Prostaglandins & other Lipid Mediators 125 (2016) 57–64

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Icosapent ethyl: Eicosapentaenoic acid concentration and triglyceride-lowering effects across clinical studies

Harold E. Bays (MD)[a,*], Christie M. Ballantyne (MD)[b], Ralph T. Doyle Jr. (BA)[c], Rebecca A. Juliano (PhD)[c], Sephy Philip (RPh, PharmD)[c]

[a] Louisville Metabolic and Atherosclerosis Research Center, 3288 Illinois Avenue, Louisville, KY 40213, USA
[b] Baylor College of Medicine and the Houston Methodist DeBakey Heart and Vascular Center, 6565 Fannin, M.S. A-601, Houston, TX 77030, USA
[c] Amarin Pharma Inc., 1430 Route 206, Suite 200, Bedminster, NJ 07921, USA

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 20 June 2016
Accepted 8 July 2016
Available online 11 July 2016

Keywords:
Eicosapentaenoic acid
Hypertriglyceridemia
Icosapent ethyl
Pharmacodynamics
Triglycerides

ABSTRACT

Icosapent ethyl is a high-purity prescription form of eicosapentaenoic acid (EPA) ethyl ester approved at a dose of 4 g/day as an adjunct to diet to reduce triglyceride (TG) levels in adult patients with severe (≥500 mg/dL) hypertriglyceridemia. This post-hoc exploratory analysis examined the relationship of icosapent ethyl dose with EPA concentrations in plasma and red blood cells (RBCs) across 3 clinical studies—a phase 1 pharmacokinetic study in healthy adult volunteers and 2 pivotal phase 3 studies (MARINE and ANCHOR) in adult patients with hypertriglyceridemia—and examined the relationship between EPA levels and TG-lowering effects in MARINE and ANCHOR. In all 3 studies, icosapent ethyl produced dose-dependent increases in the concentrations of EPA in plasma and RBCs. In both MARINE and ANCHOR, these dose-dependent EPA increases correlated with the degree of TG level lowering (all $P<0.01$). In patients with high TG levels (≥200 mg/dL) and treated with icosapent ethyl 4 g/day, the end-of-treatment plasma and RBC EPA concentrations were >170 μg/mL and >70 μg/mL, respectively. These studies support icosapent ethyl as producing predictable dose-dependent pharmacokinetics/pharmacodynamics, with TG level lowering dependent upon icosapent ethyl dose and EPA concentrations in plasma and RBCs.

1. Introduction

Icosapent ethyl (Vascepa®; Amarin Pharma, Inc., Bedminster, NJ) is a high-purity prescription form of eicosapentaenoic acid (EPA) ethyl ester approved by the Food and Drug Administration as an adjunct to diet to reduce triglyceride (TG) levels in adult patients with severe (≥500 mg/dL) hypertriglyceridemia [1]. Epidemiological and clinical data suggest that high TG levels are a risk factor for atherosclerotic cardiovascular disease (ASCVD) [2]. Genetic data also support a role for elevated TG levels in the causal pathway of ASCVD [3–12]. Compared with placebo, in the Multi-Center, Placebo-Controlled, Randomized, Double-Blind, 12-week Study with an Open-Label Extension (MARINE) and ANCHOR pivotal studies, icosapent ethyl significantly reduced TG levels in adult patients with very high TG levels (TG ≥ 500 and ≤2000 mg/dL) in MARINE and in statin-treated adult patients with high TG levels (TG≥200 and < 500 mg/dL) in ANCHOR [13,14]. In these studies, icosapent ethyl also significantly reduced the levels of other atherogenic lipid and lipoprotein parameters compared with placebo (e.g., non-high-density lipoprotein cholesterol, apolipoprotein B, apolipoprotein C-III, very-low-density lipoprotein cholesterol, remnant lipoprotein cholesterol, and low-density lipoprotein [LDL] particles) and decreased markers of inflammation (e.g., oxidized LDL particles, lipoprotein-associated phospholipase A_2, and C-reactive protein) [13–19].

While other omega-3 fatty acid products that contain both EPA and docosahexaenoic acid (DHA) may significantly raise LDL cholesterol (LDL-C) levels when administered to patients with elevated TG levels [20–23], icosapent ethyl did not raise LDL-C levels compared with placebo [13,14]. In addition to the effects on lipids, which may be expected to reduce ASCVD risk, other hypotheses behind the ongoing Reduction of Cardiovascular Events with EPA-Intervention Trial (REDUCE-IT; NCT01492361) are that EPA, when added to a statin, may favorably affect other steps in atherosclerosis, including endothelial dysfunction, oxidative stress, foam cell formation, inflammation, plaque formation and progression, platelet aggregation, thrombus formation, and plaque rupture

* Corresponding author.
E-mail addresses: hbaysmd@aol.com (H.E. Bays), cmb@bcm.tmc.edu (C.M. Ballantyne), ralph.doyle@amarincorp.com (R.T. Doyle Jr.), rebecca.juliano@amarincorp.com (R.A. Juliano), sephy.philip@amarincorp.com (S. Philip).

http://dx.doi.org/10.1016/j.prostaglandins.2016.07.007

[24,25]. It should be noted that icosapent ethyl is not approved by the US FDA 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.

In the MARINE and ANCHOR studies, 12 weeks of treatment with icosapent ethyl 4 g/day or 2 g/day significantly increased end-of-treatment plasma EPA levels and the EPA/arachidonic acid (AA) ratio [26,27]. In the Japan EPA Lipid Intervention Study (JELIS), ethyl icosapentate 1.8 g/day (another highly purified prescription EPA ethyl ester, approved in Japan but not in the US) significantly increased plasma EPA levels and the EPA/AA ratio at the 5-year follow-up, and decreased risk of major coronary events by 19% ($P = 0.011$) [28]. In a phase 1 pharmacokinetic study of healthy volunteers, plasma EPA concentration increased in a dose-dependent fashion [29]. This may be of clinical importance because ASCVD risk seems to increase with decreased EPA plasma [28] and red blood cell (RBC) membrane concentrations [30]. A low omega-3 index (the ratio of EPA plus DHA relative to other fatty acids in the RBC membrane) is a cardiovascular risk factor associated with poor ASCVD outcomes [31,32]. A lower plasma EPA/AA ratio is correlated with a higher risk of atherosclerosis progression and adverse cardiovascular outcomes [33–36]. Thus, both the dose of icosapent ethyl, as well as EPA plasma and RBC concentrations, may provide clinically meaningful insight into the potential ASCVD benefits of icosapent ethyl. This analysis included data from the MARINE and ANCHOR studies as well as the pharmacokinetic phase 1 study in healthy volunteers. The intent was to explore the effects of icosapent ethyl on EPA concentrations in plasma and RBCs in response to dose and the relationship to TG lowering across these 3 clinical studies.

2. Materials and methods

2.1. Study design

This post-hoc exploratory analysis examined pharmacokinetic and pharmacodynamic data from 3 clinical studies. The first study was an open-label, randomized, multidose, phase 1 pharmacokinetic study including a 14-day screening period followed by a 4-week treatment period [29]. The study enrolled healthy, non-smoking volunteers aged ≥ 18 and ≤55 years with a body mass index of > 18 and ≤30 kg/m^2. Use of lipid-altering medications or supplements was not allowed within 6 weeks prior to randomization until the end of the study. The study randomized eligible subjects (6 men and 6 women per group) to icosapent ethyl 2 g/day administered as one 1-g capsule twice daily, icosapent ethyl 4 g/day administered as two 1-g capsules twice daily, icosapent ethyl 2 g/day administered as two 1-g capsules once daily, or icosapent ethyl 2 g/day administered as two 0.5-g capsules twice daily. The present analysis includes data from the first 2 groups.

The other 2 studies, MARINE and ANCHOR, were pivotal, phase 3, placebo-controlled, randomized, double-blind, multicenter studies, with details previously described [13,14]. Briefly, both studies had a 4- to 6-week lead-in period of diet, lifestyle, and medication stabilization, with washout of prohibited lipid-altering medications, followed by a 12-week, double-blind treatment period. Patients aged ≥18 years with qualifying lipid levels (TGs ≥ 500 mg/dL to ≤2000 mg/dL in MARINE; TGs≥200 mg/dL to <500 mg/dL with statin-stabilized LDL-C ≥40 mg/dL and ≤115 mg/dL in ANCHOR) entered the treatment period and were randomized to receive icosapent ethyl 4 g/day (two 1-g capsules twice daily), icosapent ethyl 2 g/day (one 1-g capsule and one matched placebo capsule twice daily), or placebo (two matched capsules twice daily). In MARINE, stable statin therapy with or without ezetimibe was permitted but not required. ANCHOR required eligible patients to be at high risk for cardiovascular disease as defined by the National Cholesterol Education Program Adult Treatment Panel III guidelines [37] and to be receiving a stable dose of statin therapy (atorvastatin, rosuvastatin, or simvastatin with or without ezetimibe).

Investigators instructed subjects in each study to take study medication orally with or after their morning and evening meal, and subjects agreed to maintain a stable diet and physical activity level throughout the study. Eligible women had a negative urine pregnancy test at screening, agreed to use an effective method of contraception, could not be pregnant or breastfeeding, and could not be planning on becoming pregnant during the study. Each study was conducted in accordance with the principles originating in the Declaration of Helsinki and in accordance with Good Clinical Practice and all applicable laws and regulations. An institutional review board at each site approved the respective study protocol before any subjects were enrolled at that site. All subjects provided written informed consent.

2.2. Assessments

In the MARINE and ANCHOR studies, fasting baseline EPA measurements were taken before the first dose of study drug, and fasting trough (minimum concentration [C_{min}]) EPA concentrations were based on measurements taken before the morning icosapent ethyl dose at 12 weeks of treatment. For the time points relevant to this analysis, fasting EPA measurements were taken in the phase 1 pharmacokinetic study before the morning icosapent ethyl dose on the first day (pre-treatment baseline) and at 28 days (pre-dose trough EPA concentration).

These studies measured EPA concentrations in plasma and in RBCs using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) method (Charles River Laboratories Ltd, Elphinstone Research Center, Tranent, Edinburgh, Scotland) as previously described [29]. Total plasma EPA comprised all EPA forms, including unesterified EPA as well as that incorporated into phospholipids, triacylglycerols, and cholesteryl esters, whereas EPA in RBCs was from cell membranes, where it is mainly incorporated into phospholipids. For these measurements, lipids were isolated from plasma and RBC suspensions by acid/methanol/chloroform extraction followed by centrifugation, and purified by isohexane extraction and filtration on a solid-phase extraction column after confirmed complete lipid hydrolysis and transmethylation following an overnight incubation at 50 °C with acid/methanol. Quantitation of EPA in each sample utilized linolenic acid as an internal standard and a standard EPA calibration curve. The lower limits of quantitation (LLOQ) were 10 μg/mL for total plasma EPA and 5 μg/mL for total RBC EPA.

The MARINE and ANCHOR studies evaluated serum TG levels as previously described [26], using enzymatic colorimetric tests (Olympus AU2700 or AU5400 Analyzer, Olympus Center Valley, PA) with calibration directly traceable to US Centers for Disease Control reference procedures.

2.3. Data analysis

The phase 1 pharmacokinetic study determined EPA concentrations in plasma and RBCs for subjects in the per-protocol population, which included all randomized subjects who completed the 28-day treatment period without any protocol violations and who provided baseline and day 28 samples. The MARINE and ANCHOR studies determined EPA concentrations for patients in the intent-to-treat population (all randomized patients who took ≥ 1 dose of study drug and had valid baseline laboratory efficacy measurements and at least 1 post-randomization laboratory efficacy measurement) who had EPA values at baseline and at the week-12

endpoint. If missing, the last-observation-carried-forward method was used for the endpoint value. The EPA concentration analyses were considered descriptive and *P* values were calculated merely to help identify possible trends for hypothesis generation and a nominal alpha level of 5% was used without multiplicity adjustment. For MARINE, percent change from baseline in EPA concentration was evaluated using an analysis of covariance (ANCOVA) model with fixed effect terms including treatment, randomization stratification factors (gender and the use of statin therapy [used statin therapy, did not use statin therapy]), and baseline value as a covariate. For ANCHOR, percent change from baseline in EPA concentration was evaluated using ANCOVA with fixed effect terms including treatment, randomization stratification factors (gender, type of statin [atorvastatin, rosuvastatin, simvastatin] and presence of diabetes [present diabetes, past or no diabetes]), and baseline value as a covariate. MARINE and ANCHOR analyzed serum TG levels as previously described [26].

In MARINE and ANCHOR, outliers were identified within each treatment group as percent change values <Q1 − 1.5•IQR or >Q3 + 1.5•IQR, where Q indicates quartile and IQR indicates interquartile range. Patients with outlier values were excluded from the analysis. In MARINE and ANCHOR, when plasma EPA values were below the LLOQ of 10.0 μg/mL, 5 μg/mL was imputed for analysis; when RBC EPA values were below the LLOQ of 5.00 μg/mL, 2.5 μg/mL was imputed for analysis. In the PK study, when plasma or RBC EPA values fell below their respective LLOQs, they were set to zero and included in the analysis.

The relationship between change from baseline to week 12 endpoint in EPA concentration and percent change from baseline to week 12 endpoint in TG level was evaluated using a correlation analysis of individual patient data from MARINE and ANCHOR. As the analysis for TG concentration was non-parametric and summarized with medians as previously described [14], the Spearman correlation coefficient was calculated. A *P* value < 0.05 was the prespecified alpha for significance for exploratory analyses in MARINE and ANCHOR and was used in the present post hoc analyses.

3. Results

3.1. Study populations

Table 1 shows the demographics for each study. Reflective of the populations in each study, mean age was lower in the phase 1 pharmacokinetic study of healthy volunteers (37.4 years) than in the patients from MARINE (52.9 years) and ANCHOR (61.4 years). Mean body weight and BMI were greater in ANCHOR and MARINE patients than in the subjects in the pharmacokinetic study. Diabetes mellitus was present in 27.5% of patients in MARINE and 73.2% of patients in ANCHOR. Consistent with entry criteria, baseline median TG levels were numerically higher in MARINE (679.5 mg/dL) than in ANCHOR (259.0 mg/dL) patients (Table 2). Baseline mean EPA concentrations in plasma and RBCs were also numerically higher in the MARINE study than in the other 2 studies (Table 2).

3.2. EPA dose response

Plasma trough total EPA concentrations at baseline and end-of-treatment were available for 19 of 24 randomized subjects (79.2%) in the phase 1 pharmacokinetic study, 192 of the 229 randomized patients (83.8%) in MARINE, and 225 of the 702 randomized patients (32.1%) in ANCHOR. Treatment with icosapent ethyl produced dose-dependent increases in plasma EPA concentration in healthy volunteers (phase 1 pharmacokinetic study) and patients with hypertriglyceridemia (MARINE and ANCHOR)

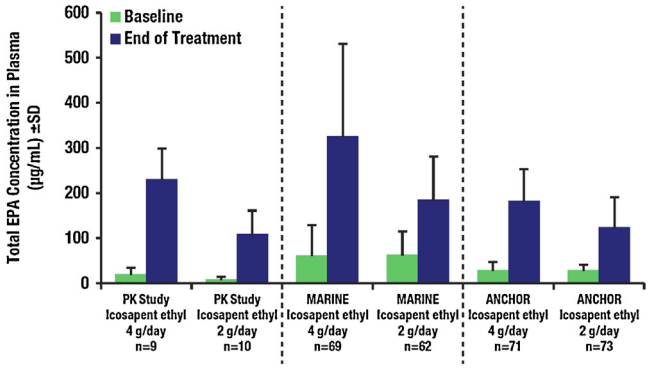

Fig. 1. Mean fasting trough total EPA concentrations in plasma at baseline and at end of treatment with icosapent ethyl in the phase 1 PK study in healthy adult volunteers (28 days) and in the MARINE and ANCHOR studies in adult patients with hypertriglyceridemia (12 weeks). End of treatment was 4 weeks for the phase 1 PK study and 12 weeks in the MARINE and ANCHOR studies. Error bars represent standard deviations. EPA = eicosapentaenoic acid; MARINE = Multi-Center, Placebo-Controlled, Randomized, Double-Blind, 12-week Study with an Open-Label Extension; PK = pharmacokinetic; SD = standard deviation.

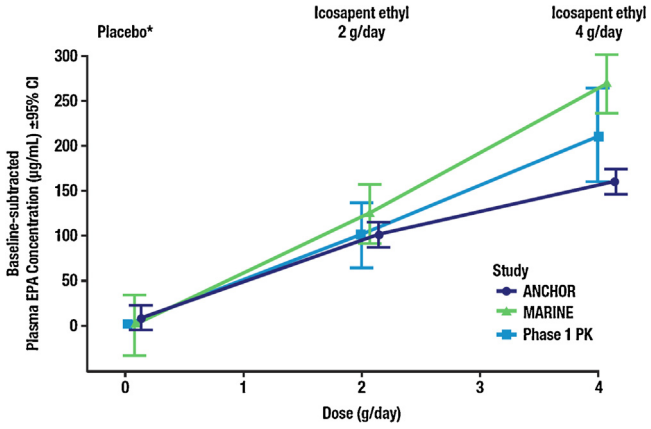

Fig. 2. Icosapent ethyl dose dependence of fasting plasma EPA concentrations in the phase 1 PK study in healthy adult volunteers (28 days) and in the MARINE and ANCHOR studies in adult patients with hypertriglyceridemia (12 weeks). Mean concentrations are based on baseline-subtracted trough concentrations of total EPA (change from baseline) at steady state. *In the PK study, there was no placebo group but a value of zero was plotted as a point of reference. CI = confidence interval; EPA = eicosapentaenoic acid; MARINE = Multi-Center, Placebo-Controlled, Randomized, Double-Blind, 12-week Study with an Open-Label Extension; PK = pharmacokinetic.

(Fig. 1). In each of these 3 studies, icosapent ethyl 4 g/day produced greater increases in plasma EPA concentration than for 2 g/day both in plasma (Fig. 2) and RBCs (Fig. 3). In MARINE, the least squares mean change ± SE from baseline to end of treatment in plasma EPA was +269.2 ± 16.3 μg/mL with icosapent ethyl 4 g/day, +124.2 ± 16.8 μg/mL with the 2-g/day dose, and −0.2 ± 17.1 μg/mL with placebo; the corresponding least squares mean changes in RBC EPA concentrations were +57.0 ± 2.7 μg/mL, +30.6 ± 2.8 μg/mL, and −1.4 ± 2.8 μg/mL, respectively ($P < 0.0001$ for each icosapent ethyl dose vs placebo for both plasma and RBC EPA). Similarly, in ANCHOR, the least squares mean change ± SE from baseline to end-of-treatment in plasma EPA was +159.5 ± 7.0 μg/mL with icosapent ethyl 4 g/day, +100.5 ± 6.8 μg/mL with the 2-g/day dose, and +8.1 ± 6.6 μg/mL with placebo; the corresponding least squares mean changes in RBC EPA concentrations were +62.8 ± 2.6 μg/mL, +34.6 ± 2.5 μg/mL, and +0.4 ± 2.4 μg/mL, respectively ($P < 0.0001$ for each icosapent ethyl dose vs placebo for both plasma and RBC EPA). The numbers of patients included in this analysis with EPA results below the LLOQ in plasma and RBCs were 6 and 17 in

Table 1
Demographics.

Characteristic	Phase 1 PK Study		MARINE			ANCHOR		
	Icosapent Ethyl 4 g/day (n = 12)	Icosapent Ethy 2 g/day (n = 12)	Icosapen Ethyl 4 g/day (n = 77)	Icosapent Ethyl 2 g/day (n = 76)	Placebo (n = 76)	Icosapent Ethyl 4 g/day (n = 233)	Icosapent Ethyl 2 g/day (n = 236)	Placebo (n = 233)
Age, mean (SD), y	37.9 (12.9)	36.9 (13.5)	51.9 (10.3)	53.4 (9.3)	53.4 (8.3)	61.1 (10.0)	61.8 (9.4)	61.2 (10.1)
Men, n (%)	6 (50.0)	6 (50.0)	59 (76.6)	58 (76.3)	58 (76.3)	142 (60.9)	144 (61.0)	145 (62.2)
Weight, mean (SD), kg	75.5 (13.4)	74.5 (14.1)	93.2 (18.3)	92.1 (15.6)	93.0 (16.9)	94.5 (18.3)	95.5 (18.3)	97.0 (19.1)
BMI, mean (SD), kg/m^2	27.2 (2.6)	26.2 (2.6)	30.4 (4.3)	30.8 (4.2)	31.0 (4.3)	32.7 (5.0)	32.9 (5.0)	33.0 (5.0)
Diabetes, n (%)	NA	NA	22 (28.6)	20 (26.3)	21 (27.6)	171 (73.4)	172 (72.9)	171 (73.4)
Statin use, n (%)	NA	NA	20 (26)	19 (25)	18 (24)	Required	Required	Required

BMI = body mass index; NA = not applicable; PK = pharmacokinetic; SD = standard deviation.

Table 2
Fasting Triglyceride and EPA Concentrations at Baseline and End of Treatment With Icosapent Ethyl.

Characteristic	Phase 1 PK Study		MARINE			ANCHOR		
	Icosapent Ethyl 4 g/day (n = 12)	Icosapent Ethyl 2 g/day (n = 12)	Icosapent Ethyl 4 g/day (n = 77)	Icosapent Ethyl 2 g/day (n = 76)	Placebo (n = 76)	Icosapent Ethyl 4 g/day (n = 233)	Icosapent Ethyl 2 g/day (n = 236)	Placebo (n = 233)
Plasma triglyceride levels, mg/dL								
			n = 76	n = 73	n = 75	n = 226	n = 234	n = 227
Baseline, median (IQR)	NA	NA	679.5 (265.3)	656.5 (303.5)	703.0 (426.5)	264.8 (93.0)	254.0 (92.5)	259.0 (81.0)
EOT, median (IQR)	NA	NA	502.0 (302.0)	605.5 (415.0)	745.5 (886.5)	220.8 (92.0)	244.3 (117.0)	269.5 (149.5)
Total plasma EPA levels, µg/mL								
	n = 9	n = 10	n = 69	n = 62	n = 61	n = 71	n = 73	n = 81
Baseline, mean (SD)	19.3 (16.1)	7.9 (7.0)	61.2 (67.4)	63.6 (51.4)	57.7 (42.7)	28.1 (18.8)	28.1 (13.7)	28.1 (28.0)
EOT, mean (SD)	231.2 (69.0)	108.9 (52.7)	326.7 (205.7)	185.0 (96.9)	52.8 (40.0)	182.6 (71.7)	123.8 (67.8)	30.6 (27.9)
RBC EPA levels, µg/mL								
	n = 9	n = 10	n = 66	n = 61	n = 64	n = 69	n = 71	n = 79
Baseline, mean (SD)	12.1 (15.7)	5.7 (4.3)	16.0 (9.2)	15.7 (9.9)	14.7 (9.3)	11.6 (5.6)	10.9 (5.2)	11.2 (6.6)
EOT, mean (SD)	76.2 (19.5)	36.9 (14.6)	71.6 (31.5)	44.9 (20.5)	11.6 (8.7)	72.7 (31.5)	43.7 (16.8)	9.9 (5.7)

EOT = end of treatment; EPA = eicosapentaenoic acid; IQR = interquartile range; NA = not applicable; RBC = red blood cell; SD = standard deviation; TG = triglyceride.

MARINE and 5 and 11 in ANCHOR, respectively; these values were imputed as described in the Methods. In the PK study, there were 7 and 4 subjects with EPA results below the LLOQ in plasma and RBCs, respectively (all were baseline values); values for these patients were set to zero and were included in the analysis. The numbers of patients with outlier values that were excluded from analysis of EPA concentrations in plasma and RBC, respectively, were 18 and 20 in MARINE and 11 and 15 in ANCHOR. There were no excluded subjects in the PK study due to outlier EPA values.

3.3. Relationship between TG-lowering response and EPA concentration

Analysis of MARINE and ANCHOR revealed a dose-dependent reduction in fasting TG levels that was also associated with increases in EPA concentrations (Fig. 4). In MARINE, icosapent ethyl 4 g/day and 2 g/day produced increases in plasma EPA levels of +679% and +309%, respectively (least squares mean percent changes from baseline), which also corresponded with TG level reductions of −27% and −7%, respectively (median percent change from baseline). In ANCHOR, icosapent ethyl 4 g/day and 2 g/day produced increases in plasma EPA levels of +666% and +398%, respectively (least squares mean percent changes from baseline), which also corresponded with TG level reductions of −18% and −6%, respectively (median percent change from baseline). Icosapent ethyl produced similar dose-dependent relationships for the reduction in median TG levels and increase in RBC EPA concentrations (Fig. 4). The Spearman correlation coefficients for the relationship between the TG-lowering response and EPA concentration were −0.22 ($P = 0.002$) in plasma and −0.34 ($P < 0.0001$) in RBCs in MARINE and −0.24 ($P = 0.0003$) in plasma and −0.33 ($P < 0.0001$) in RBCs in ANCHOR.

3.4. Factors potentially affecting icosapent ethyl pharmacokinetics

In the ANCHOR ANCOVA model for percent change from baseline to week 12 in EPA concentrations in both plasma and in RBCs, the term for treatment was statistically significant ($P < 0.0001$), while the terms for gender, type of statin, and presence of diabetes were

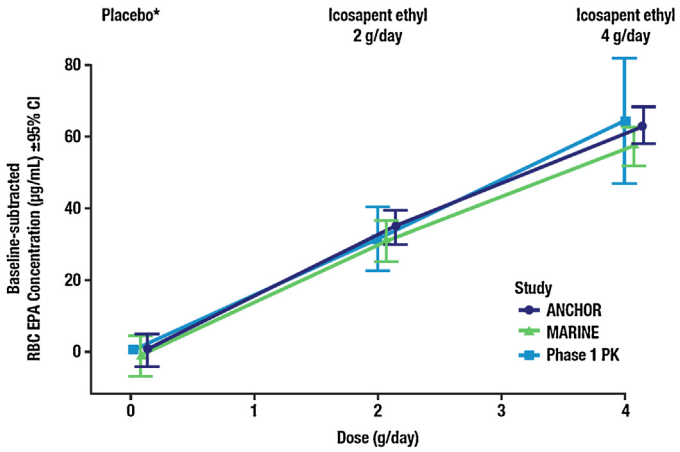

Fig. 3. Icosapent ethyl dose dependence of RBC fasting EPA concentrations in the phase 1 PK study in healthy adult volunteers (28 days) and in the MARINE and ANCHOR studies in adult patients with hypertriglyceridemia (12 weeks). Mean concentrations are based on baseline-subtracted trough concentrations of total EPA (change from baseline) at steady state. *In the PK study, there was no placebo group but a value of zero was plotted as a point of reference. CI = confidence interval; EPA = eicosapentaenoic acid; MARINE = Multi-Center, Placebo-Controlled, Randomized, Double-Blind, 12-week Study with an Open-Label Extension; PK = pharmacokinetic.

not statistically significant ($P = 0.7884$, 0.6342, and 0.4971, respectively, for plasma EPA; $P = 0.7910$, 0.5353, and 0.4722, respectively, for RBC EPA).

In the MARINE ANCOVA model for percent change from baseline to week 12 in plasma EPA concentration, the term for treatment was statistically significant ($P < 0.0001$), while the terms for gender and the use of statin therapy at randomization were not statistically significant ($P = 0.4132$ and 0.5643, respectively). In the ANCOVA model for percent change from baseline to week 12 in RBC EPA concentration, the terms for treatment and gender were statistically significant ($P < 0.0001$ and $P = 0.0063$, respectively), while the term for the use of statin therapy at randomization was not statistically significant ($P = 0.8083$). Since the gender term was statistically significant for percent change in RBC EPA, the treatment•gender interaction was added to the model and least squares means were calculated, which resulted in a statistically significant difference by gender in the 2 g/day arm ($P = 0.0268$) and a difference approaching statistical significance in the 4 g/day arm ($P = 0.0789$).

The pharmacokinetic assessments in these studies were performed in the fasting state, just prior to administration of icosapent ethyl with or following a meal or snack. Studies investigating the pharmacokinetics of icosapent ethyl when administered without food were not performed.

4. Discussion

Icosapent ethyl at doses of 4 g/day and 2 g/day increased trough EPA concentrations in plasma and RBCs compared with baseline in a dose-dependent manner across 3 clinical studies, indicating that this agent has predictable pharmacokinetics in healthy volunteers and in patients with hypertriglyceridemia. The EPA concentrations achieved with icosapent ethyl appeared to be similar to or exceed the total levels of EPA alone or EPA and DHA reported in studies of other prescription omega-3 fatty acid products [38]. For example, in patients with very high TG levels, the trough plasma EPA level measured at the end of treatment was 327 μg/mL with icosapent ethyl 4 g/day in MARINE. In comparison, plasma EPA and DHA levels were 170 μg/mL and 169 μg/mL, respectively, following treatment with omega-3-carboxylic acids 4 g/day in the Epanova for Lowering Very High Triglycerides (EVOLVE) study in patients with very high TG levels [21]. Similarly, in patients with high TG levels, the plasma EPA concentration was 183 μg/mL at end of treatment with icosapent ethyl 4 g/day in ANCHOR, whereas plasma EPA and DHA concentrations were 105 μg/mL and 100 μg/mL, respectively, following treatment with omega-3-carboxylic acids 4 g/day in the separate Epanova Combined With a Statin in Patients With Hypertriglyceridemia to Reduce Non-HDL Cholesterol (ESPRIT) study [39].

The mean trough EPA concentrations in plasma achieved with icosapent ethyl treatment for 12 weeks in patients with high TG levels in ANCHOR (2 g/day, 124 μg/mL; 4 g/day, 183 μg/mL) and very high TG levels in MARINE (2 g/day, 185 μg/mL; 4 g/day, 327 μg/mL) appear to be similar to or greater than the 5-year on-treatment plasma EPA concentration reported in JELIS (1.8 g/day, 170 μg/mL), which investigated treatment with another highly purified prescription EPA ethyl ester that is approved in Japan but not in the US [40]. JELIS demonstrated that addition of EPA ethyl ester 1.8 g/day to low-dose statin therapy significantly reduced major coronary events in hypercholesterolemic Japanese patients. Although a lower dose of EPA ethyl ester was used in JELIS, baseline plasma EPA concentrations (97 μg/mL) appeared to be notably higher than those found in ANCHOR, likely reflecting high fish consumption in the Japanese diet. The effect of icosapent ethyl 4 g/day on cardiovascular outcomes is currently being investigated in high-risk patients with baseline TG levels of 200–499 mg/dL despite statin therapy in REDUCE-IT.

This analysis revealed dose-dependent relationships between plasma and RBC EPA concentrations and the degree of TG level lowering by icosapent ethyl. These relationships are consistent with the predictable pharmacokinetic-pharmacodynamic characteristics of icosapent ethyl. Moreover, the pharmacokinetics of icosapent ethyl based on percent change increases in plasma EPA was not affected by the use/type of statins and did not appear to be affected by gender. A treatment•gender interaction was observed in the MARINE RBC EPA analysis, but it is difficult to draw any substantial conclusions regarding the strength of this interaction considering the small sample size of women in the treatment arms (13–15 per arm for EPA concentrations) and the lack of a treatment•gender interaction across plasma EPA in ANCHOR and MARINE or in RBC EPA in ANCHOR. Evaluation of icosapent ethyl 4 g/day in drug–drug interaction studies of atorvastatin, warfarin, omeprazole, and rosiglitazone found no drug–drug interactions [41–44].

The EPA levels measured in the present analyses appeared to be similar to those previously determined in MARINE and ANCHOR fatty acid analyses using a gas chromatography/flame ionization detection (GC/FID) method [26,27]. The LC–MS/MS method used herein permits calculation of EPA concentration in units of weight/volume (μg/mL), whereas the GC/FID method determines EPA in units of weight/weight (μg/g). However, with the latter method, 28 fatty acids were measured, and expressed plasma EPA levels as a percentage of total fatty acids. With the 4-g/day dose, EPA levels (percentage of total fatty acids) increased from 0.5% at baseline to 3.4% at week 12 in MARINE, and from 0.4% to 3.6% in ANCHOR [26,27]. The GC/FID method also examined ratios of anti-inflammatory omega-3 fatty acids to pro-inflammatory omega-6 fatty acids, including the EPA/AA ratio. Both omega-3/omega-6 fatty acids and EPA/AA ratios increased significantly with icosapent ethyl treatment in MARINE and ANCHOR [26,27]. Although there were differences in study design, including product administered, dose, patient populations, and study duration between JELIS and the MARINE and ANCHOR studies, it may be worth noting that in JELIS, higher EPA/AA ratios were associated with decreased risk of major coronary events; patients with EPA/AA ratios > 0.75 were at 17% lower risk compared with those having ratios < 0.75 ($P = 0.031$) [28]. Similarly, and with the same limitations noted above, the Combination Therapy of Eicosapentaenoic Acid and Pitavastatin for

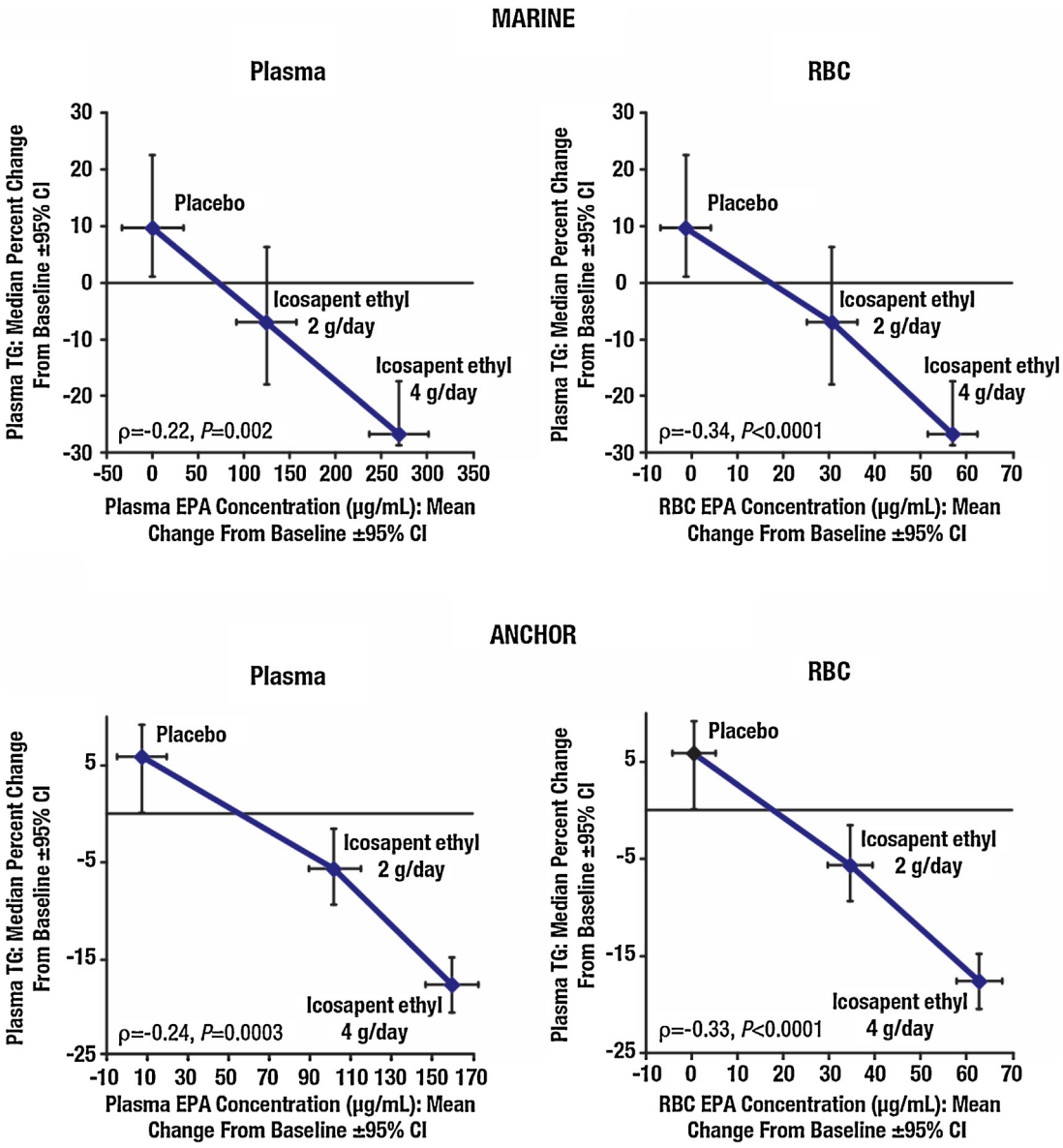

Fig. 4. Pharmacokinetics-pharmacodynamics of icosapent ethyl showing relationship between fasting plasma TG level lowering and fasting EPA concentrations in plasma and RBCs. CI = confidence interval; EPA = eicosapentaenoic acid; MARINE = Multi-Center, Placebo-Controlled, Randomized, Double-Blind, 12-week Study with an Open-Label Extension; RBC = red blood cell; ρ = Spearman correlation coefficient.

Coronary Plaque Regression Evaluated by Integrated Backscatter Intravascular Ultrasonography (CHERRY) study showed a significant inverse correlation between plasma EPA/AA ratio levels and coronary plaque volume (as plasma EPA and EPA/AA ratio levels increased, plaque volume decreased) [45].

This analysis was exploratory in nature. Limitations include differences in populations, study design, dosing regimens, and duration of treatment between the 3 separate clinical studies assessed. An additional limitation is that EPA levels were assessed at the nominal 5% level with no statistical methods used to control for the overall error rate. Therefore, results should be interpreted with caution and further investigation may be needed to confirm these findings.

5. Conclusions

Based on this post-hoc exploratory analysis of 3 clinical studies, icosapent ethyl produces dose-dependent increases in plasma and RBC concentrations of EPA and decreases TG levels. The plasma and RBC concentrations are also dose-dependently related to TG-lowering efficacy. In patients with high TG levels (≥200 mg/dL), the end-of-treatment mean plasma and RBC EPA concentrations were >170 μg/mL and >70 μg/mL with the icosapent ethyl dose of 4 g/day. Taken together, these findings indicate that icosapent ethyl has a predictable pharmacokinetic-pharmacodynamic profile.

Funding sources

These studies were designed and sponsored by Amarin Pharma Inc., Bedminster, NJ, USA. Medical writing assistance was provided by Peloton Advantage, LLC, Parsippany, NJ, USA, and was funded by Amarin Pharma Inc.

Author disclosures

In the past 12 months, Dr. Harold Bays' research site has received research grants from Amarin Pharma Inc., Amgen, Ardea, Arisaph, AstraZeneca, Bristol Myers Squibb, Catabasis, Cymabay, Eisai,

Elcelyx, Eli Lilly, Esperion, Ferrer/Chiltern, Gilead, GSK, Hanmi, Hisun, Hoffman LaRoche, Home Access, Janssen, Johnson & Johnson, Kowa, Merck, Necktar, Novartis, NovoNordisk, Omthera, Orexigen, Pfizer, Pronova, Regeneron, Sanofi, Takeda, and TIMI. In the past 12 months, Dr. Harold Bays has served as a consultant/advisor for Alnylam, Akcea, Amgen, AstraZeneca, Eli Lilly, Ionis, Merck, Novartis, Pronova, Regeneron, Sanofi, and Takeda. In the past 12 months, Dr. Harold Bays has served as a speaker for Amarin Pharma Inc., Amgen, AstraZeneca, Eisai, Regeneron, Sanofi, and Takeda.

Dr. Ballantyne has received research/grant support from Abbott Diagnostics, Amarin Pharma Inc., Amgen, Eli Lilly, Esperion, Genentech, GlaxoSmithKline, Merck, Novartis, Pfizer, Regeneron, Roche, Roche Diagnostic, Sanofi-Synthelabo, NIH, and AHA (all paid to institution, not individual), is a consultant for Abbott Diagnostics, Aegerion, Amarin Pharma Inc., Amgen, Arena, Cerenis, Esperion, Genentech, Genzyme, Kowa, Merck, Novartis, Pfizer, Resverlogix, Roche, and Sanofi-Synthelabo, and has received honoraria from Abbott, Amarin Pharma Inc., AstraZeneca, Bristol-Myers Squibb, Cerenis, Esperion, Genentech, GlaxoSmithKline, Kowa, Merck, Novartis, Omthera, Regeneron, Resverlogix, Roche, and Sanofi-Synthelabo.

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

Author roles

Study design: HEB, CMB.
Study investigator: HEB, CMB.
Enrolled patients: HEB, CMB.
Collection and assembly of data: All authors.
Data analysis: All authors.
Data interpretation: All authors.
Manuscript preparation: All authors.
Manuscript review and revisions: All authors.
Final approval of manuscript: All authors.

Acknowledgments

The authors thank Rene Braeckman, PhD, Paresh Soni, MD, PhD, and William Stirtan, PhD, for their involvement in the design of this analysis, and Lisa Jiao, PhD and Sherry Pinnell, MS for their support with statistical analysis.

References

[1] Vascepa [package insert], Amarin Pharma Inc., Bedminster, NJ, 2015.
[2] M. Miller, N.J. Stone, C. Ballantyne, et al., Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association, Circulation 123 (20) (2011) 2292–2333.
[3] C.J. Willer, E.M. Schmidt, S. Sengupta, et al., Discovery and refinement of loci associated with lipid levels, Nat. Genet. 45 (11) (2013) 1274–1283.
[4] R. Do, C.J. Willer, E.M. Schmidt, et al., Common variants associated with plasma triglycerides and risk for coronary artery disease, Nat. Genet. 45 (11) (2013) 1345–1352.
[5] A.B. Jorgensen, R. Frikke-Schmidt, A.S. West, P. Grande, B.G. Nordestgaard, A. Tybjaerg-Hansen, Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction, Eur. Heart J. 34 (24) (2013) 1826–1833.
[6] 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 (4) (2013) 427–436.
[7] 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 (12) (2013) 1298–1309.
[8] M. Thomsen, A. Varbo, A. Tybjaerg-Hansen, B.G. Nordestgaard, Low nonfasting triglycerides and reduced all-cause mortality: a Mendelian randomization study, Clin. Chem. 60 (5) (2014) 737–746.
[9] M.V. Holmes, F.W. Asselbergs, T.M. Palmer, et al., Mendelian randomization of blood lipids for coronary heart disease, Eur. Heart J. 36 (9) (2015) 539–550.
[10] A.B. Jorgensen, R. Frikke-Schmidt, B.G. Nordestgaard, A. Tybjaerg-Hansen, Loss-of-function mutations in APOC3 and risk of ischemic vascular disease, N. Engl. J. Med. 371 (1) (2014) 32–41.
[11] J. Crosby, G.M. Peloso, P.L. Auer, et al., Loss-of-function mutations in APOC3, triglycerides, and coronary disease, N. Engl. J. Med. 371 (1) (2014) 22–31.
[12] R. Do, N.O. Stitziel, H.H. Won, et al., Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction, Nature 518 (7537) (2015) 102–106.
[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 (5) (2011) 682–690.
[14] C.M. Ballantyne, H.E. Bays, J.J. Kastelein, 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 (7) (2012) 984–992.
[15] H.E. Bays, C.M. Ballantyne, R.A. Braeckman, W.G. Stirtan, P.N. Soni, Icosapent ethyl, a pure ethyl ester of eicosapentaenoic acid: effects on circulating markers of inflammation from the MARINE and ANCHOR studies, Am. J. Cardiovasc. Drugs 13 (1) (2013) 37–46.
[16] H.E. Bays, R.A. Braeckman, C.M. Ballantyne, et al., Icosapent ethyl, a pure EPA omega-3 fatty acid: effects on lipoprotein particle concentration and size in patients with very high triglyceride levels (the MARINE study), J. Clin. Lipidol. 6 (6) (2012) 565–572.
[17] C.M. Ballantyne, H.E. Bays, R.A. Braeckman, et al., Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on apolipoprotein C-III in patients from the MARINE and ANCHOR studies, J. Clin. Lipidol. 10 (3) (2016) 635–645.
[18] C.M. Ballantyne, H.E. Bays, R.A. Braeckman, et al., Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on remnant-like particle cholesterol from the MARINE and ANCHOR studies [abstract], Circulation 130 (Suppl. 2) (2014) A16803.
[19] C.M. Ballantyne, R.A. Braeckman, H.E. Bays, 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 (3) (2015) 377–383.
[20] W.S. Harris, H.N. Ginsberg, N. Arunakul, et al., Safety and efficacy of Omacor in severe hypertriglyceridemia, J. Cardiovasc. Risk 4 (5–6) (1997) 385–391.
[21] J.J.P. Kastelein, K.C. Maki, A. Susekov, 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 (1) (2014) 94–108.
[22] M.Y. Wei, T.A. Jacobson, Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis, Curr. Atheroscler. Rep. 13 (6) (2011) 474–483.
[23] T.A. Jacobson, S.B. Glickstein, J.D. Rowe, P.N. Soni, Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review, J. Clin. Lipidol. 6 (1) (2012) 5–18.
[24] K.M. Borow, J.R. Nelson, R.P. Mason, Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis, Atherosclerosis 242 (1) (2015) 357–366.
[25] D. Mozaffarian, J.H. Wu, Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events, J. Am. Coll. Cardiol. 58 (20) (2011) 2047–2067.
[26] 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), Prostaglandins Leukot. Essent. Fatty Acids 89 (4) (2013) 195–201.
[27] R. Braeckman, M.S. Manku, C.M. Ballantyne, W.G. Stirtan, P.N. Soni, Effects of AMR101, a pure eicosapentaenoic omega-3 fatty acid, on the fatty acid profile in plasma and red blood cells in statin-treated patients with persistent high triglycerides (results from the ANCHOR study) [abstract], Circulation 126 (2012) (A18549).
[28] H. Itakura, M. Yokoyama, M. Matsuzaki, et al., Relationships between plasma fatty acid composition and coronary artery disease, J. Atheroscler. Thromb. 18 (2) (2011) 99–107.
[29] R.A. Braeckman, W.G. Stirtan, P.N. Soni, Pharmacokinetics of eicosapentaenoic acid in plasma and red blood cells after multiple oral dosing with icosapent ethyl in healthy subjects, Clin. Pharmacol. Drug Dev. 3 (2) (2014) 101–108.
[30] W.S. Harris, K.F. Kennedy, J.H. O'Keefe Jr., J.A. Spertus, Red blood cell fatty acid levels improve GRACE score prediction of 2-yr mortality in patients with myocardial infarction, Int. J. Cardiol. 168 (1) (2013) 53–59.
[31] C. von Schacky, Omega-3 index and cardiovascular health, Nutrients 6 (2) (2014) 799–814.
[32] W.S. Harris, J.V. Pottala, S.M. Lacey, R.S. Vasan, M.G. Larson, S.J. Robins, Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study, Atherosclerosis 225 (2) (2012) 425–431.
[33] T. Nozue, S. Yamamoto, S. Tohyama, et al., Effects of serum n-3 to n-6 polyunsaturated fatty acids ratios on coronary atherosclerosis in statin-treated patients with coronary artery disease, Am. J. Cardiol. 111 (1) (2013) 6–11.
[34] T. Serikawa, S. Miura, M. Okabe, et al., Ratio of eicosapentaenoic acid to arachidonic acid is a critical risk factor for acute coronary syndrome in middle-aged older patients as well as younger adult patients, J. Cardiol. 63 (1) (2014) 35–40.

[35] Y. Wakabayashi, H. Funayama, Y. Ugata, et al., Low eicosapentaenoic acid to arachidonic acid ratio is associated with thin-cap fibroatheroma determined by optical coherence tomography, J. Cardiol. 66 (6) (2015) 482–488.
[36] T. Endo, H. Tomita, T. Higuma, et al., Low serum eicosapentaenoic acid level is a risk for ventricular arrhythmia in patients with acute myocardial infarction: a possible link to J-waves, Heart Vessels 29 (6) (2014) 847–854.
[37] 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 (25) (2002) 3143–3421.
[38] H.S. Weintraub, Overview of prescription omega-3 fatty acid products for hypertriglyceridemia, Postgrad. Med. 126 (7) (2014) 7–18.
[39] K.C. Maki, D.G. Orloff, S.J. Nicholls, et al., A highly bioavailable omega-3 free fatty acid formulation improves the cardiovascular risk profile in high-risk, statin-treated patients with residual hypertriglyceridemia (the ESPRIT trial), Clin. Ther. 35 (9) (2013) 1400–1411.
[40] M. Yokoyama, H. Origasa, M. Matsuzaki, et al., Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis, Lancet 369 (9567) (2007) 1090–1098.
[41] R.A. Braeckman, W.G. Stirtan, P.N. Soni, Effect of concomitant icosapent ethyl (eicosapentaenoic acid ethyl ester) on the pharmacokinetics of atorvastatin, Clin. Drug Investig. 35 (1) (2015) 45–51.
[42] R.A. Braeckman, W.G. Stirtan, P.N. Soni, Phase 1 study of the effect of icosapent ethyl on warfarin pharmacokinetic and anticoagulation parameters, Clin. Drug Investig. 34 (7) (2014) 449–456.
[43] R.A. Braeckman, W.G. Stirtan, P.N. Soni, Effect of icosapent ethyl (eicosapentaenoic acid ethyl ester) on omeprazole plasma pharmacokinetics in healthy adults, Drugs R D 14 (3) (2014) 159–164.
[44] R.A. Braeckman, W.G. Stirtan, P.N. Soni, Effects of icosapent ethyl (eicosapentaenoic acid ethyl ester) on pharmacokinetic parameters of rosiglitazone in healthy subjects, Clin. Pharmacol. Drug Dev. 4 (2) (2015) 143–148.
[45] K. Ando, T. Watanabe, H. Daidoji, et al., Combination therapy of eicosapentaenoic acid and pitavastatin for coronary plaque regression evaluated by integrated backscatter intravascular ultrasonography: a randomized controlled trial [abstract 12007], Circulation 132 (2015) (A12007).