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Special Report

Association of Apolipoprotein B and Nuclear Magnetic Resonance Spectroscopy–Derived LDL Particle Number with Outcomes in 25 Clinical Studies: Assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices

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BACKGROUND: The number of circulating LDL particles is a strong indicator of future cardiovascular disease (CVD) events, even superior to the concentration of LDL cholesterol. Atherogenic (primarily LDL) particle number is typically determined either directly by the serum concentration of apolipoprotein B (apo B) or indirectly by nuclear magnetic resonance (NMR) spectroscopy of serum LDL particle number (LDL-P).

CONTENT: To assess the comparability of apo B and LDL-P, we reviewed 25 clinical studies containing 85 outcomes for which both biomarkers were determined. In 21 of 25 (84.0%) studies, both apo B and LDL-P were significant for at least 1 outcome. Neither was significant for any outcome in only 1 study (4.0%). In 50 of 85 comparisons (58.8%), both apo B and LDL-P had statistically significant associations with the clinical outcome, whereas in 17 comparisons (20.0%) neither was significantly associated with the outcome. In 18 comparisons (21.1%) there was discordance between apo B and LDL-P.

CONCLUSIONS: In most studies, both apo B and LDL-P were comparable in association with clinical outcomes. The biomarkers were nearly equivalent in their ability to assess risk for CVD and both have consistently been shown to be stronger risk factors than LDL-C. We support the adoption of apo B and/or LDL-P as indicators of atherogenic particle numbers into CVD risk screening and treatment guidelines. Currently, in the opinion of this Working Group on Best Practices, apo B appears to be the preferable biomarker for guideline adoption because of its availability, scalability, standardization, and relatively low cost.

LDL cholesterol (LDL-C),[7] a key cardiovascular biomarker, is recommended by National Cholesterol Education Program Adult Treatment Panel-III (NCEP-ATP III) guidelines *(1)* for assessing cardiovascular disease (CVD) risk and for monitoring lipid-lowering therapy. These guidelines are over 10 years old, and there are now numerous studies demonstrating that alternative measures of LDL are superior to LDL-C for CVD risk assessment *(2)*. In addition, the heterogeneity of LDL particles and the difficulty in standardizing assays for LDL-C, an analyte that cannot be chemically well defined, has also made it difficult to develop accurate and robust assays. For example, direct assays for LDL-C from dyslipidemic samples have been shown to differ considerably from values obtained by reference method procedures *(3, 4)*, thus potentially limiting the effectiveness of the assay in the very patients requiring accurate values. Recent recommendations from the National Lipid Association described several alternative biomarkers that may be useful for managing CVD patients, including LDL particle number (LDL-P) *(5)*.

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[7] Nonstandard abbreviations: LDL-C, LDL cholesterol; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel-III; CVD, cardiovascular disease; LDL-P, LDL particle number; apo B, apolipoprotein B; NMR, nuclear magnetic resonance; IDL, intermediate density lipoprotein; Lp(a), lipoprotein(a); ITA, immunoturbidimetric assay; INA, immunonephelometric assay; LSP, Lipid Standardization Program; DCM, designated comparison method; CIMT, carotid intima media thickening; MetSyn, metabolic syndrome; VA-HIT, Veterans Affairs High-Density Lipoprotein Intervention Trial; OR, odds ratio; HR, hazards ratios; RR, relative risk; WHS, Women's Health Study; FOS, Framingham Offspring Study; HPS, Heart Protection Study; MOCE, major occlusive coronary event; VTE, venous thromboembolic disease; VTES, VTE Study; HTN, hypertension; NWLMDRL, Northwest Lipid Metabolism and Diabetes Research Laboratories.

LDL-P has been found to be a stronger predictor of CVD than LDL-C concentration *(2)*.

The focus of this special report is the comparison between the 2 main diagnostic tests for LDL-P, namely concentration of apolipoprotein B (apo B) and nuclear magnetic resonance (NMR)-derived LDL-P, in their association with CVD and related conditions as outcome measures.

apo B is the main protein component of LDL and, because each LDL particle has 1 molecule of apo B, it is considered to be a direct measure of LDL-P *(2)*. The full-length form of apo B, called apo B-100, is found on VLDL, intermediate density lipoprotein (IDL), LDL, and lipoprotein(a) [Lp(a)], whereas a truncated form, called apo B-48, is found on chylomicrons and chylomicron remnants. Because of its relatively high abundance, apo B is readily measured in the clinical laboratory by several methods. The most common commercial assays are the immunoturbidimetric assay (ITA) and the immunonephelometric assay (INA). Typically, these methods measure both apo B-100 and apo B-48, but in fasting samples greater than 95% of apo B in plasma is apo B-100 and almost all of it is associated with LDL *(6)*. Therefore, the measurement of plasma apo B concentration is essentially an estimate of LDL-apo B concentration or LDL-P *(7)*. Calibration of these assays is attained through the use of calibrators provided by the assay kit manufacturer, which may now have set points established by the CDC Lipid Standardization Program (LSP) using the WHO-IFCC reference material SP3–08 as secondary reference material and the designated comparison method (DCM). In many studies, apo B has been shown to be superior or at least equivalent to LDL-C for assessing patients for CVD risk *(2)*.

LDL-P can also be estimated by NMR of plasma *(8)*, a proprietary method that has been available since 1997 as part of the NMR LipoProfile® from LipoScience, a specialty lipid reference laboratory. The terminal methyl groups on lipoprotein-associated lipids emit a characteristic NMR spectrum, which varies depending on the size of the lipoprotein particle, allowing NMR to estimate the different size LDL and other lipoprotein subfractions *(9)*. A specific spectral region captures the NMR signal emitted by the total number of terminal methyl group protons of phospholipids, cholesterol, cholesteryl esters, and triglycerides of the different lipoprotein particle subclasses *(10)*. A least squares deconvolution process is used to calculate the individual subclass signal amplitudes. The deconvolution algorithm was developed using a library of different lipoprotein size subclasses, obtained from a diverse donor population, containing isolated VLDL, LDL, and HDL. Total LDL-P is determined by summing the concentrations of the individual LDL subfractions, including IDL and Lp(a). Numerous studies have shown the superiority of LDL-P over LDL-C for both identifying patients at CVD risk and in monitoring patients on lipid-lowering therapy *(2)*.

Ion mobility analysis has been proposed as another method to measure LDL-P directly *(11)*, although the accuracy of the method has been disputed *(12)*. This assay currently is available only at Quest Diagnostics Nichols Institute. Ion mobility analysis has been reported for only one clinical study *(13)* and has not been compared to other LDL-P assays.

Non-HDL-C has also been shown in several studies to be a predictor of CVD events *(5, 14)* and serves as a secondary treatment goal by the NCEP for those patients with hypertriglyceridemia who have met LDL-C treatment goals. Non-HDL-C is readily calculated from standard lipid panels as the difference between total cholesterol and HDL-C and, similar to apo B, encompasses all putative atherogenic lipoproteins. Although non-HDL-C reflects total atherogenic particle number better than LDL-C, unlike apo B, non-HDL-C does not accurately reflect atherogenic particle number and may leave residual CVD risk after treatment goals are met, due to the variability of the composition of the constituent lipoproteins.

In this study, we reviewed the literature on apo B and LDL-P and examined 25 clinical studies that included both parameters to compare their utility as biomarkers for CVD risk, as well as other related conditions and parameters, such as carotid intima media thickening (CIMT), diabetes and metabolic syndrome (MetSyn).

Methods

A PubMed survey of the medical literature, using the key terms apo B, LDL-P, and the name Otvos, was performed to find clinical trials of the association of both apo B and LDL-P with specific outcomes, such as CVD, the presence of carotid atherosclerosis, or metabolic diseases or conditions, including diabetes mellitus and MetSyn. A total of 26 publications was found through June 2012. One publication with an outcome of newborn body weight *(15)* was excluded from our analysis. The relevant data for each clinical study is summarized in Table 1. Each study was identified with an order number, study acronym, and year of publication, e.g., 1 Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) 2006. An assessment was made for each clinical study or relevant portion thereof whether apo B or LDL-P was statistically related to the outcome (Table 2). The study or subpart was then assigned to 1 of 4 outcome categories: 1. apo B statistically significant, LDL-P not significant; 2. LDL-P statistically significant, apo B not significant; 3. both statistically significant; or 4. neither statistically significant.

Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a]

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB	LDL-P	Matching and/or adjustment variables
Prediction of CVD or events				
1 VA-HIT 2006; Otvos et al. *(16)* (Intervention with gemfibrozil; 364 cases, 697 controls; 7 or 12 months)	On-trial values, gemfibrozil vs placebo groups, *P*	$P = 0.0002$	$P < 0.0001$	Age
	OR (95% CI) 1 SD at Baseline, *P*	1.12 (0.99–1.27), NS ($P = 0.08$)	1.20 (1.05–1.37), $P = 0.006$	Treatment group, age, HTN, smoking, BMI,[b] diabetes
	OR (95% CI) 1 SD at on-trial, *P*	1.07 (0.94–1.23), NS ($P = 0.31$)	1.28 (1.12–1.47), $P = 0.0003$	
	($r = 0.56$)	(On-trial data at 12-month visit)	(On-trial data at 7-month visit)	
2 WHS 2002; Blake et al. *(17)* (intervention with aspirin and vitamin E; 130 cases, 130 controls; 3 years follow-up)	Baseline concentrations, cases vs controls, *P*	$P = 0.002$	$P < 0.001$	
	Quartile 4 vs 1, RR (95% CI), *P* trend	2.43 (1.23–4.82), $P = 0.005$	4.17 (1.96–8.87), $P < 0.001$	Age, smoking, treatment group
	($r = 0.70$, $P < 0.001$)			
3 WHS 2009; Mora et al. *(18)* (intervention with aspirin and vitamin E; 1,015 CVD, 26,658 non-CVD; 11 years follow-up)	Baseline concentrations, CVD vs non-CVD, *P*	$P < 0.001$	$P < 0.001$	Age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, BP, diabetes mellitus, BMI
	Quintile 5 vs 1, HR (95% CI), *P* linear trend	2.57 (1.98–3.33), $P < 0.001$	2.51 (1.91–3.30), $P < 0.001$	
	($r = 0.84$)			
4 HPS 2012; Parish et al. *(19)* (intervention with simvastatin and antioxidant vitamins; 20 021 men and women; 5.3 years follow-up)	HR (95% CI) 1SD at baseline, *P*			Multiple baseline covariates
	MOCE			
	Statin arm	1.23 (1.15–1.33), $P < 0.001$	1.25 (1.16–1.35), $P < 0.001$	
	Placebo arm	1.11 (1.05–1.17), $P < 0.001$	1.11 (1.05–1.17), $P < 0.001$	
	Revascularization			
	Statin arm	1.16 (1.08–1.23), $P < 0.001$	1.15 (1.07–1.23), $P < 0.001$	
	Placebo arm	1.16 (1.10–1.23), $P < 0.001$	1.19 (1.13–1.26), $P < 0.001$	
	Other cardiac event			
	Statin arm	1.06 (0.96–1.16), NS ($P < 0.9$)	1.08 (0.98–1.19), NS ($P < 0.9$)	
	Placebo arm	1.08 (0.99–1.17), NS ($P < 0.1$)	1.10 (1.02–1.18), $P < 0.05$	
	Ischemic stroke			
	Statin arm	1.11 (1.01–1.23), $P < 0.05$	1.06 (0.96–1.18), NS ($P < 0.9$)	
	Placebo arm	1.01 (0.94–1.10), NS ($P < 0.95$)	1.01 (0.93–1.08), NS ($P < 0.95$)	
	($r = 0.84$)			

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Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a] *(Continued from page XX)*

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB	LDL-P	Matching and/or adjustment variables
5 FOS 2007; Cromwell et al. *(20)* (longitudinal population study; 1140 men, 1626 women; median 14.8 years follow-up)	Baseline sex-specific difference between study participants with or without CVD, *P*	No apo B data	Men: $P < 0.0001$ Women: $P < 0.0001$	
	HR (95% CI) 1 SD, *P*		Men: 1.24 (1.10–1.39), $P = 0.0005$ Women: 1.33 (1.17–1.50), $P < 0.0001$ Both: 1.28 (1.17–1.39), $P < 0.0001$	Age, SBP, DBP, smoking, lipid medication use
6 FOS 2007a; Ingelsson et al. *(21)* (longitudinal population study; 1562 men, 1760 women; about 15 years follow-up)	HR (95% CI) 1 SD, *P*	Men: 1.37 (1.20–1.57), $P < 0.001$ Women: 1.38 (1.15–1.67), $P < 0.001$	No LDL-P data	Age, SBP, antihypertensive treatment, diabetes, smoking
7 PEDCS 2003; Soedamah-Mutha et al. *(22)* (longitudinal study of 59 type 1 diabetic patients with CAD and 59 controls; 10 years follow-up)	Baseline concentrations, cases vs controls, univariate, *P*	$P < 0.001$	$P < 0.01$	
	Multivariate, *P*	NS	NS	6 Models
Presence of carotid atherosclerosis				
8 JHSS 2002; Post et al. *(23)* (cross-sectional study of carotid IMT in 216 African-American siblings of patients with premature CAD)	Association with increased carotid IMT on univariate analysis, *P*	$P < 0.05$	$P < 0.005$	Age, sex, duration of diabetes
	Stepwise multivariate logistic regression to predict increased carotid intima media thickening, *P*	NS ($P = 0.08$)	$P = 0.008$	Age, male sex, SBP
9 GOCADAN 2009; Masulli et al. *(24)* (cross-sectional study of CIMT in 656 Alaska Eskimos)	Association with increased carotid IMT, *P*, or	NS ($P = 0.07$)	$P = 0.037$	
	plaque score by tertile, *P*	NS ($P = 0.09$)	NS ($P = 0.432$)	Age, sex, BMI, SBP, current smoking
10 SANDS 2009; Howard et al. *(25)* (intervention trial of CIMT regression using aggressive vs standard lipid lowering in 418 type 2 diabetics; 3 years follow-up)	Change in aggressive group vs standard group at 36 months, *P*	$P < 0.0001$	$P = < 0.0001$	CIMT, LDL-C, SBP values
	Correlation to CIMT regression by quartiles, *P*	NS ($P = 0.07$)	NS ($P = 0.09$)	

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Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a] *(Continued from page XX)*

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB		LDL-P		Matching and/or adjustment variables
		Internal	Common	Internal	Common	
11 DCCT/EDIC 2006; Lyons et al. *(26)* (cross-sectional study of CIMT in 540 men and 428 women with type 1 diabetes)	Univariate multiple regression analysis of CIMT vs parameter, *P*	Men: $P < 0.0005$ Women: $P < 0.01$	$P < 0.0001$ $P < 0.05$	Men: $P < 0.001$ Women: $P < 0.0001$	$P < 0.0005$ NS ($P = 0.08$)	Raw data
	Multivariate multiple regression analysis of CIMT vs parameter, *P*	Men: $P < 0.01$ Women: $P = 0.02$	$P = 0.05$ NS	Men: $P < 0.01$ Women: $P < 0.001$	NS NS	Age, duration of diabetes, Hb A_{1c}, BMI, AER, HTN, smoking, DCCT randomization group.
	Multivariate multiple regression analysis by set of CIMT vs parameter, *P*	Men: NS ($P = 0.07$) Women: NS	NS ($P = 0.06$) NS	Men: $P = 0.01$ Women: $P < 0.0001$	NS NS ($P = 0.08$)	
12 ARIC 2011; Virani et al. *(27)* [Cross-sectional study of atherosclerosis by MRI in carotid arteries of 1,670 participants)	Association of parameters with:					Fully adjusted, see Table 2, footnote a
	Total wall volume, *P*	$P = 0.04$		$P = 0.006$		
	Maximum wall thickness, *P*	NS ($P = 0.12$)		$P = 0.008$		
	Normalized wall index, *P*	$P = 0.02$		$P = 0.002$		
	SD of wall thickness, *P*	NS ($P = 0.04$)		NS ($P = 0.32$)		
	Presence of lipid rich core:					
	OR at 1 SD, *P*	1.08, NS ($P = 0.34$)		1.15, NS ($P = 0.08$)		Fully adjusted
	OR at 1 SD, *P*	1.04, NS ($P = 0.68$)		1.07, NS ($P = 0.44$)		Fully adjusted + wall thickness
Prediction of MetSyn						
13 FOS 2006; Kathiresan et al. *(28)* (cross-sectional population study of MetSyn in 2293 study participants)	Sex-specific difference between study participants with or without MetSyn, *P*	Men: $P < 0.0001$ Women: $P < 0.0001$		Men: $P < 0.0001$ Women: $P < 0.0001$		Age
	Sex-specific trend analysis of association with number of MetSyn RF, *P*	Men: $P < 0.0001$ Women: $P < 0.0001$		Men: $P < 0.0001$ Women: $P < 0.0001$		Age
14 COMETS 2009; Rosenson et al. *(29)* [Statin treatment in 257 MetSyn patients; 12 weeks)	Relation to statin, change from placebo baseline value at 6 weeks, *P*	Rosuvatatin, $P < 0.001$ Atorvastatin, $P < 0.001$		Rosuvatatin, $P < 0.001$ Atorvastatin, $P < 0.001$		Treatment, study center, baseline value.

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Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a] *(Continued from page XX)*

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB	LDL-P	Matching and/or adjustment variables
15 METSYN 2007; Clarenbach et al. *(30)* [Cross-sectional study of 274 adults with MetSyn.)	Less than 3 MetSyn RF vs 3 or more RF, *P* Men, *P* Women	Men: $P < 0.05$ Women: NS	Men: $P < 0.05$ Women: NS	
	Trend analysis of association with number of MetSyn RF, *P*	$P = 0.039$	$P = 0.003$	
	($r = 0.73$, $P < 0.0001$)		(Conversion of LDL-P to apo B equivalents, then compared)	
Association with diabetes mellitus or diabetic complications				
16 DCCT/EDIC 2003; Jenkins et al. *(31)* (cross-sectional study of diabetic nephrophy in 540 men and 428 women with type 1 diabetes)	Univariate multiple regression analysis of log AER vs parameter by categories of normo-, micro- and albuminuria, *P* trend	Men: $P < 0.0001$ Women: $P < 0.005$ Both: $P < 0.0001$	Men: $P < 0.005$ Women: $P < 0.05$ Both: $P < 0.0001$	Raw data
	Multivariate multiple regression analysis as above, *P* trend	Men: $P < 0.01$ Women: NS ($P = 0.07$) Both: $P < 0.002$	Men: $P < 0.05$ Women: NS Both: $P < 0.05$	Age, (sex for total cohort), diabetes duration, HTN, Hb A_{1c}, BMI, WHR, DCCT randomization group
	Univariate multiple regression analysis of creatinine clearance vs parameter by categories of normal, elevated or low, *P*	Both: $P < 0.0005$	Both: $P < 0.0002$	Raw data
	Multivariate multiple regression analysis as above, *P*	Both: $P < 0.0005$	Both: $P < 0.0005$	Age, sex, diabetes duration, HTN, Hb A_{1c}, BMI, WHR, DCCT randomization group

		Intensive	Conventional	Intensive	Conventional	Raw data
17 DCCT/EDIC 2003a; Jenkins et al. *(32)* (cross-sectional study of sex and glycemia in 540 men and 428 women with type 1 diabetes)	Univariate regression analysis of sex by DCCT treatment group vs parameter, *P*	$P < 0.001$	$P < 0.001$	$P < 0.05$	NS	Raw data
	Multivariate regression analysis of sex by DCCT treatment group vs parameter, *P*	NS	NS	NS	$P < 0.05$	Age, duration of diabetes, Hb A_{1c}, BMI, AER, WHR, HTN, smoking, DCCT randomization group.

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Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a] *(Continued from page XX)*

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB		LDL-P		Matching and/or adjustment variables
		Univariate	Multivariate	Univariate	Multivariate	
	Correlation between concurrent hemoglobin A_{1c} and parameter by sex, *P*	Men: $P < 0.0001$ Women: $P < 0.0001$	$P < 0.0001$ $P < 0.001$	Men: $P < 0.0001$ Women: $P < 0.0001$	$P < 0.0001$ $P < 0.01$	Age, duration of diabetes, BMI, AER, WHR, HTN, smoking, DCCT randomization group.
18 GLOWS 2009; Rosenson et al. *(33)* and Zieve et al. *(34)* (intervention with colesevelam or placebo in 56 type 2 diabetics over 12 weeks) Association with plasma lipids and lipoproteins	Reduction in biomarker in treated group vs placebo group at 12 weeks, *P*	$P = 0.003$		$P = 0.006$		Raw data
19 SMART 2011; Baker et al. *(35)* (intervention with antiretroviral therapy in 254 study participants with HIV; 6 months)	ART Rx vs no ART Rx At 2 months At 6 months ($r = 0.75$, $P < 0.001$)	 $P = 0.03$ NS ($P = 0.60$)		 NS ($P = 0.096$) NS ($P = 0.79$)		
20 ESTROGEN 1998; Giri et al. *(36)* (intervention with estrogen in 22 elderly men over 9 weeks)	Reduction in biomarker vs baseline at 9 weeks, *P*	$P < 0.001$		$P < 0.001$		Raw data
21 HRT 1998; Vadlamudi et al. *(37)* (cross-sectional study of parameters in 8 women on and 10 not on HRT)	Comparison of plasma parameters between women on or off HRT, *P*	NS		NS		HRT use
22 FOS 2004; Freedman et al. *(38)* (cross-sectional study of sex and age effects on lipoproteins in 3266 study participants)	Male/female differences in apo B concentration and LDL particle number, *P* (r Men = 0.86; r Women = 0.88)	$P = 0.001$ NS		$P = 0.001$ NS		Age adjusted Age and lipid adjusted
23 SIMVA 2001; Miller et al. *(39)* (intervention with simvastatin for 18 weeks; 20 men and women)	Reduction from baseline vs placebo: 40 mg simvastatin, *P* 80 mg simvastatin, *P*	 $P = 0.0003$ $P < 0.0001$		 $P \leq 0.001$ $P \leq 0.001$		

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Table 1. Summary of publications comparing apo B and LDL-P for various outcomes in 25 clinical studies.[a] *(Continued from page XX)*

Study/authors/type/duration	Parameter association (correlation of apo B to LDL-P, *r*, *P*)	apoB	LDL-P	Matching and/or adjustment variables
Prediction of miscellaneous events				
24 VTES 2005; Deguchi et al. *(40)* (cross-sectional study of 49 cases with VTE from the Scripps Venous Thrombosis Registry age-matched with 49 controls)	Baseline concentrations, cases vs controls, *P*	NS ($P = 0.07$)	$P = 0.02$	
	OR (95% CI) for quartile 4 vs 1, *P*	2.1 (0.91–4.9), NS ($P = 0.08$)	2.2 (1.0–4.9), $P = 0.047$	Age, sex
	OR (95% CI) for quartile 4 vs 1, *P*	1.9 (0.63–5.8), NS ($P = 0.25$)	3.6 (1.0–16.0), $P = 0.04$	BMI, factor vs Leiden, prothrombin 20210A
	Trend across quartiles, *P* trend	NS ($P = 0.18$)	$P = 0.03$	
25 WHS 2011; Paynter et al. *(41)* (intervention with aspirin and vitamin E; 4714 incident HTN, 12 858 non-HTN)	Baseline concentrations, HTN vs non-HTN, *P*	$P < 0.001$	$P < 0.001$	Model 1: age, smoking, fasting status, use of cholesterol-lowering medication, trial treatment assignment, hormone use, race, exercise, alcohol use, BMI, diabetes, education, vegetable, fruit, sodium and grain intake.
	Association of parameter with incident HTN, quintile 5 vs quintile 1 OR (95% CI) 1 SD, *P* for trend			
	Unadjusted	2.23 (2.00–2.48) $P < 0.001$	2.82 (2.53–3.15) $P < 0.001$	Model 2: adds C-reactive protein, homocysteine, fibrinogen, soluble intercellular adhesion molecule, Hb A_{1c}
	Model 1	1.44 (1.28–1.61) $P < 0.001$	1.73 (1.54–1.95) $P < 0.001$	
	Model 2	1.39 (1.24–1.56) $P < 0.001$	1.63 (1.45–1.84) $P < 0.001$	

[a] For study 4 HPS 2012, *P* values were derived by the authors from χ^2_1 values given in the publication.

[b] BMI, body mass index; NS, not significant; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; PEDCS, Pittsburgh Epidemiology of Diabetes Complications Study; JHSS, Johns Hopkins Siblings Study; GOCADAN, Genetics of Coronary Artery Disease in Alaska Natives; SANDS, Stop Atherosclerosis in Native Diabetics Study; DCCT/EDIC, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study; ARIC, Atherosclerosis Risk in Communities; COMETS, Comparative Study with Rosuvastatin in Subjects with Metabolic Syndrome; METSYN, Study of Metabolic Syndrome; RF, risk factors; AER, albumin excretion rate; hemoglobin A_{1c}; Hb A_{1c} WHR, waist-hip ratio; GLOWS, Glucose-Lowering Effect of WelChol Study; SMART, Strategies for Management of Antiretroviral Therapy; Rx, pharmacological treatment; ESTROGEN, Study of estrogen in elderly men; HRT, hormone replacement therapy; 21 HRT 1998, Study of hormone replacement therapy on lipoproteins; SIMVA, Study of Simvastatin in Mixed Hyperlipidemia.

Table 2. Categorizations of comparisons of apo B and LDL-P in reviewed publications.

Study/authors	Only apo B statistically significant, LDL-P not significant	Only LDL-P statistically significant, apo B not significant	Both statistically significant	Neither statistically significant
Clinical parameter or outcome				
Prediction of CVD or events				
1 VA-HIT 2006 Otvos et al. *(16)*		X	X	
Gemfibrozil vs placebo		X		
OR baseline				
OR on-trial				
2 WHS 2002 Blake et al. *(17)*			X	
Baseline concentrations			X	
Quartile 4 vs 1, RR				
3 WHS 2009 Mora et al. *(18)*			X	
Baseline concentrations			X	
Quintile 5 vs 1, HR				
4 HPS 2012 Parish et al. *(19)*	X	X	X	X
MOCE			X	X
Statin arm			X	
Placebo arm			X	
Revascularization				
Statin arm				
Placebo arm				
Other cardiac event				
Statin arm				
Placebo arm				
Ischemic stroke				
Statin arm				
Placebo arm				
5 FOS 2007 Cromwell et al. *(20)*/6 FOS 2007a Ingelsson et al. *(21)*			X	
Relation to future CVD, HR, men			X	
Relation to future CVD, HR, women				
7 PEDCS 2003 Soedamah-Mutha et al. *(22)*			X	X
Baseline concentrations, UV[a]				
Baseline concentrations, MV				
Presence of carotid atherosclerosis				
8 JHSS 2002 Post et al. *(23)*		X	X	
Association with carotid IMT, UV				
Association with carotid IMT, MV				
9 GOCADAN 2009 Masulli et al. *(24)*		X		X
Association with carotid IMT				
Association with carotid plaque score				
10 SANDS 2009 Howard et al. *(25)*			X	X
Response to therapy				
Correlation to CIMT regression by quartiles				
11 DCCT/EDIC 2006 Lyons et al. *(26)*	X	X	X	X

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Table 2. Categorizations of comparisons of apo B and LDL-P in reviewed publications. *(Continued from page XX)*

Study/authors	Only apo B statistically significant, LDL-P not significant	Only LDL-P statistically significant, apo B not significant	Both statistically significant	Neither statistically significant
ICA, CIMT vs parameter, UV, men	X	X	X	X
ICA, CIMT vs parameter, UV, women			X	X
CCA, CIMT vs parameter, UV, men			X	
CCA, CIMT vs parameter, UV, women			X	
ICA, CIMT vs parameter, MV, men				
ICA, CIMT vs parameter, MV, women				
CCA, CIMT vs parameter, MV, men				
CCA, CIMT vs parameter, MV, women				
ICA, CIMT vs parameter, MV set, men				
ICA, CIMT vs parameter, MV set, women				
CCA, CIMT vs parameter, MV set, men				
CCA, CIMT vs parameter, MV set, women				
12 ARIC 2011 Virani et al. *(27)*		X	X	X
Total wall volume			X	X
Maximum wall thickness				X
Normalized wall index				
SD of wall thickness				
OR, lipid-rich core, fully adjusted				
OR, lipid-rich core, fully adjusted + wall thickness				
Prediction of MetSyn				
13 FOS 2006 Kathiresan et al. *(28)*			X	
Sex-specific difference, men			X	
Sex-specific difference, women			X	
Trend with number of risk factors, men			X	
Trend with number of risk factors, women				
14 COMETS 2009 Rosenson et al. *(29)*			X	
Change from baseline, rosuvastatin			X	
Change from baseline, atorvastatin				
15 METSYN 2007 Clarenbach et al. *(30)*			X	X
Three or more risk factors, men			X	
Three or more risk factors, women				
Trend with number of risk factors				
Association with diabetes mellitus or diabetic complications				
16 DCCT/EDIC 2003 Jenkins et al. *(31)*			X	X
AER vs parameter by category, UV, men			X	
AER vs parameter by category, UV, women			X	
AER vs parameter by category, UV, both			X	
AER vs parameter by category, MV, men			X	
AER vs parameter by category, MV, women			X	
AER vs parameter by category, MV, both			X	
Creatinine clearance vs parameter, UV, both				
Creatinine clearance vs parameter, MV, both				

Continued on page XX

Table 2. Categorizations of comparisons of apo B and LDL-P in reviewed publications. *(Continued from page XX)*

Study/authors	Only apo B statistically significant, LDL-P not significant	Only LDL-P statistically significant, apo B not significant	Both statistically significant	Neither statistically significant
17 DCCT/EDIC 2003a Jenkins et al. *(32)*	X	X	X	X
Treatment group vs parameter, UV, intensive			X	
Treatment group vs parameter, UV, conventional			X	
Treatment group vs parameter, MV, intensive			X	
Treatment group vs parameter, MV, conventional			X	
Hb A_{1c} vs parameter by sex, UV, men				
Hb A_{1c} vs parameter by sex, UV, women				
Hb A_{1c} vs parameter by sex, MV, men				
Hb A_{1c} vs parameter by sex, MV, women				
18 GLOWS 2009 Rosenson et al. and Zieve et al. *(33,34)*			X	
Colesevelam Rx				
Association with plasma lipids and lipoproteins				
19 SMART 2011 Baker et al. *(35)*	X			X
ART Rx at 2 months				
ART Rx at 6 months				
20 ESTROGEN 1998 Giri et al. *(36)*			X	
Estrogen Rx				
21 HRT 1998 Vadlamudi et al. *(37)*				X
HRT Rx				
22 FOS 2004 Freedman et al. *(38)*			X	X
Age adjusted				
Age and lipid adjusted				
23 SIMVA 2001 Miller et al. *(39)*			X	
40 mg Rx			X	
80 mg Rx				
Prediction of miscellaneous events				
24 VTES 2005 Deguchi et al. *(40)*		X		
Baseline concentrations		X		
OR, quartile 4 vs 1, UV		X		
OR, quartile 4 vs 1, MV		X		
Trend across quartiles				
25 WHS 2011 Paynter et al. *(41)*			X	
Baseline concentrations			X	
OR, quintile 5 vs 1, Unadjusted			X	
OR, quintile 5 vs 1, Model 1			X	
OR, quintile 5 vs 1, Model 2				

[a] UV, univariate; MV, multivariate; ICA, internal carotid artery, CCA, common carotid artery; other nonstandard abbreviations not defined in the text are defined in the Table 1 footnote.

Results

REVIEW OF SURVEYED PUBLICATIONS

Comprehensive summaries of the 25 surveyed clinical studies are presented in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol59/issue5. The associations of apo B and LDL-P to clinical outcomes are shown in Table 1. Depending on the study design and statistical analyses, associations are indicated by odds

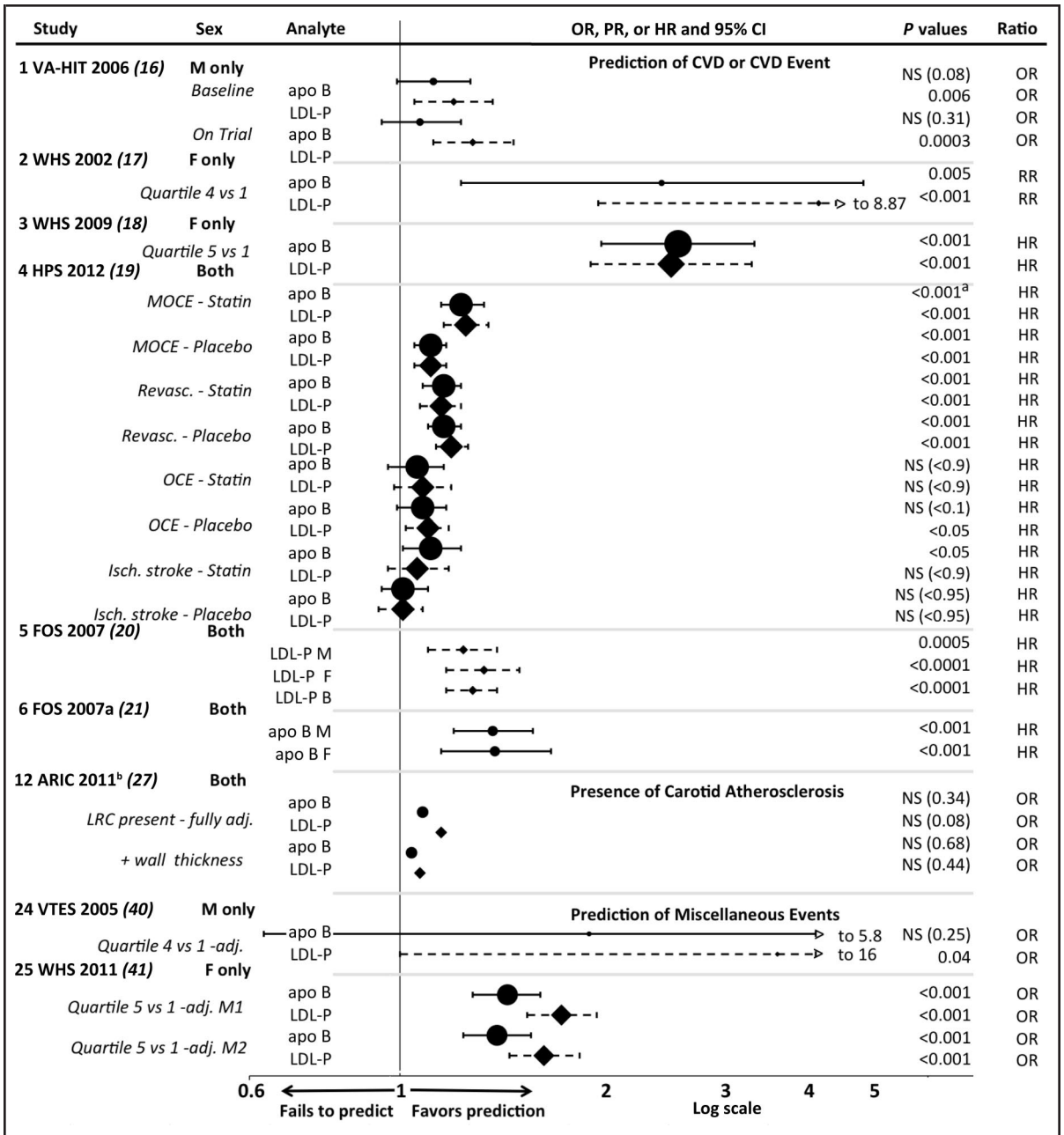

Fig. 1. Comparison of association of apo B and LDL-P to various clinical outcomes based on OR, RR or HR.

[a] For study 4 HPS 2012, *P* values were derived by the authors from X_1^2 values given in the publication. [b] Study 12 ARIC 2011 did not provide 95% CIs around the OR. Revasc., revascularization; OCE, other cardiac event; Isch., ischemic; LRC, lipid rich core; M1, model 1; M2, model 2; adj, adjusted; M, male; F, female; B, both. Other nonstandard abbreviations not defined in the text and information about study reference citations are provided in Table 1. References shown in figure: Otvos et al. *(16)*, Blake et al. *(17)*, Mora et al. *(18)*, Parish et al. *(19)*, Cromwell et al. *(20)*, Ingelsson et al. *(21)*, Virani et al. *(27)*, Deguchi et al. *(40)*, Paynter et al. *(41)*.

ratio (OR), hazard ratio (HR), relative risk (RR), regression analysis, or comparison of means. ORs, HRs, and RRs are also illustrated as a forest plot in Figure 1, providing the risk estimate ±95% CI for a 1 mean (SD) increment in the biomarker concentration. Intervals which fall below 1.0 are considered statistically not significant. The comparisons of apo B and LDL-P are shown in Table 2, based on the 4 outcome categories

described in Methods, using derived data shown in Table 1 and Figure 1. A brief summary of findings for various outcomes follows.

Prediction of CVD or events. In 15 of 19 comparisons, apo B and LDL-P either were both were associated (n = 12) or neither was associated (n = 3) with study outcomes, giving a high concordance between the 2 biomarkers (78.9%), particularly with regard to prediction of future CVD events *(16–22)*. On the basis of risk ratios, LDL-P was more strongly associated with events in the 1 VA-HIT 2006 and 2 Women's Health Study (WHS) 2002 studies, whereas apo B was a stronger predictor in the 3 WHS 2009, 4 Framingham Offspring Study (FOS) 2007, and 5 FOS 2007a studies. In 4 Heart Protection Study (HPS) 2012, both biomarkers were essentially identically associated with the occurrence of major occlusive coronary events (MOCEs) and the need for revascularization.

Because of the lack of data for non-HDL-C, a meaningful 4-way comparison with apo B and LDL-P was possible for only the 6 studies in this prediction category (see online Supplemental Table 1). In this limited data set, apo B, LDL-P, and non-HDL-C were comparable in the number of comparisons which were significant (13 to 15 of 19 total comparisons), whereas LDL-C was significant in only 9 comparisons.

Presence of carotid atherosclerosis. Both apo B and LDL-P were strongly predictive of carotid atherosclerosis *(23–27)*. For most outcomes LDL-P was a stronger predictor than apo B. Both apo B and LDL-P agreed in 17 of 24 outcomes, for a concurrence rate of 70.8%.

Prediction of MetSyn. In 9 of 9 clinical outcomes (100%), apo B and LDL-C were equivalently associated with the presence of MetSyn or with the effect of statin therapy on patients with MetSyn *(28–30)*.

Association with diabetes mellitus or diabetic complications. In general, both apo B and LDL-P were equally associated with diabetic nephropathy and distribution by sex and glycemic control in individuals with type 1 diabetes *(31–34)*. In addition, the strengths of the associations were similar. Similar reductions of both apo B and LDL-P were observed as a result of treatment of individuals with type 2 diabetes with colesevelam. Overall, 14 of 16 outcomes were in agreement between apo B and LDL-P (87.5%).

Association with plasma lipids and lipoproteins. A consistent role of apo B or LDL-P in this grouping is not evident because of the number of outcomes in which both parameters were not statistically significant; however, in instances in which both were significant, both were generally affected to the same extent *(35–39)*.

Prediction of miscellaneous associations. In the 1 small study reported for venous thromboembolic disease (VTE) [24 Venous Thromboembolic Disease Study (VTES) 2005], LDL-P was superior to apo B in predicting VTE in men; LDL-P alone was statistically significant for all outcomes (n = 4) *(40–41)*. Both apo B and LDL-P predicted incident hypertension (HTN) in initially healthy women, but the ORs were larger for LDL-P than for apo B.

ASSESSMENT OF OVERALL CONCORDANCE BETWEEN apo B AND LDL-P ACROSS STUDIES

The comparisons were grouped into 1 of 4 distinct outcome categories as described in Methods. For the 25 studies reported here, both apo B and LDL-P were significant for at least 1 outcome in 21 (84.0%) of the 25 studies. Neither was significant for any outcomes in only 1study. In 2 studies, LDL-P was significant for at least 1 outcome whereas apo B was not, and in 1 study apo B was significant for at least 1 outcome whereas LDL-P was not. In the 25 studies, there were a total of 85 outcome measures compared. Table 2 shows that, of a total of 85 observed comparisons of various types, 50 fell into the "both statistically significant" category (58.8%), 13 fell into the "LDL-P statistically significant, apo B not significant" category (15.3%), and 5 fell into the "apo B statistically significant, LDL-P not significant" category (5.9%). These data indicate that both apo B and LDL-P were generally in agreement in their association with diverse clinical outcomes (58.8%), but with a substantial amount of discordance (21.2%) in which one biomarker was statistically significant whereas the other was not. The "neither statistically significant" category (20.0%) is considered noninformative because it does not allow a comparison of one biomarker to the other. Although several comparisons showed equivalent strength of association with the outcome measures for apo B and LDL-P, in some cases one or the other biomarker appeared to be more strongly associated with the outcome variable. In these cases, LDL-P showed a significant association with a clinical outcome more often than apo B alone, and the level of statistical significance, as indicated by the *P* value, and the strength of association, as indicated by the OR, RR, and HR, was more often higher for LDL-P than it was for apo B (Table 1, Figure 1).

Discussion

At present, LDL-C is used as the primary lipoprotein indicator of cardiovascular risk in the US and serves as the primary treatment goal in the NCEP-ATP III guidelines. As new clinical studies emerge, the relative utility of biomarkers for assessment of CVD should be reevaluated and treatment guidelines revised accord-

ingly. This occurred in Canada, where apo B measurements were incorporated into the clinical guidelines for the assessment of CVD in 2003 *(42)*. Similar recommendations have recently been made in the US *(5)*.

As reviewed recently, LDL-P, as either apo B or LDL-P, has been shown to be a better predictor of future CVD events than LDL-C and a better monitor of therapy in many *(2)*, but not all, studies *(18, 21, 43)*. Although the clinical utility of the measurement of LDL-P is currently debated, the recent report from an expert panel of lipid specialists presents a reasonable use of these biomarkers for diagnostic and monitoring purposes in selected groups of patients *(5)*. Given that both apo B and LDL-P measure atherogenic indicators, primarily LDL-P, their association with various disease states or conditions would be expected to be strongly related. To determine their relative utility, we chose to review all publications that allow direct comparison of the association of apo B and LDL-P to clinical outcomes. Our review of 25 relevant publications shows that neither was consistently superior to the other (Table 2). Less clear is the lack of equivalency that is observed in some studies, which, as discussed below, may be due to differences in the analytical methods used to measure these biomarkers.

As observed in our limited comparison of 4 biomarkers (see online Supplemental Table 1), both non-HDL-C and apo B are superior to LDL-C in predicting CVD risk. Conventional wisdom is that non-HDL-C is better than LDL-C because it includes the cholesterol in VLDL. However, it has been clearly shown that the amount of cholesterol in VLDL particles cannot explain the increased RR seen with non-HDL-C versus LDL-C *(44)*. The better explanation is that non-HDL-C more accurately reflects the number of LDL particles *(44)*. In a metaanalysis of LDL-C, non-HDL-C, and apo B as risk factors for CVD, Sniderman et al. demonstrated the superiority of apo B over non-HDL-C and LDL-C, with RRs in the order apo B $>$ non-HDL-C $>$ LDL-C *(45)*. These data suggest that treating to goal with non-HDL-C leaves residual risk.

COMPARISON OF apo B ITA/INA AND LDL-P METHODS

The reliable application of risk cutpoints and treatment goals, which are based on data derived from epidemiologic trials, requires precise and accurate analytical measurements to be medically useful (Table 3). Relatively small errors in measurement can lead to the misclassification of individuals with regard to risk, with potentially serious consequences *(4)*. The accuracy of a method is established by standardization, a process involving a reference system of primary and secondary reference measurement procedures and reference materials, which assures that reported results for an analyte will agree with other laboratories over time, independent of methodology *(46)*. Bias, the measure of inaccuracy, is the difference of a measured value from the true value of an analyte. Bias among laboratories in the measurement of apo B would be minimized if they all used standardized methods, but for most of the studies reported here, it is not possible to determine whether standardized assays were used or used in the format under which they were standardized (see online Supplemental Table 2). None of the 25 publications reviewed provided a measure of bias to a reference method. Precision is a measure of the reproducibility of measurement and is expressed as % CV. An imprecise method, even though on average free of bias, will still result in excessive error on individual measurements. Together, accuracy and precision define the total error associated with a measurement process. Performance goals for accuracy and precision for an analyte are set so that the requisite medical usefulness of a biomarker is attained. At this time, no performance goals have been established by the NCEP or any other expert panel for either apo B or LDL-P *(46, 47)*. However, a 6.0% maximum bias, based on biological variation and analytical imprecision, has been suggested for apo B, which is similar to the one for LDL-C (6.8%) *(48)*. No such information is available for LDL-P. A standardization program for apo B is provided by the Northwest Lipid Metabolism and Diabetes Research Laboratories (NWLMDRL) in Seattle, WA, which determines precision and bias relative to an in-house method, accepted as a DCM for apo B by the CDC LSP *(47)*. Thus, apo B is standardized using the same CDC LSP which is used for the standardization of cholesterol, LDL-C, and HDL-C. The strategy of the LSP is to work directly with manufacturers to standardize their commercial assays, which are then used by clinical laboratories. Although there is no formal standardization program for LDL-P, it could conceivably conform to the apo B LSP, by adding the VLDL particle number to the LDL-P and converting nanomoles per liter (nmol/L) to mass units (mg/dL) based on the molecular weight of apo B (550 000 Da) *(49)*. This conversion recognizes that both total plasma apo B and LDL-P measurements presumably include IDL and Lp(a), in addition to LDL.

The methods for only 4 studies listed in online Supplemental Table 2 were stated to be standardized for apo B, and that was by verification of calibration to the apo B assay kit manufacturer, Incstar, which had taken part in the WHO-IFCC standardization program using SP3–07 as reference material *(50, 51)*. Presumably, a single laboratory performed the apo B measurements for these studies. Three other laboratories were described as standardized through the LSP for lipids, but it is not clear that they were also standardized for apo B (see online Supplemental Table 2). Standardiza-

Table 3. Comparison of apo B immunoturbidometric assay/immunonephelometric assay and NMR–LDL-P methodologies.

	apo B	NMR–LDL-P
Assay methods	Multiple vendors, not all standardized	Proprietary in-house, not standardized
Calibration	Reference material (SP3–07, SP3–08), performed by end user using kit calibrators	Not calibrated; uses proprietary library of isolated lipoprotein fractions to set concentrations
Entity measured	Antigen–antibody complex	Terminal methyl groups on lipoprotein-associated lipids
Precision, within assay (%CV)	Lab dependent	2%–4%
Precision, between assay (%CV)	Lab dependent, CV 4.1%–11.6%	2%–4%
Precision, mean within method, between laboratory (%CV)	Lab dependent, 7.2% (CAP PT[a] data)	NA
Samples	Destructive	Destructive
Fed or fasted	Either	Either
Samples frozen at −70	Stable for years	Stable for years
Stability in ongoing clinical studies	Can change over time, depends on manufacturer diligence	Probably stable over time, requires internal surveillance
Information reported	apo B concentration	Particle number and size, lipid concentrations
Standardization program	Yes, performed by manufacturer	No
Performance criteria	None	None
Potential problems	Antibody recognition of epitopes on variant LDLs, e.g. small, complex formation rate, turbidity vs detergent effect, may include VLDL, IDL, and Lp(a)	Identifies particle only by size, i.e., includes IDL and Lp(a) in LDL-P
Availability of test	Runs in typical clinical lab on common instrumentation	Run only at LipoScience on specialized instrumentation
Growth	Readily scalable; numerous vendors and applications for most analyzers	Limited by capacity of central lab
Cost	Modest (≅$17)	Modest to high (≅$30–$40)

[a] CAP PT, College of American Pathologists Proficiency Testing Program; NA, not applicable or available.

tion was not claimed for any of the remaining 17 methods. As noted by several authors, a major concern about the use of apo B for assessment and treatment of CVD is the lack of rigorous standardization among methods and laboratories *(49, 52)*. Interestingly, this concern has not been shared for LDL-P, which is not standardized to any reference system, because it is measured in only one laboratory, thereby eliminating the problems of among-method and among-laboratory variability. In fact, standardization is not a prerequisite for the establishment of an association of a biomarker with a clinical outcome within any single laboratory and study, but it is critical for comparison between laboratories and studies or if data from several laboratories will be pooled for the establishment of disease risk assessments or treatment cutpoints.

A major advantage of the NMR method vs apo B is the determination not only of LDL-P but also LDL subclasses and other major lipoprotein classes; thus NMR provides considerably more information with the analysis than does the measurement of apo B. Although additional information may contribute to the value of an assay, only that information which has been shown to provide proven clinical significance should be reported to the patient. Also, the NMR measurement of LDL-P appears to be generally more precise than that of apo B. For the few laboratories that reported % CV for the measurement of apo B, it is assumed to be a measure of between-run precision, with CV's ranging from 5% to11%, whereas the same measure for LDL-P was from 2% to 4% *(8)*. This disparity in precision may give LDL-P an advantage over apo B in discerning small differences between groups in a clinical study. According to the College of American Pathologist's proficiency testing reports (2011 Accuracy-Based Lipid Surveys, SBL-A and ABL-B) for apo B, the within-method, between-laboratory precision averaged about 5.96% for all the methods tested and the overall preci-

sion (between method/between laboratory) precision averaged about 6.75% for all methods in their program. In contrast, LDL-P has no between-laboratory precision value because it is measured in only one laboratory. In addition, the measurement of LDL-P in a single laboratory would also be expected to benefit from the long-term stability of measurement, although the deconvolution algorithm used by LipoScience has evolved over time *(53)*. In contrast, the stability of apo B measurements requires the assay kit providers to reassess the calibrator accuracy with each change in the lots of antisera and calibrators. The lack of between-laboratory variability and extended long-term stability would allow pooling of LDL-P data collected in different clinical studies over long periods of time with less variability than for apo B data, which may be impacted by the assay antibody and method as well as laboratory and assay bias issues.

Several other factors may also impact the use of apo B and LDL-P measurements (Table 3).

Other factors that may have an impact on the use of apo B and LDL-P measurements are shown in Table 3.

Samples. For both methods, serum or plasma from either fed or fasted individuals may be used. Samples have been reported to be stable for at least 10 years for NMR analysis when frozen at −70°C, allowing the analysis of reserved study samples *(8)*, whereas apo B changes that occur upon freezing and storage appear to be method dependent *(54)*. The NMR-based LDL-P assay uses 200 μL of sample and the immunoassay of apo B generally requires only about 2 to15 μL of sample (depending on the vendor).

Test availability. Increasing recognition of the superiority of measures of particle number over LDL-C will likely drive increased demand for apo B and LDL-P measurements. The number of lipid panels performed annually in the US to obtain LDL-C is estimated to be 216 000 000 *(55)*. Considering the implications of a shift to particle measurement, it must be recognized that the apo B measurement is inherently scalable, with many vendors providing assays that can be performed on almost every chemistry analyzer in any clinical laboratory and physician office (Table 3). Although the LDL-P test is performed only by the LipoScience reference laboratory, LipoScience has recently received US Food and Drug Administration clearance for distribution of its NMR analyzers for lipoprotein testing to other clinical laboratories. Nevertheless, the capital expense and logistical complications initially may still limit availability of the LDL-P assay.

Expense. Based on the Medicare fee schedule, the incremental cost of adding an apo B or LDL-P test to a lipid panel is about $17 or $30–$40, respectively, depending on the payer. There is some concern as to the cost benefit of the added testing given the additional expense *(52)*. However, according to at least one US Expert Panel the incremental expense is warranted in certain patients *(5)*. The therapeutic value relative to the incremental cost of each of the measurements is currently under aggressive debate *(5, 56)*.

SUGGESTED EXPERIMENT TO FURTHER COMPARE apo B TO LDL-P

A limitation of our study is that none of the studies reviewed were specifically designed to compare results and clinical utility of apo B vs LDL-P. Further work is recommended to compare these methods on a variety of sample types and determine their utility as predictive biomarkers in various diseases and conditions. An example of an appropriate study was recently described by Miller, et al. *(3)*. Briefly, samples were collected from healthy individuals and from individuals with a variety of dyslipidemic and cardiovascular diseases. The samples were analyzed with a panel of HDL-C and LDL-C clinical assays, as well as by the appropriate reference measurement procedures. Trueness and total error relative to the RMP were determined. A similar experiment should be conducted using a variety of standardized apo B assays and the LipoScience LDL-P assay and comparing with the CDC LSP DCM for apo B. To make a suitable comparison to plasma apo B, NMR VLDL-P values would need to be added to the LDL-P (nmol/L), which would then be converted to apo B equivalents (mg/dL) by multiplication by the factor 0.055 *(49)*. As has been previously described when comparing the clinical utility of LDL-P vs LDL-C as a biomarker *(57)*, the discordant cases between apo B vs LDL-P will likely be most revealing in terms of the relative merits of the 2 tests.

When compared for all the clinical outcomes in the 25 publications reviewed here, associations of apo B and LDL-P were generally equivalent, although when discordance between associations with outcome measures occurred, LDL-P appeared more often to be superior to apo B, based on statistical strength. The finding that the 2 biomarkers show similar CVD risk prediction is expected given that both methods measure, in addition to LDL-P, the particle numbers of IDL and Lp(a). Total plasma apo B may additionally include VLDL and chylomicron remnants, which are typically present in small quantities in fasting samples. Although a standardization program for apo B exists, it appears from an inspection of the published studies to be underutilized, particularly for measurements made in older clinical studies, thereby confounding the use of pooled data from multiple trials to set disease assessment and treatment cut-points.

RECOMMENDATIONS

Based on the preceding observations, we make the following recommendations:

1. The measurement of particle number, either as concentration of apo B or LDL-P should be incorporated into the guidelines for the assessment of CVD risk.
2. Manufacturers of analytical systems for measurement of apo B concentration or particle number should produce well-characterized, robust assays with disclosure of analytical properties, such as antibody specificity, and information regarding standardization.
3. All manufacturers should standardize their assays according to WHO-IFCC reference materials by the currently available standardization program at the NWLMDRL using the apo B DCM.
4. Researchers and laboratories using these assays in clinical studies should calibrate or verify the accuracy through the use of frozen serum samples from NWLMDRL.
5. Performance goals (precision, bias, total error) for LDL-P assays should be determined by expert consensus, as was done for other lipid/lipoprotein biomarkers.
6. Additional studies should be performed to determine the optimum specificities for apo B antibodies (e.g. apo B-100, apo B-48, apo [a]), and to the various apo B–carrying particles, to best characterize CVD risk and monitor therapy.
7. Further studies should be performed to compare apo B to LDL-P using a variety of representative specimens to better understand the inherent differences and contributors to discordance, as well as relative advantages and disadvantages of the 2 assays.

Conclusions

In the majority of studies, both apo B and LDL-P were comparable in association with clinical outcomes. Where discordant, the differences may be due to inherent methodological differences between the 2 technologies as well as the large number of methodologies employed to measure apo B and the consistency of the NMR method, which was performed using a single method in a single laboratory for all the studies. The 2 markers appear to be nearly equivalent in their ability to assess risk for CVD, and as previously published by the Lipoprotein and Vascular Diseases Division Working Group on Best Practices, both measures have consistently been shown to be stronger risk factors than LDL-C *(2)*. We continue to support the adoption of apo B and/or LDL-P as indicators of atherogenic particle numbers into cardiovascular risk screening and treatment guidelines. Currently, in the opinion of this Working Group on Best Practices, apo B appears to be the preferred biomarker for guideline adoption because of its widespread availability, scalability, standardization, and relatively low cost. As LDL-P is expected to become more readily available in the near future, with a possible lowering of cost and potentially superior performance in direct comparison with apo B, it may become the preferred test as a cardiovascular biomarker. Although this study primarily was aimed at comparing apo B and LDL-P, results of our limited evaluation of non–HDL-C and current literature *(44, 45)* suggest that the high performance of non-HDL-C likely is related to being an indirect way of estimating apo B.

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