Circulation

ORIGINAL RESEARCH ARTICLE

Effects of Randomized Treatment With Icosapent Ethyl and a Mineral Oil Comparator on Interleukin-1β, Interleukin-6, C-Reactive Protein, Oxidized Low-Density Lipoprotein Cholesterol, Homocysteine, Lipoprotein(a), and Lipoprotein-Associated Phospholipase A2: A REDUCE-IT Biomarker Substudy

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BACKGROUND: REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl—Intervention Trial) reported a 25% relative risk reduction in major adverse cardiovascular events with use of icosapent ethyl compared with pharmaceutical grade mineral oil. The mechanisms underlying this benefit remain uncertain. We explored whether treatment allocation in REDUCE-IT might affect a series of biomarkers in pathways known to associate with atherosclerosis risk.

METHODS: Serum levels of interleukin-1β, interleukin-6, high-sensitivity C-reactive protein, oxidized low-density lipoprotein cholesterol, homocysteine, lipoprotein(a), and lipoprotein-associated phospholipase A2 (Lp-PLA2) were measured at baseline, at 12 months, at 24 months, and at the end-of-study visit among REDUCE-IT participants with triglyceride levels ≥135 mg/dL and <500 mg/dL who were randomly allocated to treatment with either 4 grams daily of icosapent ethyl or mineral oil used as a comparator.

RESULTS: At baseline, median levels of each biomarker were similar in the 2 treatment groups. The levels of biomarkers associated with atherosclerosis increased over time among those allocated to mineral oil treatment; in this group at 12 months, the median percent increases from baseline were 1.5% for homocysteine, 2.2% for lipoprotein(a), 10.9% for oxidized low-density lipoprotein cholesterol, 16.2% for interleukin-6, 18.5% for lipoprotein-associated phospholipase A2, 21.9% for high-sensitivity C-reactive protein, and 28.9% for interleukin-1β (all *P* values <0.001), with similar changes at 24 months. In the icosapent ethyl group, there were minimal changes in these biomarkers at 12 and 24 months. As such, at study conclusion, between-group treatment differences largely reflected increases in the mineral oil group with median percent differences of 2.4% for lipoprotein(a), 3.0% for homocysteine, 4.2% for oxidized low-density lipoprotein cholesterol, 19.8% for interleukin-6, 26.2% for Lp-PLA2, 38.5% for high-sensitivity C-reactive protein, and 48.7% for interleukin-1β (all *P* values ≤0.007). These data are consistent with previous REDUCE-IT results in which the median percent change for low-density lipoprotein cholesterol at 12 months was −1.2% among those allocated to icosapent ethyl and 10.9% among those allocated to the mineral oil comparator.

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CONCLUSIONS: Among participants in REDUCE-IT, allocation to icosapent ethyl had minimal effects on a series of biomarkers associated with atherosclerotic disease, whereas levels increased among those allocated to mineral oil. The effect of these findings on interpretation of the overall risk reductions in clinical events observed within REDUCE-IT is uncertain.

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Key Words: atherosclerosis ■ biomarkers ■ clinical trial ■ mineral oil

ORIGINAL RESEARCH ARTICLE

Editorial, see p 380

Clinical Perspective

What Is New?

- In an evaluation of biomarkers within REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial), random allocation to icosapent ethyl had little to no effect on lipoprotein(a), homocysteine, oxidized low-density lipoprotein, interleukin-6, Lp-PLA2 activity, interleukin-1β, high-sensitivity C-reactive protein, or low-density lipoprotein cholesterol levels at 12 months, whereas increases in these biomarkers were observed among patients randomly allocated to a mineral oil comparator.

What Are the Clinical Implications?

- Our findings may have implications for understanding the biologic pathways associated with clinical outcomes observed in REDUCE-IT.
- The effect of these findings on interpretation of the overall risk reductions in clinical events observed within REDUCE-IT is uncertain.

Nonstandard Abbreviations and Acronyms

EPA	eicosapentaenoic acid
hsCRP	high-sensitivity C-reactive protein
Lp-PLA2	lipoprotein-associated phospholipase A2
OxLDL	oxidized low-density lipoprotein cholesterol
REDUCE-IT	Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial

REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial) demonstrated a 25% reduction in first major adverse ischemic events (hazard ratio, 0.75 [95% CI, 0.68–0.83]; $P<0.001$) and a 30% reduction in the combination of first and recurrent ischemic events (hazard ratio, 0.70 [95% CI, 0.62–0.78]; $P<0.001$) among patients with elevated triglyceride levels (≥135 mg/dL and <500 mg/dL) treated with icosapent ethyl 4 grams daily (2 grams twice per day) or a pharmaceutical grade mineral oil comparator.[1,2] The mechanisms underlying these clinical benefits remain uncertain. In particular, whereas icosapent ethyl lowered triglyceride levels by 20%, observed event reductions within REDUCE-IT were similar across baseline triglyceride tertiles as well as across on-treatment levels of triglycerides.[3]

Hypothesized mechanisms that could provide insight into the clinical benefit of icosapent ethyl include potential effects on inflammation, particularly inhibition of the central interleukin-1β to interleukin-6 to C-reactive protein pathway of innate immunity that is important for atherosclerosis development[4,5] and where treatment with a targeted interleukin-1β inhibitor has demonstrated clinical benefit.[6] Other potential mechanisms of effect include hypothesized beneficial effects of icosapent ethyl on biomarkers such as oxidized low-density lipoprotein cholesterol (OxLDL), homocysteine, lipoprotein(a), and lipoprotein-associated phospholipase A2 (Lp-PLA2).[7-9] Furthermore, in light of neutral effects in a large outcomes trial of eicosapentaenoic acid (EPA) combined with docosahexaenoic acid,[10,11] there has been controversy regarding the use of mineral oil as a comparator within REDUCE-IT, an issue where biomarker and outcome data are limited.[12-14]

To begin addressing these issues, levels of interleukin-1β, interleukin-6, high-sensitivity C-reactive protein (hsCRP), OxLDL, homocysteine, lipoprotein(a), and Lp-PLA2 were measured at baseline, at 12 months, at 24 months, and at the end-of-study visit among REDUCE-IT participants using their archived serum samples.

METHODS

REDUCE-IT randomly allocated 8179 statin-treated patients with triglyceride levels >135 mg/dL and <500 mg/dL to treatment with 2 grams twice daily of icosapent ethyl or mineral oil used as a comparator. Participants with established atherosclerotic disease or diabetes plus additional cardiovascular risk factors were included and followed over a median period of 4.9 years (maximum 6.2 years) for major adverse cardiovascular events (myocardial infarction, stroke, coronary revascularization, hospitalization for unstable angina, or cardiovascular death). The methods used for participant screening, randomization, follow-up, and end point ascertainment as well as descriptions of the

cohort baseline characteristics and overall effects of icosapent ethyl compared with mineral oil on the trial primary end point have been presented previously.[1-3] REDUCE-IT was approved globally by institutional review committees and all participants gave informed consent before enrollment. The data that support the findings of this study may be made available from the corresponding author on reasonable request.

Per protocol, serum samples were archived at randomization and on an annual basis throughout the trial, including at the end-of-study visit. For this analysis, biomarker levels were measured post hoc in up to 8175 trial participants who had adequate serum samples available at baseline, at 12 months, at 24 months, and at the end-of-study visit. Graphical displays of the distribution of exposure time for trial participants, overall and by treatment group, are provided in Figures S1 and S2. Samples within REDUCE-IT have been stored in a commercial freezer facility at −70 °C since original collection starting at time of randomization through trial completion (minimum of 2 and maximum of 9 years before analysis).

Levels of interleukin-1β, interleukin-6, and OxLDL were measured in the Laboratory of Clinical Chemistry, Children's Hospital Medical Center in Boston, Massachusetts, a facility that has served as a core reference laboratory for multiple inflammation biology trials. Levels of hsCRP at 48 months and end of study as well as all measures of homocysteine, lipoprotein(a), and Lp-PLA2 activity were measured by Nexelis Laboratories. Levels of low-density lipoprotein (LDL) cholesterol and triglycerides were measured during the trial by Covance Laboratories, as well as levels of hsCRP at baseline and at 24 months. Details of each assay are provided in the Supplemental Material.

Statistical Analysis

Following the trial prespecified statistical analysis plan, the effects of treatment with icosapent ethyl or mineral oil were evaluated as the median percent change from baseline at 12 months, 24 months, and the end-of-study visit. Analyses are presented both within group and between groups. Tests for significance of within-group percent change from baseline were computed using Wilcoxon signed-rank tests and between-group differences in percent change were computed using Wilcoxon rank-sum tests. The median differences between groups were computed using the Hodges-Lehmann estimation as described in the Supplemental Material.

To further understand interrelationships between biomarkers, Spearman correlation matrices for the change in biomarker levels over time (pooled follow-up data minus baseline data) were constructed between each pair of biomarkers, stratified by treatment.

All *P* values are 2-sided and all CIs computed at the 95% level without multiplicity adjustment.

RESULTS

At baseline, median levels of all biomarkers were similar in the 2 treatment groups. The levels of biomarkers associated with atherosclerosis increased over time among those allocated to mineral oil treatment; in this group at 12 months, the median percent increases from baseline were 1.5% for homocysteine, 2.2% for lipoprotein(a), 10.9% for OxLDL, 16.2% for interleukin-6, 18.5% for Lp-PLA2, 21.9% for hsCRP, and 28.9% for interleukin-1β (all *P* values <0.001) with similar changes at 24 months. In the icosapent ethyl group, there were minimal changes in these biomarkers at 12 and 24 months. As such, at study conclusion, between-group treatment differences largely reflected increases in the mineral oil group with median percent differences of 2.4% for lipoprotein(a), 3.0% for homocysteine, 4.2% for OxLDL, 19.8% for interleukin-6, 26.2% for Lp-PLA2, 38.5% for hsCRP, and 48.7% for interleukin-1β (all *P* values ≤0.007). These biomarker data are consistent with previous REDUCE-IT results, in which the median percent change for LDL cholesterol at 12 months was −1.2% among those allocated to icosapent ethyl and 10.9% among those allocated to the mineral oil comparator (Table). As also shown in the Table for completeness, the median percent change in triglycerides at 12 months was −18.3% in the icosapent ethyl group as compared with 2.2% in the mineral oil group.

Correlation matrices between each pair of biomarkers evaluating the changes over time (pooled 12-month, 24-month, and end-of-study value minus baseline value) are presented in the Figure for the icosapent ethyl group (top) and for the mineral oil group (bottom). As shown, moderately positive pairwise correlations over time (*r* values between 0.32 and 0.53) were observed between the change in hsCRP and the change in interleukin-6, the change in Lp-PLA2 and the change in OxLDL, the change in LDL cholesterol and the change in Lp-PLA2, and the change in LDL cholesterol and OxLDL (all *P* values <0.001) with effect sizes similar in the mineral oil comparator and icosapent ethyl groups.

The distribution of trial participants by time from randomization is shown for all participants in Figure S1 and stratified by treatment assignment in Figure S2.

DISCUSSION

Among patients with elevated triglyceride levels on primary and secondary prevention treatment with statins, REDUCE-IT has previously demonstrated statistically significant reductions in major adverse cardiovascular events in a randomized treatment trial of 4 grams daily of icosapent ethyl and mineral oil used as a comparator.[1,2] These effects, however, are not tied closely to baseline or achieved triglyceride levels[3] and the causal mechanisms underlying the clinical benefits observed in REDUCE-IT remain uncertain.

The current data indicate that within REDUCE-IT, allocation to icosapent ethyl had minimal effects on a series of biomarkers associated with atherosclerotic disease, whereas levels increased among those allocated to mineral oil. These data are consistent with pervious REDUCE-IT results in which the median percent change for LDL cholesterol at 12 months was −1.2%

Table. Effects of Treatment With Icosapent Ethyl and a Pharmaceutical-Grade Mineral Oil Comparator on Measured Biomarkers and Low-Density Lipoprotein Cholesterol and Triglycerides in REDUCE-IT

	Mineral oil (n=4090)				Icosapent ethyl (n=4089)				Between-group difference	
Marker	Median (IQR)	Median change	Median change, %*	*P*†	Median (IQR)	Median change	Median change, %*	*P*†	Median difference, %‡	*P*§
hsCRP, mg/L										
Baseline (n=4089:4086)	2.15 (1.07–4.50)				2.18 (1.07–4.49)					
12 months (3077:3089)	2.80 (1.3–5.2)	0.32	21.95	<0.0001	1.90 (0.9–3.9)	−0.18	−12.41	0.003	−30.48	<0.0001
24 months (3229:3322)	2.79 (1.3–5.8)	0.47	32.26	<0.0001	1.79 (0.86–4.01)	−0.18	−13.86	0.04	−39.91	<0.0001
Last visit‖ (3113:3198)	2.79 (1.33–5.49)	0.42	30.12	<0.0001	1.69 (0.81–3.99)	−0.19	−13.25	0.58	−38.48	<0.0001
Interleukin-6, pg/mL										
Baseline (n=3133:3203)	3.27 (2.16–5.17)				3.23 (2.14–5.02)					
12 months (2875:2907)	3.76 (2.42–6.09)	0.44	16.22	<0.0001	3.09 (2.05–5.06)	−0.08	−2.60	0.005	−16.29	<0.0001
24 months (2819:2933)	3.86 (2.53–6.04)	0.50	18.21	<0.0001	3.08 (2.04–4.98)	−0.05	−1.98	0.0004	−19.47	<0.0001
Last visit‖ (2491:2654)	3.97 (2.56–6.49)	0.73	26.25	<0.0001	3.24 (2.05–5.16)	0.09	3.01	<0.0001	−19.82	<0.0001
Interleukin-1β, pg/mL										
Baseline (n=3134:3204)	0.06 (0.03–0.10)				0.06 (0.03–0.10)					
12 months (2875:2908)	0.08 (0.04–0.13)	0.02	28.89	<0.0001	0.06 (0.03–0.10)	0.00	0.00	<0.0001	−26.06	<0.0001
24 months (2820:2934)	0.08 (0.04–0.13)	0.02	30.68	<0.0001	0.05 (0.03–0.09)	0.00	0.00	<0.0001	−34.47	<0.0001
Last visit‖ (2492:2655)	0.09 (0.05–0.15)	0.03	48.28	<0.0001	0.05 (0.03–0.09)	0.00	0.00	<0.0001	−48.65	<0.0001
OxLDL, mU/L										
Baseline (n=3134:3204)	45 879 (37 523–54 088)				44 641 (36 863–53 483)					
12 months (2875:2908)	50 457 (40 986–61 384)	4877.57	10.94	<0.0001	45 594 (37 888–56 627)	1293.21	2.94	<0.0001	−7.37	<0.0001
24 months (2820:2934)	48 725 (39 607–59 661)	3493.94	7.81	<0.0001	45 410 (36 819–55 576)	400.70	0.81	<0.0001	−6.46	<0.0001
Last visit‖ (2492:2655)	47 838 (38 710–58 877)	2301.78	5.06	<0.0001	45 251 (36 669–55 529)	59.68	0.15	<0.0001	−4.23	<0.0001
Homocysteine, μmol/L										
Baseline (n=3509:3514)	12.50 (10.38–15.13)				12.64 (10.36–15.38)					
12 months (3073:3087)	12.55 (10.40–15.64)	0.18	1.46	<0.0001	12.55 (10.38–15.51)	0.05	0.39	0.0007	−1.22	0.02
24 months (2912:2987)	13.05 (10.70–16.04)	0.53	4.44	<0.0001	12.87 (10.52–15.8)	0.32	2.70	<0.0001	−2.19	0.0004
Last visit‖ (2576:2725)	13.61 (11.00–16.95)	1.12	9.51	<0.0001	13.40 (11.02–16.63)	0.75	6.17	<0.0001	−2.98	0.0001
Lipoprotein(a), mg/dL										
Baseline (n=3511:3515)	11.40 (5.00–36.80)				11.60 (5.10–37.90)					
12 months (3077:3089)	12.60 (5.20–39.90)	0.40	2.17	<0.0001	12.50 (5.40–40.00)	0.00	0.00	<0.0001	−0.91	0.07
24 months (2920:2991)	12.60 (5.10–39.45)	0.40	2.81	<0.0001	12.40 (5.30–39.00)	0.00	0.00	<0.0001	−2.45	0.0003
Last visit‖ (2579:2726)	13.60 (5.60–41.20)	1.15	7.6	<0.0001	13.45 (5.70–41.50)	0.70	4.41	<0.0001	−2.43	0.007
Lp-PLA2, nmol/min/mL										
Baseline (n=3485:3480)	134.00 (113.00–159.00)				134.00 (113.00–157.00)					
12 months (3032:3057)	157.90 (131.40–185.60)	24.00	18.46	<0.0001	129.8 (107.50–153.30)	−4.50	−3.50	<0.0001	−21.13	<0.0001
24 months (2894:2970)	159.65 (132.50–191.00)	26.60	20.18	<0.0001	128.2 (106.80–152.10)	−5.90	−4.42	<0.0001	−23.92	<0.0001
Last visit‖ (2543:2705)	166.90 (136.90–202.60)	33.40	25.81	<0.0001	133.2 (111.60–159.30)	−1.70	−1.30	0.72	−26.17	<0.0001
LDL-C, Hopkins, mg/dL										
Baseline (n=4089:4086)	86.7 (75.0–98.2)				85.8(74.1–97.2)					
12 months (3618:3672)	95.9 (81.2–112.9)	9.29	10.94	<0.0001	85.3(70.9–102.9)	−1.12	−1.19	0.06	−11.42	<0.0001

(*Continued*)

Table. Continued

Marker	Mineral oil (n=4090)				Icosapent ethyl (n=4089)				Between-group difference	
	Median (IQR)	Median change	Median change, %*	*P*†	Median (IQR)	Median change	Median change, %*	*P*†	Median difference, %‡	*P*§
24 months (3240:3339)	96.1 (80.6–113.9)	9.50	11.41	<0.0001	85.5(71.4–103.3)	−0.15	−0.20	0.0001	−11.12	<0.0001
Last visit‖ (3146:3235)	91.5 (74.7–109.0)	4.86	5.99	<0.0001	83.8(69.0–102.8)	−1.43	−1.65	0.52	−7.01	<0.0001
Triglycerides, mg/dL										
Baseline (n=4089:4086)	216.0 (175.5–274.0)				216.5 (176.5–272.0)					
12 months (3633:3689)	221.0 (164.0–298.0)	4.50	2.24	<0.0001	175.0 (132.0–238.0)	−39.00	−18.32	<0.0001	−19.72	<0.0001
24 months (3257:3352)	220.0 (164.0–294.0)	4.25	2.09	<0.0001	173.0 (129.0–238.0)	−38.50	−18.86	<0.0001	−19.68	<0.0001
Last visit‖ (3152:3243)	200.5 (148.5–275.0)	−15.50	−7.57	<0.0001	169.0 (124.0–234.0)	−46.00	−22.22	<0.0001	−13.82	<0.0001

hsCRP indicates high-sensitivity C-reactive protein; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp-PLA2, lipoprotein-associated phospholipase A2; OxLDL, oxidized low-density lipoprotein cholesterol; and REDUCE-IT, Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial.

*Change and percent change from baseline at each postbaseline visit are calculated for patients with both baseline and postbaseline values.

†Median percent change *P* value was from Wilcoxon signed-rank test.

‡Median percent difference was on the basis of Hodges-Lehmann estimation.

§Median percent difference *P* value was from Wilcoxon rank-sum test. Biomarker (lower limit of quantification): hsCRP, 0.3 mg/L; interleukin-6, 0.01 pg/mL; interleukin-1β, 0.030 pg/mL; OxLDL, 1 mU/L; homocysteine, 2.00 μmol/L; lipoprotein(a), 3.3 mg/dL; Lp-PLA2, 10.0 nmol/min/mL.

‖The analysis at last visit includes all available data for patients who completed the protocol-specified last visit.

among those allocated to icosapent ethyl compared with a 10.9% median increase among those allocated to mineral oil.[1]

It is unclear why multiple biomarkers increased over time among REDUCE-IT participants allocated to mineral oil. No substantive biomarker changes were observed in the placebo groups over periods of 3 to 5 years for measures of LDL cholesterol, interleukin-1β, interleukin-6, or hsCRP in the large-scale JUPITER (Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin), CIRT (Cardiovascular Inflammation Reduction Trial), CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), or SPIRE (Evaluation of Bococizumab in Reducing the Occurrence of Major Cardiovascular Events in High-Risk Subjects),[6,15-17] nor did biomarker levels increase over time in STRENGTH (Outcomes Study to Assess Statin Residual Risk Reduction With Epanova In High CV Risk Patients With Hypertriglyceridemia), which used a corn oil rather than a mineral oil placebo.[10,11] Furthermore, no change in Lp-PLA2 over time was observed in the placebo arms of trials evaluating potential inhibitors of this pathway[18,19] or in studies of stability of circulating Lp-PLA2 levels over time.[20]

The core design of REDUCE-IT does not make it possible to resolve convincingly whether any adverse effects associated with mineral oil use as a comparator may have affected clinical outcomes. Resolution of this controversy can only be addressed formally by undertaking a biomarker trial randomly allocating patients to pharmaceutical grade mineral oil and a fully neutral placebo or by a second icosapent ethyl trial using a non–mineral oil comparator. Whereas the former is unlikely to be conducted for ethical reasons, the latter would help resolve ongoing controversy. The only other data in this arena are a 19% reduction in risk of major vascular events observed in the open-label JELIS (Japan EPA Lipid Intervention Study), using icosapent ethyl, in which no placebo was used.[21] We are not aware of other prospective intervention data demonstrating that mineral oil at a quantity of 4 grams daily compared with corn oil or other forms of placebo has effects, direct or indirect, on the biomarkers measured here. Such data would be helpful.

What net clinical effect the current data, if taken in combination, might have had on outcomes in REDUCE-IT is difficult to estimate. Regulatory agencies evaluating REDUCE-IT estimated that approximately 3% of the net clinical benefit observed with icosapent ethyl might have been a consequence of adverse biomarker effects on LDL cholesterol and hsCRP attributable to mineral oil.[12] In the context of an overall 25% relative risk reduction in first events and a 30% reduction in total ischemic events observed, a potential bias of this magnitude, even if doubled in size, would be unlikely to fully attenuate the overall benefit of icosapent ethyl observed. At the same time, we are not aware of a simple or widely accepted method to assess in an unbiased manner what the potential magnitude might be of a combination of the multiple effects observed here, an issue that again requires prospective comparison data for resolution.

Despite our large sample size and randomized double-blind design, there are limitations to our analyses that merit consideration. First, care must be taken not to generalize these findings beyond what is presented here; for example, whereas we evaluated for effects in the canonical interleukin-1 to interleukin-6 to C-reactive protein pathway of innate immunity,[4,5] we did not evaluate

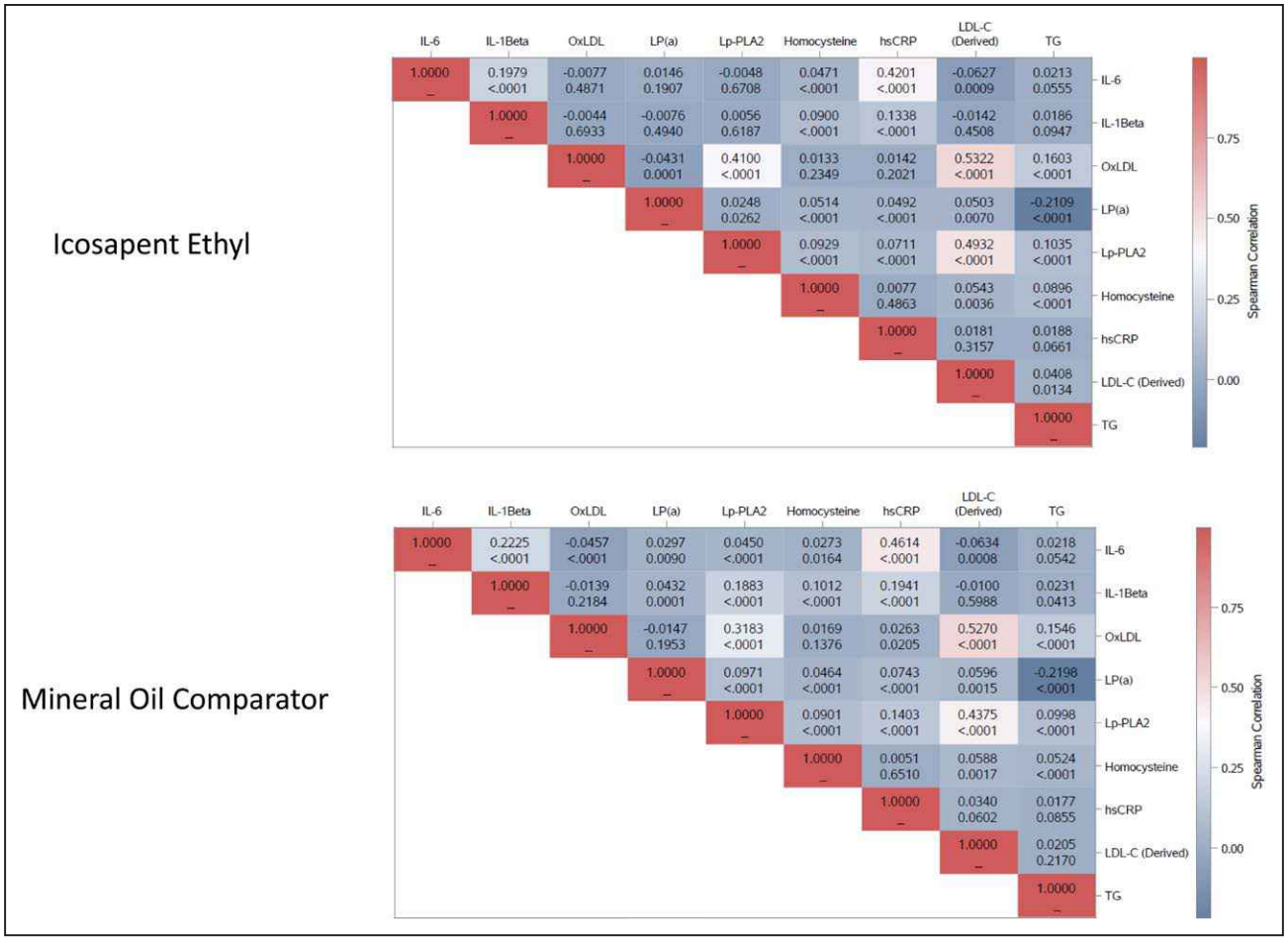

Figure. Spearman correlation matrices between biomarker changes over time in REDUCE-IT.
Spearman correlation matrices between biomarker changes over time in REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl—Intervention Trial) for patients allocated to icosapent ethyl (top) and patients allocated to the mineral oil comparator (bottom). hsCRP indicates high-sensitivity C-reactive protein; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; Lp-PLA2, lipoprotein-associated phospholipase A2; LP(a), lipoprotein(a); OxLDL, oxidized low-density lipoprotein cholesterol; and TG, triglycerides.

alternative pathways of inflammation that might also be involved in atheroprotection. Specialized pro-resolving and other anti-inflammatory mediators, for example, are powerful anti-inflammatory molecules produced from EPA, and EPA can reduce production of proinflammatory factors from arachidonic acid competitively. None of these, however, has been measured in REDUCE-IT; nor have alternative biomarkers such as ceramides and glycoprotein acetylation. Second, large-scale randomized trials are designed to address clinical outcomes; as such, analyses of surrogate biomarker changes within trials, although of pathophysiologic interest, should not generally alter core clinical or therapeutic observations. Third, whereas pharmacologic interventions have proven that lowering of biomarkers such as LDL cholesterol and hsCRP associate with reduced risk, the contrapositive conclusion that elevations of these or other biomarkers necessarily increase risk is not proven, nor is the magnitude of any such effect known, even if the effects of the mineral oil comparator used in REDUCE-IT are taken to be causal.[12-14]

In sum, among participants in REDUCE-IT, allocation to icosapent ethyl had minimal effects on a series of biomarkers associated with atherosclerotic disease, whereas levels increased over time among those allocated to placebo, findings consistent with previous data in this cohort for LDL cholesterol. The effect of these findings on the interpretation of the REDUCE-IT trial results remains unclear and will require further investigation.[22]

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J. MacFadyen reports no conflicts.

Supplemental Material

Supplemental Methods

Figures S1 and S2

REFERENCES

1. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT, Juliano RA, Jiao L, Granowitz C, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med.* 2019;380:11–22. doi: 10.1056/NEJMoa1812792
2. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT, Juliano RA, Jiao L, Granowitz C, et al. Effects of icosapent ethyl on total ischemic events: from REDUCE-IT. *J Am Coll Cardiol.* 2019;73:2791–2802. doi: 10.1016/j.jacc.2019.02.032
3. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Jiao L, Tardif JC, Gregson J, Pocock SJ. Ballantyne CM and REDUCE-IT Investigators. Reduction in first and total ischemic events with icosapent ethyl across baseline triglyceride tertiles. *J Am Coll Cardiol.* 2019;74:1159–1161. doi: 10.1016/j.jacc.2019.06.043
4. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. *Circ Res.* 2016;118:145–156. doi: 10.1161/CIRCRESAHA.115.306656
5. Ridker PM. Anticytokine agents: targeting interleukin signaling pathways for the treatment of atherothrombosis. *Circ Res.* 2019;124:437–450. doi: 10.1161/CIRCRESAHA.118.313129
6. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med.* 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914
7. Bays HE, Ballantyne CM, Braeckman RA, Stirtan WG, Soni PN. 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.* 2013;13:37–46. doi: 10.1007/s40256-012-0002-3
8. Bays HE, Ballantyne CM, Kastelein JJ, Isaacsohn JL, Braeckman RA, Soni PN. 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.* 2011;108:682–690. doi: 10.1016/j.amjcard.2011.04.015
9. Ballantyne CM, Bays HE, Kastelein JJ, Stein E, Isaacsohn JL, Braeckman RA, Soni PN. 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.* 2012;110:984–992. doi: 10.1016/j.amjcard.2012.05.031
10. Nicholls SJ, Lincoff AM, Garcia M, Dash D, Ballantyne CM, Barter PJ, Davidson MH, Kastelein JJP, Koenig W, McGuire DK, et al. Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events

in patients at high cardiovascular risk: the STRENGTH randomized clinical trial. *JAMA.* 2020;324:2268–2280. doi: 10.1001/jama.2020.22258
11. Nissen SE, Lincoff AM, Wolski K, Ballantyne CM, Kastelein JJP, Ridker PM, Ray KK, McGuire DK, Mozaffarian D, Koenig W, et al. Association between achieved omega-3 fatty acid levels and major adverse cardiovascular outcomes in patients with high cardiovascular risk: a secondary analysis of the STRENGTH trial. *JAMA Cardiol.* 2021;6:910–917. doi: 10.1001/jamacardio.2021.1157
12. Olshansky B, Chung MK, Budoff MJ, Philip S, Jiao L, Doyle RT, Copland C, Giaquinto A, Juliano RA, Bhatt DL. Mineral oil: safety and use as placebo in REDUCE-IT and other clinical studies. *Eur Heart J Suppl.* 2020;22:J34–J48. doi: 10.1093/eurheartj/suaa117
13. Doi T, Langsted A, Nordestgaard BG. A possible explanation for the contrasting results of REDUCE-IT vs. STRENGTH: cohort study mimicking trial designs. *Eur Heart J.* 2021;42:4807–4817. doi: 10.1093/eurheartj/ehab555
14. Steg PG, Bhatt DL. The reduction in cardiovascular risk in REDUCE-IT is due to eicosapentaenoic acid in icosapent ethyl. *Eur Heart J.* 2021;42:4865–4866. doi: 10.1093/eurheartj/ehab760
15. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Kastelein JJP, Koenig W, Libby P, Lorenzatti AJ, MacFadyen J, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med.* 2008;359:2195–2207. doi: 10.1056/NEJMoa0807646
16. Ridker PM, Everett BM, Pradhan A, MacFadyen J, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, et al. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med.* 2019;380:752–762. doi: 10.1056/NEJMoa1809798
17. Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F, Flather M, Glynn RJ, Gregoire J, Jukema JW, et al. Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med.* 2017;376:1527–1539. doi: 10.1056/NEJMoa1701488
18. The STABILITY Investigators. Darapladib for preventing ischemic events in stable coronary heart disease. *N Engl J Med.* 2014;370:1702–1711. doi: 10.1056/NEJMoa1315878
19. O'Donoghue ML, Braunwald E, White HD, Steen DL, Lukas MA, Tarka E, Steg PG, Hochman JS, Bode C, Maggioni AP, et al. Effect of darapladib on major coronary events after an acute coronary syndrome. The SOLID-TIMI 52 randomized clinical trial. *JAMA* 2014;312:1006–1015. doi: 10.1001/jama.2014.11061
20. Elkind MSV, Leon V, Moon YP, Paik MC, Sacco RL. High-sensitivity C-reactive protein and lipoprotein-associated phospholipase A2 stability before and after stroke and myocardial infarction. *Stroke* 2009;40:3233–3237. doi: 10.1161/STROKEAHA.109.552802
21. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;369:1090–1098. doi: 10.1016/S0140-6736(07)60527-3
22. Mason RP, Libby P, Bhatt DL. Emerging mechanisms of cardiovascular protection for the omega-3 fatty acid eicosapentaenoic acid. *Arterioscler Thromb Vasc Biol.* 2020;40:1135–1147. doi: 10.1161/ATVBAHA.119.313286