Advanced Lipid Testing: LDL Particles for the Clinician

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Disclosures

- Advisory board: Merck, Sanofi/Regeneron
- Consultant: Amgen
- Chief Science Officer for the National Lipid Association

What kills you

May not always be preventable!!

INTERHEART:

52 countries, 15,152 cases and 14,820 controls; 9 Risk Factors
Population Attributable Risk (PAR)

- **Smoking** (current vs. never)
  - OR 2·87 PAR 35·7%
- **ApoB/ApoA1** (top vs. bottom 1/5)
  - OR 3·25 PAR 49·2%
- **History of HTN**
  - OR 1·91 PAR 17·9%
- **DM**
  - OR 2·37 PAR 9·9%
- **Abd. obesity** (top vs bottom 1/3)
  - OR 1·12 PAR 20·1%
- **Psychosocial factors**
  - OR 2·67 PAR 32.5%
- **Daily fruits and vegetables**
  - OR 0·70 PAR 13·7%
- **Regular alcohol consumption**
  - OR 0·91 PAR 6·7%
- **Regular physical activity**
  - OR 0·86 PAR 12·2%

	Men		Women	
	≤55 yo	>55 yo	≤65 yo	>65 yo
PAR (%)	93.1	88.3	96.5	87.7

So why would any other Biomarkers be needed?

Lancet 2004; 364: 937–52

Usefulness of Biomarkers

- Indicators of disease state
  - Subclinical: Screening
  - Clinical: Diagnosis [recognizing overt disease]
  - Surrogate for specific disease
- Indicators of disease rate
  - Staging: categorizing disease severity
  - Prognostic: predicting course of disease, response to therapy, monitoring efficacy of therapy
- Indicators of disease trait
  - **Risk Factors** (causative - e.g., smoking)

    vs

    Risk Marker (associative - e.g., age)

Circulation 2006;113:2335-2362

Advanced Lipid Testing: There is Value

- Not certain that "advanced lipid testing" is a helpful clinical term:

  Does this mean a "size" or "density" measurement?

  Does it mean compositional changes – lipid content, protein content, charge, or oxidation?

  Does it mean functional measurements?

6

ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults

- None of the RCTs titrated drug therapy to specific LDL-C (or nonHDL-C) goals to improve ASCVD outcomes. The benefit was dependent on using maximally tolerated statin intensity.
  - Therefore, "there is no evidence to suggest" that we need targets of treatment.
  - What does this mean? That our beloved LDL-C biomarker is not useful?
- Of course not! The guideline constantly refers to knowing the LDL-C level in decision-making.
  - I believe that the EBM term "there is no evidence to suggest" really means that the "scientific evidence is inconclusive, however intuition, experience and other knowledge suggests" that knowing the amount of atherogenic lipid particles, and how I treat it, would be crucial.

JAMA 2013;310:2149

7

Advanced Lipid Testing: There is Value

- I propose we focus on tests that can be helpful to the clinician for assessing baseline risk and for on-treatment decisions.

<u>Particle number</u>: 1."Evolved LDL-Chol" testing
- NonHDL-C
- Apoprotein B
- LDL-P

2. "Evolved HDL-Chol" testing
- HDL-P
- Apoprotein A1

3. Lipoprotein (a)

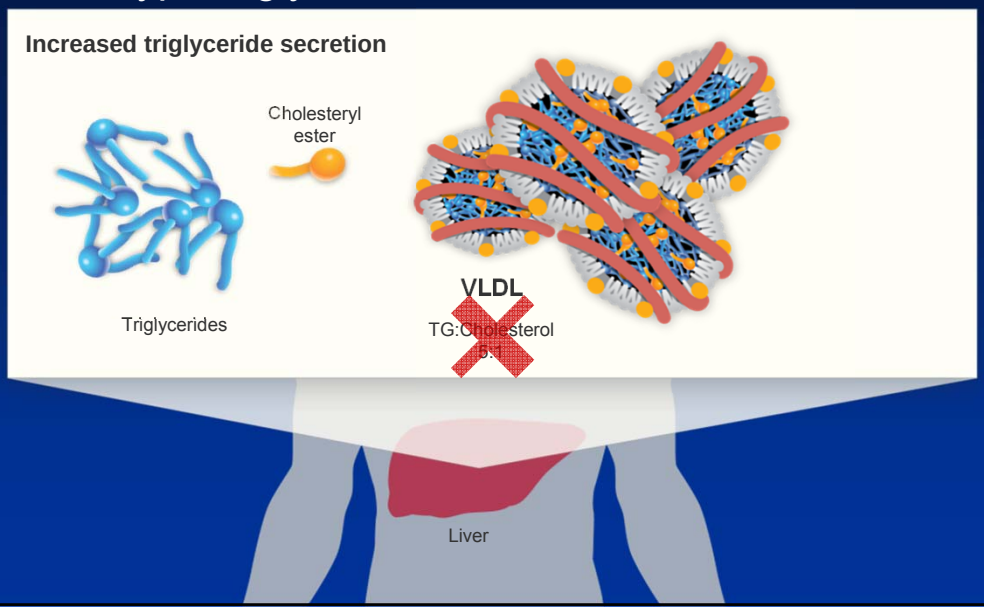
8

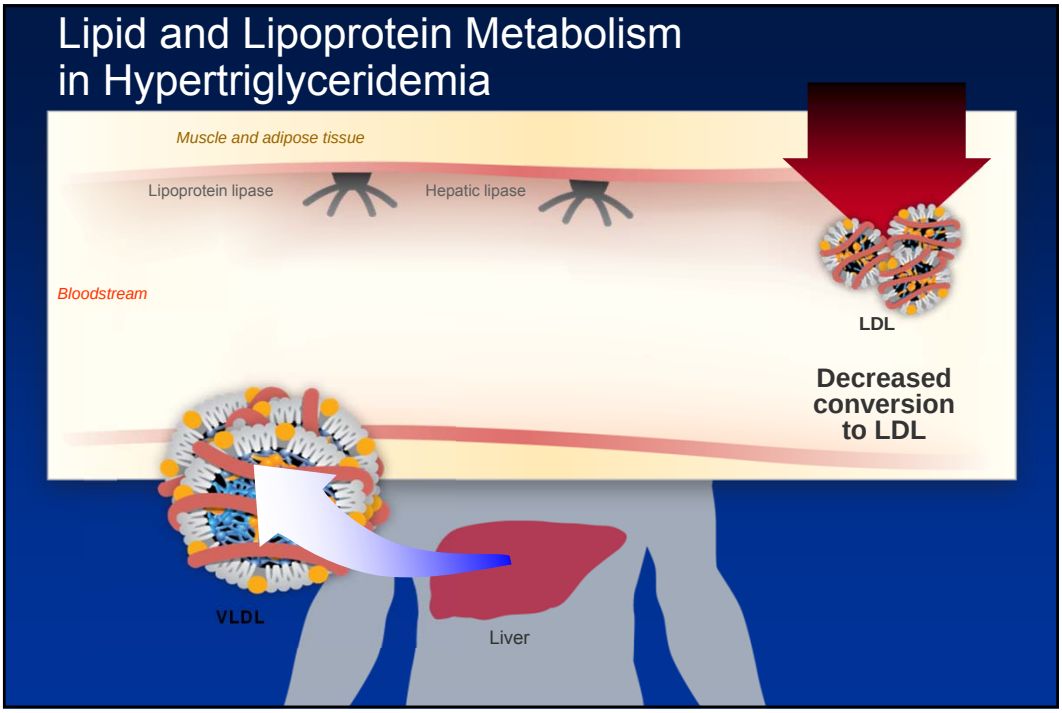
What is the value in doing "evolved LDL-C" testing?

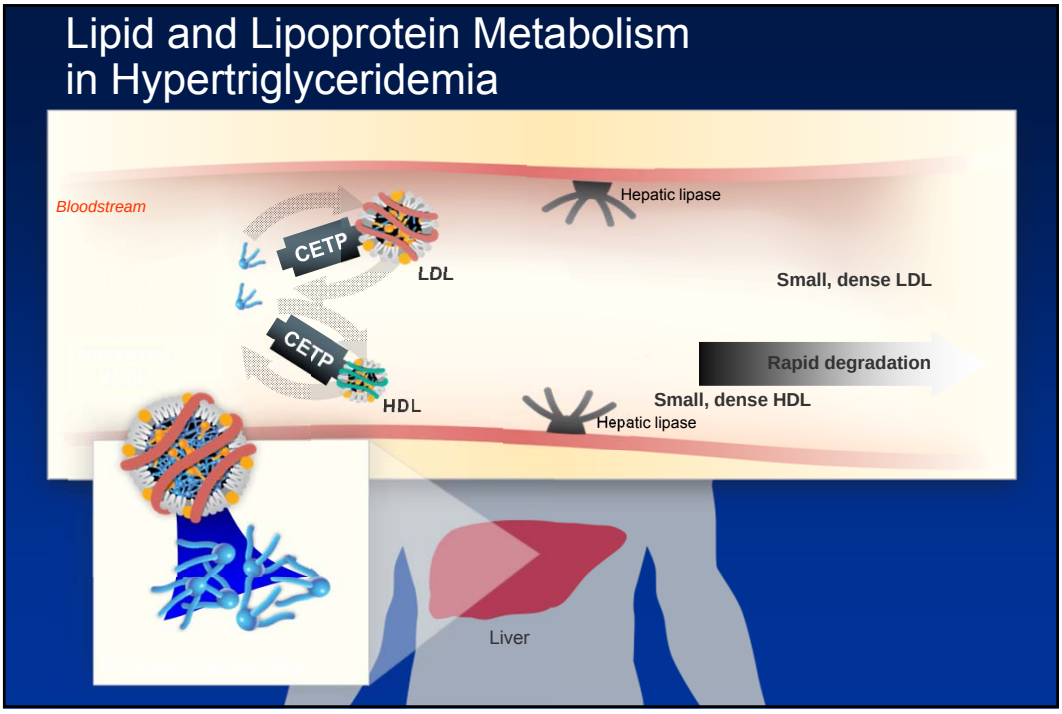
- It would lead to a better understanding of the lipid metabolism disturbances that may underlie the "residual risk" in many patients on optimal statin therapy. Disturbed TG-rich LP metabolism (e.g. in IR and T2DM) results in discordance between the cholesterol content of LDL and particle number.
- As a result, intensification of medical therapies that correct the lipid disturbance, and the discordance, could further reduce the risk.

9

Lipid and Lipoprotein Metabolism in Hypertriglyceridemia




Lipid and Lipoprotein Metabolism in Hypertriglyceridemia
Muscle and adipose tissue
Lipoprotein lipase
Hepatic lipase
Bloodstream
LDL
Decreased conversion to LDL
VLDL
Liver


Lipid and Lipoprotein Metabolism in Hypertriglyceridemia
Bloodstream
CETP
LDL
Hepatic lipase
Small, dense LDL
CETP
Rapid degradation
HDL
Small, dense HDL
Hepatic lipase
Liver

Clinical Quantification of LDL Particles

- NMR Lipoprofile
  - LDL-P (Liposcience)
- Fluorescence Analytical Ultracentrifugation
  - LPP (Spectracell)
- Apolipoprotein B
  - Surrogate for particle number

13

Population Equivalent Cut-points for Alternate LDL Measures

(LDL-C, Measured Apo B and NMR LDL-P)

Biomarker	Population	Percentile Equivalent Concentration			
		< 5th	20th	50th	80th
LDL-C (mg/dL)	Framingham[1]	< 70	100	130	160
Measured **Apo B** (mg/dL)	Framingham[1]	< 60	80	100	120
LDL-P (nmol/L)	Framingham[1]	< 850	1100	1400	1800
	MESA[2]	< 700	1000	1300	1600

14

[1] Contois JH, et al. *Clinical Chemistry.* 2009;55:407-419.
[2] Mora S, et al. AHA/ADA Met Syn/Met Risks, San Francisco, May 3-5, 2006.

Common Commercially Available Lipoprotein Tests Size Separation Methods

Method	Vendor	Values Reported			
		LDL	HDL	VLDL	Selected Other Tests (different technology)
Gradient Gel Electrophoresis	Berkeley	Total LDL-C, % LDL-C in 7 regions (I, IIa, IIb, IIIa, IIIb, IVa, IVb)	Total HDL-C, % HDL-C in 5 regions (2b, 2a, 3a, 3b, 3c)		Apo B, Lp(a), ApoE genotype, Kif-6 genotype, Lp-PLA2
	Quantimetrix	Total LDL-C, (Cholesterol in 3 "Mid" regions from VLDL - LDL plus 7 LDL regions [1-7])	Total HDL-C, HDL-C in 3 regions (small, intermediate, Large)	Total VLDL-C	

Common Commercially Available Lipoprotein Tests Density Separation Methods

Method	Vendor	Values Reported			
		LDL	HDL	VLDL	Selected Other Tests
Ultra-centrifugation Vertical spin	Atherotech	Total LDL-C, (LDL-R, Lp[a]-C, IDL-C), LDL Density (LDL_{1+2} buoyant, LDL_{3+4} dense)	Total HDL-C, (HDL_2-C, HDL_3-C)	Total VLDL-C, ($VLDL_{1+2}$-C, $VLDL_3$-C)	Triglycerides, Non-HDL-C, Remnant-C ($IDL+VLDL_3$), Calculated Apo AI, Calculated Apo B, Calculated Apo B/AI
Continuous Gradient Ultra-centrifugation Florescent Marker	SpectraCell	Particle Numbers Total LDL, RLP, Dense LDL_{III}, Dense LD_{IV},+LP(a)	Particle Numbers Total HDL, Buoyant HDL_{2b}	Particle Number Total VLDL	Total-C, LDL-C, HDL-C, Triglycerides, Lp(a) LDL Density HDL Density

Common Commercially Available Lipoprotein Tests Non Size/Density Separation Methods

Method	Vendor	Values Reported			
		LDL	HDL	VLDL	Selected Other Tests
Nuclear Magnetic Resonance Spectroscopy	Lipo-Science	Total LDL Particle Number (includes IDL-P), Small LDL Particle Number, LDL Particle Size	Total HDL Particle Number, Large HDL Particle Number, HDL Particle Size	Large VLDL Particle Number, VLDL Particle Size	LP-IR Score (Insulin Resistance) Lipid Panel Total C, HDL-C, TG, LDL-C (calculated)
Apolipoprotein	Multiple	Apo B	Apo AI	Multiple Alternate Assays	

- I will admit that a population-based approach to the use of apo B or LDL-P would suggest that there is little effect of these factors on the c-statistic (ROC) of traditional RF, or on the Net Reclassification Index (NRI)
- However, I say that as clinicians, we don't treat populations – we treat people one at a time, and we need guidance on individual treatment decisions

18

Calculated LDL-C Misclassifies Particle Number at the Low End of LDL Spectrum

- Evaluation of 1.3 million patients with VAP test 2009-2011
- Calculated LDL-C (TC – HDL – TG/5) vs direct LDL measurement
- Calc LDL-C 70 – 99 mg/dL:
  - Direct LDL-C 7 mg/dL higher if TG 150-200 mg/dL
  - Direct LDL-C 14 mg/dl higher if TG > 200 mg/dL
- Calc LDL-C < 70 mg/dL:
  - Direct LDL-C 9 mg/dL higher if TG 150-200
  - Direct LDL-C 18 mg/dL higher if TG > 200
- NonHDL-C is a better measure of particle number at low calculated LDL-C

JACC 2013; 62:732

Meta-analysis of 13 epidemiology studies: overall vascular relative risk ratios per standard deviation increase

Marker	RRR (95% CI)	P-value
LDL-C	1.25* (1.18 to 1.33)	<0.001
Non-HDL-C	1.34 (1.24 to 1.44)	<0.001
ApoB	1.43 (1.35 to 1.51)	<0.001

Sniderman AD, et al. *Circ Cardiovasc Qual Outcomes. May 2011;4(3):337-345.*

- So I would say that the most cost-efficient “advanced” lipid test is nonHDL-C. It can be done fasting or nonfasting. It is the recommended target of therapy from IAS and NLA.
- For clinical decisions, when both LDL-C is < 70 mg/dL and nonHDL-C is < 100 mg/dL (**concordant**) on optimal statin doses in high risk patients, there is no need to assess apo B or LDL-P.

  AIM HIGH
  HPS 2 THRIVE
  ACCORD Lipid

Mixed Dyslipidemia: Discordance Between LDL-C and ApoB / non-HDL-C

TC	198 mg/dL
LDL-C	130 mg/dL
TG	90 mg/dL
HDL-C	50 mg/dL
Non-HDL-C	148 mg/dL
ApoB	95 mg/dL

Cholesterol
ApoB

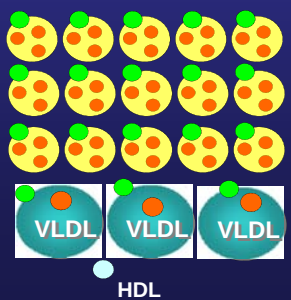
TC	210 mg/dL
LDL-C	130 mg/dL
TG	250 mg/dL
HDL-C	30 mg/dL
Non-HDL-C	180 mg/dL
ApoB	118 mg/dL

Large LDL *(pattern A)*

Same LDL-C

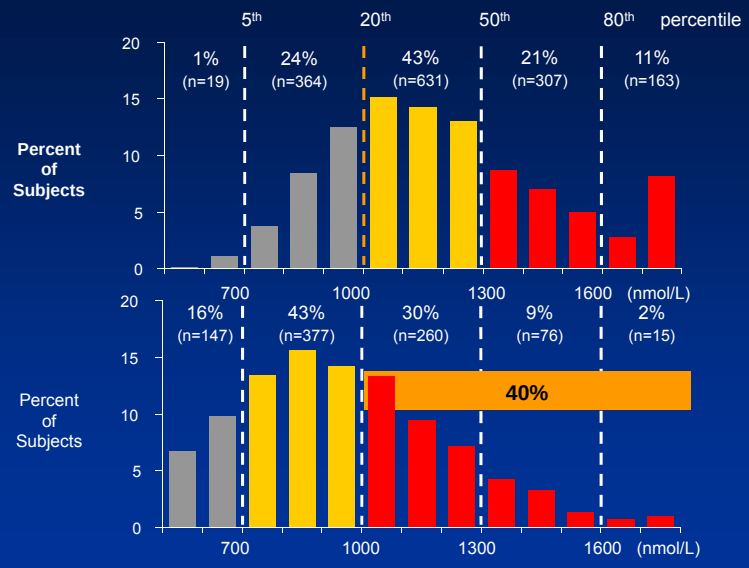
Different non-HDL-C, ApoB

Small, dense LDL (pattern B)



Size doesn’t matter – particle number is more important!

LDL Particle Number Distribution in T2DM Subjects in MESA



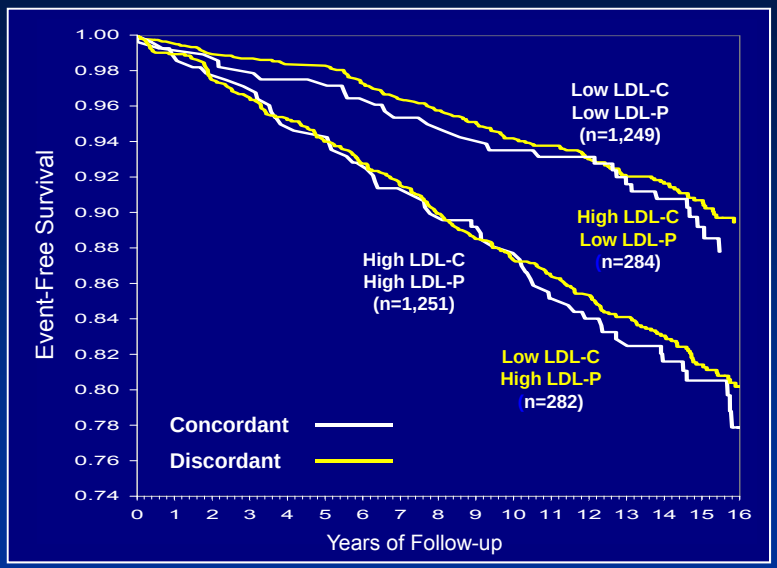
LDL-C
70-99 mg/dL
(n=1484)

LDL-C
<70 mg/dL
(n=871)

Cromwell WC, Otvos JD. *Am J Cardiol.* 2006;98(12):1599-1602.

CHD Event Associations of LDL-P versus LDL-C

Framingham Offspring Study (n=3,066)



J Clin Lipidology 2007;1(6):583-592.

Lipoprotein Particle Measures for Assessing CHD Risk: MESA

- 4679 MESA participants frre of CHD followed for 8.5 years
- Apo B and LDL-P (highest quartile vs lowest) significantly predicted CHD (HR 1.84 and 1.77)
- After adjustment for non-HDL-C, the prediction of both was not significant
- Net reclassification index (NRI) was not improved by apo B or LDL-P over non-HDL-C
- Conclusion: Routine measurement of apo B or LDL-P not recommended. There was not enough outcomes data available to adequately determine if "discordant" phenotypes would be different.

ATVB 2015;35:448

- Therefore, when LDL-C and nonHDL-C are **discordant**, risk tracks with particle number (which can be determined by non-HDL-C, apo B or LDL-P).

Post hoc subgroup analysis from AIM HIGH, HPS 2 THRIVE and ACCORD suggest that additional lipid drug therapy to reduce non-HDL (or LDL-P or apo B) could provide incremental risk reduction under this clinical situation.

2015 AACE Practical T2DM Guidelines

- **Dyslipidemia management**:

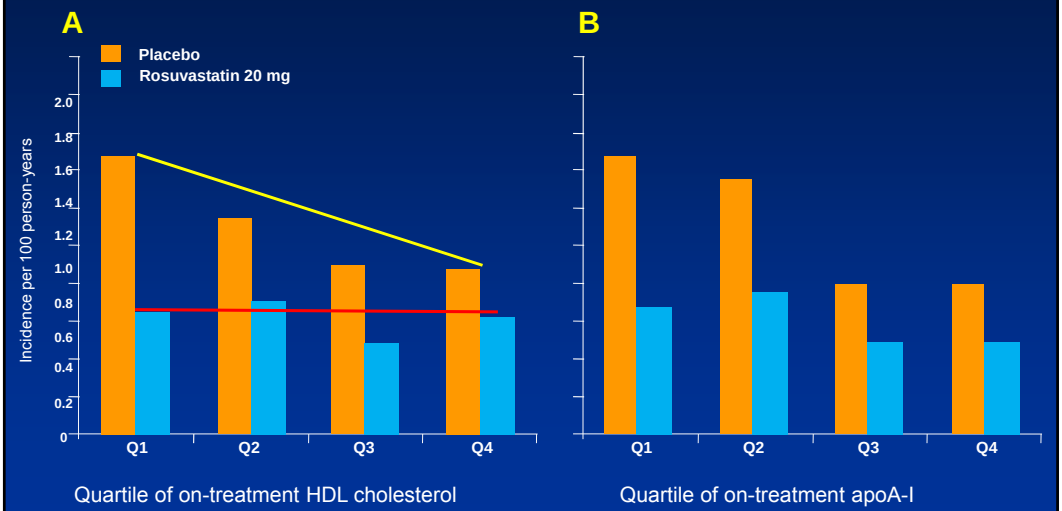
  Moderate risk (no RF, age < 40):

  LDL-C < 100 mg/dl
  non-HDL-C < 130 mg/dL
  apo B < 90 mg/dL
  LDL-P < 1200 nmol/L

  High risk (+ CVD, multiple RF):

  LDL-C < 70 mg/dL
  non-HDL-C < 100 mg/dL
  apo B < 80 mg/dL
  LDL-P < 1000 nmol/L

Primary Endpoint Incidence Rates in JUPITER According to On-Treatment HDL-C and apoA-I Quartiles



Ridker PM, et al. *Lancet.* 2010;376(9738):333-339. Copyright © 2010 Elsevier Ltd.

CVD Risk After Statin Treatment (JUPITER): HDL-C, HDL Size and HDL-P Effects

- NMR measurements of HDL size and HDL-P in 10,886 subjects in JUPITER
- Findings:
  1. HDL-P correlated better with apo A1 than HDL-C
  2. In PBO group, on-treatment HDL-C, apo A1 and HDL-P had similar inverse relationship to CV events
  3. In RSV group, on-treatment HDL-P was better than apo A1 and HDL-C in predicting CV events. HDL size had no relationship to CV events

Circulation 2013;128:1189

HDL-C, HDL-P and Apo A1 as Risk Predictors

Meta- analysis of 8 trials; n = 38,153 participant-level data

Findings:

HDL-C and apo A1 were inversely predictive of events, even with LDL-C < 50 mg/dL.

The change (increases) in apo A1, but not HDL-C, showed incremental risk benefit on statin treatment.

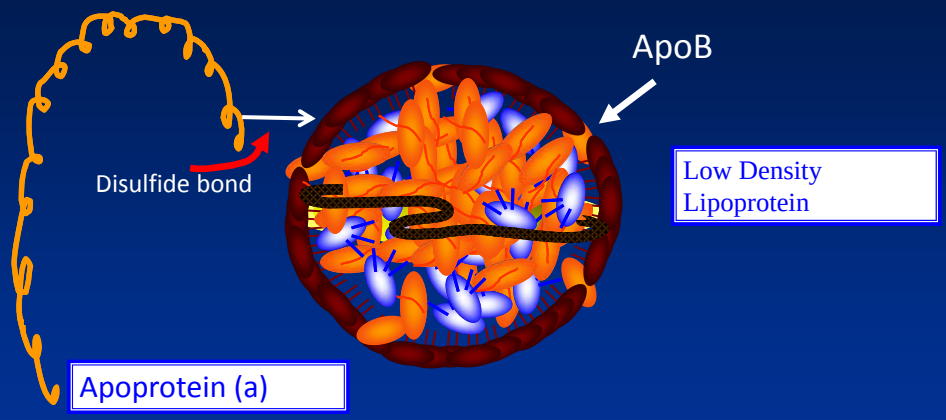
Dallas Heart Study: n = 1977 subjects followed for 9 yr.; incident CHD and CAC score.

HDL-C was not associated with CAC (overall) nor incident CHD in AA.

HDL-P was inversely associated with both CAC and CHD, independent of ethnicity.

Circulation 2013; on line August
Am J Cardiol 2015;115:890

Lipoprotein (a)



Lp(a) is an LDL particle to which a large glycoprotein, apolipoprotein (a), is covalently bonded to apoB.

Lipoprotein (a)

- LPA gene is causally related to CVD risk. Lp(a) is predictive of outcomes post PCI (AJC 2015;115:157) and post CABG (Atherosclerosis 2014;235:477)
- LPA gene is associated with greater prevalence of aortic stenosis (NEJM 2013; 368:503, JAMA 2014; online 10/2014))
- **AIM HIGH** study showed that baseline and on-treatment Lp(a) predicted CV events in both treatment arms (JACC 2013;62:1575). The **LIPID** trial also found similar baseline and on-treatment relationship of highest quartile Lp(a) to events (ATVB 2013;33:2902). Finally, **JUPITER** showed that highest Lp(a) tertile predicted CVD in primary prevention subjects at baseline and on-treatment (Circulation 2014;129:635)
- Standardized measurement is not established:
  - Lp(a) cholesterol
  - Lp(a) protein
  - Lp(a) total particle in mg/dL (favored by EAS)
  - Lp(a) particle number in nmol/L (favored by NIH)

EAS Screening Recommendatons for Lp(a)

- Premature CVD
- Familial hypercholesterolemia
- Family history premature CVD or increased Lp(a)
- Recurrent CVD events despite optimal statin treatment
- High risk if > 50 mg/dL (> 75 nmol/L)

Eur Heart J 2010; 31:2844

What to do with high risk Lp(a)?

- If Lp(a) > 75 nmol/L (or > 50 mg/dL):

  High intensity statin +/- other LLT
  Consider niacin ER 2 gm/day if patient has FH and/or CHD (EAS recommendation)
  PCSK9 monoclonal antibodies [reduce LDL and Lp(a)]

Eur Heart J 2010; 31:2844

Practical Value of Evolved Lipid Testing

- NonHDL-C should be the cost efficient test to assess baseline risk, and on-treatment decisions.

  1. Concordant LDL-C and nonHDL-C on optimal statin dosing does not require any further particle number testing.

  2. Persistent discordance in LDL-C and nonHDL-C (> 30 mg/dL) on optimal statin doses MAY be a case for knowing the apo B or LDL-P. Many of these patients are high risk, and have T2DM or IR. Subgroup analysis of AIM HIGH and ACCORD suggest that further LLT could provide incremental benefit.

Practical Value of Evolved Lipid Testing

- Assess Lp(a) in patients with personal premature CHD, FHx of premature CHD, FH or recurrent events on high-intensity statin.

  If Lp(a) > 75 nmol/L (50 mg/dL), high intensity statin use is mandatory in primary prevention cases. Consider niacin ER 2 gm/day as add-on therapy if FH and/or CHD

- If assessing baseline risk, HDL-P is better than HDL-C.

  No clinical need to assess **on-treatmen**t HDL-P since therapy directed at changing it has not been confirmed to provide incremental benefit. (Eur Heart J 2015;36:10-12)

NLA Summary Recommendations for Initial Clinical Assessment

	Initial Clinical Assessment					
	CRP	**Lp-PLA$_2$**	**Apo B**	**LDL-P**	**Lp(a)**	**HDL or LDL Subfractions**
Low risk	Consider for selected patients	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Intermediate risk	Recommended for routine measurement	Consider for selected patients	Reasonable for many patients	Reasonable for many patients	Consider for selected patients	Not recommended
CHD Equivalent	Consider for selected patients	Consider for selected patients	Consider for selected patients	Consider for selected patients	Consider for selected patients	Not recommended
Family History	Reasonable for many patients	Consider for selected patients	Reasonable for many patients	Reasonable for many patients	Reasonable for many patients	Not recommended
Recurrent Events	Reasonable for many patients	Consider for selected patients	Reasonable for many patients	Reasonable for many patients	Reasonable for many patients	Not recommended

NLA Summary Recommendations for On-Treatment Management Decisions

	On-Treatment Management Decisions					
	CRP	**Lp-PLA$_2$**	**Apo B**	**LDL-P**	**Lp(a)**	**HDL or LDL Subfractions**
Low risk	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Intermediate risk	Reasonable for many patients	Not recommended	Reasonable for many patients	Reasonable for many patients	Not recommended	Not recommended
CHD Equivalent	Reasonable for many patients	Not recommended	Reasonable for many patients	Reasonable for many patients	Consider for selected patients	Not recommended
Family History	Consider for selected patients	Not recommended	Consider for selected patients	Consider for selected patients	Consider for selected patients	Not recommended
Recurrent Events	Reasonable for many patients	Not recommended	Reasonable for many patients	Reasonable for many patients	Consider for selected patients	Not recommended

Take Home Messages

- 1. Basic lipid panel (preferably fasting), with LDL-C and non-HDL-C, is required. A nonfasting non-HDL-C can still be useful.
- 2. Further measures of atherogenic particle number (apo B, LDL-P) are not necessary since non-HDL-C is equivalent and sufficient, even in discordant situations
- 3. Do not measure Lp(a) routinely. You should measure it for patient with a personal history of premature CVD, a FHx of premature CVD, or FH. A high risk level is > 50 mg/dL (> 75 nmol/L)
- 4. Lipid particle size and/or density is not helpful if you know the overall particle number

Case Study

66 year old non-diabetic Hispanic woman with history of Stage 1 hypertension, not on meds

Height	5'6"
Waist	36"
Weight	188 lb
BMI	30.3
BP	146/84

Smoker?	No
TC	231
TG	240
HDL	57
LDL	126
nonHDL	174

What is her risk for CHD?

Framingham Risk Score and Pooled Cohort Risk

Age 66	12
TC	2
Nonsmoker	0
HDL-C	0
SBP	3
Total	17

5% 10-year FRS

8.4% 10-year Pooled

Since she is “low risk” by FRS and at her LDL-C goal of < 130 mg/dL, no lipid therapy is needed.

By Pooled risk, she is candidate for moderate-intensity statin

. *JAMA* 2001;285:2486-2497

My.americanheart.org/cvriskcalculator.

Case Study

66 year old non-diabetic Hispanic woman with history of Stage 1 hypertension, not on med.

TC	231	Apo B	118 mg/dL
TG	240	LDL-P	1600
HDL	57		
LDL	126		
nonHDL	174		

If you had these special lipid tests, would you have given a statin?

Case Study

Based on the Pooled Cohort Risk and the new guideline recommendation, you initiate atorvastatin 10 mg without doing the extra lipid tests.

Follow up lab:

TC	177
TG	210
HDL	57
LDL	78
nonHDL	120

Apo B	118 to 83
LDL-P	1600 to 1120

Would you now move to high-intensity statin (atorvastatin 40 mg) if you knew the Apo B or LDL-P? Would the nonHDL-C have been enough to do that?

On AT 40, nonHDL-C is 105, apo B 75 and LDL-P 986