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.
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 A₂, 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.

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Preamble

Since the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) I in 1988, 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. 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.

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 and major knowledge gaps on the calculation of non-HDL-C and the goals of therapy. The National Lipid Association official policy has advocated the inclusion of non-HDL-C on all lipid profile laboratory reports. 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. 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. 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.

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
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-PLA2), 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). 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 1 Summary recommendations for measurement of inflammatory markers and advanced lipoprotein/subfraction testing in initial clinical assessment and on-treatment management decisions

<table>
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<tr>
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<th>Initial Clinical Assessment</th>
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<tr>
<td></td>
<td>CRP</td>
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<tr>
<td>Low risk (≥5% 10-year CHD event risk)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Intermediate risk (5-20% 10-year CHD event risk)</td>
<td>Recommended for routine measurement</td>
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<tr>
<td>CHD or CHD Equivalent</td>
<td>Consider for selected patients</td>
</tr>
<tr>
<td>Family History</td>
<td>Reasonable for many patients</td>
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<td>Recurrent Events</td>
<td>Reasonable for many patients</td>
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<th>On-Treatment Management Decisions</th>
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<td></td>
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<td>Recurrent Events</td>
<td>Reasonable for many patients</td>
</tr>
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Apo, apolipoprotein; CHD, coronary heart disease; CRP, C-reactive protein; HDL, high-density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; LDL, low-density lipoprotein; LDL-P, LDL particle number/concentration; Lp(a), lipoprotein (a).
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 established with 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”).

**Table 2** Laboratory values of CRP, Lp-PLA2, Apo B, LDL-P, and Lp(a) according to lower-, intermediate-, and greater-risk categories, approximated from population studies

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Population-based approximations</th>
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<tr>
<td></td>
<td>Lower risk</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Lp-PLA2, mg/mL</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Apo B, mg/dl</td>
<td>&lt;80</td>
</tr>
<tr>
<td>LDL-P, nmol/L</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Lp(a), mg/dl</td>
<td>&lt;5</td>
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</tbody>
</table>

Apo, apolipoprotein; CRP, C-reactive protein; LDL-P, low-density lipoprotein particle number/concentration; Lp(a), lipoprotein (a); Lp-PLA2, lipoprotein-associated phospholipase A2.

*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-PLA2 in Human Plasma and Serum product insert information (diaDexus, Inc., South San Francisco, CA). Results from several studies have suggested that population cutoffs 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 cut-points of <100 mg/dL (20th percentile) and ≥160 mg/dL (80th percentile).

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

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-PLA2: initial clinical assessment**

1. LP-PLA2 testing should generally not be performed in low-risk patients for the purpose of reclassification (rating: “not recommended”).

2. LP-PLA2 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-PLA2: on-treatment management decisions**

1. Measurement of LP-PLA2 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. 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%.

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. 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.

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). Elevated CRP has also been associated with increased vascular event rates among patients with acute coronary ischemia, stable angina pectoris, stable coronary artery disease, and a history of MI. 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.

**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. 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 and is produced de novo in atherosclerotic lesions.

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; (2) reduced endothelial nitric oxide synthase expression, nitric oxide release; (3) increased coronary vasoreactivity; (4) increased expression of endothelial plasminogen activator inhibitor-1, a protein that inhibits fibrinolysis and increases thrombotic risk; (5) promotion of the production of endothelin-1, a potent vasoconstrictor and inducer of vessel wall inflammation, abnormal cell growth, and thrombosis; (5) increased monocyte chemoattractant protein-1 expression, which promotes the influx of monocytes into the subendothelial space; (6) activation of complement by binding to partly degraded non-oxidized LDL cholesterol, and colocalization with the terminal membrane attack complex in early atherosclerotic lesions; (7) stimulation of macrophage scavenging for oxidized LDL, a principal step in foam cell formation; and (8)
up-regulation of angiotensin type 1 receptors in vascular smooth muscle,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{56} Similar results were found in a post-hoc analysis of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS).\textsuperscript{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\textsuperscript{58} and women.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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-1$\beta$ 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 <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

### 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 A₂ (Lp-PLA₂)

**Does Lp-PLA₂ predict risk, over and above traditional risk factors?**

The authors of several prospective epidemiological studies have identified Lp-PLA₂ 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-PLA₂ in the upper tertile or quartile. Lp-PLA₂ predicts risk independent of, and complementary to, CRP.73 Notably, in the ARIC Study, when both Lp-PLA₂ and CRP were in the top tertile, the risks for CHD events and stroke increased 4-fold and 11-fold.
respectively, compared with those in the lowest tertile for both markers. Unlike for CHD risk, epidemiological studies fail to show a consistent relationship between elevated LDL-C and stroke risk. However, elevation in Lp-PLA2 confers approximately a 2-fold increase in both first and recurrent strokes.

A recent meta-analysis of almost 80,000 patients in 32 prospective studies evaluated associations of Lp-PLA2 mass and activity with risk of CHD, stroke, and mortality (Fig. 3). RR ratios adjusted for conventional risk factors and expressed per one standard deviation increment in Lp-PLA2 activity or mass at baseline were as follows: 1.10 (95% CI 1.05–1.16) with Lp-PLA2 activity and 1.11 (1.07–1.16) with Lp-PLA2 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-PLA2 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-PLA2 predicts risk for CHD events and stroke, the NLA Biomarkers Expert Panel recommends that the measurement of Lp-PLA2 may assist with the risk assessment of intermediate-risk and some high-risk patients.

What is the physiological rationale for the link between Lp-PLA2 and adverse CV outcome?

Lp-PLA2 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-PLA2 activity has been shown to be up-regulated in atherosclerotic lesions and in rupture-prone fibrous caps. Lp-PLA2 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

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**Figure 3** Risk ratios for CHD, ischemic stroke, and vascular, and nonvascular mortality per 1 standard deviation greater Lp-PLA2 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. *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 non-fatal events. Permission to reuse figure granted by Elsevier.
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 has been identified in early atherosclerosis and are associated with 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.\(^{88}\) 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.\(^{89}\)

### 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.\(^{90}\))

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\(^{90}\) 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-PLA₂ inhibitors that are currently in clinical development. Darapladib, a potent selective inhibitor of Lp-PLA₂, produced sustained inhibition of plasma Lp-PLA₂ 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-PLA₂ 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-PLA₂ 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-PLA₂ for on-treatment risk management decisions.

What are the main areas of controversy and research questions regarding Lp-PLA₂ and its use in clinical practice?

The main areas of controversy regarding Lp-PLA₂ center on cost-effectiveness and whether the measurement of Lp-PLA₂ 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-PLA₂ 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 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. 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. 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. 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.

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. 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.

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. By contrast, the measurement of Apo B is standardized, and can be performed relatively inexpensively and reliably in clinical laboratories. 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, 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. Both Apo B48 and Apo B100 are recognized by most clinically available immunoassays. Apo B48 particles, even postprandially, contribute minimally to the total number of Apo B particles in plasma. 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.

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, 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{124} On the basis of this definition, in a cohort presenting with premature MI, Wiesbauer et al\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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
risk. 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 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, 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 data have 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.150 Although subject...
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, a more recent meta-analysis that included a larger number of studies showed superiority of Apo B over non-HDL-C and LDL-C.

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, 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.

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. 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.

An alternative way to quantify LDL is to assess the concentration of LDL particles, either by measures of Apo B or LDL-P. 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).

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). Individuals with elevated triglycerides or low HDL-C manifest progressively greater elevations of LDL-P concentrations at a given level of LDL-C. 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. Similar results have also been shown for patients with type 2 diabetes mellitus and LDL-C <70 mg/dL. 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.

When one evaluates evidence bearing on the potential clinical utility of a reference standard laboratory measure...
versus a new measure (LDL-P), it is useful to focus attention on cases of disagreement (discordance) between the measures. 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. 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.

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. 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. 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.

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, 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, as well as those with low HDL-C and/or elevated triglycerides.

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 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. 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, post-hoc analyses demonstrate that on-trial levels of LDL-P may be more predictive of residual risk than LDL-C. In addition, given that statin therapy reduces LDL-C and non-HDL-C to a greater extent than it lowers LDL-P, 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. 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]). 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]).

**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, 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]). Medications routinely used for lipid optimization have well documented effects on LDL-P. 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.

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.” According to a recent review of the available evidence by Nordestgaard et al., Lp(a) is specifically associated with increased risk for CHD in a continuous nonthreshold manner (Fig. 8). 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.

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). 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. 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). Variation in the promoter combines with the sequence differences to create highly variable plasma concentrations (approximately 1000-fold). Lp(a) collects in the arterial wall where it is taken up by scavenger receptors on monocyte/macrophages. It also binds to fibrin and may interfere with the conversion of plasminogen to plasmin. 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.

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
should Lp(a) affect treatment decisions?

Should Lp(a) be a target of therapy? If not, how valuable?

In which patients would Lp(a) testing be most determinant of related CVD risk.

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. Therefore, a very strong family history of vascular events, suggesting an autosomal dominant pattern, should lead to assessment. Because elevated concentrations are additive to risk, 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. A variety of other compounds can change Lp(a), but none is truly suitable as therapeutic agents. 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.

Retropective evidence suggests that aggressive reduction of LDL-C has a very significant effect on those with both elevated Lp(a) and elevated LDL-C. Therefore, many have recommended more aggressive management of LDL-C, with treatment to lower target values in patients with elevated Lp(a). 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.

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. 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. Correlations between analytic methods for determination of LDL size vary widely and concordance in identifying LDL patterns ranges from 7% to 94%. 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,\textsuperscript{226,233,234} whereas others have failed to find a relationship.\textsuperscript{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.\textsuperscript{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 proteome,\textsuperscript{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


**C-reactive protein**


can C-reactive protein be used to target statin therapy in primary prevention? Am J Cardiol. 2006;97(2A):33A–341A.


Lipoprotein-associated Phospholipase A2


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.


155. Grundy SM. Low-density lipoprotein, non-high density lipoprotein and apolipoprotein B as targets of lipid-lowering therapy. Circulation. 2002;106:2526–2529.


161. 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;99:1599–1602.


171. 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.


## Lipoprotein (a)


## Low-density lipoprotein subfractions


High-density lipoprotein subfractions
