

Relationship between plasma apolipoprotein B concentrations and LDL particle number

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Abstract: Many subjects with relatively normal, or even optimal, levels of low-density lipoprotein cholesterol (LDL-C) have increased atherogenic lipoprotein particle concentrations (apolipoprotein B [apoB] and LDL particle number [LDL-P] determined by nuclear magnetic resonance spectroscopy [NMR]). Numerous analyses have demonstrated that apoB and LDL-P predict the risk of future cardiovascular events more robustly than LDL-C, as estimated using the Friedewald equation. Little is known about the relationship between an individual's apoB and LDL-P level, and whether the relationship is comparable at different levels of LDL-C. The aim of this study was to examine the correlation between plasma apoB and LDL-P levels and specifically to evaluate the heterogeneity of LDL-P at low levels of apoB (< the 20th population percentile [78 mg/dL]). Data were derived from a group of consecutive patients added to a large, single laboratory database (LipoScience, Inc.) during a 1-week period in which a standard lipid profile, apoB, and LDL-P levels were available. When risk categories were assigned to the subjects using the Framingham Offspring Study's population percentiles for apoB and LDL-P, there was good agreement between the two measures when LDL-C levels were high (≥ 160 mg/dL). However, among individuals with low LDL-C and apoB, NMR analysis could identify a subgroup of individuals with potentially greater cardiovascular risk, as suggested by unexpected elevations in LDL-P.

Keywords: apolipoprotein B, LDL particle number, nuclear magnetic resonance spectroscopy, lipoproteins

Introduction

Low-density lipoprotein cholesterol (LDL-C) has been used as a predictor of cardiovascular risk and a marker of the efficacy of therapy for several decades. Recent evidence has demonstrated that LDL-C frequently underestimates risk among patients with metabolic disorders.¹ Alternate measures of atherogenic apoB-containing lipoprotein particles, including LDL particle number (LDL-P) determined by nuclear magnetic resonance (NMR) spectroscopy or apolipoprotein B (apoB) levels, have been shown to provide a more reliable assessment of cardiovascular disease (CVD) risk, particularly in patients with metabolic dyslipidemic disorders who have achieved optimal LDL-C levels.²⁻⁴ Based on these findings, numerous expert consensus documents and international guidelines have endorsed the use of apoB and LDL-P in select populations.^{3,4} While both apoB and LDL-P are highly correlated with each other, these two markers may be affected differently by various confounding factors that influence their ability to predict CVD.

In this report, we examined the relationship between plasma apoB and LDL-P levels in a cross-sectional study of 1,027 men and women; evaluating the heterogeneity of

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LDL-P at low and high levels of apoB (< the 20th percentile [78 mg/dL] and > the 80th percentile [118 mg/dL] in the Framingham Offspring Study population).⁵

Methods

Plasma samples were collected from fasting patients seen at physicians' offices during a 1-week period (December 29, 2010–January 5, 2011), according to sample preparation specifications required for *NMR LipoProfile*[®] (LipoScience Inc., Raleigh, NC, USA) analysis. Samples (one per patient for a total of 1,027) were shipped to LipoScience, Inc., and analyzed within 48 hours of collection. The information available for each patient was limited to sex and age; the patient's medical history and/or medication use was not available.

Plasma lipids (total cholesterol, triglycerides, and high-density lipoprotein cholesterol [HDL-C]) were determined by enzymatic methods, using an Olympus AU680[®] autoanalyzer, with reagent and calibrator kits supplied by Beckman Coulter Inc. (Brea, CA, USA). The LDL-C was calculated from total cholesterol and HDL-C using the Friedewald formula.⁶ The apoB concentrations were determined by an immunoturbidimetric method using reagents and standards from Beckman Coulter Inc. The lipoprotein particle concentrations were analyzed by NMR spectroscopy using a 400-MHz proton NMR analyzer, as previously described.⁷

To allow comparisons between apoB and LDL-P, levels of each were expressed as population percentiles based on data from the Framingham Offspring Study.^{2,5} The Framingham Offspring Study was chosen because it is the only large observational study in which both apoB and LDL-P population percentiles were reported. Discordance was defined as a mismatch between apoB and LDL-P percentiles. For example, an individual whose apoB level is in the <20th percentile and LDL-P level is in the 50th percentile would be considered discordant.

Results

Table 1 presents the clinical characteristics of the 533 men and 494 women included in this cross-sectional observational study. The mean LDL-C across the entire population was 112.7±39.4 mg/dL, mean LDL-P was 1,590±554 nmol/L, and mean apoB was 97.7±25.1 mg/dL. Figure 1 illustrates the linear relationship between the concentration of apoB determined by immunoassay and LDL-P assessed by NMR spectroscopy for men (Figure 1A) and women (Figure 1B). As shown, the relationship between these two measurements was comparable, independent of sex.

Table 1 Subject characteristics, mean (standard deviation)

Parameters	Women	Men	All
Sample size	494	533	1,027
Age (years)	57.2 (14.2)	60.0 (14.6)	58.5 (14.5)
Total chol (mg/dL)	203.0 (47.0)	185.2 (43.4)	193.7 (46.0)
TG (mg/dL)	158.8 (87.4)	175.6 (154.0)	167.5 (126.7)
HDL-C (mg/dL)	53.5 (12.6)	45.3 (10.3)	49.3 (12.4)
Non-HDL-C (mg/dL)	149.4 (44.8)	139.7 (42.5)	144.4 (43.9)
Calc LDL-C (mg/dL)	117.7 (39.9)	107.8 (38.4)	112.7 (39.4)
ApoB (mg/dL)	100.5 (25.8)	95.1 (24.2)	97.7 (25.1)
LDL-P (nmol/L)	1,630 (563)	1,554 (544)	1,590 (554)

Abbreviations: total chol, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, nonhigh-density lipoprotein cholesterol; calc LDL-C, calculated low-density lipoprotein cholesterol; apoB, apolipoprotein B; LDL-P, low-density lipoprotein particle number.

Figure 2 shows the distribution of LDL-P values in subjects with an apoB concentration <78 mg/dL which corresponds to the 20th Framingham Offspring Study population percentile (n=228). At these low apoB concentrations, only 64% had comparably low LDL-P concentrations of <1,100 nmol/L;

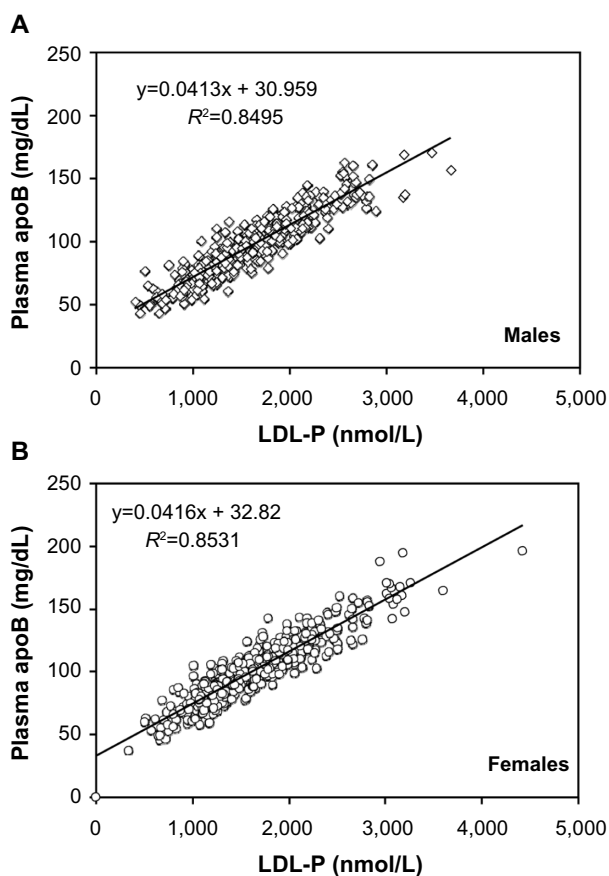


Figure 1 Relationship between apoB concentrations and LDL-P is independent of sex.

Notes: Relationship between plasma apoB determined by immunoassay and LDL-P determined by NMR spectroscopy. Linear regression between apoB and LDL-P for men (A) and women (B).

Abbreviations: apoB, apolipoprotein B; LDL, low-density lipoprotein; LDL-P, low-density lipoprotein particle number; NMR, nuclear magnetic resonance.

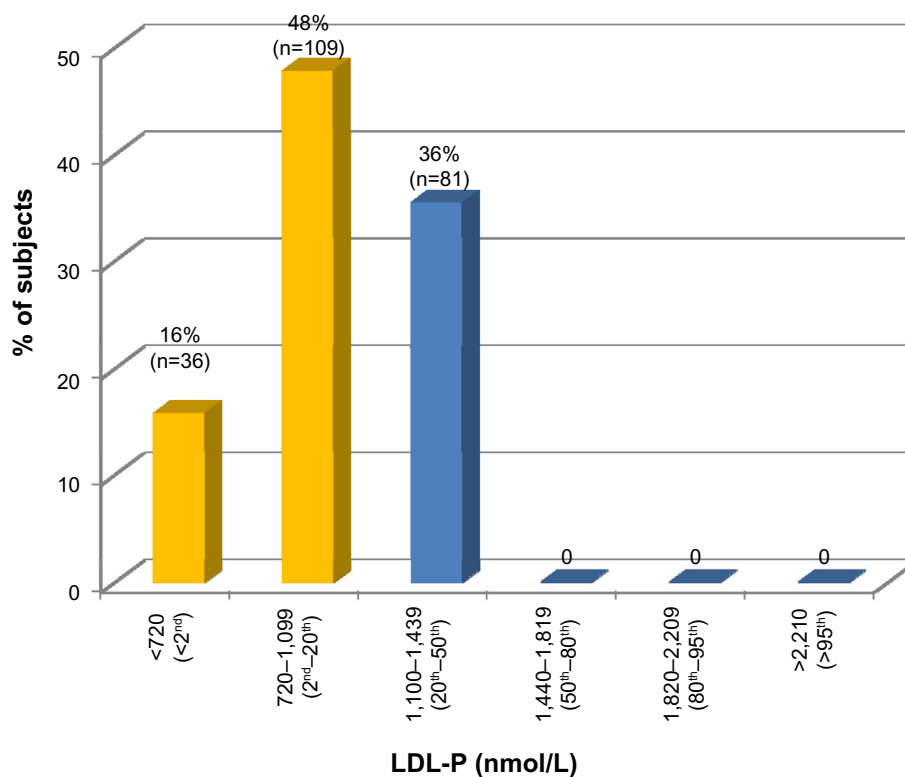


Figure 2 LDL-P distribution in subjects with apoB levels <78 mg/dL.

Note: Numbers in brackets refer to the percentile ranks within the Framingham Offspring Study population.

Abbreviations: LDL-P, low-density lipoprotein particle number; apoB, apolipoprotein B.

36% had LDL-P levels of 1,100–1,439 nmol/L, which corresponds to the 20th–50th Framingham Offspring Study population percentile. In contrast, in subjects with apoB concentrations >118 mg/dL (which corresponds to the 80th Framingham Offspring Study population percentile [n=230]), 93% had comparably high LDL-P values ($\geq 1,820$ nmol/L) (Figure 3).

Figure 4 compares the distribution of various measures of LDL (LDL-C, LDL-P, and apoB) across Framingham Offspring Study population percentiles. While 40% of the subjects had LDL-C levels <100 mg/dL (20th Framingham Offspring Study population percentile), only 17% and 22% had comparably low LDL-P and apoB levels (<1,100 nmol/L and <78 nmol/L, respectively). Figure 5 shows the distribution of LDL-P levels among a subset of patients in the database (subjects) that had LDL-C levels <100 mg/dL, 61% were found to have discordantly high LDL-P levels; whereas, 46% had discordantly high apoB levels.

Discussion

Recent studies suggest that apoB⁸ may be a better predictor of CVD risk than total cholesterol, LDL-C,^{9,10} and non-HDL-C.^{11,12} ApoB is the primary protein component of LDL particles, but it is also found in plasma in association

with triglyceride-rich very low density lipoproteins, intermediate-density lipoproteins (IDL) and lipoprotein(a). The concentration of apoB in plasma is most commonly determined in commercial clinical laboratories by immunoturbidimetric and immunonephelometric methods that provide a single measure for both the liver-derived and intestinally derived forms of apoB (apoB100 and apoB48, respectively). In nonfasting samples, the measured apoB may also include protein associated with chylomicrons and their remnants. It is not clear whether all apoB-containing lipoprotein particles have the same atherogenic potential as LDL particles.

Furthermore, this heterogeneity in apoB-containing particles may potentially interfere with the immunological recognition of the antigenic sites used for apoB quantitation. It is possible that variations in the folding of the apoB molecule between small and large LDL particles and differences in the concentrations of other lipids, such as phospholipids on the particle surface, may interfere with detection by immunoassay.

NMR spectroscopy offers an alternate method for the direct determination of the number of LDL particles in plasma. When placed in a magnetic field, the terminal methyl groups on lipoprotein-associated lipids emit a characteristic

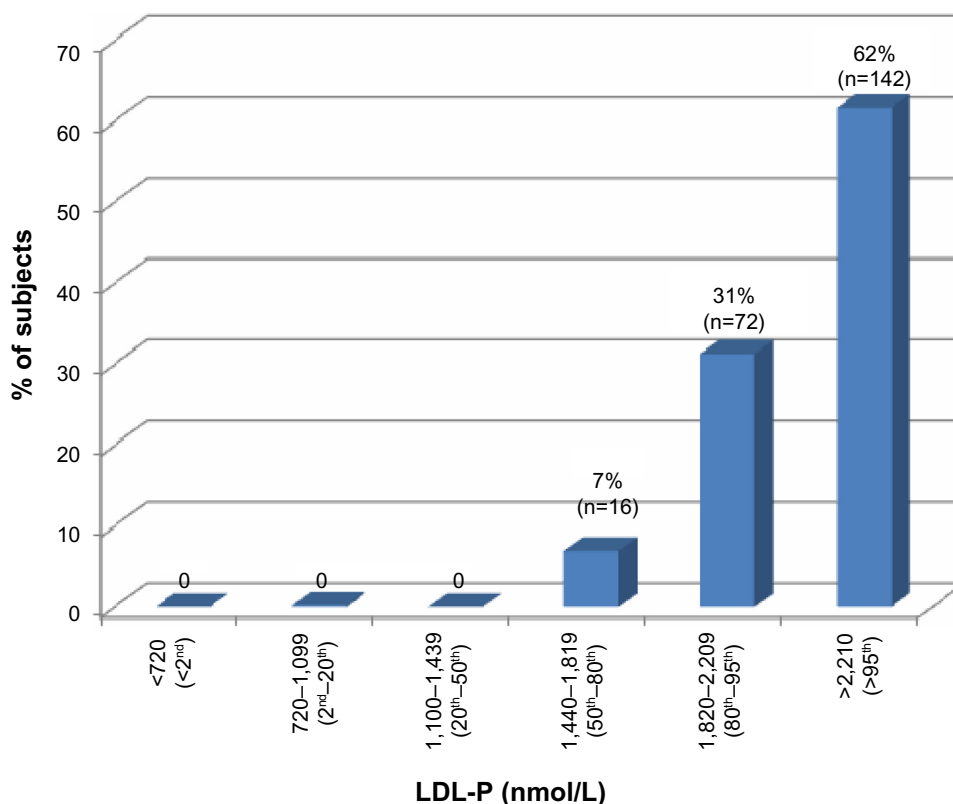


Figure 3 LDL-P distribution in subjects with apoB levels > 118 mg/dL.
Note: ApoB levels > 118 mg/dL correspond to the =80th percentile for the Framingham Offspring Study population.
Abbreviations: LDL-P, low-density lipoprotein particle number; apoB, apolipoprotein B.

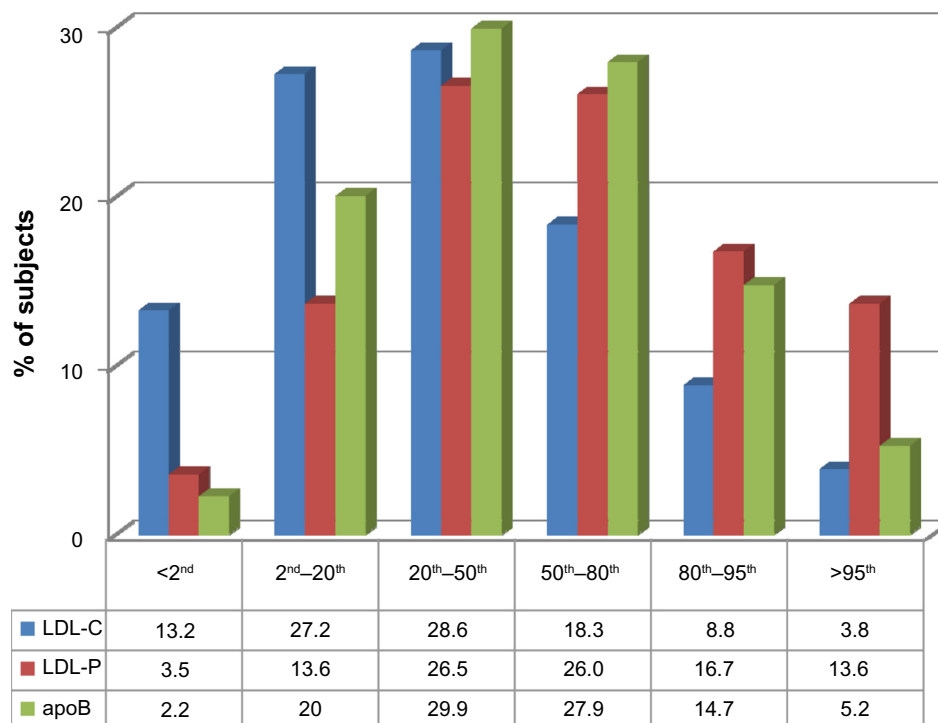


Figure 4 Distribution of LDL-C, LDL-P, and apoB levels across subjects based on population percentiles for the Framingham Offspring Study population.
Abbreviations: LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle number; apoB, apolipoprotein B.

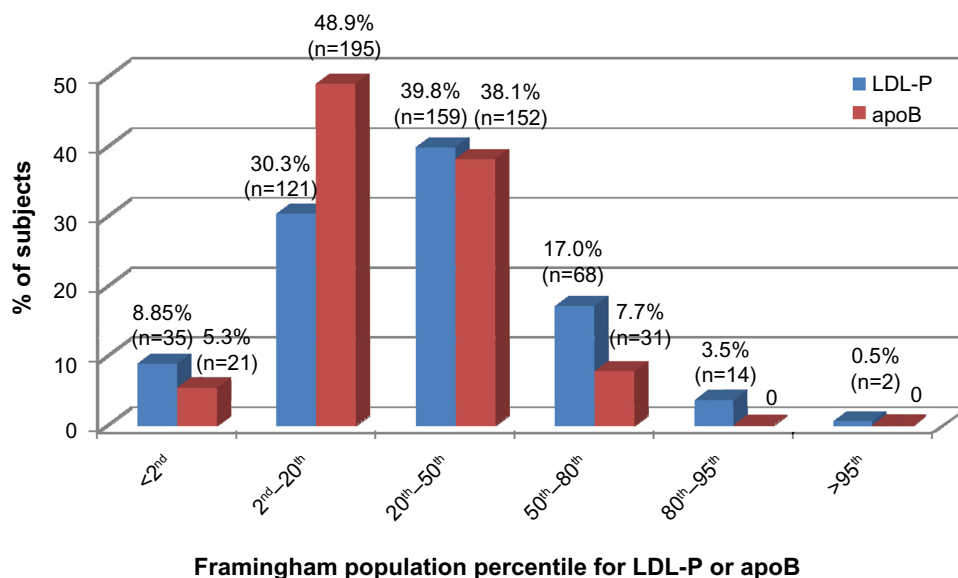


Figure 5 Distribution of LDL-P and apoB levels in subjects with LDL-C levels <100 mg/dL based on population percentiles for Framingham Offspring Study population. **Abbreviations:** LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle number; apoB, apolipoprotein B.

NMR signal which varies depending on the size of the lipoprotein particle.⁷ The amplitude of the signal is directly proportional to the number of particles.⁷ Total LDL-P is determined by summing the concentrations of the individual LDL subclasses, including IDL. Numerous studies have reported that LDL-P is superior to LDL-C for the identification of patients at CVD risk as well as adjudicating the efficacy of lipid-modifying therapy.¹ The American Association of Clinical Chemistry Lipoprotein and Vascular Diseases Division Working Group on Best Practices recently published a review of 25 clinical studies in which the strength of association of apoB and LDL-P with specific cardiovascular outcomes was evaluated.³ LDL-P measured by NMR was more strongly and more frequently associated with the clinical outcomes than apoB.³ The working group noted several advantages of LDL-P by NMR over apoB. NMR can provide information on other major lipoprotein classes, such as very low density lipoproteins, IDL, and HDL – in addition to LDL-P.

Furthermore, the NMR measurement of LDL-P appears to be more precise than that of apoB. The typical intra-assay coefficient of variation for apoB ranges from 5%–11%; whereas, LDL-P has a coefficient of variation that ranges from 2%–4%.³

In the recently published *Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Risk in Adults*, issued by the American College of Cardiology and the American Heart Association,¹³ LDL lowering remains a

central tenet of clinical practice. The measurement of LDL continues to be relevant to guide the clinical judgment of patient adherence to treatment, individual response to therapy, and the need for adjustment in medications to achieve an appropriate individual response. This requires highly reliable LDL measures that correlate with clinical outcomes.

In the current report, we evaluated the relationship between apoB and LDL-P and their associations with conventional lipid biomarkers in a group of patients being followed at typical medical offices. Although many subjects had concordant levels of LDL-P and apoB, a considerable percentage with low apoB levels (<78 mg/dL) had higher than desirable LDL-P levels. These patients may retain considerable potential LDL-attributable CVD risk, with 36% of them having an LDL-P concentration >1,100 nmol/L. This discrepancy between LDL-P and apoB was not observed in subjects with high apoB levels (>118 mg/dL), as a similar percentage had comparably high LDL-P levels.

Further evidence that the apoB and LDL-P levels may provide different information related to the CVD risk was confirmed when the population percentiles for each of the measures from the Framingham Offspring Study were used to classify risk. Among the subjects with safe LDL-C levels (<100 mg/dL; the 20th Framingham Offspring Study percentile cut-point), 46% had discordantly high apoB levels; whereas, 61% had discordantly high LDL-P levels. This finding suggests that both markers uncovered excessive atherogenic particles in subjects who attained an

LDL-C <100 mg/dL and that management of this risk with more aggressive lipid-modifying therapy may be considered. This study additionally highlights the fact that apoB and LDL-P are not comparable measures of atherogenic lipoproteins, particularly at low levels of apoB, and suggests that one measure cannot simply be substituted for the other. LDL-P appears to identify significantly more individuals with excessive concentrations of atherogenic lipoproteins at low levels of both LDL-C and apoB and thus may have the potential to identify more subjects with residual cardiovascular risk. The hypothesis that LDL-P is a more robust predictor of atherogenic lipoprotein-related risk than apoB merits further investigation.

A limitation of this study is the lack of clinical information from study subjects, including medication utilization and associated comorbidities. Additionally, while this study demonstrates discordance between these two parameters at low levels of apoB, this investigation did not assess the superiority of one measure over the other at predicting CVD risk. Despite these limitations, this study clearly establishes that apoB and LDL-P are not comparable measures of atherogenic lipoproteins, particularly in individuals who might otherwise be considered low-risk having achieved optimal LDL-C levels.

Disclosure

Pamela B Morris is a member of the speaker's bureau for Abbott Laboratories, LipoScience, Inc., and Merck & Co. Hector A Malave is a member of the speaker's bureau for LipoScience, Inc., and Abbott Laboratories. James A Underberg is a consultant for Aegerion Pharmaceuticals, Amarin Corporation, Genzyme, LipoScience Inc., and Sanofi; a member of the speaker's bureau for Abbott Laboratories, Amarin Corporation, AstraZeneca, Daiichi Sankyo Inc., Eli Lilly and Company, Forest Pharmaceuticals Inc., GlaxoSmithKline, Kowa Pharmaceuticals, and Merck & Co; and the recipient of research grants from Kowa Pharmaceuticals and LipoScience, Inc. Ngoc-Anh Le received a research grant from Merck & Co and is a consultant for LipoScience, Inc. Michael D Shapiro is a member of the speaker's bureau for

LipoScience, Inc. Deborah A Winegar and Ray Pourfarzib are both full-time employees of LipoScience, Inc. Kellie H McLain reports no conflicts of interest in this work.

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