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ORIGINAL INVESTIGATIONS

Effect of Pelacarsen on Lipoprotein(a) Cholesterol and Corrected Low-Density Lipoprotein Cholesterol

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ABSTRACT

BACKGROUND Laboratory methods that report low-density lipoprotein cholesterol (LDL-C) include both LDL-C and lipoprotein(a) cholesterol [Lp(a)-C] content.

OBJECTIVES The purpose of this study was to assess the effect of pelacarsen on directly measured Lp(a)-C and LDL-C corrected for its Lp(a)-C content.

METHODS The authors evaluated subjects with a history of cardiovascular disease and elevated Lp(a) randomized to 5 groups of cumulative monthly doses of 20-80 mg pelacarsen vs placebo. Direct Lp(a)-C was measured on isolated Lp(a) using LPA4-magnetic beads directed to apolipoprotein(a). LDL-C was reported as: 1) LDL-C as reported by the clinical laboratory; 2) LDL-C_{corr} = laboratory-reported LDL-C − direct Lp(a)-C; and 3) LDL-$C_{corrDahlén}$ = laboratory LDL-C − [Lp(a) mass × 0.30] estimated by the Dahlén formula.

RESULTS The baseline median Lp(a)-C values in the groups ranged from 11.9 to 15.6 mg/dL. Compared with placebo, pelacarsen resulted in dose-dependent decreases in Lp(a)-C (2% vs −29% to −67%; $P = 0.001$-<0.0001). Baseline laboratory-reported mean LDL-C ranged from 68.5 to 89.5 mg/dL, whereas LDL-C_{corr} ranged from 55 to 74 mg/dL. Pelacarsen resulted in mean percent/absolute changes of −2% to −19%/−0.7 to −8.0 mg/dL ($P = 0.95$-0.05) in LDL-C_{corr}, −7% to −26%/−5.4 to −9.4 mg/dL ($P = 0.44$-<0.0001) in laboratory-reported LDL-C, and 3.1% to 28.3%/0.1 to 9.5 mg/dL ($P = 0.006$-0.50) increases in LDL-$C_{corrDahlén}$. Total apoB declined by 3%-16% ($P = 0.40$-<0.0001), but non-Lp(a) apoB was not significantly changed.

CONCLUSIONS Pelacarsen significantly lowers direct Lp(a)-C and has neutral to mild lowering of LDL-C_{corr}. In patients with elevated Lp(a), LDL-C_{corr} provides a more accurate reflection of changes in LDL-C than either laboratory-reported LDL-C or the Dahlén formula. (J Am Coll Cardiol 2022;79:1035-1046) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Approximately 60 years since its discovery, lipoprotein(a) [Lp(a)] is now accepted as a genetic, independent, and likely causal risk factor for cardiovascular disease.[1-3] Lp(a) is a risk factor in primary prevention settings,[4,5] as well as in patients on statins and those enrolled in PCSK9 inhibitor trials.[6,7] In particular, alirocumab provides significant risk reduction in recurrent events

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ABBREVIATIONS AND ACRONYMS

apoB = apolipoprotein B-100

LDL-C = low-density lipoprotein cholesterol

LDL-C_{corr} = low-density lipoprotein cholesterol estimated by measuring Lp(a)-C directly

LDL-$C_{corrDahlén}$ = low-density lipoprotein cholesterol estimated by the Dahlén formula

Lp(a) = lipoprotein(a)

Lp(a)-C = lipoprotein(a) cholesterol

MACE = major adverse cardiovascular events

OxPL = oxidized phospholipids

following acute coronary syndromes, with each 5-mg/dL reduction in Lp(a) predicted to reduce the event rate by 2.5%.[8] Similarly, the reduction in Lp(a) was associated with reduced risk for recurrent events with evolocumab.[6] There are now multiple RNA-based therapeutics in clinical development, with the antisense oligonucleotide pelacarsen the most advanced.[9] The Lp(a) HORIZON (A Randomized Double-blind, Placebo-controlled, Multicenter Trial Assessing the Impact of Lipoprotein (a) Lowering With TQJ230 on Major Cardiovascular Events in Patients With Established Cardiovascular Disease) trial is currently enrolling subjects with Lp(a) ≥70 mg/dL (~≥175 nmol/L) and history of prior myocardial infarction, stroke, or significant, symptomatic peripheral arterial disease to pelacarsen 80 mg monthly vs placebo.

In prior studies of pelacarsen, it was been observed that not only does it lower Lp(a) but it also lowers oxidized phospholipids (OxPL) on apolipoprotein(a) [apo(a)] and apolipoprotein B-100 (apoB) by 70%-88% as well modestly lowering laboratory-reported low-density lipoprotein levels (LDL-C) up to 26% and total apoB levels up to 16% at highest doses used.[10-12] The reductions noted on OxPL-apo(a) and OxPL-apoB were not unexpected, because it has been shown that Lp(a) is a preferential lipoprotein carrier of OxPL.[13,14] However, the effects on LDL-C and apoB were somewhat surprising, because pelacarsen has no known direct effect on hepatic apoB production.[15] Whether the changes in LDL-C are a real physiological effect or a byproduct of the fact that laboratory measures of LDL-C include both true LDL-C plus Lp(a) cholesterol [Lp(a)-C],[16] which might also be expected to decline, is not known.

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Laboratory-reported LDL-C methods, including Friedewald or Martin-Hopkins formulas, ultracentrifugation, or direct LDL-C measurements, all have the limitation that they cannot measure LDL-C independent of Lp(a)-C, and thus, the reported "LDL-C" is a combination of true LDL-C plus Lp(a)-C (and also intermediate density lipoprotein cholesterol if it is present in fasting samples).[16] This is likely of little clinical relevance in subjects with normal Lp(a) levels (<30 mg/dL), but in subjects with elevated Lp(a) that may represent 20%-30% of the population, Lp(a)-C may constitute a substantial proportion of the reported LDL-C.[17] Currently, a major limitation of interpreting such studies is that Lp(a)-C has to be mathematically estimated, most commonly with the Dahlén formula,[18,19] because of the lack of a validated, quantitative method to measure Lp(a)-C.

We recently described a novel, quantitative, sensitive, high-throughput method to directly measure Lp(a)-C on isolated Lp(a) holoparticles.[20] We now apply this method to assess the effect of pelacarsen on directly measured Lp(a)-C and on corrected LDL-C without the interference of Lp(a)-C.

METHODS

STUDY POPULATION. The phase 2B trial of pelacarsen was previously reported.[12] In brief, it was a randomized, double-blind, placebo-controlled, dose-ranging trial involving 286 patients with established cardiovascular disease and screening Lp(a) levels ≥60 mg/dL (≥150 nmol/L). Patients received the hepatocyte-directed antisense oligonucleotide pelacarsen at 20, 40, or 60 mg every 4 weeks; 20 mg every 2 weeks; or 20 mg every week (cumulative doses of 20, 40, 60, and 80 mg monthly), or they received saline placebo subcutaneously for 6 to 12 months. The primary endpoint was the percent change in Lp(a) levels from baseline to month 6 of exposure (week 25 in the groups that received monthly doses and week 27 in the groups that received more frequent doses).

MEASUREMENT OF Lp(a)-C. The methodology and early clinical experience of the method for directly measuring Lp(a)-C was recently described.[20] In brief, plasma is added to monoclonal antibody LPA4-conjugated magnetic beads (MyOne Epoxy, Life Technologies) in the presence of 200 mmol/L proline and 200 mmol/L epsilon amino caproic acid in U-bottom 96-well plates.[20,21] The Lp(a)-LPA4-dynabeads are then extracted from each well using a magnetic bead extraction replicator and released into parallel 96-well plates containing 200 μL of PBS 1% BSA, 200 mmol/L proline and 200 mmol/L epsilon amino caproic acid in each well and any nonspecifically bound cholesterol is wash off. The Lp(a)-LPA4-dynabeads are then transferred to a parallel, clear, flat-bottom, 96-well plate containing 200 μL of enzymatic cholesterol reagent (Pointe Scientific) and analyzed for absorbance at 500 nm (primary) and 700 nm (background). The amount of Lp(a)-C is determined against a standard curve, adjusting for the input volume of plasma. The 2 major advances of this assay compared with prior methods is the high sensitivity to <1 mg/dL cholesterol and the expanded linear range of up 747 nmol/L Lp(a) that captures >99% of the population Lp(a) levels. In prior studies with electrophoretic methods, Lp(a)-C could only be quantitated in ~one-third of subjects whose levels were generally >30 mg/dL.[22]

TABLE 1 Laboratory Characteristics of the Patients at Baseline

	Pelacarsen					
	20 mg/Q4W (n = 48)	40 mg/Q4W (n = 48)	60 mg/Q4W (n = 47)	20 mg/Q2W (n = 48)	20 mg/QW (n = 48)	Pooled Placebo (n = 47)
Lp(a), nmol/L	246.6 (179.2-300.8)	220.0 (176.5-283.3)	204.5 (163.8-286.5)	238.2 (183.7-298.4)	233.7 (193.1-275.3)	231.6 (194.9-317.7)
Direct Lp(a)-C, mg/dL	15.0 (11.8-19.6)	12.3 (9.4-17.0)	11.9 (10.0-14.8)	14.1 (10.2-18.0)	14.3 (11.7-18.5)	15.6 (11.5-20.2)
Lp(a)-$C_{Dahlén}$, mg/dL	29.6 (21.5-36.1)	26.4 (21.2-34.0)	24.5 (19.7-34.4)	28.6 (22.0-35.8)	28.0 (23.2-33.0)	27.8 (23.4-38.1)
Laboratory-reported LDL-C,[a] mg/dL	89.5 ± 37.1	77.5 ± 39.5	68.5 ± 27.2	74.5 ± 28.8	76.2 ± 28.5	78.7 ± 29.8
LDL-C_{corr}, mg/dL	73.5 ± 33.9	63.3 ± 37.1	55.2 ± 26.9	59.2 ± 27.1	61.5 ± 27.9	62.5 ± 29.0
LDL-$C_{corrDahlén}$, mg/dL	56.5 ± 39.5	49.9 ± 39.2	40.9 ± 28.3	45.3 ± 27.7	46.9 ± 30.1	47.7 ± 30.1
Total apoB, mg/dL	80.7 ± 23.6	71.9 ± 23.4	68.5 ± 18.8	69.3 ± 19.8	70.6 ± 19.1	73.8 ± 16.9
Total apoB, nmol/L	1,466.7 ± 429.4	1,306.4 ± 424.6	1,245.3 ± 341.4	1,259.8 ± 359.5	1,283.7 ± 348.2	1,342.4 ± 307.7
Non-Lp(a) apoB, nmol/L	1,204.1 ± 426.7	1,066.0 ± 426.2	1,012.6 ± 355.8	1,004.9 ± 356.9	1,043.6 ± 367.3	1,091.6 ± 306.2
Total cholesterol, mg/dL	166.0 ± 38.6	154.4 ± 52.1	142.9 ± 32.4	146.7 ± 36.3	154.4 ± 34.7	154.4 ± 33.6
Triglycerides, mg/dL	97 (44-230)	97 (35-283)	106 (53-567)	106 (35-204)	89 (35-266)	106 (35-576)
HDL-C, mg/dL	54.2 ± 15.9	55.7 ± 21.0	48.9 ± 12.8	52.8 ± 18.8	57.6 ± 18.4	51.6 ± 18.2

Values are median (IQR) or mean ± SD. Lp(a) in mg/dL was calculated by dividing values in molar concentration by 2.5. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. [a]Direct LDL-C measurements were made when triglycerides exceeded 400 mg/dL, which only occurred in 3 subjects at baseline and 1 subject at the primary analysis time point.

apoB = apolipoprotein B-100; LDL-C = low-density lipoprotein cholesterol; LDL-C_{corr} = low-density lipoprotein cholesterol estimated by measuring Lp(a)-C directly; LDL-$C_{corrDahlén}$ = low-density lipoprotein cholesterol estimated by the Dahlén formula; Lp(a) = lipoprotein(a); Lp(a)-C = lipoprotein(a) cholesterol; non-Lp(a) apoB = total apoB − Lp(a)–apoB; Q2W = every 2 weeks; Q4W = every 4 weeks; QW = once a week; TIA = transient ischemic attack.

In the current study, Lp(a)-C was measured at baseline, week 13, the primary analysis time point (week 25/27), and week 69/final analysis time point, which represents 16 weeks off pelacarsen in all subjects. The study protocol was approved by the UCSD Human Protections Program and conforms to the Declaration of Helsinki.

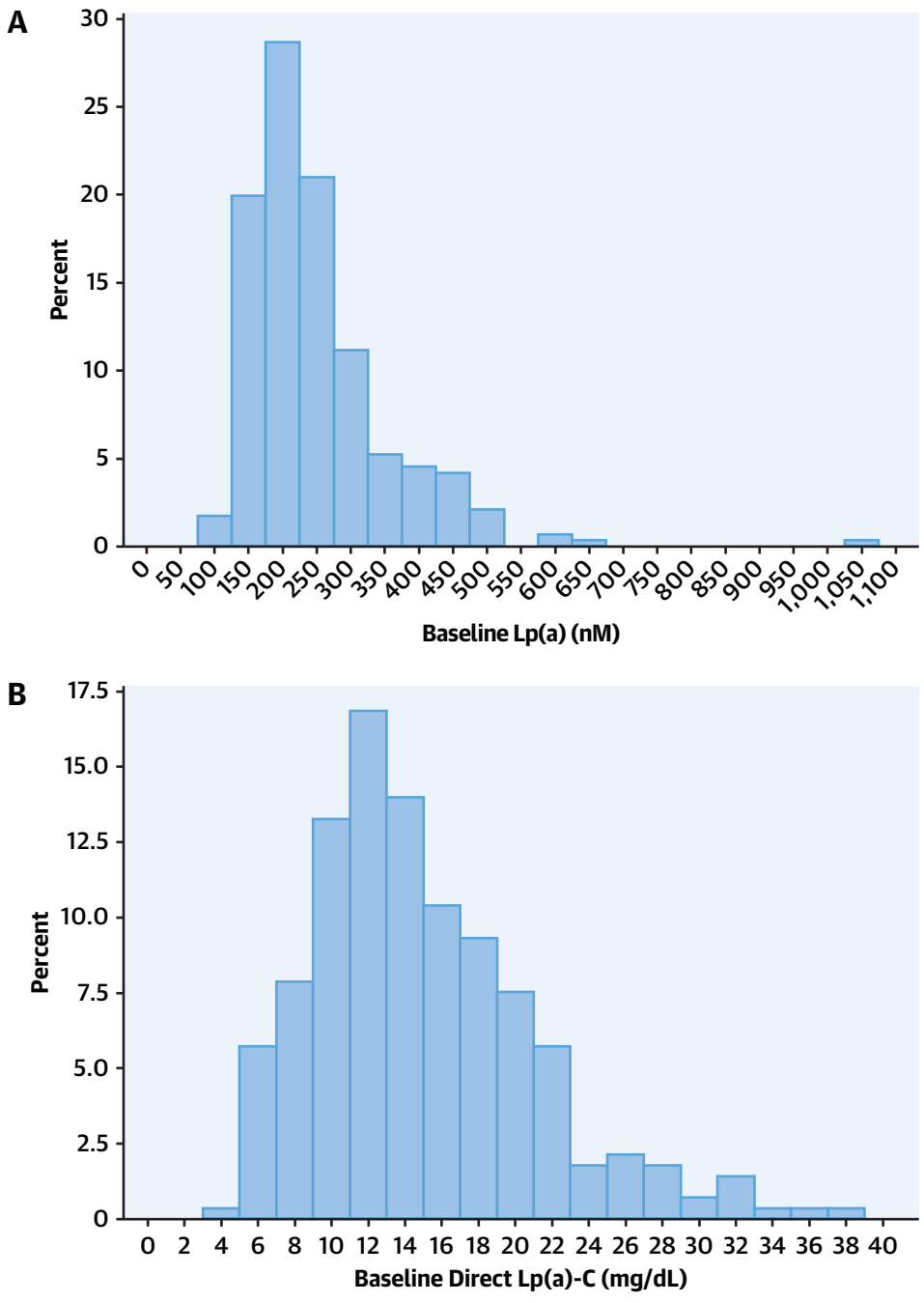
LABORATORY MEASUREMENTS. Relevant laboratory variables were measured as previously described.[12] In brief, Lp(a) molar concentrations (nmol/L), representing apolipoprotein(a) particle number, were measured with an isoform-independent assay at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington. All other laboratory measurements, including LDL-C and total apoB, were measured with commercially available kits at Medpace Reference Laboratories. Laboratory-reported LDL-C was calculated by the Friedewald formula or measured by ultracentrifugation if triglyceride levels exceeded 400 mg/dL. Laboratory measured LDL-C values were further corrected by 2 methods: 1) by the current method, as LDL-C_{corr} = laboratory LDL-C − directly measured Lp(a)-C; and 2) by the Dahlén formula, as LDL-$C_{corrDahlén}$ = laboratory LDL-C − [Lp(a) mass × 0.30].[18]

Levels of apoB not associated with Lp(a) [non-Lp(a)-apoB] were calculated by converting total apoB mass in mg/dL to nmol/L by multiplying by 19.9493 (using molecular weight of apoB at 550 kDa) and then subtracting Lp(a) molar concentration [non-Lp(a)-apoB = total ApoB − Lp(a)-apoB]. Because apoB and apo(a) are in a 1:1 molar relationship, when the molar concentration of Lp(a) is known, the Lp(a)-apoB concentration is identical.

Oxidized phospholipids on apoB (OxPL-apoB) and apo(a) [OxPL-apo(a)] were measured as previously described.[23]

STATISTICAL ANALYSIS. All summaries and analyses were conducted in the full analysis set, defined as all patients who had undergone randomization and had received at least 1 dose of the study drug or placebo. The baseline data were summarized using descriptive statistics. Continuous data are expressed as mean ± SD or median (IQR). Correlations for the day 1 predose data were assessed by the Spearman's rank correlation coefficient. Correlations for the data at the primary analysis time point were assessed by the Spearman's partial rank order correlation coefficients controlling for treatment group and log-transformed baseline values. The percent changes in Lp(a)-C, Lp(a), laboratory-reported LDL-C, LDL-C_{corr}, LDL-$C_{corrDahlén}$, total apoB, and non-Lp(a) apoB levels were analyzed with the use of an analysis of covariance model with treatment group as factor and the log-transformed baseline value for each respective measure as covariate. Missing data were handled with a multiple-imputation model containing baseline and postbaseline values, stratified according to treatment group. The imputations

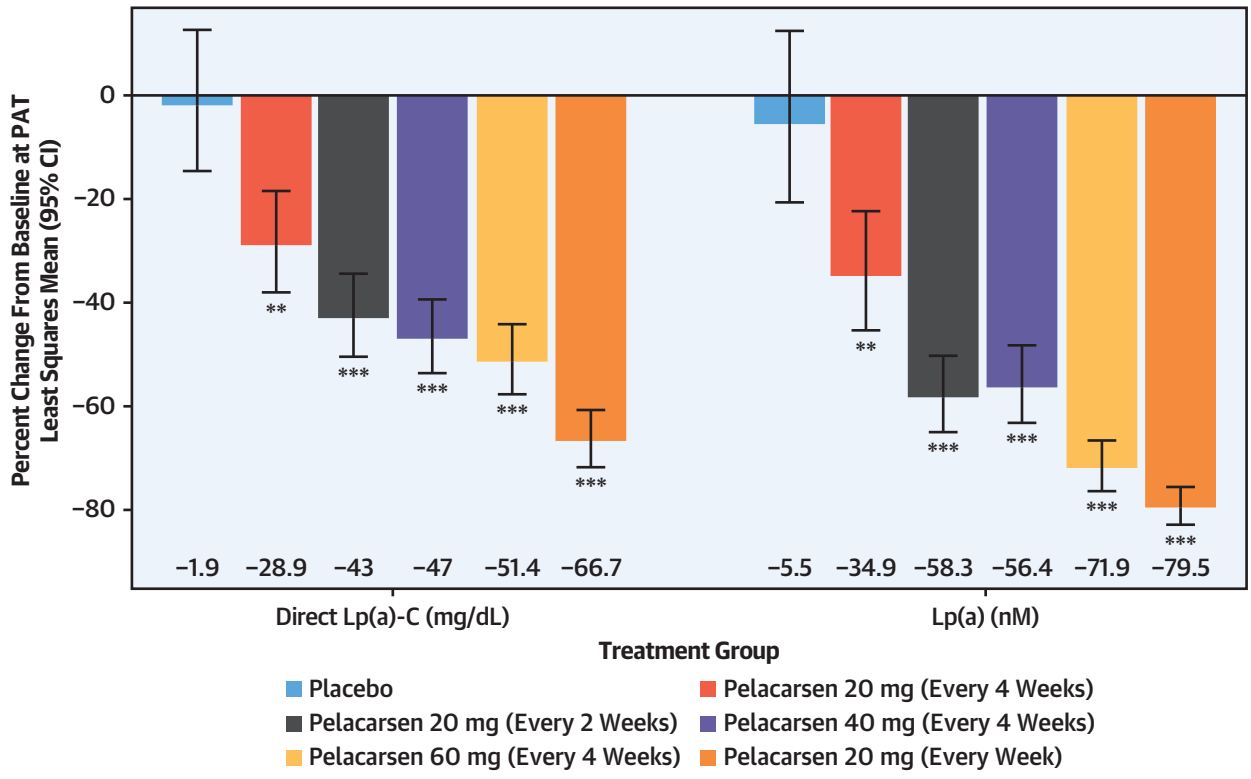
FIGURE 1 Frequency Distribution of Lp(a) and Lp(a)-C



Frequency distribution of **(A)** Lp(a) molar concentration and **(B)** direct Lp(a)-C at the baseline time point in the combined groups. Lp(a) = lipoprotein(a); Lp(a)-C = lipoprotein(a)-cholesterol.

were performed for postbaseline values by the Markov chain Monte Carlo method. Due to the exploratory nature of this analysis, the *P* values and widths of the 95% CIs presented in this report have not been adjusted for multiplicity, and therefore, inferences drawn from these statistics may not be reproducible. The analyses were performed with SAS version 9.4.

FIGURE 2 Change in Lp(a)-C and Lp(a)



The effect of pelacarsen on Lp(a) molar concentration and Lp(a)-C at the primary analysis time point. The figure shows the least-squares mean percent changes and 95% CIs from baseline to the primary analysis time point (PAT). PAT was at 6 months of exposure: week 25 in the groups that received monthly doses and week 27 in the groups that received more frequent doses. $^{**}P < 0.01$. $^{***}P < 0.001$. No corrections for multiple testing were applied. Abbreviations as in Figure 1.

RESULTS

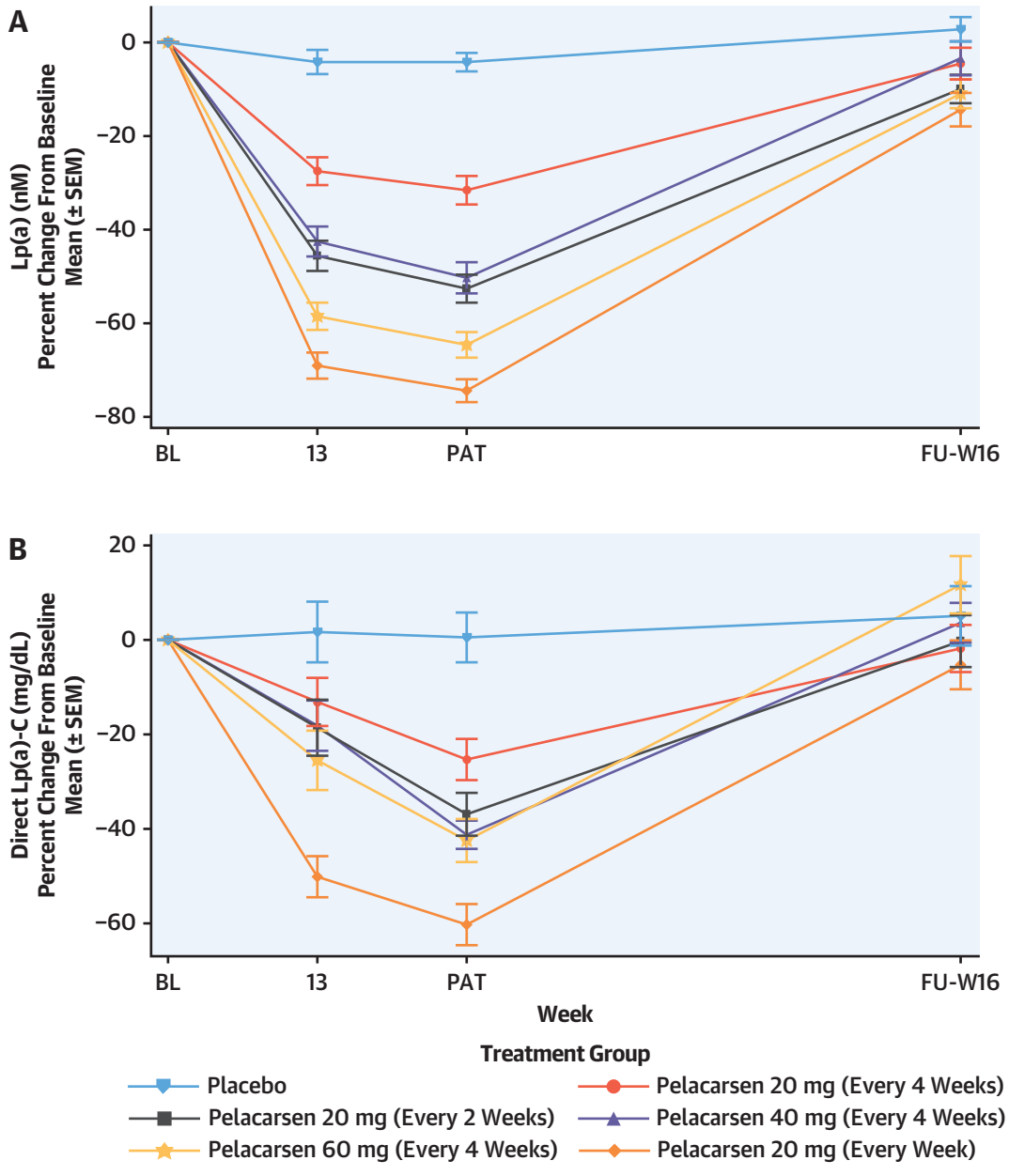
BASELINE LEVELS OF LABORATORY PARAMETERS IN THE TREATMENT GROUPS. The demographic and clinical characteristics of the study groups were previously described.[12] Table 1 displays the relevant laboratory variables of the current analysis. The baseline Lp(a) levels ranged from ~205 to 247 nmol/L and the median Lp(a)-C values ranged from 11.9 to 15.6 mg/dL. The mean laboratory-reported LDL-C ranged from 68.5 to 89.5 mg/dL in the 6 groups. The LDL-C_{corr} was ~13 to 16 mg/dL lower than the laboratory reported LDL-C. In contrast, the LDL-$C_{corrDahlén}$ was ~27 to 33 mg/dL lower than the laboratory measured LDL-C, consistent with LDL-$C_{corrDahlén}$ significantly overestimating Lp(a)-C and underestimating true LDL-C.

The frequency distribution of baseline levels of Lp(a)-C (Figure 1B) are right skewed and similar to Lp(a) molar concentration (Figure 1A).

THE EFFECT OF PELACARSEN ON LP(A) AND LP(A)-C LEVELS. Pelacarsen resulted in statistically significant dose-dependent mean percent decreases in Lp(a)-C compared with 2% decrease in pooled placebo (29%-67%; $P = 0.001$-<0.0001 at the primary analysis time point) (Figure 2, left). The effect of pelacarsen on Lp(a) molar concentration was previously reported[12] and is shown here for comparison to Lp(a)-C levels (Figure 2, right). The temporal changes of direct Lp(a)-C and Lp(a) from baseline to the primary analysis time point and to 16 weeks after the last dose are shown in Figures 3A and 3B. The extent and temporal changes in directly measured Lp(a)-C are consistent with change in Lp(a) molar concentration.

The absolute changes in Lp(a)-C are shown in Supplemental Table 1. The absolute decline in Lp(a)-C values ranged from −5.4 to −9.4 mg/dL from lowest to highest cumulative monthly doses.

FIGURE 3 Change in Lp(a) and Lp(a)-C

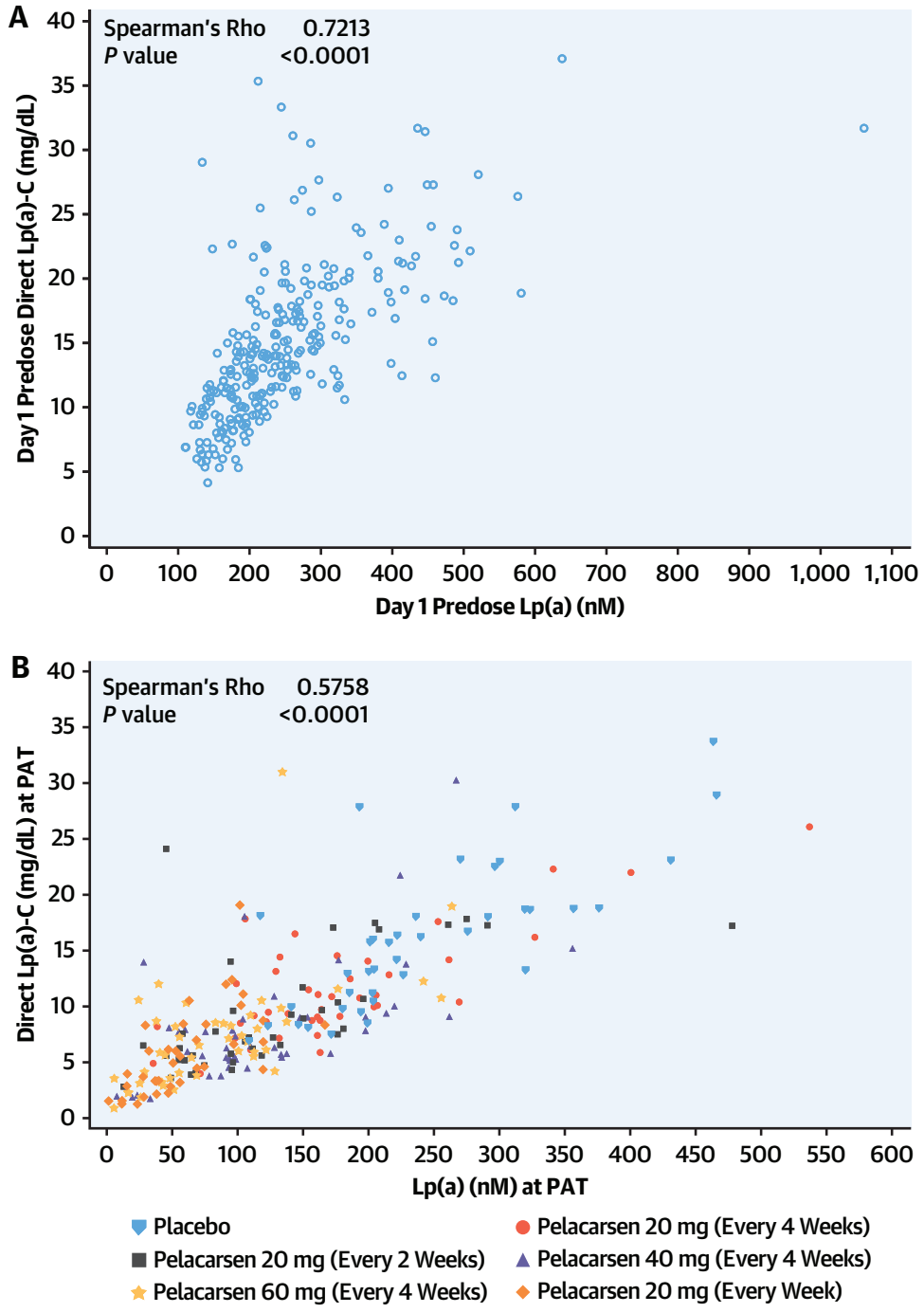


Temporal changes of **(A)** Lp(a) and **(B)** direct Lp(a)-C from baseline to week 13, to the PAT, and to 16 weeks after the last dose. Abbreviations as in Figures 1 and 2.

Spearman ρ between Lp(a)-C and Lp(a) molar concentration at baseline and the primary analysis time point (week 25/27) were 0.72 ($P < 0.0001$) and 0.58 ($P < 0.0001$) (Figures 4A and 4B), respectively.

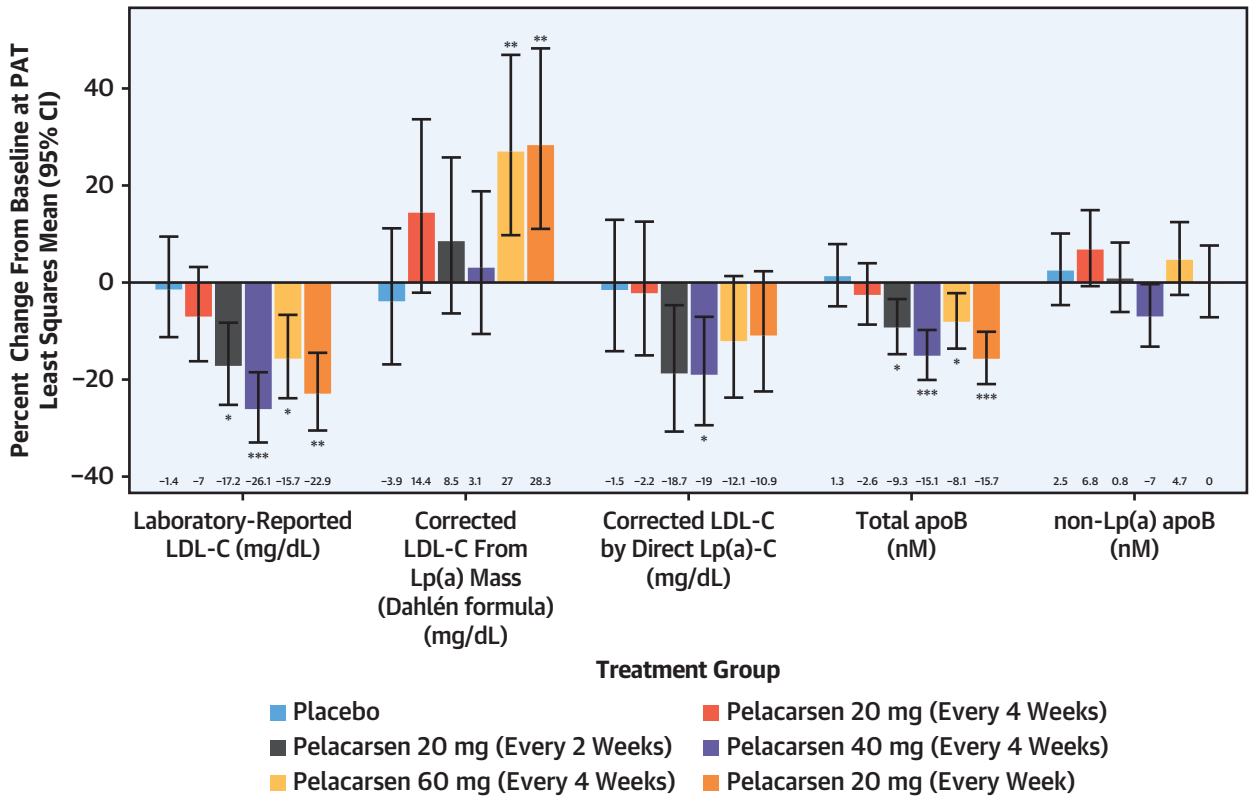
THE EFFECT OF PELACARSEN ON LABORATORY-REPORTED LDL-C, LDL-C_{CORR}, AND LDL-$C_{corrDahlén}$ AT THE PRIMARY ANALYSIS TIME POINT. The mean percent changes from baseline to the primary analysis time point in selected laboratory variables are shown in Figure 5. Compared with the laboratory-derived LDL-C that was previously reported, the LDL-C_{corr} trended in the same direction, albeit with a smaller effect and with loss of statistical significance in some of the groups. In contrast, the estimated levels of LDL-$C_{corrDahlén}$ were significantly increased from baseline in most of the cohorts compared with placebo. Reflecting the limitations of the Dahlén formula,

FIGURE 4 Scatter Plots of Direct Lp(a)-C and Lp(a)



Scatter plots of direct Lp(a)-C and Lp(a) molar concentration at day 1 predose **(A)** and primary analysis time point **(B)**. PAT was at 6 months of exposure: week 25 in the groups that received monthly doses and week 27 in the groups that received more frequent doses. Abbreviations as in Figures 1 and 2.

FIGURE 5 Effect of Pelacarsen on Lipid Variables



The effect of pelacarsen on laboratory-reported LDL-C, LDL-C_{corr}, and LDL-$C_{corrDahlén}$ apoB and non-Lp(a) apoB at the primary analysis time point. The figure shows the least-squares mean percent changes and 95% CIs from baseline to the primary analysis time point. PAT was at 6 months of exposure: week 25 in the groups that received monthly doses and week 27 in the groups that received more frequent doses. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. No corrections for multiple testing were applied. Abbreviations as in Figures 1 and 2.

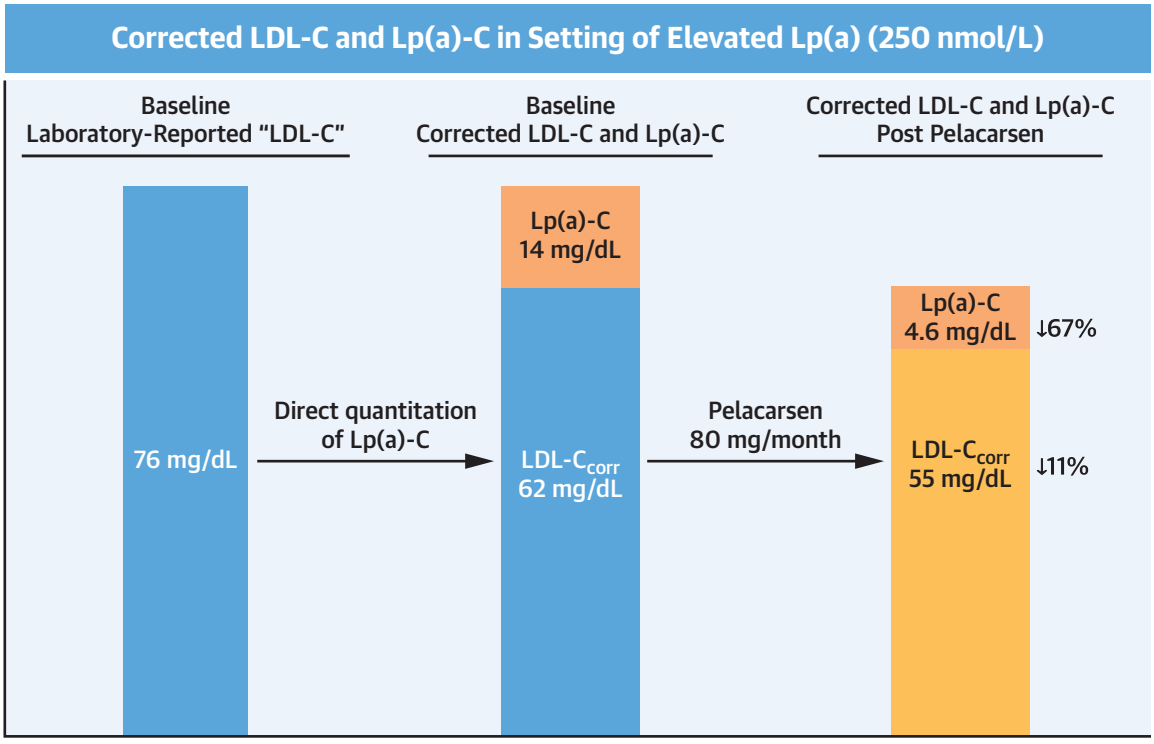
there were 8 patients who had only baseline LDL-$C_{corrDahlén}$ ≤0, 2 patients who had LDL-$C_{corrDahlén}$ at the PAT ≤0, and 4 patients who had LDL-$C_{corrDahlén}$ at both baseline and PAT ≤0. Previously reported total apoB levels were significantly decreased in all cohorts except the lowest dose at 20 mg per 4 weeks. However, the non-Lp(a) apoB levels were not significantly reduced in any cohorts (Figure 5).

The total apoB declined across all doses in range from −2.3 to −10.8 mg/dL, but there was variability in the changes in non-Lp(a) apoB with no dose response noted. The absolute changes in Lp(a)-related lipid variables are shown in Supplemental Table 1. The decrease in LDL-C_{corr} ranged from −0.7 to −8.0 mg/dL, whereas the laboratory-reported LDL-C decreased from −5.6 to −6.7, and LDL-$C_{corr\ Dahlén}$ increased by 0.1-9.5 mg/dL.

There were no clinically relevant differences in the previously mentioned results based on whether patients were treated with statins or PCSK9 inhibitors (data not shown).

CORRELATIONS BETWEEN LP(A)-RELATED VARIABLES. In all groups combined at baseline, direct Lp(a)-C correlated strongly with Lp(a) molar concentration ($P < 0.0001$); modestly with laboratory-reported LDL-C, total apoB, OxPL-apoB, and triglycerides ($P < 0.0001$ for all); and weakly with LDL-C_{corr} ($P = 0.0077$) (Supplemental Table 2). LDL-C_{corr} did not correlate with Lp(a) or direct Lp(a)-C, but did correlate strongly with total apoB and non-Lp(a) apoB. In all groups combined at the primary analysis time

CENTRAL ILLUSTRATION **Lipoprotein(a) Cholesterol and Corrected Low-Density Lipoprotein Cholesterol Before and After Pelacarsen Therapy**



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A hypothetical patient is presented with a lipoprotein(a) molar concentration of 250 nmol/L treated with pelacarsen 80 mg cumulative monthly. The baseline laboratory-reported low-density lipoprotein cholesterol is 76 mg/dL. However, following direct measurement of the lipoprotein(a) cholesterol component of "low-density lipoprotein cholesterol," the lipoprotein(a) cholesterol is 14 mg/dL and the corrected low-density lipoprotein cholesterol is 62 mg/dL. Following treatment with pelacarsen, a 67% reduction in lipoprotein(a) cholesterol and 11% reduction on lipoprotein(a) cholesterol is noted, achieving final concentrations of 54 mg/dL and 4.6 mg/L, respectively. LDL-C = low-density lipoprotein cholesterol; LDL-C_{corr} = low-density lipoprotein cholesterol estimated by measuring Lp(a)-C directly; Lp(a)-C = lipoprotein(a) cholesterol.

point, generally similar trends in statistical associations were noted (Supplemental Table 2).

DISCUSSION

The current study demonstrates several novel findings derived from the recent phase 2 study of pelacarsen. First, it demonstrates that pelacarsen produced a robust, dose-dependent reduction in directly measured Lp(a)-C that is consistent with its effect on Lp(a) molar concentration. Second, it shows that in patients with elevated Lp(a), corrected LDL-C is 13 to 16 mg/dL lower than laboratory-reported LDL-C (**Central Illustration**). This observation should be further evaluated for clinical significance in differentiating major adverse cardiovascular events (MACE) driven by Lp(a)-C vs LDL-C vs both combined.[24] Third, it confirms that the Dahlén formula, which uses a fixed 30% correction of Lp(a) mass to estimate Lp(a)-C for every individual, significantly overestimates the Lp(a)-C and underestimates the LDL-C in subjects with elevated Lp(a) and its use should be discontinued. The clinical implications of each finding are discussed in the following text.

The robust, dose-dependent reduction in Lp(a)-C is a novel observation and mirrored the reduction in Lp(a) molar concentration, but was of slightly lower magnitude. The reduction in Lp(a)-C is not unexpected, because all patients had elevated Lp(a) levels and pelacarsen has been shown to potently lower Lp(a) with no nonresponders identified to date.[12,25] The correlations between the baseline and primary endpoint of Lp(a)-C and Lp(a) molar concentration were strong and statistically significant but not near unity. This is consistent with recent observations that Lp(a) particles in subjects with highly elevated Lp(a)

are heterogeneous in their cholesterol content, ranging from 6% to 57% in an inverse and curvilinear fashion as recently shown.[20] More research is required to identify the factors that lead to such heterogeneity, but besides the *LPA* gene, possibilities include the *APOE, CETP*, and APOH genes, which influence Lp(a) levels as recently described from the UK Biobank.[26] Such variables may mediate fluxes of triglyceride-rich lipoproteins and cholesterol esters, as well as oxidized phospholipids, between Lp(a) and other lipoproteins or tissues.

We observed that baseline LDL-C_{corr} levels were ~13 to 16 mg/dL lower than the laboratory-reported LDL-C, a clinically significant difference based on the CTT meta-analysis.[27] Additionally, we recently showed in a meta-analysis of landmark statin trials including 18,043 patients, 5,390 events, and 4.7 years median follow-up that mathematically removing Lp(a)-C from the laboratory-reported LDL-C in a bracket of 20% to 45% of Lp(a) mass resulted in corrected LDL-C no longer being predictive of MACE.[24] The current study, as best as can be estimated with the limitations of converting Lp(a) molar concertation to Lp(a) mass, suggests that many patients have <20% Lp(a)-C of Lp(a) mass as cholesterol. Future studies using the quantitative method described here will be needed to validate the findings of the previously mentioned meta-analysis. With the emergence of Lp(a)-lowering therapeutics,[9] the ability to differentiate a more accurate LDL-C from Lp(a)-C may allow the ability to assess which pool of cholesterol will be responsive to LDL-targeted vs Lp(a)-targeted therapies and to choose the most appropriate types and dosage of concomitant therapies.

The trends in changes in LDL-C_{corr} are largely reflected in the laboratory LDL-C, but with a attenuation of the effect size leading to lack of statistical significance in all but 1 treatment group. This suggests that the laboratory-reported LDL-C mildly overestimates the LDL-C-lowering effect of pelacarsen, likely because it includes the Lp(a)-C, which was reduced in significantly greater proportion than true LDL-C. We believe the current technique of directly measuring Lp(a)-C and deriving the LDL-C_{corr} most closely estimates the most accurate LDL-C changes caused by pelacarsen and that both laboratory-reported LDL-C and the Dahlén formula are less accurate. If the Lp(a) HORIZON trial (NCT04023552) shows a reduction in MACE, it will allow an opportunity to examine the relative contributions of the reductions in laboratory-reported LDL-C, LDL-C_{corr}, Lp(a), and oxidized phospholipids in response to pelacarsen.

It was also demonstrated here that the Dahlén formula, by assuming that Lp(a)-C is universally a fixed 30% of Lp(a) mass, overestimates Lp(a)-C and underestimates true LDL-C in patients with elevated Lp(a), where it is most crucial to differentiate the proportions of each. The original description of this formula was reported in textbook format[18,19] and appears to have been studied in a small number of subjects without subsequent rigorous biochemical or clinical validation. Although Lp(a)-C measured using an indirect method in 55 Japanese individuals suggested an average Lp(a)-C mg/dL to Lp(a) mg/dL ratio of 0.3, significant interindividual variation existed.[28] Lp(a) mass assays (mg/dL) measure apo(a) immunologically and not total particle mass and use calibrators not traceable to any validated primary standard; thus, the denominator of this equation is a source of error. Based on the current findings, we recommend that the Dahlén formula be discontinued as an estimate corrected LDL-C. We also discourage the reporting of Lp(a)-C as a percentage of Lp(a) mass. Instead, when an Lp(a)-C mass is needed, a directly measured Lp(a)-C should be used to allow determination of Lp(a)-C and LDL-C.

The observation that the significant reduction in total apoB levels appeared to be driven by reduction in Lp(a)-apoB implies enhanced apoB plasma clearance, because pelacarsen is not known to affect *APOB* mRNA levels.[15] It is known that clearance of Lp(a) particles is slower than LDL particles,[29-31] likely caused by weaker recognition of Lp(a)-apoB by LDL receptors. Pelacarsen inhibits hepatic apo(a) synthesis and Lp(a) release from the liver, effectively diverting apo(a) from complexing with apoB. This would imply the same amount of apoB is secreted from the liver but in a higher proportion of LDL than Lp(a), which would have faster removal from plasma compared with the same apoB as part of an Lp(a). Other potential mechanisms for the reduction in plasma apoB include a change in the competition for hepatic LDL receptors with a decreasing plasma Lp(a) favoring higher LDL-apoB clearance. It has also been suggested by some but not all kinetic studies that apo(a) may disassociate and reassociate at least once with another apoB-100 particle during its plasma residence,[29] which would favor faster clearance of LDL-apoB particles not encumbered by covalently attached apo(a). Finally, the modest divergence of true LDL-C from non-Lp(a) apoB may also imply changes in CETP activity[32] or apoE function[33] in the pelacarsen groups, both of which can affect LDL-C and Lp(a) levels. Additional studies, including kinetic studies with and without pelacarsen with methodologies recently described studying Lp(a)

catabolism with PCSK9 inhibitors,[34] will be required to define the underlying mechanisms.

CLINICAL IMPLICATIONS. As therapies to lower Lp(a) are developed, understanding the relative contribution of Lp(a)-C to the laboratory-reported LDL-C in patients with elevated Lp(a) may affect clinical decision making, both in terms of aggressiveness as well as choice in therapy. For example, as shown here in patients with elevated Lp(a), the laboratory-reported LDL-C is incorrectly reported as significantly higher than the corrected LDL-C. Although guidelines for targets of therapy are based on laboratory-reported LDL-C, for subjects with elevated Lp(a), it does not fully capture the risk of the respective components of "LDL-C." Furthermore, because statins tend to increase Lp(a) levels, only the true LDL-C portion responds to statins.[35] Therefore, if the proportion of true LDL-C is lower than realized,[36] increasing the dose or adding additional LDL-C–lowering agents may not necessarily treat all of the underlying risk. Persistently elevated LDL-C despite maximal therapy, ie, statin resistance, may also alert clinicians to the hidden presence of elevated Lp(a).[17] Ultimately, reporting both LDL-C and Lp(a) molar concentration, or Lp(a)-C, may allow a more personalized approach to addressing residual risk. One may also measure apoB-100 levels; however, they do not easily discriminate what portion is on LDL particles vs other particles, and therefore, it reflects a different measurement.

The current method to quantitate Lp(a)-C may be useful in clinical research studies to understand changes in lipid components affecting both Lp(a)-C and LDL-C. For example, we recently showed in a meta-analysis that included several doses of different statins that most statins increase Lp(a) 8%-24%.[35] In those studies, Lp(a)-C was not measured, but it would be interesting to assess if Lp(a)-C also increases with increases in Lp(a) or is unchanged or decreases. Because statins affect cholesterol composition of LDL particles, it would be difficult to predict the effect and any clinical relevance of the increase in Lp(a) without a direct measure of the true LDL-C.

STUDY LIMITATIONS. Limitations of this study include that the Lp(a)-C and LDL-C_{corr} methods have not been validated against a gold standard, which does not exist. Therefore, validation will need to proceed with additional studies, particularly those that have clinical outcomes. The studies to date measuring direct Lp(a)-C have been performed in subjects with relatively normal triglyceride levels, and further studies are needed to assess whether deriving a corrected LDL-C in patients with elevated triglyceride-rich lipoproteins allows accurate correction of LDL-C. These limitations, however, provide a rationale to continue to develop and validate sensitive and quantitative methods to more accurately measure both Lp(a) and LDL-C.

CONCLUSIONS

Direct Lp(a)-C assessment in subjects with elevated Lp(a) shows that the LDL-C_{corr} is significantly lower than can be appreciated by the clinical laboratory. Pelacarsen significantly decreases in Lp(a)-C with neutral to modest effects on corrected LDL-C. Determining LDL-C_{corr} by directly quantitating Lp(a)-C and subtracting it for the laboratory LDL-C provides a more accurate reflection of the baseline and change in LDL-C following pelacarsen in patients with elevated Lp(a).

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL OUTCOMES: Laboratory-reported LDL-C levels reflect both true LDL-C and Lp(a) cholesterol, and corrected LDL-C may be 17-26 mg/dL lower than the reported LDL-C. Pelacarsen reduces Lp(a) cholesterol mainly by reducing Lp(a) rather than LDL-C.

TRANSLATIONAL OUTLOOK: Differentiating true LDL-C from Lp(a) cholesterol would facilitate more precise, personalized decisions on risk assessment and guide therapy.

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APPENDIX For supplemental tables, please see the online version of this paper.