

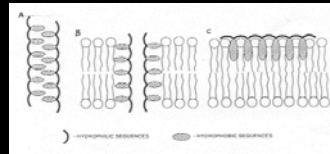
ApoB / Apo AI ratio and related laboratory issues

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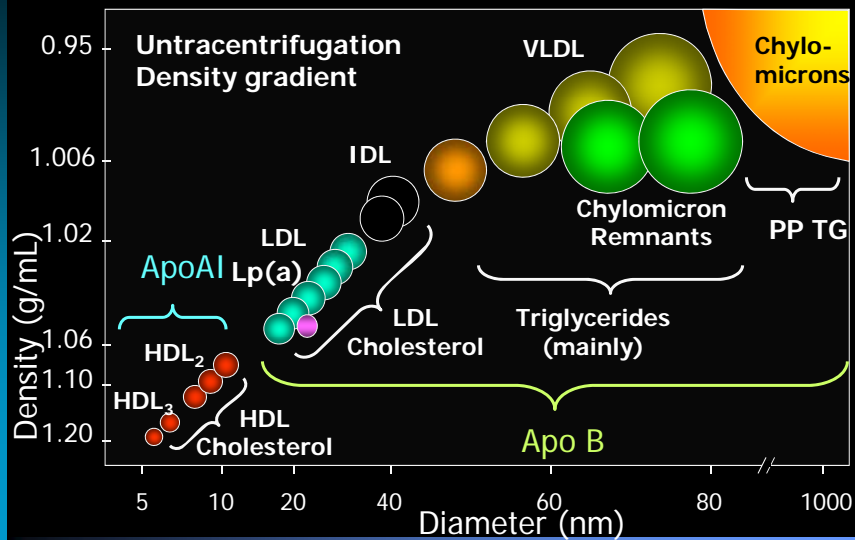
Apolipoproteins, ratios etc
Non-fasting samples in CVD risk assessment
What is the worst thing for arteries
(particles or cholesterol)?

Lipids, Lipoproteins and Apolipoproteins

- "Lipids" – substances with poor water solubility.
- Dispersed in blood by amphipathic proteins.
- Laws of physical chemistry: in a hydrophilic environment (eg blood) - lipoproteins spheres or discs.
- "Apo" means "part of", so "apolipoprotein" is a protein that is part of a lipoprotein



Lipoprotein Subclasses



Major Apolipoproteins

Apo	Location	Function	Plasma Levels	Athero
A-I	HDL (Chyl)	Multi anti-athero	High	↓↓↓
A-II	HDL	??	Moderate	↓?
B-48	Chyl	Exog. TG & Ch transp	Moderate (post-prandial only)	↑?
B-100	VLDL, LDL	Deliver endog. cholesterol	High	↑↑↑
C-II	VLDL, HDL	↑ LPL activity	Low	↓
C-III	VLDL, HDL	↓ LPL, plq rupt?	Low	↑↑
E	VLDL, HDL	Remn Lp Catab, Chol Efflux?	Low	↑↑↑/↓?
(a)	Lp(a)	Ox FFA scaveng	Low	↑↑↑

Apo B-100 and Apo A-I are most important clinically, but all are ~important.

Interheart: Case-control study of MI

- 52 Countries across all continents (N = approx 30,000)
- Risk Factor Odds Ratio Population attributable risk
-
- **ApoB/ApoA1** **3.25** **49%**
- **Smoking** **2.87** **36%**
- **Hypertension** **1.91** **18%**
- **Diabetes** **2.37** **10%**
- **Abdominal obesity** **1.12 – 1.62** **20%**
- **Psychosocial** **2.67** **33%**
- **Diet (fruit & veg)** **0.70** **14%**
- **Activity** **0.86** **12%**
- **Alcohol** **0.91** **7%**

ApoB/Apo A1 (especially Apo B) was superior in TNT and IDEAL trials

Univariate analysis	β		z	p	
apoB/A1	1.08110		11.871	<0.0001	
TC/HDL-C	0.19188		10.804	<0.0001	
LDL-C/HDL-C	0.25683		10.029	<0.0001	
apoB	0.00705		8.598	<0.0001	
Non-HDL-C	0.00546		8.400	<0.0001	
LDL-C	0.00502		6.275	<0.0001	
Multivariate analysis	β	p	β	p	
LDL-C	-0.00344	0.036	apoB	0.00945	0.0001
LDL-C/HDL-C	-0.14129	0.026	apoB/A1	1.42836	0.0001

β = regression coefficient for effect on MCVE from CPHRM; $Z = \beta / \text{StdErr}\beta$ In summary, pooled analyses of year 1 data from 17987 patients on statin therapy in IDEAL and TNT found that although LDL-C, non-HDL-C, apoB, LDL-C/HDL-C, TC/HDL-C, and apoB/A1 were all significant predictors of MCVE risk, apoB/A1 was the best predictor. These findings add rationale to the debate of whether apoB/A1 ratio as a predictor of risk should be incorporated into clinical guidelines.

Apo B was the best predictor of recurrent CVD events in LIPID

Circulation

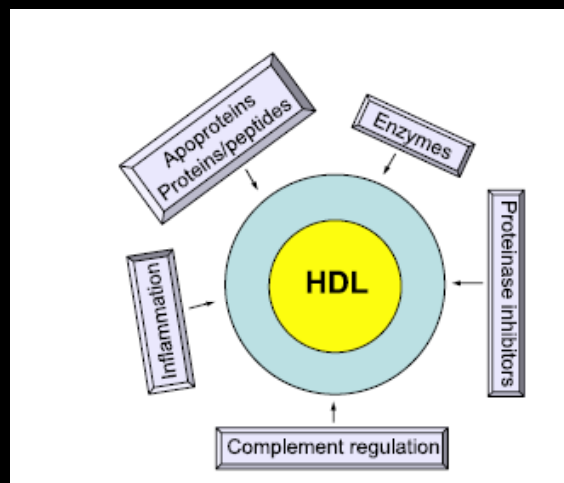
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association
Learn and Live™

Relationship Between Lipid Levels and Clinical Outcomes in the Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) Trial: To What Extent Is the Reduction in Coronary Events With Pravastatin Explained by On-Study Lipid Levels?

R. John Simes, Ian C. Marschner, David Hunt, David Colquhoun, David Sullivan, Ralph A.H. Stewart, Wendy Hague, Anthony Keech, Peter Thompson, Harvey White, John Shaw and Andrew Tonkin

HDL: Variable numbers of Apo A1 and many other important proteins



Prospective and Intervention Studies Assessing the Relationship of Apo AI versus HDL-C with CHD Events *

Study [Reference]	Baseline Age Range (Years)	Primary Outcome	Number of Subjects		Plasma Apo AI Relationship with Coronary Heart Disease Outcomes		
			Affected (M/F)	Unaffected (M/F)	Apo AI Relative To Outcome	p Value	Apo AI versus HDL-C
Primary Nested Case Control							
BUPA [20]	35-64	CHD Death	2280M	1145M	Lower in Affected Subjects	<0.05	No Difference
Primary Epidemiologic							
ARIC [21]	45-64	Nonfatal MI, CHD Death, Revascularization	509M/216F	4023M/6691F	Lower in Affected Subjects	<0.005	HDL-C Superior
Cunipilly [22]	40-65	Nonfatal MI or CHD Death	2612M	1964M	Lower in Affected Subjects	<0.02	HDL-C Superior
Dubbo [23]	60+	Nonfatal MI or CHD Death	441F/438M	1067F/839M	Lower in Affected Subjects	<0.001	HDL-C Superior
PRIME [24]	50-59	Fatal or Nonfatal MI, Revascularization, Angina	2892M	8794M	Lower in Affected Subjects	<0.0001	Apo AI Superior
Clinical Intervention Primary Prevention							
AFCAP/TexCAPS [3]	45-73	Fatal or Nonfatal MI, Unstable Angina, Sudden CHD Death	Placebo 170M/13F Lovastatin 109M/7F	Placebo 2633M/487F Lovastatin 2690M/492F	Lower in Affected Subjects	0.013	Apo AI Superior
Clinical Intervention Secondary Prevention							
Van Lenep [25]	51-75	Nonfatal MI or All-Cause Mortality	77M/21F (All Treated)	598M/133F (All Treated)	Lower in Affected Subjects	0.0003	Apo AI Superior

AFCAP/TexCAP, Airforce/Texas Coronary Atherosclerosis Prevention Study; ARIC, Atherosclerosis Risk in Communities Study; BUPA, British Union Provident Association; Caerphilly, Caerphilly and Speedwell Collaborative Heart Disease Studies; DUBBO, Prospective Dubbo Study of Australian Elderly; PRIME, Prospective Epidemiological Study of Myocardial Infarction.

Cromwell WC. *J Clin Lipidology* 2007;1:57-64.

Ability of traditional lipid ratios and apolipoprotein ratios to predict cardiovascular risk in people with type 2 diabetes (The FIELD Trial).

- RESULTS:** In the placebo group, the variables best predicting CVD events were non-HDL-cholesterol:HDL-cholesterol, total cholesterol:HDL-cholesterol (HR 1.21, $p < 0.001$ for both), ApoB:ApoA-I (HR 1.20, $p < 0.001$), LDL-cholesterol:HDL-cholesterol (HR 1.17, $p < 0.001$), HDL-cholesterol (HR 0.84, $p < 0.001$) and ApoA-I (HR 0.85, $p < 0.001$). In the fenofibrate group, the first four predictors were very similar (but ApoB:ApoA-I was fourth), followed by non-HDL-cholesterol and ApoB. Lipid ratios and ApoB:ApoA-I performed better than any single lipid or apolipoprotein in predicting CVD risk.

Cheating! Ratios usually involve 2 independent risk factors.

- Justified as “lipid risk”
- Really 2 independent components
- Separate clinical problems
- Separate treatment requirements
 - Increased LDL-C (or apo B)
 - Decreased HDL-C (or apo AI)
- Blood pressure x Plasma glucose ?
 - Would outperform single risk factors
 - Lacks any logical basis


Most studies rely on a single central lab Need to consider interlaboratory variability

Poor prior to
standardization
programme

Now probably
adequate

Consider suitability
of light-scattering
methods in lipaemic
serum

The Journal Of The International Federation Of Clinical Chemistry
And Laboratory Medicine



How to Cite this article: Reference Materials for the Standardization of the Apolipoproteins A-I and B, and Lipoprotein(a), Dati F, Tate J, eJIFCC vol 13 no 3: <http://www.ijfccc.org/ijfccc/vol13no3/130301003.htm>

Reference Materials for the Standardization of the Apolipoproteins A-I and B, and Lipoprotein(a)

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disease risk. Until recently, the lack of internationally accepted standardization impeded the broad application of apolipoproteins in laboratory medicine. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) through its Committee on Apolipoproteins and Working Group on Lp(a), and together with research institutions and several diagnostic companies have succeeded in their effort to achieve a consensus on a practical standardization procedure. This included the preparation of suitable secondary reference preparations needed for calibrating all commercially available immunoassays for measurement of apo A-I and B, and Lp(a).

Lipoproteins and apolipoproteins: Classification and properties

Professional and Public awareness: conveying concepts

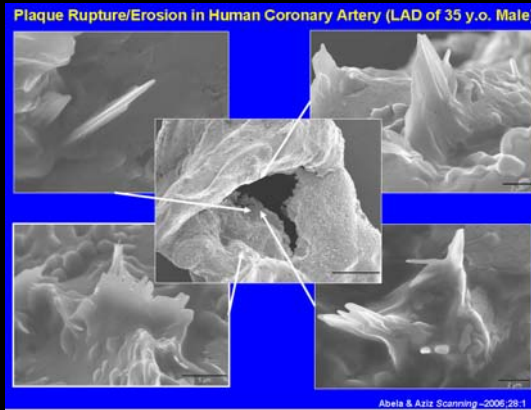
Plumbing:

Blocked arteries and
reduced flow

Banking or Accounting:

Cholesterol in the
artery wall
accumulates or
diminishes

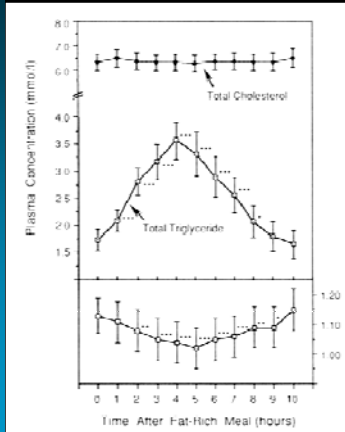
Apolipoproteins don't
fit the picture as easily



Summary re Apo B : Apo A1

- Apo A1 has little benefit over HDL-C
- Ratios only superior to single risk factors due to amalgamation
- Trials do not allow for inter-laboratory variability
- Apo B may have advantages over other single lipid risk factors such as LDL-C

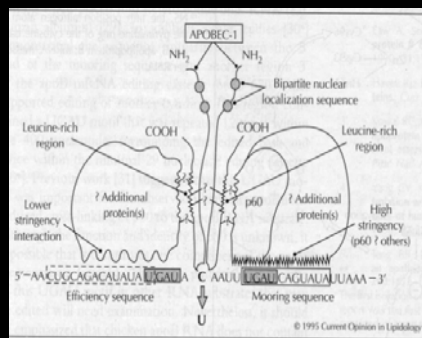
What difference does a meal make? Non-fasting samples



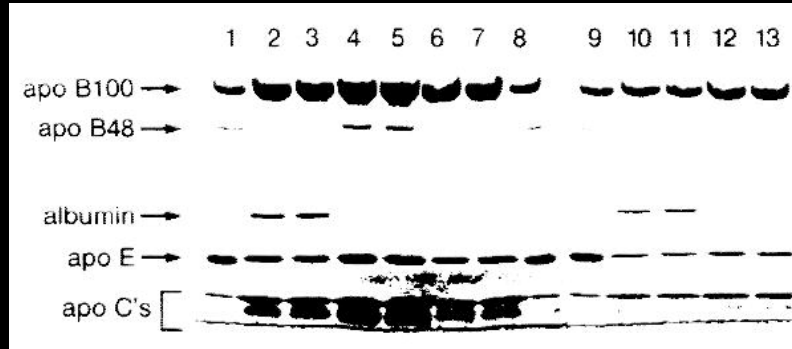
Fasting status affects TG, and to some extent HDL-C. It alters calculation of LDL-C rather than LDL-C itself (or total cholesterol).

Post-prandial apo B: Editing apo B100 to apo B48

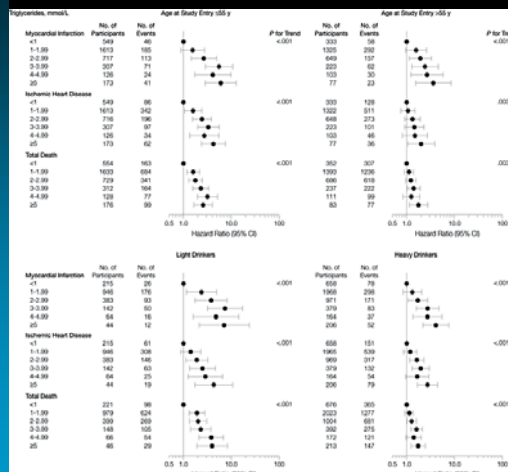
- Single gene (apo B100) produces 2 functionally different proteins
 - a) Hepatic B100 and
 - b) Intestinal (edited) B48 due to intestinal expression of editing enzyme (APOBEC1).



The number of apo B48 particles is very small compared to that of apo B100



Does fasting matter?



Standardization versus sensitivity:

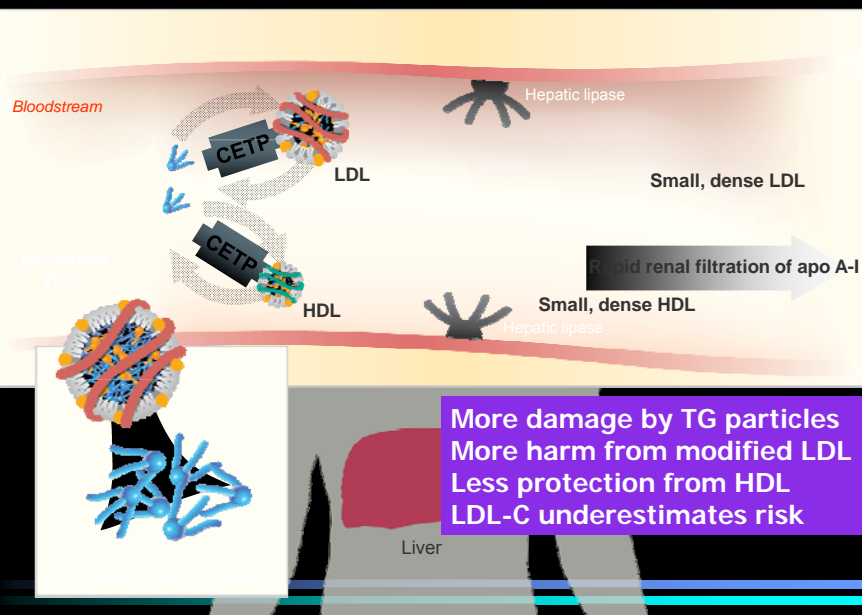
Age adjusted hazard ratios for MI, IHD and mortality stratified for alcohol and age at entry according to non-fasting TG level

Nordestgaard, B. G. et al. JAMA 2007;298:299-308

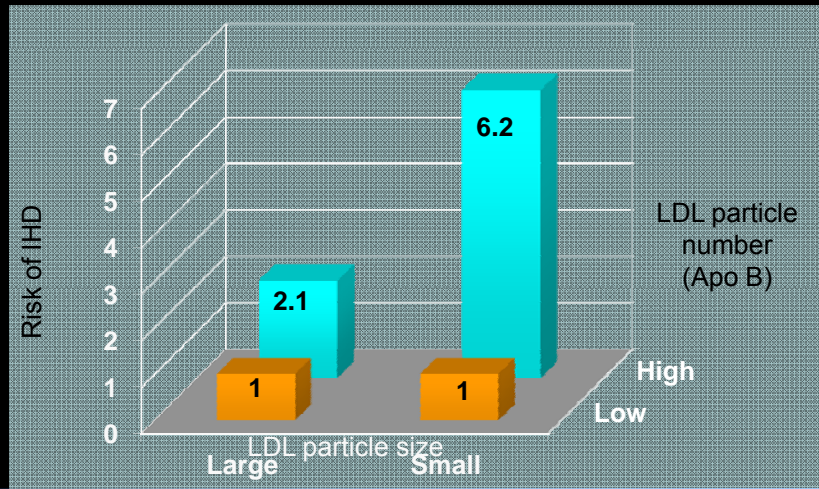
Summary re fasting

- Apo B 48 increases markedly after food intake, but this is quantitatively insignificant against a background of much higher Apo B100
- LDL-C doesn't change very much, but its calculation is invalidated by increased TG
- Non-fasting TG is a sensitive indicator of CVD risk

Does triglyceride matter?

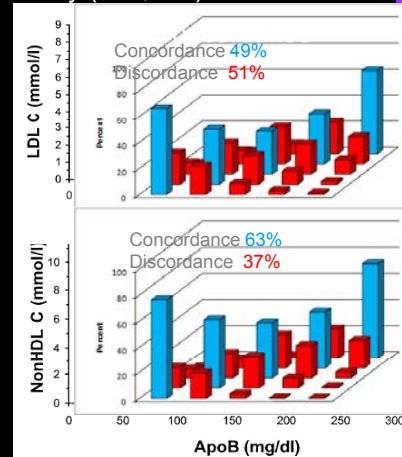
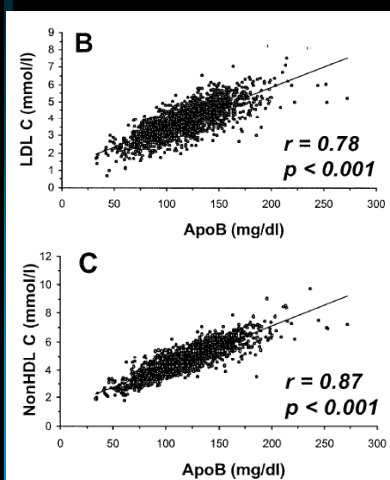


Quantitatively, CVD risk is proportional to the number of atherogenic lipoproteins rather than their cholesterol content (or composition).

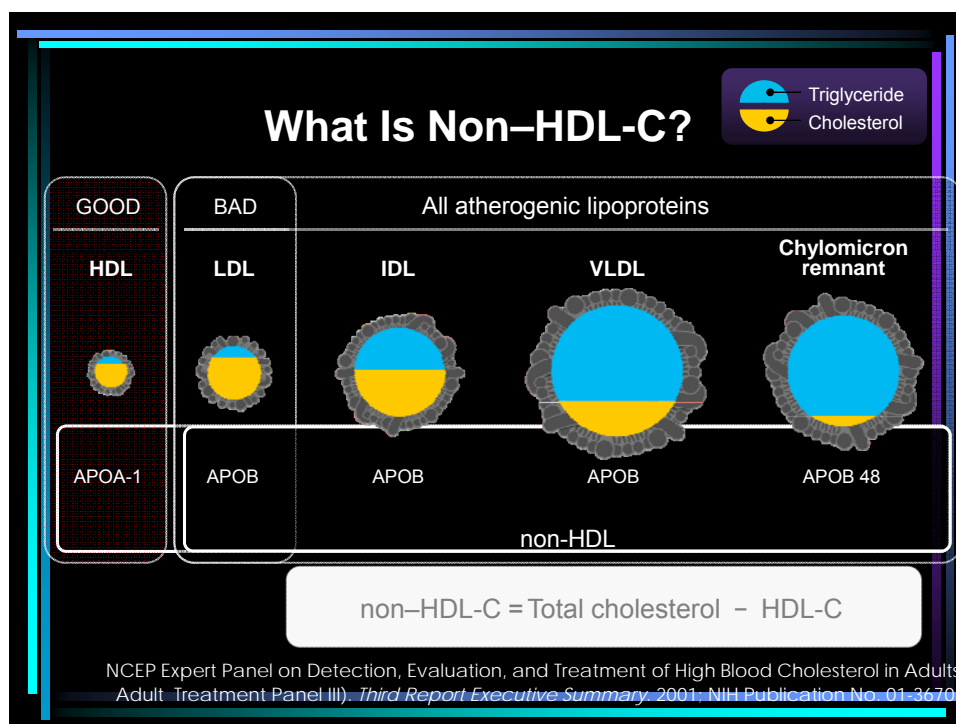


Relations of Apo B and Lipid Measures

Quebec Cardiovascular Study (n=2,103)



Adapted from Sniderman AD, et al. *Am J Cardiol* 2003;91:1173-1177



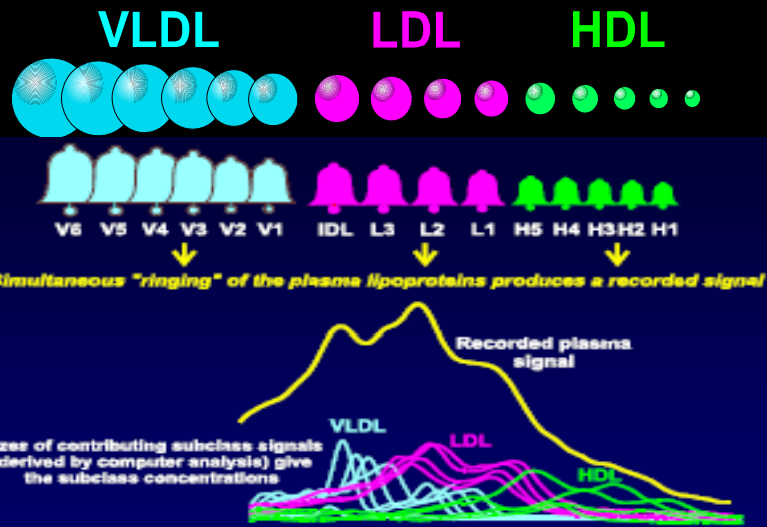
US Health Professionals Study

Biomarker	CHD RR, 95% CI	P-Trend
LDL-C	2.07 (1.24 – 3.45)	< 0.001
Non-HDL-C	2.75 (1.62 – 4.67)	< 0.001
Apo B	2.98 (1.76 – 5.06)	< 0.001

•Quintile 5 vs. Quintile 1
 •P-Trend is a test for a rise or fall in RR from Q1 to Q5

Pischon et al. *Circulation* 2005;112:3375-3383.

Counting particles by NMR

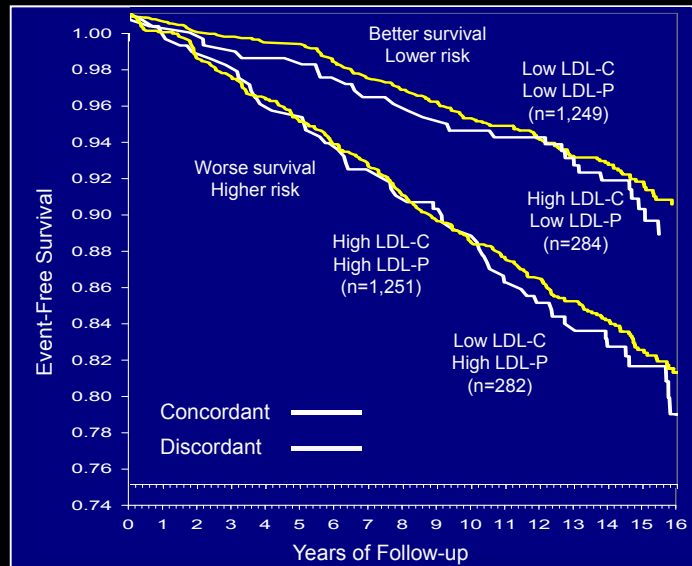


Outcome Associations of LDL Particle Number (LDL-P) versus LDL Cholesterol (LDL-C)

Study	CHD Status	Atherosclerotic Endpoint	Associations Stronger for LDL-P than LDL-C *
Women's Health Study <i>Circulation</i> 2002; 106:1930-1937 <i>Circulation</i> 2009;119:931-939	Primary Prevention	Incident MI, CHD death, CVA	YES
VA-HDL Intervention Trial <i>Circulation</i> 2006;113:1556-63	Secondary Prevention	Non-fatal MI or CHD Death	YES
MESA Trial <i>Atherosclerosis</i> 2007;192:211-217.	Primary Prevention	Carotid IMT	YES
Framingham Heart Study <i>J Clin Lipidology</i> 2007;1(6):583-592	Primary Prevention	Incident CVD Events	YES
Cardiovascular Health Study <i>ATVB</i> 2002; 22:1175-1180	Primary Prevention	Incident MI or Angina	YES
PLAC-I <i>AJC</i> 2002;90:89-94	Secondary Prevention	Angiographic MLD	YES
Health Women Study <i>AJC</i> 2002;90(suppl): 711-771	Primary Prevention	EBCT Coronary Calcium Score	YES

* Significant and independent after multivariate modeling (lipids and established risk factors)

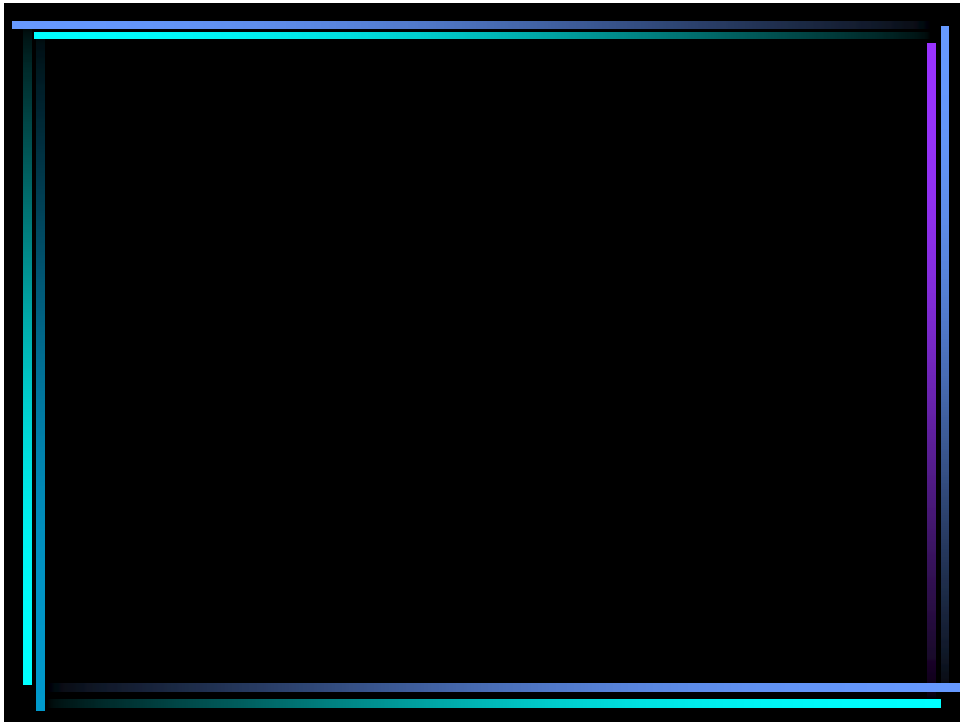
CHD Event Associations of LDL-P versus LDL-C Framingham Offspring Study (n=3,066)



Cromwell WC et al. *J Clin Lipidology* 2007;11(6):583-592

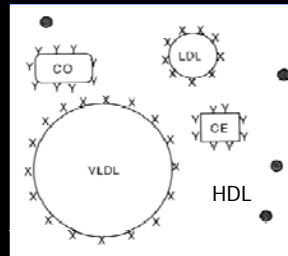
Summary

- Apo B has advantages over LDL-C, particularly when TG is elevated
- Non-HDL cholesterol is a "poor man's" substitute for Apo B
- Apo B reflects particle numbers, which can be quantified by NMR
- It may be difficult to change public and professional concepts about lipids and CVD.



"Direct" HDL-C measurement

- Automated methods use specific reagents to selectively expose and "directly" measure the HDL-C
- Selectivity not complete
 - (HDL > VLDL,
Chylomicrons > LDL)



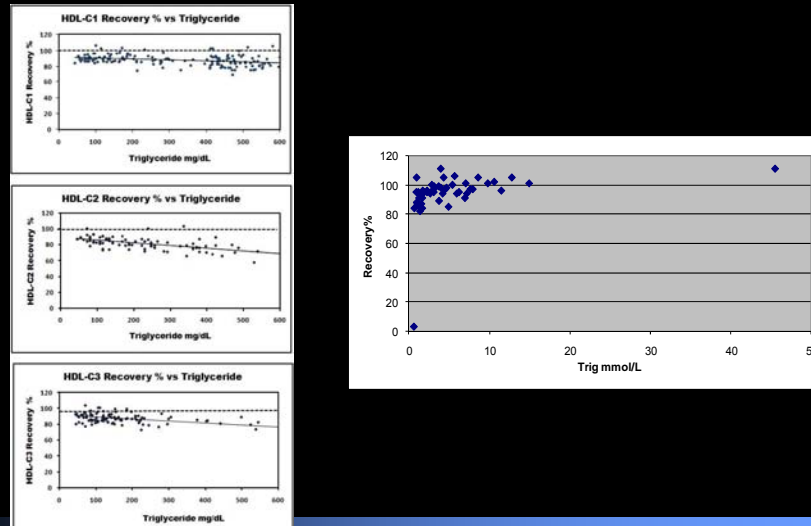
Can TG-rich lipoproteins interfere with direct HDL methods?

- Free of TG interference up to lipaemia index > 1200
 - Tested with Intralipid *which does not contain cholesterol*
- Free of interference from native TG up to 13.7 mmol/l
 - but...
- Package insert:
 - "To date, there is no model available that can mimic interference by triglycerides as triglyceride levels in patient specimens behave unpredictably,... Therefore customers cannot verify interference by triglycerides in patient specimens"

Hypothesis

- TRL cholesterol positively interferes with "direct" HDL-C methods
- Current "direct" measurements are confounded in many patients with high TG leading to
 - Failure to recognise Metabolic Syndrome
 - Artificial reduction in calculated LDL-C
 - Under-estimation of calculated CHD risk
 - Paradoxical TG and HDL-C responses to appropriate interventions.

Relationship between HDL-C recovery (UC-direct) and TG



Results: 20% over-estimation of HDL-C causes...

- 30-45% reduction in identification of metabolic syndrome (by various IDF definitions)
- 30 - 40% under-estimate of 10 year CHD risk according to Framingham equation.
- Studies in which triglyceride-rich lipoprotein levels are reduced may appear to also reduce HDL-C when direct HDL measurement is used (reported in response to fibrate, glitazone and niacin)

Conclusion

- Routine measurements of HDL-C may be confounded by the cholesterol content of triglyceride-rich lipoproteins thereby confounding assessment of metabolic syndrome, calculated LDL-C, CVD risk and treatment response