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Special Report

Apolipoprotein B and Cardiovascular Disease Risk: Position Statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices

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BACKGROUND: Low-density lipoprotein cholesterol (LDL-C) has been the cornerstone measurement for assessing cardiovascular risk for nearly 20 years.

CONTENT: Recent data demonstrate that apolipoprotein B (apo B) is a better measure of circulating LDL particle number (LDL-P) concentration and is a more reliable indicator of risk than LDL-C, and there is growing support for the idea that addition of apo B measurement to the routine lipid panel for assessing and monitoring patients at risk for cardiovascular disease (CVD) would enhance patient management. In this report, we review the studies of apo B and LDL-P reported to date, discuss potential advantages of their measurement over that of LDL-C, and present information related to standardization.

CONCLUSIONS: In line with recently adopted Canadian guidelines, the addition of apo B represents a logical next step to National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) and other guidelines in the US. Considering that it has taken years to educate physicians and patients regarding the use of LDL-C, changing perceptions and practices will not be easy. Thus, it appears prudent to consider using apo B along with LDL-C to assess LDL-related risk for an interim period until the superiority of apo B is generally recognized.

LDL cholesterol (LDL-C)[7] has been the cornerstone measurement for the assessment of cardiovascular disease (CVD) risk and for guiding lipid-lowering therapy for nearly 2 decades, and remains so today. When the lipoproteins were identified mid-twentieth century, the common practice was to quantify them based on their cholesterol content *(1)*. Later, as the apolipoprotein constituents were recognized and characterized, awareness gradually developed that apolipoprotein B (apo B), occurring as 1 molecule per LDL particle, was a more representative indicator of the concentration of LDL. Nevertheless, most of the early population and intervention studies measured LDL in terms of its associated cholesterol. Hence, as national guidelines were developed and promulgated, patient characterization and treatment continued to be based primarily on LDL-C. In recent years, as immunoassays for apo B have improved and become more readily available, increasing numbers of studies have included both apo B and LDL cholesterol.

Consequently, considerable debate and controversy have developed regarding the relative merits of monitoring LDL in terms of cholesterol content or particle concentration, as measured by apo B, to assess risk and monitor therapy. Nuclear magnetic resonance (NMR) has more recently been introduced as another means of quantifying LDL particle number (LDL-P) concentration *(2)*. Results from prospective studies generally demonstrate the superiority of apo B or LDL-P over LDL-C measurement for the assessment of

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[7] Nonstandard abbreviations: LDL-C, LDL cholesterol; CVD, cardiovascular disease; apo B, apolipoprotein B; NMR, nuclear magnetic resonance; LDL-P, LDL particle number; CHD, coronary heart disease; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; Lp(a), lipoprotein(a); HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; mRNA, messenger RNA; MTP, microsomal triglyceride transfer protein; FH, familial hypercholesterolemia; HTG, hypertriglyceridemia; FCHL, familial combined hyperlipidemia; AMORIS, Apolipoprotein-Related Mortality Risk Study; MI, myocardial infarction; TC/HDL-C, total/HDL cholesterol ratio; 4S, Scandinavian Simvistatin Survival Study; LIPID, Long-term Intervention with Pravastatin in Ischaemic Disease; VAHIT, Veterans Affairs High-Density-Lipoprotein Cholesterol Intervention Trial; MESA, Multi-Ethnic Study of Atherosclerosis; AFCAPS/TexCAPs, Air Force/Texas Coronary Atherosclerosis Prevention Study; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III.

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risk. Thus, the addition of apo B measurement to the routine lipid panel for assessing and monitoring patients at risk for CVD could enhance patient management. This position was supported in a recent review advocating apo B measurement, with the panel of experts concluding that risk is more directly related to the number of circulating atherogenic particles than to the cholesterol content of lipoproteins *(3)*. Further, a consensus conference report from the American Diabetes Association and the American College of Cardiology concluded that measurement of apo B with a standardized assay is warranted in patients with metabolic syndrome, especially to assist with therapeutic monitoring *(4)*. Such an approach would be in line with guidelines already adopted in Canada *(5)*.

On the other hand, physicians and patients recognize and understand LDL-C as the "bad" cholesterol. Changing practice to replace LDL-C would require considerable education and might also cause confusion. Thus, there is a tradeoff between superior predictive power and the potential to complicate efforts to intervene and ameliorate the risk associated with LDL.

In this report, we review the studies of apo B and LDL-P reported to date and discuss potential advantages of their measurement over that of LDL-C, including standardization issues. In light of the mounting evidence, the members of this working group of the Lipoproteins and Vascular Diseases Division of the AACC believe that apo B and alternate measures of LDL particle concentration should be recognized and included in guidelines, rather than continuing to focus solely on LDL-C.

apo B-CONTAINING LIPOPROTEINS AND CVD RISK

It is now evident that an increased serum apo B concentration is an important coronary heart disease (CHD) risk factor. apo B is a component of all atherogenic or potentially atherogenic particles, including very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and lipoprotein(a) [Lp(a)], and each particle contains 1 molecule of apo B. Therefore, apo B provides a direct measure of the number of atherogenic lipoprotein particles in the circulation. Even in hypertriglyceridemic patients, however, most of the total plasma apo B is associated with LDL, making apo B a good surrogate for LDL particle concentration *(6)*. The larger apo B-carrying particles may be less atherogenic than the smaller LDL particles, suggesting that specific measurement of apo B in LDL might be a better predictor than total serum apo B, although this has not been demonstrated conclusively *(7–11)*.

LDL particles, not simply LDL-C, play a central role in atherogenesis. The initiating process is the subendothelial retention of intact apo B-containing particles *(12)*. LDL particles move into the arterial intima through a gradient-driven process, and the rate of passive diffusion is increased when the concentration of circulating LDL particles is increased *(13)*. Once inside the intima, the LDL particles bind to proteoglycans and initiate a process whereby the LDL particles become oxidized or otherwise modified and are taken up by monocytes or macrophages to form foam cells *(14)*. The cholesterol molecules contained in the LDL are "passengers," but the intact particles drive the atherosclerotic process.

Cholesterol has served as a useful surrogate for estimating LDL-related risk, but LDL-C concentration can vary widely between individuals with the same LDL particle concentration *(2, 15)*. LDL-C content does not reflect LDL particle concentration because metabolic reactions involving lipids can alter both lipoprotein size and lipid composition. The relative amounts of cholesterol and triglycerides in LDL particles can vary widely between individuals. In 1 study of 118 healthy men and women, the ratio of cholesterol to triglycerides in LDL ranged from 1.8 to 11.5 *(16)*. The majority of subjects had large LDL, with the expected ratio of cholesterol to triglycerides >4. Surprisingly, 21% of subjects had LDL particles that were cholesterol-depleted (cholesterol/triglycerides ratio <4), indicating that even an accurately measured LDL-C will underestimate LDL particle concentration and presumably CHD risk, as well.

Numerous prospective epidemiologic studies have shown apo B and LDL-P to be statistically significant predictors of heart disease (Supplemental Tables 1 and 2, which accompany the online version of this article at www.clinchem.org/content/vol55/issue3). apo B or LDL-P measurement to assess CHD risk is especially important in the large and rapidly growing subset of the population with diabetes or with characteristics of the metabolic syndrome. Individuals with metabolic syndrome or diabetes tend to have an increased number of small, dense LDL particles but relatively normal LDL-C concentrations. Because therapies with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors reduce LDL-C to a greater extent than they do LDL particles *(17)*, apo B or LDL-P appear to provide a better assessment of on-treatment residual risk than LDL-C measurement *(18)*.

apo B STRUCTURE AND FUNCTION

Apolipoproteins, the protein components of lipoproteins *(19, 20)*, collectively have 3 major functions. They are involved in (1) modulating the activity of enzymes that act on lipoproteins, (2) maintaining the structural integrity of the lipoprotein complex, and (3) facilitating the uptake of lipoprotein by acting as ligands for specific cell-surface receptors.

apo B is a large amphipathic glycoprotein with 2 isoforms: apo B-100, which is synthesized in the hepatocytes, and apo B-48, an abridged version that is also derived from the apo B-100 gene [but from a modified messenger RNA (mRNA) transcript] and synthesized in the small intestine *(21)*. apo B-48 is the structural protein of chylomicrons and is responsible for their formation and secretion.

Amphipathic helices are common structural motifs that are shared in most apolipoproteins, enabling them to bind and solubilize lipids in the aqueous circulation. The lipid-associating domains of apo B-100 consist of 2 regions of amphipathic β-strands alternating with 2 regions of amphipathic α-helices and a third N-terminal amphipathic α-helical domain resulting in a pentapartite structure *(22)*.

Domains within the pentapartite model have been shown to be vital for lipoprotein assembly *(23)*. Studies with apo B lacking this domain due to missense mutations show impaired secretion of apo B *(24, 25)*. In addition, interaction with microsomal triglyceride transfer protein (MTP) appears to be vital for lipid recruitment by apo B *(26, 27)*.

apo B GENETICS

The gene coding for apo B, located on the short arm of chromosome 2, consists of 29 exons. The gene codes for apo B-100, with 4536 amino acids (550 kDa) and apo B-48 (265 kDa)—only about half the length of the native apo B-100 molecule *(28, 29)*. For apo B-48, only the first 2152 N-terminal amino acids get translated from the B-100 gene transcript; therefore, the smaller apolipoprotein lacks the C-terminal LDL receptor binding region. Several common polymorphisms within the apo B gene have been described, with variable effects on lipid concentrations *(24)* and others with detrimental effect on the binding properties with the LDL receptor *(30)*. Three of the most frequently investigated in the literature are the T2488T, E4154K, and the signal peptide insertion/deletion polymorphism (SpIns/Del), which deletes 3 amino acids.

Previous metaanalyses have suggested that the SpIns/Del is the only apo B polymorphism that is consistently associated with increased risk of CHD together with increased LDL-C and apo B *(31, 32)*. Although the other 2 polymorphisms seem to change LDL-C and apo B concentrations, they do not significantly change the risk of CHD. This is in contrast to 1 large study that found a 3- to 5-fold reduced risk of ischemic cerebrovascular disease associated with homozygous carriers of E4154K *(33)*. These findings suggest either a complex relationship between CVD and changes in lipid concentrations caused by the apo B polymorphisms or that the known polymorphisms are in linkage with the true causative regions of the apo B gene *(34)*.

DISORDERS OF apo B

Many lipoprotein disorders are characterized by increased serum apo B concentrations. Apo B mediates the uptake of LDL particles by liver and peripheral tissue via a specific interaction with the LDL receptor. Familial hypercholesterolemia (FH) is due to a defect in the LDL receptor that prevents the clearance of LDL particles from the circulation. An increased number of LDL particles is therefore a hallmark of FH. Familial defective apo B is a related disorder due to a mutation in apo B that prevents binding of apo B to the LDL receptor, resulting in a clinical phenotype similar to FH. Sporadic or polygenic hypercholesterolemia is likely due to overproduction of LDL particles. Hypertriglyceridemia (HTG) with increased LDL particle number (and therefore apo B) may be the most common dyslipidemia. HTG without increased LDL particle concentration is probably not atherogenic. Similarly, individuals with Lp(a) excess also appear to have an excess of small, dense LDL particles *(35)*.

The most common and perhaps underdiagnosed lipoprotein disorder, familial combined hyperlipidemia (FCHL), was originally defined as a total cholesterol and/or triglycerides concentration $\geq$95th percentile in probands with premature CHD and at least 1 affected first-degree relative. Subsequent research has identified an association of FCHL with an increase in small, dense LDL particles and determined that FCHL is most accurately diagnosed with a panel that includes measurement of apo B *(36)*. Because apo B is directly involved with defects of LDL synthesis or clearance, it is expected to play a central role in diagnosis and monitoring of these disorders.

PROSPECTIVE STUDIES OF apo B IN PRIMARY AND SECONDARY PREVENTION

Thompson and Danesh *(37)* performed a metaanalysis of prospective studies of apo B. It is clear from their analysis that apo B is a significant predictor of CHD, with an overall relative risk of about 2.0 for the upper vs the lower tertile (Fig. 1). Among the more compelling studies is AMORIS (Apolipoprotein-Related Mortality Risk Study) *(38)*. More than 175 000 men and women over the age of 60 were followed for about 5 years. Cases of fatal myocardial infraction (MI) included 864 men and 359 women. After adjusting for age and traditional lipid risk factors, including LDL-C, apo B remained a significant predictor of MI, with relative risks of 1.33 (CI 1.17–1.51) and 1.53 (1.25–1.88) for a 1SD increase in men and women, respectively. Importantly, LDL-C was an insignificant risk factor in women and only modestly associated with MI in men.

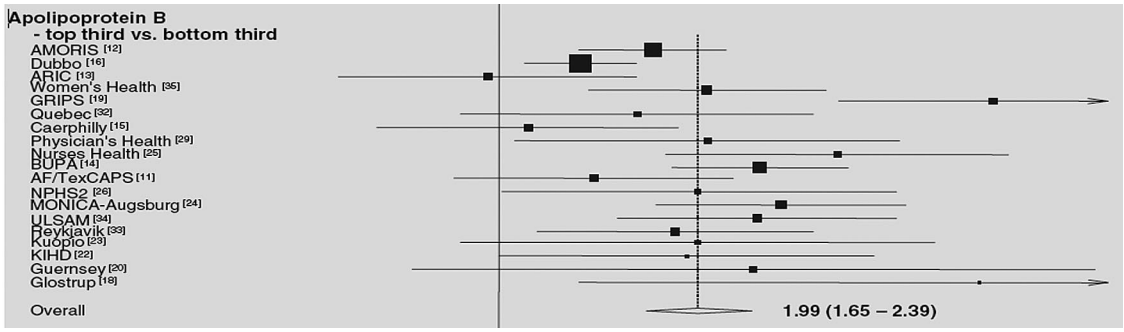

Fig. 1. Relative risk of CHD [adapted from Thompson and Danesh *(37)*].

The Quebec Cardiovascular Study followed 2039 men, ages 45–76, for 5 years *(39)*. apo B was a strong, independent predictor of future cardiac events even after adjustment for age, smoking, systolic blood pressure, diabetes, and medication use. Interestingly, the investigators found a synergistic relationship between apo B and the total/HDL cholesterol ratio (TC/HDL-C). When TC/HDL-C was low, increased apo B was associated with a 60% increased risk of CHD, but when TC/HDL-C was high, increased apo B was associated with a 2.6-fold increased risk. A 13-year follow-up of the Quebec Cardiovascular Study participants also suggested a similar synergy between LDL-C and apo B *(40)*. Among the men with increased LDL-C but a low concentration of apo B, <128 mg/dL (1.28 g/L), the relative risk for CHD was a modest 1.5, but when both LDL-C and apo B were increased, the relative risk was 2.2.

Among the many published prospective studies of apo B in primary prevention *(38–60)*, all but one found a statistically significant association with CHD, even after adjustment for nonlipid risk factors (Table 1). Of the 13 primary prevention studies that also provided data for LDL-C, only 9 reported a significant relationship between LDL-C and CHD in both men and women. Among the studies reporting both apo B and LDL-C, apo B was consistently the stronger risk factor (Table 1).

Secondary prevention studies reported similar results. Baseline value of apo B was a significant predictor of recurrent cardiovascular events in the Scandinavian Simvistatin Survival Study (4S), Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID), and other studies *(*61–65*)*. Neither apo B nor LDL-C was a significant predictor of recurrent events in the Veterans Affairs High-Density-Lipoprotein Cholesterol Intervention Trial (VAHIT); however, subjects were preselected to have relatively low LDL-C concentrations (≤140 mg/dL; 3.63 mmol/L) *(66)*.

There is a wide variation in the reported apo B relative risks for CHD in various epidemiologic studies, largely dependent on adjustment for other lipids and lipoproteins. Thus, the debate has focused on statistics rather than biological plausibility. As the Quebec Cardiovascular Study and AMORIS have shown, however, in large-scale studies with precise and standardized apo B measurement, apo B does retain statistical significance even when traditional lipids and lipoproteins are covariates in the regression models. This is also evident in the Health Professionals Follow-up Study *(58)*. When apo B and LDL-C were both simultaneously included in the model, relative risk was strongly associated with apo B, whereas LDL-C and non–HDL-C were no longer statistically significant.

LDL-P has also been measured in several primary and secondary prospective studies (online Supplemental Table 2) *(44, 59, 66–70)*, and the data are consistent with the apo B findings (Table 2). LDL-P is consistently more predictive of cardiovascular disease than is LDL-C, most noticeably in VAHIT *(66)*, the Women's Health Study *(47, 59)*, and the Framingham Heart Study *(70)*, where LDL-P was more strongly predictive of cardiovascular events than other lipid parameters. In the Multi-Ethnic Study of Atherosclerosis (MESA), LDL-P was associated with preclinical atherosclerosis (carotid intima-media thickness), even in subjects with LDL-C <100 mg/dL (2.59 mmol/L) *(71)*.

LDL-LOWERING TREATMENT AND RESIDUAL CVD RISK

The statin trials have consistently shown a remarkable lowering of LDL-C associated with a substantial lowering of relative CHD risk. In terms of absolute risk, however, the reduction is far less dramatic *(72)*. This has led many lipid experts to conclude that LDL-C targets need to be set much lower. However, it appears that a reduction in apo B or LDL particles, rather than LDL choles-

Table 1. Prospective studies of apo B and LDL-P: comparison with LDL-C.

Study	Comparison	apo B	LDL-C	Matching and/or adjustment variables
Salonen et al. *(48)*		NS[a]	No data	Sex, age, TC, smoking, CVD history, mean arterial BP
Stampfer et al. *(49)*	Quintile 5 vs 1	2.50 (1.31–4.75)	No LDL-C	Age, smoking
Sigurdsson et al. *(50)*	1 SD	1.32 ($P < 0.001$)	No LDL-C	
Coleman et al. *(51)*	Tertile 3 vs 1	2.4 (1.0–4.7)	No LDL-C	
Wald et al. *(52)*	Quintile 5 vs 1	7.02 (3.96–12.5)	No LDL-C	
Lamarche et al. *(39)*	1 SD	1.44 (1.22–1.67)	No LDL-C	Age, SBP, diabetes, smoking, medications
Cremer et al. *(53)*	Quintile 5 vs 1	8.7 (5.2–14.5)	13.2 (7.4–23.6)	Age, smoking, alcohol use, family history
Sweetnam et al. *(54)*	1 SD	1.20 (1.05–1.37)	No data	DBP, smoking, BMI, ischemia at baseline
Gotto et al. *(46)*	Baseline 1 year treatment	$P = 0.002$	NS	Treatment group, age, sex, marital status, hypertension, smoking, family history
		$P < 0.001$	NS	
Walldius et al. *(38)*	1 SD	M, 1.33 (1.17–1.51); W, 1.53 (1.25–1.88)	M, 1.14 (1.01–1.28); W, 0.85 (0.69–1.05)	Age, TC, TG, apo B, LDL-C
Simons et al. *(55)*	1 SD	1.28 (1.15–1.42)	1.28 (1.14–1.42)	Age, BMI, sex, family history, SBP, BP medications, smoking, diabetes, TC, HDL-C, TG
Sharrett et al. *(45)*	Quintile 5 vs 1	M, 2.4; W, 2.8	M, 2.5; W, 2.7	Smoking, BP, diabetes, medications
Talmud et al. *(41)*	1 SD	1.42 (1.19–1.70)	1.31 (1.12–1.52)	Age, clinic, HDL-C
Blake et al. *(44)*	Quartile 4 vs 1	2.43 (1.23–4.82)	2.06 (1.03–4.12)	Age, smoking, treatment group
Shai et al. *(42)*	1 SD, quintile 5 vs 1	1.8 (1.5–2.2); 4.7 (2.5–8.9)	1.4 (1.2–1.6); 2.7 (1.6–4.6)	Fasting status, age, smoking, month of blood draw
Jiang et al. *(43)*	Quartile 4 vs 1	2.31 (1.23–4.35)	1.74 (0.99–3.06)	Age, BMI, family history of MI, smoking, physical activity, alcohol intake, fasting status, hypertension, aspirin use, Hb A_{1c}
St-Pierre et al. *(40)*	Tertile 3 vs 1	7 years, 2.4 (1.5–3.8); 13 years, 1.6 (1.0–2.5)	No data	Age, BMI, SBP, diabetes, smoking, medications, TG, HDL-C
Ridker et al. *(47)*	Quintile 5 vs 1	2.50 (1.68–3.72)	1.62 (1.17–2.25)	Age, smoking, BP, diabetes, BMI
Meisinger et al. *(56)*	1 SD	M, 1.49 (1.25–1.78); W, 1.73 (1.32–2.27)	M, 1.49 (1.25–1.78); W, 1.79 (1.40–2.30)	Diabetes, smoking, BMI, hypertension, age, alcohol use
Pischon et al. *(58)*	Quintile 5 vs 1	2.98 (1.76–5.06)	2.07 (1.24–3.45)	Age, smoking, month of blood draw, BMI, family history of premature MI, diabetes, alcohol use, physical activity

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Table 1. Prospective studies of apo B and LDL-P: comparison with LDL-C. (*Continued from page 411*)

Study	Comparison	apo B	LDL-C	Matching and/or adjustment variables
Ingelsson et al. *(57)*	1 SD	M, 1.35 (1.18–1.55); W, 1.42 (1.18–1.73)	M, 1.10 (0.96–1.27); W, 1.19 (0.98–1.45)	Age, SBP, antihypertension medications, diabetes, smoking
Benn et al. *(60)*	Tertile 3 vs 1	M, 1.4 (1.1–1.8); W, 1.5 (1.1–2.1)	"less predictive"	Age, TC, LDL-C, HDL-C, TG, BMI, hypertension, diabetes, smoking
Mora et al. *(59)*	Quintile 5 vs 1	2.57 (1.98–3.33)	1.74 (1.40–2.16)	Age, treatment group, smoking, menopausal hormone use, BP, BMI, diabetes
Pedersen et al. *(61)*	–10 mg/dL	−5.3% risk (placebo)	−3.3% risk (placebo)	Sex, age, qualifying MI, smoking, hypertension
		−5.1% risk (baseline)	2.9% risk (baseline)	
		−8.8% risk (on-trial)	−7.2% risk (on-trial)	
Moss et al. *(62)*	Quartile 4 vs 1–3	1.82 (1.10–3.00)	No data	Diabetes, MI, electrocardiogram infarct type, pulmonary congestion, sex, ejection fraction
van Lennep et al. *(63)*	On-trial, 1 unit	3.21 (1.10–9.35)	1.16 (0.80–1.67)	Age [LDL-C, mmol/L; apo B, g/L]
Simes et al. *(64)*	Baseline, 1 unit	2.07 (1.32–3.22)	1.28 (1.10–1.46)	Age, sex, hypertension, diabetes, smoking, stroke or TIA, PVD, previous revascularization, stable angina, and qualifying event. [LDL-C, mmol/L; apo B, g/L]
	On-trial, 1 unit	2.10 (1.21–3.64)	1.20 (1.00–1.45)	
Corsetti et al. *(65)*	1 unit	2.02 (1.10–3.69)	No data	Calcium channel blockers
Otvos et al. *(66)*	Baseline, 1 SD	1.12 (0.99–1.27)	1.10 (0.97–1.25)	Treatment group, age, hypertension, smoking, BMI, diabetes
	On-trial, 1 SD	1.07 (0.94–1.23)	1.08 (0.95–1.23)	

[a] NS, not significant; TC, total cholesterol; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TG, triglycerides; Hb A_{1c}, hemoglobin A1c; TIA, transient ischemic attack; PVD, peripheral vascular disease.

terol, is a better target for monitoring therapeutic effectiveness and residual risk.

Statins are highly effective in reducing serum cholesterol through inhibition of HMG-CoA reductase, which upregulates LDL receptors and leads to increased clearance of LDL particles from the circulation. Statins also reduce the production of both VLDL-apo B and LDL-apo B. As indicated in Table 3, however, the reduction in serum apo B or LDL-P concentration is not as dramatic as the reduction in LDL-C or non–HDL-C *(17)*. As a result, patients treated to goal for LDL-C may not have achieved correspondingly low LDL particle concentrations, leaving them with potential residual risk *(17, 18)*.

In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPs), apo B at baseline and following 1 year on therapy was a strong predictor of future cardiovascular events, whereas LDL-C failed to reach significance ($P > 0.05$ at baseline and on therapy) *(46)*. The LIPID study provided similar results *(64)*. The reason is apparent: LDL-related risk is not captured by LDL-C measurement alone. Results from both primary and secondary statin trials suggest that on-therapy concentrations of apo B better predict future CHD events than does LDL-C.

Similarly, on-treatment concentrations of LDL-P reflect residual risk better than LDL-C, as indicated by VAHIT data, where on-trial LDL-P concentration was

Table 2. Prospective studies of LDL-P in comparison with LDL-C.

Study	Comparison	LDL-P	LDL-C	Matching and/or adjustment variables
Blake et al. *(44)*	Quartile 4 vs 1	4.17 (1.96–8.87)	2.06 (1.03–4.12)	Age, smoking, treatment group
Kuller et al. *(67)*	Quartile 4 vs 1	M, NS; W, 2.59	M, NS[a]; W, 3.34	Age, race
Rosenson et al. *(68)*	Above vs below median	2.1 (0.7–5.8)	1.4 (0.5–3.9)	Age, race, baseline lumen diameter
El Harchaoui et al. *(69)*	Quartile 4 vs 1	1.78 (1.34–2.37)	1.22 (0.92–1.61)	Smoking, SBP, LDL-C or LDL-P
Otvos et al. *(66)*	Baseline, 1 SD	1.20 (1.05–1.37)	1.10 (0.97–1.25)	Treatment group, age, hypertension, smoking, BMI, diabetes
	On-trial, 1 SD	1.28 (1.12–1.47)	1.08 (0.95–1.23)	
Cromwell et al. *(70)*	1 SD	M, 1.24 (1.10–1.39); W, 1.33 (1.17–1.50)	M, 1.06 (0.94–1.20); W, 1.18 (1.02–1.37)	Age, SBP, DBP, smoking, medications
Mora et al. *(59)*	Quintile 5 vs 1	2.51 (1.91–3.30)	1.74 (1.40–2.16)	

[a] NS, not significant; SBP, systolic blood pressure; BMI, body mass index; DBP, diastolic blood pressure.

a significant predictor of future CVD events, although subjects with increased LDL-C were excluded *(66)*. In the Framingham Offspring Study, cardiovascular disease event rates among subjects with low LDL-P or LDL-C (<25th percentile) were 59 vs 81 events per 1000 person-years, respectively, suggesting that residual risk is higher among individuals with low LDL-C concentrations compared with LDL-P *(70)*.

MANAGEMENT OF LDL-RELATED RISK

Although it is often considered to be a distinct risk factor, apo B is better considered an alternate measure of LDL-related risk because it largely reflects LDL particle concentration. LDL-C, non–HDL-C, LDL-P, and total apo B are all, to varying degrees, measures of LDL-related risk. These cholesterol and particle measures are highly intercorrelated, which explains why they have all been implicated as predictors of CVD risk in epidemiologic studies, but biologically they reflect different entities. Despite a high correlation, these markers are only modestly concordant, indicating that one cannot simply substitute a marker for another in classifying patients into risk categories.

National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines suggest an LDL-C goal <100 mg/dL (2.59 mmol/L) and a non–HDL-C goal of <130 mg/dL (3.37 mmol/L) in high-risk patients. An equivalent goal for apo B, <90 mg/dL (0.90 g/L), has been proposed *(73)*—a concentration that has been endorsed by the Canadian Cardiovascular Society as a primary target of therapy *(5)*. Stein et al. *(74)* have assessed the comparability of these goals using a database of more than 22 000 individuals from various clinical trials. In 14 425 subjects with "normal" triglycerides (<200 mg/dL, 2.26 mmol/L), 58% and 66% met the LDL-C and non–HDL-C goals, respec-

Table 3. Effectiveness of statin treatment at reducing LDL-C, non–HDL-C, apo B, and LDL-P.[a]

	Reduction on therapy, %	Mean on-treatment concentration	Mean on-treatment percentile
ApoB studies (n = 17 035)			
LDL-C	42.1	99.2 mg/dL	21
Non–HDL-C	39.6	127.0 mg/dL	29
apo B	33.1	101.6 mg/dL	55
LDL-P Studies (n = 889)			
LDL-C	35.9	105.2 mg/dL	27
LDL-P	30.6	1459 nmol/L	51

[a] From Sniderman *(17)*.

Table 4. Population distributions of LDL-C, non–HDL-C, apo B, and LDL-P in the Framingham Offspring Study.

Percentile	LDL-C, mg/dL	Non–HDL-C, mg/dL	LDL-P, nmol/L	apo B, mg/dL
2	70	83	720	54
5	78	94	850	62
10	88	104	940	69
20	100	119	1100	78
30	111	132	1220	85
40	120	143	1330	91
50	130	153	1440	97
60	139	163	1540	103
70	149	175	1670	110
80	160	187	1820	118
90	176	205	2020	130
95	191	224	2210	140

tively; however, only 30% of these same individuals met the apo B goal. In 7611 subjects with increased triglycerides, only 17% met the apo B goal, whereas 60% and 51% of subjects met the LDL-C and non–HDL-C goals, respectively. Interestingly, the subjects who met the apo B goal were virtually assured of meeting both the LDL and non–HDL-C goals.

We believe that the medical decision cutpoints should be set so that the apo B and LDL-P cutpoints are equivalent to those for LDL-C in terms of population percentiles. Table 4 presents population distribution data for LDL-C, non–HDL-C, LDL-P, and apo B from the Framingham Offspring Study *(75, 76)*. An LDL-C concentration of 100 mg/dL (2.59 mmol/L) falls at the 20th percentile. Corresponding values for non–HDL-C, apo B, and LDL-P are approximately 120 mg/dL (3.11 mmol/L), 80 mg/dL (0.80 g/L), and 1100 nmol/L, respectively. Therefore, the suggested apo B cutpoint of <90 mg/dL (0.90 g/L), as discussed above, is not equivalent to an LDL-C of 100 mg/dL (2.59 mmol/L) in terms of population distribution. We provide recommended cutpoints for non–HDL-C, LDL-P, and apo B in Table 5, equivalent to LDL cutpoints of 100 mg/dL (2.59 mmol/L) and 130 mg/dL (3.37 mmol/L). We do not believe that an apo B cutpoint equivalent to an LDL-C of <70 mg/dL (1.81 mmol/L) is necessary at this time. We believe that a focus on reduction of LDL particles in very-high-risk patients is appropriate, and data are needed to determine optimal apo B and LDL-P target concentrations. However, a goal that is less than the 5th percentile of the population, as is an LDL-C <70 mg/dL (1.81 mmol/L), may be unreasonable or unnecessary.

Table 5. Suggested treatment goal for apo B and non–HDL-P with "equivalent" cutoffs for LDL-C.

apo B, mg/dL	LDL-C, mg/dL	Non–HDL-C, mg/dL	LDL-P, nmol/L
	<70	<80	
<80	<100	<120	<1100
<100	<130	<150	<1400

There are certain flaws with using the cycle 4 dataset from the Framingham Offspring Study to determine population equivalent cutpoints. The specimens were collected between 1988 and 1991, the vast majority of Framingham subjects were white, and the dataset excludes subjects with triglycerides >400 mg/dL (4.52 mmol/L) in order to calculate LDL-C. There has likely been a shift in the distribution of lipids and lipoproteins over time so that what was once the 20th percentile is now the 30th percentile; however, the equivalence between a given percentile of apo B and LDL-C is unlikely to shift significantly. Also, although the relative risk associated with a given concentration of apo B or LDL-C may vary somewhat with race, the relationship between apo B and LDL-C with CVD risk is strong for all racial groups. Therefore, we believe that these recommended cutpoints remain valid.

NON-HDL CHOLESTEROL

NCEP ATPIII recommends non–HDL-C as a secondary target of therapy in patients with increased triglycerides *(73)*. After LDL-C concentrations have reached goal, intensification of therapy to reach non–HDL-C goals is recommended. The recent consensus conference report on Lipoprotein Management in Patients with Cardiometabolic Risk from the American Diabetes Association and the American College of Cardiology also recognized the importance of non–HDL-C and recommended that non–HDL-C be calculated on all lipid panel reports *(4)*. The National Lipid Association Taskforce on Non-HDL Cholesterol also came to the conclusion that non–HDL-C is an inclusive measure of atherogenic lipoproteins and predicts cardiovascular disease *(77)*. They further recommend that non–HDL-C should be reported on all lipid profiles, as it is robust from a laboratory standpoint, incurs no additional expense (since it can be calculated from the lipid panel), and is treatable with existing lipid-lowering agents *(77)*.

We agree that a greater emphasis on non–HDL-C rather than LDL-C will improve patient care. Data from several prospective studies show non–HDL-C to be a better predictor of cardiovascular events than LDL-C *(42–44, 47, 57–59, 69, 70)*. In terms of relative

risk, non–HDL-C is consistently stronger than LDL-C and, in many studies, equivalent to apo B or LDL-P *(44, 78, 79)*. However, apo B has been more extensively validated in epidemiological studies and clinical trials than non–HDL-C *(80)*, and non–HDL-C, like LDL-C, reflects the cholesterol content of atherogenic particles and not the number of atherogenic particles. Importantly, on-treatment non–HDL-C concentrations may not reflect residual risk associated with increased LDL particle number *(17, 18)*.

The NCEP-recommended cutpoints for non–HDL-C were arbitrarily set 30 mg/dL higher than LDL cutpoints because the VLDL cholesterol associated with a triglyceride concentration of 150 mg/dL is 30 mg/dL. In terms of population equivalence to LDL-C goals, however, lower cutpoints appear more appropriate (see Table 5).

MEASUREMENT ISSUES

Although LDL-C measurement remains the de facto standard for assessing LDL-related risk, calculations and assays are not without flaws. Even the definition of LDL is ambiguous. Traditionally, LDL was defined by sequential density ultracentrifugation as the lipoprotein fraction in the density range from 1.019 to 1.063 kg/L. Lp(a) particles, with a density range of 1.045–1.080 kg/L, overlap with LDL. Later, the β-quantification method developed at the NIH defined LDL as the cholesterol in the density fraction of >1.006 kg/L minus the cholesterol in the HDL fraction isolated by precipitation. Therefore, "β-quant" measures IDL and Lp(a) cholesterol along with LDL-C.

The Friedewald formula, which estimates LDL-C, also includes the IDL and Lp(a) cholesterol components and makes assumptions of a standard VLDL triglycerides/cholesterol ratio, a lack of chylomicrons, and a lack of excessive remnant lipoproteins. It should not be used if patients are nonfasting, if triglycerides are >400 mg/dL (4.52 mmol/L), or if the patient has type III hyperlipoproteinemia. The equation is increasingly inaccurate with triglycerides >200 mg/dL (2.26 mmol/L) *(81, 82)* and at relatively low LDL-C concentrations *(83)*. The Friedewald formula is based on the measurement of total cholesterol, triglycerides, and HDL-C. The equation is therefore affected by the lack of standardization of triglycerides and HDL-C measurements. Homogeneous methods measure LDL-C directly without the need for triglyceride and HDL-C measurement and offer the potential advantages of accurately measuring LDL-C when triglyceride concentrations are >400 mg/dL (4.52 mmol/L) and not requiring individuals to fast. Although initial evaluations of these methods demonstrated an ability to meet NCEP requirements for accuracy and precision and outperform calculated LDL-C in samples with triglycerides >400 mg/dL (4.52 mmol/L), questions were raised regarding their reliability in unusual specimens or in individuals with conditions that may alter lipoprotein characteristics, such as diabetes, liver disease, and kidney disease *(84)*. Subsequently, comparison of a number of homogeneous methods to the β-quantification reference method in 100 patient samples that covered a wide range of cholesterol and triglyceride concentrations demonstrated an inability of the methods to satisfy the NCEP goal for total error of <12%, and the homogeneous LDL-C results did not improve on the performance of calculated LDL-C using the Friedewald equation *(85)*. The principle limitation of the homogeneous methods was nonspecificity for the LDL fraction over the range of lipoprotein compositional differences encountered in clinical practice. The observed intermethod differences also highlight the need for standardization of homogeneous methods before consideration of implementation in clinical practice. Regardless, any measure of LDL-C, including the β-quantification reference method, suffers from the fact that measurement of the cholesterol component of LDL does not consistently reflect the concentration of LDL particles in serum/plasma. A summary of issues regarding LDL-C and apo B quantification is presented in Table 6.

Programs to standardize LDL-C, HDL-C, and triglycerides have met with only modest success, despite the widespread belief that these assays are accurate and reliable. apo B standardization has fared much better with the success of the IFCC standardization project to improve apo A-I and apo B measurements *(86–88)*. The standardization committee recognized that bias between manufacturers was due to a lack of common calibration, and they identified suitable reference materials to be used by manufacturers for calibrator value assignment. Subsequent studies reported a respectable between-laboratory CV of 3.1%–6.7% with a variety of assays using fresh-frozen patient sera and common calibrators *(89)*. LDL-C assays are not standardized by a common reference material, but by comparison to a reference method. The problems with the direct LDL-C assays appear to relate more to varying specificity due to inherent assay design rather than to differences in calibration.

Fasting per se is not required for apo B measurement, and despite the historic objection that apo B assays are not widely available, commercially available immunonephelometric and immunoturbidimetric assays are now available for use on a wide variety of automated platforms. LDL-P measurement by NMR also does not require fasting samples. LDL-P measurement using alternative modalities such as ion mobility analysis may be available in the future *(90)*. Nevertheless, because apo B and LDL-P measurements have been

Table 6. Comparison of LDL-C and apo B.

Parameter	LDL-C	apo B
Nature of target analyte	LDL is not a unique molecular species but a heterogeneous and polydisperse population of particles with varying chemical composition and physicochemical properties. Therefore, LDL is defined functionally in terms of the method used to separate it from other lipoproteins.	apo B is well defined as a molecular species (apo B-100 and apo B-48). Although methods for measuring apo B-48 are available, routine "apo B" methods measure either apo B-100 or total apo B.
Reference material	Standard Reference Material (SRM) 1951b (frozen human serum preparations) certified by the National Institute of Standards and Technology (NIST), Gaithersburg, MD. LDL-C determined by β-quantification (see below) at CDC, USA. Level I, 113.2 (3.1) mg/dL or 2.93 (0.08) mmol/L; level II, 152.6 (3.0) mg/dL. Note: Direct comparison with the "reference method" β-quantification (see below) is considered the only reliable accuracy test for an LDL-C method at present.[a]	International Reference Material SP3–07 (a human serum preparation in liquid-stabilized form) developed by IFCC Standardization Project and endorsed by WHO.[b] Accuracy-based mass value of 1.22 g/L [3.95 (0.08) mmol/L] assigned to apo B.[b]
Comparison methods	Various ultracentrifugation methods sometimes combined with chemical precipitation agents [e.g., dextran sulfate or phosphotungstate with $MgCl_2$, heparin with $MnCl_2$, and polyethylene glycol (PEG) 6000].[a,c]	Behring (now Siemens) Nephelometer at the Northwest Lipid Research Laboratories (NWLRL), University of Washington, Seattle, WA.[d]
Reference method	β-Quantification.[a,c] Widely accepted (including CDC in USA) but not formally credentialed.[a,c] Defines LDL as a population of particles with hydrated density $\geq$1.006 kg/L and precipitation by polyanion-metal ions.	Not defined.
Definitive method	Not defined.	Not defined.
Principle of analytical methods for quantitation	Different methodologies are based on different physicochemical properties of LDL particles	All methodologies are based on the antigenicity of apo B and involve the use of specific anti-apo B antibodies.

[a] Nauck et al. *(84)*.
[b] Marcovina et al. *(88)*.
[c] Rifai et al. *(91)*.
[d] Marcovina et al. *(86)*.

used primarily in a limited market supporting research studies rather than in clinical laboratories, the manufacturers may not have fully optimized the assays. With increasing recognition of the superiority of apo B and LDL-P over cholesterol as indicators of CVD risk, and more widespread application, manufacturers will likely make further improvements in the assay technologies. Measurement of apo B as a discrete molecular entity is inherently more amenable to standardization than approximation of a heterogeneous population of LDL particles in terms of their cholesterol content.

Several practical issues must be addressed when considering implementing apo B or LDL particle measurement into routine clinical practice. The use of LDL-C to assess cardiovascular risk and guide therapy is firmly entrenched in current guidelines and routine practice, and therefore, simply replacing LDL-C with apo B is not likely. Thus, measurement of both apo B and LDL-C will likely be necessary, at least for an interim period of time. This may result in increased cost for reagents and labor associated with apo B analysis, but considering the advantages of apo B, this should be worthwhile. Some have expressed concern that introducing apo B into clinical practice will result in confusion to both physicians and patients, and that the public may lose confidence in the healthcare system if cholesterol, which has been emphasized for decades, is challenged as the primary means of risk assessment.

However, the concern for patient confusion is likely unfounded as apo B is a single measurement that, with specific guidelines for measurement and follow-up, could easily be incorporated into patient care. This consideration, however, does stress the need for future versions of the NCEP guidelines to address apo B and LDL-P measurement, as we are recommending. Deferring action, in spite of the accumulating evidence that apo B is the superior measure of LDL-related risk, does increase risk of eventually losing public trust.

An equally important concern is reimbursement. Whereas LDL-C is generally accepted among government and private payers, reimbursement policies for apo B are inconsistent.

A wealth of evidence has now accumulated demonstrating the superiority of apo B measurement over that of LDL cholesterol for assessment of CVD risk. Accordingly, addition of apo B to the routine lipid panel for assessing and monitoring patients at risk for adverse outcomes should enhance patient management. The next logical step is the addition of apo B to NCEP and other guidelines in the US. Changing perceptions and practice will not be easy, considering that physicians and patients are accustomed to LDL-C. Significant education efforts will be required, and it appears prudent at this point to consider using both apo B (or LDL-P) and LDL-C to assess LDL-related risk for an interim period until the superiority of apo B is generally recognized.

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