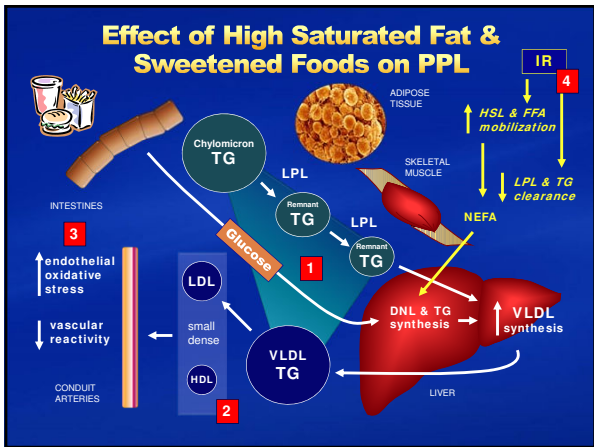
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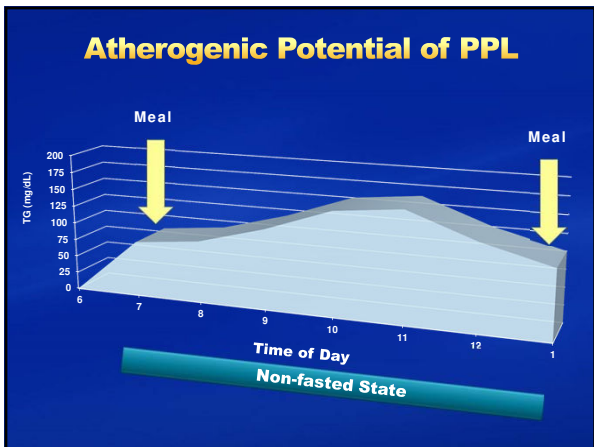
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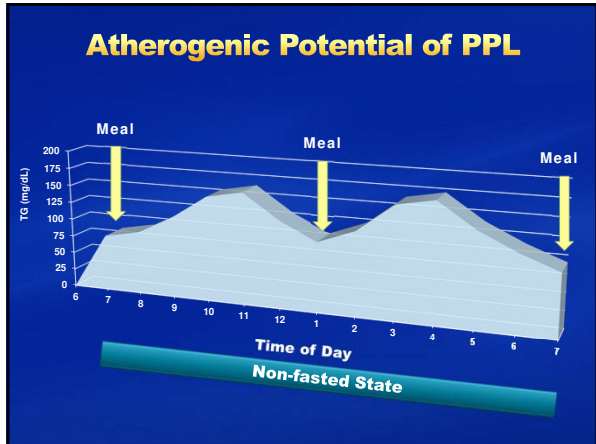
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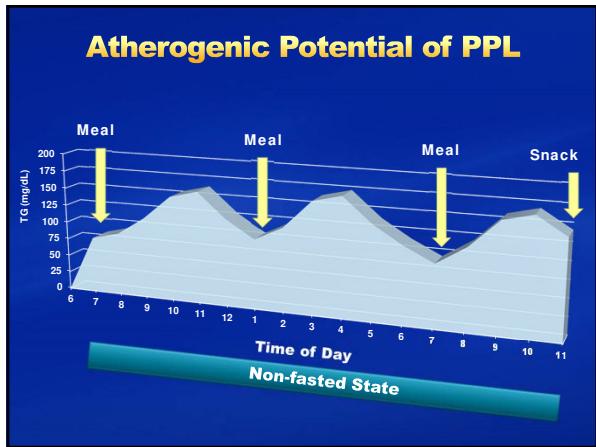
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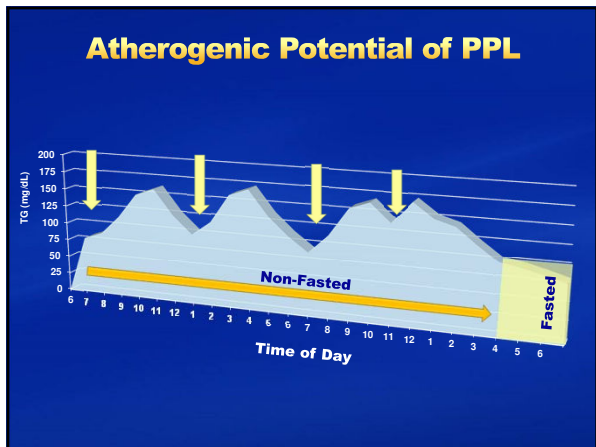
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### Atherogenic Potential of PPL

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.




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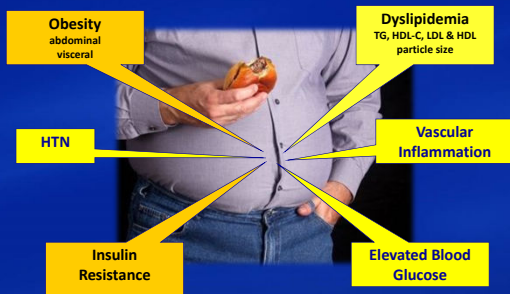
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### Metabolic Syndrome




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### Measuring Postprandial Lipid Responses




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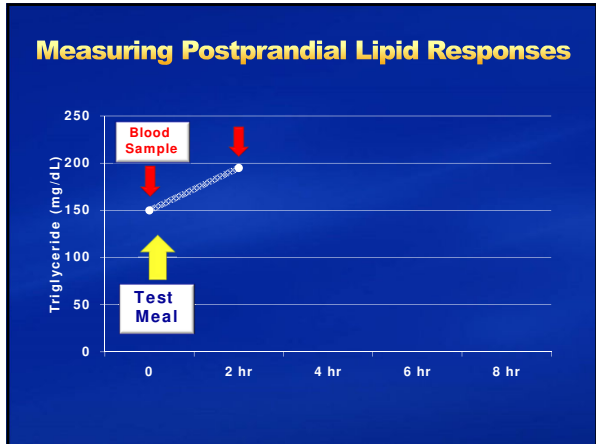
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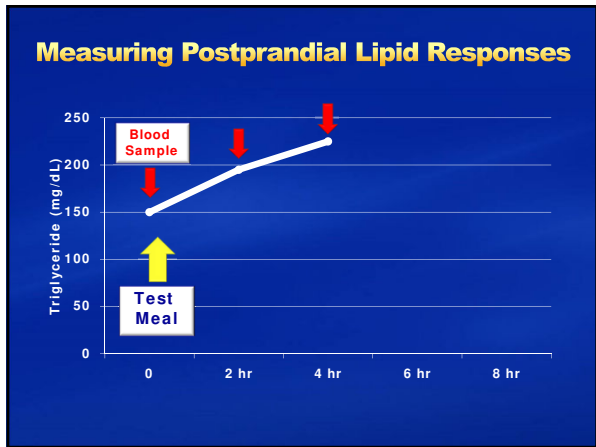
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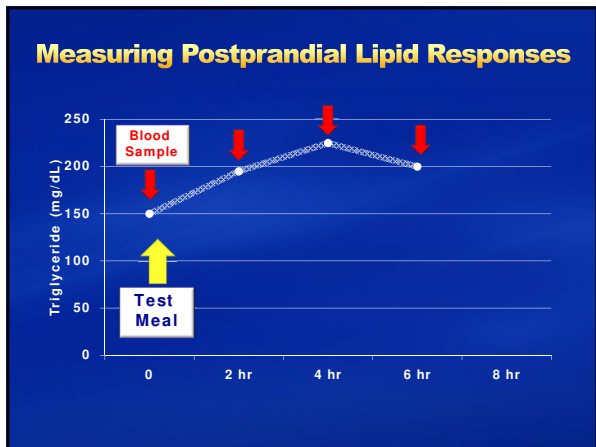
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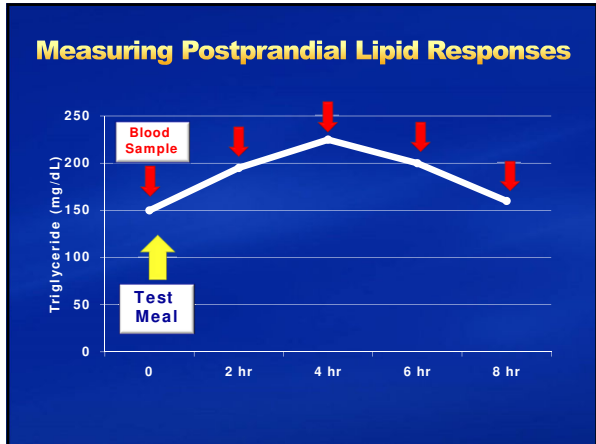
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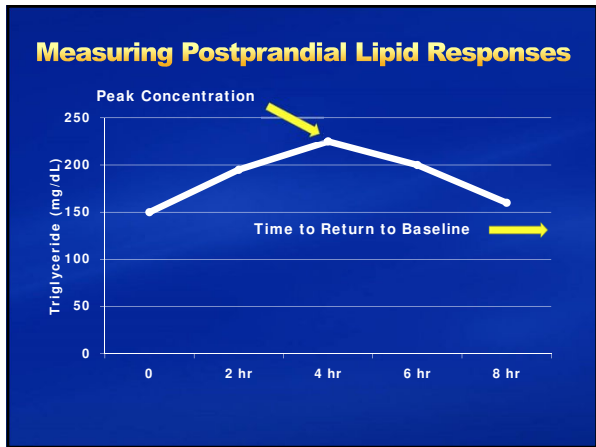
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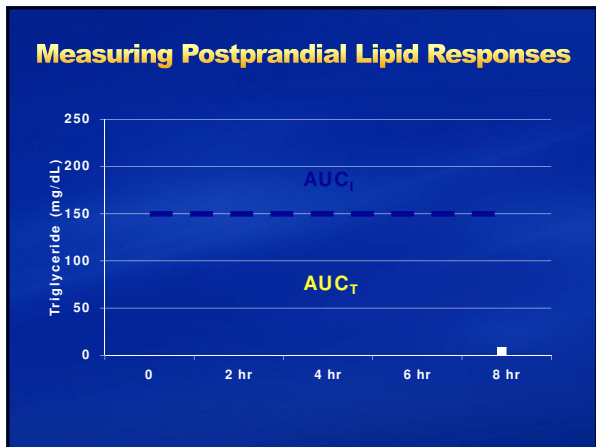
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## Non-Fasting Lipids & Lipoproteins

**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 AI apolipoproteins

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

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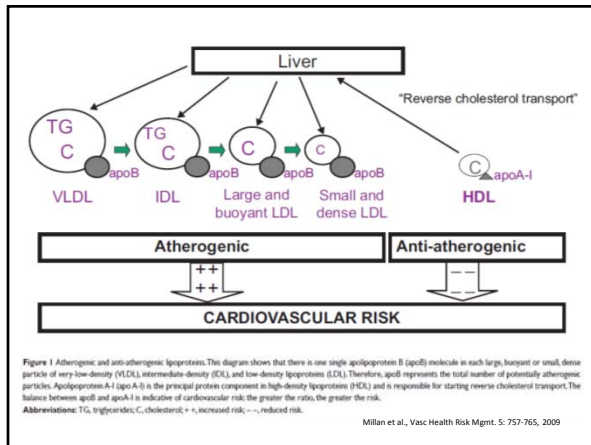
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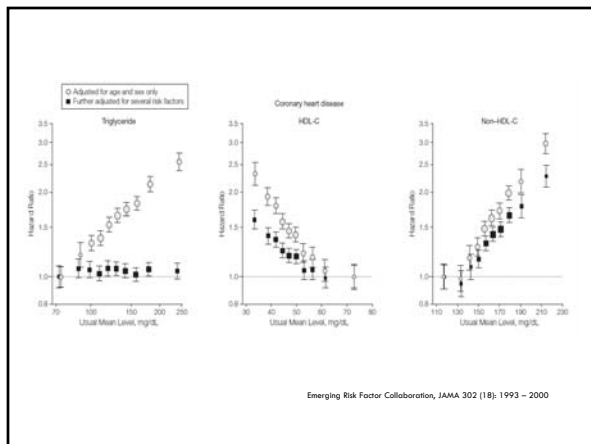
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## Non-HDL cholesterol & CAD Risk

Non-HDL-C = serum TC – HDLC

NCEP cut points are arbitrarily set at 30 mg/dL above LDL-C and assume a [triglyceride] of  $\leq 150$  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

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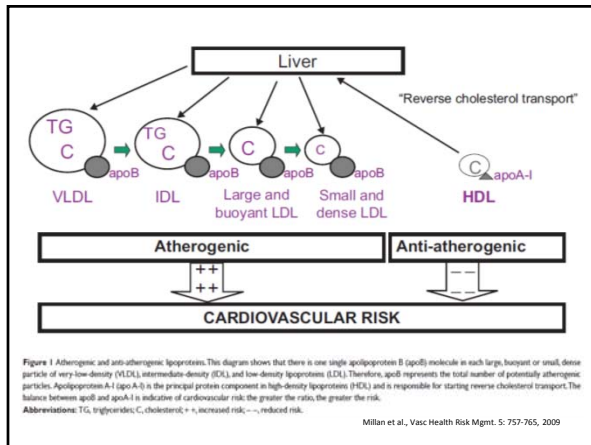
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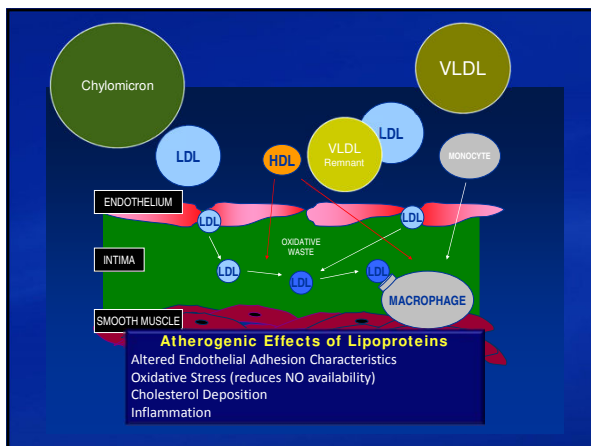
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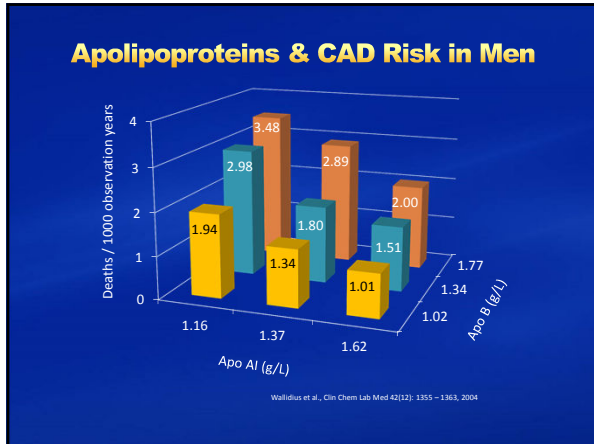
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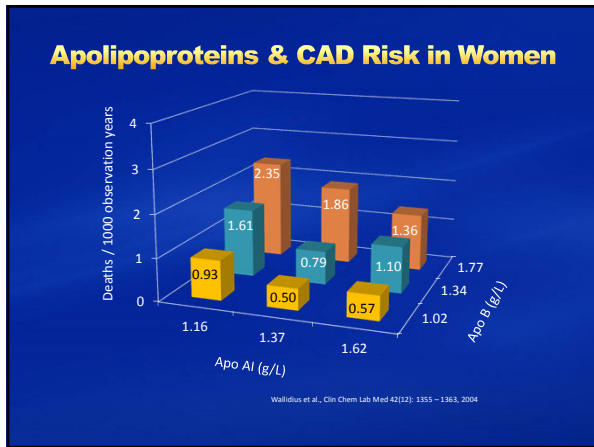
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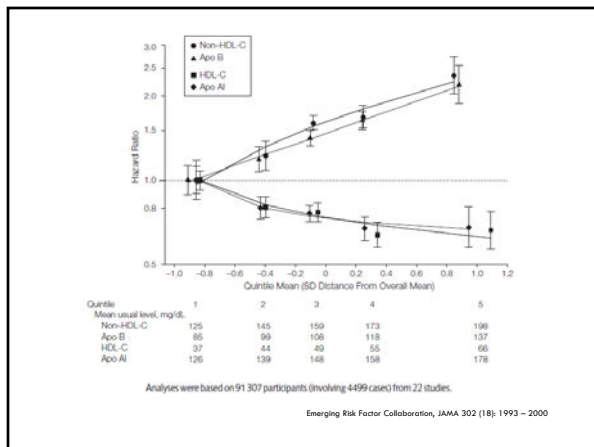
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### Apolipoprotein B & CAD Risk

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

**LDL-C < 70 mg/dL; non-HDL-C < 100 mg/dL; apo B < 80 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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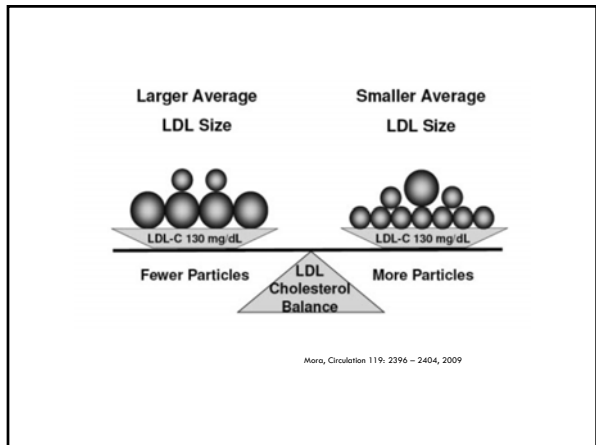
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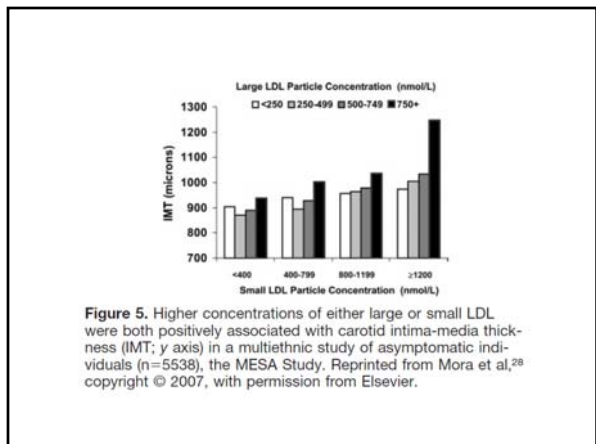
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**Figure 5.** Higher concentrations of either large or small LDL were both positively associated with carotid intima-media thickness (IMT; y axis) in a multiethnic study of asymptomatic individuals (n=5538), the MESA Study. Reprinted from Mora et al.,<sup>28</sup> copyright © 2007, with permission from Elsevier.

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### Advanced Lipoprotein Testing

**Gradient Gel Electrophoresis (Berkeley 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

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

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Lack of standardization and comparability of information provided by various tests

Information overload can be minimized by focusing on several key lipoprotein measures

Lack of accessibility

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 (eg, those with cardiometabolic risk factors)

Favorable cost-benefit ratio has not been demonstrated

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Moro, Circulation 119: 2396 – 2404, 2009

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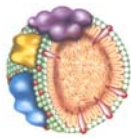
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### High Density Lipoprotein



**Promotes cholesterol efflux**

**Anti-oxidant**

**Anti-inflammatory** →

**Anti-fibrotic**

**Anti-thrombotic**

**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 TNF $\alpha$**

**Protects against endotoxins**

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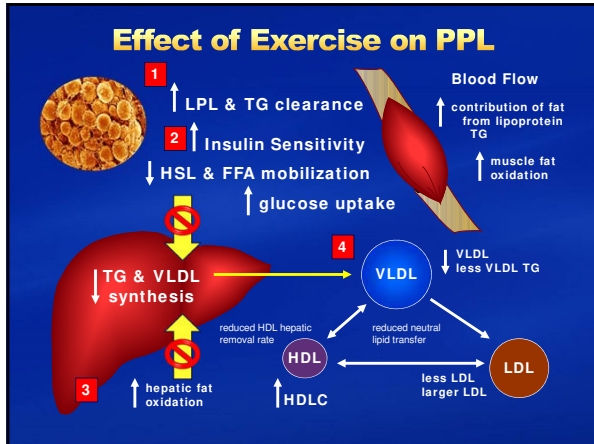
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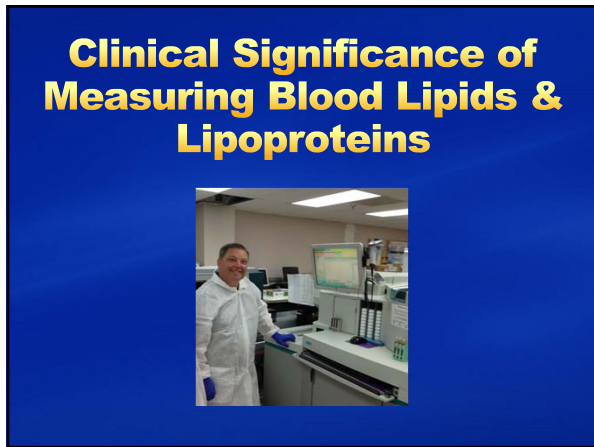
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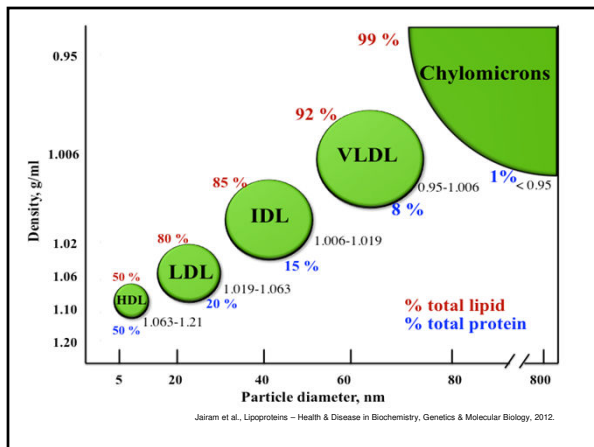
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