CrossMark

Icosapent ethyl (eicosapentaenoic acid ethyl ester): Effects on remnant-like particle cholesterol from the MARINE and ANCHOR studies

Christie M. Ballantyne [a, *], Harold E. Bays [b], Sephy Philip [c], Ralph T. Doyle Jr. [c], Rene A. Braeckman [c, 1], William G. Stirtan [c, 2], Paresh N. Soni [c, 3], Rebecca A. Juliano [c]

[a] Baylor College of Medicine and the Houston Methodist DeBakey Heart and Vascular Center, 6565 Fannin St. MSA 601, Houston, TX 77030, USA
[b] Louisville Metabolic and Atherosclerosis Research Center, Louisville, KY, USA
[c] Amarin Pharma Inc., Bedminster, NJ, USA

ARTICLE INFO

ABSTRACT

Background and aims: Remnant-like particle cholesterol (RLP-C) is atherogenic and may increase atherosclerotic cardiovascular disease risk. Icosapent ethyl is a high-purity prescription eicosapentaenoic acid ethyl ester (approved as an adjunct to diet to reduce triglyceride [TG] levels in adult patients with TGs ≥500 mg/dL [≥5.65 mmol/L] at 4 g/day). In the MARINE and ANCHOR studies, icosapent ethyl reduced TG and other atherogenic lipid parameter levels without increasing low-density lipoprotein cholesterol (LDL-C) levels. This exploratory analysis evaluated the effects of icosapent ethyl on calculated and directly measured RLP-C.
Methods: MARINE (TGs ≥500 and ≤2000 mg/dL [≥5.65 mmol/L and ≤22.6 mmol/L]) and ANCHOR (TGs ≥200 and <500 mg/dL [≥2.26 and <5.65 mmol/L] despite statin-controlled LDL-C) were phase 3, 12-week, double-blind studies that randomized adult patients to icosapent ethyl 4 g/day, 2 g/day, or placebo. This analysis assessed median percent change from baseline to study end in directly measured (immunoseparation assay) RLP-C levels (MARINE, n = 218; ANCHOR, n = 252) and calculated RLP-C levels in the full populations.
Results: Icosapent ethyl 4 g/day significantly reduced directly measured RLP-C levels −29.8% (p = 0.004) in MARINE and −25.8% (p = 0.0001) in ANCHOR versus placebo, and also reduced directly measured RLP-C levels to a greater extent in subgroups with higher versus lower baseline TG levels, in patients receiving statins versus no statins (MARINE), and in patients receiving medium/higher-intensity versus lower-intensity statins (ANCHOR). Strong correlations were found between calculated and directly measured RLP-C for baseline, end-of-treatment, and percent change values in ANCHOR and MARINE (0.73–0.92; p < 0.0001 for all).
Conclusions: Icosapent ethyl 4 g/day significantly reduced calculated and directly measured RLP-C levels versus placebo in patients with elevated TG levels from the MARINE and ANCHOR studies.
© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Triglyceride (TG)-rich apolipoprotein B (ApoB)-containing lipoproteins released from the intestine or liver undergo lipolysis at the surface of endothelial cells, creating TG-poor and cholesterol-rich remnant particles (e.g., chylomicron remnants in the non-fasting state and dense subfractions of very-low-density lipoprotein [VLDL] and intermediate-density lipoprotein in the fasting and non-fasting states) [1,2]. An indirect approximation of remnant lipoprotein cholesterol (RLP-C) levels can be readily achieved by subtracting the cholesterol carried by low-density lipoproteins (LDL-C) from the cholesterol carried by all atherogenic lipoproteins, as reflected by non-high-density lipoprotein cholesterol (non-HDL-C), which in turn is calculated by subtracting high-density

lipoprotein cholesterol (HDL-C) from total cholesterol (TC) (i.e., RLP-C = TC − [HDL-C] − [LDL-C]) [3]. More direct measures of RLP-C levels include immunoseparation and vertical auto profile testing [4]. Direct isolation of remnants allows for a more accurate measurement of the atherogenic cholesterol within the TG-rich lipoprotein subpopulation than calculated RLP-C, but such direct measures are not readily available to practicing clinicians [5].

High RLP-C levels appear to be a risk factor for atherosclerotic cardiovascular disease in patients with coronary artery disease, diabetes, or metabolic syndrome, as well as those on statin therapy [6–10]. Overall, current evidence suggests that elevated remnant cholesterol levels are one of the causal factors of residual ischemic heart disease risk [3] and cardiovascular disease [11].

Icosapent ethyl (Vascepa®; Amarin Pharma Inc., Bedminster, NJ, USA) is a high-purity prescription form of eicosapentaenoic acid (EPA) ethyl ester approved by the US Food and Drug Administration as an adjunct to diet to reduce TG levels in adult patients with severe hypertriglyceridemia (≥500 mg/dL [≥5.65 mmol/L]) at a dose of 4 g/day [12]. Two randomized, double-blind, placebo-controlled, multicenter, phase 3 studies (MARINE and ANCHOR) demonstrated the efficacy and safety of icosapent ethyl 2 g/day and 4 g/day. In both studies, icosapent ethyl 4 g/day significantly reduced TG levels and improved other lipid parameters including non-HDL-C and ApoB, without raising LDL-C levels compared with placebo [13,14]. To gain a better understanding of the lipid effects of icosapent ethyl, this exploratory analysis evaluated the impact of icosapent ethyl on directly measured and calculated RLP-C levels in patients from the MARINE and ANCHOR studies.

2. Materials and methods

2.1. Study design

Details of the MARINE and ANCHOR studies have been published previously [13,14]. Briefly, both studies included a 4–6 week lead-in period of diet, lifestyle, and medication stabilization with washout of prohibited lipid-altering medications. Both studies randomized patients aged >18 years with qualifying lipid levels (TGs ≥500 and ≤2000 mg/dL [≥5.65 and ≤22.6 mmol/L] in MARINE; statin-stabilized TGs ≥200 and <500 mg/dL [≥2.26 and <5.65 mmol/L] and LDL-C ≥40 and <100 mg/dL [≥1.04 and <2.59 mmol/L] in ANCHOR) to receive icosapent ethyl 4 g/day, icosapent ethyl 2 g/day, or matching placebo during a 12-week double-blind treatment period. The MARINE study permitted, but did not require stable statin therapy with or without ezetimibe. Enrollment in the ANCHOR study required patients to be at high risk of cardiovascular disease as defined by the National Cholesterol Education Program Adult Treatment Panel III guidelines [15] and on a stable statin dose (atorvastatin, rosuvastatin, or simvastatin with or without ezetimibe) prior to baseline initiation of icosapent ethyl or placebo. The protocols for each study were approved by the appropriate institutional review boards, and all patients provided written informed consent before enrollment [13,14].

2.2. Assessments and measurements

The MARINE and ANCHOR studies measured serum RLP-C as a prespecified exploratory endpoint using an immunoseparation assay from Polymedco (Cortlandt, NY, USA) on a Daytona chemistry analyzer (Randox Laboratories, Crumlin, UK) [16–18]. A central laboratory measured other lipid parameters as previously described, including LDL-C measurements by preparative ultracentrifugation (beta-quantification) [13,19]. Plasma lipid parameter evaluations included patients from the intent-to-treat population, defined as all randomized patients who had a baseline TG

measurement, received ≥1 dose of the study drug, and had ≥1 post-randomization efficacy measurement.

2.3. Statistical analysis

Medians and interquartile ranges of RLP-C were calculated for each treatment group at baseline and at week 12, and for the percent changes from baseline. The median differences in percent changes from baseline for RLP-C were estimated between the icosapent ethyl and placebo groups using the Hodges-Lehmann method, with the Wilcoxon rank sum test used to calculate *p* values. Subgroup analyses were conducted by baseline TG level in each study, by statin use in the MARINE study, and by the statin intensity (lower vs medium/higher) in the ANCHOR study. This post hoc analysis computed Pearson correlation coefficients to assess the relationships between calculated RLP-C and directly measured RLP-C for baseline, week 12, and percent change from baseline values. Each analysis utilized pooled data from the icosapent ethyl treatment and placebo groups. A *p* value of 0.05 was the pre-specified alpha for significance for exploratory endpoints in the MARINE and ANCHOR studies and was used in all analyses, including post hoc analyses in this report. The formula of RLP-C (TC − [HDL-C] − [LDL-C]; LDL-C being measured or calculated) was employed for correlation analyses involving calculated RLP-C levels [3]. For these RLP-C calculations, LDL-C was either directly measured by preparative ultracentrifugation (beta-quantification) or, when applicable, calculated. When calculated LDL-C was included in the RLP-C equation, the Friedewald equation (LDL-C = TC − [HDL-C] − [TG/5]) was applied, but only for patients with TG levels <400 mg/dL (<4.52 mmol/L) [20]. When applicable, resulting calculated RLP-C values are reported for both LDL-C methods.

3. Results

3.1. Patients

Baseline demographics and lipid parameters were comparable among treatment groups within each study (Table 1). In MARINE, 57 of 229 patients (25%) were receiving statin therapy, whereas in ANCHOR, all 702 patients were on statins, of whom 654 (93%) were on medium- or higher-intensity statin regimens (definitions of medium- and higher-intensity statin regimens are listed in the legend of Table 2). An immunoseparation assay was used to assess total RLP-C levels in MARINE and ANCHOR. In MARINE, RLP-C measurements were available from 218 patients (95% of study cohort); in ANCHOR, as prespecified in the study protocol, RLP-C measurements were to be conducted in (approximately) the first 240 patients enrolled (actual n = 252; 36% of study cohort).

Within each study, treatment groups showed comparable median baseline levels of TGs, non-HDL-C, LDL-C, VLDL-C, VLDL-TG, and ApoB in the subset of patients with RLP-C measurements (Table 1) as well as in the intent-to-treat populations of each study [13,14]. Each study also had comparable median RLP-C levels at baseline across treatment groups, ranging from 43.0 to 47.0 mg/dL (1.11–1.22 mmol/L) in MARINE and from 13.5 to 15.0 mg/dL (0.35–0.39 mmol/L) in ANCHOR (Table 2).

3.2. MARINE

At the approved dose of 4 g/day, icosapent ethyl significantly reduced median levels of directly measured RLP-C by −29.8% (*p* = 0.004) (Fig. 1 and Table 2) *versus* placebo. When evaluated by statin use, icosapent ethyl 4 g/day significantly reduced directly measured RLP-C levels by −21.4% in patients not receiving statins

Table 1
Baseline characteristics (randomized populations) and baseline lipid parameters (patients from ITT populations with RLP-C measurements).

	Icosapent ethyl 4 g/day	Icosapent ethyl 2 g/day	Placebo
Baseline characteristics			
MARINE	**n = 77**	**n = 76**	**n = 76**
Age, mean (SD), y	51.9 (10.27)	53.4 (9.34)	53.4 (8.34)
Male, n (%)	59 (77)	58 (76)	58 (76)
Weight, mean (SD), kg	93.2 (18.27)	92.1 (15.57)	93.0 (16.92)
BMI, mean (SD), kg/m^2	30.4 (4.29)	30.8 (4.24)	31.0 (4.25)
Diabetes, n (%)	22 (29)	20 (26)	21 (28)
Statin use, n (%)	20 (26)	19 (25)	18 (24)
ANCHOR	**n = 233**	**n = 236**	**n = 233**
Age, mean (SD), y	61.1 (10.03)	61.8 (9.42)	61.2 (10.05)
Male, n (%)	142 (61)	144 (61)	145 (62)
Weight, mean (SD), kg	94.5 (18.30)	95.5 (18.29)	97.0 (19.14)
BMI, mean (SD), kg/m^2	32.7 (4.99)	32.9 (4.98)	33.0 (5.04)
Diabetes, n (%)	171 (73)	172 (73)	171 (73)
Baseline lipid parameters[a]			
MARINE	**n = 75**	**n = 70**	**n = 73**
TG, mg/dL	679.5 (268.0)	660.5 (304.0)	706.0 (413.0)
LDL-C, mg/dL	90.0 (45.0)	81.0 (61.0)	81.0 (56.0)
Non-HDL-C, mg/dL	225.0 (93.0)	211.5 (77.0)	230.0 (85.0)
VLDL-C, mg/dL	122.0 (101.0)	121.5 (59.0)	130.0 (81.0)
VLDL-TG, mg/dL	521.0 (352.0)	506.0 (447.0)	549.0 (453.0)
ApoB, mg/dL	121 (34.0)	117.5 (35.0)	118.0 (39.0)
ANCHOR	**n = 82**	**n = 84**	**n = 86**
TG, mg/dL	255.8 (90.0)	262.5 (83.3)	264.3 (77.5)
LDL-C, mg/dL[b]	78.0 (22.0)	83.0 (22.0)	80.0 (29.0)
Non-HDL-C, mg/dL	122.0 (32.0)	124.5 (31.5)	124.0 (29.0)
VLDL-C, mg/dL[b]	43.5 (19.0)	41.0 (20.0)	41.0 (16.0)
VLDL-TG, mg/dL[b]	195.5 (102.0)	192.0 (86.0)	197.0 (89.0)
ApoB, mg/dL	88.5 (24.0)	91.0 (19.0)	92.0 (22.0)

ITT, intent to treat.

[a] Data are presented as medians (interquartile ranges) for baseline lipid parameter values. Baseline RLP-C levels are shown in Table 2. To convert from mg/dL to the International System unit of mmol/L, multiply cholesterol by 0.0259 and multiply TG by 0.0113. To convert ApoB levels to the International System unit of g/L, multiply by 0.01.

[b] Data shown for 82 patients in the icosapent ethyl 4 g/day group, 83 patients in the icosapent ethyl 2 g/day group, and 85 patients in placebo group.

($p = 0.05$; n = 56) and by −56.8% in those receiving statins ($p = 0.02$; n = 19) *versus* placebo (Fig. 2 and Table 2). When grouped by baseline TG level, icosapent ethyl 4 g/day significantly reduced directly measured RLP-C levels by −37.5% in patients with baseline TG levels >750 mg/dL (>8.48 mmol/L; $p = 0.02$; n = 28) *versus* placebo; icosapent ethyl 4 g/day also reduced directly measured RLP-C levels in patients with baseline TG levels ≤750 mg/dL (≤8.48 mmol/L) *versus* placebo, although this reduction only approached significance (−26.1%; $p = 0.06$; n = 47) (Table 2).

3.3. ANCHOR

Icosapent ethyl 4 g/day significantly reduced median levels of directly measured RLP-C by −25.8% ($p = 0.0001$) overall compared with placebo (Fig. 1 and Table 2). The effect of icosapent ethyl 4 g/day on directly measured RLP-C levels was statistically significant *versus* placebo in patients with TG values below or above the study-wide baseline median of 259 mg/dL (2.93 mmol/L); icosapent ethyl 4 g/day reduced directly measured RLP-C levels by −22.2% in patients with TG levels below the median ($p = 0.03$; n = 42) and by −30.6% in those with TG levels above the median ($p = 0.001$; n = 40) (Table 2). When evaluated by statin regimen intensity, icosapent ethyl 4 g/day significantly reduced directly measured RLP-C levels by −26.7% ($p < 0.001$; n = 77) *versus* placebo when used with a medium- or higher-intensity statin regimen, whereas changes were not statistically significant *versus* placebo when icosapent ethyl 4 g/day was used with a lower-intensity statin regimen ($p = 0.5$; n = 5) (Table 2).

3.4. Correlation analyses

Calculated RLP-C levels from both the MARINE and ANCHOR

studies correlated with directly measured RLP-C levels (r = 0.63−0.92; $p < 0.0001$ for all) in analyses of values at baseline, week 12, and percent change from baseline. In ANCHOR, directly measured RLP-C had higher correlations with calculated values that were derived from LDL-C measured with beta-quantification at baseline and follow-up (0.79 and 0.91, respectively) compared with the LDL-C derived by the Friedewald equation (0.63 and 0.76) (Table 3). In MARINE and ANCHOR, similar percent changes from baseline *versus* placebo were seen for directly measured and calculated RLP-C using LDL-C measured by beta-quantification, with a larger discrepancy between results observed in ANCHOR between directly measured and calculated RLP-C when using LDL-C derived by the Friedewald equation (Table 3).

4. Discussion

High RLP-C or remnant cholesterol levels are strong markers of coronary risk and are likely causal factors for ischemic heart disease [3,6,21,22]. The present analysis examined the effects of icosapent ethyl on directly measured and calculated RLP-C levels in patients with very high TG levels with or without concomitant statin therapy from the MARINE study and in patients with high TG levels despite statin therapy from the ANCHOR study. Previous analyses reported that icosapent ethyl 4 g/day significantly lowered TG, non-HDL-C, ApoB, and ApoC-III levels and decreased LDL particle number compared with placebo (i.e., potentially reducing LDL atherogenicity) without increasing LDL-C levels [13,14,23,24]. The present report demonstrates that icosapent ethyl significantly reduces RLP-C compared with placebo in patients from the MARINE and ANCHOR studies as measured directly via an immunoseparation assay or when calculated. In these studies, icosapent ethyl 4 g/day reduced RLP-C across a wide range of baseline TG levels, with

Table 2
Change from baseline to week 12 in directly measured RLP-C levels in patients from the MARINE and ANCHOR studies.

| | Directly measured RLP-C concentration[a] | | | | | | | | | Median change from baseline in RLP-C concentration *vs.* placebo[b] | |
| | Icosapent ethyl 4 g/day | | | Icosapent ethyl 2 g/day | | | Placebo | | | Icosapent ethyl 4 g/day | Icosapent ethyl 2 g/day |
	Baseline, mg/dL	End of treatment, mg/dL	Change, %	Baseline, mg/dL	End of treatment, mg/dL	Change, %	Baseline, mg/dL	End of treatment, mg/dL	Change, %	%, *p*	%, *p*
MARINE	n = 76			n = 73			n = 75				
ITT[c]	n = 75			n = 70			n = 73				
	45.0	38.0	−16.1	43.0	44.5	+5.8	47.0	58.0	+14.2	−29.8	−14.9
	(53.0)	(45.0)	(86.5)	(37.0)	(38.0)	(85.5)	(58.0)	(90.0)	(105.4)	0.0041	0.1528
Current statin use											
No	n = 56			n = 52			n = 56				
	45.0	39.5	−16.6	45.0	44.5	+3.0	50.5	54.5	+7.0	−21.4	−8.5
	(55.5)	(48.5)	(85.9)	(34.5)	(36.0)	(67.6)	(61.0)	(77.5)	(107.0)	0.0456	0.4421
Yes	n = 19			n = 18			n = 17				
	37.0	33.0	−16.1	31.5	46.5	+29.2	40.0	65.0	+54.3	−56.8	−41.6
	(45.0)	(27.0)	(136.4)	(47.0)	(50.0)	(108.0)	(38.0)	(124.0)	(116.9)	0.0198	0.1511
Baseline TG[d]											
≤750 mg/dL	n = 47			n = 42			n = 41				
	34.0	29.0	−8.8	27.5	32.5	+18.2	30.0	33.0	+19.0	−26.1	−7.3
	(30.0)	(28.0)	(100.0)	(23.0)	(23.0)	(85.8)	(21.0)	(31.0)	(95.5)	0.0570	0.6554
>750 mg/dL	n = 28			n = 28			n = 32				
	89.5	68.5	−25.4	66.0	65.0	−10.7	90.5	100.5	+5.7	−37.5	−25.4
	(109.0)	(73.0)	(59.7)	(51.5)	(35.5)	(72.5)	(75.5)	(89.5)	(111.1)	0.0196	0.1016
ANCHOR	n = 226			n = 234			n = 227				
ITT[c]	n = 82			n = 84			n = 86				
	13.5	10.0	−24.0	15.0	11.0	−11.1	14.0	13.0	+8.0	−25.8	−16.7
	(6.0)	(6.0)	(45.5)	(7.0)	(7.0)	(40.0)	(7.0)	(9.0)	(66.9)	0.0001	0.0153
Statin intensity[e]											
Lower	n = 5			n = 7			n = 6				
	16.0	15.0	−29.2	15.0	11.0	−11.1	15.0	11.0	−19.4	−11.9	2.7
	(3.0)	(5.0)	(33.3)	(6.0)	(9.0)	(21.1)	(6.0)	(24.0)	(105.1)	0.5228	0.8303
Medium and higher	n = 77			n = 77			n = 80				
	13.0	10.0	−23.1	15.0	11.0	−11.1	14.0	13.0	−8.7	−26.7	−17.3
	(6.0)	(6.0)	(45.5)	(7.0)	(7.0)	(42.3)	(7.0)	(8.5)	(66.5)	0.0002	0.0135
Study-wide median TG (259 mg/dL)[f]											
<259 mg/dL	n = 42			n = 40			n = 37				
	11.0	9.0	−16.0	11.5	11.0	−8.1	11.0	12.0	+9.1	−22.2	−16.4
	(4.0)	(5.0)	(47.6)	(5.0)	(3.5)	(37.4)	(5.0)	(7.0)	(58.2)	0.0263	0.1092
≥259 mg/dL	n = 40			n = 44			n = 49				
	17.0	11.0	−31.3	18.0	13.5	−17.4	16.0	16.0	+7.7	−30.6	−18.0
	(5.5)	(6.5)	(37.6)	(8.0)	(7.0)	(37.2)	(4.0)	(11.0)	(64.1)	0.0010	0.0658

ITT, intent to treat.

[a] Data are presented as medians (interquartile ranges) for endpoint values. To convert from mg/dL to the International System unit of mmol/L, multiply cholesterol by 0.0259 and multiply TG by 0.0113.

[b] Median percent changes vs placebo are Hodges-Lehmann medians.

[c] Patients from ITT populations with RLP-C measurements.

[d] Per study protocol, patients in MARINE were stratified at randomization by baseline TG level ≤750 mg/dL and >750 mg/dL.

[e] Lower-intensity statin regimens: simvastatin 5–10 mg; medium- and higher-intensity statin regimens: rosuvastatin 5–10 mg, atorvastatin 10–20 mg, simvastatin 20–40 mg, simvastatin 10–20 mg plus ezetimibe 5–10 mg, rosuvastatin 20–40 mg, atorvastatin 40–80 mg, simvastatin 80 mg, simvastatin 40–80 mg plus ezetimibe 5–10 mg.

[f] Study-wide median represents the median of patients assessed in the 4 g/day, 2 g/day, and placebo groups.

reductions in RLP-C greatest among patients with higher baseline TG levels.

As statins are the cornerstone of lipid-lowering therapy, it is worth noting that icosapent ethyl 4 g/day reduced RLP-C with or without concomitant statin therapy in the MARINE study and in the statin-treated patients in the ANCHOR study. In the latter study, the reductions in RLP-C with icosapent ethyl 4 g/day appeared greater in patients receiving medium-to higher-intensity statin regimens than in patients receiving lower-intensity statin regimens. However, small sample size may be a limiting factor in the subgroup analyses of patients receiving lower-intensity statin regimens in the ANCHOR study, and of patients receiving statin therapy in the MARINE study. The medium- and higher-intensity statin regimens used in this analysis correspond with current recommendations for statin therapy [25,26].

Results of the present analysis expand on previously reported results in patients with type 2 diabetes in the ANCHOR study, showing that treatment with icosapent ethyl 4 g/day reduced directly measured RLP-C levels by −25% *versus* placebo ($p < 0.01$) in this patient subset [27]. Other prescription omega-3 fatty acid products, including another pure EPA product from Japan, as well as products containing both EPA and docosahexaenoic acid, have also been reported to reduce RLP-C levels in plasma at moderate to high doses [27–33].

Residual cardiovascular risk can persist in patients with elevated TG and RLP-C levels even after lowering of LDL-C levels [1,6,7]. Some of this residual risk may be explained by elevated remnant cholesterol levels. Notably, standard measurements of TG levels or LDL-C calculations do not account for this atherogenic cholesterol content [34]. Cholesterol-rich remnant lipoprotein particles may
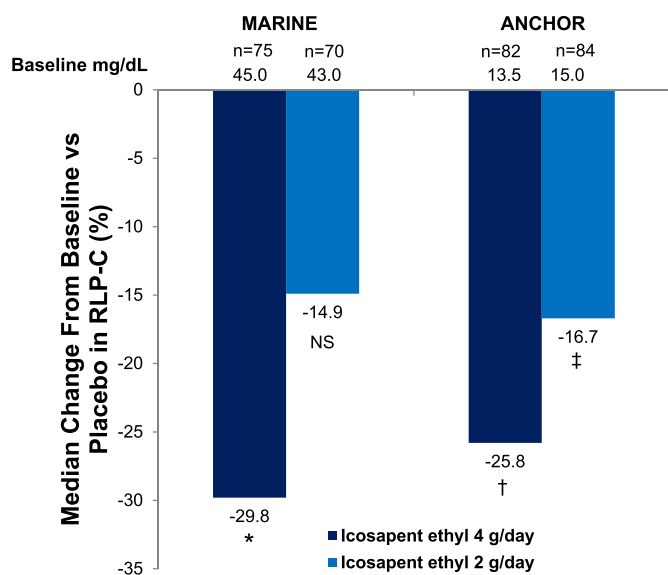
Fig. 1. **Median percent change from baseline to week 12 *vs*. placebo in directly measured RLP-C levels in patients from the MARINE and ANCHOR studies**. Median differences in percent changes *vs*. placebo are Hodges-Lehmann medians. *$p < 0.01$; †$p = 0.0001$; ‡$p < 0.05$; NS, not significant; RLP-C, remnant-like particle cholesterol.
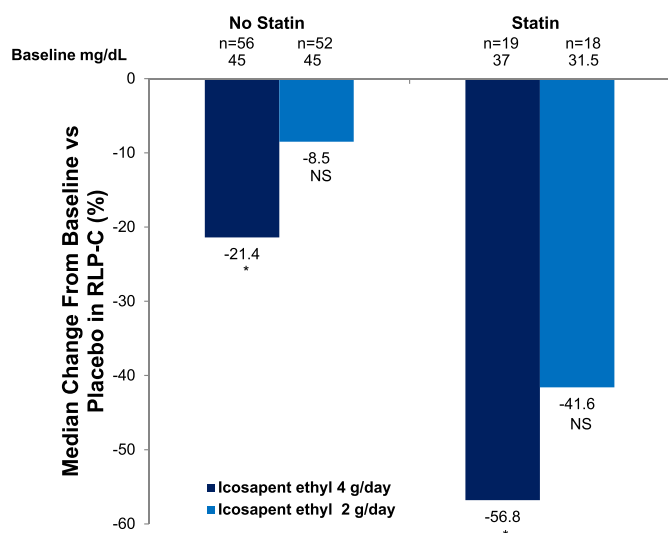
Fig. 2. **Median percent change from baseline to week 12 *vs*. placebo in directly measured RLP-C levels by statin use in patients from the MARINE study**. Median differences in percent changes *vs*. placebo are Hodges-Lehmann medians. *$p < 0.05$; NS, not significant; RLP-C, remnant-like particle cholesterol.

contain more than 5 times the cholesterol per particle compared with low-density lipoproteins (LDLs) [35]. RLP-C crosses the endothelial barrier and may become trapped in the subendothelial space preferentially to LDL-C, possibly due to the larger size of the remnant particles [34], but unlike LDL particles, remnant particles do not require oxidative modification for uptake and can be taken up in an unregulated fashion by macrophages [1]. RLP-C then contributes to multiple steps in the development and progression of atherosclerosis including endothelial dysfunction, foam cell formation, and vascular inflammation [35—37]. RLP-C may have pro-inflammatory properties and may cause endothelial dysfunction via actions on endothelial nitric oxide synthase [37]. Remnant-like lipoprotein particles can induce endothelial dysfunction, including impaired endothelial vasodilatation and abnormal

endothelial secretion, thereby contributing to development of atherosclerosis [37]. RLP-C levels also correlate with the vulnerability of coronary plaques for rupture [38]. Thus, add-on therapy to a statin may be needed to address residual cardiovascular risk. The recent IMPROVE-IT outcomes study demonstrated that statin add-on therapy can help address residual cardiovascular risk and that lower LDL-C levels are associated with lower risk [39,40]. The present results show that, compared with placebo, icosapent ethyl lowers RLP-C levels in statin-treated patients in addition to its previously reported reductions in TG, non-HDL-C, ApoB, and ApoC-III levels and LDL particle number without increasing LDL-C levels. These findings suggest that icosapent ethyl 4 g/day may provide a beneficial option in a statin-treated patient population such as that of ANCHOR. The ongoing REDUCE-IT trial (NCT01492361) is evaluating whether icosapent ethyl 4 g/day will reduce cardiovascular events when used as an adjunct to statin therapy in patients with persistently high TG levels who have a high risk of future cardiovascular events [41]. It should be noted that icosapent ethyl is not approved by the United States Food and Drug Administration to reduce the risk of coronary heart disease; the effect of icosapent ethyl on the risk of cardiovascular mortality and morbidity has not been determined.

As noted earlier, different methods (calculated, vertical auto profile testing, and immunoseparation) may be used to measure RLP-C, but these methods currently lack standardization. A recent study found that reductions observed with one method can be expected to occur across all methods, but RLP-C defined by immunoseparation and vertical auto profile testing may differ in mass and response to pharmacologic intervention [4]. Thus, standardization of measurement methods may be needed for use of RLP-C in assessing cardiovascular risk [4]. In our correlation analyses of calculated RLP-C *versus* directly measured RLP-C, we found strong correlations in baseline, 12-week, and percent change from baseline analyses, despite differences in the amount of RLP-C reported by each method. Furthermore, we also found statistically significant correlations between directly measured RLP-C and calculated RLP-C when RLP-C was derived from LDL-C values based on either the Friedewald equation for patients with TG levels <400 mg/dL (<4.52 mmol/L) or based on beta-quantification wherein the cholesterol content of LDL is directly measured. While direct RLP-C measurements require a specific test that utilizes a two-step immunoseparation procedure, clinicians can quickly calculate RLP-C from a standard lipid panel by subtracting LDL-C from non-HDL-C [3]. These methods, however, do not produce identical values; yet, in clinical practice, change in calculated RLP-C may still provide a useful and readily accessible estimation of how interventions such as icosapent ethyl may be impacting a patient's remnant cholesterol burden. Although direct measurements of RLP-C in this study had a better correlation to calculated RLP-C when using LDL-C measured with beta quantification, RLP-C calculated using Friedewald-derived LDL-C may represent an easily accessible and inexpensive means in clinical practice to monitor approximate changes in RLP-C for patients with TG < 400 mg/dL (<4.52 mmol/L). Large prospective clinical outcome studies are needed to fully assess the effects of icosapent ethyl on remnant cholesterol.

Mechanisms by which icosapent ethyl may decrease RLP-C have not yet been fully elucidated but may result from a reduction in VLDL particle number through reduced hepatic release and/or increased plasma clearance. The potential mechanisms of action of EPA include increased β-oxidation; inhibition of acyl-CoA:1,2-diacylglycerol acyltransferase; decreased lipogenesis in the liver; and increased plasma lipoprotein lipase activity [42].

In addition to limitations mentioned earlier, it should be noted that although the data are not pooled, they come from two different

Table 3
Correlation analysis of calculated RLP-C *vs.* directly measured RLP-C levels.

Population	Correlation between calculated and directly measured RLP-C[a]			Median change from baseline in RLP-C concentration for icosapent ethyl 4 g/day vs. placebo[b]	
	Baseline r value, p value	End of treatment r value, p value	% Change r value, p value	Directly measured[c] %, p value	Calculated %, p value
MARINE					
Calculated RLP-C based on measured LDL-C[d]	n = 224 0.88 <0.0001	n = 218 0.92 <0.0001	n = 218 0.84 <0.0001	n = 76[e] −29.8 0.0041	n = 76[g] −28.9 0.0002
ANCHOR					
Calculated RLP-C based on measured LDL-C[d]	n = 250 0.79 <0.0001	n = 250 0.91 <0.0001	n = 250 0.73 <0.0001	n = 82[e] −25.8 0.0001	n = 82[g] −31.7 <0.0001
Calculated RLP-C based on calculated LDL-C[f]	n = 239 0.63 <0.0001	n = 224 0.76 <0.0001	n = 216 0.74 <0.0001		n = 74[g] −16.6 0.0009

[a] Correlation analyses were conducted on pooled data from the icosapent ethyl and placebo groups. RLP-C was calculated as TC − (HDL-C) − (LDL-C) using measured or calculated LDL-C.

[b] Median differences in percent changes with icosapent ethyl 4 g/day vs placebo are Hodges-Lehmann medians.

[c] Directly measured values are carried over from Table 2 for comparison.

[d] Calculated RLP-C levels were based on LDL-C levels as directly measured using beta-quantification.

[e] MARINE: n = 76 for icosapent ethyl 4 g/day, n = 75 for placebo; ANCHOR: n = 82 for icosapent ethyl 4 g/day, n = 86 for placebo.

[f] Calculated RLP-C levels were based on LDL-C levels attained via the Friedewald equation (LDL-C = TC − [HDL-C] − [TG/5]) in patients with TG levels <400 mg/dL (<4.52 mmol/L).

[g] MARINE (LDL-C measured): n = 76 for icosapent ethyl 4 g/day, n = 75 for placebo; ANCHOR (LDL-C measured): n = 82 for icosapent ethyl 4 g/day, n = 85 for placebo; ANCHOR (LDL-C calculated): n = 74 for icosapent ethyl 4 g/day, n = 68 for placebo.

studies with patient populations that differed in terms of lipids (e.g., baseline TG levels were higher in MARINE than in ANCHOR by design), demographics (e.g., MARINE was an international study, ANCHOR was conducted solely in the United States), and medication use (e.g., statin use was allowed in MARINE and required in ANCHOR).

In summary, icosapent ethyl 4 g/day, as compared with placebo, significantly reduced RLP-C in addition to its previously described effects in lowering TG, non-HDL-C, ApoB, and ApoC-III levels and LDL particle number without raising LDL-C levels. This analysis extends the findings to date regarding the potentially beneficial lipid effects of icosapent ethyl in patients with elevated TG levels, including those on statin therapy. The ongoing REDUCE-IT trial will help to clarify whether the improvements in RLP-C and other atherogenic parameters seen with high-purity prescription EPA at 4 g/day will translate to a reduction in major cardiovascular events in statin-treated patients with persistent hypertriglyceridemia.

Conflict of interest

Dr. Ballantyne has received research/grant support from Abbott Diagnostics, Amarin Pharma Inc., Amgen, Eli Lilly, Esperion, Ionis, Novartis, Otsuka, Pfizer, Regeneron, Roche Diagnostic, Sanofi-Synthelabo, Takeda, NIH, ADA, and AHA (all paid to institution, not individual), and is a consultant for Abbott Diagnostics, Amarin Pharma Inc., Amgen, AstraZeneca, Eli Lilly, Esperion, Genzyme, Matinas Biopharma, Merck, Novartis, Pfizer, Regeneron, Roche Diagnostics, and Sanofi-Synthelabo.

Dr. Harold Bays owns no pharmaceutical stocks or patents. In the past 36 months, Dr. Harold Bays' research site has received research grants from Amarin Pharma Inc., Amgen, Ardea, Arisaph, AstraZeneca, Bristol-Meyers Squibb, Catabasis, Cymabay, Eisai, Elcelyx, Eli Lilly, Esperion, Ferrer/Chiltern, Gilead, GSK, Hanmi, Hisun, Hoffman LaRoche, Home Access, Janssen, Johnson & Johnson, Kowa, Merck, Nektar, Novartis, NovoNordisk, Omthera, Orexigen, Pfizer, Pronova, Regeneron, Sanofi, Takeda, and TIMI. In the past 36 months, Dr. Harold Bays has served as a consultant/advisor for Akcea, Alnylam, Amgen, AstraZeneca, Catabasis, Eli Lilly, Ionis,

Janssen, Johnson & Johnson, Merck, Novartis, Pronova, Regeneron, Sanofi, and Takeda. In the past 36 months, Dr. Harold Bays has served as a speaker for Amarin Pharma Inc., Amgen, Astra Zeneca, Eisai, Orexigen, Regeneron, Sanofi, and Takeda.

Dr. Philip, Dr. Juliano, and Mr. Doyle are employees and stock holders of Amarin Pharma Inc.

Drs. Braeckman, Stirtan, and Soni are former employees and stock holders of Amarin Pharma Inc.

References

[1] A. Varbo, M. Benn, B.G. Nordestgaard, Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment, Pharmacol. Ther. 141 (2014) 358–367.

[2] S.R. Jones, S.S. Martin, E.A. Brinton, Letter by Jones et al regarding article, "Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation", Circulation 129 (2014) e655.

[3] A. Varbo, M. Benn, A. Tybjaerg-Hansen, A.B. Jorgensen, R. Frikke-Schmidt, B.G. Nordestgaard, Remnant cholesterol as a causal risk factor for ischemic heart disease, J. Am. Coll. Cardiol. 61 (2013) 427–436.

[4] P.P. Toth, H. Bays, W. Brown, J. Tomassini, C. Wang, A. Polis, et al., Cholesterol in remnant-lipoproteins as measured by different methods [abstract], J. Am. Coll. Cardiol. 65 (2015) A1569.

[5] A. Varbo, M. Benn, A. Tybjaerg-Hansen, B.G. Nordestgaard, Reply to letters regarding article, "elevated remnant cholesterol causes both low-grade

inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation, Circulation 129 (2014) e656.

[6] T. Nakamura, J.E. Obata, M. Hirano, Y. Kitta, D. Fujioka, Y. Saito, et al., Predictive value of remnant lipoprotein for cardiovascular events in patients with coronary artery disease after achievement of LDL-cholesterol goals, Atherosclerosis 218 (2011) 163–167.

[7] S.V. Nguyen, T. Nakamura, K. Kugiyama, High remnant lipoprotein predicts recurrent cardiovascular events on statin treatment after acute coronary syndrome, Circ. J. 78 (2014) 2492–2500.

[8] S.V. Nguyen, T. Nakamura, M. Uematsu, D. Fujioka, K. Watanabe, Y. Watanabe, et al., Remnant lipoproteinemia predicts cardiovascular events in patients with type 2 diabetes and chronic kidney disease, J. Cardiol. (2016) (Epub ahead of print).

[9] P.H. Joshi, A.A. Khokhar, J.M. Massaro, S.T. Lirette, M.E. Griswold, S.S. Martin, et al., Remnant lipoprotein cholesterol and incident coronary heart disease: the jackson heart and framingham offspring cohort studies, J. Am. Heart Assoc. 5 (2016).

[10] A.K. Jepsen, A. Langsted, A. Varbo, L.E. Bang, P.R. Kamstrup, B.G. Nordestgaard, Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease, Clin. Chem. 62 (2016) 593–604.

[11] P.P. Toth, Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease, Vasc. Health Risk Manag. 12 (2016) 171–183.

[12] Vascepa [package insert], Bedminster, NJ, Amarin Pharma Inc., 2015.

[13] H.E. Bays, C.M. Ballantyne, J.J. Kastelein, J.L. Isaacsohn, R.A. Braeckman, P.N. Soni, Eicosapentaenoic acid ethyl ester (AMR101) therapy in patients with very high triglyceride levels (from the Multi-center, plAcebo-controlled, Randomized, double-blINd, 12-week study with an open-label Extension [MARINE] trial), Am. J. Cardiol. 108 (2011) 682–690.

[14] C.M. Ballantyne, H.E. Bays, J.J. Kastelein, E. Stein, J.L. Isaacsohn, R.A. Braeckman, et al., Efficacy and safety of eicosapentaenoic acid ethyl ester (AMR101) therapy in statin-treated patients with persistent high triglycerides (from the ANCHOR study), Am. J. Cardiol. 110 (2012) 984–992.

[15] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report, Circulation 106 (2002) 3143–3421.

[16] S. Lamon-Fava, D.M. Herrington, D.M. Reboussin, M. Sherman, K.V. Horvath, L.A. Cupples, et al., Plasma levels of HDL subpopulations and remnant lipoproteins predict the extent of angiographically-defined coronary artery disease in postmenopausal women, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 575–579.

[17] J.R. McNamara, P.K. Shah, K. Nakajima, L.A. Cupples, P.W. Wilson, J.M. Ordovas, et al., Remnant lipoprotein cholesterol and triglyceride reference ranges from the Framingham Heart Study, Clin. Chem. 44 (1998) 1224–1232.

[18] K. Nakajima, T. Saito, A. Tamura, M. Suzuki, T. Nakano, M. Adachi, et al., Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels, Clin. Chim. Acta 223 (1993) 53–71.

[19] R.A. Braeckman, M.S. Manku, H.E. Bays, W.G. Stirtan, P.N. Soni, Icosapent ethyl, a pure EPA omega-3 fatty acid: effects on plasma and red blood cell fatty acids in patients with very high triglyceride levels (results from the MARINE study), Prostagl. Leukot. Essent. Fat. Acids 89 (2013) 195–201.

[20] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.

[21] T. Nakamura, H. Takano, K. Umetani, K. Kawabata, J.E. Obata, Y. Kitta, et al., Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome, Atherosclerosis 181 (2005) 321–327.

[22] A. Varbo, M. Benn, A. Tybjaerg-Hansen, B.G. Nordestgaard, Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation, Circulation 128 (2013) 1298–1309.

[23] C.M. Ballantyne, H.E. Bays, R.A. Braeckman, W.G. Stirtan, R.T. Doyle Jr., R. Juliano, et al., Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on apolipoprotein C-III in patients from the MARINE and ANCHOR studies [abstract 122], J. Clin. Lipidol. 8 (2014) 313–314.

[24] C.M. Ballantyne, R.A. Braeckman, H.E. Bays, J.J. Kastelein, J.D. Otvos, W.G. Stirtan, et al., Effects of icosapent ethyl on lipoprotein particle concentration and size in statin-treated patients with persistent high triglycerides (the ANCHOR Study), J. Clin. Lipidol. 9 (2015) 377–383.

[25] N.J. Stone, J. Robinson, A.H. Lichtenstein, C.N. Bairey Merz, D.M. Lloyd-Jones, C.B. Blum, et al., 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, J. Am. Coll. Cardiol. 63 (2014) 2889–2934.

[26] T.A. Jacobson, M.K. Ito, K.C. Maki, C.E. Orringer, H.E. Bays, P.H. Jones, et al., National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1-executive summary, J. Clin. Lipidol. 8 (2014) 473–488.

[27] E.A. Brinton, C.M. Ballantyne, H.E. Bays, J.J. Kastelein, R.A. Braeckman, P.N. Soni, Effects of icosapent ethyl on lipid and inflammatory parameters in patients with diabetes mellitus-2, residual elevated triglycerides (200–500 mg/dL), and on statin therapy at LDL-C goal: the ANCHOR study, Cardiovasc. Diabetol. 12 (2013) 100.

[28] N. Nakamura, T. Hamazaki, M. Kobayashi, M. Ohta, K. Okuda, Effects of eicosapentaenoic acids on remnant-like particles, cholesterol concentrations and plasma fatty acid composition in patients with diabetes mellitus, In Vivo 12 (1998) 311–314.

[29] M. Ando, T. Sanaka, H. Nihei, Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients, J. Am. Soc. Nephrol. 10 (1999) 2177–2184.

[30] N. Satoh, A. Shimatsu, K. Kotani, N. Sakane, K. Yamada, T. Suganami, et al., Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome, Diabetes Care 30 (2007) 144–146.

[31] J.J.P. Kastelein, K.C. Maki, A. Susekov, M. Ezhov, B.G. Nordestgaard, B.N. Machielse, et al., Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: the EpanoVa fOr Lowering Very high triglyceridEs (EVOLVE) trial, J. Clin. Lipidol. 8 (2014) 94–108.

[32] M.H. Davidson, K.C. Maki, H. Bays, R. Carter, C.M. Ballantyne, Effects of prescription omega-3 ethyl esters on lipoprotein particle concentrations, apolipoproteins AI and CIII, and lipoprotein-associated phospholipase A2 mass in statin-treated subjects with hypertriglyceridemia, J. Clin. Lipidol. 3 (2009) 332–340.

[33] H.E. Bays, J. McKenney, K.C. Maki, R.T. Doyle, R.N. Carter, E. Stein, Effects of prescription omega-3-acid ethyl esters on non–high-density lipoprotein cholesterol when coadministered with escalating doses of atorvastatin, Mayo Clin. Proc. 85 (2010) 122–128.

[34] K.T. White, M.V. Moorthy, A.O. Akinkuolie, O. Demler, P.M. Ridker, N.R. Cook, et al., Identifying an optimal cut point for the diagnosis of hypertriglyceridemia in the nonfasting state, Clin. Chem. 61 (2015) 1156–1163.

[35] R. McPherson, Remnant cholesterol: "Non-(HDL-C + LDL-C)" as a coronary artery disease risk factor, J. Am. Coll. Cardiol. 61 (2013) 437–439.

[36] S.A. Khetarpal, D.J. Rader, Triglyceride-rich lipoproteins and coronary artery disease risk: new insights from human genetics, Arterioscler. Thromb. Vasc. Biol. 35 (2015) e3–e9.

[37] X.Y. Zheng, L. Liu, Remnant-like lipoprotein particles impair endothelial function: direct and indirect effects on nitric oxide synthase, J. Lipid Res. 48 (2007) 1673–1680.

[38] N. Matsuo, T. Matsuoka, S. Onishi, H. Yamamoto, A. Kato, Y. Makino, et al., Impact of remnant lipoprotein on coronary plaque components, J. Atheroscler. Thromb. 22 (2015) 783–795.

[39] C.P. Cannon, M.A. Blazing, R.P. Giugliano, A. McCagg, J.A. White, P. Theroux, et al., Ezetimibe added to statin therapy after acute coronary syndromes, N. Engl. J. Med. 372 (2015) 2387–2397.

[40] R. McPherson, R.A. Hegele, Ezetimibe: rescued by randomization (clinical and Mendelian), Arterioscler. Thromb. Vasc. Biol. 35 (2015) e13–e15.

[41] A Study of AMR101 to Evaluate its Ability to Reduce Cardiovascular Events in High Risk Patients with Hypertriglyceridemia and on Statin (REDUCE-IT), ClinicalTrials. gov, 2015.

[42] H.E. Bays, A.P. Tighe, R. Sadovsky, M.H. Davidson, Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications, Expert Rev. Cardiovasc. Ther. 6 (2008) 391–409.