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Biomarkers

Clinical utility of inflammatory markers and advanced lipoprotein testing: Advice from an expert panel of lipid specialists

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Abstract: The National Cholesterol Education Program Adult Treatment Panel guidelines have established low-density lipoprotein cholesterol (LDL-C) treatment goals, and secondary non-high-density lipoprotein (HDL)-C treatment goals for persons with hypertriglyceridemia. The use of lipid-lowering therapies, particularly statins, to achieve these goals has reduced cardiovascular disease (CVD) morbidity and mortality; however, significant residual risk for events remains. This, combined with the rising prevalence of obesity, which has shifted the risk profile of the population toward patients in whom LDL-C is less predictive of CVD events (metabolic syndrome, low HDL-C, elevated triglycerides), has increased interest in the clinical use of inflammatory and lipid biomarker assessments.

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Lipoprotein(a);
Lipoprotein subfractions

Furthermore, the cost effectiveness of pharmacological intervention for both the initiation of therapy and the intensification of therapy has been enhanced by the availability of a variety of generic statins. This report describes the consensus view of an expert panel convened by the National Lipid Association to evaluate the use of selected biomarkers [C-reactive protein, lipoprotein-associated phospholipase A_2, apolipoprotein B, LDL particle concentration, lipoprotein(a), and LDL and HDL subfractions] to improve risk assessment, or to adjust therapy. These panel recommendations are intended to provide practical advice to clinicians who wrestle with the challenges of identifying the patients who are most likely to benefit from therapy, or intensification of therapy, to provide the optimum protection from CV risk.

Preamble

Since the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) I in 1988,[1] low-density lipoprotein cholesterol (LDL-C) has been the principal target of cholesterol treatment to reduce cardiovascular (CV) risk. The NCEP treatment guidelines have established LDL-C goals on the basis of risk stratification, with the lowest LDL-C targets for the patients at the greatest absolute risk for coronary heart disease (CHD) events. This strategy has successfully resulted in lower LDL-C levels and a significant reduction in the incidence of CV morbidity and mortality. Subsequently, non-high-density lipoprotein (HDL)-C goals were incorporated into the ATP III guidelines for patients with hypertriglyceridemia as a secondary target once LDL-C goals are achieved.[2] Post-hoc analyses of clinical trial datasets support the inclusion of non-HDL-C as a target of therapy, with the authors of most studies demonstrating that non-HDL-C is superior to LDL-C as a predictor of recurrent events on statin therapy.[3]

Unfortunately, measurements of non-HDL-C and the treatment to non-HDL-C goals have not been widely implemented, with surveys showing poor adherence to the recommended non-HDL-C targets[4] and major knowledge gaps on the calculation of non-HDL-C and the goals of therapy.[5] The National Lipid Association official policy has advocated the inclusion of non-HDL-C on all lipid profile laboratory reports.[6] The National Lipid Association believes that if clinicians are made more aware of a patient's non-HDL-C level, achievement of the non-HDL-C goals will improve in practice and ultimately result in further CV outcomes benefit.

Surveys of National Lipid Association members have demonstrated a major interest in the clinical utility of biomarkers to improve CV risk prediction and as potential novel targets of therapy. Three major factors are driving an increased interest in the use of biomarkers to potentially improve patient outcomes. First, although statins and LDL-C reduction reduce CV events, a significant residual risk for events remains in both primary and secondary prevention populations receiving statin therapy. The residual risk is most prominent in patients with metabolic syndrome and/or diabetes.[7,8] Second, a sharp increase in the prevalence of obesity has occurred during the last three decades, thereby markedly shifting the risk profile of the population toward patients with the metabolic syndrome features such as low HDL-C and elevated triglycerides. This is the same population at the greatest residual risk for events on statin therapy, and LDL-C is less predictive of CVD events in this group. Therefore, clinicians express considerable interest in the use of biomarkers, such as C-reactive protein (CRP), and lipid parameters, such as apolipoprotein (Apo) B or LDL particle concentration (LDL-P), that are elevated in this population and are frequently discordant with other traditional risk factors, particularly the level of LDL-C.

The Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) was the first major clinical trial in which investigators tested the hypothesis that a novel biomarker such as CRP could be used to identify patients who would benefit from statin treatment but would have been considered "healthy" and not candidates for cholesterol-lowering therapy on the basis of existing guidelines.[9] Finally, the use of generic statins has made the cost of treatment very low and, therefore, enhanced the cost-effectiveness of the use of biomarkers to identify additional patients at increased absolute risk of CV events for whom more aggressive intervention may ultimately improve morbidity and mortality.[10,11]

The National Lipid Association convened a panel of clinical experts to evaluate the use of selected biomarkers in clinical practice as either tools to improve risk assessment or as markers to adjust therapy once a decision to treat had been made (Table 1). Five clinical scenarios were considered by the panel that accounted for the vast majority of patients in whom clinicians would consider the use of biomarkers. These clinical scenarios were defined as follows: (1) low risk (patients with <5% 10-year CHD event risk on the basis of NCEP ATP III Framingham risk scoring); (2) intermediate risk (patients with 5%–20% 10-year CHD event risk on the basis of NCEP ATP III Framingham risk scoring); (3) CHD or CHD equivalent (ie, diabetes, atherosclerotic CV disease [CVD], or more than 20% 10-year CHD event risk by ATP III Framingham risk scoring); (4) patients with a family history of premature CHD; and (5) patients with CVD and recurrent events despite apparently "optimal" medical therapy. Risk categories are classified in this document according to estimated Framingham 10-year CHD event risk to provide an objective standard for demarcation of low, intermediate (moderate to moderately-high), and high risk in those without CHD or CHD risk equivalents; however, the panel recognizes the role of clinical

Table 1 Summary recommendations for measurement of inflammatory markers and advanced lipoprotein/subfraction testing in initial clinical assessment and on-treatment management decisions

	Initial Clinical Assessment					
	CRP	**Lp-PLA$_2$**	**Apo B**	**LDL-P**	**Lp(a)**	**HDL or LDL Subfractions**
Low risk (<5% 10-year CHD event risk)	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Intermediate risk (5-20% 10-year CHD event risk)	Recommended for routine measurement	Consider for selected patients	Reasonable for many patients	Reasonable for many patients	Consider for selected patients	Not recommended
CHD or 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

	On-Treatment Management Decisions					
	CRP	**Lp-PLA$_2$**	**Apo B**	**LDL-P**	**Lp(a)**	**HDL or LDL Subfractions**
Low risk (<5% 10-year CHD event risk)	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended
Intermediate risk (5-20% 10-year CHD event risk)	Reasonable for many patients	Not recommended	Reasonable for many patients	Reasonable for many patients	Not recommended	Not recommended
CHD or 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

Apo, apolipoprotein; CHD, coronary heart disease; CRP, C-reactive protein; HDL, high-density lipoprotein; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; LDL, low-density lipoprotein; LDL-P, LDL particle number/concentration; Lp(a), lipoprotein (a).

judgment in risk categorization and acknowledges that Framingham risk scoring may not be necessary for many patients with 0 or 1 major CHD risk factors.

The panel limited its assessment to the following biomarkers and lipid markers: CRP, lipoprotein-associated phospholipase A2 (Lp-PLA$_2$), Apo B, LDL-P, lipoprotein(a) [Lp(a)], and LDL and HDL subfractions. This list of biomarkers evaluated was not intended to be comprehensive, and the panel acknowledges that additional biomarkers may be used in some clinical practices. The specific biomarkers selected were those that, in the collective opinion of the organizers, have penetrated into clinical practice to at least a moderate degree, and for which sufficient evidence from epidemiological and clinical studies has accumulated for the panel to provide recommendations relevant to clinical practice (Table 2).[12–17] Additional panels may be organized in the future to address other biomarkers and/or to update recommendations for the biomarkers covered herein as new information becomes available.

The recommendations of the panel should not be considered guidelines or official policy of the National Lipid Association. They represent the consensus of opinions of clinicians considered to be experts in the field of clinical lipidology. In the development of a consensus, there are always compromises that are reached and, therefore, individuals on the panel may have points of view that are different from the consensus opinion. The expert panel believed that the recommendations should be both practical and clearly defined. Thus, the panel identified four categories of recommendations:

1. recommended for routine measurement in this population,
2. reasonable for many patients,
3. consider in selected patients, or
4. not recommended.

The panel weighed the available clinical evidence and heard testimony from other experts in the field; voting was used to establish the consensus position for each biomarker

Table 2 Laboratory values of CRP, Lp-PLA$_2$, Apo B, LDL-P, and Lp(a) according to lower-, intermediate-, and greater-risk categories, approximated from population studies

	Population-based approximations		
Biomarker	Lower risk	Intermediate risk	Greater risk
CRP, mg/L[12]	<1.0	1.0−3.0	>3.0
Lp-PLA$_2$, ng/mL[13,*]	<200	200−259	≥260
Apo B, mg/dL[14,†]	<80	80−119	≥120
LDL-P, nmol/L[15,16,‡]	<1000	1000−1559	≥1600
Lp(a), mg/dL[17,§]	<5	5−49	≥50

Apo, apolipoprotein; CRP, C-reactive protein; LDL-P, low-density lipoprotein particle number/concentration; Lp(a), lipoprotein (a); Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$.

*Values for lower, intermediate, and greater risk represent approximate tertiles of population distribution values obtained from a sample of 425 healthy men and women as described in the PLAC® Enzyme Immunoassay for the Quantitative Determination of Lp-PLA$_2$ in Human Plasma and Serum product insert information (diaDexus, Inc., South San Francisco, CA). Results from several studies have suggested that population cutpoints may vary markedly depending on the assay used.

†Values for lower, intermediate, and greater risk taken from the Framingham Offspring Study correspond approximately to Apo B population percentiles consistent with those from NCEP ATP III LDL-C cut-points of <100 mg/dL (20th percentile) and ≥160 mg/dL (80th percentile).

‡Values for lower, intermediate, and greater risk taken from the Multi-Ethnic Study of Atherosclerosis (MESA) population correspond approximately to LDL-P population percentiles consistent with those from NCEP ATP III LDL-C cutpoints of <100 mg/dL (20th percentile) and ≥160 mg/dL (80th percentile).

§Values for lower risk represent <22nd percentile and greater risk represent ≥80th percentile of the general population. Many laboratories use ≥30 mg/dL as a cutpoint for indicating an elevated Lp(a) concentration; this represents approximately the top tertile of the general population.

considered for each of the risk categories defined. Within a large population, risk for many will be similarly classified whether or not novel risk factors are included in the assessment. However, there was a consensus among the panel members that there are occasions when novel risk factor evaluation can provide useful insight into an individual patient's CV risk, particularly in cases where clinical judgment leads one to suspect that a patient may be at higher risk than suggested by traditional risk factor evaluation. The objective of this report is to provide practical advice to clinicians who wrestle with the challenges of identifying the patients who are most likely to benefit from therapy or intensification of therapy, to provide the optimum protection from CV risk.

Executive summary of recommendations

CRP: initial clinical assessment

1. In patients with low risk (10-year CHD event risk <5% on the basis of Framingham scoring), CRP measurement is not recommended for routine use but may be of value in selected patients, particularly those who have multiple mild disturbances, including those with the metabolic syndrome (**rating: "not recommended"**).
2. In patients with intermediate risk (5%–20% 10-year risk), it is recommended that CRP be measured routinely in men >50 years of age and women >60 years of age given its capacity to enhance risk prediction, especially when used with Reynolds risk scoring (**rating: "recommended for routine measurement"**).
3. In certain patients with CHD and risk equivalents, CRP measurement may be considered (**rating: "consider for selected patients"**).
4. In patients with a premature family history of CHD or in patients with established CHD with a history of recurrent events despite appropriate therapy, CRP measurement is a reasonable option to help determine if therapy should be: (1) started in the case of premature family history; or (2) intensified, or effort be made to identify other ancillary risk factors that may be impacting the progression or stability of established atherosclerotic plaque (**rating: "reasonable for many patients"**).

CRP: on-treatment management decisions

1. Among patients on treatment, there is insufficient evidence to support CRP measurement in patients with low risk and it is not recommended (**rating: "not recommended"**).
2. In patients with intermediate risk, CHD (or a CHD risk equivalent), or a history of recurrent coronary events, CRP measurement is reasonable and can help to guide the intensity of therapy (**rating: "reasonable for many patients"**).
3. Among patients with family history of premature CHD, CRP measurement can be considered and may have value, but its clinical utility in guiding therapy in this setting is less certain pending further investigation (**rating: "consider for selected patients"**).

Lp-PLA$_2$: initial clinical assessment

1. Lp-PLA$_2$ testing should generally not be performed in low-risk patients for the purpose of reclassification (**rating: "not recommended"**).
2. Lp-PLA$_2$ testing may be considered in intermediate-risk patients, as well as certain higher risk subgroups, such as those with CHD or a CHD risk equivalent, patients with family history of premature CHD, and patients with recurrent CHD events (**rating: "consider for selected patients"**).

Lp-PLA$_2$: on-treatment management decisions

1. Measurement of Lp-PLA$_2$ is not recommended for on-treatment risk management decisions in low-risk or intermediate-risk patients or in those with CHD or a

CHD risk equivalent, family history of premature CHD, or with recurrent CHD events (**rating: "not recommended"**).

Apo B: initial clinical assessment

1. In patients at low risk, <5% 10-year CHD event risk, the likelihood of markedly elevated Apo B is low. Hence, use of Apo B is not recommended in this category (**rating: "not recommended"**).
2. In patients at intermediate risk, those with premature family history, and those with recurrent events, measurement of Apo B would enable the best possible management of modifiable factors for vascular risk (**rating: "reasonable for many patients"**).
3. Once a patient with CHD or CHD risk equivalent has achieved his or her LDL-C and/or non-HDL-C goals, obtaining an Apo B measurement might be useful for determining whether further intensification of lipid-lowering therapy should be considered, as might be the case for discordant individuals with residual Apo B elevation (**rating: "consider for selected patients"**).

Apo B: on-treatment management decisions

1. There is no clear benefit of measuring Apo B in patients at low risk receiving lipid-altering therapy, and therefore it is not recommended in this group of patients (**rating: "not recommended"**).
2. In patients at intermediate risk, with CHD or CHD risk equivalent, and in those with recurrent events, measurement of Apo B is reasonable for many patients (**rating: "reasonable for many patients"**)
3. In patients with a family history of premature CHD, measurement of Apo B should be considered for selected patients (**rating: "consider for selected patients"**).

LDL-P: initial clinical assessment

1. Treatment decisions are unlikely to be altered by use of LDL-P among low-risk patients. Hence, use of LDL-P was not recommended for this patient group (**rating: "not recommended"**).
2. There is a substantial number of patients for whom LDL-C may not accurately reflect CVD risk, and data show that discordantly elevated LDL-P is more strongly associated with incident CVD risk than LDL-C. When LDL-P is discordantly elevated, consideration should be given to initiating LDL-lowering therapy. Thus, the use of LDL-P is thought to be reasonable for many patients at intermediate risk (5%–20%), those with a family history of CHD, and those with recurrent events, all of whom have the potential for discordantly elevated LDL-P (**rating: "reasonable for many patients"**).
3. Because of high CV risk, patients with known CHD or a CHD risk equivalent are candidates for aggressive lipid-altering therapy, and it is unclear whether additional LDL-P information would alter initial therapeutic decisions, but measurement might be considered for selected patients (**rating: "consider for selected patients"**).

LDL-P: on-treatment management decisions

1. Treatment decisions are unlikely to be altered by use of LDL-P among low-risk patients. Hence, use of LDL-P is not recommended for this patient group (**rating: "not recommended"**).
2. Use of LDL-P measurement is reasonable for many patients at intermediate risk treated to LDL-C and non-HDL-C goal, among patients with CHD or CHD risk equivalents on lipid-lowering therapy, and in those with recurrent CHD events, to adjudicate the adequacy of LDL lowering therapy. When LDL-P is discordantly elevated, consideration should be given to intensifying LDL lowering therapy (**rating: "reasonable for many patients"**).
3. Increased LDL-P is commonly encountered among patients with a family history of premature CHD. Once on therapy, use of LDL-P should be considered for selected patients treated to LDL-C and non-HDL-C goal to adjudicate the adequacy of LDL lowering therapy (**rating: "considered for selected patients"**).

Lp(a): initial clinical assessment

1. In patients with low risk (<5% 10-year CHD event risk), Lp(a) measurement is not recommended for routine use (**rating: "not recommended"**).
2. In patients with intermediate risk (5%–20% 10-year CHD event risk) or CHD or a CHD equivalent, it is recommended that Lp(a) measurement be considered for selected patients (**rating: "consider for selected patients"**).
3. Because elevated Lp(a) is additive to CHD risk, measurement of Lp(a) in patients with a premature family history of CHD or in patients with established CHD with a history of recurrent events despite appropriate therapy is a reasonable option (**rating: "reasonable for many patients"**).

Lp(a): on-treatment management decisions

1. Among patients with low-risk or intermediate-risk for CHD receiving treatment, there is insufficient evidence to support Lp(a) measurement and it is not recommended (**rating: "not recommended"**).
2. Lp(a) measurement may be considered for assistance with on-treatment management decisions in selected patients with CHD (or a CHD risk equivalent), premature family history, or a history of recurrent coronary events, on the basis of the rationale that aggressive LDL-C reduction is beneficial in those with elevated Lp(a) and LDL-C,

and that there is no evidence that reducing Lp(a) is harmful (**rating: "consider for selected patients"**).

LDL subfractions: initial clinical assessment and on-treatment management decisions

1. In patients with low risk (<5% 10-year CHD event risk), intermediate risk (5%–20% 10-year CHD event risk), CHD or CHD risk equivalent, premature family history of CHD in the absence of other risk factors, and in patients with established CHD who experience recurrent events despite appropriate therapy there is insufficient evidence to support LDL subfraction measurement for initial clinical assessment or on-treatment management decisions (**rating: "not recommended"**).

HDL subfractions: initial clinical assessment and on-treatment management decisions

1. In patients with low risk (<5% 10-year CHD event risk), intermediate risk (5%-20% 10-year CHD event risk), CHD or CHD risk equivalent, premature family history of CHD in the absence of other risk factors, and in patients with established CHD who experience recurrent events despite appropriate therapy there is insufficient evidence to support HDL subfraction measurement for initial clinical assessment or on-treatment management decisions (**rating: "not recommended"**).

C-reactive protein (CRP)

Does CRP predict risk, over and above traditional risk factors?

CRP is a marker of risk for CV events and reflects the intensity of inflammation.[18,19] In most cases, the term CRP indicates high-sensitivity CRP (ie, measured with a high-sensitivity assay), which is recommended for use in clinical practice. Serum high-sensitivity CRP levels of <1.0, 1.0–3.0, and >3.0 mg/L, representing approximate tertiles of values in the U.S. population, indicate lower, moderate, and greater relative risk for CV events, independent of serum LDL-C levels. CRP levels are most useful to refine risk estimates in patients with 10-year CHD event risk in the intermediate range of 5%–20%.[20]

In the Women's Health Study, CRP measurements were more predictive of CV events than lipids or apolipoproteins, including LDL-C, HDL-C, total cholesterol (TC)/HDL-C ratio, and Apo B, or other inflammatory markers such as interleukin (IL)-6 and serum amyloid A.[21]A number of prospective observational studies have demonstrated that the serum level of CRP is a strong, independent predictor of risk for myocardial infarction (MI), stroke, peripheral arterial disease, and CV mortality.[21–30]

A meta-analysis by the Emerging Risk Factors Collaboration (ERFC) of 54 prospective cohorts demonstrated that the hazard ratio (HR) for a one standard deviation change in CRP after adjustment for traditional risk factors was 1.37 (95% confidence interval [CI] 1.27–1.48), a HR that was equal to or greater in magnitude than that of non-HDL-C (1.28, 95% CI 1.16–1.40) or systolic blood pressure (1.35, 95% CI 1.25–1.45), and results were consistent in men and women (Fig. 1).[31] Elevated CRP has also been associated with increased vascular event rates among patients with acute coronary ischemia,[32–35] stable angina pectoris,[36] stable coronary artery disease,[37] and a history of MI.[38] In a study of 27,939 healthy women, baseline CRP measurements were predictive of CV events, including MI, stroke, and death, and risk for CV events increased in a linear fashion from the lowest to the highest serum levels of CRP.[39]

What is the physiological rationale for the link between CRP and adverse CV outcome?

Elevation in serum CRP was first associated with host immunity in patients with streptococcal pneumonia. On a molecular level, CRP is an annular, pentameric disk that belongs to the pentraxin family of proteins whose physiological role is to bind to phosphocholine present on pneumococci, oxidized LDL, and apoptotic and dying cells, suggesting it is part of the innate immune response to phosphocholine-bearing antigens. CRP is produced by the liver as part of the acute phase response.[40] During an infection, CRP binds to microbes and promotes their destruction by activating complement. CRP binds to the lectin-like oxidized LDL receptor-1 on endothelial cells[41] and is produced de novo in atherosclerotic lesions.[42]

At the present time, a clinical trial has not been completed to demonstrate that targeted, specific CRP lowering with an anti-inflammatory agent beneficially impacts CV outcomes. However, CRP has been hypothesized to directly promote atherogenesis by a number of potential mechanisms, including its role in: (1) endothelial cell adhesion molecule expression, which potentiates intravascular inflammation by increasing the influx of inflammatory white cells such as monocytes and T cells[41]; (2) reduced endothelial nitric oxide synthase expression,[43] nitric oxide release,[44] and increased coronary vasoreactivity[45]; (3) increased expression of endothelial plasminogen activator inhibitor-1, a protein that inhibits fibrinolysis and increases thrombotic risk[46]; (4) promotion of the production of endothelin-1,[47] a potent vasoconstrictor and inducer of vessel wall inflammation, abnormal cell growth, and thrombosis[48]; (5) increased monocyte chemoattractant protein-1 expression, which promotes the influx of monocytes into the subendothelial space[49]; (6) activation of complement by binding to partly degraded non-oxidized LDL cholesterol,[50] and colocalization with the terminal membrane attack complex in early atherosclerotic lesions[51]; (7) stimulation of macrophage scavenging for oxidized LDL, a principal step in foam cell formation[52]; and (8)

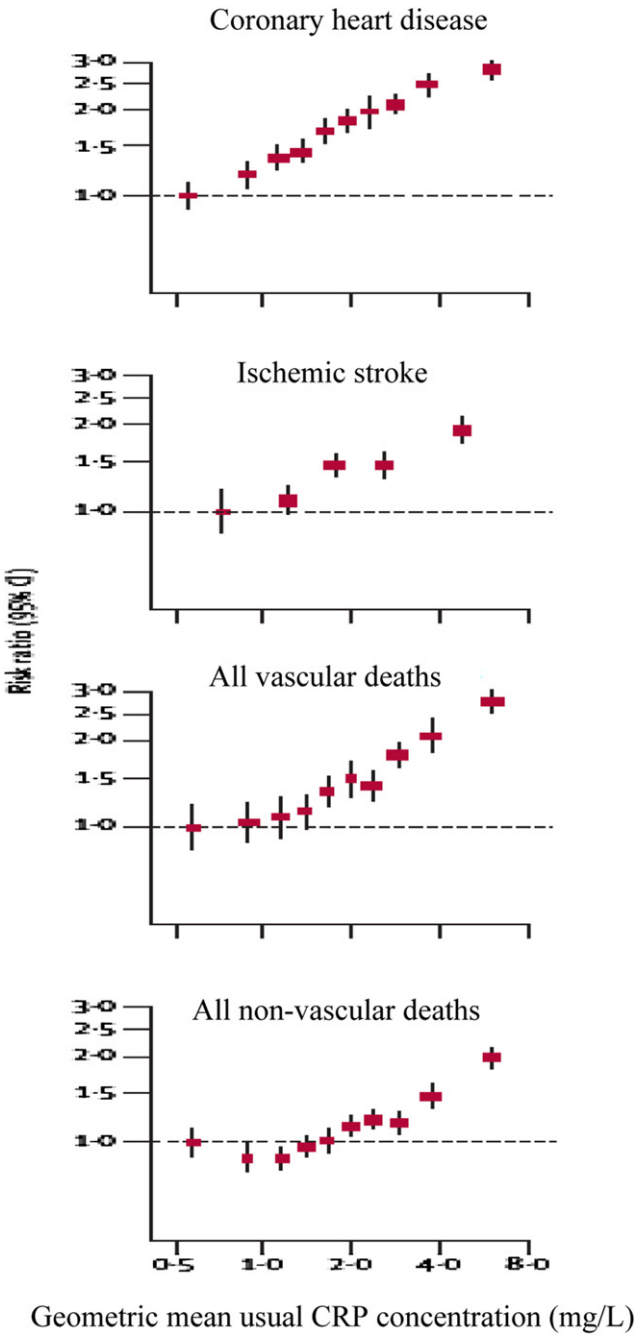

Figure 1 Risk ratios for major vascular and nonvascular outcome by quartiles of CRP concentration, adjusted for age, sex, and study, from a meta-analysis of 54 prospective cohort studies from the Emerging Risk Factors Collaboration.[31] Permission to reuse figure granted by Elsevier.

up-regulation of angiotensin type 1 receptors in vascular smooth muscle,[53] among other functions. On the basis of these investigations, CRP appears to have the potential to directly and indirectly activate inflammation and cytotoxicity, resulting in progressive vessel wall injury and atherosclerotic plaque formation. Consequently, it is possible, though not proven, that CRP may not only be a marker of CV risk, but may also directly promote its development and progression.

In which patients would CRP testing be most valuable?

Consistent with the results of JUPITER, it is appropriate to measure CRP in men at least 50 years and women at least 60 years of age who have an LDL-C $<$130 mg/dL and at least one other major CHD risk factor. If CRP is $\geq$2.0 mg/L in these patients, statin therapy for lipid lowering may be strongly considered.[54] JUPITER demonstrated benefit in patients at intermediate or greater risk on the basis of global risk scoring, whether they did, or did not, have metabolic syndrome or a family history of CHD.[55,56]

Among younger patients, there is no clear consensus as to the role of measuring CRP. As shown in the Atherosclerosis Risk in Communities (ARIC) study, the absolute risk for CV events is low in patients with low LDL-C and low CRP; however, absolute risk is greater and underestimated by Framingham scoring in patients with low LDL-C but elevated CRP.[56] Similar results were found in a post-hoc analysis of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS).[57] Because traditional risk scoring and routine cholesterol screenings miss a significant percentage of patients at risk for events, consideration might be given to routine inclusion of CRP when evaluating global CV risk among patients with two or more major CHD risk factors in primary prevention. CRP measurement and family history for CV disease are important components of the Reynolds risk score (http://www.reynoldsriskscore.org) that are not included in the Framingham risk scoring system used in the NCEP ATP III guidelines. The Reynolds risk score has been shown to more accurately predict CV risk than Framingham risk scoring in both men[58] and women.[59]

When considering whether to measure CRP, it is important to avoid measurement in the setting of an active infection because CRP production increases as part of the acute phase response. In addition, if the patient has a malignancy or chronic inflammatory disease, CRP should not be measured for CV risk prediction. Postmenopausal hormone therapy with oral estrogen is associated with increased serum levels of CRP.[60] Moreover, because CRP shows substantial intraindividual variability (test-retest coefficient of variation $\sim$40%), it is ideal to obtain and average at least two measurements when assessing CRP level in clinical practice.[20]

Should CRP be a target of therapy? If not, how should CRP affect treatment decisions?

1. CRP is a risk marker and is not presently considered a proven direct factor in the causal pathway for CV disease. CRP measurements assist health care providers in evaluating the adequacy of therapeutic intensity. It is not currently recommended that CRP be considered a treatment target. A clinical trial is underway which will help to determine whether the addition of an anti-inflammatory agent (interleukin-1β antagonist) in

high-risk patients after MI who continue to have elevated CRP levels will improve CV outcomes.

2. Investigators from JUPITER and other studies suggest that CRP levels predict outcomes in patients on statin therapy in both primary and secondary prevention settings. If the CRP level remains elevated with lipid therapy, then comprehensive CV risk management can be intensified through lifestyle modification and pharmacologic intervention as indicated for dyslipidemia, hypertension, insulin resistance, etc. In JUPITER, the patients who achieved the largest reductions in relative risk (RR; 79%) were those who achieved the dual targets of LDL-C <70 mg/dL and a CRP <1.0 mg/L (HR 0.21; 95% CI 0.49–0.84).[61] Patients who achieved LDL-C <70 mg/dL and CRP <2.0 mg/L achieved a 65% RR reduction for the primary composite end point (HR 0.35; 95% CI, 0.23–0.54; Fig. 2).[62] Among patients who achieved neither of these targets, the risk reduction with rosuvastatin was significantly attenuated to 36% (HR 0.64; 95% CI 0.49–0.84; $P < .0001$).

The potential importance of achieving dual targets for LDL-C and CRP is highlighted by additional studies. In the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction (PROVE-IT-TIMI) trial, the effects of intensive (atorvastatin 80 mg) compared with standard (pravastatin 40 mg) statin therapy on the prevention of secondary coronary events among 4162 patients demonstrated that those patients achieving an LDL-C <70 mg/dL and a CRP level <1.0 mg/L on therapy had the lowest risk for events compared with patients unable to achieve either or both of these levels.[63] These results were corroborated by the Aggrastat-to-Zocor (A to Z) trial, which compared early intensive statin treatment (simvastatin 40 mg/d for 30 days followed by 80 mg/d) to a delayed conservative statin strategy (placebo for 4 months followed by 20 mg/d simvastatin).[64] In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, intensive lipid lowering (atorvastatin 80 mg) compared with moderate lipid lowering (pravastatin 40 mg) in 654 patients with stable coronary artery disease demonstrated that the rate of progression of coronary artery atheroma volume was significantly and independently associated with the magnitude of reduction in CRP.[65] Atheroma regression was only observed in patients with CRP less than the median, irrespective of whether achieved LDL-C was above or below the median.[66] Although there is no specific anti-inflammatory drug currently available for use in clinical practice that reproducibly reduces serum levels of CRP, patients in primary and secondary prevention settings with CRP levels ≥2.0 mg/L may benefit from intensification of both lifestyle modification (weight loss, smoking cessation, dietary modification) and statin therapy, which have been shown to lower serum levels of CRP.[54,67–70]

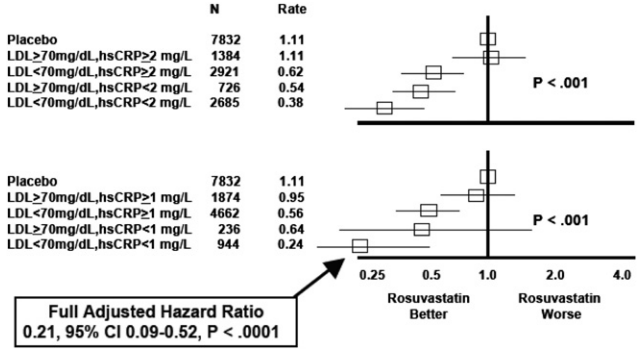

Figure 2 A prospective assessment of the effects of 20 mg of rosuvastatin versus placebo on rates of nonfatal MI, nonfatal stroke, admission for unstable angina, arterial revascularization, or cardiovascular death according to on-treatment concentrations of LDL-C and high-sensitivity CRP in JUPITER.[62] Permission to reuse figure granted by Elsevier.

What are the main areas of controversy and research questions regarding CRP and its use in clinical practice?

One major area of controversy relates to whether CRP itself is a direct contributor to the atherothrombotic process, or a marker for other processes that are within the causal pathways leading to clinical events. A second important issue is whether CRP should be a treatment target. Results from multiple statin intervention trials suggest that those with low levels of LDL-C and CRP during treatment have better CV outcomes than those with a low on-treatment level of one or the other. To date, no trial has been completed in which the policy of treating to specific target levels of CRP has been tested. Recently, a trial of an anti-inflammatory agent (interleukin-1β antagonist) that lowers CRP without reducing atherogenic lipoprotein levels has been started in post-MI patients with elevated CRP. This trial is expected to help establish whether reducing inflammation in high-risk patients, as reflected in the change in CRP concentration, leads to reduced CV morbidity and mortality.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)

Does Lp-PLA_2 predict risk, over and above traditional risk factors?

The authors of several prospective epidemiological studies have identified Lp-PLA_2 as a significant predictor of CV events and stroke.[71,72] In both primary and secondary prevention trials, an approximate 2-fold increase in risk for CV events, after multivariate adjustment for traditional risk factors, is associated with Lp-PLA_2 in the upper tertile or quartile. Lp-PLA_2 predicts risk independent of, and complementary to, CRP.[73] Notably, in the ARIC Study, when both Lp-PLA_2 and CRP were in the top tertile, the risks for CHD events and stroke increased 4-fold and 11-fold,

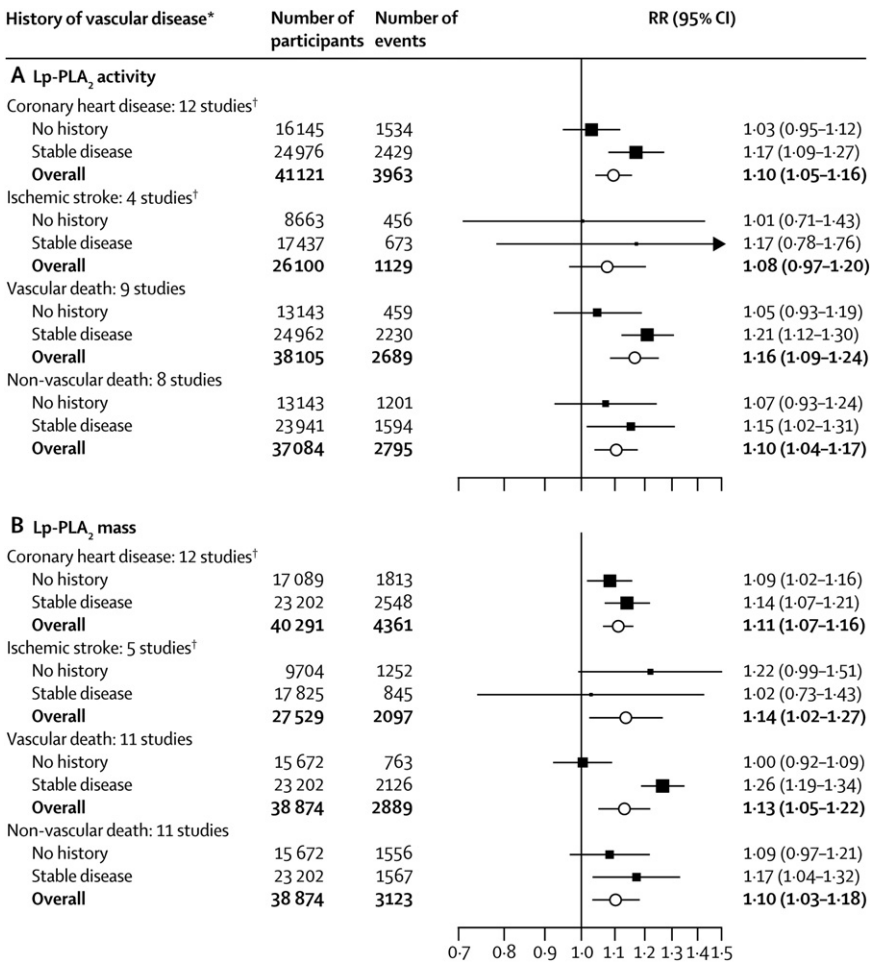

Figure 3 Risk ratios for CHD, ischemic stroke, and vascular, and nonvascular mortality per 1 standard deviation greater Lp-PLA_2 activity or mass at baseline, adjusted for several risk factors. Error bars represent 95% CIs. The sizes of the boxes are proportional to the inverse of the variance of risk ratios.[78] *Diagnosis more than 30 days before baseline of myocardial infarction, angina, other coronary heart disease, stroke (including transient ischemic attack), peripheral vascular disease, or coronary surgery (including revascularizations). †Fatal and nonfatal events. Permission to reuse figure granted by Elsevier.

respectively, compared with those in the lowest tertile for both markers.[74,75] Unlike for CHD risk, epidemiological studies fail to show a consistent relationship between elevated LDL-C and stroke risk.[76] However, elevation in Lp-PLA_2 confers approximately a 2-fold increase in both first and recurrent strokes.[77]

A recent meta-analysis of almost 80,000 patients in 32 prospective studies evaluated associations of Lp-PLA_2 mass and activity with risk of CHD, stroke, and mortality (Fig. 3).[78] RR ratios adjusted for conventional risk factors and expressed per one standard deviation increment in Lp-PLA_2 activity or mass at baseline were as follows: 1.10 (95% CI 1.05–1.16) with Lp-PLA_2 activity and 1.11 (1.07–1.16) with Lp-PLA_2 mass for CHD; 1.08 (0.97–1.20) and 1.14 (1.02–1.27) for ischemic stroke; and 1.16 (1.09–1.24) and 1.13 (1.05–1.22) for vascular mortality, respectively. The association between baseline Lp-PLA_2 and CHD risk was similar in magnitude to those for non-HDL-C and systolic blood pressure.

As a result of consistent epidemiological data showing that elevated Lp-PLA_2 predicts risk for CHD events and stroke, the NLA Biomarkers Expert Panel recommends that the measurement of Lp-PLA_2 may assist with the risk assessment of intermediate-risk and some high-risk patients.

What is the physiological rationale for the link between Lp-PLA_2 and adverse CV outcome?

Lp-PLA_2 primarily circulates bound to LDL particles, although it also resides on HDL particles, lipoprotein (a) [Lp(a)], and triglyceride-rich remnant lipoproteins. It is produced by macrophages, monocytes, T lymphocytes, and mast and liver cells. Lp-PLA_2 activity has been shown to be up-regulated in atherosclerotic lesions and in rupture-prone fibrous caps.[79,80] Lp-PLA_2 is an enzyme that is responsible for the hydrolysis of oxidized phospholipids on LDL particles within the arterial intima, thus producing two highly inflammatory mediators, lysophosphatidylcholine and oxidized fatty acids. These products result in a cascade of events that have been linked to atherosclerotic plaque formation: up-regulation of adhesion molecules, expression of cytokines, recruitment of monocytes to the

intimal space, and differentiation of monocytes into macrophages that engulf oxidized LDL, producing foam cells.[81–84] Foam cells aggregate to form a fatty streak covered by a fibrous cap. Cytokines and proteases secreted by the plaque destroy the collagen within the fibrous cap, making it prone to rupture and resulting in an acute coronary event.

Lp-PLA_2 and its byproduct, lysophosphatidylcholine, have been identified in early atherosclerosis and are associated with endothelial dysfunction.[85] Furthermore, Lp-PLA_2 expression in carotid artery plaques predicted long-term cardiac events in 162 consecutive patients who underwent elective carotid endarterectomy and were then followed for approximately 4 years. Carotid plaque expression of Lp-PLA_2 above the median was associated with markedly increased risk for cardiac events (HR 3.39; 95% CI 1.13–10.17; $P = .03$).[86]

The rationale for Lp-PLA_2 as a key inflammatory biomarker is attractive because this enzyme is produced in atherosclerotic plaques and is specifically linked to plaque inflammation, and presumably, rupture, suggesting a possible causal pathway leading to clinical events. In preclinical studies investigators have shown that inhibition of Lp-PLA_2 attenuates the inflammatory response and slows atherosclerotic plaque progression.[87] Lp-PLA_2 shows less variability than CRP, making it a practical tool for CVD risk assessment.[81] However, clinical trials are necessary to support the proposition that blocking or reducing Lp-PLA_2 activity will interrupt the sequence of events leading to atherosclerotic plaque formation and/or rupture.[88]

In which patients would Lp-PLA_2 testing be most valuable?

Recently, a consensus panel of investigators recommended how to use Lp-PLA_2 along with guideline-endorsed CVD risk assessment to better stratify individuals who might be at greater CVD risk than suggested by traditional risk factors and thus benefit from more aggressive management strategies.[89] The consensus panel endorsed the use of Lp-PLA_2 for the assessment of CHD event and stroke risk in intermediate- or moderate-risk populations, and specifically recommended testing in the following patients:

- any patient with two or more major CHD risk factors;
- any patient 65 years of age or older with one additional risk factor, given that risk for CHD events and strokes increase with age;
- smokers;
- individuals with an elevated fasting glucose; and
- patients with diagnostic criteria for metabolic syndrome who are generally at moderate risk (it has been shown that elevated Lp-PLA_2 further increases CVD risk in these patients.[88])

According to that consensus panel, moderate-risk individuals with an elevated Lp-PLA_2 level (>200 ng/mL) should be reclassified as high risk, and the LDL-C goal adjusted from <130 mg/dL to <100 mg/dL. The panel[89] also recommended Lp-PLA_2 testing for patients with known CHD or a CHD risk equivalent, such as diabetes or ischemic stroke. An elevated Lp-PLA_2 would place these patients in the very high-risk category, and therefore, the LDL-C goal is <70 mg/dL.

Results from epidemiological studies have suggested that Lp-PLA_2 predicts risk independent of, and complementary to, CRP.[73] Therefore, it might be reasonable to measure both inflammatory markers in intermediate- and high-risk individuals. Given that CRP is an acute-phase reactant, its elevation can be caused by acute infections, chronic inflammatory conditions and obesity, as well as certain medications such as oral estrogens. Lp-PLA_2, on the other hand, appears to be related specifically to vascular inflammation, shows significantly less variability than CRP, and may be causally linked to plaque rupture.

Similar to the previous consensus panel, members of the NLA Biomarkers Expert Panel recommends that Lp-PLA_2 testing may be considered in intermediate-risk patients, as well as certain greater-risk subgroups, such as those with CHD or a CHD risk equivalent, patients with family history of premature CHD, and patients with recent CHD events, to identify patients who might benefit from more intensive lipid therapy. Lp-PLA_2 testing should generally not be performed in low-risk patients for the purpose of reclassification. An elevated level of Lp-PLA_2 measured 1 month after a patient started statin therapy in PROVE IT–TIMI 22 was associated with increased CV event risk, with an adjusted HR of 1.33 (95% CI 1.01–1.74) for the top versus bottom Lp-PLA_2 quintile.[90] The association between the Lp-PLA_2 level and the primary CV event outcome appeared somewhat attenuated in the group receiving high-dose atorvastatin, HR 1.29 (95% CI 0.87−1.92), compared with the group receiving pravastatin, HR 1.63 (95% CI 1.03–2.58), but the test for interaction did not reach statistical significance. Although the on treatment data for Lp-PLA_2 level were not presented for the Heart Protection Study, the vascular protection produced by simvastatin did not vary significantly by baseline level of Lp-PLA_2.[91] Because of the paucity of data examining the predictive value of Lp-PLA_2 during lipid-modifying therapy, Lp-PLA_2 testing is not recommended for these patients.

Should Lp-PLA_2 be a target of therapy? If not, how should Lp-PLA_2 affect treatment decisions?

Although Lp-PLA_2 has been shown to be a significant predictor of risk for CHD events, stroke, and mortality in primary and secondary prevention studies, there are no randomized trials in which the authors examine the benefits of lowering Lp-PLA_2 with any specific therapies. Lipid-altering medications, including statins, fenofibrate, ezetimibe, and prescription omega-3 fatty acids, as well as weight loss, have been shown to reduce inflammatory markers, including Lp-PLA_2[92–96]; however, the degree of inflammatory marker reduction typically correlates with the extent of lipid lowering. It is currently unknown whether lowering Lp-PLA_2 per se, will have a direct benefit on CVD events and mortality.

This question may be answered in the near future by investigations of selective Lp-PLA_2 inhibitors that are currently in clinical development. Darapladib, a potent selective inhibitor of Lp-PLA_2, produced sustained inhibition of plasma Lp-PLA_2 activation in patients on atorvastatin therapy. In a clinical trial with 95% of patients having CHD or CHD risk equivalents, darapladib at 40, 80, and 160 mg produced dose-related reductions of 43%, 55%, and 66% in Lp-PLA_2 activity.[97] At the greatest dose of 160 mg of darapladib, there were changes in IL-6 and CRP at 12 weeks that suggest a possible reduction in total inflammatory burden. A study in a hyperlipidemic, diabetic pig model showed a marked reduction in atherosclerosis.[98] In a proof-of-concept trial in which the authors used intravascular ultrasound with virtual histology in 330 patients with coronary disease, darapladib prevented necrotic core expression versus placebo ($P = .012$) but did not significantly modify the primary endpoint (plaque deformability).[99]

On the basis of these preliminary studies suggesting a beneficial effect of Lp-PLA_2 inhibition on the atherosclerotic process, a large morbidity and mortality trial was initiated in 2008 to evaluate the long-term safety and efficacy of darapladib versus placebo in patients with chronic high-risk CHD, receiving standard of care, including lipid-lowering and antiplatelet therapies. In the Stabilization of Atorvastatin Plaque by Initiation of Darapladib Therapy (STABILITY) trial,[100] 15,828 patients were randomized to receive darapladib 160 mg or placebo for 3 years. The primary end point is the composite of major adverse CV events: CV death, nonfatal MI, and nonfatal stroke. Until the STABILITY Trial results are known, the NLA Biomarkers Expert Panel cannot recommend the measurement of Lp-PLA_2 for on-treatment risk management decisions.

What are the main areas of controversy and research questions regarding Lp-PLA_2 and its use in clinical practice?

The main areas of controversy regarding Lp-PLA_2 center on cost-effectiveness and whether the measurement of Lp-PLA_2 after the institution of lipid-altering therapy is warranted to help guide therapy. The results from the ongoing STABILITY trial are expected to provide evidence relevant to these questions. With the development of automated assays for Lp-PLA_2 mass and activity, there need to be additional studies to examine population distributions, as there has been a considerable range of median values reported in studies using different assays.[78]

Apolipoprotein B (Apo B)

Does Apo B predict risk, over and above traditional risk factors?

A wealth of epidemiological and clinical trial evidence justifies LDL as the cornerstone of lipid management. A body of evidence has evolved that supports the view that LDL-C is not the best indicator of the risk attributable to LDL because risk correlates more closely with the number of circulating atherogenic particles than with the quantity of cholesterol carried by those particles.[101–113] The LDL-P concentration is the major determinant of plasma Apo B because ~90% of the total circulating Apo B is associated with LDL particles in both normotriglyceridemic and hypertriglyceridemic patients.[114] Type III hyperlipoproteinemia, an uncommon but important disorder because it carries a very high risk of vascular disease, is one of the few exceptions because large numbers of remnant Apo B48 and Apo B100 particles account for almost half of the total Apo B particles in these patients.[115,116]

If the amount of cholesterol per LDL particle was constant, the LDL-C concentration would consistently reflect the number of LDL particles. However, the amount of cholesterol per LDL particle varies substantially.[117] In individuals whose LDL particles, on average, contain the normal amount of cholesterol, the LDL-C level will accurately reflect the LDL burden. In these patients, Apo B and LDL-C levels are concordant and are equivalent markers of risk and the adequacy of therapy. However, in individuals whose LDL particles, on average, contain less cholesterol than normal, the LDL-C concentration will underestimate the number of LDL particles. In these individuals, the Apo B concentration will more accurately reflect the number of LDL particles and LDL-related CVD risk.

Similarly, in individuals whose LDL particles, on average, contain more cholesterol than normal, the LDL-C concentration will overestimate the number of LDL particles. In these patients as well, the Apo B concentration will more accurately indicate the number of LDL particles than will the LDL-C concentration.[116] This variance in the composition of LDL particles[117] is important clinically because small, cholesterol-poor LDL particles are the dominant form of LDL in a substantial proportion of patients in all the major clinical risk groups for vascular disease. It is these groups in which Apo B level amplifies the capacity to estimate more accurately the LDL-related risk of vascular disease in an individual patient.

Thus, a high proportion of patients with diabetes or the metabolic syndrome,[118] abdominal obesity,[119] hypertriglyceridemia,[120] or with low HDL-C but otherwise-normal lipids,[121,122] will have increased numbers of LDL particles that contain less cholesterol than average. The LDL-C concentration is often normal in these patients despite an elevated level of LDL particles, and hence an elevated circulating concentration of Apo B. An increased number of cholesterol-poor LDL particles is also the hallmark abnormality of the most common familial dyslipoproteinemia associated with coronary disease, familial combined hyperlipidemia (FCH).[123–125] Notably, in familial hypercholesterolemia, LDL particles contain greater-than-average quantities of cholesterol, but both LDL-C and Apo B concentrations are markedly elevated.

In many prospective studies, investigators have demonstrated that the risk of vascular disease relates more closely

to the level of Apo B than LDL-C.[101–113] The non-HDL-C concentration reflects the sum of the cholesterol in all Apo B-containing particles and also predicts risk better than LDL-C in both normotriglyceridemic and hypertriglyceridemic individuals.[107,126] The evidence comparing Apo B and non-HDL-C as markers of risk is mixed, with results from some studies suggesting them to be equivalent and others supporting the view that Apo B is superior. A subset of the ERFC project database (22 of the 68 studies) was analyzed and the hazard ratio for non-HDL-C was equivalent to that for Apo B.[127] However, most of these studies were unpublished and, within the ERFC analysis, the hazard ratios for LDL-C and non-HDL-C were indistinguishable, a finding that contrasts with much prior experience. A more recent meta-analysis of the published studies that include risk estimates for non-HDL-C and Apo B suggests a hierarchy of outcome among the markers, with Apo B being the best predictor, LDL-C the worst, and non-HDL-C intermediate.[128]

Another advantage Apo B has over LDL-C is accuracy of measurement, a critical issue in therapeutic decision-making. The limitations of the Friedewald method used to estimate LDL-C have been well documented.[129] The introduction of direct LDL-C measurement methods may have improved precision for normolipidemic samples, but not for hyperlipidemic sera, and these assays also suffer from the disadvantage of not being standardized.[130]

The switch to direct HDL-C measurement brings with it a similar set of problems as those noted for the direct LDL-C assays, leading to error in the calculation of non-HDL-C.[129] By contrast, the measurement of Apo B is standardized, and can be performed relatively inexpensively and reliably in clinical laboratories.[129,131]As with non-HDL-C, fasting is not required for Apo B measurement, a major advantage in clinical practice.

What is the physiological rationale for the link between Apo B and adverse CV outcome?

Each lipoprotein particle secreted by the intestine or the liver contains one molecule of Apo B,[132] which is embedded within the phospholipid monolayer that encircles the particle. The Apo B molecule provides external structural integrity for the particle and, in contrast to all the other apolipoproteins, which can associate transiently with lipoproteins, Apo B stays with the lipoprotein particle for its lifetime.

Because each particle contains one molecule of Apo B, the plasma Apo B concentration is a direct indication of the total number of circulating Apo B-containing lipoprotein particles. The intestinal Apo B particles contain Apo B48, whereas the hepatic particles contain the full-length form of the protein, Apo B100.[133] Both Apo B48 and Apo B100 are recognized by most clinically available immunoassays.[129]Apo B48 particles, even postprandially, contribute minimally to the total number of Apo B particles in plasma.[129,134] Measurement of Apo B, like non-HDL-C, does not require a fasting blood draw.

Atherosclerosis is initiated and advanced by the trapping of Apo B-containing lipoprotein particles within the subintimal space of the arterial wall. The cholesterol that is deposited within the arterial wall, which leads over time to the development of a complex plaque, is transported into the arterial wall within an Apo B-containing lipoprotein particle. LDL Apo B particles are considered to be far more important than VLDL Apo B particles in driving atherogenesis because, in most cases, the serum LDL particle concentration is roughly nine times that of the VLDL particle concentration. Also, LDL particles are substantially smaller than VLDL particles, so are able to enter the arterial wall more readily.

The number of LDL particles entering the arterial wall is directly related to the concentration of LDL particles in plasma. A greater number of Apo B particles entering the arterial wall will increase the number that becomes trapped in the subendothelial space. This, in turn, increases the number of particles susceptible to modification via oxidation and other pathways, leading to unregulated uptake by macrophages, further promoting the development and progression of atherosclerosis.[135]

In which patients would Apo B testing be most valuable?

Low risk

Apo B was **"not recommended"** in this category of patients because the characteristic that defines this group, ie, <5% 10-year CHD event risk, makes the likelihood of a markedly elevated Apo B low.

Intermediate risk

In this category, Apo B received a **"reasonable for many patients"** recommendation because a large portion of the patients, if not the majority, belong to one of the classes in which discordance between Apo B and LDL-C has been well-documented. These include patients with hypertriglyceridemia, abdominal obesity, the metabolic syndrome or insulin resistance, and patients with otherwise-normal lipids but low HDL-C. In patients with LDL-C and/or non-HDL-C above NCEP ATP III cutpoints for initiation of lipid therapy, the measurement of Apo B would not be required to make the decision to initiate treatment, and therefore would not be necessary. On the other hand, given the analytical imprecision in the laboratory determination of LDL-C,[136] it could be argued that the decision to commit a patient to a prolonged course of therapy or, conversely, not to treat when treatment might be of value, should be confirmed by an independent and more reliable laboratory parameter, such as Apo B. That is a question of clinical judgment.

For those at intermediate risk with an LDL-C and/or non-HDL-C below the NCEP ATP III cutpoints for initiation of therapy, the NLA Biomarkers Expert Panel accepts that Apo B is a more reliable measure of the quantity of

LDL in plasma than LDL-C and measurement of Apo B would be reasonable to identify patients with an elevated LDL particle burden who might benefit from LDL-lowering treatment. The choices of threshold levels of Apo B for initiation of therapy have generally been determined on the basis of population percentile equivalents of the NCEP ATP III LDL-C cutpoints. For example, the Canadian Guidelines selected a level of Apo B of 100 mg/dL to correspond to an LDL-C level of 130 mg/dL.[137]

CHD or CHD risk equivalent

In CHD patients, the decision to substantially lower LDL is based on clinical criteria, and statin therapy would be indicated no matter the level of any of the markers: LDL-C, non-HDL-C or Apo B.[138] Because the decision to treat is not based on the level of any of these markers, it could be reasonably argued that it is not necessary to measure them before instituting the therapy. Once a patient has been treated to his or her LDL-C and/or non-HDL-C goal(s), obtaining an Apo B measurement would help to determine whether further intensification of lipid lowering therapy might be considered, as might be the case for discordant individuals with residual elevation in Apo B concentration despite having attained cholesterol goals.

In those with a CHD risk equivalent such as clinical evidence of non-CHD atherosclerosis or diabetes mellitus, the same argument could be made. In patients with diabetes, for example, the LDL-C concentration is often normal, and only a low or moderate dose of statin might be thought necessary to achieve target levels, but in a substantial number of these patients, Apo B is markedly elevated, notwithstanding the normal level of LDL-C. Therefore, the panel decided on a **"consider for selected patients"** recommendation for patients with CHD or risk equivalents.

Family history of premature CHD

This panel accepted the ATP III definition of a premature family history, namely of the presence of CHD before age 55 years in a male and before age 65 years in a female first-degree relative.[139]Apo B received a **"reasonable for many patients"** recommendation based, in part, on the fact that FCH is the most common atherogenic dyslipoproteinemia associated with premature CHD, far more common, in fact, than familial hypercholesterolemia. Moreover, the clinical risk associated with FCH is similar to the clinical risk associated with heterozygous familial hypercholesterolemia.

The fact that the genetic basis of FCH has not been clearly defined does not reduce its clinical importance. Until recently, a reliable diagnosis of FCH has not been possible in routine clinical care. However, a diagnostic algorithm has been developed and validated to identify individuals affected with FCH. The FCH phenotype has been defined as triglycerides >150 mg/dL and an Apo B >120 mg/dL.[124] On the basis of this definition, in a cohort presenting with premature MI, Wiesbauer et al[140] demonstrated that 38% had a lipoprotein phenotype consistent with FCH and 76% of these families were shown to have FCH. Thus, just as an individual presenting with familial hypercholesterolemia is an opportunity to identify other affected family members, so a patient presenting with FCH is an important opportunity to identify other affected family members.

Recurrent events

Apo B received a **"reasonable for many patients"** recommendation for this category of patients, whose very high level of risk requires the best possible management of all the modifiable factors for vascular risk. Accurately assessing LDL burden by measuring Apo B will often be useful for aiding difficult therapeutic choices.

Should Apo B be a target of therapy? If not, how should Apo B affect treatment decisions?

Statins reduce clinical vascular event rates in nearly every category of patients in which they have been tested. Consensus groups have recommended targets for LDL-C principally on the basis of the levels achieved in these trials for different classes of patients, matching the intensity of lipid treatment to the absolute risk for an event. However, objection has been raised to this practice because the major statin trials were designed as tests of different therapeutic regimens, not as tests of different target levels of LDL-C.[141]

Lowering of Apo B by statins is as directly related to the fundamental metabolic mechanism of statin action as is lowering of LDL-C because enhanced clearance of Apo B particles is the principal basis for the reduction in both. In addition, in none of the statin trials in which Apo B was measured was there an imbalance at baseline between the levels of Apo B in the groups compared.[142–149] No statin treatment trial has failed to find a significant relation between on-treatment Apo B and residual risk of vascular disease, whereas a number have found no significant relation between on-treatment LDL-C to the residual risk of vascular disease.[142,150,151] Such results validate the use of Apo B as a target of statin therapy.

Clinicians should be aware that statins lower LDL-C and non-HDL-C levels more than they lower Apo B.[152] Measurement of Apo B provides a more direct assessment of the residual number of atherogenic particles, which could potentially modify therapeutic decisions. In the large subgroup of patients with cholesterol-depleted LDL particles, the LDL-C level underestimates LDL particle concentration. Treatment with statins exaggerates this discordance, thus some patients at target levels of LDL-C (and non-HDL-C) still have concentrations of LDL particles above desirable levels.[153] If Apo B is measured, therapeutic adjustments can be made when such discordant patients are identified.

Although the clinical benefits of statin therapy are unequivocal, the evidence for clinical gains from combination therapy is incomplete. Adding an additional agent to

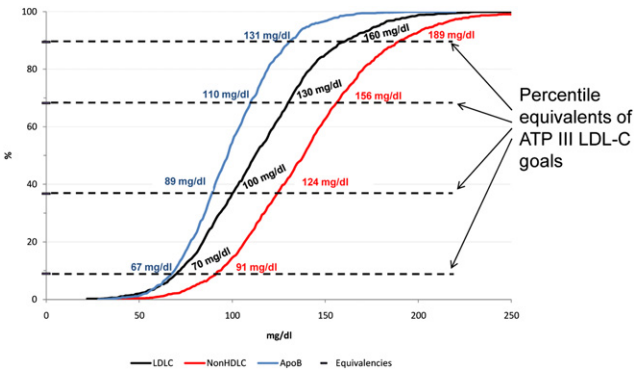

Figure 4 Cumulative distributions of Apo B, LDL-C, and non-HDL-C from National Health and Nutrition Examination Survey, 2005-2006.

statin therapy will help to further lower LDL-C, non-HDL-C, and Apo B. However, whether that results in substantial additional clinical benefit remains unknown. Until the appropriate large-scale clinical outcomes trials are completed, each clinician must use his or her clinical judgment as to whether any form of combination therapy to further lower LDL burden is warranted in a particular patient. Also, it is not universally appreciated that the relationships of LDL-C, non-HDL-C and Apo B to CV risk are curvilinear (ie, log-linear), not linear. Accordingly, risk increases and decreases exponentially upon changes in the concentration of LDL-C or Apo B. This means that the absolute gain becomes less and less as the initial levels of LDL-C or Apo B become lower and lower. Accordingly, combination therapy will be of greatest potential benefit to those who are farthest from target levels.

The American Diabetes Association/American College of Cardiology Foundation consensus panel recommended measurement of Apo B in patients at elevated cardiometabolic risk.[154] In high-risk patients, including those with lipoprotein abnormalities without diabetes or clinical CVD, but with at least two more major CVD risk factors, an Apo B target <90 mg/dL is recommended. In patients categorized as being at the greatest risk, including those with known clinical CVD, or diabetes, and at least one other cardiometabolic risk factor, an Apo B concentration <80 mg/dL is recommended. The Canadian guidelines recommend a target Apo B of <80 mg/dL in moderate-to-high risk patients as a secondary optional treatment target once LDL-C is at goal.[137]

One approach to selection of treatment goals is to use Apo B values that are equivalent to LDL-C and non-HDL-C targets based on population percentiles. Figure 4 compares the percentile distributions and ATP III cutpoints of LDL-C, non-HDL-C, and Apo B in NHANES III. On the basis of this figure, Apo B values of 90 mg/dL and 67 mg/dL would be equivalent to LDL-C concentrations of 100 mg/dL and 70 mg/dL, respectively,[155] whereas on the basis of the Framingham Offspring Study, the equivalent values of Apo B would be 80 and 55 mg/dL, respectively.

An alternative basis for the choice of treatment goals is evaluation of the levels of Apo B achieved in trials of interventions that reduced clinical events. Table 3 lists the mean or median baseline and on-treatment levels of LDL-C and Apo B in the major statin trials for which Apo B has been reported.[143–146,149,156–159] On-treatment levels of Apo B in these trials ranged from 67 to 98 mg/dL, compared with 55 to 115 mg/dL for LDL-C. Notably, two studies achieved mean or median on-treatment levels of Apo B well below 80 mg/dL, ie, PROVE-IT, with 67 mg/dL, and JUPITER, with 71 mg/dL. The majority of these studies, with the exception of the IDEAL study, showed a significant reduction in the primary CV event outcome variable.

Figure 5 shows the relationship between Apo B levels and CHD event rates in primary and secondary prevention studies of lipid-altering drug therapies.[160] Although subject

Table 3 Baseline and on-treatment levels of LDL-C and Apo B and RR or HR vs the comparator for the primary outcome in clinical trials of statin therapy

Study	Statin and dosage, mg/d	LDL-C, mg/dL		Apo B, mg/dL		HR or RR (95% CI) vs comparator*
		Baseline	On-treatment	Baseline	On-treatment	
LIPID[156]	Pravastatin 40	150 (130−170)	107 (NR)	132 (NR)	98 (NR)	0.76 (0.65−0.88)
AFCAPS/TexCAPS[157]	Lovastatin 20	150 (17)	115 (20)	120 (NR)	96 (NR)	0.63 (0.50−0.79)
HPS[146]	Simvastatin 40	132 (31)	80 (NR)	114 (23)	78 (NR)	0.87 (0.81−0.94)
CARDS[143]	Atorvastatin 10	118 (28)	82 (27)	117 (24)	80 (19)	0.63 (0.48−0.83)
TNT[144,149]	Atorvastatin 80	152 (NR)	75 (23)	111 (NR)	91 (21)	0.78 (0.69−0.89)
IDEAL[158]	Atorvastatin 80	122 (33)	84 (25)	119 (NR)	90 (NR)	0.89 (0.78−1.01)
PROVE-IT[159]	Atorvastatin 80	106 (89−128)	62 (50-79)	102 (NR)	67 (NR)	0.84 (0.74−0.95)
JUPITER[145]	Rosuvastatin 20	108 (94−119)	55 (44-70)	108 (NR)	71 (NR)	0.56 (0.46−0.69)

AFCAPS/TexCaps, Air Force/Texas Coronary Atherosclerosis Prevention Study; Apo, apolipoprotein; CARDS, Collaborative Atorvastatin Diabetes Study; CI, confidence interval; HPS, Heart Protection Study; HR, hazard ratio; IDEAL, Incremental Decrease in End Points through Aggressive Lipid Lowering; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; LDL-C, low-density lipoprotein cholesterol; LIPID, Long-term Intervention with Pravastatin In Ischemic Disease; NR, not reported; PROVE-IT, Pravastatin or Atorvastatin Evaluation and Infection Therapy; RR, relative risk; SD, standard deviation; SEM, standard error of the mean; TNT, Treating to New Targets.

Values are mean (SD) or median (interquartile limits) values.

*Hazard ratio (HR) or relative risk (RR) and 95% confidence intervals (CI) for the primary cardiovascular event outcome for the statin group indicated vs its comparator group (placebo or less-aggressive statin therapy).

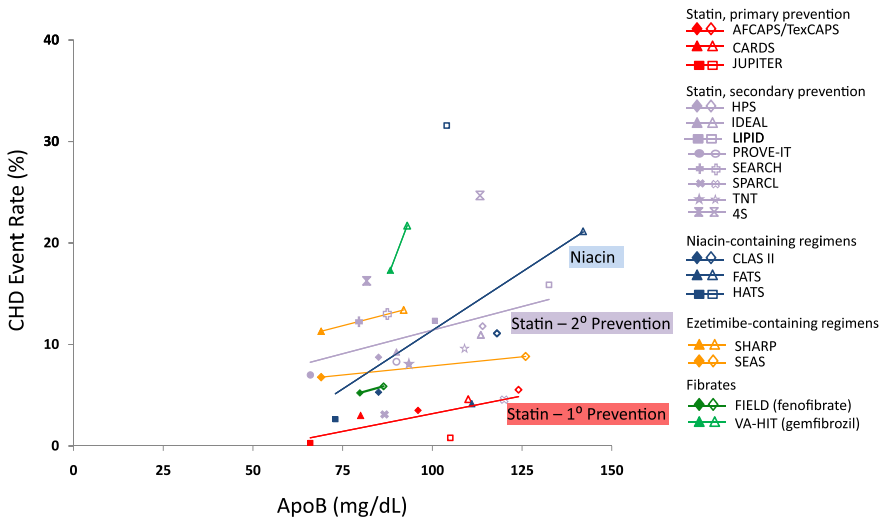

Figure 5 Relationship between mean Apo B concentration and CHD event rate in primary and secondary prevention trials with lipid-altering drug therapies. Statin trials are shown in red and purple, niacin (nicotinic acid) in blue, fibrates (fibric acid derivatives) in green, and ezetimibe in orange. Filled symbols represent the treated group, and empty symbols represent the control or placebo group.[160] AFCAPS/TexCAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; CARDS, Collaborative Atorvastatin Diabetes Study; CLAS-II, Cholesterol-Lowering Atherosclerosis Study; FATS, Familial Atherosclerosis Treatment Study; FIELD, Fenofibrate Intervention and Event Lowering in Diabetes; HATS, HDL Atherosclerosis Treatment Study; HPS, Heart Protection Study; IDEAL, Incremental Decrease in End Points Through Aggressive Lipid Lowering; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; LIPID, Long-Term Intervention With Pravastatin in Ischemic Disease; PROVE-IT, Pravastatin or Atorvastatin Evaluation and Infection, high-dose atorvastatin group; 4S, Scandinavian Simvastatin Survival Study, simvastatin group; SEARCH, Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine; SEAS, Simvastatin and Ezetimibe Aortic Stenosis, simvastatin-ezetimibe group; SHARP, Study of Heart and Renal Protection; SPARCL, Stroke Prevention by Aggressive Reduction in Cholesterol Levels; TNT, Treating to New Targets; VA-HIT, Veterans Affairs High-Density Lipoprotein Intervention Trial.

selection was not based on Apo B for the majority of these studies, none of the trials showed a significant imbalance between groups for baseline Apo B concentration. Also, these studies varied with regard to the therapeutic regimen tested, clinical population, and Apo B assay used. Nevertheless, a clear and approximately linear association is present, suggesting that a lower on-treatment Apo B concentration is associated with a lower CHD event rate, as has been shown previously for LDL-C and non-HDL-C.[161,162] The association is particularly robust for statin therapy, which has the largest evidence base for risk reduction.

Thus, although data on the effects of lowering Apo B to values <80 mg/dL are limited, the available results from clinical trials are consistent with the potential for further risk reduction. Accordingly, in patients at very high risk, it may be reasonable to consider more aggressive lowering of Apo B to <70 mg/dL. Additional research is needed to more clearly define optimal treatment targets for Apo B, as well as LDL-C and non-HDL-C.

What are the main areas of controversy and research questions regarding Apo B and its use in clinical practice?

The major areas of controversy regarding the use of Apo B in clinical practice relate to the relative merits of Apo B versus non-HDL-C for assessing risk and adequacy of treatment, as well as patient and clinician awareness and knowledge regarding Apo B. The panel concluded that previous controversies regarding measurement accuracy, standardization, and availability of measurement at relatively low cost on automated chemistry analyzers, were no longer of concern.

The majority view of the NLA Biomarkers Expert Panel was that the available evidence clearly supports the conclusion that Apo B is a better indicator of risk and treatment adequacy than LDL-C. However, its superiority over non-HDL C for these purposes has been less well established. In epidemiological studies that have compared Apo B with non-HDL-C for risk prediction, a majority suggest Apo B to be superior or equivalent to non-HDL-C, whereas very few have found superiority for non-HDL-C. Although the results of the analysis by the Emerging Risk Factor Collaboration suggested equivalence of Apo B and non-HDL-C for risk prediction,[127] a more recent meta-analysis that included a larger number of studies showed superiority of Apo B over non-HDL-C and LDL-C.[128]

The issue is complicated because the potential superiority of Apo B to non-HDL-C (and LDL-C) may not be constant across all subgroups of the population. Both LDL-C and non-HDL-C may underestimate atherogenic particle burden in subsets of the population in whom the cholesterol content of LDL particles is lower than average (greater Apo B than predicted by non-HDL-C or LDL-C concentrations), such as those with hypertriglyceridemia, the metabolic

syndrome, diabetes, or low HDL-C. More studies are needed to better define risk in "discordant" patients whose Apo B (or LDL-P) level is higher or lower than would be predicted based on measurement of lipoprotein cholesterol (LDL-C and non-HDL-C) concentrations.

Because measurement of Apo B is associated with additional cost and complexity compared with the standard lipoprotein lipid profile, whereas non-HDL-C can be calculated from the standard lipoprotein lipid profile at essentially no additional cost, important questions remain regarding whether Apo B should be incorporated into routine clinical evaluation,[163] or reserved for measurement in subgroups for whom the prevalence of discordance is high. Research is needed to further model the cost-effectiveness of routine versus targeted use of Apo B measurements for risk assessment, as well as for evaluation of residual risk and treatment adequacy in patients receiving lipid-altering therapies.

As reviewed previously, there is also debate about the methods that should be used for establishing Apo B treatment goals. Lipid-altering drug therapy often reduces non-HDL-C and LDL-C to a greater degree than Apo B. Thus, more aggressive therapy would be required to attain Apo B levels that correspond to population percentiles similar to those for the recommended LDL-C and non-HDL-C treatment goals. More aggressive therapy is associated with incremental costs and risks, which must be balanced against potential therapeutic gains. Thus, additional work is needed to develop and test models that justify treatment targets.

Finally, education of clinicians regarding the value and clinical application of new treatment targets, such as Apo B, remains a significant challenge. The magnitude and difficulty of the task has been illustrated by the experience following introduction of non-HDL-C into treatment guidelines. A decade after the NCEP ATP III report, clinician knowledge and use of non-HDL-C treatment goals in clinical practice remains low.[164]

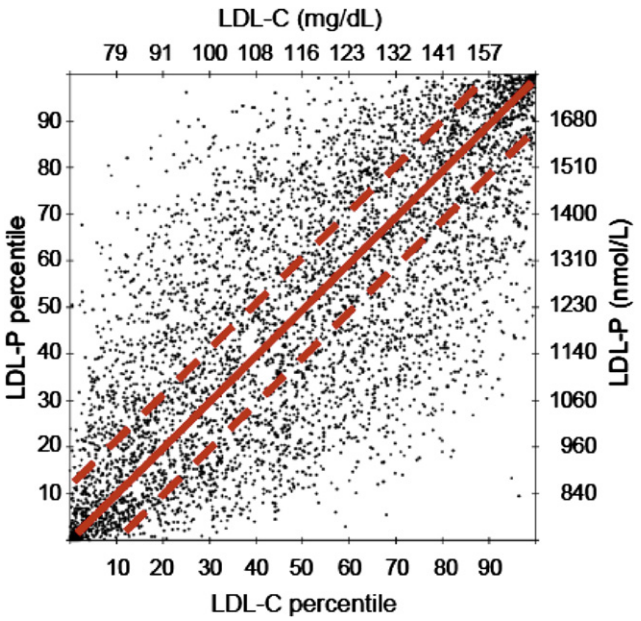

Figure 6 Relationship of LDL-C and LDL-P levels given in percentile units. The dashed lines bracket discordant LDL-C and LDL-P values defines in those with ± 12 percentile units.[173] Permission to reuse figure granted by Elsevier.

Low-density lipoprotein particle number/ concentration (LDL-P)

Does LDL-P predict risk, over and above traditional risk factors?

LDL measurements have two important clinical applications: (1) risk assessment, with LDL levels used, along with other risk markers, to identify patients at increased risk of CVD, and (2) risk management via LDL lowering, with target levels serving as treatment goals and indicators of the success of LDL-lowering therapies. The quantitative measure of LDL used traditionally for both of these applications is LDL-C, the amount of cholesterol carried in a person's LDL particles. However, the cholesterol content of LDL particles is not constant, varying more than 2-fold between individuals.[165–168] Furthermore, the cholesterol content of a given patient's LDL particles is not fixed, but can change over time in response to lipid-altering treatments.[169]

An alternative way to quantify LDL is to assess the concentration of LDL particles, either by measures of Apo B or LDL-P.[170] For many patients, levels of LDL-C and LDL-P (as well as Apo B) are concordant. But for many others, because of the variability of the cholesterol content of LDL particles, LDL-C and LDL-P levels are discordant (one LDL measure being higher or lower than the other on the basis of population percentiles; Fig. 6).[165,171–174]

In the general population, ~50% of subjects demonstrate discordance between LDL-C and LDL-P defined as a differential in population percentile of 12% or more (Fig. 7).[173] Individuals with elevated triglycerides or low HDL-C manifest progressively greater elevations of LDL-P concentrations at a given level of LDL-C.[166,175] In observational studies of patients with type 2 diabetes mellitus or metabolic syndrome and LDL-C <100 mg/dL (<20th percentile), discordantly elevated LDL-P levels greater than the 20th percentile (>1000 nmol/L) occur in 75% of subjects.[172,174] Similar results have also been shown for patients with type 2 diabetes mellitus and LDL-C <70 mg/dL.[172] In addition, discordance between LDL-C and LDL-P levels frequently occurs in patients receiving statin therapy because statins lower the LDL-C concentration to a greater degree than the LDL-P concentration.[169]

When one evaluates evidence bearing on the potential clinical utility of a reference standard laboratory measure

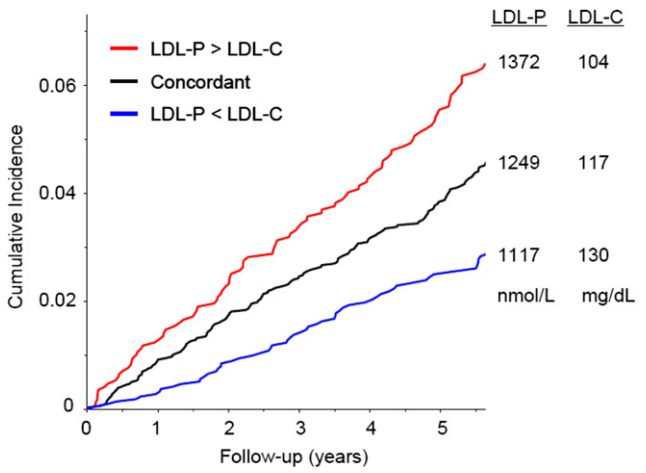

Figure 7 Cumulative incidence of cardiovascular events in subgroups with concordant or discordant levels of LDL-C and LDL-P, from proportional hazards models adjusted for age, sex, and race.[173] Permission to reuse figure granted by Elsevier.

(LDL-C) versus a new measure (LDL-P), it is useful to focus attention on cases of disagreement (discordance) between the measures.[176] To address questions regarding the practical implications of concordance versus discordance in LDL measures, this NLA Biomarkers Expert Panel report considered specific clinical circumstances to appraise the potential utility of LDL-P in clinical practice. Recently, an American College of Cardiology Foundation/American Heart Association task force issued recommendations in which the use of lipoprotein measures beyond a standard fasting lipid profile for CV risk assessment in asymptomatic adults was not recommended.[177] However, the report did not examine CV outcomes when alternate LDL measures (LDL-C vs LDL-P) are discordant. Although similar outcome associations are observed for the two measures when LDL-C and LDL-P are concordant, CV risk is more strongly associated with LDL-P when these measures are discordant.[167,173]

What is the physiological rationale for the link between LDL-P concentration and adverse CV outcome?

The key role played by LDL particles in the pathogenesis of CHD is well established. LDL particles move into the arterial wall via a gradient-driven process; the greater the circulating concentration of LDL particles, the greater the rate of passive diffusion into the arterial wall.[178–180] Once inside the intima, LDL particles that bind to arterial wall proteoglycans are retained, oxidized or otherwise modified, and subsequently taken up by macrophages to form foam cells.[181] When the serum LDL-P level is low (ie, fewer LDL particles are present in the circulation), fewer particles enter the arterial wall resulting in less propensity for initiation and promotion of atherosclerosis.[181]

In which patients would LDL-P testing be most valuable?

Use of LDL-P concentration in initial clinical assessment

Low risk (<5% 10-year CHD event risk)

It is the consensus of the NLA Biomarkers Expert Panel that treatment decisions are unlikely to be altered by use of LDL-P among low risk patients. Hence, measurement of LDL-P was **"not recommended"** for this patient group.

Intermediate risk (5–20% 10-year CHD event risk)

It is the consensus of the NLA Biomarkers Expert Panel that there are a substantial number of patients for whom LDL-C may not accurately reflect CVD risk. On the basis of the data showing that discordantly elevated LDL-P is more strongly associated with incident CVD risk than LDL-C level,[167,173] measurement of LDL-P is thought to be **"reasonable for many patients."** When LDL-P is discordantly elevated, consideration should be given to initiating or intensifying LDL lowering therapy. Conversely, a more conservative treatment approach could be considered for patients with lower LDL-P values than predicted based on their LDL-C (or non-HDL-C) concentrations. Populations known to manifest increased prevalence of discordance (elevated LDL-P for the level of LDL-C or non-HDL-C) include patients with metabolic syndrome,[171,174] as well as those with low HDL-C and/or elevated triglycerides.[165–167]

CHD or CHD risk equivalent

Because of high CV risk, patients with known CHD or a CHD risk equivalent are candidates for aggressive lipid-altering therapy. Given the clinical benefit of treating these patients with appropriate medical therapy, it is unclear whether additional LDL-P information would alter initial therapeutic decisions. Hence, it is the consensus of the NLA Biomarkers Expert Panel that use of LDL-P should be **"considered for selected patients only"** to identify individuals who might benefit. An example of such a patient might be an individual with type 2 diabetes in the absence of other major CHD risk factors who has LDL-C <100 mg/dL and non-HDL-C <130 mg/dL before treatment. In this setting, discordantly elevated LDL-P is commonly present[172] and could reasonably be used to justify more aggressive LDL lowering.

Family history of premature CHD (male <55 years, female <65 years)

Increased LDL-P concentration is often encountered among patients with a family history of premature CHD, including patients with FCH.[182,183] Because of the presence of cholesterol-depleted LDL particles, LDL-C levels are frequently unremarkable and fail to indicate the presence and degree of elevated LDL-P. Hence, it is the consensus of the NLA Biomarkers Expert Panel that measurement of LDL-P would be **"reasonable for many patients"** with a family history of premature CHD. When LDL-P is

discordantly elevated, consideration should be given to initiating LDL-lowering therapy.

Recurrent CHD events

Despite therapeutic lifestyle and pharmacologic therapy, some patients continue to have CHD progression and recurrent CHD events. Given the potential for discordantly elevated LDL-P among such individuals, it is the consensus of the NLA Biomarkers Expert Panel that use of LDL-P would be **"reasonable for many patients"** with recurrent CHD events. Discordantly elevated LDL-P could lead to more aggressive LDL lowering therapy which might reduce risk for future events.

Use of on-therapy LDL-P concentration to aid in clinical management

Lowering LDL is a key strategy in managing CVD risk. The authors of numerous clinical trials of statin agents, which up-regulate LDL receptors, resulting in reduced levels of circulating LDL-P, have shown significant reductions in CVD events among a wide range of patients. Although these data collectively reveal that greater LDL reduction is significantly associated with greater relative CVD event reduction, statin trials were not designed to evaluate the impact of adjusting individual therapy to achieve a specific LDL-C or LDL-P target of therapy. Rather, statin trials have generally used a fixed dose of statin compared with an alternative dose or placebo without titration to a specific treatment goal.

Consistent with data showing that CHD risk tracks with LDL-P, not LDL-C, when these two measures are discordant,[167,173] post-hoc analyses demonstrate that on-trial levels of LDL-P may be more predictive of residual risk than LDL-C.[184,185] In addition, given that statin therapy reduces LDL-C and non-HDL-C to a greater extent than it lowers LDL-P,[169] recent expert recommendations suggest that LDL-P may provide a better assessment of on-treatment residual risk than LDL-C or non-HDL-C measurement.[186] Thus, it was suggested that intensification of therapy would be a reasonable consideration when residually elevated LDL-P concentration is present. To adjudicate response to therapy, LDL-P targets were proposed as an optional therapeutic goal (in addition to LDL-C and non-HDL-C). LDL-P values advocated as targets of therapy were selected based on population equivalent levels for LDL-C targets in the Framingham Offspring cohort (<20th percentile for very high and high risk patients [LDL-P <1100 nmol/L], <50th percentile for moderately high- and moderate-risk patients [LDL-P <1440 nmol/L]).[170,186] Slightly lower population equivalent LDL-P levels have been reported from the Multi-Ethnic Study of Atherosclerosis (MESA; LDL-P values <1000 nmol/L [20th percentile], <1300 nmol/L [50th percentile]).[170,173]

Low risk (<5% 10-year CHD event risk)

It is the consensus of the NLA Biomarkers Expert Panel that treatment decisions are unlikely to be altered by use of LDL-P among low risk patients. Hence, use of LDL-P was **"not recommended"** for this group.

Intermediate risk (5–20% 10-year CHD event risk)

Because of the heterogeneity of the cholesterol content of LDL particles, and frequent LDL-P elevation among patients on lipid lowering therapy,[169] it is the consensus of the NLA Biomarkers Expert Panel that measurement of LDL-P would be **"reasonable for many patients"** at intermediate risk treated to LDL-C and non-HDL-C goal to adjudicate the adequacy of LDL lowering therapy. When the LDL-P concentration is discordantly elevated, consideration should be given to intensifying LDL lowering therapy. Conversely, a more conservative approach could be considered for patients with low LDL-P values.

CHD or CHD risk equivalent

Because of LDL-P heterogeneity among CHD or CHD risk equivalent patients on lipid-lowering therapy, it is the consensus of the NLA Biomarkers Expert Panel that use of LDL-P would be **"reasonable for many patients"** treated to LDL-C and non-HDL-C goal to adjudicate the adequacy of LDL lowering therapy.

Family history of premature CHD (male <55 years, female <65 years, first-degree relative)

As previously noted, increased LDL-P is commonly encountered among patients with a family history of premature CHD, including patients with FCH. Because of the presence of cholesterol-depleted LDL particles, LDL-C levels are often unremarkable and fail to indicate the presence of elevated LDL-P concentration. Once on therapy, it is the consensus of the NLA Biomarkers Expert Panel that measurement of LDL-P should be **"considered for selected patients"** treated to LDL-C and non-HDL-C goal to adjudicate the adequacy of LDL lowering therapy.

An example of such a selected patient could be a patient with significant family history of premature CHD and LDL-P elevation on lipid-lowering therapy. In this setting, LDL-P would be reasonable to adjudicate the adequacy of LDL-lowering therapy.

Recurrent CHD events

Given the very high risk inherent to patients with recurrent CHD events, it is the consensus of the NLA Biomarkers Expert Panel that use of LDL-P would be **"reasonable for many patients"** treated to LDL-C and non-HDL-C goal to adjudicate the adequacy of LDL lowering therapy.

Should LDL-P be a target of therapy? If not, how should LDL-P affect treatment decisions?

If elevated LDL-P is present in patients at LDL-C and non-HDL-C goals, intensification of therapy would be a reasonable consideration. Furthermore, LDL-P has been

proposed as an optional therapeutic goal with LDL-P targets advocated at population equivalent levels used for LDL-C targets (<20th percentile for very high and high risk patients [LDL-P <1100 nmol/L], <50th percentile for moderately high- and moderate-risk patients [LDL-P <1440 nmol/L]).[170,186] Medications routinely used for lipid optimization have well documented effects on LDL-P.[187] Because of changes in the cholesterol content of LDL particles on therapy, some treatments lower LDL-C more than they lower LDL-P concentration (statins, statin combination with ezetimibe and bile acid sequestrates), whereas other therapies lower LDL-P more than they lower LDL-C concentration (niacin, fibrates, or statin combination with niacin or fibrates). Accordingly, clinicians have several options for adjusting medication selection, dosage or combination therapy in response to elevated LDL-P.

What are the main areas of controversy and research questions regarding LDL-P and its use in clinical practice?

As is the case for Apo B concentration, which is also a reflection of the number of circulating atherogenic particles, the superiority of LDL-P concentration to non-HDL-C for CVD risk stratification and for guiding therapy has not been fully documented. Accordingly, the cost-effectiveness of using LDL-P in clinical practice, as an adjunct to or replacement of the traditional cholesterol measures, has not been established. Furthermore, the relative merits of measuring LDL-P versus Apo B remains uncertain and, at present, the decision about which to use remains a matter determined by availability, cost and clinician preference. The greatest usefulness of LDL-P (and Apo B) appears to reside in subgroups of patients for whom LDL-C, and to a lesser degree, non-HDL-C, do not provide a reliable indication of the burden of circulating atherogenic particles. In such patients, available data and expert panel recommendations support consideration of LDL-P (or Apo B) as a target of therapy (in addition to LDL-C and non-HDL-C) to adjudicate the adequacy of LDL-lowering therapy. Population equivalent values <20th percentile (<1100 nmol/L) for very high and high risk patients, or <50th percentile (<1440 nmol/L) for moderately high- and moderate-risk patients have been advocated for this purpose. Additional research is needed to more clearly define optimal treatment targets for LDL-P. Given the prevalence and magnitude of discordance between cholesterol and particle number measures of LDL burden, additional research is needed to more clearly define settings in which a policy of treating to LDL-P (or Apo B) goals might produce more favorable outcomes than the alternative of treating to LDL-C and non-HDL-C goals.

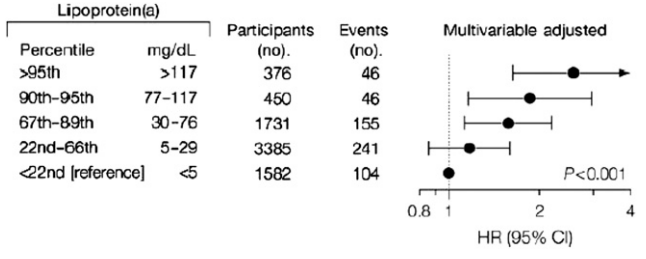

Figure 8 Risk of MI by levels of lipoprotein(a) in the general population assessed from the 1991–1994 examination of Copenhagen City Heart Study (n = 7524). HRs were adjusted for cardiovascular risk factors, and *P* value is for trend of HR [increasing lipoprotein(a) levels].[191,192] Permission to reuse figure granted by Oxford University Press.

Lipoprotein (a)

Does lipoprotein (a) [Lp(a)] predict risk, over and above traditional risk factors?

Lp(a) has positive predictive power that is additive to other measures of lipoprotein risk factors and to the classical "Framingham Risk Factors."[188–190] According to a recent review of the available evidence by Nordestgaard et al,[191] Lp(a) is specifically associated with increased risk for CHD in a continuous nonthreshold manner (Fig. 8).[191,192] Furthermore, the association between Lp(a) and CHD risk is independent of LDL-C, non-HDL-C, and the presence of other CV risk factors.[191]

What is the physiological rationale for the link between Lp(a) and adverse CV outcome?

Lp(a) represents a modification of LDL by addition of the "lipoprotein antigen," a protein made in the liver that binds to LDL in the plasma compartment and forms a disulfide bond with Apo B (Fig. 9).[193,194] The lipoprotein antigen is highly variable in its molecular weight because of the duplication of a sequence in the coding region of the gene that creates repeat amino acid sequences.[195] The large number of alleles causes a high variability of the molecular weight of Lp(a) in the population (from approximately 300,000 to 800,000 Daltons).[193] Variation in the promoter combines with the sequence differences to create highly variable plasma concentrations (approximately 1000-fold).[193] Lp(a) collects in the arterial wall where it is taken up by scavenger receptors on monocyte/macrophages.[196–199] It also binds to fibrin and may interfere with the conversion of plasminogen to plasmin.[194,200,201] This would, in theory, enhance clotting triggered by endothelial damage or plaque rupture, providing for a larger thrombus, a greater probability of arterial blockage, and a resulting acute clinical event. It may also promote monocyte adhesion to the endothelium, and carry a significant amount of potentially atherogenic oxidized phospholipids in human plasma.[196–199]

The molecular weight of apolipoprotein (a) can vary from approximately 300,000 to more than 800,000 D, depending on the number of K-IV units produced by the allele of a given genotype. This variability in structure changes the properties of Lp(a) and affects the mass in the

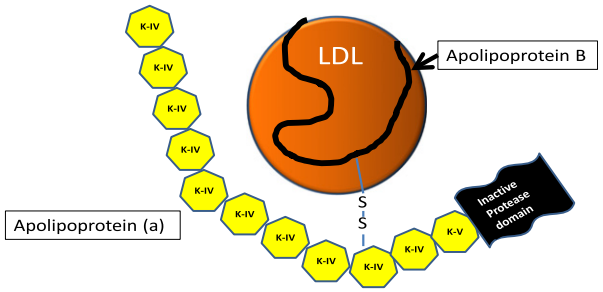

Figure 9 Diagram of Lp(a) structure. Lp(a) is composed of a LDL-P to which the apo(a) is attached by a disulfide bond through a cysteine side chain in Apo B. The apo(a) contains three basic regions depicted in the figure. From the carboxyl end, there is an inactive protease domain that is preceded by a series of folded structures that are reminiscent of a Danish pastry referred to as a kringle. Two different kringle structures are found in apo(a). This recapitulates the structure of plasminogen which has an initial active protease region preceded by five different forms of kringle structures designated by Roman numerals (I through V). Apo(a) contains a kringle V and a highly variable number of kringle IV repeats. There are no structures of the plasminogen type kringle I through III. The kringle IV repeats in apo(a) have sequence variability producing some 10 different types (KIV-1 through KIV-10). The disulfide bond with Apo B exists between a KIV-9 component in apo(a). KIV-7 and KIV-8 have non-covalent binding properties for regions of Apo B.

plasma of individuals. Smaller molecules are associated with higher synthesis rates in population data and it is the number of molecules of Lp(a) that seems to be the strongest determinant of related CVD risk.

In which patients would Lp(a) testing be most valuable?

Elevated plasma concentrations are controlled primarily by features of the Lp(a) gene.[202] Therefore, a very strong family history of vascular events, suggesting an autosomal dominant pattern, should lead to assessment.[192,203] Because elevated concentrations are additive to risk,[192] any patient with early disease that is not explained by the composite of other risk factors should be assessed. However, because family history is often inaccurate and the impact of other risk factors is variable, one could argue that anyone presenting with vascular disease should have this measurement. Because it is a very stable parameter, unaffected by diet and most drugs, a single measure that is well within normal limits (<25 mg/dL) is usually adequate to rule out Lp(a) as an important contributor to CVD risk in an individual patient. Many laboratories use ≥30 mg/dL as a cutpoint for indicating an elevated Lp(a) concentration; this represents approximately the top tertile of the general population.

Should Lp(a) be a target of therapy? If not, how should Lp(a) affect treatment decisions?

Lp(a) can be reduced by niacin therapy and, in women, by estrogen therapy.[204,205] A variety of other compounds can change Lp(a), but none is truly suitable as therapeutic agents.[206] The reduction of events in patients so treated has not been determined to relate specifically to changes in Lp(a). Therefore, although there is a strong theoretical reason to believe that lowering an elevated Lp(a) concentration would be beneficial, the clinical rationale for lowering Lp(a) with these agents has not been established.

Retrospective evidence suggests that aggressive reduction of LDL-C has a very significant effect on those with both elevated Lp(a) and elevated LDL-C.[207] Therefore, many have recommended more aggressive management of LDL-C, with treatment to lower target values in patients with elevated Lp(a).[207] Because there is no evidence that reducing Lp(a) is harmful, some lipidologists will use niacin in the effort to treat other lipoprotein abnormalities and to also achieve a lower Lp(a) value.

What are the main areas of controversy and research questions regarding Lp(a) and its use in clinical practice?

The absence of clear evidence that treating Lp(a) will change risk has prevented recommendations that this be used in screening all patients. The occurrence of high risk related to this is relatively uncommon; however, the authors of several studies have suggested that values sufficient to add significant risk occur in up to one fourth of the population.[191]

Important clinical questions remaining to be answered include the following:

1. Will reduction of plasma levels in those with elevated plasma concentrations reduce recurrent clinical events?
2. Will pharmacologic reduction of Lp(a) levels among individuals without manifest disease but high Lp(a) concentrations result in lower risk of CV events?
3. Can we develop a specific inhibitor of the synthesis of the "little a" protein such as an antisense oligonucleotide specific for the mRNA would provide a tool for specific reduction without changing other parameters. Because other current agents that reduce Lp(a) markedly alter other lipoprotein concentrations, they are not interventions that can give a clear answer to these questions.

Low-density lipoprotein subfractions

Do LDL subfractions predict risk, over and above traditional risk factors?

LDL particles are heterogeneous in size, density, and cholesterol/lipid content. Multiple analytic methods have been developed to classify LDL particles into various subfractions.[208–210] These subfractions can be individually quantitated or can be expressed as LDL particle patterns depending on the size of the predominant subfraction (Pattern

A or B if large or small LDL particles predominate, respectively). A gold standard for such analyses does not exist, and few comparative studies have been performed with highly variable statistical analysis methods.[210–212] Correlations between analytic methods for determination of LDL size vary widely and concordance in identifying LDL patterns ranges from 7% to 94%.[211,212] Furthermore, comparability of methods appears to vary by type of patient population.[212]

Studies have linked large LDL particles to atherosclerosis in nonhuman primates,[213] in patients with familial hypercholesterolemia (who have an elevated concentration of predominantly large LDL particles),[214] in participants of the population-based MESA study,[215] in normolipidemic men with CHD,[216] and among patients after MI in the Cholesterol And Recurrent Events (CARE) study.[217]

Predominantly small LDL particles are often present in patients with CHD, in individuals with type 2 diabetes mellitus, in those with low HDL-C and high triglycerides, and in individuals with insulin resistance and other features of the metabolic syndrome.[208] Many studies document links between small dense LDL particles and atherosclerotic CVD.[208–210,218–222] However, these statistical associations between small, dense LDL and CV outcomes are either significantly attenuated or abolished when the analyses are adjusted for the overall number of circulating LDL particles (LDL-P) either by adjustment for Apo B levels or by adjustment for nuclear magnetic resonance-derived LDL-P.[208,210]

What is the physiological rationale for the link between LDL subfractions and adverse CV outcome?

All lipoprotein particles in the LDL fraction are atherogenic, independent of size. LDL particles become trapped in the arterial wall and are internalized by macrophages through scavenger receptors on the macrophage surface, resulting in foam cell formation, activation of these foam cells and expansion of the inflammatory response.[223] It has been proposed that small, dense LDL particles are more atherogenic than larger particles due to longer residence time in plasma, increased susceptibility to oxidation, enhanced arterial proteoglycan binding, and increased permeability through the endothelial barrier.[208,209]

In which patients would LDL subfraction testing be most valuable?

The NLA Biomarkers Expert Panel was unable to identify any patient subgroups in which LDL subfractionation is recommended.

Should LDL subfraction be a target of therapy? If not, how should LDL subfractions affect treatment decisions?

Several investigators have suggested that lifestyle change and pharmacologic treatment can change LDL particle distribution.[208,209,224,225] However, such shifts are always accompanied by changes in LDL-C concentration and/or change in LDL-P, and often by changes in other lipoprotein fractions (eg, HDL-C and triglyceride levels) or nonlipid risk factors (eg, weight loss, improved insulin sensitivity, improved blood pressure with lifestyle modification). To date, there is no evidence that the shift in LDL subfractions directly translates into change in disease progression or improved outcome.

What are the main areas of controversy and research questions regarding LDL subfractions and its use in clinical practice?

Major areas of uncertainty can be summarized as follows:

- There is no agreed-upon gold standard for measurement of LDL subfractions and comparability of methods is limited.
- There are no studies to formally assess the incremental risk prediction achieved by measurement of LDL subfractions above and beyond traditional lipid measures and nonlipid risk factors.
- There are no prospective studies to show that a treatment strategy of changing LDL subfractions is superior to traditional lipid-lowering therapy in terms of atherosclerosis progression or CV morbidity and mortality.

High-density lipoprotein subfractions

Do HDL subfractions predict risk, over and above traditional risk factors?

HDL particles are heterogeneous in size, charge, density, and cholesterol/lipid content, and contain a large number of surface proteins which determine metabolic fate and function.[226] Although many aspects of reverse cholesterol transport have been elucidated in recent years, other antiatherosclerotic functions of HDL remain poorly understood.[227] Several analytic methods have been developed to classify HDL particles into various subfractions, but only recently has a unified nomenclature been proposed.[226]

HDL-C levels are strongly inversely associated with CV outcomes in population-based studies.[228] Most, but not all, analyses suggest that both baseline and on-trial HDL-C levels are also prognostically useful among patients on lipid-lowering therapy.[229–232]A number of studies have

shown that HDL subfractions also correlate with risk,[226,233,234] whereas others have failed to find a relationship.[235]

What is the physiological rationale for the link between HDL subfractions and adverse CV outcome?

HDL particles are involved in reverse cholesterol transport and have additional antioxidant and anti-inflammatory properties believed to be antiatherogenic.[226,227]

In which patients would HDL subfraction testing be most valuable?

The NLA Biomarkers Expert Panel was unable to identify any patient subgroups in which HDL subfractionation would be recommended.

Should HDL subfractions be a target of therapy? If not, how should HDL subfractions affect treatment decisions?

Several investigators have suggested that lifestyle change and pharmacologic treatment can change HDL particle distribution and the HDL proteonome,[226,236] but such changes are always accompanied by a change in HDL-C concentration and/or in HDL particle number, and often by changes in other lipoprotein fractions (eg, LDL-C levels and triglyceride levels) or nonlipid risk factors, especially when changes are achieved with comprehensive lifestyle modification. To date, there is no evidence that such a shift in HDL subfractions translates into change in disease progression or improved outcome.

What are the main areas of controversy and research questions regarding HDL subfractions and its use in clinical practice?

Major areas of uncertainty can be summarized as follows:

- HDL structure, metabolism, and function are very complex and not well understood.
- There is no consensus regarding a gold standard for measurement of HDL subfractions and comparability of methods is limited.
- There are no studies to formally assess the incremental risk prediction achieved by measurement of HDL subfractions above and beyond traditional lipid measures and non-lipid risk factors.
- There are no prospective studies in which authors demonstrate that a treatment strategy of changing HDL subfractions is superior to traditional lipid-lowering therapy in terms of atherosclerosis progression or CV morbidity and mortality.

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References

Preamble

1. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. *Arch Intern Med*. 1988;148:36–69.
2. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation*. 2002;106:3143–3421.
3. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. *J Am Coll Cardiol*. 2009;53:316–322.
4. Davidson MH, Maki KC, Pearson TA, et al. Results of the National Cholesterol Education Program (NCEP) evaluation project utilizing novel E-technology (NEPTUNE) II survey and implications for treatment under the recent NCEP Writing Group recommendations. *Am J Cardiol*. 2005;96:556–563.
5. Virani SS, Woodard LD, Landrum CR, et al. Institutional, provider, and patient correlates of low-density lipoprotein and non-high-density lipoprotein cholesterol goal attainment according to the Adult Treatment Panel III guidelines. *Am Heart J*. 2011;161:1140–1146.
6. Blaha MJ, Blumenthal RS, Brinton EA, Jacobson TA, National Lipid Association Taskforce on Non-HDL Cholesterol. The importance of non-HDL cholesterol reporting in lipid management. *J Clin Lipidol*. 2008;2:267–273.
7. Drexel H, Aczel S, Marte T, Vonbank A, Saely CH. Factors predicting cardiovascular events in statin-treated diabetic and non-diabetic patients with coronary atherosclerosis. *Atherosclerosis*. 2010;208:484–489.
8. Rosenson RS, Davidson MH, Pourfarzib R. Underappreciated opportunities for low-density lipoprotein management in patients with cardiometabolic residual risk. *Atherosclerosis*. 2010;213:1–7.
9. Ridker PM, Danielson E, Fonseca FA, et al. JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;259:2195–2207.
10. Blake GJ, Ridker PM, Kuntz KM. Potential cost-effectiveness of C-reactive protein screening followed by targeted statin therapy for the primary prevention of cardiovascular disease among patients without overt hyperlipidemia. *Am J Med*. 2003;114:485–494.
11. Biasucci LM, Biasillo G, Stefanelli A. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. *Clin Chem Lab Med*. 2010;48:1685–1691.

12. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107:499–511.
13. PLAC® Enzyme Immunoassay for the Quantitative Determination ofLp-PLA2 in Human Plasma and Serum product insert information (diaDexus, Inc. South San Francisco, CA). Available at http://www.framinghamheartstudy.org/share/protocols/lppla2m1_7s_protocol.pdf. Accessed May 16, 2011.
14. Contois JH, McConnell JP, Sethi AA, et al. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. *Clin Chem*. 2009;55:407–419.
15. Mora S, Szklo M, Otvos JD, et al. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2007;192:211–217.
16. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle numbers. *J Clin Lipidol*. 2011;5:105–113.
17. Nordestgaard BG, Chapman MJ, Ray K et al, European Atherosclerosis Society Consensus Panel. Lipoprotein (a) as a Cardiovascular Risk Factor. *Eur Heart J*. 2010;31:2844–2853.

C-reactive protein

18. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107:363–369.
19. Ridker PM, Bassuk SS, Toth PP. C-reactive protein and risk of cardiovascular disease: evidence and clinical application. *Curr Atheroscler Rep*. 2003;5:341–349.
20. Pearson TA, Mensah GA, Alexander RW, et al. Centers for Disease Control and Prevention; American Heart Association: Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107:499–511.
21. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836–843.
22. Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation*. 1998;98:839–844.
23. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation*. 1998;97:425–428.
24. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979 Erratum appears in *N Engl J Med*. 1997;337:356.
25. Koenig W, Sund M, Fröhlich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation*. 1999;99:237–242.
26. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. *Am J Epidemiol*. 1996;144:537–547.
27. Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol*. 1997;17:1121–1127.
28. Roivainen M, Viik-Kajander M, Palosuo T, et al. Infections, inflammation, and the risk of coronary heart disease. *Circulation*. 2000;101:252–257.
29. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106:506–512.
30. Mendall MA, Strachan DP, Butland BK, et al. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J*. 2000;21:1584–1590.
31. Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010;375:132–140.
32. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. *N Engl J Med*. 1994;331:417–424.
33. Morrow DA, Rifai N, Antman EM, et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. Thrombolysis in Myocardial Infarction. *J Am Coll Cardiol*. 1998;31:1460–1465.
34. Biasucci LM, Liuzzo G, Grillo RL, et al. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. *Circulation*. 1999;99:855–860.
35. Toss H, Lindahl B, Siegbahn A, Wallentin L, Prognostic influence of increased fibrinogen and C-reactive protein levels in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *Circulation*. 1997;96:4204–4210.
36. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *N Engl J Med*. 2000;343:1139–1147.
37. Sabatine MS, Morrow DA, et al, PEACE Investigators. Prognostic significance of the Centers for Disease Control/American Heart Association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease. *Circulation*. 2007;115:1528–1536.
38. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet*. 1997;349:462–466.
39. Ridker PM, Cook N. Clinical usefulness of very high and very low levels of C-reactive protein across the full range of Framingham Risk Scores. *Circulation*. 2004;109:1955–1959.
40. Chang MK, Binder CJ, Torzewski M, Witztum JL. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. *Proc Natl Acad Sci U S A*. 2002;99:13043–13048.
41. Shih HH, Zhang S, Cao W, Hahn A, Wang J, Paulsen JE, Harnish DC. CRP is a novel ligand for the oxidized LDL receptor LOX-1. *Am J Physiol Heart Circ Physiol*. 2009;296:H1643–H1650.
42. Yasojima K, Schwab C, McGeer EG, McGeer PL. Generation of C-reactive protein and complement components in atherosclerotic plaques. *Am J Pathol*. 2001;158:1039–1051.
43. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation*. 2002;106:1439–1441.
44. Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation*. 2002;106:913–919.
45. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation*. 2000;102:1000–1006.
46. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation*. 2003;107:398–404.
47. Verma S, Li SH, Badiwala MV, et al. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation*. 2002;105:1890–1896.

48. Luscher TF, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. *Circulation*. 2000;102:2434–2440.
49. Pasceri V, Cheng JS, Willerson JT, Yeh ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. *Circulation*. 2001; 103:2531–2534.
50. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol*. 1999; 19:2348–2354.
51. Torzewski J, Torzewski M, Bowyer DE, et al. C-reactive protein frequently colocalizes with the terminal complement complex in the intima of early atherosclerotic lesions of human coronary arteries. *Arterioscler Thromb Vasc Biol*. 1998;18:1386–1392.
52. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation*. 2001;103:1194–1197.
53. Wang CH, Li SH, Weisel RD, et al. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation*. 2003;107:1783–1790.
54. Ridker PM, Danielson E, Fonseca FA, et al, JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.
55. Koenig W, Ridker PM. Rosuvastatin for primary prevention in patients with European systematic coronary risk evaluation risk ≥ 5% or Framingham risk >20%: post hoc analyses of the JUPITER trial requested by European health authorities. *Eur Heart J*. 2011;32:75–83.
56. Yang EY, Nambi V, Tang Z, et al. Clinical implications of JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) in a U.S. population insights from the ARIC (Atherosclerosis Risk in Communities) study. *J Am Coll Cardiol*. 2009;54:2388–2395.
57. Ridker PM, Rifai N, Clearfield M, et al, Air Force/Texas Coronary Atherosclerosis Prevention Study Investigators. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med*. 2001;344:1959–1965.
58. Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. *Circulation*. 2008;118:2243–2251.
59. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. *JAMA*. 2007;297:611–619.
60. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation*. 1999;100:17–22.
61. Ridker PM. The JUPITER trial: results, controversies, and implications for prevention. *Circ Cardiovasc Qual Outcomes*. 2009;2: 279–285.
62. Ridker PM, Danielson E, Fonseca FA, et al, JUPITER Trial Study Group. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet*. 2009;373:1175–1182.
63. Ahmed S, Cannon CP, Murphy SA, Braunwald E. Acute coronary syndromes and diabetes: Is intensive lipid lowering beneficial? Results of the PROVE IT-TIMI 22 trial. *Eur Heart J*. 2006;27:2323–2329.
64. Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation*. 2006;114:281–288.
65. Nissen SE. Aggressive lipid-lowering therapy and regression of coronary atheroma—reply. *JAMA*. 2004;292:39–40.
66. Nissen SE, Tuzcu EM, Schoenhagen P, et al, Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med*. 2005;352:29–38.
67. Mora S, Ridker PM. Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)—can C-reactive protein be used to target statin therapy in primary prevention? *Am J Cardiol*. 2006;97(2A):33A–341A.
68. Shen J, Ordovas JM. Impact of genetic and environmental factors on hsCRP concentrations and response to therapeutic agents. *Clin Chem*. 2009;55:256–264.
69. Belalcazar LM, Reboussin DM, Haffner SM, et al, Look AHEAD Research Group. A 1-year lifestyle intervention for weight loss in individuals with type 2 diabetes reduces high C-reactive protein levels and identifies metabolic predictors of change: from the Look AHEAD (Action for Health in Diabetes) study. *Diabetes Care*. 2010;33: 2297–2303.
70. Horiuchi Y, Hirayama S, Soda S, et al. Statin therapy reduces inflammatory markers in hypercholesterolemic patients with high baseline levels. *J Atheroscler Thromb*. 2010;17:722–729.

Lipoprotein-associated Phospholipase A_2

71. Toth PP, McCullough PA, Wegner MS, Colley KJ. Lipoprotein-associated phospholipase A2: role in atherosclerosis and utility as a cardiovascular biomarker. *Exp Rev Cardiovasc Ther*. 2010;8:425–438.
72. Braun LT, Davidson MH. Lp-PLA2: a new target for statin therapy. *Curr Atheroscler Rep*. 2010;12:29–33.
73. Anderson JL. Lipoprotein-associated phospholipase A_2: an independent predictor of coronary artery disease events in primary and secondary prevention. *Am J Cardiol*. 2008;101:23F–33F.
74. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation*. 2004;109: 837–842.
75. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arch Intern Med*. 2005; 165:2479–2484.
76. Shahar E, Chambless LE, Rosamond WD, et al, Atherosclerosis Risk in Communities Study. Plasma lipid profile and incident ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. *Stroke*. 2003;34:623–631.
77. Gorelick PB. Lipoprotein-associated phospholipase A2 and risk of stroke. *Am J Cardiol*. 2008;101(12A):34F–40F.
78. Lp-PLA(2) Studies Collaboration, Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet*. 2010;375:1536–1544.
79. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. *Arterioscler Thromb Vasc Biol*. 2006;26:1586–1593.
80. Häkkinen T, Luoma JS, Hiltunen MO, et al. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 1999;19:2909–2917.
81. Anderson JL. Lipoprotein-associated phospholipase A_2: an independent predictor of coronary artery disease events in primary and secondary prevention. *Am J Cardiol*. 2008;101(12A):23F–33F.
82. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated A2 as a cardiovascular risk marker. *Am J Cardiol*. 2008;101(12A):41F–450F.
83. Lerman A, McConnell JP. Lipoprotein-associated phospholipase A2: a risk marker or a risk factor? *Am J Cardiol*. 2008;101(12A): 11F–122F.
84. Macphee CH, Moores KE, Boyd HF, et al. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates

two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem J*. 1999;338(Pt 2):479–487.
85. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation*. 2007;115:2715–2721.
86. Herrmann J, Mannheim D, Wohlert C, et al. Expression of lipoprotein-associated phospholipase A(2) in carotid artery plaques predicts long-term cardiac outcome. *Eur Heart J*. 2009;30:2930–2938.
87. Wilensky RL, Shi Y, Mohler ER 3rd, et al. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nat Med*. 2008;14:1059–1066.
88. Garcia-Garcia HM, Serruys PW. Phospholipase A2 inhibitors. *Curr Opin Lipidol*. 2009;20:327–332.
89. Davidson MH, Corson MA, Alberts MJ, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A2 testing into cardiovascular disease risk assessment guidelines. *Am J Cardiol*. 2008;101(12A):51F–57F.
90. O'Donoghue M, Morrow DA, Sabatine MS, et al. Lipoprotein-associated phospholipase A_2 and its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction) trial. *Circulation*. 2006;113:1745–1752.
91. Heart Protection Study Collaborative Group. Lipoprotein-associated phospholipase A_2 activity and mass in relation to vascular disease and nonvascular mortality. *J Intern Med*. 2010;268:348–358.
92. Davidson MH. Clinical significance of statin pleiotropic effects: hypotheses versus evidence. *Circulation*. 2005;111:2280–2281.
93. Schaefer EJ, McNamara JR, Asztalos BF, et al. Effects of atorvastatin versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A2 in patients with coronary heart disease versus control subjects. *Am J Cardiol*. 2005;95: 1025–1032.
94. Muhlestein JB, May HT, Jensen JR, et al. The reduction of inflammatory biomarkers by statin, fibrate, and combination therapy among diabetic patients with mixed dyslipidemia. the DIACOR (Diabetes and Combined Lipid Therapy Regimen) Study. *J Am Coll Cardiol*. 2006; 48:396–401.
95. Saougos VG, Tambaki AP, Kalogirou M, et al. Differential effect of hypolipidemic drugs on lipoprotein-associated phospholipase A2. *Arterioscler Thromb Vasc Biol*. 2007;27:2236–2243.
96. Tzotzas T, Filippatos TD, Triantos A, Bruckert E, Tselepis AD, Kiortsis DN. Effects of a low-calorie diet associated with weight loss on lipoprotein-associated phospholipase A2 (Lp-PLA2) activity in healthy obese women. *Nutr Metab Cardiovasc Dis*. 2008;18: 477–482.
97. Mohler ER 3rd, Ballantyne CM, Davidson MH, et al, Darapladib Investigators. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study.J. *Am Coll Cardiol*. 2008;51: 1632–1641.
98. Wilensky RL, Shi Y, Mohler ER 3rd, et al. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nat Med*. 2008;14:1059–1066.
99. Serruys PW, García-García HM, Buszman P, et al, Integrated Biomarker and Imaging Study-2 Investigators. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation*. 2008;118: 1172–1182.
100. White H, Held C, Stewart R, et al. Study design and rationale for the clinical outcomes of the STABILITY Trial (STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY) comparing darapladib versus placebo in patients with coronary heart disease. *Am Heart J*. 2010;160:655–661.

Apolipoprotein B

101. Ingelsson E, Schaefer EJ, Contois JH, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. *JAMA*. 2007;298:776–785.
102. Shai I, Rimm EB, Hankinson SE, et al. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. *Circulation*. 2004;110:2824–2830.
103. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*. 2009;119:931–939.
104. Benn M, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study. *Arterioscler Thromb Vasc Biol*. 2007;27:661–670.
105. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. *JAMA*. 2005;294:326–333.
106. Jiang R, Schulze MB, Li T, Rifai N, Stampfer MJ, Rimm EB, Hu FB. Non-HDL cholesterol and apolipoprotein B predict cardiovascular disease events among men with type 2 diabetes. *Diabetes Care*. 2004;27:1991–1997.
107. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation*. 2005;112: 3375–3383.
108. Bruno G, Merletti F, Biggeri A, et al. Effect of age on the association of non-high-density-lipoprotein cholesterol and apolipoprotein B with cardiovascular mortality in a Mediterranean population with type 2 diabetes: the Casale Monferrato study. *Diabetologia*. 2006; 49:937–944.
109. Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB. Apolipoprotein B and non-high density lipoprotein cholesterol and the risk of coronary heart disease in Chinese. *J Lipid Res*. 2007;48: 2499–2505.
110. Holme I, Aastveit AH, Jungner I, Walldius G. Relationships between lipoprotein components and risk of myocardial infarction: age, gender and short versus longer follow-up periods in the Apolipoprotein MOrtalityRISk study (AMORIS). *J Intern Med*. 2008;264: 30–38.
111. McQueen MJ, Hawken S, Wang X, et al, INTERHEART study investigators. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *Lancet*. 2008;372:224–233.
112. Parish S, Peto R, Palmer A, et al., International Studies of Infarct Survival Collaborators. The joint effects of apolipoprotein B, apolipoprotein A1, LDL cholesterol, and HDL cholesterol on risk: 3510 cases of acute myocardial infarction and 9805 controls. *Eur Heart J*. 2009;30:2137–2146.
113. Talmud PJ, Hawe E, Miller GJ, Humphries ST. Nonfasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. *Arterioscler Thromb Vasc Biol*. 2002;22:1918–1923.
114. Sniderman AD, de Graaf J, Couture P. ApoB and the atherogenic ApoB dyslipoproteinemias. In: Kwiterovich PO Jr, editor. *The Johns Hopkins Textbook of dyslipidemia*. Philadelphia, PA: Lippincott Williams & Wilkins, 2009. p. 196–210.
115. Blom DJ, O'Neill FH, Marais AD. Screening for dysbetalipoproteinemia by plasma cholesterol and apolipoprotein B concentrations. *Clin Chem*. 2005;51:904–907.
116. Sniderman A, Tremblay A, Bergeron J, Gagné C, Couture P. Diagnosis of type III hyperlipoproteinemia from plasma total cholesterol, triglyceride, and apolipoprotein B. *J Clin Lipidol*. 2007;1: 256–263.

117. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res*. 1982;23:97–104.
118. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med*. 2001;135:447–459.
119. Lamarche B, Lemieux I, Després JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. *Diabetes Metab*. 1999;25: 199–211.
120. Krauss RM, Siri PW. Metabolic abnormalities: triglyceride and low-density lipoprotein. *Endocrinol Metab Clin North Am*. 2004;33: 405–415.
121. Genest J Jr., Bard JM, Fruchart JC, Ordovas JM, Schaefer EJ. Familial hypoalphalipoproteinemia in premature coronary artery disease. *Arterioscler Thromb*. 1993;13:1728–1737.
122. Sniderman AD, Dagenais GR, Cantin B, Després JP, Lamarche B. High apolipoprotein B with low high-density lipoprotein cholesterol and normal plasma triglycerides and cholesterol. *Am J Cardiol*. 2001; 87:792–793.
123. Sniderman AD, Castro Cabezas M, Ribalta J, et al. A proposal to redefine familial combined hyperlipidaemia - third workshop on FCHL held in Barcelona from 3 to 5 May 2001, during scientific sessions of the European Society for Clinical Investigation. *Eur J Clin Invest*. 2002;(32):71–73.
124. Veerkamp MJ, de Graaf J, Hendriks JC, Demacker PN, Stalenhoef AF. Nomogram to diagnose familial combined hyperlipidemia on the basis of results of a 5-year follow-up study. *Circulation*. 2004;109:2980–2985.
125. Sniderman A, Couture P, deGraaf J. Diagnosis and treatment of apolipoprotein B dyslipoproteinemias. *Nat Rev Endocrinol*. 2010;6:335–346.
126. Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM. Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. *Am J Cardiol*. 2006;98:1363–1368.
127. Emerging Risk Factors Collaboration. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302:1993–2000.
128. Sniderman AD, Williams K, Contois JH, et al. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcomes*. 2011;4:337–345.
129. Contois JH, McConnell JP, Sethi AA, et al, AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. *Clin Chem*. 2009;55:407–419.
130. Contois JH, Warnick GR, Sniderman AD. Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol and apolipoprotein B measurement. *J Clin Lipidol*. 2011;5:264–272.
131. Marcovina SM, Albers JJ, Kennedy H, Mei JV, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International Reference Material. *Clin Chem*. 1994;40:586–592.
132. Elovson J, Chatterton JE, Bell GT, et al. Plasma very low density lipoproteins contain a single molecule of apolipoprotein B. *J Lipid Res*. 1988;29:1461–1473.
133. Kane JP. Apolipoprotein B: structural and metabolic heterogeneity. *Annu Rev Physiol*. 1983;45:637–650.
134. Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58,434 individuals from the Copenhagen General Population Study. *Clin Chem*. 2011;57:482–489.
135. Smith EB, Staples EM. Intimal and medial plasma protein concentrations and endothelial function. *Atherosclerosis*. 1982;41:295–308.
136. Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clin Chem*. 2010;56:977–986.
137. Genest J, McPherson R, Frohlich J, et al. 2009 Canadian Cardiovascular Society/Canadian Guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult—2009 recommendations. *Can J Cardiol*. 2009;25:567–579.
138. Grundy SM, Cleeman JI, Merz CN, et al, Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J Am Coll Cardiol*. 2004;44: 720–732.
139. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;285: 2486–2497.
140. Wiesbauer F, Blessberger H, Azar D, et al. Familial-combined hyperlipidaemia in very young myocardial infarction survivors (<or =40 years of age). *Eur Heart J*. 2009;30:1073–1079.
141. Hayward R, Hofer TP, Vijan S. Narrative review: lack of evidence for recommended low-density lipoprotein treatment targets: a solvable problem. *Ann Intern Med*. 2006;145:520–530.
142. Gotto AM Jr., Whitney E, Stein EA, et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation*. 2000;101:477–484.
143. Colhoun HM, Betteridge DJ, Durrington PN, et al, CARDS Investigators. Primary preventions of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet*. 2004;364:685–696.
144. LaRosa JC, Grundy SM, Waters DD, et al, Treating to New Targets (TNT) Investigators. intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med*. 2005;352: 1425–1435.
145. Ridker PM, Danielson E, Fonseca FAH, et al, JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359: 2195–2207.
146. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:7–22.
147. Ray KK, Cannon CP, Cairns R, Morrow DA, Ridker PM, Braunwald E. Prognostic utility of Apo B/AI, total cholesterol/HDL, non-HDL cholesterol, or hs-CRP as predictors of clinical risk in patients receiving statin therapy after acute coronary syndromes: results from PROVE IT TIMI 22. *Arterioscler Thromb Vasc Biol*. 2009;29: 424–430.
148. Roeters van Lennep JE, Westerveld HT, Roeters van Lennep HWO, Zwinderman AH, Erkelens W, van der Wall EE. Apolipoprotein concentrations during treatment and recurrent coronary artery disease events. *Arterioscler Thromb Vasc Biol*. 2000;20:2408–2413.
149. Kastelein JJ, van der Steeg WA, Holme I, et al, TNT Study Group, IDEAL Study Group. Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation*. 2008;117:3002–3009.
150. Simes RJ, Marschner IC, Hunt D, et al, LIPID Study Investigators. 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? *Circulation*. 2002;105: 1162–1169.
151. Shepherd J, Blauw GJ, Murphy MB, et al, PROSPER study group, PROspective Study of Pravastatin in the Elderly at Risk. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet*. 2002;360:1623–1630.

152. Sniderman AD. Differential response of cholesterol particle measures of atherogenic lipoproteins to LDL-lowering therapy: implications for clinical practice. *J Clin Lipidol*. 2008;2:36–42.
153. Stein EA, Sniderman A, Laskarzewski P. Assessment of reaching goal in patients with combined hyperlipidemia: low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, or apolipoprotein B. *Am J Cardiol*. 2005;96:36K–43K.
154. Brunzell JD, Davidson M, Furberg CD, et al, American Diabetes Association. American College of Cardiology Foundation. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care*. 2008;31: 811–822.
155. Grundy SM. Low-density lipoprotein, non-high density lipoprotein and apolipoprotein B as targets of lipid-lowering therapy. *Circulation*. 2002;106:2526–2529.
156. The Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med*. 1998;339: 1349–1357.
157. Downs JR, Clearfield M, Weis S, et al, AFCAPS/TexCAPS Research Group. Primary prevention of acute coronary events with Lovastatin in men and women with average cholesterol levels. Results of AFCAPS/TexCAPS. *JAMA*. 1998;279:1615–1622.
158. Pedersen TR, Faergeman O, Kastelein JJP, et al, Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group. High-dose atorvastatin vs usual dose simvastatin for secondary prevention after myocardial infarction. The IDEAL Study: a randomized controlled trial. *JAMA*. 2005;294:2437–2445.
159. Cannon CP, Braunwald E, McCabe CH, et al, Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*. 2004; 350:1495–1504.
160. Jacobson TA. Opening a new lipid "apo−thecary": incorporating apolipoproteins as potential risk factors and treatment targets to reduce cardiovascular risk. *Mayo Clin Proc*. 2011;86:762–780.
161. Robinson JG, Smith B, Maheshwari N, Schrott H. Pleiotropic effects of statins: benefit beyond cholesterol reduction? *J Am Coll Cardiol*. 2005;46:1855–1862.
162. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density cholesterol reduction and coronary heart disease risk. *J Am Coll Cardiol*. 2009;53:316–322.
163. Ramjee V, Sperling LS, Jacobson TA. Non-HDL versus Apo B in cardiovascularrisk stratification: do the math. *J Am Coll Cardiol*. 2011; 58:457–463.
164. Virani SS, Woodard LD, Landrum CR, et al. Institutional, provider, and patient correlates of low-density lipoprotein and non-high-density lipoprotein cholesterol goal attainment according to the Adult Treatment Panel III guidelines. *Am Heart J*. 2011;161:1140–1146.

Low-density lipoprotein particle number/ concentration

165. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. *Am J Cardiol*. 2002;90:22i–29i.
166. Cromwell WC, Otvos JD. Low-density lipoprotein particle number and risk for cardiovascular disease. *Curr Atheroscler Rep*. 2004;6: 381–387.
167. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—Implications for LDL management. *J Clin Lipidol*. 2007;1: 583–592.
168. Sniderman AD, Furberg CD, Keech A, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. *Lancet*. 2003;361:777–780.
169. Sniderman AD. Differential response of cholesterol and particle measures of atherogenic lipoproteins to LDL-lowering therapy: implications for clinical practice. *J Clin Lipidol*. 2008;2:36–42.
170. Cromwell WC, Barringer TA. Low-density lipoprotein and apolipoprotein B: clinical use in patients with coronary heart disease. *Curr Cardiol Rep*. 2009;11:468–475.
171. Kathiresan S, Otvos JD, Sullivan LM, et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation*. 2006; 113:20–29.
172. Cromwell WC, Otvos JD. Heterogeneity of low-density lipoprotein particle number in patients with type 2 diabetes mellitus and low-density lipoprotein cholesterol <100 mg/dL. *Am J Cardiol*. 2006; 98:1599–1602.
173. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J Clin Lipidol*. 2011;5:105–113.
174. Otvos J, Cromwell W, Shalaurova I, et al. LDL particles, but not LDL cholesterol, are highly elevated in the metabolic syndrome—Results from the Framingham Offspring Study. *Circulation*. 2003;108 IV−740.
175. Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes*. 2003;52: 453–462.
176. Glasziou P, Irwig L, Deeks JJ. When should a new test become the current reference standard? *Ann Intern Med*. 2008;149:816–821.
177. Greenland P, Alpert JS, Beller GA, et al, American College of Cardiology Foundation, American Heart Association. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2010;56:e50–e103.
178. Nielsen LB. Transfer of low density lipoprotein into the arterial wall and risk of atherosclerosis. *Atherosclerosis*. 1996;123:1–15.
179. Rudd JHDJ, Weissberg PL. Atherosclerotic biology and epidemiology of disease. In: Topol R, editor. *Textbook of Cardiovascular Medicine*. Philadelphia, PA: Lippincott, Williams & Wilkins, 2002. p. 2–12.
180. Nordestgaard BG, Wootton R, Lewis B. Selective retention of VLDL, IDL, and LDL in the arterial intima of genetically hyperlipidemic rabbits in vivo. Molecular size as a determinant of fractional loss from the intima-inner media. *Arterioscler Thromb Vasc Biol*. 1995; 15:534–542.
181. Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation*. 2007;116:1832–1844.
182. Sniderman AD, Castro Cabezas M, et al. A proposal to redefine familial combined hyperlipidaemia—third workshop on FCHL held in Barcelona from 3 to 5 May 2001, during the scientific sessions of the European Society for Clinical Investigation. *Eur J Clin Invest*. 2002;32:71–73.
183. Gaddi A, Cicero AF, Odoo FO, Poli AA, Paoletti R, Atherosclerosis and Metabolic Diseases Study Group. Practical guidelines for familial combined hyperlipidemia diagnosis: an up-date. *Vasc Health Risk Manag*. 2007;3:877–886.
184. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. *Am J Cardiol*. 2002;90:89–94.
185. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation*. 2006;113:1556–1563.
186. Contois JH, McConnell JP, Sethi AA, et al, AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices.

Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. *Clin Chem*. 2009;55: 407–419.

187. Cromwell WC, Bays HE, Toth PP. Lipoprotein subfraction analysis using nuclear magnetic resonance spectroscopy. In: Adams JE, Apple F, Jaffe AS, editors. *Markers in Cardiology: A Case-Oriented Approach*. London: Blackstone, 2007. p. 217–250.

Lipoprotein (a)

188. Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA*. 2009;302: 412–423.
189. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation*. 2000;102: 1082–1085.
190. Bennet A, Di Angelantonio E, Erqou S, et al. Lipoprotein(a) levels and risk of future coronary heart disease: large-scale prospective data. *Arch Intern Med*. 2008;168:598–608.
191. Nordestgaard BG, Chapman MJ, Ray K, et al, European Atherosclerosis Society Consensus Panel. Lipoprotein (a) as a cardiovascular risk factor. *Eur Heart J*. 2010;31:2844–2853.
192. Kamstrup PR, Tybjærg-Hansen A, Steffensen R, Nordestgaard BG. Geneticallyelevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA*. 2009;301:2331–2339.
193. Rader DJ, Cain W, Ikewaki K, et al. The inverse association of plasma lipoprotein(a) concentrations with apolipoprotein(a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. *J Clin Invest*. 1994;93:2758–2763.
194. Koschinsky ML, Marcovina SM. Structure-function relationships in apolipoprotein(a): insights into lipoprotein(a) assembly and pathogenicity. *Curr Opin Lipidol*. 2004;15:167–174.
195. Kronenberg F, Kronenberg MF, Kiechl S, et al. Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis: prospective results from the Bruneck study. *Circulation*. 1999;100:1154–1160.
196. Sotiriou SN, Orlova VV, Al-Fakhri N, et al. Lipoprotein(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin. *FASEB J*. 2006;20:559–561.
197. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann CJ, Oberthaler S, Xu Q, Witzum JL, Tsimikas S. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: Prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol*. 2007;27:1788–1795.
198. Bergmark C, Dewan A, Orsoni A, Merki E, Miller ER, Shin MJ, Binder CJ, Horkko S, Krauss RM, Chapman MJ, Witztyn JL, Tsimikas S. A novel function of lipoprotein (a) as a preferential carrier of oxidized phospholipids in human plasma. *J Lipid Res*. 2008; 49:2230–2239.
199. Tsimikas S, Mallat Z, Talmud PJ, Kastelein JJ, Wareham NJ, Sandhu MS, Miller ER, Benessiano J, Tedgui A, Qitzum JL, Khaw KT, Boekholdt SM. Oxidation-specific biomarkers, lipoprotein(a), and risk of fatal and nonfatal coronary events. *J Am Coll Cardiol*. 2010;56:946–955.
200. Rouy D, Grailhe P, Nigon F, Chapman J, Anglés-Cano E. Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. In vitro studies in a plasma milieu. *Arterioscler Thromb.* 1991;11:629–638.
201. Boonmark NW, Lou XJ, Yang ZJ, et al. Modification of apolipoprotein(a) lysine binding site reduces atherosclerosis in transgenic mice. *J Clin Invest*. 1997;100:558–564.
202. Utermann G. Lipoprotein(a). In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York, NY: McGraw-Hill, 2001. p. 2753–2787.
203. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*. 2004;33:30–42.
204. Koschinsky M, Marcovina SM. Lipoprotein(a). In: Ballantyne C, editor. *Clinical Lipidology: A Companion to Braunwauld's Heart Disease*. Philadelphia, PA: Saunders Elsevier, 2009. p. 130–143.
205. Suk Danik J. Rifai N, Buring JE, Ridker PM. Lipoprotein(a), hormone replacement therapy, and risk of future cardiovascular events. *J Am Coll Cardiol*. 2008;52:124–131.
206. Tziomalos K, Athyros VG, Wierzbicki AS, Mikhailidis DP. Lipoprotein a: where are we now? *Curr Opin Cardiol*. 2009;24:351–357.
207. Brown WV, Ballantyne CM, Jones PH, Marcovina S. Management of Lp(a). *J Clin Lipidol*. 2010;4:240–247.

Low-density lipoprotein subfractions

208. Sacks FM, Campos H. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. *J Clin Endocrinol Metab*. 2003;88:4525–4532.
209. Superko HR. Advanced lipoprotein testing and subfractionation are clinically useful. *Circulation*. 2009;119:2383–2395.
210. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. *Ann Intern Med*. 2009;150:474–484.
211. Ensign W, Hill N, Heward CB. Disparate LDL phenotypic classification among 4 different methods assessing LDL particle characteristics. *Clin Chem*. 2006;52:1722–1727.
212. Chung M, Lichtenstein AH, Ip S, Lau J, Balk EM. Comparability of methods for LDL subfraction determination: a systematic review. *Atherosclerosis*. 2009;205:342–348.
213. Rudel LL, Parks JS, Johnson FL, Babiak J. Low density lipoproteins in atherosclerosis. *J Lipid Res*. 1986;27:465–474.
214. Patsch W, Ostlund R, Kuisk I, Levy R, Schonfeld G. Characterization of lipoprotein in a kindred with familial hypercholesterolemia. *J Lipid Res*. 1982;23:1196–1205.
215. Mora S, Szklo M, Otvos JD, et al. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2007;192:211–217.
216. Campos H, Roederer GO, Lussier-Cacan S, Davignon J, Krauss RM. Predominance of large LDL and reduced HDL2 cholesterol in normolipidemic men with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1995;15:1043–1048.
217. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. *JAMA*. 2001;286:1468–1474.
218. Lamarche B, Després JP, Moorjani S, Cantin B, Dagenais GR, Lupien PJ. Prevalence of dyslipidemic phenotypes in ischemic heart disease (prospective results from the Québec Cardiovascular Study). *Am J Cardiol*. 1995;75:1189–1195.
219. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation*. 2006;113:1556–1563.
220. Kuller L, Arnold A, Tracy R, et al. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. *Arterioscler Thromb Vasc Biol*. 2002;22: 1175–1180.
221. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation*. 2002;106:1930–1937.
222. Hallman DM, Brown SA, Ballantyne CM, Sharrett AR, Boerwinkle E. Relationship between low-density lipoprotein subclasses and asymptomatic atherosclerosis in subjects from the Atherosclerosis Risk in Communities (ARIC) Study. *Biomarkers*. 2004;9:190–202.
223. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999;340:115–126.

224. Williams PT, Krauss RM, Vranizan KM, Wood PD. Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation*. 1990;81:1293–1304.
225. Vakkilainen J, Steiner G, Ansquer JC, et al, DAIS Group. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease. The Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation*. 2003;107: 1733–1737.

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226. Rosenson RS, Brewer HB Jr., Chapman MJ, et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin Chem*. 2011;57:392–410.
227. Reilly MP, Tall AR. HDL proteomics: pot of gold or Pandora's box? *J Clin Invest*. 2007;117:595–598.
228. Emerging Risk Factors Collaboration. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins and risk of vascular disease. *JAMA*. 2009;302:1993–2000.
229. Baigent C, Keech A, Kearney PM, et al., Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366: 1267–1278.
230. Barter P, Gotto AM, LaRosa JC, et al, Treating to New Targets Investigators. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med*. 2007;357:1301–1310.
231. Ridker PM, Genest J, Boekholdt SM, et al, JUPITER Trial Study Group. HDL cholesterol and residual risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the JUPITER trial. *Lancet*. 2010;376:333–339.
232. Briel M, Ferreira-Gonzalez I, You JJ, et al. Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. *BMJ*. 2009;338:b92.
233. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation*. 2006;113:1556–1563.
234. Williams PT, Feldman DE. Prospective study of coronary heart disease vs. HDL2, HDL3, and other lipoproteins in Gofman's Livermore Cohort. *Atherosclerosis*. 2011;214:196–202.
235. Asztalos BF, Demissie S, Cupples LA, et al. LpA-I, LpA-I: A-II HDL and CHD-risk: The Framingham Offspring Study and the Veterans Affairs HDL Intervention Trial. *Atherosclerosis*. 2006;188:59–67.
236. Green PS, Vaisar T, Pennathur S, et al. Combined statin and niacin therapy remodels the high-density lipoprotein proteome. *Circulation*. 2008;118:1259–1267.