July 16, 2019

Submitted via Regulations.gov

Divisions of Docket Management
Department of Health and Human Services
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

CITIZEN PETITION

Medical Research Collaborative, LLC submits this petition under 21 C.F.R. § 10.30 and 21 C.F.R. § 10.31 of the Federal Food, Drug, and Cosmetic Act (FDCA), to request that the Commissioner of Food and Drugs (Commissioner) take the actions identified in section A below.

A. Actions Requested

Medical Research Collaborative respectfully requests that the Commissioner delays approval of the supplemental New Drug Application (sNDA) for icosapent ethyl, aka "Vascepa," which is currently under review for a broad label based on the results of the REDUCE-IT trial, until such time as reliable data are presented to the agency from multiple clinical drug interaction studies, proving that concomitant dosing of mineral oil with cardiac medications does not attenuate the absorption/efficacy of the latter.

We also recommend that such studies mimic the actual dosing patterns of subjects in the placebo group of REDUCE-IT, by requiring that the sponsor use the services of a third-party organization to poll such participants if living, and family members if deceased, to ascertain the most common dosing pattern of such subjects (i.e. With food? Without food? Part of polypharmacy in evening, hours after last meal? Etc.), so that drug-drug interaction studies can be designed to accurately reflect what actually occurred in the trial, not just what was recommended (*"2 g twice a day with food"*).

Should the above proposed DDI studies present compelling evidence the REDUCE-IT trial design, which utilized what appears to be a placebo that is not inert and may attenuate the efficacy of concomitant cardiac medications, was faulty to answer the central question of the study (i.e. "Does dosing with 4 g/d icosapent ethyl reduce the risk of ASCVD in subjects with elevated triglyceride levels who are also at increased risk of a cardiac event?"), we recommend the special protocol assessment

(SPA) agreement for the REDUCE-IT trial be rescinded, and a complete response letter (CRL) be issued to the sponsor, requesting reliable efficacy data from a new cardiovascular outcomes trial (CVOT) designed with a truly inert placebo before expanding the label for Vascepa to include patients with elevated triglycerides (~135 mg/dL – 499 mg/dL) and at increased risk of ASCVD.

We further request the Commissioner place a clinical hold on the EVAPORATE trial (ClinicalTrials.gov Identifier: NCT02926027), which is being funded by Amarin Corp.,[1] and in which the same 4 g/d mineral oil dose as in REDUCE-IT is currently being administered to its placebo group subjects, who are on similar background therapies and of similar ASCVD risk as the REDUCE-IT trial subjects, and any other ongoing trials that utilize a mineral oil placebo where concomitant therapies might also be attenuated, until such time as mineral oil is proven harmless in this regard, as such subjects could be exposed to immediate and serious harm.

Lastly, we recommend a warning be required on the label of all largely undigestible, synthetic fats or fat-substitutes for sale in the US that are for internal use (i.e. mineral oil), or are a main ingredient in such products for consumption (i.e. olestra), mentioning the possibility that synthetic or semi-synthetic, poorly digested lipids may interfere with the absorption of orally administered drugs—unless and until the results of numerous DDI studies exonerate these specific oils in this regard.

B. Statement of Grounds

Impact of Light Paraffin Oil Placebo on the Interpretation of REDUCE-IT Trial Results

1.1 Summary

The ability to confidently interpret data from drug trials is of the utmost importance in patient care and drug regulation.

There is now moderately strong evidence that light paraffin oil adversely impacts the utility of cardiac medications, calling into question the degree of risk reduction observed in the REDUCE-IT trial.

We conclude that multiple DDI studies are warranted to determine the degree to which statins and other concomitant therapies may be attenuated by light paraffin oil before a label expansion for icosapent ethyl is granted—and further, that studies utilizing light paraffin oil placebo should be placed on clinical hold until the issue is resolved, as subjects could be exposed to immediate and serious harm.

[1] Budoff M, Brent Muhlestein J, Le VT, May HT, Roy S, Nelson JR. Effect of Vascepa (icosapent ethyl) on progression of coronary atherosclerosis in patients with elevated triglycerides (200-499 mg/dL) on statin therapy: Rationale and design of the EVAPORATE study. *Clin Cardiol.* 2018. [link]

1.2 Introduction

The REDUCE-IT trial results were presented at the AHA Scientific Sessions on November 10th, 2018, along with a paper released in the NEJM on the study, sponsored by Amarin Corp. Data were glowingly positive on the primary and nearly all secondary endpoints. The relative risk reduction (RRR) of ASCVD events was 25% for treatment group, heralding the therapy (icosapent ethyl, a highly purified fish oil composed of >95% eicosapentaenoic acid (EPA), brand name "Vascepa") as a potential landmark treatment for secondary cardiovascular disease patients with persistently elevated triglycerides (150 mg/dL - 499 mg/dL).

However, in the Discussion section of the NEJM paper, there was a mention expressing the potential that light paraffin oil (aka "mineral oil," hereafter abbreviated "MO") placebo may have adversely impacted statin absorption in some unknown number of subjects.

> "Our trial has certain limitations. First, at the time the trial was designed, there was relatively little use of ezetimibe or data supporting its use. However, subgroup analyses do not suggest a differential benefit for patients taking ezetimibe. Similarly, proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibitors were not available for the majority of the patients in the trial. Second, **if mineral oil in the placebo affected statin absorption in some patients, this might have contributed to differences in outcomes between the groups.** However, the relatively small differences in LDL cholesterol levels between the groups would not be likely to explain the 25% lower risk observed with icosapent ethyl, and a post hoc analysis suggested a similar lower risk regardless of whether there was an increase in LDL cholesterol level among the patients in the placebo group. Although JELIS was designed as an open-label study that did not use a mineral oil placebo, it showed a 19% lower risk of ischemic events with statin therapy plus EPA than with statin therapy alone." [2]

This is a perplexing statement, because it casts the results into ambiguity. The question arises, "To what extent is the stated 25% RRR overblown?" The study authors attempt to answer this with three points, the first being, *"The relatively small differences in LDL-C levels between groups would not be likely to explain the 25% lower risk observed with icosapent ethyl."* Elsewhere the Global Principal Investigator, Dr. Bhatt, provided color to this statement, suggesting the increase in LDL-C in MO placebo group infers at most a lowering of the MCE composite RRR from 25% to 20%.[3] But is this speculation sanguine?

1.3 Effect of Between-Group Differences in Biomarkers on ASCVD Event Risk

The 2013 ACC/AHA guidelines[4] introduced a paradigm shift in our perspective on LDL-C targets in favor of matching intensity of statin therapy with a patient's risk category over achieving LDL-C goals, due to a lack of RCTs testing ASCVD risk reduction based on numeric level of LDL-C achieved (only meta-analyses

[2] Bhatt DL, Steg G, Miller M, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. *N Eng J Med*. 2018. [link]
[3] Bhatt DL interview w/ Walton-Shirley MK. CV Risk: Can Prescription Omega-3 REDUCE-IT? *Medscape*. 2018. [link]
[4] Stone NJ, Robinson JG, Lichtenstein AH, et al. *Circulation*. 2013. [link]

provide these extrapolated data). Instead, all statin trials compared a set intensity of therapy against placebo or another, usually lower intensity, statin. These data suggest that two patients of similar risk strata will achieve similar risk reductions with similar percent reductions in LDL-C as a result of statin therapy, more or less regardless of baseline values. The updated guidelines (Nov 2018) reiterate the same, summarizing current thinking below:

> "In large RCTs of cholesterol-lowering therapy, LDL-C lowering has been consistently shown to reduce the risk of ASCVD. One large meta-analysis (S2.4-1) of statin clinical trials showed a progressive reduction in risk of major ASCVD events with lower on-treatment LDL-C levels. In another larger meta-analysis (S2.4-2) of 14 statin trials, it was observed that a 38.7 mg/dL (1-mmol/L) reduction of LDL-C levels is accompanied by a 21% reduction in ASCVD risk. In clinical practice, however, absolute responses in LDL-C to statin therapy depend on baseline LDL-C concentrations**. A given dose of statins produces a similar percentage reduction in LDL-C levels across a broad range of baseline LDL-C levels. For this reason, a more reliable indicator of statin efficacy is percentage reduction**. In the present document, the percentage reduction is used in follow-up monitoring of patients to estimate the efficacy of statin therapy. As a rough guide, **a lowering of LDL-C levels of 1% gives an approximate 1% reduction in the risk of ASCVD**—somewhat more at higher baseline LDL-C levels and somewhat less at lower baseline levels (S2.4-1)."

Thus, if one patient has a 5-year ASCVD risk of 20% and a baseline LDL-C of 240 mg/dL, and experiences a 3 mmol/L reduction in LDL-C as a result of statin therapy, they will obtain a similar risk reduction as another patient with a 5-year risk of 20% and a baseline LDL-C of 160 mg/dL that experiences a 2 mmol/L reduction in LDL-C—because they both achieved the same percentage lowering (~48%). The difference in absolute point reduction in LDL-C between the two patients is less relevant.

Percentage reductions in LDL-C across various statin regimens and dosages are predictive, regardless of pre-statin levels:[5]

[5] Bjorn W. Karlson, Olov Wiklund, Michael K. Palmer, et al. Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: results from VOYAGE. *European Heart Journal*. 2016. [link]

Table 3 **Mean (SD) percent change in LDL-C in response to atorvastatin 10–80 mg, rosuvastatin 5–40 mg, and simvastatin 10–80 mg**

	n	LDL-C reduction (%)	
		Mean (SD)	Median (IQR)
Atorvastatin			
10 mg	7804	−35.7 (16.0)	−38.3 (−46.1, −28.8)
20 mg	3896	−43.1 (14.5)	−45.5 (−52.0, −37.2)
40 mg	1324	−47.9 (13.8)	−49.6 (−56.1, −42.4)
80 mg	2070	−49.2 (17.3)	−52.6 (−59.7, −43.4)
Rosuvastatin			
5 mg	668	−41.4 (12.8)	−43.6 (−49.5, −35.3)
10 mg	11 650	−43.5 (17.9)	−47.0 (−55.3, −36.1)
20 mg	3551	−49.4 (17.5)	−52.5 (−59.8, −43.4)
40 mg	2981	−55.5 (14.8)	−58.1 (−64.8, −49.6)
Simvastatin			
10 mg	165	−28.4 (13.8)	−29.4 (−37.6, −22.5)
20 mg	2923	−33.5 (15.8)	−35.8 (−43.9, −26.1)
40 mg	542	−40.3 (13.0)	−42.3 (−49.0, −33.2)
80 mg	478	−45.7 (13.1)	−47.6 (−54.7, −39.6)

IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.

An analysis of various dose-effects of atorvastatin showed that a 9.8-percentage point difference in LDL-C reduction between subjects represented an approximate 4-fold difference in statin dose.[6]

> "Atorvastatin 2.5 to 80 mg/d causes a linear dose-response reduction in percent change from control of blood total cholesterol and LDL-cholesterol. Manufacturer-recommended atorvastatin doses of 10 to 80 mg/d resulted in 37.1% to 51.7% decreases in LDL-cholesterol**.** From the slope of the lines, it can be seen that **for every two-fold increase, a 3.6% and 4.9% decrease in blood total cholesterol and LDL-cholesterol, respectively**, was noted."

A meta-analysis of statin trials that examined data from over 32,000 randomized subjects ascertained a range of 4 – 7 percentage point further lowering of LDL-C for every doubling of dose of various statins.[7]

---

[6] Adams SP, Tsang M, Wright JM. Atorvastatin for lowering lipids. *Cochrane Database of Systematic Reviews*. 2015. Issue 3. [link]

[7] Nicholls SJ1, Brandrup-Wognsen G, Palmer M, Barter PJ. Meta-analysis of comparative efficacy of increasing dose of Atorvastatin versus Rosuvastatin versus Simvastatin on lowering levels of atherogenic lipids (from VOYAGER). *Am J Cardiol*. 2010. [link]

Conversely, an approximate 4 – 7 percentage point decreased reduction in LDL-C from baseline would be observed if the dose was cut in half, and an approximate 8 – 14 percentage point decreased reduction would be observed if the dose was quartered. As mentioned in ACC/AHA guidelines, these percent changes are consistent across patient populations, irrespective of baseline values.

For example, in a study by Huang et al. (2012), two groups of subjects were given doses of the same statin in a 1:4 ratio. Those that were randomized to receive 10 mg/d atorvastatin experienced a reduction from baseline LDL-C levels of 2.75 mmol/L to 2.55 mmol/L, whereas those randomized to 40 mg/d saw levels fall from the same 2.75 mmol/L baseline to 2.19 mmol/L. The difference in absolute change in LDL-C levels between groups at the end of the study was 12%. In this instance that correlated with a 4-fold reduction in statin dose.[8]

In the robust sized REAL-CAD trial, subjects were randomized to recieve 1 mg/d or 4 mg/d pitavastatin. The difference in absolute change in LDL-C between groups was approximately 15%.[9]

Other atherogenic markers likewise show predictable changes between statin dosages. For example, rosuvastatin has been shown to reduce non-HDL-C by 42.0% at the 10 mg dose and 50.9% at the 40 mg dose. Therefore, an approximate 9-percentage point greater reduction resulted from 4-fold the statin dose. With atorvastatin there was a 14-percentage point greater reduction in non-HDL-C between the 10 mg and 80 mg dose, and an 8-percentage point greater reduction between the 10 mg and 40 mg dose. Thus, a 4 – 5 percentage point greater reduction in non-HDL-C for every doubling of dose (similar to LDL-C). The same was observed with simvastatin as well.

Rosuvastatin also reduced apoB by 36.7% to 45.3% between the 10 mg and 40 mg dose, demonstrating an 8.6-percentage point greater reduction from 4-fold the dose. The differential effects of various dosages of atorvastatin on apoB were somewhat more attenuated (~7% absolute reduction from 4-fold the dose). Simvastatin produced similar percent changes in apoB between dosages as atorvastatin.

Total cholesterol and triglyceride levels, meanwhile, showed a lesser reduction between lower and higher statin dosages. For example, rosuvastatin 10 mg versus 40 mg reduced TC by 32.9% and 40.2%, and TG by 19.8% and 26.1%, respectively. This equates to a 3.6 and 3.1 percentage point further reduction in TC and TG for every doubling of statin dose, respectively.[10]

The increase in LDL-C in placebo group in REDUCE-IT, concurrent with highly significant increases in all other atherogenic markers (representative of statin malabsorption), could therefore infer a multi-fold reduction in the effect of the administered statin dose.

---

[8] Bingsheng Huang MD Ying Cheng MD Qiang Xie BSM, et al. Effect of 40 mg Versus 10 mg of Atorvastatin on Oxidized Low-Density Lipoprotein, High-Sensitivity C-Reactive Protein, Circulating Endothelial-Derived Microparticles, and Endothelial Progenitor Cells in Patients With Ischemic Cardiomyopathy. *Clinical Cardiology*. 2012. Vol 35, Issue 2. [link]

[9] Isao Taguchi , Satoshi Iimuro , Hiroshi Iwata, et al. High-Dose Versus Low-Dose Pitavastatin in Japanese Patients With Stable Coronary Artery Disease (REAL-CAD). *Circulation*. 2018. [link]

[10] Jones, Peter H. et al. Effects of rosuvastatin versus atorvastatin, simvastatin, and pravastatin on non-high-density lipoprotein cholesterol, apolipoproteins, and lipid ratios in patients with hypercholesterolemia: additional results from the STELLAR trial. *Clinical Therapeutics*. 2004. [link]

1.4 Extrapolating the Potential Attenuation of Statin Efficacy in the REDUCE-IT Trial

In REDUCE-IT, the percentage increase in LDL-C from baseline in placebo group at 4 months, 1 year, 2 years, and 3 years was 8.7%, 10.9%, 11.4% and 10.5%, with corresponding between-group elevations in LDL-C of 10.1 mg/dL, 10.5 mg/dL, 10.6 mg/dL, and 11.1 mg/dL respectively. Not only LDL-C, but apoB, non-HDL-C, and CRP increased significantly from baseline (+7.8%, +10.4%, +32.3% at 1 year, respectively). Changes in total cholesterol were not reported.

Triglyceride levels, meanwhile, showed only a slight increase of 2.7% from baseline in placebo group. However, the full potential impact of dosing with 4 g/d MO concurrent with statin therapy on TG levels may have been masked by a contrariwise regression to the mean,[11] as the study preferentially selected for those with elevated levels at baseline (150 – 499 mg/dL). Those with a temporary elevation in TG levels would have experienced a normalization of levels soon after randomization, with the net effect being a reduction in median values. The 2.7% increase in placebo group occurred despite this tendency, and it is probable we would have observed a greater increase in TG without it.

The elevations in biomarkers in the REDUCE-IT placebo group are uncannily similar to what would be expected between groups given different intensity statins. And by the 5th and 6th years of the study, these elevated levels finally began to lower somewhat, presumably due to dropouts ceasing their MO therapy.

However, in order to determine what degree of statin malabsorption the ~11% increase in LDL-C in placebo group might represent, we would need to know the REDUCE-IT subjects' pre-statin LDL-C levels. Fortunately, this can be calculated with a fair degree of accuracy, given the reliable percent decreases observed across statin trials.

In REDUCE-IT it was divulged that 6.5%, 63%, and 30% of subjects randomized to placebo group were on background low-, moderate-, and high-intensity therapies respectively (0.5% data missing), defined as:

- simvastatin 5-10mg (low-intensity);
- rosuvastatin 5-10mg/ atorvastatin 10-20mg/ simvastatin 20-40mg/ simvastatin 10-20mg + ezetimibe 5-10mg (moderate-intensity);
- rosuvastatin 20-40mg/ atorvastatin 40-80mg/ simvastatin 80mg/ simvastatin 40-80mg + ezetimibe 5-10mg (high-intensity)

A calculation of the above study-specific dosages reveals an average LDL-C reduction corresponding with each intensity level as follows:

- Low intensity: 26%
- Moderate intensity: 38%
- High intensity: 49%

Therefore, 6.5% of the placebo group would have had their pre-statin LDL-C reduced by approximately 26% as a result of background therapy, 63% would have had their LDL-C reduced by ~38% due to

[11] Adrian G Barnett, Jolieke C van der Pols, Annette J Dobson. Regression to the mean: what it is and how to deal with it. *International Journal of Epidemiology*. 2005. [link]

background therapy, and 30% would have had their LDL-C reduced by ~49%. A net reduction of 40.5% in the group can thus be calculated ([6.5 * 26] + [63 * 38] + [30 * 49] / 99.5 = 40.5).

With a baseline LDL-C in the supplement to the REDUCE-IT study of 86.7 mg/dL (Hopkins) for placebo group, and all subjects on background statin therapy, a calculation using the above equates to a pre-statin LDL-C value of ~146 mg/dL. Thus, a return and stabilization to around 96 mg/dL in this group represents a reduction of 34% from pre-statin levels, and an absolute difference in LDL-C of ~7% between arms. This could in effect infer a 3-fold or more decrease in statin dose, considering data presented in the previous section.

It is accepted that the benefits of statin therapy are multifactorial, and go beyond LDL-C reduction, including: oxLDL reduction, decrease in LDL-P concentration, increase in LDL particle size, non-HDL-C reduction, stabilization and potential reduction of atherosclerotic plaque, inflammation reduction (Lp-PLA2, CRP, etc.), improved endothelial function, and inhibition of thrombogenesis.[12]

Higher intensity statins reduce and stabilize atherosclerotic plaque to a greater degree than lower intensity regimens. In one study, patients treated with rosuvastatin 10 mg/d showed a significant decrease in necrotic core volume (15.5 ± 8.4 mm^3 at baseline and 13.0 ± 9.4 mm^3 at follow-up, p = 0.015) and an increase in fibrofatty plaque volume (4.5 ± 4.0 mm^3 at baseline and 5.9 ± 3.5 mm^3 at follow-up, p = 0.017), whereas there were no significant changes in the simvastatin 20 mg/d arm. Meanwhile, the absolute difference in LDL-C reduction between groups was 11%.[13]

In the Treating to New Targets (TNT) Study, subjects with stable CHD were randomized to either 10 mg or 80 mg atorvastatin groups. All subjects began on 10 mg/d and experienced a 35% reduction in LDL-C from 152 mg/dL to 98 mg/dL. Half of the subjects were then randomized to 80 mg/d, and experienced a further reduction in LDL-C to 77 mg/dL, representing a total reduction from baseline of ~49%. Thus, the absolute difference in LDL-C reduction between groups was ~14%.[14] In the STELLAR trial, an approximate 14-percentage point greater reduction in LDL-C also resulted from the 80 mg dose vs the 10 mg dose (36.8% vs 51.1% with atorvastatin). In both instances, the moderate discrepancy in LDL-C reduction corresponded with an 8-fold difference in statin dose. The ~7-percentrage point difference in LDL-C reduction from pre-statin levels between groups in REDUCE-IT could thus infer as much as a 4-fold reduction in their administered statin dose.

If the placebo group in REDUCE-IT had a significantly lower level of their statin medication absorbed as a result of concurrent dosing with MO, then an apt comparison is not the relative risk between groups with lower versus higher LDL-C levels alone, but rather that between two groups of predominantly CHD patients administered lower versus higher intensity statins, with the resultant differential effects on atherogenic markers, inflammation, atherosclerotic plaque, and ultimately, ASCVD risk.

---

[12] De Groot LJ, Chrousos G, Dungan K, et al., editors. South Dartmouth (MA): *MDText.com, Inc.*; 2000. [link]

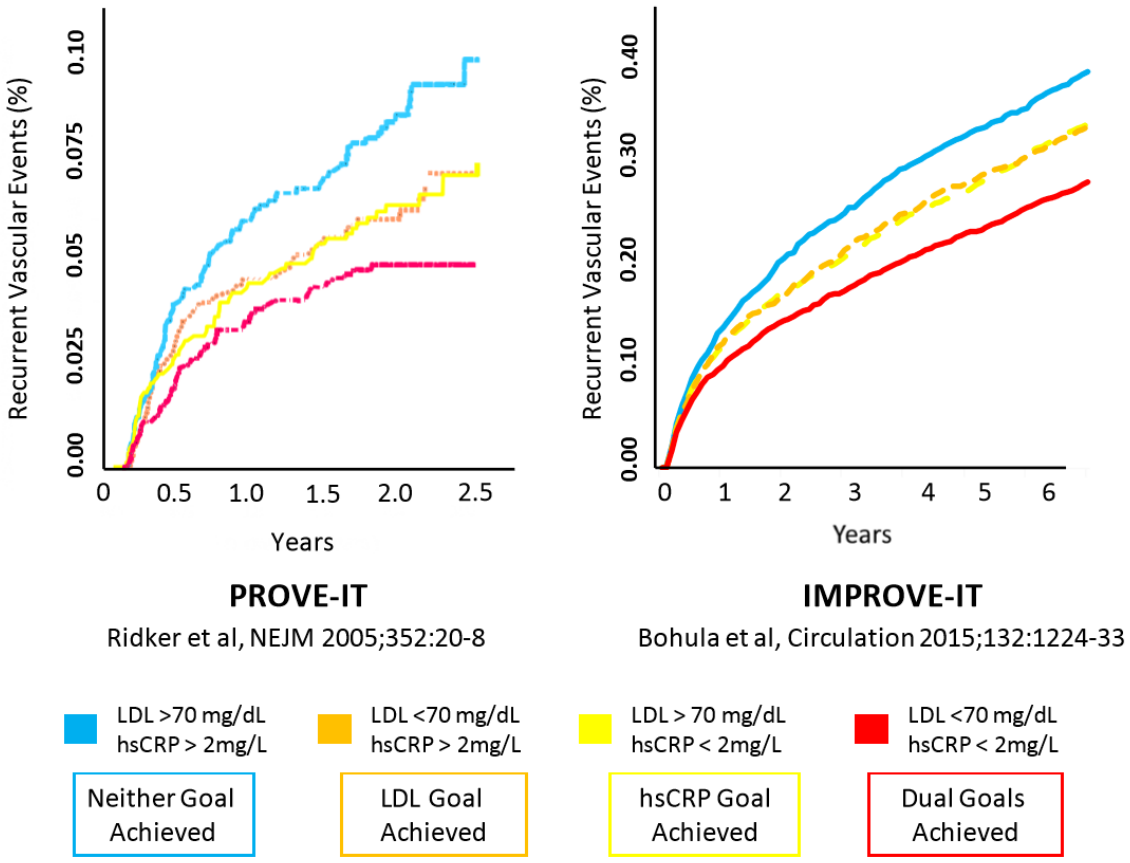
[13] Myeong-Ki Hong MD, Duk-Woo Park MD, Cheol-Whan Lee MD, et al. *JACC: Cardiovascular Interventions*, Volume 2, Issue 7, July 2009, Pages 679-688. [link]

[14] John C. LaRosa JC, Grundy SM, Waters DD, et al. Intensive Lipid Lowering with Atorvastatin in Patients with Stable Coronary Disease. *N Eng J Med*. 2005. [link]

1.5 The Role of CRP in Evaluating ASCVD Event Risk in REDUCE-IT

Going beyond extrapolations based on differences in atherogenic markers, the sharp increase in hs-CRP levels in MO placebo group in REDUCE-IT, which was observed early on and was maintained until the end of the study, provides further evidence of the heightened risk of this group.

Data have routinely demonstrated an increased prevalence of ASCVD events amongst patients with elevated levels of CRP—particularly in those with hs-CRP levels above 2.0 mg/L. This is independent of and additive to extrapolations based on LDL-C.[15]



Ridker et al, Eur Heart J 2016;37:1729-22

An analysis of the EXAMINE trial showed a similar finding.[16]

---

[15] Weber M, Bhatt DL, Brennan DM, et al. High-sensitivity C-reactive protein and clopidogrel treatment in patients at high risk of cardiovascular events: a substudy from the CHARISMA trial. *Heart*. 2011. [link]

[16] You-Cheol Hwang MD David A. Morrow MD Christopher P. Cannon MD, et al. High-sensitivity C-reactive protein, low-density lipoprotein cholesterol and cardiovascular outcomes in patients with type 2 diabetes in the EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care) trial. *Diabetes, Obesity, and Metabolism*. 2017. [link]

"Materials and methods

Study participants enrolled in the EXAMINE trial and were stratified by baseline hsCRP levels (<1, 1-3 and >3 mg/L). They were also sub-divided into 4 groups according to baseline hsCRP (≤3 or >3 mg/L) and achieved LDL-C (<70 or ≥70 mg/dL) levels. Among 5380 patients, the MACE rate, a composite of cardiovascular death, non-fatal acute myocardial infarction and non-fatal stroke, was evaluated during the 30 months of follow-up.

Results

Cumulative incidence of MACE was 11.5% (119 events), 14.6% (209 events) and 18.4% (287 events) in patients with hsCRP levels of <1, 1 to 3 and >3 mg/L, respectively (P < .001). In patients with hsCRP >3 mg/L, the adjusted hazard ratio (95% confidence interval) was 1.42 (1.13, 1.78; P = .002) for MACE compared with patients with hsCRP <1 mg/L. MACE cumulative incidences were 11.0% (128 events), 14.4% (100 events), 15.6% (194 events) and 21.3% (182 events) in patients with low LDL-C and low hsCRP, low LDL-C and high hsCRP, high LDL-C and low hsCRP, and high LDL-C and high hsCRP levels, respectively (P < .001).

Conclusions

Levels of hsCRP were associated with recurrent cardiovascular events in patients with type 2 diabetes and recent acute coronary syndrome, and this association appears to be independent of and additive to the achieved LDL-C level."

An adjusted analysis of the above data also showed that those with levels > 3.0 mg/L had a markedly increased risk of an ASCVD event, irrespective of other risk factors:

"In patients with baseline hsCRP >3 mg/L, the adjusted hazard ratio (HR) (95% confidence interval [CI]) was 1.42 (95% CI, 1.13, 1.78; P = .002) for MACE, 1.40 (95% CI, 1.04, 1.89; P = .025) for non-fatal myocardial infarction, 2.04 (95% CI, 1.34, 3.11; P < .001) for hospitalization following heart failure and 1.77 (95% CI, 1.29, 2.42; P < .001) for death from any cause, compared to patients with baseline hsCRP <1 mg/L, and were independent of treatment group, age, sex, body mass index, current smoking status, total cholesterol, estimated GFR, blood pressure, glycated haemoglobin and duration of diabetes."

This was also confirmed in an analysis of the A to Z trial.[17]

"Patients with hsCRP >3 mg/L at 30 days had significantly higher 2-year mortality rates than those with hsCRP 1 to 3 mg/L or hsCRP <1 mg/L (6.1% versus 3.7% versus 1.6%, P<0.0001). Results were similar with hsCRP measured at 4 months. After adjusting for age, gender, diabetes, smoking, cardiovascular history, index event, lipid levels, and randomly assigned treatment, patients with hsCRP >3 mg/L were at more than 3-fold higher risk of death (HR, 3.7; 95% CI, 1.9 to 7.2) compared with those with hsCRP <1 mg/L. "Average" levels of hsCRP (1 to 3 mg/L) were also associated with increased risk compared with those with hsCRP <1 mg/L (HR, 2.3; 95% CI, 1.2 to 4.6)."

[17] Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation*. 2006. [link]

This is similar to what was observed in the FOURIER trial:[18]

> "METHODS: Patients (n=27 564) with stable atherosclerotic cardiovascular disease and LDL-C ≥70 mg/dL on a statin were randomly assigned to evolocumab versus placebo and followed for a median of 2.2 years (1.8–2.5). The effects of evolocumab on the primary end point of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina or coronary revascularization, and the key secondary end point of cardiovascular death, myocardial infarction, or stroke were compared across strata of baseline hsCRP (<1, 1–3, and >3 mg/dL). Outcomes were also assessed across values for baseline hsCRP and 1-month LDL-C in the entire trial population. Multivariable models adjusted for variables associated with hsCRP and 1-month LDL-C were evaluated.
>
> RESULTS: A total of 7981 (29%) patients had a baseline hsCRP<1 mg/L, 11 177 (41%) had a hsCRP 1 to 3 mg/L, and 8337 (30%) had a hsCRP >3 mg/L. Median (interquartile range) baseline hsCRP was 1.8 (0.9–3.6) mg/L and levels were not altered by evolocumab. In the placebo arm, patients in higher baseline hsCRP categories experienced significantly higher 3-year Kaplan-Meier rates of the primary and key secondary end points: 12.0%, 13.7%, and 18.1% for the primary end point (Ptrend<0.0001) and 7.4%, 9.1%, and 13.2% for the key secondary end point (Ptrend<0.0001) for categories of <1, 1 to 3, and >3 mg/dL, respectively. In adjusted analyses of the association between LDL-C and hsCRP levels and cardiovascular risk, both LDL-C and hsCRP were independently associated with the primary outcome (P<0.0001 for each)."

In a substudy of the SEAS trial, a multivariable Cox regression analysis adjusting for traditional risk factors showed that the risk of a MCE was still significantly higher between the second (hs-CRP 0.9 – 2.15 mg/L) and the third (hs-CRP 2.16 – 4.5 mg/L) quartiles (HR 1.28, p<0.05), and remained elevated.[19] Subjects were followed a minimum of 4 years.

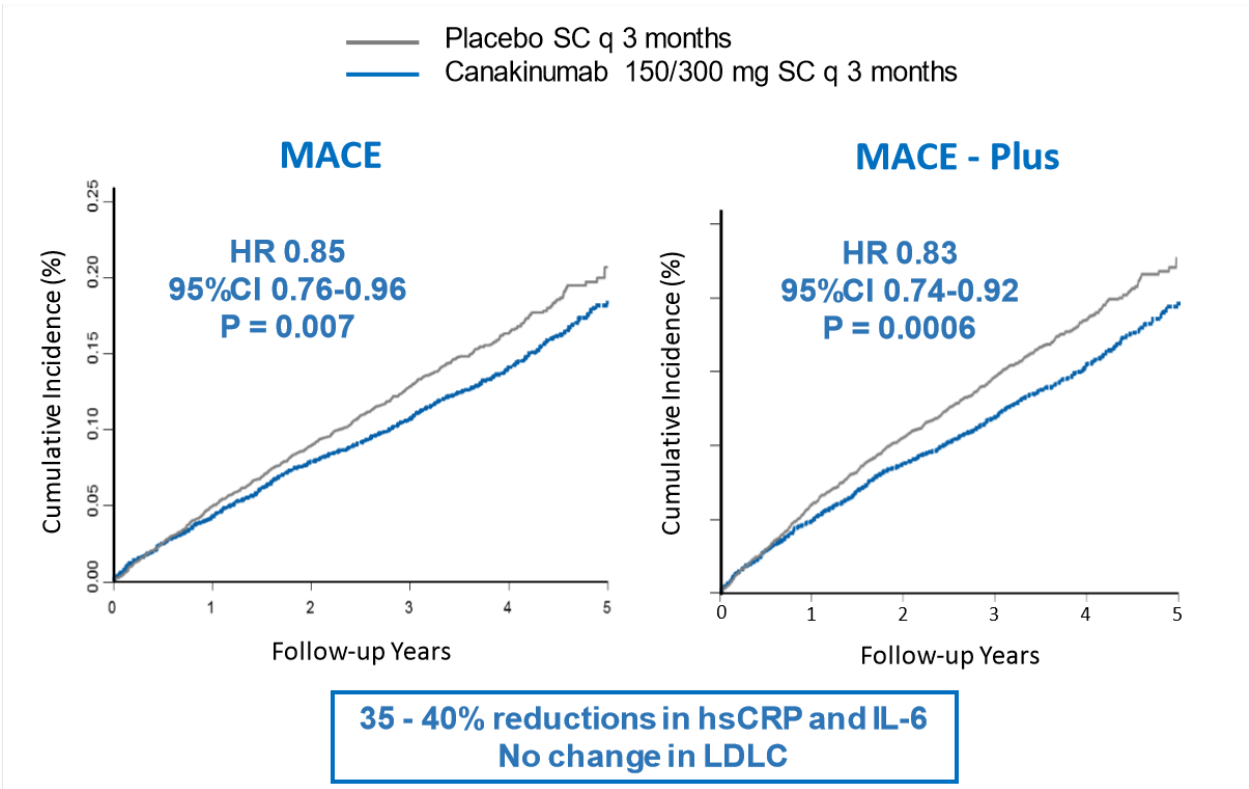
Although these examples offer strong evidence that an elevated CRP level is closely correlated with increased ASCVD event risk, a direct test of the inflammatory hypothesis had been lacking. However, recently, a significant reduction in event risk as a result of reducing inflammation directly, without impacting LDL-C or other lipid/ lipoprotein levels, was demonstrated in the CANTOS trial—particularly in those that achieved hs-CRP < 2.0 mg/L.[20]

---

[18] Erin A. Bohula, Phil Robert P. Giugliano, Lawrence A. Leiter, et al. Inflammatory and Cholesterol Risk in the FOURIER Trial. *Circulation*. 2018. [link]

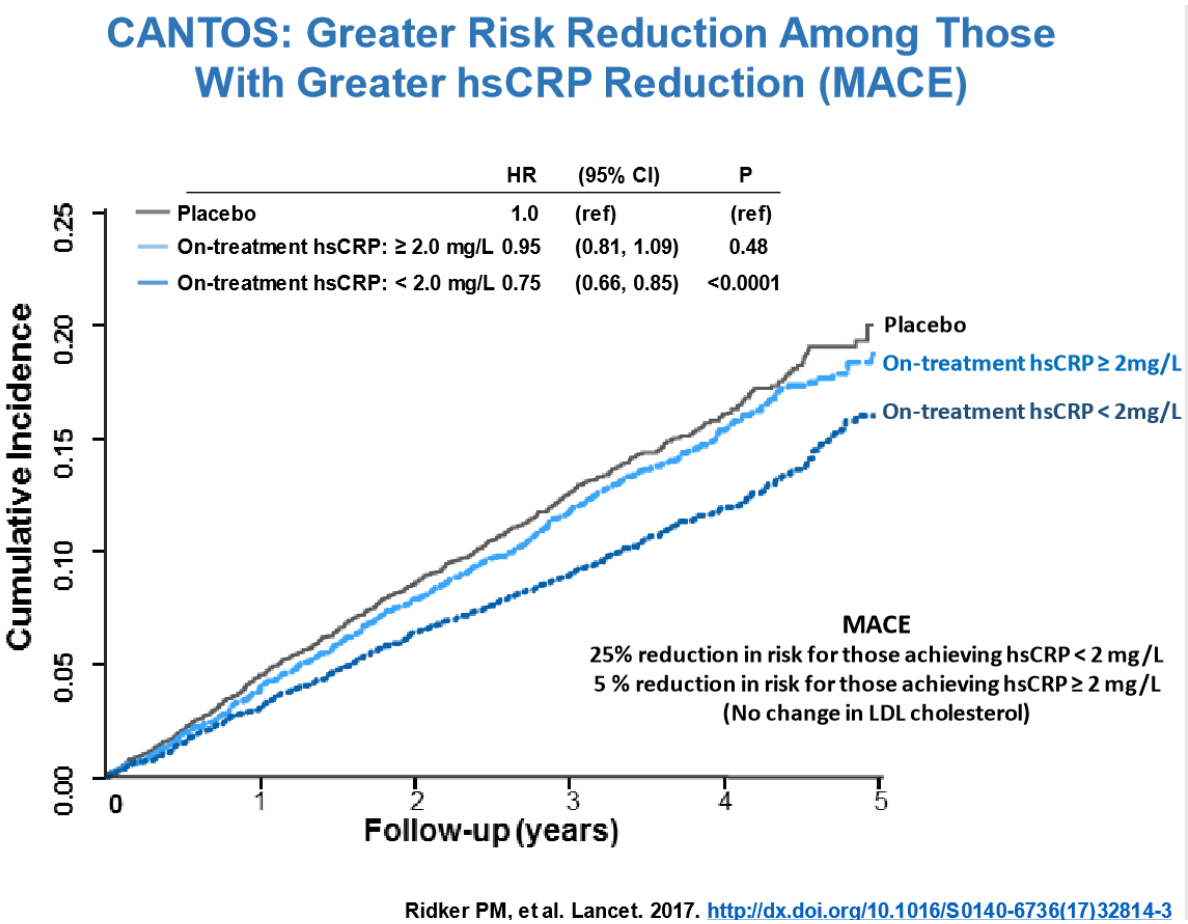
[19] Adam Blyme, Camilla Asferg, Olav W Nielsen, Thomas Sehestedt, et al. High sensitivity C reactive protein as a prognostic marker in patients with mild to moderate aortic valve stenosis during lipid-lowering treatment: an SEAS substudy. *Open Heart*. 2015. [link]

[20] Prof. Paul M. Ridker: The CANTOS trial: Implications for the management of patients with residual risk. *Pace-Cme.* 2017. [link]



35 - 40% reductions in hsCRP and IL-6
No change in LDLC

Ridker PM et al. N Engl J Med. 2017;377:1119-31



The following was enumerated in the NEJM paper on the trial:[21]

> "At 48 months, the median reduction from baseline in the high-sensitivity C-reactive protein level was 26 percentage points greater in the group that received the 50-mg dose of canakinumab, 37 percentage points greater in the 150-mg group, and 41 percentage points greater in the 300-mg group than in the placebo group. Canakinumab did not reduce lipid levels from baseline.
>
> The hazard ratios as compared with placebo were as follows: in the 50-mg group, 0.93 (95% confidence interval [CI], 0.80 to 1.07; P=0.30); in the 150-mg group, 0.85 (95% CI, 0.74 to 0.98; P=0.021); and in the 300-mg group, 0.86 (95% CI, 0.75 to 0.99; P=0.031).
>
> CANTOS was designed to test directly the inflammatory hypothesis of atherothrombosis. In this trial, in patients with a history of myocardial infarction, the levels of high-sensitivity C-reactive protein and interleukin-6 were significantly reduced from baseline by canakinumab, as compared with placebo, with no significant reduction in lipid levels from baseline. Although the 50-mg dose of canakinumab did not have a significant effect on the primary cardiovascular end

[21] Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Eng J Med*. 2017. [link]

point as compared with placebo, patients in the 150-mg group had a risk of the primary end point that was 15% lower than the risk in the placebo group (3.86 vs. 4.50 events per 100 person-years) and a risk of the key secondary cardiovascular end point that was 17% lower than that in the placebo group (4.29 vs. 5.13 events per 100 person-years).

Although the patients in CANTOS had generally well-controlled levels of LDL cholesterol, rates of both the primary end point and the secondary cardiovascular end point in the placebo group were high, with cumulative incidences of more than 20% at 5 years. Our data thus affirm that statin-treated patients with residual inflammatory risk as assessed by means of a high-sensitivity C-reactive protein level of 2 mg or more per liter at baseline have future event rates that are at least as high as, if not higher than, those among statin-treated patients with a residual risk due to LDL cholesterol level."

Both the 2013 and 2018 ACC/AHA guidelines state a hs-CRP level of 2.0 mg/L as a level above which to consider introducing moderate-intensity statin treatment for intermediate-risk patients, further establishing its clinical significance.

In REDUCE-IT, median baseline levels of hs-CRP were 2.2 mg/L for icosapent ethyl (IPE) group and 2.1 mg/L for MO placebo group. At year 2, those levels were 1.8 mg/L and 2.8 mg/L, with a 13.9% reduction ($p=0.04$) and 32.3% increase ($p<0.001$) noted, respectively.

Much of the reduction in hs-CRP levels from baseline in IPE group may be explainable as regression to the mean, as one of the potential additional risk factors listed in the inclusion criteria for primary prevention subjects was a hs-CRP level > 3.0 mg/L. The study thus preferentially selected for those with acutely elevated as well as chronically elevated CRP levels in its primary prevention segment (~30% of all subjects). We would therefore expect values in both groups to lower somewhat as inflammatory markers returned to normal in those with a temporary elevation.

Also, in two separate studies testing 4 g/d IPE in subjects for 12 weeks after an extensive stabilization period (largely controlling for regressions to the mean), hs-CRP levels had only decreased insignificantly by 3 – 4% from baseline in either study.[22] Furthermore, no significant effect on CRP has been noted from nearly every clinical trial testing EPA or EPA+DHA formulations of various dosages, durations, and in various patient populations to date.[23] These observations increase the likelihood that the 13.9% significant reduction in hs-CRP levels from baseline in IPE arm observed in REDUCE-IT was largely the result of a regression to the mean rather than a treatment effect of EPA.

A similar regression to the mean in hs-CRP levels was also seen in the JUPITER and CANTOS trials, wherein all subjects were required to have elevated levels for study entry (hs-CRP > 2.0 mg/L). Interestingly, in both trials, a baseline value of about 4.2 mg/L was noted across arms, which subsequently regressed to ~3.5 mg/L soon after randomization. This level was then maintained for years until the end of both studies.

In the placebo group in REDUCE-IT, hs-CRP levels *increased* by 32.3% following randomization. And the post-randomization hs-CRP levels of both arms were likewise maintained for years until the end of the

[22] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf

[23] Buoite Stella A, Gortan Cappellari G, Barazzoni R, Zanetti M. Update on the Impact of Omega 3 Fatty Acids on Inflammation, Insulin Resistance and Sarcopenia: A Review. *Int J Mol Sci*. 2018. [link]

trial (-12.6% for IPE group, +29.9% for placebo at final). If a regression to the mean of ~10 percentage points is taken into account, the 32.3% increase in hs-CRP levels from baseline noted in the REDUCE-IT placebo group could be even more pronounced, representing a 40% increase or more.

The evidence is compelling that chronically elevated CRP levels, particularly hs-CRP > 2.0 mg/L—but especially > 3.0 mg/L—is associated with an increased risk of ASCVD events in those with established CHD as well as in high-risk primary prevention patients, and that a large (>30%), sustained increase in CRP levels in such patients already optimally treated is likely clinically meaningful—even if it is only evidence of some other unidentified, but meaningful, harm(s). This is independent of and additive to the adverse impact from elevated atherogenic markers.

In REDUCE-IT, those randomized to placebo group experienced a sharp increase in CRP levels despite the potential for a regression to a lower mean, with nearly half of these subjects subsequently relegated to the hs-CRP > 3.0 mg/dL category. In contrast, the majority of those randomized to IPE arm found themselves in the hs-CRP < 2.0 mg/L category, which, as elaborated upon above, probably had little to do with the effects of 4 g/d EPA. This shift in inflammatory markers also occurred rapidly and was maintained for years until the end of the trial. That likely had, either directly or indirectly, a significant and differential impact on each group's future ASCVD event risk. Thus, we can no longer say with any confidence that these groups are prognostically well-balanced.

1.5.1 Log-transformed hs-CRP Data

A line in the supplement to the NEJM paper on REDUCE-IT cites the result of an analysis of log-transformed hs-CRP data, which showed a highly significant 21.8% reduction in IPE group, and no change from baseline in the placebo group. The sponsor's website provides the following explanation:[24]

> "Extreme outliers due to infections caused by temporary illness or other factors can heavily influence summary statistics of hsCRP, even beyond what is handled by using a non-transformed data approach (e.g., a conventional mean or median on a nominal scale). These individual outlier results can affect a mean or median population measurement in a way that can convey a misleadingly skewed result for the population studied. For this reason, a more reliable log transformation of hsCRP is used to incorporate outlier data appropriately within the context of the entire data set."

However, with a study as large as REDUCE-IT, and with nine separate fasting lipid panels performed on average per subject to ascertain levels,[25] log-transforming biomarker data is unnecessary, and analyses based on such data could be misleading.

The Central Limit Theorem (CLT) states that the sampling distribution of the mean of any independent, random variable will be near normal if the sample size is large enough. Thus, the means of large samples will closely follow a normal distribution, whatever the distribution of the observations themselves.[26]

---

[24] https://amarincorp.gcs-web.com/static-files/97e31858-1936-43da-80ea-2f78bb7e7260
[25] https://www.nejm.org/doi/suppl/10.1056/NEJMoa1812792/suppl_file/nejmoa1812792_appendix.pdf
[26] Douglas G Altman. Statistics notes: The normal distribution. *BMJ* 1995;310:298. [link]

Generally, this is considered true in samples of modest size where skewness and kurtosis are low, but certainly in samples of over 500 subjects when skewness and kurtosis are high.[27]

As the CLT stipulates that for large samples the mean can be approximated by a normal distribution even if the population is non-normal (i.e. data are skewed), then if the sample size is large, it is appropriate to use a t-test.[28] With over 4,000 subjects per arm in REDUCE-IT, and with the added benefit of so many repeat lipid panels performed, untransformed hs-CRP data will follow a normal distribution (mean and median approximately equal). Essentially, REDUCE-IT is over-sized and over-powered when doubling as a biomarker trial, i.e. there were 1,612 major coronary events, but >8,000 biomarker "events." The percent changes noted in the study are based on median values, which reflect the central tendencies of the robustly sized groups. And because of the large size of each treatment group, the geometric mean and median hs-CRP values will be near equal, and the kurtosis near 3.[29] Skewness will also affect both groups approximately equally, and thus, any subsequent regression to the mean will equally impact both arms.

As the previous citation also enumerates, log-transformation has been shown to have major drawbacks. It can cause right-skewed data to become left-skewed, and may even increase skewness; it can often increase—instead of reduce—the variability of data, whether or not there are outliers; it can lead to inaccurate estimates of the true population mean of the original data; it can cause significant errors in hypothesis testing, and is prone to numerous user errors as well. There is also little value in comparing the variability of original versus log-transformed data because they are on different scales. Log-transformed data therefore cannot usually facilitate inferences concerning the original data, since it shares little in common with the original data.[30] Therefore, an analysis based on classical statistical methods or generalized estimating equations is most appropriate.

Lastly, and as was also seen in JUPITER and CANTOS, hs-CRP values were highly consistent between lipid panel tests over years in both arms of REDUCE-IT (after the initial post-randomization changes had occurred). It has previously been shown that hs-CRP levels are as consistent over time as total cholesterol and blood pressure.[31] This adds to the reliability of interpretations based on observed levels.

The above considerations make drawing conclusions based on log-transformed hs-CRP data from the REDUCE-IT trial potentially misleading. Comparing the percent median change between groups using untransformed hs-CRP data is an accurate and reliable way to analyze these data.[32]

---

[27] Thomas Lumley, Paula Diehr, Scott Emerson, and Lu Chen. The Importance of the Normality Assumption in Large Public Health Data Sets. *Annual Review of Public Health.* 2002 23:1, 151-169 [link]

[28] Hui Jin, Xuejun Zhao. Transformation and Sample Size. *Hogskolan Dalarna*. 2009. [link]

[29] DeCarlo LT. On the Meaning and Use of Kurtosis. *APA*. 1997. [link]

[30] Feng C, Wang H, Lu N, et al. Log-transformation and its implications for data analysis. *Shanghai Arch Psychiatry*. 2014. [link]

[31] Robert J. Glynn, Jean G. MacFadyen, Paul M Ridker, et al. Tracking of High-Sensitivity C-Reactive Protein after an Initially Elevated Concentration: The JUPITER Study. *Clinical Chemistry*. 2009. [link]

[32] Median percent change: a robust alternative for assessing temporal trends.
Geraci M1, Alston RD, Birch JM. Median percent change: a robust alternative for assessing temporal trends. *Cancer Epidemiol*. 2013. [link]

1.5.2 To What Extent Does the Increased CRP Levels in the REDUCE-IT Placebo Group Confound Results?

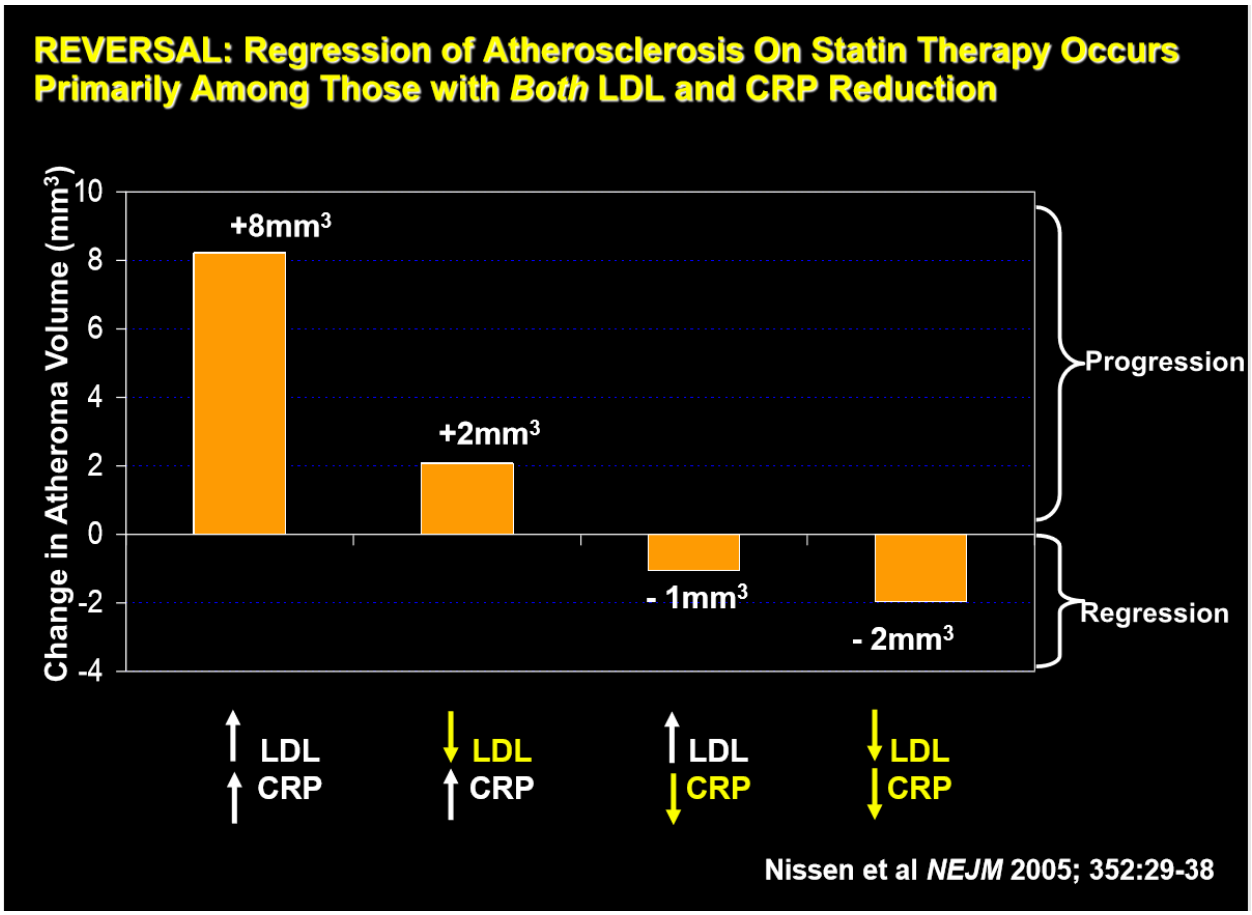
In the TNT study, the 80 mg/d atorvastatin arm achieved a 30.2-percentage point greater reduction in hs-CRP compared with the 10 mg/d arm, along with a 14-percentage point greater reduction in LDL-C. This translated to a 22% RRR in MCE. In the REAL-CAD trial, the 4 mg/d pitavastatin arm achieved a 20-percentage point greater reduction in hs-CRP than the 1 mg/d arm, along with a 15-percentage point greater reduction in LDL-C. This translated to a 19% RRR in MCE. And in CANTOS, a 37% reduction in hs-CRP compared to placebo—with no difference in LDL-C, apoB, or non-HDL-C between groups—yielded a 15% RRR in MCE.

It is reasonable to deduce that the greater CRP reduction in the TNT study lent itself to the greater RRR seen there, largely accounting for the 1.6% relative reduction in MCE for every 1% reduction in LDL-C noted in the study. In the REAL-CAD trial, the 4 mg/d group experienced a less pronounced reduction in hs-CRP levels versus the 1 mg/d arm than the between-group differences in hs-CRP in the TNT study (although still robust), and demonstrated a 1.3% RRR for every 1% decrease in LDL-C. Both examples are a good deal higher than the 1%/1% enumerated in ACC/AHA guidelines. In addition, CANTOS proved that a marked and sustained reduction in CRP levels favorably impacts ASCVD event risk.

In the REVERSAL study, the 80 mg/d atorvastatin arm achieved a 31.2-percentage point greater reduction in hs-CRP than the 40 mg/d pravastatin arm. This, combined with other positive changes in atherogenic markers (especially LDL-C), resulted in significant differences in progression of coronary atherosclerosis between groups.[33]

In adjusted analyses, researchers found a significant correlation between extent of progression relative to the median hs-CRP value; those that achieved a greater decrease in CRP levels than the median value saw regression of atherosclerosis, and contrariwise, those that had higher CRP levels than the median value saw progression of atherosclerosis. This was once again additive to and independent of changes in LDL-C.

[33] Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA*. 2004. [link]



One cannot rule out the potential for the ~7% difference in absolute LDL-C reduction between groups in REDUCE-IT to infer a more than 7-percentage point correction to the stated 25% RRR, considering the sharp increase in hs-CRP (+32.3%) in placebo group, which was observed soon after randomization and was maintained for the length of the trial—and despite what appears to be a contrariwise regression to the mean in hs-CRP levels in IPE arm (that should have equally impacted placebo group). Using the 1.6%/1% ratio seen in TNT, that would equate to an 11.2-percentage point removal from the 25% RRR in MCE, resulting in a *13.8%* corrected RRR.

Although even a HR of 0.88 (12% RRR) in MCE would still be significant from 1,612 events, the closer to 1.0 the actual HR is, the fewer the secondary endpoints that would remain significant. This ambiguity creates difficult problems in determining what exactly to put in an expanded label for IPE (aka "Vascepa"). How can it be explained that the stated 25% RRR may actually be nearly twice inflated in a more pessimistic view? And that few to none of the secondary endpoints may after all be significant?

However, it may be a good deal worse than that. We have extrapolated the above detraction from the reported 25% RRR based on changes in values in the MO placebo group, which comprised over 4,000 subjects. But, the 25% RRR in actuality only involved some 900 of these 4,000 subjects, or a little less than 1/4th. Just what were the changes in LDL-C, non-HDL-C, LDL-P, apoB, TG, hs-CRP, etc. in these ~900 subjects that had an event? Were these levels more elevated in the placebo group subjects that had an event vs those that did not? What if LDL-C increased over 20% and hs-CRP over 40% in such subjects, a

disproportionate percentage of whom were on high-intensity statins (higher baseline risk)? These are questions that only the sponsor and the FDA will be able to answer, but one that may shed much-needed light on the likelihood that MO inhibited the absorption of statin drugs, in particular if those ~900 placebo group subjects that had an event showed an even more pronounced increase in atherogenic/inflammatory markers relative to the rest.

While this discussion on statin malabsorption by itself is troubling, it appears another potential confounder has just presented itself: if MO quite possibly inhibited statin absorption in placebo group (observable by highly significant increases in LDL-C, apoB, non-HDL-C and CRP from baseline), does that not increase the likelihood of malabsorption of other cardiac medications, such as antithrombotics and antihypertensives? If so, how might that appear in the data?

1.6 Potential Impact of Mineral Oil on Cardiac Medications

Antithrombotics (aspirin, clopidogrel, ticagrelor, dipyridamole, etc.) have a consistent track record of increased bleeding risk in patients.[34] In the REDUCE-IT trial, there was a borderline significant increase in bleeding events in IPE arm (111 vs 85 events, p=0.06). This observed increase could infer a decreased absorption of antithrombotics in MO placebo arm. The likelihood that 4 g/d EPA caused an increased incidence of bleeding-related disorders appears to be low.[35]

> "In all the patients considered (over 600 subjects treated with the active product in total), with moderate to severe disease, with or without concomitant use of antithrombotic agents, at home or in an Intensive Care Unit (ICU), no evidence of increased risk of bleeding with use of n-3 LC-PUFAs was observed. Furthermore, there were no statistically significant changes from baseline in measured coagulation parameters.
>
> These findings further support the safe consumption of n-3 LC-PUFAs, even at short-term doses up to 10 g/day of eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) or consumed for up to 52 weeks above 1.5 g/day, in selected vulnerable and sensitive populations such as subjects with gastrointestinal cancer or patients in an ICU. We found no evidence to support any concern raised with regards to the application of n-3 LC-PUFAs and the potentially increased risk for the occurrence of adverse bleeding manifestations in these selected patient populations consuming fish oil enriched medical nutrition."

The National Institutes of Health Office of Dietary Supplements states:

> "Fish oil can have antiplatelet effects at high doses, although it appears to be less potent than aspirin [177,178]. Fish oil might prolong clotting times, as indicated by an elevated international normalized ratio (INR), when it is taken with warfarin [179], but most research indicates that doses of 3–6 g/day fish oil do not significantly affect the anticoagulant status of patients taking

[34] Sandeep Nathan, MD, FACC. Bleeding Risks with Triple Antithrombotic Therapy. *ACC*. 2013. [link]
[35] Stephanie Jeansen, Renger F.Witkamp, Jossie A.Garthoff et al. Fish oil LC-PUFAs do not affect blood coagulation parameters and bleeding manifestations: Analysis of 8 clinical studies with selected patient groups on omega-3-enriched medical nutrition. *Clinical Nutrition*, Volume 37, Issue 3, June 2018, Pages 948-957. [link]

> warfarin [180]. The authors of a 2014 review concluded that omega-3s do not affect the risk of clinically significant bleeding [181], and the FDA-approved package inserts for omega-3 pharmaceuticals state that studies with omega-3s have not produced clinically significant bleeding episodes."[36]

An application review by the EMEA for a different indication originally sought in 2003 by Amarin corp., noted the following:[37]

> "A review of AE data of 44 subjects exposed to 4 g/day ethyl-EPA for up to 12 weeks in studies LA01.01.0001 and LA01.01.0002 revealed no apparent effect on bleeding time."

An FDA review of Vascepa noted a similar observation.[38]

> "The anticoagulation pharmacodynamic parameters of warfarin and their comparisons with and without ethyl-EPA were also evaluated. The ratio of the geometric means of INRmax following administration of warfarin with and without ethyl-EPA was 0.87, while the same ratio for AUCINR was 0.94. The 90% confidence intervals were all between 80% and 125%. It is concluded that 4 g/day ethyl-EPA does not significantly affect the anticoagulation parameters of warfarin."

And, according to the European Food Safety Authority, long-term consumption of EPA and DHA supplements at combined doses of up to about 5 g/day have not been shown to cause bleeding problems.[39]

The borderline significant increase in bleeding events in the IPE arm of REDUCE-IT is thus suspect, and could potentially be the result of an inhibition of antithrombotics in placebo group, causing a decrease in bleeding events in that arm.

There is also exploratory evidence from a study examining the possibility of laxatives causing over-anticoagulation that MO may have inhibited coagulative properties of antithrombotics, having shown an inverse association with over-anticoagulation. In a time-dependent analysis, liquid paraffin caused an adjusted 30% decreased risk of an INR > 6.0 compared with control, eluding to a reduction in the effectiveness of acenocoumarol or phenprocoumon.[40]

The 28% RRR in stroke and the 31% RRR in MI in REDUCE-IT could be related to this phenomenon, as antithrombotics exhibit strong protection against both kinds of events in ACS patients.[41]

The robust reduction in strokes with 4 g/d EPA seen in REDUCE-IT is most difficult to harmonize with the published literature. For example, in the JELIS trial, over 18,000 subjects were randomized 1:1 and

---

[36] https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/

[37] https://www.ema.europa.eu/documents/withdrawal-report/withdrawal-assessment-report-ethyl-eicosapent-soft-gelatin-capsules_en.pdf

[38] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000ClinPharmR.pdf

[39] EFSA Panel on Dietetic Products NaA. Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*. 2012. [link]

[40] Loes E Visser, Fernie J A Penning-van Beest, J H P Wilson, et al. Overanticoagulation associated with combined use of lactulose and acenocoumarol or phenprocoumon. *BJCP*. 2004. [link]

[41] Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients, *BMJ*, 2002. [link]

administered either 1.8 g/d highly purified EPA or no treatment, and the trend was worse for EPA group in strokes (166 vs 162 events).

In a meta-analysis that included an examination of higher dose prescription omega-3 (POM3) studies for treatment effect, there was likewise a negative trend in stroke incidence (HR 1.13).[42]

The recently published ASCEND (stroke HR 1.01) and VITAL (stroke HR 1.03) studies also showed no trend for benefit with POM3.

In a study that attempted to isolate the effect of each fatty acid from diet and examine its impact on stroke, a significant inverse relationship between DHA and DPA serum levels and stroke incidence was found—but not EPA, regardless of quartile.[43]

The above negative trends in stroke incidence in POM3 and EPA-only supplementation studies may be related to the increased incidence in atrial fibrillation associated with such therapy at higher dosages, which may be more related to EPA than DHA.[44] Indeed, in REDUCE-IT, there was noted a highly significant increase in AFib in the 4 g/d IPE arm (215 vs 159 events, p=0.003). A causal relationship between AFib and stroke incidence is well established, particularly in older adults.[45] The consistent trends in an adverse direction in strokes with POM3 or EPA-only dosing in the literature further removes the likelihood that either can significantly reduce strokes.

The 28% reduction in nonfatal stroke seen with 4 g/d Vascepa in REDUCE-IT is therefore quite surprising, and one might suggest dubious. If concurrent dosing with MO inhibits the effectiveness of antithrombotics, that could explain much of the finding. Although an inhibition of other cardiac medications could also help explain it.

One class of antihypertensives known as calcium channel blockers, such as amlodipine, felodipine, and nifedipine, have been strongly linked to incidence of peripheral edema.[46] Additionally, ACE inhibitors and angiotensin receptor blockers (ARBs) are implicated in the incidence of angioedema (it is unclear whether or not these are included with peripheral edemas in the list of adverse events in the NEJM paper). If MO inhibited the absorption of these, we could expect an increase in edemas in the group that did not experience this inhibition, having effectively received greater doses of these drugs. In REDUCE-IT there was a highly significant increase in the incidence of edema in IPE arm compared to placebo group (267 vs 203 events, p=0.002), confirming this hypothesis.

As further confirmation, in response to commentary to the editor published in the NEJM in April 2019, hypothesizing that 4 g/d EPA may have reduced blood pressure in subjects, lending to the observed

---

[42] Sang Mi Kwak, MD; Seung-Kwon Myung, MD; Young Jae Lee, MD, MS; et al. Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease, A Meta-analysis of Randomized, Double-blind, Placebo-Controlled Trials. *JAMA*. 2012. [link]

[43] Saber H, Yakoob MY, Shi, P, et al. Omega-3 Fatty Acids and Incident Ischemic Stroke and Its Atherothrombotic and Cardioembolic Subtypes in 3 US Cohorts. *Circulation*. 2017. [link]

[44] Rix TA, Joensen AM, Riahi S, et al. A U-shaped association between consumption of marine n-3 fatty acids and development of atrial fibrillation/atrial flutter—a Danish cohort study. *EP Europace*. 2014. [link]

[45] Warren J Manning. Stroke in patients with atrial fibrillation. *UpToDate*. Cited as of 2018. [link]

[46] Domenic A. Sica, MD. Calcium Channel Blocker-Related Peripheral Edema: Can It Be Resolved? *Medscape*. [link]

benefit in MCE risk reduction (which, incidentally, is curious, as it has been understood for some time that DHA, but not EPA, has any effect on blood pressure[47, 48]), Dr. Bhatt responded with the following:[49]

> "Regarding blood pressure, prespecified exploratory analyses of icosapent ethyl with no adjustment for multiple comparisons have shown average placebo-corrected reductions from baseline in systolic blood pressure of 1.3 mm Hg (95% CI, 0.9 to 1.6) and in diastolic blood pressure of 0.5 mm Hg (95% CI, 0.3 to 0.7). These differences appear to be modest, but it is possible that they contributed to the benefits of icosapent ethyl.[3] Additional analyses of data from REDUCE-IT, including biomarkers (e.g., the ratio of EPA to arachidonic acid) and blood pressure, are in process to increase our understanding of the effects of icosapent ethyl and potential mechanisms for the observed reduction in cardiovascular risk."

The key wording being "placebo-corrected." Dr. Bhatt did not divulge more detail, but given the many studies that show no effect of EPA on systolic or diastolic hypertension, it is likely that the noted "reductions" above relate only to their relative increases in placebo group subjects. And further, the above represents data across all patients randomized, even though not all were on antihypertensives. Thus, the true impact is diluted and understated. The FDA will likely examine data specific to those on antihypertensives randomized to placebo vs IPE group, looking for signs of such confoundment.

It is well-known that antihypertensives decrease the risk of ASCVD events in high-risk primary prevention and CHD patients with hypertension.[50] This observation (highly significant increase in incidence of edema—or even any observable increase in edema of any kind) is completely absent from previous studies testing EPA and DHA, separate or in combination, as far as our exhaustive searches revealed. If anything, POM3 dosing tends to show a reduction in some markers of inflammation,[51] and we found no evidence POM3 may cause fluid retention. This further removes the probability that IPE induced this phenomenon, and increases the likelihood of it being the result of inhibition of antihypertensives via concurrent doing with MO in placebo group.

Furthermore, as antihypertensives are known to lower circulating CRP levels,[52] there could be a connection in the 32.3% elevation of hs-CRP in the placebo group in REDUCE-IT with an attenuation of these therapies beyond what might be expected from statin malabsorption alone.

Lastly, there was a significant increase in anemia in placebo group (191 vs 236 events, p=0.03). Those with borderline anemia may have experienced anemia if dosing with MO concurrent with iron

---

[47] Mori T, Bao DQ, Burke V, et al. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension*. 1999. [link]

[48] Lee J, Notay K, Klingel S, et al. Docosahexaenoic acid reduces resting blood pressure but increases muscle sympathetic outflow compared with eicosapentaenoic acid in healthy men and women. *Am J Physiol Heart Circ Physiol*. 2019. [link]

[49] https://www.nejm.org/doi/full/10.1056/NEJMc1902165

[50] Krishna K. Patel, MD; Suzanne V. Arnold, MD, MHA; Paul S. Chan. MD, et al. Personalizing the Intensity of Blood Pressure Control Modeling the Heterogeneity of Risks and Benefits From SPRINT (Systolic Blood Pressure Intervention Trial). *Circulation.* 2017. [link]

[51] Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients*. 2010;2(3):355-74. [link]

[52] Madej A, Dąbek J, Majewski M, Szuta J. Effect of perindopril and bisoprolol on IL-2, INF-γ, hs-CRP and T-cell stimulation and correlations with blood pressure in mild and moderate hypertension. *Int J Clin Pharmacol Ther*. 2018. [link]

supplementation/iron-containing foods. Anemia is associated with increased risk of ASCVD events.[53] Also, by extension, if mineral oil placebo inhibited the absorption of vitamin D, that may also have played a role in increased risk in those with a deficiency.[54]

A potential scenario deduced from changes in atherogenic and inflammatory markers due to perceived statin malabsorption would put the corrected RRR in the primary endpoint in REDUCE-IT at about 13.5% for IPE group—though still a significant result. However, if one or more other cardiac medications were attenuated in addition to statin malabsorption, the prospective RRR could well fall to a level that imputes insignificance. And, once again, a granular view of the data that focuses on the ~900 placebo group subjects that had an event may highlight even more compelling evidence that multiple cardiac medications were attenuated (i.e. greater increases in blood pressure, greater number of bleeding events, sharper increases in atherogenic/inflammatory markers, higher incidence of edema, etc. than the rest of the group).

1.6.1 Additional Observations That Infer Drug Malabsorption in REDUCE-IT

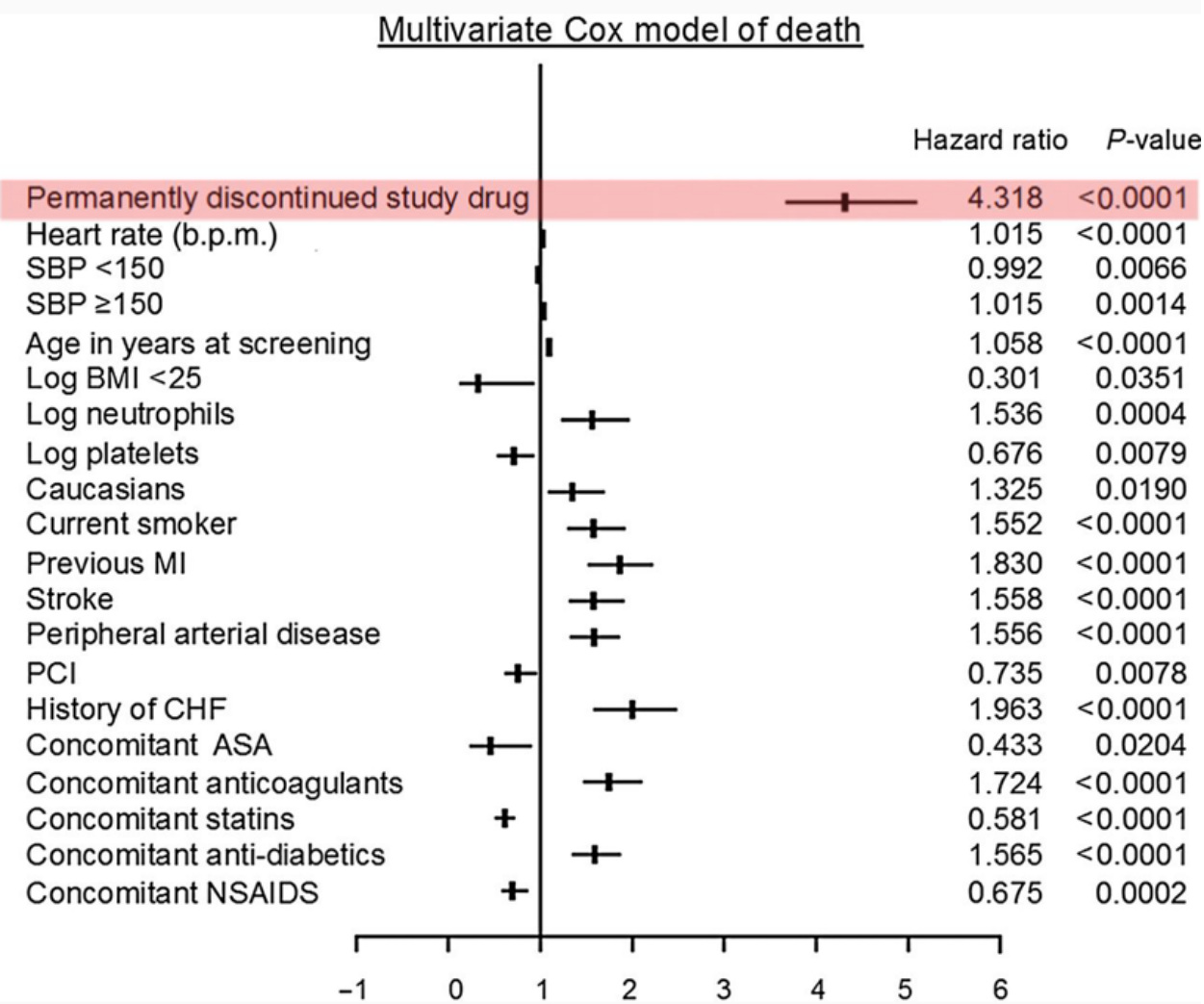
The greatest ASCVD event risk reduction observed between groups in REDUCE-IT was seen in those in IPE arm on high-intensity statins (HR 0.69 from 542 events), followed by those on moderate-intensity statins (HR 0.76 from 967 events), whereas there was no trend in risk reduction observed in subjects on low-intensity statins (HR 1.12 from 93 events). The clearest evidence of benefit from cardiac medications are in high-risk and CHD patients, and thus an attenuation of these therapies of greatest detriment to them.[55, 56]

> "In PREMIER, mortality hazard ratios for [secondary prevention] patients discontinuing treatment when compared with those continuing ranged from 1.82 (95% CI: 1.09–3.03) for aspirin to 2.86 (9 5% CI: 1.47–5.55) for statins. Absolute mortality for those stopping all medications was five-fold higher, rising from 2.3 to 11.5% (P < 0.001). Another study showed one in six patients receiving DES failed to fill clopidogrel prescriptions on discharge day, correlating with a 1.8-fold increased risk of death or myocardial infarction (14.2 vs. 7.9%; P < 0.001).
>
> In the EFFECT trial, composed of an older population, 1-year absolute mortality of patients failing to fill prescriptions was 30.4% as compared with 20.5 or 12.8% in patients who filled some or all prescriptions. These are global trends. In the REACH registry spanning 44 countries, risk of cardiovascular death, myocardial infarction, or stroke increased from 13.4 to 17.4% in as

[53] Lee PC, Kini AS, Ahsan C, et al. Anemia Is an Independent Predictor of Mortality After Percutaneous Coronary Intervention. *JACC*. 2004. [link]
[54] Kheiri B, Abdalla A, Osman M, Ahmed S, Hassan M, Bachuwa G. Vitamin D deficiency and risk of cardiovascular diseases: a narrative review. *Clin Hypertens*. 2018. [link]
[55] Kumaran Kolandaivelu, Benjamin B. Leiden, Patrick T. O'Gara, Deepak L. Bhatt; Non-adherence to cardiovascular medications, *European Heart Journal*. 2014. [link]
[56] Tsivgoulis G, Safouris A, Kim DE, Alexandrov AV. Recent Advances in Primary and Secondary Prevention of Atherosclerotic Stroke. *J Stroke*. 2018. [link]

little as 1-year for patients non-adherent at baseline, being even worse in patients who went from taking to not taking medications (HR: 1.36; 95% CI, 1.17–1.57).

Non-adherence is not only associated with poorer outcomes, but can dominate risk. In CHARISMA, which considered addition of clopidogrel to aspirin in high-risk, stable [CHD] populations, patients discontinuing clopidogrel therapy experienced a 4.3-fold increased hazard of death—eclipsing risk factors including uncontrolled blood pressure, tobacco use, or prior MI (hazard ratios 1.02, 1.55, and 1.83 respectively; P ≤ 0.0014."



To put the above in context, a 4.32 HR from total non-compliance equates with a HR of 0.23 with total drug compliance, or a 77% RRR in mortality in patients with established CHD. Assuming a 5-year risk of CV mortality of ~7% for compliant individuals > age 50 with well-controlled LDL-C, the absolute risk reduction (ARR) compared with non-compliant CHD patients would be 23%, and a NNT of 5 over 5-years. If even a minor degree of the effects of these therapies are attenuated, the elevation in risk of CV

mortality (and by extension any ASCVD event) in such patients could be dramatic. Treatment vs non-treatment with statin therapy alone confers an ~11% absolute risk reduction in ASCVD events in such patients with established CHD, and an NNT of 9 over 5-years.[57]

As the more recent ARRIVE, ASPREE, and ASCEND trials have shown, the use of antithrombotics in primary prevention patients is of little utility in the modern era of statin prevalence.[58] Also, there are no data that show higher intensity statins produce a greater ASCVD risk reduction compared with lower intensity regimens in primary prevention patients, as there are no head-to-head statin RCTs in this subset of patients (as of 2018). And if MO hindered the absorption of antihypertensives, it would also not impact this group as appreciably as those of secondary prevention.[59] Therefore, if the RRR seen in IPE group is mostly the result of a detrimental impact on drug absorption in placebo group, we could expect to see a lesser adverse impact in primary prevention subjects, and greater impact in secondary prevention subjects.

In line with this, primary prevention subjects in REDUCE-IT showed a much-lessened risk reduction with IPE (HR 0.88) compared with those of secondary prevention (HR 0.73), despite the robust size of this pre-specified subgroup (~30% of all subjects). The p-value for interaction was 0.14 ($p<0.15$ was significant for interaction, as enumerated in the SAP[60]).

Other observations that might be expected if concomitant therapies were inhibited in MO placebo group in REDUCE-IT suggest themselves. For example, those from Eastern Europe (n=2106/8179) where background therapy is less extensive[61] saw a smaller risk reduction (HR 0.84) compared with those treated in the US (HR 0.69; $p<0.15$). Those > 65 yrs of age, in whom statin therapy has been shown to be less effective,[62] saw a smaller risk reduction (HR 0.87) compared with those < 65 (HR 0.65; $p=0.004$). The hazard ratio in women, who have been shown to derive somewhat less benefit from statin and antiplatelet therapy,[63, 64, 65] and thus, less detriment from a reduction in these therapies, was also less pronounced (HR 0.82), however, this interaction did not reach statistical significance.

---

[57] Brent M. Egan, Jiexiang Li, Kellee White, et al. 2013 ACC/AHA Cholesterol Guideline and Implications for Healthy People 2020 Cardiovascular Disease Prevention Goals. *JAHA*. 2016. [link]

[58] Joseph Meyer, Kelly Arps, Roger S. Blumenthal, et al. New Data on Aspirin Use in the Era of More Widespread Statin Use. *ACC*. 2018. [link]

[59] Kimberly N. Hong, Valentin Fuster, Robert S. Rosenson, Clive Rosendorff, Deepak L. Bhatt. How Low to Go With Glucose, Cholesterol, and Blood Pressure in Primary Prevention of CVD. *Journal of the American College of Cardiology*. 2017. [link]

[60] https://www.nejm.org/doi/suppl/10.1056/NEJMoa1812792/suppl_file/nejmoa1812792_appendix.pdf

[61] Martin Bødtker Mortensen, Børge G. Nordestgaard, Shoaib Afzal, Erling Falk. ACC/AHA guidelines superior to ESC/EAS guidelines for primary prevention with statins in non-diabetic Europeans: the Copenhagen General Population Study. *European Heart Journal*. 2017. [link]

[62] Benjamin H. Han, David Sutin, Jeff D. Williamson, et al. Effect of Statin Treatment vs Usual Care on Primary Cardiovascular Prevention Among Older Adults; The ALLHAT-LLT Randomized Clinical Trial. *JAMA*. 2017. [link]

[63] Karp I, Chen SF, Pilote L. Sex differences in the effectiveness of statins after myocardial infarction. *CMAJ*. 2007. [link]

[64] Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet*. 2010. [link]

[65] Yerman T, Gan WQ, Sin DD. The influence of gender on the effects of aspirin in preventing myocardial infarction. *BMC Med*. 2007. [link]

Contrariwise, the risk reduction in subjects that are more likely to derive greater benefit from statin therapy and other cardiac medications (and thus greater detriment from an inhibition of the same), such as those with low HDL-C/high TG (HR 0.62; p=0.04), male gender (HR 0.73; p=0.33), and those who as a result of therapy had achieved a hs-CRP < 2.0 mg/L (HR 0.68; p=0.07), was more pronounced, potentially due to a greater detriment to their placebo group counterparts. Importantly, none of the above trends were noted previously in statin add-on or POM3 trials, other than those with mixed dyslipidemia (low HDL-C/high TG) in fibrate studies.[66]

The 5-year ASCVD event rate in the REDUCE-IT placebo group also appears disproportionately high. In CANTOS, the placebo group subjects were all secondary prevention patients (100% prior MI), with 54% having had a STEMI, 79% hypertensive, and 75% male; median BMI was about 30, age, 61, 40% had type 2 diabetes, median baseline hs-CRP was 4.1 mg/L, and median LDL-C was about 83 mg/dL. These patients were enrolled in similar institutions and in a similar timeframe as the REDUCE-IT trial subjects. And their cumulative incidence of ASCVD events was just over 20% at 5-years.

Comparatively, in the REDUCE-IT placebo group, 71% were secondary prevention, 72% were male, avg BMI was 30.8, age, 63, 58% had type 2 diabetes (though half were primary prevention), baseline median hs-CRP was 2.1 mg/L, and derived LDL-C was 76 mg/dL. Yet, although a lower risk group than that in CANTOS, the REDUCE-IT placebo group experienced an even greater percentage of cumulative events at 5-years at 22%. The IPE group's 5-year cumulative MCE rate was 17%, which appears more closely in line with this group's baseline 5-year risk, especially considering nearly a third of the subjects were primary prevention (also, type 2 diabetics over age 55 with TG > 150 mg/dL, but no additional risk factors, could be admitted entry into this primary prevention segment).

There is also compelling evidence from a study by Blackwood et al. (2015)[67] that concurrent dosing with ezetimibe and the omega-3 fatty acid ALA causes severe malabsorption of the latter:

> "BACKGROUND AND AIMS:
>
> Elevated levels of circulating omega-3 polyunsaturated fatty acids like alpha linolenic acid (ALA) may be beneficial for cardiovascular health. Circulating ALA concentrations are elevated dramatically by a cholesterol supplemented diet which increases ALA bioavailability through enhanced micelle formation in the intestines. Conversely, it is possible that drugs which inhibit cholesterol metabolism in the intestine may also inhibit fatty acid absorption. The purpose of this study is to determine if a cholesterol absorption inhibitor, ezetimibe, will decrease circulating levels of ALA.
>
> METHODS AND RESULTS:
>
> Cardiac patients (n = 34) between 44 and 80 years old, requiring statin therapy to regulate blood cholesterol levels, were randomly assigned to one of four groups for a 6 week trial: 1) placebo; 2) ezetimibe therapy; 3) a supplement of flaxseed oil (containing 1.0 g ALA in 2.0 g of flaxseed oil); or 4) ezetimibe and flaxseed oil supplementation. Ingestion of flaxseed oil resulted in a

[66] Moutzouri E, Kei A, Elisaf MS, Milionis HJ. Management of dyslipidemias with fibrates, alone and in combination with statins: role of delayed-release fenofibric acid. *Vasc Health Risk Manag*. 2010. [link]

[67] D.P. Blackwood, R.K. LaVallée, A. Al Busaidi. et al. A randomized trial of the effects of ezetimibe on the absorption of omega-3 fatty acids in cardiac disease patients: A pilot study. *Clin Nutr ESPEN*. 2015. [link]

significant increase in circulating ALA levels (6 ug/dl) in patients who were not given ezetimibe. However, in the presence of ezetimibe, circulating ALA levels did not increase significantly even in the presence of flax oil supplementation (a decrease of 4 ug/dl). There were no significant differences amongst the groups in terms of circulating total cholesterol, LDL, HDL, triglyceride levels in the blood.

CONCLUSION:

Ezetimibe therapy inhibited the absorption of omega-3 fatty acids. Patients receiving ezetimibe therapy may not receive the expected cardiovascular benefits from dietary supplementation with omega-3 fatty acids."

In the above study, dosing with ezetimibe completely inhibited omega-3 fatty acid absorption. A similar finding was shown in a study by Labonte et al. (2008) wherein ezetimibe significantly reduced absorption of dietary saturated fatty acids in mice.[68]

It is suspicious, then, that the pre-specified subgroup analysis showed those on ezetimibe + IPE achieved an 18% risk reduction in MCE compared with those using ezetimibe + MO. If the IPE was effectively nullified by concurrent dosing with ezetimibe, how did they still achieve an 18% RRR? Unless it wasn't due to the benefits of being given 4 g/d IPE at all, but rather the de facto result of an adverse effect of MO placebo on cardiac medication absorption in the placebo group.

The observations in this section add to the evidence that cardiac medications may have been attenuated in the REDUCE-IT placebo group, substantially increasing the group's ASCVD event risk.

1.7 Confirmation of an Adverse Impact of Mineral Oil on Drug Absorption

The elevation of all atherogenic and inflammatory markers measured in MO placebo group in the REDUCE-IT trial was previously observed to a similar degree in a separate study—the ANCHOR trial, also sponsored by Amarin Corp.

In ANCHOR, over 700 subjects (all of whom were on background statin therapy) were randomized 1:1:1 to either 4 g/d MO arm, 2 g/d EPA arm (which included 2 g/d MO in addition to 2 g/d EPA[69]), or 4 g/d EPA arm. The trial was designed with an extensive lead-in period to stabilize lipid and lipoprotein values before randomization. The median percent changes in parameters from baseline to 12-weeks, showing stark contrast in atherogenic/inflammatory markers between groups in unexpected ways, were as follows:

[68] Labonté ED, Camarota LM, Rojas JC, et al. Reduced absorption of saturated fatty acids and resistance to diet-induced obesity and diabetes by ezetimibe-treated and Npc1l1-/- mice. *Am J Physiol Gastrointest Liver Physiol*. 2008. [link]

[69] Bays HE, Ballantyne CM, Doyle RT, et al. Icosapent ethyl: Eicosapentaenoic acid concentration and triglyceride lowering effects across clinical studies. *Elsevier*. 2016. [link]

	Median % Change from Baseline to Week 12		
	4g/d MO:	2g/d EPA (+2g/d MO):	4g/d EPA:
TG	+5.90%	-5.60%	-17.50%
LDL-C	+8.80%	+2.40%	+1.50%
LDL-P	+11.90%	+4.90%	+3.80%
ox-LDL	+11.60%	+2.60%	-4.80%
non-HDL-C	+9.80%	+2.40%	-5.00%
VLDL-C	+15.00%	+1.60%	-12.10%
apoB	+7.10%	+1.60%	-2.20%
RLP-C	+8.00%	-11.10%	-24.00%
CRP	+17.10%	+10.30%	-2.40%
LpPLA2	+6.70%	-1.80%	-12.80%
Tot. Chol.	+9.10%	+2.10%	-3.20%

In fact, and compellingly, the cumulative *increases* in markers from baseline in the 4 g/d MO group outshine the cumulative *decreases* in markers in the 4 g/d Vascepa group.

An extensive wash-out and stabilization period should have helped control against such errant swings in markers between the three robustly sized groups (approximately 230 randomized per arm). The lead-in period protocol details from the trial are outlined below:

> "Eligible patients who wished to participate provided written informed consent, underwent a fasting blood draw, received dietary counseling on implementing the NCEP Therapeutic Lifestyle Changes diet, and initiated either a 4- or 6-week lead-in period depending on whether either a washout of a non-statin lipid-lowering therapy or an adjustment to the background statin was necessary.
>
> Patients who did not require washout of non-statin lipid-lowering therapy: The screening visit occurred at Visit 1 (Week -6). Eligible patients entered a 4-week diet lead-in period and continued on their current dose of statin before the first TG/LDL-C qualifying visit (Visit 2/Week-2). Patients who required a change in their statin dose during the 2 weeks following Visit 1 entered a statin stabilization period so that the statin dose was stable for at least 4 weeks before the first TG/LDL-C qualifying visit (Visit 2/Week -2). At the discretion of the investigator, patients could be switched from a non-study statin to an allowed statin at Visit 1.
>
> Patients who required washout of non-statin lipid-lowering therapy: The screening visit occurred at Visit 1 (Week -8). Eligible patients began a 6-week washout period before the first TG/LDLC qualifying visit (Visit 2/Week-2).
>
> Qualifying period: At the end of either the 4-week or 6-week lead-in period, eligible patients had fasting LDL-C (calculated with Friedewald equation) and TG levels measured at Visit 2 (Week -2) and Visit 3 (Week -1)."

After examining the ANCHOR trial data, an FDA reviewer stated the following in the briefing documents:

> "The changes in lipid and lipoprotein parameters from baseline to Week 12 in the mineral oil placebo group are rather atypical for a trial that included a stabilization period for diet and lipid-lowering therapy, raising the possibility that mineral oil may not be as inert as assumed. If true, the treatment effects observed with AMR101 [Vascepa] may be overestimated."

In his book Dyslipidemia: Pathophysiology Evaluation and Management, 2015., Dr. Garg writes,

> "Compared to placebo, LDL-C increased by 3.5% in the COMBOS trial using Lovaza, and decreased by 6.2% in the ANCHOR study using Vascepa 4 g/d. Compared to baseline, however, there were no differences in LDL-C in either trial. In other words, the difference in LDL-C response between Lovaza and Vascepa is due to the different LDL-C responses to placebo. In the Lovaza studies, lipids in the placebo arms were either unaffected by treatment or decreased to a small extent, whereas in the Vascepa studies, lipids increased more than would be expected (e.g. 9% increase in LDL-C and 6% increase in TGs in the placebo arm of the ANCHOR trial). The reason for the differences in placebo responses in unknown. It is possible that the Vascepa study populations became less adherent to lifestyle and/or pharmacologic therapy over the course of the trial. However, the elevation of atherogenic lipids in the placebo arms of the Vascepa trials raises the question of whether the light paraffin oil could have interfered with effectiveness of concomitant therapies.
>
> …in ANCHOR, apoB increased 4 mg/dL on the 2 g/d dose *[comprised of 2 g/d EPA + 2 g/d MO],* decreased 3 mg/dL on the 4 g/d dose*,* and increased 7 mg/dL on the placebo. As noted previously, the reason for the consistent and substantial increase in apoB while on placebo is not apparent. Thus, one can conclude that either Vascepa (EPA-only) has apoB lowering effects not seen with EPA + DHA, or more likely, the placebo in these trials (light paraffin oil) was not wholly inert."

Although the most profound elevations in markers were observed in the 4 g/d MO arm, the 2 g/d arm exhibited a muted effect compared to what might be expected from this dose of concentrated EPA. For example, in the ORD study, comparing TAK-085 (a POM3) with EE-EPA, the 1.8 g/d EPA arm (n=195) showed an 11.2% reduction in triglycerides at the end of 12 weeks of dosing.[70] However, in ANCHOR, 2 g/d EPA combined with 2 g/d MO resulted in a more tepid 5.6% reduction in TG. Importantly, mean TG levels at baseline were similar between the two studies (272 mg/dL in the ORD study and 262 mg/dL in ANCHOR).

In fact, the 5.6% reduction in TG in the 2 g/d arm is similar to the reduction seen in the 4 g/d olive oil placebo arm in the ESPIRIT trial, which enrolled subjects of similar background therapy and baseline TG levels as ANCHOR. There, a 5.9% reduction from baseline in TG level was observed in placebo group.[71]

---

[70] Tatsuno, Ichiro et al. Efficacy and safety of TAK-085 compared with eicosapentaenoic acid in Japanese subjects with hypertriglyceridemia undergoing lifestyle modification: The omega-3 fatty acids randomized double-blind (ORD) study. *Journal of Clinical Lipidology*. 2013. [link]

[71] Maki KC1, Orloff DG, Nicholls SJ, 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*. 2013. [link]

Was concurrent dosing with MO somehow negatively impacting the efficacy of EPA, or perhaps hindering statin absorption, thus attenuating the overall TG lowering effects observed in the ANCHOR 2 g/d group? The same incongruency is observed in other markers as well, for example non-HDL-C was reduced by 5.7% in ORD (1.8 g/d arm), but *increased* by 2.4% in ANCHOR (2 g/d arm).

From the MARINE trial data (~25% of whom were on background statin therapy), which also tested 4 g/d EPA against 4 g/d MO or 2 g/d MO+2 g/d EPA in subjects with very high triglycerides (>500 mg/dL), an FDA reviewer commented on the wild fluctuations in mean triglyceride levels in the two arms that received MO, while no fluctuation in the 4 g/d EPA arm that received none. This is consistent with the notion that MO causes some form of interference with drug absorption.[72]

> "Reviewer Comment: Although the Vascepa 2g dose reduced TG, there were wide fluctuations in TG levels. By Week 11, the slight improvements in TG levels achieved at Week 4 were reduced back to almost the Baseline TG. Within one week (from Week 11 to Week 12) the mean percent change in TG changed from 0.20% to -9.78%. Wide fluctuations in TG were also observed in the Placebo group.
>
> The Vascepa 4g dose reached maximum effectiveness by Week 4 and despite a slight decrease at Week 11 and Week 12, showed none of the wide fluctuations seen in Vascepa 2g or Placebo."

Interestingly, in the ANCHOR and REDUCE-IT placebo groups there was a 4.8% and 4.7% increase in HDL-C respectively, both significant. Counter-intuitively, HDL-C has been shown to increase more from a lower versus higher atorvastatin regimen.[73]

> "A meta-analysis of 32,258 patients of dyslipidemia was done by Philip J. Barter et al. It included 37 randomized studies of different types of statin (Rosuvastatin, atorvastatin, and simvastatin, etc.). Effect of these statins on HDL cholesterol level was assessed in this meta-analysis. They found that increase in serum HDL cholesterol was inversely related to dose of atorvastatin. There was 4.5% increase in serum HDL cholesterol with 10 mg dose while there was a 2.3% increase in serum HDL cholesterol with 80 mg dose of atorvastatin. It was concordant with our study which showed percentage increase in HDL-Cholesterol in the range of 9.52 ± 30.07 and 11.36 ± 28.62 at 3 and 6 months follow-up respectively in 40 mg group. While in 80 mg group, percentage increase in HDL-Cholesterol was 7.74 ± 26.43 and 9.02 ± 27.47 at 3 and 6 months follow-up respectively."

The increase in HDL-C in both placebo groups might therefore be expected if the dose was effectively lowered due to malabsorption. In the MARINE trial, where the vast majority were not on statin therapy, HDL-C was unaffected in either the 4 g/d MO or 2 g/d MO+2 g/d EPA groups.

In the REDUCE-IT trial, highly significant increases in atherogenic markers also appeared in the MO placebo group quickly (at the next blood draw 4 months later) and remained elevated throughout the study. Meanwhile, in those randomized to IPE arm, there was little change (i.e. LDL-C moved down slightly from 85.8 mg/dL at baseline to 83.6 mg/dL at month 4, then back up to 85.3 mg/dL at year 1 and

---

[72] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf

[73] Agrawal D, Manchanda SC, Sawhney JP, et al. To study the effect of high dose Atorvastatin 40 mg versus 80 mg in patients with dyslipidemia. *IHJ*. 2018. [link]

to 85.5 mg/dL at year 2)—except in markers known to be impacted by EPA, such as TG and, to a lesser extent, non-HDL-C.

Regarding the differences in risk reduction seen between subjects on low (HR 1.12), medium (HR 0.76) and high-intensity (HR 0.69; p=0.12, significant for interaction) statins in the REDUCE-IT study, a related phenomenon surfaced in ANCHOR.

> "Compared to placebo in ANCHOR, IPE 4 g/day significantly decreased hsCRP levels in patients receiving higher (-29 %, $p < 0.05$) and medium (-23 %, $p < 0.01$) but not lower-efficacy statin regimens (+4 %, $p > 0.05$)."

However, this was "compared to placebo." The "decreased hs-CRP" as stated was not due to an observed reduction in hs-CRP levels in IPE group from baseline (-2.4%, p>0.05), but instead due to the highly significant increase in hs-CRP levels in the MO placebo arm. The breakdown is given as follows:

> "In ANCHOR, the changes from baseline in hsCRP for IPE 4 g/day and placebo in patients treated with atorvastatin were -12 and +31 %, respectively, resulting in a statistically significant placebo-adjusted reduction of 37 % ($p = 0.0475$). The changes from baseline in hsCRP for IPE 4 g/day and placebo in patients treated with rosuvastatin were -1.2 % and +15.2 %, respectively, resulting in a statistically significant placebo-adjusted reduction of 31 % ($p = 0.0217$). The changes from baseline in hsCRP for IPE 4 g/day and placebo in patients treated with simvastatin were 0.0 and +13.2 % respectively, resulting in a statistically non-significant placebo-adjusted reduction of 13.6 % ($p = 0.0755$)."[74]

But why would IPE—compared to MO placebo—have such differential effects on CRP, depending on the intensity of the background statin therapy of the subject? We can see that IPE had no measurable impact on CRP levels from baseline in those dosed with it; therefore, whatever impact on CRP is likely coming not from IPE, but from an adverse effect of MO on placebo group subjects.

High-intensity statin regimens produce the largest reductions in CRP levels, followed by moderate and then low-intensity regimens.[75, 76] Therefore, if MO attenuated the efficacy of statin therapy, we would expect those on high-intensity statins to show the largest increase in CRP levels, followed by those on moderate-intensity, with the least change in those on low-intensity regimens. And that is what the ANCHOR trial data show.

Regarding the difference in changes in hs-CRP levels between the ANCHOR (+17% at 12 weeks) and REDUCE-IT (+32.3% at year 2) studies, Bonnet et al. (2008)[77] showed that after an initial sharp decrease in CRP levels from statin therapy, high-dose therapy further lowered levels over a 26-week follow-up

---

[74] 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. [link]

[75] Zamani B, Saatlo BB, Naghavi-Behzad M, Taqizadeh-Jahed M, Alikhah H, Abbasnezhad M. Effects of high versus low-dose atorvastatin on high sensitive C-reactive protein in acute coronary syndrome. *Niger Med J*. 2014. [link]

[76] Scott Kinlay , Gregory G. Schwartz , Anders G. Olsson, et al. High-Dose Atorvastatin Enhances the Decline in Inflammatory Markers in Patients With Acute Coronary Syndromes in the MIRACL Study. *Circulation*. 2003. [link]

[77] Bonnet J, McPherson R, Tedgui A, et al. Comparative effects of 10-mg versus 80-mg Atorvastatin on high-sensitivity C-reactive protein in patients with stable coronary artery disease: results of the CAP (Comparative Atorvastatin Pleiotropic effects) study. *Clin Ther*. 2008. [link]

period; i.e. atorvastatin 10 mg/d produced a 25% decrease in hs-CRP over a 5-week period, which was maintained at 26 weeks (-24.3%), whereas the 80 mg dose produced a 36.6% decrease over 5-weeks, which then continued on to a 57.1% decrease at 26 weeks. Thus, even in this regard, the ANCHOR and REDUCE-IT studies mirror what might happen at different time points if statin therapy was attenuated in one group of randomized high-risk subjects, but not the other.

Having two clinical trials testing the same therapies (IPE vs MO) in back to back succession, reproducing the findings in one other, adds to the reliability of the evidence presented. In this case, the evidence suggests that MO adversely impacted the efficacy of concomitant therapies.

1.8 Direct Evidence of an Inhibitory Effect of Mineral Oil on Drug Absorption

Beyond these observations in the REDUCE-IT and ANCHOR trials, there are also more direct evidences of an inhibitory effect of MO on drug absorption.

In their exhaustive text, Herb, Nutrient, and Drug Interactions, 2008., the authors state the following,

> "Mineral oil, as a lipid solvent, interferes with normal absorption of vitamin D (and other nutrients) and increases its elimination from the body. Some disagreement surrounds the degree of clinical significance, particularly with regard to vitamin D, but most research has found that mineral oil interferes with the absorption of many nutrients, including beta-carotene, calcium, phosphorus, potassium, and vitamins A, D K, and E. Chronic use of mineral oil can cause a deficiency of vitamins A, D, K, and E.
>
> Malabsorption of fat-soluble vitamins can be minimized by administering mineral oil on an empty stomach or consuming vitamin and mineral supplements at least 2 hours before or after the use of mineral oil."

Toxnet.nlm.nih.gov states the following peer-reviewed warning:

> "Concurrent use of /anticoagulants, coumarin- or indandione-derivative, oral, or contraceptive, oral, or digitalis glycosides or vitamins, fat-soluble, such as A, D, E, and K/ with mineral oil may interfere with the proper absorption of these or other medications and reduce their effectiveness." (USPDI-drug information for the Healthcare Professional, 1994., p. 1706)

In *"Functions of Lipids for Enhancement of Oral Bioavailability of Poorly Water-Soluble Drugs"* by Nanjwade et al. (2011), the authors note the following:

> "Non-digestible lipids such as mineral oil, e.g. liquid paraffin and sucrose polyesters, essentially remain unabsorbed in the intestinal lumen and can actually limit or even reduce drug absorption by retaining a considerable amount of co-administered drug."[78]

[78] Nanjwade BK, Patel DJ, Udhani RA, Manvi FV. Functions of lipids for enhancement of oral bioavailability of poorly water-soluble drugs. *Sci Pharm*. 2011. [link]

This is in concordance with the study, *"Effects of Oils on Drug Absorption*" (Palin, 1981).[79] Below is an extract from this study, with the most pertinent components due to their relevance to the present discussion included.

***

"The results of numerous studies indicated that whilst polar, digestible lipids increased the bioavailability of lipophilic, poorly water-soluble drugs without increasing the absorption rate, nonpolar, nondigestible lipids [such as light paraffin oil] generally did not affect the bioavailability of these drugs, although they did appear to reduce the absorption rate.

Talbot & Meade (1971) reported that the ingestion of potato chips containing small amounts of methyl polysiloxane, a lipid-like agent that enhances crispness, apparently significantly reduced the absorption of warfarin and phenindione in patients taking these drugs.

In addition, if cholesterol is administered in a hydrocarbon oil, absorption is decreased almost to zero (Sylven & Borgstrom 1969,Mattson et al.1976) because cholesterol partitions in favour of the oil which is not absorbed and the cholesterol is therefore retained within the intestinal lumen.

1.1 Introduction

The aim of the present study was to determine the potential for enhancing oral drug absorption by stimulating lymphatic absorption in the presence of a suitable oily vehicle. The effect of different oily vehicles on the oral absorption of a model compound (DDT) was investigated, the influence of lymphatic absorption and gastro-intestinal transit being determined. Three oils that are used in pharmaceutical preparations but which have chemically different structures were selected as vehicles for oral administration of DDT to rats:

a)Arachis oil B.P.I (peanut oil), a natural oil consisting of triglycerides the fatty acid constituents of which are chiefly oleic acid (C 18 containing one double bond) and linoleic acid (C 18 containing two double bonds) with smaller amounts of palmitic, arachidic, lignoceric and stearic acids.

b)Miglyol 812, a synthetic oil produced by hydrolysis, fractionation and re-esterification of coconut oil, consisting of a mixture of triglycerides of saturated fatty acids of medium chain length (C8 - C12).

c)Liquid paraffin B.P., a mineral oil consisting of a mixture of liquid hydrocarbons.

The oral absorption of DDT from solution in each oil was investigated in rats, to determine whether DDT absorption could be altered by the presence of an oily vehicle and whether the nature of the oil used was an important consideration.

[79] Palin, K.J. (1981) Effect of oils on drug absorption. PhD thesis, *University of Nottingham*. [link]

2.3.2 Preparation of Dosage Forms.

a) Oily solutions - in all experiments in which at least 1ml oil was administered the same dose of DDT (100mg/kg) was used. Oily solutions containing this dose in 2ml and in 1ml of each oil were prepared. To determine the effect of 30pl oil on DDT absorption oily solutions containing 7.5mg/kg DDT in 30pl were prepared.

b)Emulsions - oil-in-water emulsions were prepared from each oil using the Silverson blender and the QPR hand homogenisor and the same basic formula:

-Oil (containing 20mg/ml DDT)

-Tween 80

-water to 100%

Each rat was dosed with 2mls emulsion.

2.4 Results

FIGURE 2.3

THE EFFECT OF DIFFERENT VEHICLES (1 ml volumes) ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/ kg) IN RATS (Mean + S.E.M., n= 4 per group).

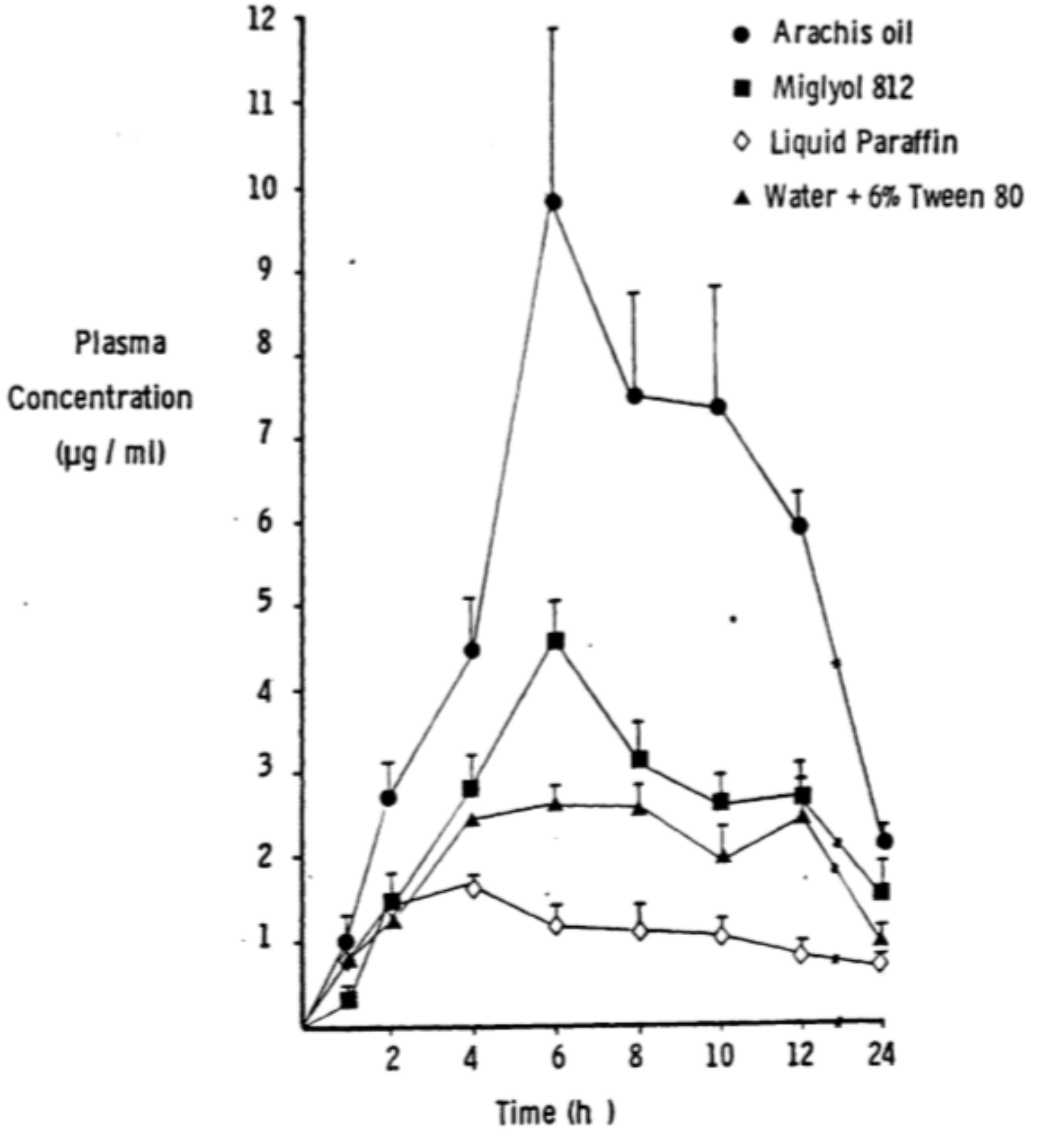


FIGURE 2.6

THE EFFECT OF DIFFERENT LIQUID PARAFFIN FORMULATIONS ON THE ABSORPTION OF ORALLY ADMINISTERED DDT (100 mg/kg) IN RATS (Mean + S.E.M., n= 4 per group)

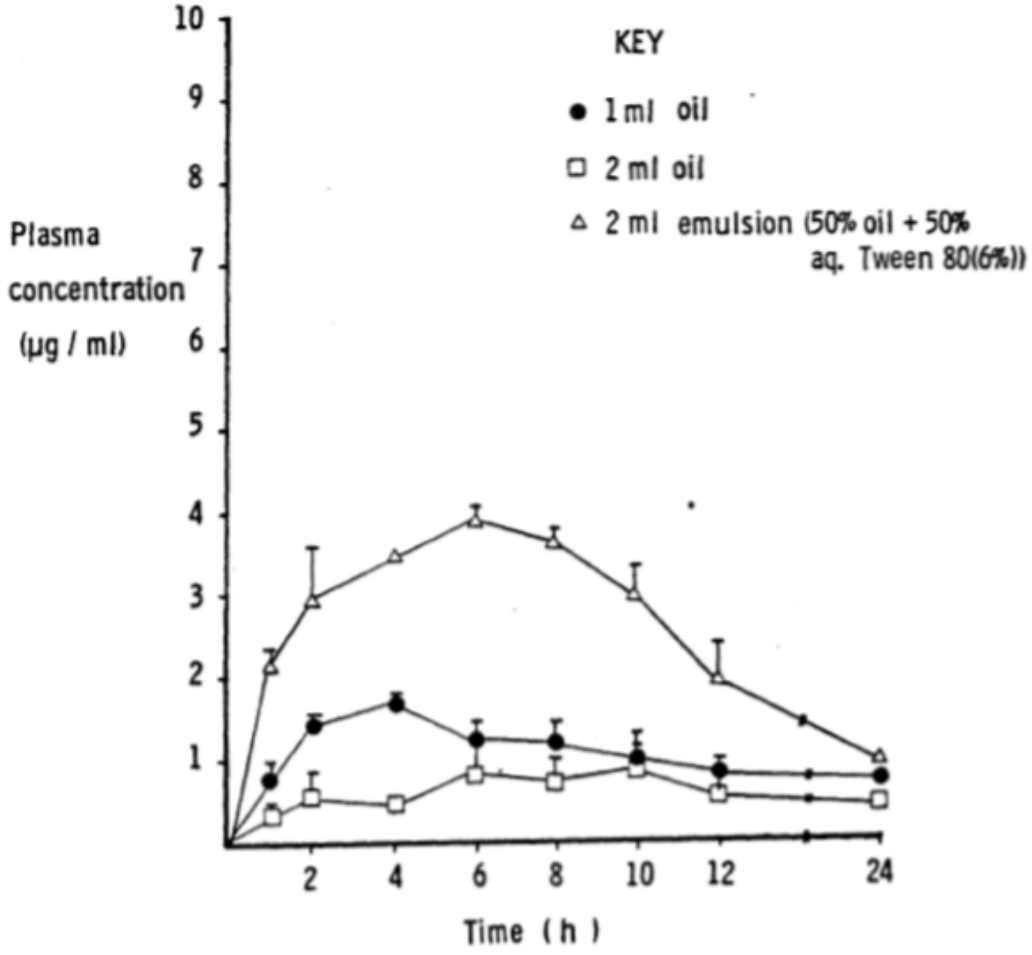


Table 2.2 - Absorption Data Following Oral Administration of DDT in Different Vehicles to Rats

(Mean ± S.D., n=4 per group).

	Arachis Oil			Miglyol 812			Liquid Paraffin		
	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.	C_{pmax} ug/ml	T_{max} h.	AUC_{0-24h} mg ml/h.
1ml oil	11.08 ±1.95	7.0 ±2.0	118.3 ±6.6	4.67 ±0.74	6.0 ±0.0	57.91 ±10.22	1.72 ±0.05	4.0 ±0.0	23.90 ±4.23
2ml oil	10.24 ±3.62	10.0 ±2.3	112.17 ±14.74	4.21 ±1.88	11.5 ±1.0	54.52 ±23.61	1.39 ±0.59	6.5 ±3.4	13.25 ±7.36
2ml emulsion	9.18 ±2.07	4.0 ±0.0	105.81 ±12.54	3.61 ±0.59	5.5 ±5.5	54.52 ±3.42	4.25 ±0.42	5.0 ±2.0	54.27 ±8.20
2ml oil (containing 6% Tween 80)	9.99 ±2.52	7.0 ±1.41	112.21 ±26.11	3.67 ±0.99	9.0 ±3.46	55.97 ±19.02	1.87 ±0.38	8.0 ±1.63	27.11 ±5.25
Predosing with Propantheline	/	/	/	3.56 ±0.73	10.0 ±0.0	46.08 ±15.44	1.99 ±0.52	9.0 ±1.16	31.80 ±6.15
Predosing with Metoclopramide	5.52 ±1.58	5.75 ±0.5	76.76 ±12.23	/	/	/	/	/	/

Control C_{pmax} = 2.88±0.37ug/ml T_{max} = 10.0±2.0h. AUC_{0-24h} = 44.59±5.32mg ml/h.

2.5 Discussion.

The results of these studies showed clearly that the oral absorption of DDT is dependent on the nature and the formulation of the vehicle in which it is administered. The plasma DDT concentration versus time profiles for DDT administered in 1ml volumes of arachis oil, Miglyol 812, liquid paraffin and water containing 6% Tween 80, exhibit significant differences (see figure 2.3). Whilst arachis oil promoted absorption ($p<0.05$ for CPmax and AUCO-24h values), liquid paraffin gave rise to a shorter absorption phase resulting in lower total drug absorption and earlier peak plasma concentrations than for the other vehicles ($p<0.05$ for Tmax and AUCO-24h values).

DDT was also administered in 30ul volumes of oil (approximately equivalent to a 10ml dose for a 70kg man on a body weight basis) to determine whether the differences in absorption from the different oils were still observed at low oil volumes (see figure 2.7). The total absorption of DDT over 24 hours was still less ($p<0.05$) from liquid paraffin than from arachis oil or Miqlyol 812.

6.1 FINAL DISCUSSION AND CONCLUSIONS.

In the present investigation the oral absorption of DDT in rats was shown to be altered by the presence of lipids. The plasma DDT concentration versus time profiles following administration of DDT in solution in 1ml volumes of different vehicles varied considerably (see figure 2.3), with the rank order for total DDT absorption over 0-24 hours being arachis oil > Miglyol 812 = water containing 6% Tween 8Q > liquid paraffin.

The variation in these physiological mechanisms may be attributed to differences in the chemical composition of the oils. As stated in the introduction arachis oil is comprised of long chain saturated and unsaturated fatty acids, Miglyol 812 of saturated medium chain fatty acids, whereas liquid paraffin is a non-digestible mineral oil. Such differences in structure may also affect the physicochemical processes occurring within the gut during absorption. A lipid digestion mixture within the small intestinal lumen is a 3-phase system comprised of an oil phase, an aqueous phase and a micellar phase. Drug absorption can occur from one or more of these phases (see figue 6.1), although reports in the literature have suggested that drug is absorbed from either the aqueous or the micellar phase; absorption from the oil phase being negligible (Armstrong & James 1980). Absorption of DDT probably occurs only from the micellar phase as it is a highly lipophilic, water insoluble structure, with an octanol:water partition coefficient of approximately one million. The presence of different lipids within the digestion mixture can alter the partitioning of DDT between the three phases and hence absorption.

Liquid paraffin is non-digestible and a large proportion of the oil is retained within the small intestinal lumen. However, as a small quantity is normally absorbed (Albro & Fishein 1970), DDT may be absorbed directly with the oil phase or following partitioning into the micellar phase. As the volume of oil absorbed is small and partitioning into bile salt micelles is limited, not being enhanced by the formation of mixed bile salt micelles, a relatively large proportion of DDT is probably retained in the liquid paraffin and excreted in the feces. Similar findings have been

> reported for cholesterol and DDT in the presence of sucrose polyester, a non-absorbable lipid (Mattson et al.1976,Vonenhein et al.1980)."

***

The above study provides evidence in its direct experiment, as well as via citations of numerous other trials testing the impact of digestible and non-digestible lipids on drug uptake (such as the inhibition of warfarin and phenindione absorption when taken along with a non-digestible lipid), that light paraffin oil is implicated in the suppression of drug absorption. To quote one of the main conclusions of the study, *"a relatively large proportion of [the drug] is probably retained in the liquid paraffin and excreted in the feces."*

Finally, there is good reason to suggest concomitant dosing of MO with prescribed therapies took place in the ANCHOR and REDUCE-IT studies. The instructions on dosing with 4 g/d of the study drug is as follows:

> "Patients took 2 capsules (IPE and/or matching placebo) in the morning and 2 capsules in the evening for a total of 4 capsules per day. Patients were instructed to take study drug with food."

The three most commonly prescribed statins are atorvastatin, simvastatin and rosuvastatin. Recommendations for dosing are as follows:[80]

- Atorvastatin is usually taken once a day, with or without food. Take the medicine at the same time each day.
- Simvastatin is usually taken at bedtime or with an evening meal. If you take simvastatin more than once daily, take it with meals.
- Rosuvastatin is usually taken once a day, with or without food. Take the medicine at the same time each day.

The AARP states the ideal time to take statin and antihypertensive therapies as "at bedtime," citing the British Heart Foundation recommendation.[81] Data suggest this is particularly important for antihypertensives.[82] Antithrombotics are also often recommended to be taken at bedtime, supported by efficacy data.[83, 84, 85]

As patients most commonly group medications together regardless of instructions to do otherwise (polypharmacy),[86] and considering the above similar instructions across therapies, it is probable that

---

[80] Drugs.com, atorvastatin dosing information, medically reviewed by Sanjai Sinha, MD. 2018. [link]
[81] https://www.aarp.org/health/drugs-supplements/info-12-2013/timing-of-daily-medications-key.html#quest1
[82] Rajesh Mohandas, A Ahsan Ejaz. Evening dosing of antihypertensive medications results in better blood pressure control and decreases cardiovascular morbidity and mortality in patients with Type 2 diabetes. *BMJ.* 2012. [link]
[83] https://www.consultant360.com/articles/warfarin-and-atrial-fibrillation
[84] Bonten TN1, Snoep JD2, Assendelft WJ. Time-dependent effects of aspirin on blood pressure and morning platelet reactivity: a randomized cross-over trial. *Hypertension*. 2015. [link]
[85] Li Z1, Liu F, Cui W, et al. Impact of application time of aspirin and clopidogrel on platelet aggregation in patients with acute coronary syndrome. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2010. [link]
[86] Qato DM, Wilder J, Schumm LP, Gillet V, Alexander GC. Changes in Prescription and Over-the-Counter Medication and Dietary Supplement Use Among Older Adults in the United States, 2005 vs 2011. *JAMA Intern Med*. 2016. [link]

they were taken concurrently—either two 1g capsules in the morning/evening, or all four 1g capsules at once—with other medications.

1.9 Drug-Drug Interaction Studies

DDI studies are commonly conducted to ensure the active pharmaceutical ingredient (API) of an experimental drug does not interfere with commonly co-prescribed medications. Before Vascepa was approved for severe hypertriglyceridemia, multiple DDI studies involving the prodrug were also required to be conducted.[87]

One such study with atorvastatin is outlined below:

4.2.5 AMR-01-01-0023: A Phase 1, Open-Label, Crossover Drug-Drug Interaction Study to Evaluate the Effect of Icosapent Ethyl (AMR101) on the Pharmacokinetics of Atorvastatin in Healthy Subjects

Objectives:

The objective of this study was to investigate the effect of AMR101 on the pharmacokinetics (PK) of atorvastatin in healthy subjects.

Study design:

This was a Phase 1, single-center, open-label, cross-over, drug-drug interaction study between AMR101 and atorvastatin in healthy subjects. Thirty subjects were enrolled to receive:

- Atorvastatin (80 mg/day) once per day (QD) on Days 1-7
- AMR101 (4 g/day, twice per day (BID)) on Days 8-35
- Atorvastatin (80 mg/day) QD on Days 29-35

Atorvastatin was provided as Lipitor® tablets (80 mg). AMR101 was provided as 1 g liquid-filled gelatin capsules. Atorvastatin was administered approximately 1 hour before breakfast, while ethyl-EPA was administered with food.

[87] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000ClinPharmR.pdf

Blood samples for the determination of atorvastatin and metabolites 2-hydroxyatorvastatin and 4-hydroxyatorvastatin plasma concentrations were obtained on Days 1 and 29 at time 0 (prior to dose) and on Days 7 and 35 at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 hours after the atorvastatin dose.

Blood samples for the determination of unesterified and total EPA plasma concentrations were obtained prior to the morning dose of AMR101 on Days 8, 29 and 35, and 5 hours following the morning AMR101 dose on Days 29 and 35.

Pharmacokinetic Endpoints:

• Atorvastatin (and metabolites): AUC_{0-24}, C_{max} and T_{max} of atorvastatin (and metabolites) when administered alone compared to when administered with AMR101.
• EPA: Total and unesterified EPA plasma concentration on Days 8, 29 and 35 (descriptive statistics only of the concentrations per day and time).

Pharmacokinetic Analyses:

Atorvastatin, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin PK parameters AUC_{0-24hr}, C_{max} and T_{max} were computed for each subject per day (without and with AMR101) with non-compartmental methods using the actual sampling times. Descriptive statistics were calculated for the PK parameters per day (without and with AMR101).

The effect of AMR101 on the exposure of atorvastatin was determined by a PK comparison between atorvastatin, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin when atorvastatin was administered alone versus atorvastatin administered with AMR101. The primary PK comparison was based on the AUC_{0-24hr} and C_{max} of atorvastatin. Analysis of variance (ANOVA) models under the cross-over design were used for analyzing all AUC and C_{max} parameters, and based on natural log-transformed values. This included the effects for treatment (without and with AMR101) as a random effect. The estimate of the ratio between two treatments for these parameters and the corresponding 90% confidence intervals (CIs) for the ratio were obtained by exponentiating the difference in logarithms, and used for assessing similarity between atorvastatin (without and with AMR101) within each PK comparison. The treatment of atorvastatin alone was the reference treatment for the purpose of statistical comparisons.

Secondary comparisons included comparisons between treatments for AUC_{0-24hr} and C_{max} of 2-hydroxyatorvastatin and 4-hydroxyatorvastatin that were similar to the primary comparison; and nonparametric analysis of T_{max} of atorvastatin, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin using the Wilcoxon Signed Rank Test. The corresponding 95% CIs for the difference in medians was reported using Walsh Average and appropriate quantile of the Wilcoxon Signed Rank Test Statistic. Significant difference for the treatment comparison was concluded if the resulting p value was <0.05.

Study results:

Mean (SD) atorvastatin, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin plasma concentrations

without and with AMR101 versus time are displayed on linear scales in Figure 20, Figure 21 and Figure 22, respectively. The PK parameters of atorvastatin and its metabolites and their comparisons with and without ethyl-EPA are listed in Table 15. The ratios of the geometric means of C_{max} for atorvastatin and its 2-hydroxy and 4-hydroxy metabolites, following administration of atorvastatin with and without ethyl-EPA were 1.08, 1.04 and 0.97, respectively. The ratio of the geometric means of AUC_{τ} for atorvastatin and its 2-hydroxy and 4-hydroxy metabolites, following administration of atorvastatin with and without ethyl-EPA were 0.99, 0.91, and 0.92, respectively. The 90% confidence intervals for C_{max} and $AUC_{0-\infty}$ were between 80% and 125% for atorvastatin and its metabolites. It is concluded that 4 g/day ethyl-EPA does not inhibit the metabolism of atorvastatin.

Figure 20 Mean (SD) Atorvastatin Plasma Concentration without and with AMR101 versus Time

Figure 20 Mean (SD) Atorvastatin Plasma Concentration without and with AMR101 versus Time

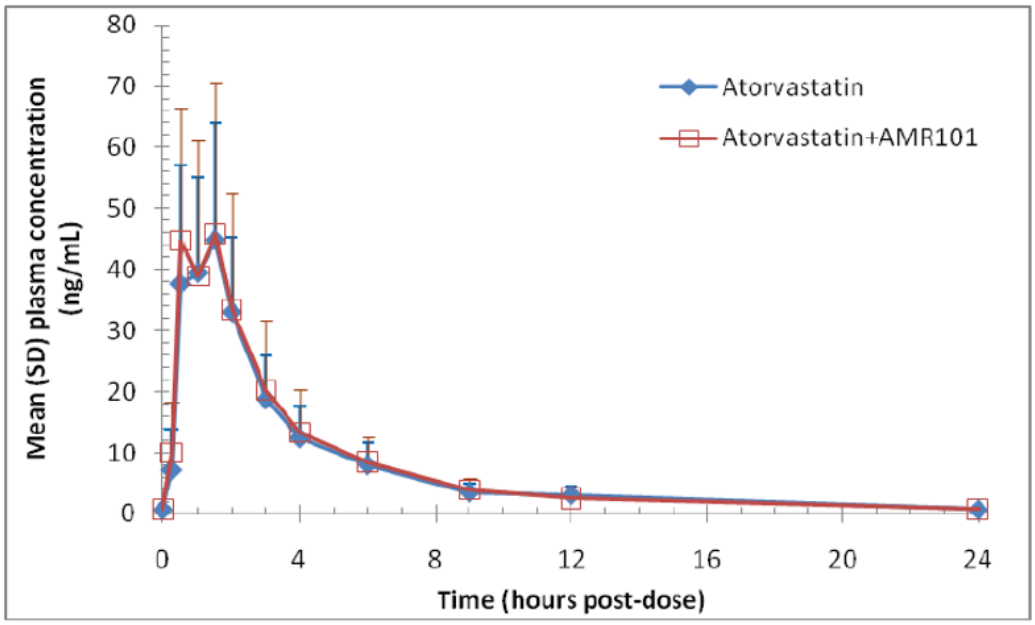


Figure 21 Mean (SD) 2-hydroxyatorvastatin plasma concentration without and with AMR101 versus time

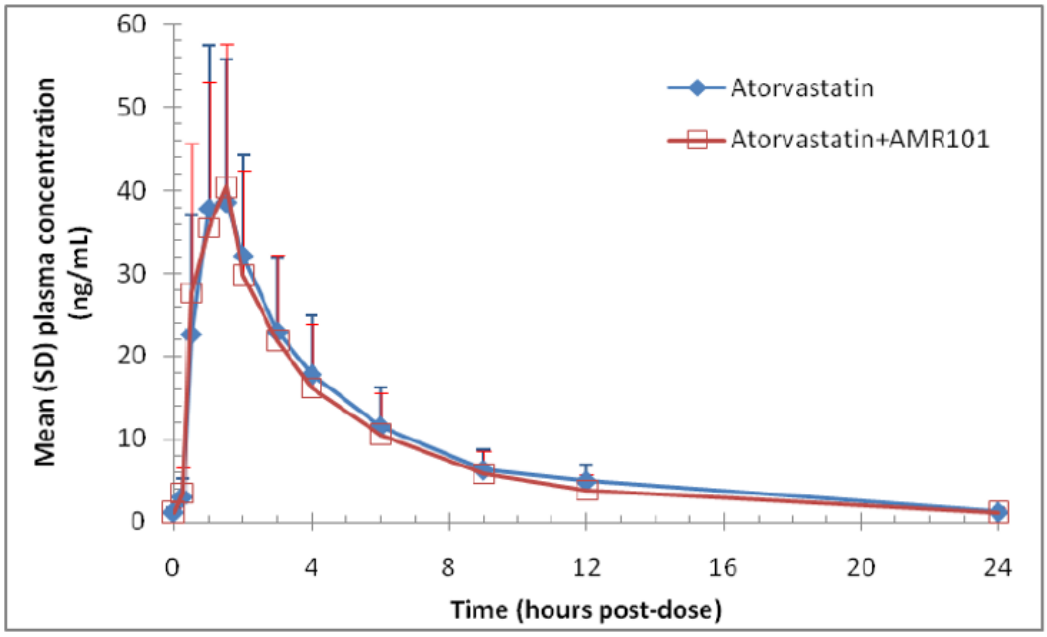


Figure 22 Mean (SD) 4-hydroxyatorvastatin plasma concentration without and with AMR101 versus time

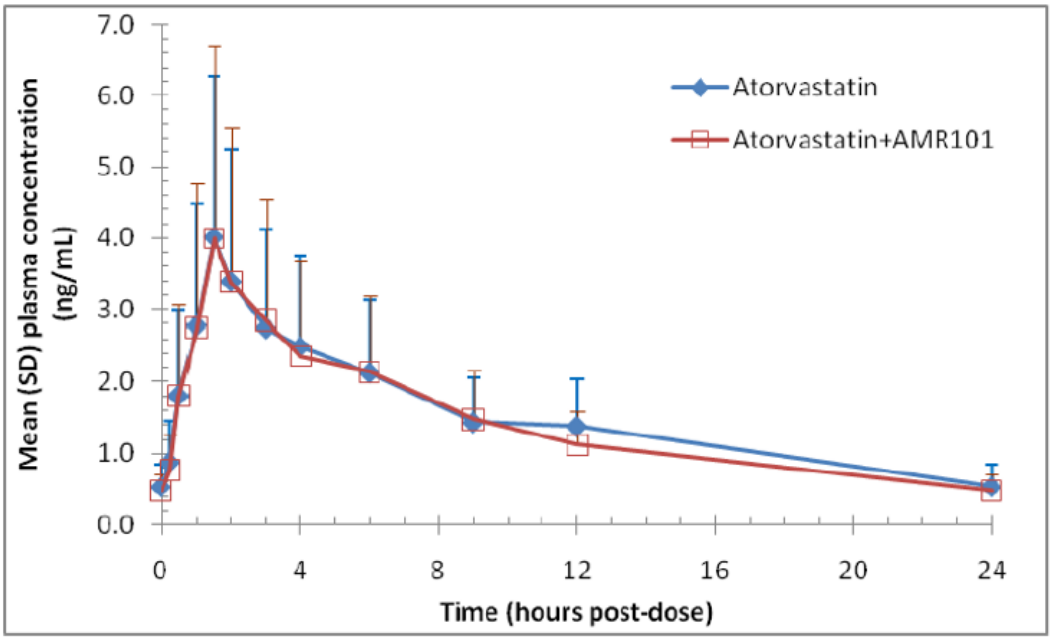


Table 15 Pharmacokinetic Parameters of Atorvastatin, 2-Hydroxy Atorvastatin, and 4-Hydroxy Atorvastatin in the Presence and Absence of Ethyl-EPA

Analyte	Vascepa	C_{max} (ng/mL)	AUC_{τ} (ng*hr/mL)	T_{max} (hr)	C_{max} Ratio (90% CI)	AUC_{τ} Ratio (90% CI)
atorvastatin	No	52.7 (19.3)	179.8 (59.5)	1.0 (0.5, 1.0)	1.08 (94.9, 122.1)	0.99 (90.2, 108.9)
	Yes	57.1 (21.9)	184.3 (79.3)	0.5 (0.5, 1.5)		
2-hydroxy atorvastatin	No	43.2 (18.8)	213.1 (73.0)	1.25 (0.5, 3.0)	1.04 (89.1, 121.3)	0.91 (82.8, 99.7)
	Yes	44.5 (17.4)	196.7 (77.0)	1.25 (0.5, 2.0)		
4-hydroxy atorvastatin	No	4.14 (2.21)	36.7 (16.4)	1.5 (1.0, 3.0)	0.97 (78.0, 117.3)	0.92 (82.3, 102.8)
	Yes	4.20 (2.66)	34.3 (16.4)	1.5 (1.5, 6.0)		

Mean (SD) displayed for all PK parameters except T_{max} displayed as median (min, max).
Ratios are geometric mean ratios of treatment with Vascepa / treatment without Vascepa.
τ = 24 hr; CI = confidence interval in percent
Source: Study AMR-01-01-0023 CSR

***

As can be seen above, concurrent dosing of atorvastatin with Vascepa had no effect on the absorption of atorvastatin. The only way to validate the REDUCE-IT trial data as unconfounded is if multiple DDI studies are conducted, testing coadministration of 4 g/d mineral oil with statins, antihypertensives, antithrombotics, and anti-ischemia agents. The one difference we have ascertained should be enacted in these proposed studies is that 4 g/d MO be administered at night, before bed, concurrent with each relevant medication.

As outlined previously, evidence suggests it is quite common that patients group all medications together (polypharmacy), rather than take some with a meal and others at bedtime, despite instructions to the contrary. We think it is likely such was done with subjects in the REDUCE-IT trial. At the very least, subjects should be polled to determine exactly how (2 g/d twice a day, or all 4 g/d at once, with or without other cardiac medications) and when they tended to take the study drug/placebo. DDI studies should be designed around what actually took place in REDUCE-IT, not merely what was instructed.

All living participants (or if deceased, their family members) could be contacted and surveyed by a disinterested third-party, with information compiled and organized, answering the questions: “What time of day did you most often take your study drug? Was it normally with food or without food? Was is normally with other medications at the same time?” With a mention on the anonymity of the information, and that there are no repercussions for having taken the study drug other than as instructed, and that absolute honesty is essential to inform regulators on this important public health matter. This should help determine what DDI-study design to require of the sponsor.

1.10 Why Didn't the DMC Recommend the REDUCE-IT Study be Halted for Safety?

In Amarin Corp.'s 2018 annual report[88] can be found the following risk factor (in bold/italics) and explanation:

> ***"[T]he REDUCE-IT trial[] may not provide sufficient safety and efficacy data to obtain the requisite regulatory approvals for product candidates or to achieve a level of market acceptance by physicians, patients, healthcare payors and others in the medical community necessary for commercial success…***
>
> …[A]s regulators such as the FDA review and analyze REDUCE-IT study data, dialogue is expected to continue with respect to the reliability of REDUCE-IT data and the study quality that could adversely affect our product development, regulatory review, market or medical community acceptance, and level of payor reimbursement in the event of an expansion of the Vascepa label…
>
> …For example… the median change in LDL cholesterol level from baseline was… 10.2% (+7.0 mg/dL) in the placebo group. Increases in the placebo group relative to the Vascepa group were also observed in other parameters classically measured in such studies but with uncertain relevance to cardiovascular outcomes…
>
> …If light liquid paraffin oil, or mineral oil, used as the placebo in REDUCE-IT adversely affected statin absorption or other parameters in some patients as is asserted by certain critics of the study [*and we would add, the study's own investigators], this could be theorized to have contributed to differences in outcomes between the groups and leave open the possibility that the placebo used in the trial was not biologically inert. These and other observations… may negatively impact how these trial results are interpreted by regulators, the medical community and third-party payors.
>
> …Consistent with our SPA for REDUCE-IT agreed to with the FDA, the trial subjects in the placebo arm of REDUCE-IT were given light liquid paraffin oil, or mineral oil, to mimic the colour and consistency of Vascepa. We also used mineral oil in the placebo arms of our MARINE and ANCHOR trials. During the public advisory committee meeting held by FDA as part of its review of our ANCHOR sNDA, a discussion regarding observed, nominally statistically significant changes from baseline in an adverse direction, while on background statin therapy, in certain lipid parameters, including triglycerides, in the placebo group, led to further discussion about the possibility that the mineral oil placebo used in the ANCHOR trial (and in the REDUCE-IT trial) might not be biologically inert and might be viewed as artificially exaggerating the clinical effect of Vascepa when measured against placebo in the ANCHOR trial. Ultimately, no strong evidence for biological activity of mineral oil was identified by the FDA before its approval of Vascepa after review of the MARINE and ANCHOR trials and consideration of other data regarding mineral oil. The FDA, early on in the course of the REDUCE-IT trial, directed the independent data monitoring committee, or DMC, for REDUCE-IT to periodically review unblinded lipid data to monitor for signals that the placebo might not be inert. After each such quarterly unblinded

[88] https://investor.amarincorp.com/static-files/f0b8f0dd-6bd8-4344-ba45-f13322a0df84

safety analysis and review meeting to date, the DMC recommended to continue the REDUCE-IT study as planned. Each of these DMC recommendations has been shared with FDA. In addition, following discussions on this topic in October 2013 in connection with the FDA's review of our supplemental new drug application for our ANCHOR study, the FDA did not seek to require that we include any qualification related to the use of mineral oil as a placebo in REDUCE-IT at the time of our March 2016 amendment to the REDUCE-IT SPA.

…If… the potential effects of the mineral oil used in the placebo arm of REDUCE-IT remains uncertain… the perception of REDUCE-IT results and Vascepa may suffer and could adversely affect our product development, regulatory review, market or medical community acceptance… any of which could have a material adverse effect on our business and financial condition and our stock price may decline."

Some background should help elucidate the above. From the clinical review of the NDA submission for Vascepa covering the MARINE trial indication (TG >/= 500 mg/dL) we have the following summary of events:[89]

"2.5 Summary of Presubmission Regulatory Activity Related to Submission

- A preIND meeting was held on 14 July 2008. During the discussion, it was agreed that a single study could potentially suffice for the indication "as an adjunct to diet to reduce triglycerides in adult patients with very high >500 mg/dL triglyceride levels" provided that the results are robust.
- A Special Protocol Assessment for the Phase 3, 12-Week study AMR-01-0016 (MARINE) was completed and accepted on 01 May 2009. Key agreements included raising the upper limit of TG entry criterion from 1500 mg/dL to 2000 mg/dL.
- IND 102,457 was opened on 22 May 2009 with the study AMR-01-0016 (MARINE).
- A Special Protocol Assessment for the Phase 3, 12-Week study AMR-01-0017 (ANCHOR) was completed and accepted on 06 July 2009.
- A preNDA meeting [*for the MARINE trial indication] was held on 16 March 2011.
- A Special Protocol Assessment for the cardiovascular outcomes trial AMR-01-01-0019 (REDUCE-IT) was completed and accepted on 05 August 2011.

A few things to note on the above; a Special Protocol Assessment (SPA) was accepted for all three trials (MARINE, ANCHOR, and REDUCE-IT) before the NDA for the first of these trials (MARINE) was submitted on 23 September 2011.[90] Vascepa's one and only approval came after the review of this NDA in July of 2012, being indicated as adjunct therapy to diet for patients with severe hypertriglyceridemia (TG >/= 500 mg/dL), as a way to circumvent pancreatitis. The FDA first noticed something peculiar about those treated with mineral oil from the lipid data from the MARINE trial, stating:[91]

"During the review of the MARINE data, the Division noted that several lipid parameters (including TG) increased from baseline to week 12 in the placebo group, treated with mineral oil. The available literature regarding potential effects of mineral oil was considered. Similar

[89] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf
[90] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000SumR.pdf
[91] http://epadruginitiative.com/files/FDA_Briefing_Document_for_ADCOM.pdf

> increases in TG levels observed in the placebo groups from the Lovaza (omega-3 EE) clinical trials of hypertriglyceridemic patients were noted, and these trials did not use a mineral oil placebo. Because no strong evidence for biological activity of mineral oil was identified, ultimately it was concluded that the between-group differences likely provided the most appropriate descriptions of the treatment effect of AMR101 and that whatever factor(s) led to the within-group changes over time in the placebo group were likely randomly distributed to all treatment groups. Taken together, along with the statistical robustness in primary and sensitivity analyses of AMR101 4g/day on TG lowering, the Division concluded that AMR101 4g/day is an effective TG-lowering agent for patients with severe hypertriglyceridemia."

Notice that the above mentions, *"no strong evidence for biological activity of mineral oil was identified,"* and *"it was concluded that the between-group differences likely provided the most appropriate descriptions of the treatment effect of AMR101,"* which Amarin prefers to recite in its annual report in a manner that implies it also refers to a review of the ANCHOR data, were relevant to the MARINE trial efficacy data only, and just 25% of the subjects in MARINE were on background statin therapy.[92] Although MARINE data did show unusual changes in some markers in its placebo group, it was not nearly to the extent of that seen in the ANCHOR, and later REDUCE-IT, trials.

Amarin had submitted some of the data from ANCHOR along with the MARINE trial data for consideration under this review, though it was not the full dataset, but a clinical study report:[93]

> "This application included clinical data (both study report and datasets) from the MARINE trial. This study investigated 229 patients with TG between 500 mg/dL to 2000 mg/dL. Patients were randomized to one of three treatment arms: Placebo, Vascepa 2g, or Vascepa 4g for 12 weeks.
>
> This application also contained a study report, but not the dataset, for the ANCHOR trial. This trial was not considered pivotal to the efficacy claims of Vascepa for this NDA. The ANCHOR trial investigated patients with TG between 200 mg/dL and 499 mg/dL despite statin therapy. The applicant was told prior to this NDA submission that data from the ANCHOR trial would not be mentioned in the Vascepa labeling until, at a minimum, 50% enrollment of a cardiovascular outcomes trial [REDUCE-IT] was reached.
>
> … The MARINE trial is the focus of the efficacy review in Section 6, whereas the safety review focuses on both the pooled data from the MARINE and the ANCHOR trials (the Hypertriglyceridemic Placebo-Controlled Dataset)."

The review team evidently did not scrutinize the ANCHOR trial study report much beyond safety data during their review of the MARINE trial dataset, as referenced above, and also because once the ANCHOR trial dataset was submitted (February of 2013) and FDA reviewed it, they much more vociferously voiced concerns over mineral oil possibly not being inert, especially given the significant increases observed across all atherogenic/inflammatory parameters in the placebo group, and given that all subjects were on background statin therapy:[94]

---

[92] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf
[93] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf
[94] http://epadruginitiative.com/files/FDA_Briefing_Document_for_ADCOM.pdf

"…[N]ote that for each of these parameters, with the exception of HDL-C and apo A1, the placebo group demonstrated nominally statistically significant changes from baseline in an adverse direction, while on background statin therapy. If these within-group changes were the result of factors that were randomly distributed across treatment groups, the comparisons to placebo should represent the best estimates of the treatment effect. If it is possible, however, that the mineral oil placebo was not biologically inert (e.g., could it have partially inhibited statin absorption if concomitantly ingested?), then the comparisons with placebo could produce biased treatment effects…

…[T]he magnitude of the changes in several lipid and lipoprotein parameters, as well as biomarkers of inflammation, between baseline and Week 12 in the placebo group are rather atypical for lipid-lowering trials. These trials, including ANCHOR, often include a several-week lead-in period to stabilize diet and concomitant lipid-altering medications well before baseline measurements. Although even highly statistically significant within-group changes can certainly result from factors other than the intended experimental intervention, one concerning possibility is that the mineral oil placebo may not be biologically inert. If this were true, the estimated treatment effects may be biased.

Thus, the review team sought evidence that might help explain the changes observed in the mineral oil group. These included considering the plausibility that treatment assignment could have been unmasked due to physical differences in study drug appearance or manufacture; reviewing the literature for mineral oil-specific effects on lipid parameters or absorption of fat-soluble vitamins; evaluating whether the statin-treated subjects in the placebo group from MARINE demonstrated a similar pattern; and considering elements of the ANCHOR study design that may have contributed. Finally, the Division reviewed lipid changes observed in the placebo groups of other lipid-lowering trials. The Chemistry, Manufacturing, and Controls data do not suggest that blinding would have been compromised. The only difference between the active capsules and the placebo capsules was that the drug substance (icosapent ethyl) was replaced with mineral oil. All other formulation components and composition remained the same and were added in an identical fashion. The submitted certificates of analysis for the AMR101 and placebo lots used in this trial describe identical appearances of the blister packs and capsules. Admittedly, even if study subjects were able to discern their assignment to placebo or AMR101, it is difficult to predict what direction bias would be introduced (e.g., how might treatment assignment influence one's adherence to dietary instruction?).

Three studies using mineral oil as a placebo and reporting baseline and end-of-treatment lipid values were reviewed to determine if similar changes were observed to those that occurred in ANCHOR. 19,20,21 The population of patients studied varied greatly: dyslipidemic women with type 2 diabetes, patients infected with HIV, and healthy volunteers. The exposure to mineral oil placebo ranged from 10 days to 2 months with daily doses of 6 grams or less. Despite these differences, in general, the effect of the mineral oil placebo on lipid parameters was small. For example, after 8 weeks of mineral oil (6g/day), the median percent change of TG from baseline in HIV-infected patients was +1%. 22

Studies from the 1940s suggested that mineral oil may block the absorption of fat-soluble vitamins.23,24 Articles submitted by the applicant and independent review of the available

medical literature on this issue were reviewed. Although initial studies suggested possible malabsorption with mineral oil, subsequent studies using large volumes of mineral oil (up to 150 mL/day) over a long period of time called these findings into question.25,26,27,28 Of course, patients in the ANCHOR trial's placebo group ingested far smaller volumes of mineral oil than this as well (approximately 4 mL/day), which weakens but does not eliminate the possibility of a local intestinal effect of mineral oil on statin absorption.

Whether mineral oil affects statin absorption has not been formally tested to our knowledge. The applicant submitted data regarding patients who were taking concomitant statin therapy in the MARINE trial and who were randomized to the mineral oil group. Only 18 patients in the mineral oil group were taking a statin. The median percent change in LDL-C was -8% in the statin-treated mineral oil group, with large variability (Q1 -36.0%, Q3 +30.8%); the median change was 0% in LDL-C among the 57 patients not taking statins in the mineral oil group. The applicant contends that if mineral oil reduced statin exposure, then LDL-C should have increased after 12 weeks of treatment, not decreased. While the reduction in LDL-C in this group is somewhat reassuring, the small number of statin-treated patients and the large intra-subject variability do not allow definitive conclusions from this subgroup.

Patient compliance (indirect measures): There was no dietary compliance assessment or measurement of physical activity in the ANCHOR trial. However, indirect measurements of diet and physical activity, i.e., weight, waist circumference, and BMI did not demonstrate significant changes between the placebo and AMR101 treatment groups, suggesting that physical and dietary habits between groups were not dramatically different throughout the trial and are unlikely to have contributed to the effects observed in the placebo group.

Regression to the mean: Subjects enrolled in ANCHOR were selected non-randomly from a broader population of subjects of which 70% failed to be randomized. The applicant contends that the asymmetric selection process may have contributed to a regression-to-the mean phenomenon apparent across the lipoprotein lipids and other biomarkers within the placebo group. If true, this would be a design element expected to affect all treatment groups similarly and the between-group differences should provide unbiased estimates of the treatment effects. Averaging two qualifying values separated by one week, all following a ≥4-week lead-in stabilization period, should have reduced the contribution of regression to the mean, although its possible contribution cannot be ruled out.

Considering the 609 subjects who were excluded at the end of the screening period because of ineligible lipid values, the majority (65%) had TG levels that were too low. Although one cannot determine with certainty, this suggests that the study might have been more likely to enroll patients who had "random highs" rather than "random lows," and if this were the case, TG levels would be expected to regress downward rather than upward. Regarding LDL-C, most (60%) of the subjects excluded for lipid reasons had LDL-C in range with the remainder more likely to be excluded for low LDL-C than high LDL-C.

Therapeutic changes during Lead-in period: The applicant has put forward the hypothesis that changes in lipid-lowering regimens and wash-out of non-statin therapy during the lead-in period may have increased variability of TG levels after randomization. Although this could occur, it

doesn't seem that "larger variability" would explain the highly statistically significant changes observed in the placebo group between baseline and Week 12.

Lipid changes in patients randomized to placebo: The applicant provided a table of studies (see Appendix) listing the lipid changes observed in placebo-treated patients from baseline in studies of patients with high or very high TG levels to compare with ANCHOR. In reviewing the trajectory of lipids in a placebo group, it is important to consider if the placebo group was on background statin therapy and if all lipid-lowering drugs were stopped during a washout period prior to randomization, as this may affect the degree and direction of lipid alterations. For example, if a placebo group was not on any lipid-lowering medications, it may be reasonable to expect a worsening of lipid parameters over time. However, if a placebo group was on statin therapy that required at least 4 weeks of consistency, it might to reasonable to expect lipid parameters to remain stable over time with minor fluctuations. Acknowledging the limitations of cross-comparisons, two studies (COMBOS and FIRST) had patient populations and study designs with lead-in periods of diet and background statin stabilization similar to ANCHOR. The placebo groups in COMBOS and FIRST had small reductions from baseline in TG at the 8 week and 13 week time points (-6.3% and -2.0%, respectively). The placebo-treated patients in COMBOS also had reductions in other measured lipid parameters. These results suggest the changes in ANCHOR are atypical, but the etiology of this remains unclear.

…The changes in lipid and lipoprotein parameters from baseline to Week 12 in the mineral oil placebo group are rather atypical for a trial that included a stabilization period for diet and lipid-lowering therapy, raising the possibility that mineral oil may not be as inert as assumed. If true, the treatment effects observed with AMR101 may be overestimated."

Ultimately, the FDA rescinded their SPA agreement with Amarin regarding the indication sought from the ANCHOR trial (TG 200 mg/dL – 499 mg/dL on background statin therapy at elevated risk of ASCVD). The FDA declared that *"a substantial scientific issue essential to determining the effectiveness of Vascepa in the studied population was identified after testing began,"* due to the evidence from multiple failed trials testing triglyceride-lowering therapies that TG-lowering therapies are not proven to reduce ASCVD risk. However, because of the open-ended manner in which the FDA's discussion was left off, the possibility that an adverse impact of mineral oil placebo had overstated the efficacy of Vascepa remains.

According to Amarin, after becoming aware of this possibility following their critical review of the ANCHOR dataset, the FDA *"directed the independent data monitoring committee, or DMC, for REDUCE-IT to periodically review unblinded lipid data to monitor for signals that the placebo might not be inert,"* highlighting that the issue was far from resolved, and that the SPA for REDUCE-IT (granted before MARINE or ANCHOR trial data were examined) could also be in doubt should the mineral oil concern present itself in the REDUCE-IT study as well (and it apparently has). Yet, by the time FDA had reviewed ANCHOR data and expressed its concerns (late 2013/early 2014), the REDUCE-IT trial already had around 6,000 subjects randomized[95] and had been ongoing for nearly 2-years.[96]

[95] https://www.globenewswire.com/news-release/2013/09/25/575674/10049767/en/Amarin-Announces-Enrollment-of-the-REDUCE-IT-Cardiovascular-Outcomes-Study-Surpasses-6-000-Patients.html
[96] https://investor.amarincorp.com/news-releases/news-release-details/amarin-announces-dosing-first-patient-cardiovascular-outcomes

But why didn't the FDA of its own volition decide to put a clinical hold on REDUCE-IT there and then, requiring clinical drug interaction studies testing mineral oil with statins before releasing the hold? Perhaps they deemed the evidence insufficient for such a move, reasoning the increases in LDL-C etc. as insufficient to seriously effect ASCVD event rates. It was only around that time that the ACC/AHA changed their stance on LDL-C to percentage change/statin-intensity being relevant to reducing risk of ASCVD instead of the previously held notion that every 1 mmol/L (38.7 mg/dL) reduction in LDL-C correlated with a 22% reduction in risk of major vascular events.[97] Using the old guidelines would equate the ~7.0 mg/dL increase in LDL-C in the REDUCE-IT placebo group with a modest 4% relative risk of a major vascular event. And it may simply not have occurred to FDA staff that if mineral oil inhibits statin absorption, it might be inhibiting other cardiac medications also, causing a disastrously harmful cumulative effect that is not immediately apparent by examining biomarker levels alone.

Instead, the FDA, according to the following testimony,[98] charged the sponsor to then charge the DMC to monitor levels closely:

> Mary Roberts testified (at 138-41) that the FDA had discussed its concern that mineral oil was not inert with Amarin: *"with the exception of HDL, the changes observed [in lipid and lipoprotein endpoints] within the placebo group went in an adverse direction from baseline to week 12…. In the case of ANCHOR … the changes observed within the placebo group stood out as atypical for similarly designed lipid-lowering trials that we have reviewed, which gave us pause, especially since the changes went in an unfavorable direction…. For REDUCE-IT, our primary concern was whether or not there was a possibility that the mineral oil could attenuate the effect of a statin, perhaps by inhibiting absorption. Because we do not have any hard evidence for an interaction, such as a formal drug-drug interaction study, we discussed our concerns with the sponsor and asked that they task the REDUCE-IT data monitoring committee with evaluating the accruing lipid data with this concern in mind."*

And, as the sponsor has reiterated, the DMC at each review, apparently "with this concern in mind," recommended the study continue without alteration. But just who is this "DMC?"

The Data Monitoring Committee (DMC), also commonly referred to as the "Data and Safety Monitoring Board" (DSMB), for the REDUCE-IT trial consisted of three individuals with expertise deemed adequate to fulfill this essential role in monitoring data from the trial by the sponsor, Amarin Corp. It is wholly within the judgement of the sponsor to choose their trial's DMC members, though they should be independent of the sponsor, with no clear financial ties or interests in the study outcome, and have expertise in the subject matter involving the question(s) the study is designed to answer.

The NIH has this to say about DSMB membership:[99]

> "The membership of the DSMB should reflect the disciplines and medical and dental specialties necessary to interpret the data from the clinical trial and to fully evaluate participant safety. The number of DSMB members depends on the phase of the trial, range of medical issues,

---

[97] Ziaeian B, Dinkler J, Guo Y, Watson K. The 2013 ACC/AHA Cholesterol Treatment Guidelines: Applicability to Patients with Diabetes. *Curr Diab Rep*. 2016. [link]

[98] http://securities.stanford.edu/filings-documents/1051/AMRN00_01/2016814_r01c_13CV06663.pdf

[99] https://www.nidcr.nih.gov/research/human-subjects-research/interventional-studies/data-and-safety-monitoring-board-guidelines

complexity in design and analysis, and potential level of risk but generally consists of three to seven members including, at a minimum:

- Expert(s) in the clinical aspects of the disease/patient population being studied;
- One or more biostatisticians; and,
- Investigators with expertise in current clinical trials conduct and methodology."

The DMC that examined safety and efficacy data from REDUCE-IT was on the smaller side, having three voting members and one independent statistician. Two of these members had a medical background. The members and their specialties/training are as follows:[100]

1. Brian Olshansky, MD (DMC Chair)[101]

"Dr. Brian Olshansky, a renowned **electrophysiologist** and professor emeritus at the University of Iowa Hospitals, has directed the Clinical Cardiac Electrophysiology and the Fellowship Training Programs at University of Iowa, Iowa City and Loyola University, Maywood, Illinois…

Dr. Olshansky is recognized globally for **expertise in** evaluation and management of **syncope, the assessment of arrhythmia mechanisms, autonomic control of arrhythmias and cardiovascular function, multi-center randomized clinical arrhythmia trials, management of atrial and ventricular arrhythmias using device, ablation and pharmacological strategies and approaches towards anticoagulation**. He has served key roles in managing the athlete with suspected arrhythmia-related problems. Dr. Olshansky was the principal investigator of one of the largest randomized controlled clinical trials of **implantable defibrillators**…"

2. Mina Chung, MD[102]

"Mina K. Chung, MD, is a Staff Cardiologist **in the Section of Pacing and Electrophysiology**, The Robert and Suzanne Tomsich Department of Cardiovascular Medicine, at Cleveland Clinic. Dr. Chung is board-certified in **internal medicine** and in the subspecialties of cardiovascular disease and **clinical cardiac electrophysiology, which is her specialty interest**.

Dr. Chung did her undergraduate work at the University of California at San Diego, graduating with a major in chemistry, and she was inducted into Phi Beta Kappa, the national honor society. She received her medical degree from Washington University School of Medicine in St. Louis, where she completed her internship and residency in internal medicine at the Jewish Hospital of the Washington University School of Medicine, becoming Chief Resident. She received fellowships in research and in cardiology from the Jewish Hospital, followed by **a fellowship in cardiac electrophysiology** from Barnes Hospital,

[100] http://www.natap.org/2018/HIV/nejmoa1812792_appendix.pdf
[101] http://www.innovationsincrm.com/cardiac-rhythm-management/articles-2015/january/673-interview-with-brian-olshansky-md
[102] https://my.clevelandclinic.org/staff/1211-mina-chung

Washington University School of Medicine, during which she received the **Michael Bilitch Fellowship Award in Cardiac Pacing and Electrophysiology from the North American Society of Pacing and Electrophysiology**.

Dr. Chung has been principal investigator or co-investigator in a number of important clinical trials **studying atrial fibrillation, supraventricular and ventricular arrhythmias, cardioversion, pacemaker and defibrillator therapy, biventricular pacing for heart failure and catheter ablation approaches**. Along with colleagues at Cleveland Clinic, she reported the first association of inflammation with persistence of atrial fibrillation. She has also been a Principal Investigator for AFFIRM (Atrial Fibrillation Follow-up Investigation of Rhythm Management), a multicenter randomized trial of rate-control versus rhythm-control strategies for atrial fibrillation. In this study, she has led analyses that demonstrate functional status benefits that help to justify and rationalize maintenance of sinus rhythm in patients with symptomatic atrial fibrillation. She is also investigating the genetics of arrhythmias, including atrial fibrillation…"

3. Alfred Hallstrom, PhD[103]

"Research Interests:

-Clinical trial methodologies, especially in cardiovascular (chronic) applications and emergency services applications.

Education:

-PhD Mathematics, Brown University, 1968

-MSc Mathematics, Brown University, 1962

-BS Physics, University of Washington, 1959"

4. Lesly A. Pearce, MS[104]

-non-voting independent statistician (normally not considered a member of the DMC).

We are surprised at the choice of medical doctors to serve on the DMC for the REDUCE-IT trial being those that have extensive expertise in a field not directly related to the indications actually being treated in the REDUCE-IT trial. Although certainly a branch of cardiology, electrophysiology deals with electric activity of the heart and its relation to arrythmias,[105] often treated with devices such as pacemakers or surgical ablation techniques, something not of import to the central question of REDUCE-IT, which involved the impact of an experimental drug on atherogenic/inflammatory markers and (although not directly measured) on atherosclerotic plaque, and thereby on ASCVD risk. Both doctors

[103] https://sph.washington.edu/faculty/facbio/Hallstrom_Alfred
[104] https://www.researchgate.net/scientific-contributions/39875808_Lesly_A_Pearce
[105] https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=134&contentid=240

were trained in internal medicine, which has at least some relevance to REDUCE-IT, but did their fellowships in and have spent the entirety of their careers focused on electrophysiology and arrythmias. These two have even co-authored a book together encompassing the topic, entitled Arrythmia Essentials.[106] Looking across the studies they were investigators in reveals that they all involved testing various modalities in treating arrythmias.[107, 108]

The question comes to mind, just how many times have either of the only two MDs that served on the REDUCE-IT DMC prescribed a statin or other lipid-altering drug, and/or monitored the effects of such drugs on their patient's levels? Whatever the number is, it is probably low. Would not an endocrinologist or cardiologist with extensive experience studying and treating ASCVD with statins and other cardiac medications been a better fit for the study? And in particular at relaying to the FDA their interpretation of changes in atherogenic/inflammatory markers observed in MO-treated subjects in the placebo group?

At first blush it may not appear to be a very serious issue to notice a change from low 80s mg/dL in LDL-C to low 90s mg/dL—until it is realized that such an apparently modest increase could infer as much as a 4-fold reduction in the absorption of statins, and that an inhibition of one medication would logically signify the malabsorption of others, less visible to data on lipids (antithrombotics, antihypertensives, etc.). The cumulative impact is highly deleterious. An experienced endocrinologist or cardiologist could have picked up on that; an excellent electrophysiologist (or two) would be, in our view, less likely to.

Finally, we find the sponsor's ambiguous statement that *"the FDA did not seek to require that **we include any qualification** [?] related to the use of mineral oil as a placebo in REDUCE-IT at the time of our March 2016 amendment to the REDUCE-IT SPA,"* to be unconvincing in the poorly hidden suggestion that the FDA thus has no qualms with mineral oil placebo (i.e. believes it to be truly inert) and views the changes in levels from baseline seen in the placebo group in ANCHOR and confirmed in REDUCE-IT as unproblematic. Common for the FDA in such a scenario would be to assert that it is "a review issue," as was revealed to have consistently been the case in its previous communications with Amarin regarding the ANCHOR SPA in a class-action lawsuit.[109] Further, the FDA has routinely issued CRLs to sponsors of clinical trials with confounded data or safety issues that had received continuation recommendations from its DMC at every review, and had gotten a "pass," if you will, by FDA regarding protocol/SPA amendments.

The SPA for REDUCE-IT was granted before the FDA had examined data from MARINE, and especially ANCHOR, that thereafter raised suspicion. We do not know of any examples in which the FDA retracted a SPA during a study. It is always a "review issue," contingent upon its thorough review of all data from the trial used as the basis for a submission for a 505(b)(1) or 505(b)(2) application (as was the case with ANCHOR). Although it is rare for the FDA to rescind a SPA agreement after reviewing data from the applicable trial, the evidence that mineral oil placebo hindered cardiac medications in two back-to-back studies (ANCHOR and then REDUCE-IT) is compelling, and at the very least warrants DDI studies to be performed to answer the question resolutely before approving the efficacy supplement for Vascepa. Thus, we think there is a real possibility the FDA will rescind the SPA agreement for REDUCE-IT after

[106] https://www.amazon.com/Arrhythmia-Essentials-Brian-Olshansky-MD/dp/0323399681
[107] https://www.doximity.com/pub/brian-olshansky-md
[108] https://www.researchgate.net/scientific-contributions/38938812_Mina_K_Chung
[109] http://securities.stanford.edu/filings-documents/1051/AMRN00_01/2016814_r01c_13CV06663.pdf

thorough review of the data, just as the regulatory body had after its review of ANCHOR. Indeed, we are of the opinion they have a responsibility to.

1.11 Evidence Vascepa Can Cause Organ Damage

Vascepa and Lovaza (as well as the less well-known "Omtryg," which is very similar to Lovaza[110]) are ethyl ester forms of omega 3 fatty acids available by prescription only. Vascepa contains a very high concentration (at least 96%) of EPA-E, i.e. the fatty acid "EPA" in ethyl ester form, with the remaining composition being an unidentified mix of DHA-E, DPA-E, ALA-E, etc.[111] Fatty acid ethyl esters (FAEE) have been implicated in organ damage, primarily as the end result of chronic alcoholism. This was elaborated on in detail in "*Fatty Acid Ethyl Esters: Ethanol Metabolites Which Mediate Ethanol-Induced Organ Damage and Serve as Markers of Ethanol Intake*," Laposata, 1998:[112]

> "Fatty acid ethyl esters (FAEEs), esterified products of fatty acids and ethanol, were discovered and rediscovered in different cells and tissues through the 1960's and 1970's. In the early 1980's, interest in FAEE metabolism and their pathologic effects increased significantly and information began to emerge on FAEE synthesis and the role of FAEE in mediating ethanol induced organ damage. The last three years have witnessed the emergence of in vitro and in vivo evidence that FAEE contribute to ethanol-induced organ damage, with a variety of different mechanisms proposed for mediation of this toxic effect.
>
> Multiple enzymatic activities associated with FAEE formation have now been described. Independent of their role in mediating cell injury, it has very recently been shown that FAEE are useful short-term and long-term serum markers of ethanol intake, given their appearance in the blood rapidly after ethanol ingestion and their presence when ethanol is no longer detectable. Despite the fact that alcoholism is a major cause of disease in society, little is known about the mechanism by which ethanol abuse induces organ damage. One increasingly compelling hypothesis is that the FAEE are at least partly responsible for the observed pattern of organ damage in alcoholics.
>
> III. THE TOXIC EFFECTS OF FATTY ACID ETHYL ESTERS
>
> In 1986, a hypothetical connection was established between FAEE and ethanol abuse. Using tissues obtained postmortem from humans acutely intoxicated at the time of death, the organs commonly damaged by ethanol abuse—pancreas, liver, heart, and brain—were found to have high levels of enzyme activity for the synthesis of FAEE and the highest concentrations of FAEE among many different organs and tissues tested. Adipose tissue was also found to have an accumulation of FAEE and measurable levels of FAEE synthetic activity. The organs not typically damaged by ethanol abuse showed little or no FAEE and correspondingly little ethyl ester synthase activity. FAEE were implicated as mediators of ethanol toxicity because FAEE and the

[110] https://www.pbm.va.gov/PBM/clinicalguidance/drugmonographs/Omega_3_Acid_Ethyl_Esters_A_OMTRYG_Monograph.pdf
[111] https://www.hindawi.com/journals/bmri/2013/284329/
[112] https://www.sciencedirect.com/science/article/pii/S0163782798000137?via%3Dihub

enzyme(s) responsible for their synthesis were distributed primarily in organs damaged by ethanol abuse.

In 1988, Hungund et al. found ethyl esters, predominantly ethyl oleate and ethyl linoleate, in the livers of mice treated with ethanol by inhalation. They were also able to detect ethyl esters in the brains of these animals, further suggesting a role for FAEE in impairment of CNS function. When the FAEE reached steady state levels in neural tissues after 3±4 days of alcohol treatment, the authors found that the FAEE were incorporated into synaptosomal plasma membranes. In vitro studies revealed that the ethyl esters disordered the membrane bilayer.

In 1993, Haber et al. demonstrated that rat pancreatic lysosomes incubated for 20 min with ethyl oleate become unstable and leak their enzymes into the surrounding medium. The hypothesis was raised that increased pancreatic lysosomal fragility mediated by FAEE is associated with ethanol abuse.

A 1994 study by Ponappa and colleagues involving toxic effects of FAEE in the pancreas showed that isolated rat pancreatic acinar cells incubated with ethanol generated FAEE endogenously and concomitantly experienced a 20 – 30% decrease in protein synthesis.

Szczepiorkowski et al. provided the first demonstration that FAEE in a physiologic particle, low density lipoprotein, exert toxic effects on an intact cell… LDL reconstituted with ethyl oleate (400 mM) decreased protein synthesis in intact HepG2 cells by 40%. Electron microscopy revealed significant changes in cell morphology, with accumulation of intracellular lipids, a distortion of the intracellular lipids and a distortion of the nuclear membrane. FAEE delivered in reconstituted LDL were rapidly hydrolyzed, and the fatty acids reesterified into phospholipids, triacylglycerols and cholesteryl esters, with preference for triacylglycerols. These findings provided evidence that FAEE are toxic for intact human hepatoblastoma cells and that they or their metabolites may be a causative agent in ethanol-induced liver damage.

A report by Gubitosi-Klug and Gross examined the effect of FAEE on a human brain potassium channel in SF9 cells expressing the recombinant channel. They found that physiologically relevant FAEE concentrations accelerated the kinetics of activation of the channel, and raised the possibility that this in vitro observation may be one of the pathologic consequences of ethanol abuse in the central nervous system.

The in vitro toxicity studies still left unanswered the question of whether FAEE are toxic in vivo. This led us to perform experiments to assess the toxicity of FAEE in reconstituted LDL in vivo in rats. In these studies, rats received FAEE in reconstituted LDL at FAEE concentrations which are physiologically attainable (10 – 30 μM) after ethanol ingestion. The FAEE were delivered as a bolus and then by continuous infusion for 1 hr through a cannula placed in the carotid artery and advanced through the aorta to the superior mesenteric artery. This placement was made to maximize the likelihood for observing FAEE induced cytotoxicity in the pancreas. The rats were sacrificed 3 – 24 hr after infusion of FAEE and biochemical makers of organ damage were measured. Histology was performed for multiple organs, and the wet/dry ratio of the pancreas determined to assess pancreatic edema. Control rats received LDL which was reconstituted with cholesterol ester. The wet/dry ratio for the rats receiving the LDL containing FAEE was significantly higher than that of the rats receiving LDL reconstituted with cholesterol ester. In

addition, by 3 hr, the level of typsinogen activating peptide, a biochemical marker for pancreatic cell damage, was 3-fold higher in the animals receiving the LDL reconstituted with FAEE relative to controls. Bora et al. also performed an in vivo experiment in which FAEE were injected directly into the left ventricle of rats undergoing thoracotomy and observed significant myocardial cell damage, while control animals not receiving FAEE did not. Taken together, many studies now show that there is substantial evidence, in vitro and in vivo, that FAEE have significant capacity to induce cell injury."

Beckemeier et al. (1998) made the following observation:[113]

> "The chronic consumption of alcohol has proven detrimental to heart tissue and can lead to alcohol-induced heart muscle disease, a condition which may result in arrhythmias, cardiomegaly, and congestive heart failure. A search for the molecular mechanism underlying observed alcohol-induced end-organ damage, such as that seen in heart, has led to the discovery of a nonoxidative pathway for the metabolism of alcohol in several human tissues including heart, brain, pancreas, and liver.
>
> It has been revealed that nonesterified fatty acids are esterified with ethanol to produce fatty acid ethyl esters (FAEE), neutral molecules which can accumulate in mitochondria and impair cell function. The observation that FAEEs are synthesized at high rates in the heart, and other organs that lack oxidative ethanol metabolism, provides a plausible link between the observed tissue damage, the ingestion of alcohol, and the subsequent development of alcohol-induced heart muscle disease.
>
> The synthesis of FAEEs are catalyzed by FAEE synthase enzyme, four of which have been characterized and purified to homogeneity from the human myocardium. Further analysis of these FAEE synthase enzymes opens up a new possibility to characterize and map a gene for alcohol-induced end-organ damage, such as that observed in heart and other organs. FAEEs have been found to be important metabolites of alcohol and are most commonly accumulated in those organs which are damaged by alcohol abuse, i.e. heart, liver."

Diczfalusy et al. (2001) elaborated on the lack of correlation with acetaldehyde and acetate and organ injury from chronic alcoholism, and focused the blame instead on FAEE.[114]

> "Ingested alcohol is mainly metabolized in the liver by oxidation to acetaldehyde and acetate. Three enzymes are involved in the oxidation of ethanol to acetaldehyde: alcohol dehydrogenase, catalase, and cytochrome P-450 IIE1, the last two enzymes being of minor importance. Further oxidation to acetate is mediated by acetaldehyde dehydrogenase(s). Many of the pathological consequences associated with ethanol abuse have been attributed to high concentrations of acetaldehyde and acetate. However, experiments in male Wistar rats fed ethanol and treated with specific acetaldehyde dehydrogenase inhibitors, which led to sustained elevated levels of acetaldehyde, resulted in prevention of hepatic inflammation and necrosis (2), suggesting that acetaldehyde may not be involved in ethanol-induced organ injury. In addition, several organs with a limited capacity to oxidize ethanol such as heart, pancreas, and brain are

[113] https://www.jmmc-online.com/article/S0022-2828(98)90812-4/pdf
[114] http://www.jlr.org/content/42/7/1025.full

also injured in alcoholics, suggesting that factors other than oxidation may be responsible for the ethanol-induced damages in these organs.

More recently, several studies have demonstrated that fatty acid ethyl esters (FAEE), nonoxidative ethanol metabolites, may be involved in organ injury. FAEE have been shown to uncouple oxidative phosphorylation in rabbit heart mitochondria (3), to inhibit protein synthesis and cell proliferation in human hepatoblastoma (HepG2) cells (4), and to increase fragility of pancreatic lysosomes (5). FAEE have been detected in several human tissues at autopsy after acute alcohol intoxication and in chronic alcoholics (6). After acute alcohol intoxication, the highest concentrations were found in adipose tissue, liver, pancreas, and heart…"

It appears the reason why ethyl ester omega-3s have been largely exonerated in this regard by healthcare professionals and the FDA (although FDA does require a warning on ALT increases in drug labels) is three-fold:

1) Numerous beneficial effects of ingesting OM3-EE have been observed, potentially obscuring the full scope of effects. These include a beneficial impact on serum triglyceride, non-HDL-C, and VLDL-C levels, and in some older studies a reduction in the incidence of major adverse coronary events. These benefits were also observed along with a lack of serious adverse events (SAE).

2) The vast majority of studies conducted testing OM3-EE were either short-term (4-12 weeks) or medium term (1-5 yrs) in length, and given that the end-result of organ damage from FAEE deposition/synthesis as a result of chronic alcoholism would take a longer period of time (15 -20 yrs on average[115]), so too would it likely take a longer time frame to observe organ damage from chronic OM3-EE use.

3) Subjects were strictly advised to take their OM3-EE with "a high-fat meal" in the majority of these studies; meanwhile, the labels for Vascepa and Lovaza only stipulate that they should be taken "with a meal." Thus, the studies were biased in this regard, and subjects therein were more likely to have had their ingested OM3's re-esterified to glycerol molecules before being subsequently absorbed as part of the high-fat meal, with a lesser percentage of ethanol still cleaved to a fatty acid. In a real-world scenario, many patients would consume a low or lower fat meal concurrent with OM3-EE dosing (or at times no meal at all), and the OM3-EE (aka "FAEE") they consumed would remain in large part unabsorbed, abiding in its FAEE form and deposited/synthesized in various organs, wherein such processes could exert a deleterious effect on organ functioning over time.

However, and especially due to its still low bioavailability, even when taken with a high-fat meal, elevations in ALT have been observed with OM3-EE dosing, and in particular the AST/ALT ratio was impacted:[116]

"In patients with hepatic impairment, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels should be monitored periodically during therapy with LOVAZA. In some patients, increases in ALT levels without a concurrent increase in AST levels were observed."

[115] https://www.rehabspot.com/alcohol/effects-of-alcohol-abuse/
[116] https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021654s039lbl.pdf

A similar warning can be found for Vascepa, although "livertox.nih.gov" states that all such observations of elevation in ALT are low-risk phenomena (which we think is misguided, and will explain why).

> "In a pooled analysis of studies, **mild** elevations in ALT (up to **2 times the upper limit of normal**) occurred in 12.8% of icosapent-ethyl [Vascepa] treated subjects compared to 10.3% of placebo treated subjects."

We think a 20% increase in relative risk of ALT elevation of "up to 2 times the upper limit of normal" is more than a "mild" elevation, especially when considering the likely harm that this *chronic* phenomenon would represent.[117] In another section of the Report (sect. 1.5) we go into detail on the destructive impact a relatively "mild" but chronically increased CRP level represents—both directly and as a marker of disease. A little bit of harm (i.e. smoking one cigarette) in an isolated sense may be relatively benign; compounded over many years, however, it can markedly worsen morbidity and mortality.

Even when examining the hepatic effect of acute intoxication, extreme elevations in ALT and/or AST are often not observed. But, once again, the ratio can become altered, with ALT levels increasing beyond increases in AST.

Hall and Cash, 2012, state the following on the AST:ALT ratio:[118]

> "A normal AST:ALT ratio should be <1. In patients with alcoholic liver disease, the AST:ALT ratio is >1 in 92% of patients, and >2 in 70%."

This phenomenon was observed in Binder et al. (2016):[119]

> "During the 8-year study period, 249 children and adolescents with the diagnosis "acute alcohol intoxication" were admitted, 132 (53%) girls and 117 (47%) boys. The mean age was 15.3 ± 1.2 years and the mean blood alcohol concentration was 0.201 ± 0.049%. Girls consumed significantly less alcohol than boys (64 g vs. 90 g), but reached the same blood alcohol concentration (girls: 0.199 ± 0.049%; boys: 0.204 ± 0.049%). The mean values of liver parameters were in normal ranges, but AST was increased in 9.1%, ALT in 3.9%, and γGT in 1.4%. In contrast, the mean value of AST/ALT ratio was increased and the ratio was elevated in 92.6% of all patients. Data of the present study showed significant differences in the AST/ALT ratio ($p < 0.01$) in comparison to a control group. Data of the present study indicate that there might be an effect of acute alcohol intoxication on transaminase levels. The AST/ALT ratio seems to reflect the damage in hepatocytes after intensive alcohol consumption."

A similar observation was also noted in Yue et al. (2006):[120]

> "After the intake of 80 g ethanol, various symptoms occurred in volunteers while the concentration of blood alcohol peaked at 1 hour and normalized within 24 hours. The ratio of alanine aminotransferase (ALT) to aspartate aminotransferase (AST) increased significantly when the venous alcoholic concentration increased from 0 g/L to 1.2 g/L and the levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (gamma-GT) were elevated

[117] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609680/
[118] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609680/
[119] https://www.ncbi.nlm.nih.gov/pubmed/26992701
[120] https://www.ncbi.nlm.nih.gov/pubmed/16481283

when the alcoholic concentration reached 0.4 g/L. No significant changes were noticed in ALT, AST or cholinesterase (CHE). Acute alcohol intoxication may cause changes in hepatic enzymes and prove the existence of reversible hepatic injury."

Here too, a dramatic impact on ALT or AST was not observed with acute intoxication, but the impact was reflected in the increase of ALT to a greater extent than (or absent of) an increase in AST. As stated, this is a common observation in alcohol-induced liver injury:[121]

> "AST and ALT enzymes have low sensitivity and specificity for screening excessive alcohol consumption, but they are highly sensitive and specific for detecting alcohol-induced liver damage.[7] The AST/ALT ratio increases with alcohol consumption; an AST/ALT ratio >1 is considered suggestive of alcohol as the cause of liver dysfunction.[8] "

The same has also been observed in numerous OM3-EE trials, leading to the following warning appearing in all such drug labels:[122]

> "In some patients, increases in alanine aminotransferase (ALT) levels without a concurrent increase in aspartate aminotransferase (AST) levels were observed. Alanine aminotransferase levels should be monitored periodically during therapy with LOVAZA."

In Tobin et al. (2018), and despite significant changes to diet in both groups, ALT decreased significantly only in the placebo group, and increasing insignificantly in OM3-EE group (but compared to placebo the increase was significant):[123]

> "2. Materials and Methods
>
> 2.1. Study Design This was a randomized double-blind placebo-controlled study conducted at 21 investigative sites across the U.S. All procedures involving human participants were approved by Quorum Review IRB.
>
> One hundred and seventy-six subjects were subsequently randomized to receive either MF4637 (n = 87) or placebo (n = 89) (Figure 1). The modified intention to treat population was defined as all subjects who took at least a 1-day dose of omega-3 fatty acids or placebo and underwent at least 1 post-randomization primary efficacy assessment.
>
> The omega-3 fatty acid medical food (MF4637; BASF AS, Lysaker, Norway) was provided as soft gel capsules, with each 1 g capsule containing marine-sourced EPA and DHA as ethyl esters (460 mg and 380 mg, respectively). Placebo capsules were identical in size and appearance to MF4637 and contained 1 g of olive oil. The investigational products were administered in a double-blinded fashion. Study participants were required to take three capsules per day of either MF4637 or placebo with food for 24 weeks.
>
> In addition to the investigational product, study participants were advised to reduce normal caloric intake as recommended by the American Association for the Study of Liver Disease (AASLD) standard-of-care guidelines for NAFLD [1], and to maintain stable physical activity levels

[121] https://arupconsult.com/content/alcohol-abuse
[122] https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021654s023lbl.pdf
[123] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6115838/

throughout the study. To provide the American Heart Association (AHA) recommended dietary intake of omega-3 fatty acids [29], participants were required to consume two meals of omega-3 rich fish per week (from a choice of salmon, herring, whitefish, sardines, bluefish and trout) and to reduce foods rich in trans-and omega-6 fatty acids (fried foods and snacks, fast foods, bacon, turkey bacon, hams, nuts, peanut butter, sesame seeds, sunflower seeds, pumpkin seeds, vegetable oils and margarine (including soybean oil and corn oil), mayonnaise and salad dressing). Dietary intake was monitored regularly throughout the study via participant's food diaries.

Table 2 details the main anthropometric and biochemical variables for participants randomized to the placebo and MF4637 groups at baseline and after 24 weeks of intervention. The overall weight of subjects in either intervention group remained unchanged from study start to study end. Triglyceride levels at baseline were similar in both groups and would be clinically regarded as borderline raised. At the end of the study, a statistically significant reduction in triglycerides was only seen in the MF4637 group. Interestingly, the liver enzymes ALT, AST and GGT showed statistical reductions at the end of study in the placebo group and not in the MF4637 group."

Table 2. Anthropometric and biochemical variables of participants randomized to placebo and MF4637 groups at baseline and study completion.

Variables	Placebo [1]			MF4637 [2]			Two-Sample Test for Change from Baseline *p*-Value [#]
	Baseline	End of Study	*p*-Value *	Baseline	End of Study	*p*-Value *	
Age, years	55.1 (10.9)			55.3 (13.3)			
Sex, M/F	44/42			36/45			
Weight, kg	90.1 (18.8)	89.5 (18.5)	0.84	88.4 (18.4)	89.0 (18.8)	0.082	0.23
Waist circumference, cm	105.9 (13.1)	104.7 (13.9)	0.005	106.3 (13.2)	105.5 (12.2)	0.72	0.18
Hip circumference, cm	110.5 (12.1)	110.0 (11.7)	0.31	110.9 (11.8)	110.4 (11.9)	0.81	0.42
Waist to hip ratio	0.96 (0.1)	0.95 (0.1)	0.081	0.96 (0.08)	0.96 (0.1)	0.66	0.58
BMI, kg/m^2	32.4 (5.0)	32.3 (4.8)	0.74	32.1 (4.8)	32.3 (5.0)	0.078	0.18
SBP, mm Hg	127.0 (10.7)	128.8 (15.0)	0.23	128.0 (11.9)	126.6 (10.6)	0.29	0.11
DBP, mm Hg	80.3 (7.2)	79.9 (9.9)	0.25	79.8 (7.6)	77.8 (8.4)	0.032	0.53
Heart rate, beats/min	74.7 (8.7)	74.1 (8.2)	0.59	73.2 (9.1)	74.2 (9.8)	0.17	0.17
Statin use, %	34.9			30.9			
Diabetes, %	39.5			35.0			
Fasting glucose, mg/dL	120.1 (48.5) [3]	125.4 (56.8) [3]	0.39	119.4 (38.1) [3]	127.5 (55.2) [3]	0.0616	0.38
Fasting insulin, µIU/mL	30.2 (41.3) [4]	30.1 (35.8) [4]	0.51	20.8 (18.2) [5]	24.0 (24.3) [5]	0.21	0.63
HbA1c, %	6.5 (1.5) [6]	6.6 (1.7) [6]	0.67	6.3 (1.4) [7]	6.3 (1.4) [7]	0.83	0.87
Triglycerides, mg/dL	199.1 (123.0) [2]	185.7 (118.0) [2]	0.52	192.0 (125.1) [5]	157.8 (84.2) [5]	0.0008	0.053
BUN, mg/dL	14.7 (4.9) [2]	15.0 (4.5) [2]	0.88	15.4 (5.0) [5]	16.2 (4.9) [5]	0.25	0.46
Creatinine, mg/dL	0.8 (0.2) [2]	0.82 (0.2) [2]	0.51	0.3 (0.2) [5]	0.84 (0.2) [5]	0.26	0.19
TSH µIU/mL	1.9 (1.0) [2]	2.5 (2.7) [2]	0.025	1.7 (0.9) [7]	1.8 (0.9) [7]	0.043	0.86
Hs-CRP, mg/L	6.4 (9.2) [2]	5.5 (5.8) [2]	0.46	8.1 (17.5) [8]	6.7 (10.9) [8]	0.75	0.82
Albumin, g/dL	4.29 (0.3) [2]	4.3 (0.3) [2]	0.69	4.29 (0.3) [5]	4.3 (0.3) [5]	0.62	0.91
ALT, IU/L	35.6 (24.0)	29.8 (21.2)	0.005	37.5 (39.0)	38.1 (37.7)	0.48	0.015
AST, IU/L	25.8 (12.2)	23.9 (13.5)	0.036	27.1 (20.3)	28.7 (24.6)	0.37	0.036
ALP, IU/L	81.5 (31.0)	76.3 (21.5)	0.01	85.5 (45.3)	83.2 (40.3)	0.09	0.73
GGT, IU/L	47.1 (49.0)	37.1 (32.2)	<.0001	62.2 (151.4)	57.1 (108.7)	0.37	0.058
Bilirubin, mg/dL	0.5 (0.2)	0.47 (0.2)	0.70	0.5 (0.3)	0.5 (0.2)	0.88	0.72

[1] Data for n = 86 participants unless otherwise specified. [2] Data for n = 81 participants unless otherwise specified. [3] Data for n = 72 participants. [4] Data for n = 82 participants. [5] Data for n = 78 participants. [6] Data for n = 74 participants. [7] Data for n = 76 participants. [8] Data for n = 79 participants. Values are expressed as Mean (SD). Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; GGT, gamma-glutamyl transferase; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure.* Within-group differences were assessed using paired *t*-tests or Wilcoxon signed rank tests. [#] Intergroup differences were assessed using two-sample *t*-tests or two-sample Wilcoxon tests.

Table 3. RBC fatty acid content at baseline and after 12- and 24-week intervention with placebo or MF4637.

	Placebo (n = 86)				MF4637 (n = 81)				p-Value [1]
	Baseline	T = 12 weeks	T = 24 weeks	Change from Baseline	Baseline	T = 12 weeks	T = 24 weeks	Change from Baseline	
RBC omega-3 index, %	4.9 (1.2)	5.8 (1.3)	5.3 (1.1)	0.4 (1.0)	4.8 (1.1)	8.7 (2.3)	8.0 (2.6)	3.2 (2.7)	<0.0001
RBC EPA + DHA, µg/mL	32.3 (26.4)	34.9 (21.0)	33.1 (20.5)	1.2 (14.9)	29.6 (17.5)	51.5 (38.9)	52.9 (40.7)	21.2 (28.7)	<0.0001
RBC EPA, %	0.54 (0.3)	0.54 (0.2)	0.54 (0.2)	0.002 (0.3)	0.53 (0.2)	1.6 (0.9)	1.4 (0.9)	0.9 (1.0)	<0.0001
RBC EPA, µg/mL	3.8 (6.6)	3.7 (2.6)	4.1 (5.7)	0.4 (7.0)	3.0 (2.6)	10.4 (10.7)	10.6 (12.0)	7.1 (10.5)	<0.0001
RBC DHA, %	4.3 (1.1)	5.2 (1.2)	4.8 (1.0)	0.4 (0.9)	4.3 (1.0)	7.1 (1.5)	6.6 (1.8)	2.3 (1.9)	<0.0001
RBC DHA, µg/mL	28.5 (21.0)	31.2 (18.6)	29.0 (16.2)	0.7 (10.7)	26.6 (15.3)	41.0 (28.8)	42.4 (29.8)	14.1 (19.5)	<0.0001
RBC omega-6: omega-3	4.9 (1.1)	4.5 (0.9)	4.7 (0.8)	−0.2 (0.7)	4.9 (1.2)	3.0 (0.9)	3.3 (1.5)	−1.6 (1.8)	<0.0001

[1] p-value is for the mean percentage change from baseline to 24 weeks between placebo and MF4637 groups using ANCOVA. Values are expressed as Mean (SD). Abbreviations: RBC, red blood cell; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; T: Time.

Table 4. MRI-PDFF liver fat percentage at baseline and after 24-week intervention with placebo or MF4637.

	Placebo [1]				MF4637 [1]				p-Value
	T = 0 weeks	T = 24 weeks	Change from Baseline		T = 0 weeks	T = 24 weeks	Change from Baseline		
			Absolute	Relative			Absolute	Relative	
Liver fat, %	17.4 (10.4)	12.6 (8.0)	−4.4 (6.9)	−27.6	14.4 (10.1)	10.7 (7.6)	−2.8 (5.8)	−25.7	0.1838

[1] As assessed for modified ITT population (Placebo, n = 60; MF4637, n = 60). Values expressed as Mean (SD); T: Time.

Given the ample size of each group, significant dietary changes as instructed by investigators should have impacted both groups about equally, and thus an approximately equal reduction in ALT and AST between them. Instead, the OM3-EE group experienced a mild *increase* in ALT and AST despite making the same significant lifestyle changes.

Scorletti et al. (2014),[124] which randomized 103 subjects with diagnosed NAFLD in a 1:1 ratio to either 4 g/d Omacor (aka Lovaza) or placebo for 15-18 months, displayed a similar phenomenon, with placebo group showing a near significant reduction in ALT (p=0.06) and a significant reduction in AST (p=0.04), while Omacor group showed no change in either ALT (p=0.70) or AST (p=0.83).

Another study by Sanyal et al. (2014) showed a variety of unfavorable trends with increasing dose of EPA-E compared with placebo:[125]

[124] https://aasldpubs.onlinelibrary.wiley.com/doi/full/10.1002/hep.27289
[125] https://www.sciencedirect.com/science/article/pii/S0016508514006040

Table 2. Primary and Secondary Histological End Points Assessed in Efficacy Evaluable Dataset

Histologic end points	Placebo (n = 55)	EPA-E (1800 mg/d) (n = 55)	EPA-E (2700 mg/day) (n = 64)	*P* value
Primary, %				
Proportion of responders[a]	40.0	37.0	35.9	NS
Proportion meeting criteria[b] 1	36.4	33.3	31.3	NS
Proportion meeting criteria[b] 2	32.7	27.8	29.7	NS
Secondary, median (IQR)				
NAS	−1 (−2, 0)	−1 (−2, 0)	−1 (−2, 0)	NS
Steatosis	0 (−1, 0)	0 (−1, 0)	0 (−1, 0)	NS
Lobular inflammation	0 (−1, 0)	0 (−1, 0)	0 (−1, 0)	NS
Hepatocyte ballooning	0 (−1, 0)	−0.5 (−1, 0)	0 (−1, 0)	NS
Fibrosis	0 (0, 0)	0 (−1, 0)	0 (0, 1)	NS

IQR, interquartile range.
[a]Proportion of subjects who met primary efficacy end point at 1 year in (end of study) Primary end point was defined by meeting either or both of the following criteria: an NAS ≤3 with no worsening of fibrosis and a decrease in NAS by 2 or more with contribution from more than 1 parameter without worsening of fibrosis. Significance determined using a Cochrane-Armitage test.
[b]Secondary histologic end points were expressed as changes in histologic scores (scores at end of study minus scores at baseline), median (25th percentile, 75th percentile). Significance determined using Kruskal-Wallis tests. These analyses were part of the planned analysis of the study.

CLINICAL LIVER

Table 3. Secondary Study End Points: Changes in Laboratory Data Assessed in Efficacy Evaluable Dataset

Variable	Change from baseline to month 12			*P* value[a]
	Placebo (n = 55)	EPA-E, 1800 mg/d (n = 55)	EPA-E, 2700 mg/d (n = 64)	
Body weight, *kg*	−1.0 (−2.7, 1.8)	0.5 (−3.0, 3.2)	0.0 (−3.7, 2.5)	NS
BMI	−0.3 (−0.9, 0.6)	0.2 (−1.1, 1.0)	0.0 (−1.4, 0.9)	NS
AST, *IU/L*	−8 (−25, 0)	−6.5 (−21.5, 11.3)	−2 (−18.3, 14.8)	NS
ALT, *IU/L*	−20 (−42, 3)	−5.5 (−23.5, 13.3)	−5.5 (−24.8, 25.5)[b]	NS
Alkaline phosphatase, *IU/L*	1 (−11, 8)	−1 (−8.5, 8.3)	−1.5 (−9, 5.8)	NS
Bilirubin, *mg/dL*	0.02 (−0.08, 0.08)	0 (−0.08, 0.09)	0.03 (−0.14, 0.13)	NS
Albumin, *mg/dL*	0.1 (−0.2, 0.3)	0.1 (−0.1, 0.2)	0.0 (−0.1, 0.2)	NS
Hemoglobin A1C, %	0.0 (−0.1, 0.2)	0.1 (−0.2, 0.2)	0.0 (−0.1, 0.3)	NS
Glucose, *mg/dL*	−2 (−8, 5)	2.5 (−7, 10)	0.5 (−10, 7.8)	NS
HOMA-IR[c]	−0.2 (−1.7, 1.5)	0.7 (−1.9, 4.0)	0.4 (−1.9, 3.2)	NS
Adiponectin, *μg/mL*[c]	0.16 (−0.28, 0.97)	−0.01 (−0.43, 0.71)	0.01 (−0.49, 0.77)	NS
Keratin 18, *U/L*[c]	−151.3 (−311.2, 17.1)	−30.4 (−232.5, 88.1)	−15.4 (−251.1, 140.8)	NS
Hyaluronic acid, *ng/mL*[c]	1 (−17, 17)	1 (−6.5, 12)	1 (−9.3, 12)	NS
Procollagen III peptide, *ng/mL*[c]	−0.7 (−2.9, 0.9)	−0.7 (−2.2, 0.4)	−0.6 (−2.2, 0.5)	NS
Collagen IV, *ng/mL*[c]	−17.4 (−37.4, 15.1)	−6.2 (−30.8, 6.1)	−1.8 (−22.3, 14.9)	NS
Total cholesterol, *mg/dL*	8 (−12, 22)	3 (−15, 23)	4 (−11, 20)	NS
LDL-cholesterol, *mg/dL*	4 (−14, 17)	8.5 (−4.5, 20)	8 (−13, 18)	NS
HDL-cholesterol, *mg/dL*	1 (−4, 6)	0 (−3.3, 4)	0.5 (−2.8, 4)	NS
Triglycerides, *mg/dL*	12 (−25, 36)	1 (−45, 26)	−6.5 (−38.3, 20.3)[b]	NS
hsCRP, *mg/L*[c]	0.0 (−1.4, 1.0)	−0.5 (−2.5, 0.6)	−0.6 (−3.0, 0.7)	NS
Ferritin, *ng/mL*[c]	−27 (−66, 7)	−11 (−52.3, 20.5)	−10 (−72.3, 27.8)	NS

NOTE. Data are expressed as median (25th percentile, 75th percentile).
AST, aspartate aminotransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.
[a]Kruskal-Wallis test.
[b]Pairwise comparison (vs placebo) was also performed using Wilcoxon rank-sum test. *P* value <.05 in the pairwise comparison. For ALT (EPA-E 2700 mg/d vs placebo); *P* value = .03. For triglycerides (EPA-E 2700 mg/d vs placebo); *P* = .04.
[c]These were analyzed using fewer subjects than the efficacy evaluable dataset because of some missing values.

Thus, there are various studies testing Lovaza and Vascepa that showed an increase in ALT, and to a greater degree than increases in AST, causing the ALT:AST ratio to increase—and there are various NAFLD studies in which subjects were all instructed to adopt healthy diet and lifestyle changes that show a much attenuated reduction (or even increase) in ALT and AST in OM3-EE group compared with placebo.

An example now of medium-term effects of repeat FAEE dosing over a 5-year period can be found in the robust-sized JELIS trial (>18,000 subjects), which showed the following by the end of study:[126]

Table 4. Serious Adverse Events (SAEs)

SAE	Control (%)	EPA (%)	*P* Value
Hemorrhage	3.4	3.8	.172
Abnormal liver function test	3.5	4.1	.032
Cancer	2.4	2.6	.263
Joint, lumbar, muscle pain	2.0	1.6	.043
Gastrointestinal disorder	1.7	3.8	< .0001
Skin rash/itching	0.7	1.7	< .0001

EPA = eicosapentaenoic acid

A significant increase in abnormal liver function test results was observed in EPA-E group, indicative of hepatic injury.[127] It is noteworthy too that despite a significant reduction in unstable angina in EPA-E group, and trends in lower CV events overall, the trend in mortality was inverted, with 265 events vs 286 in EPA-E arm (HR 1.09; p=0.33). It is conceivable that EPA-E had a negative effect on the mortality of this group due to the impact of various organs having to synthesize unabsorbed FAEE. The incidence of cancer was also trending in an adverse direction in the study, and cellular damage is oft implicated in the disease.[128]

We noticed that the published results from the REDUCE-IT study purposely omit any reporting of these important adverse event data.[129] If in JELIS we saw a significant elevation in abnormal liver function tests from 1.8 g/d EPA-E dosing, then it is reasonable to expect 4 g/d of the same concentration of EPA-E to have an even more deleterious effect. Hopefully the extent to which this occurred in REDUCE-IT will in time be revealed.

In contrast to the above examples, various studies testing non-ethyl-ester omega-3 dosing have shown a lack of adverse effect on ALT or AST levels or the ratio thereof (versus placebo). For example, such

[126] https://www.medscape.org/viewarticle/518574
[127] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609680/
[128] http://science.sciencemag.org/content/266/5192/1821
[129] https://www.nejm.org/doi/full/10.1056/NEJMoa1812792

elevations were completely absent in studies testing Epanova, an FFA omega-3 formulation devoid of ethanol:[130]

> "Omega-3 Carboxylic Acids
>
> In preregistration clinical trials in patients with hypertriglyceridemia, liver test abnormalities were no more frequent among patients receiving omega-3 carboxylic acids than in those on placebo, and there were no reports of clinically apparent liver injury. In a pooled analysis of studies, mild elevations in ALT (up to 2 times the upper limit of normal) occurred in less than 1% of omega-3 carboxylic acid treated subjects and a similar proportion of those on placebo. There were no elevations above 5 times the upper limit of normal and no patient developed jaundice or symptoms and the abnormalities apparently resolved without dose adjustment. Since its approval and more widespread clinical use, there have been no published reports of hepatotoxicity attributable to Epanova. Nevertheless, the product labels of all prescription omega-3 fatty acid products recommend monitoring aminotransferase levels in patients with pre-existing hepatic impairment."

And in Zhu et al. (2008),[131] administration of 6 g/d of seal oil containing a high concentration of omega-3 fatty acids, but absent of ethanol, correlated with a significant reduction in ALT and AST levels, and with ALT to a greater extent than diet modification alone; the dietary habits of all subjects were also tightly regulated.

Determining the long-term effects that a substance can have based on abnormal function tests is not a straightforward process. Unfortunately, much of what we know of substances causing organ damage is only learned many years and many lives after the fact. FAEE have only been approved in the US since 2004 (and only since 2012 for Vascepa), and did not enjoy widespread use in the supplement industry until sometime thereafter. It can take anywhere from 10 – 20 years for these damaging effects in the form of cellular insult to organs from FAEE synthesis (especially liver, heart and skeletal muscles[132]) to culminate in manifestations of ill-health. And those with severe hypertriglyceridemia treated with Lovaza who developed liver disease or otherwise organ impairment could readily be misdiagnosed due to their health status and lack of awareness of the potential for FAEE to exacerbate organ injury.

1.12 Additional Apologetics

Returning to the initial apologetics offered by the REDUCE-IT trial investigators, the remaining two points are as follows:

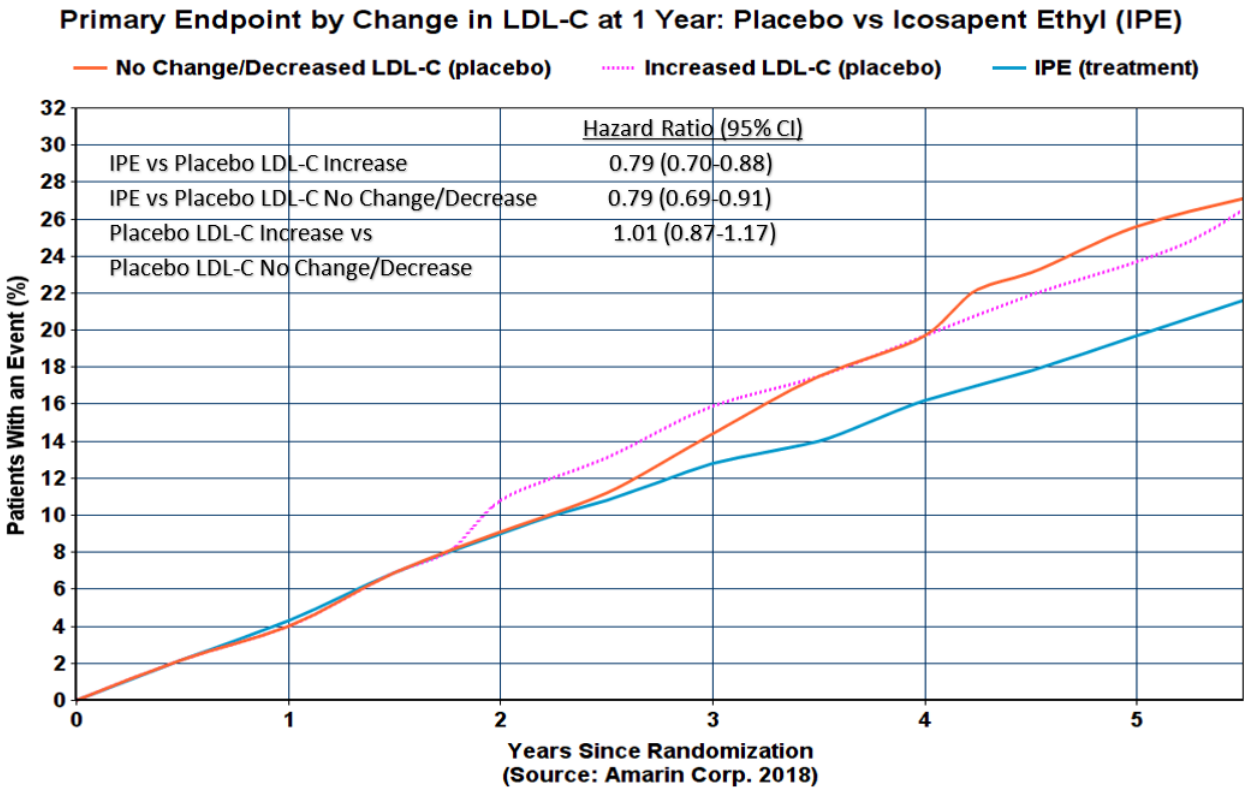
- A post hoc analysis suggested a similar lower risk [for icosapent ethyl group] regardless of whether [or not] there was an increase in LDL cholesterol level among the patients in the placebo group.

[130] https://livertox.nih.gov/Omega3FattyAcids.htm
[131] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2766124/
[132] https://academic.oup.com/alcalc/article/41/6/598/157739

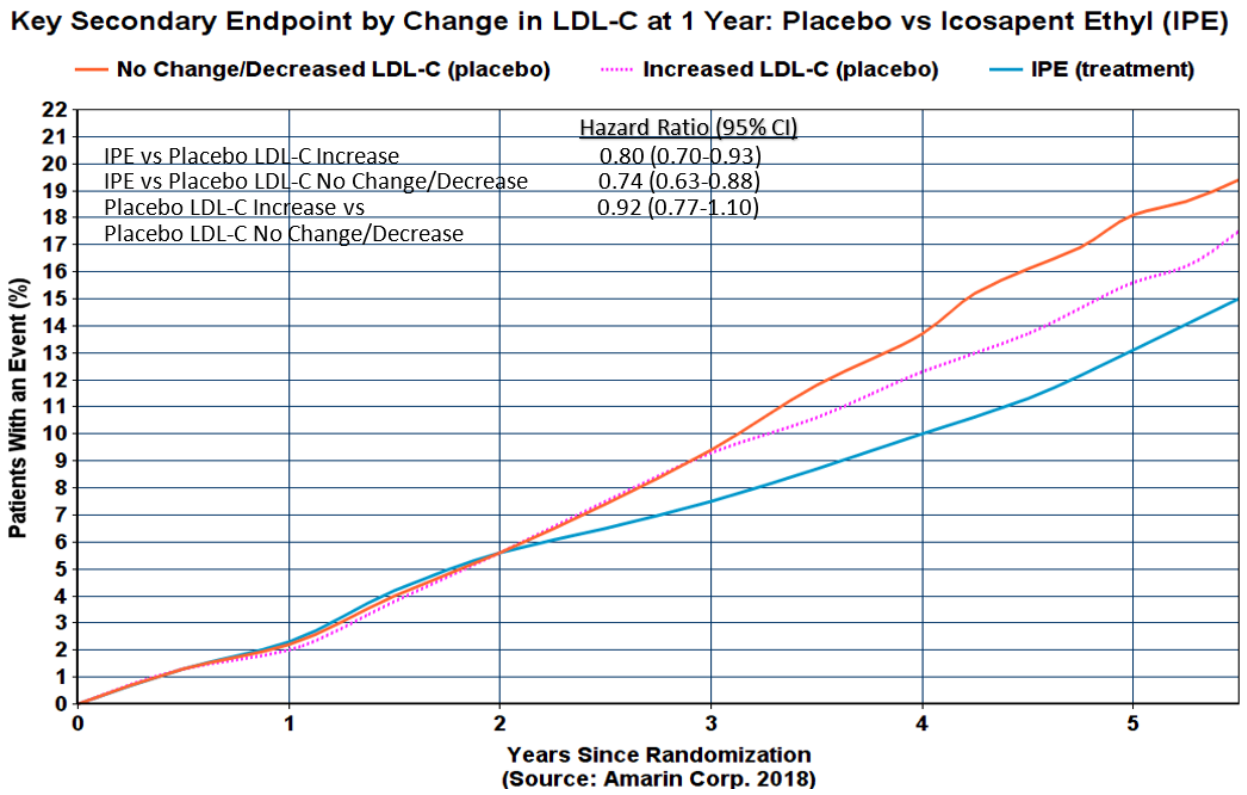
- Although JELIS was designed as an open-label study that did not use a mineral oil placebo, it showed a 19% lower risk of ischemic events with statin therapy plus EPA than with statin therapy alone.

The above is elaborated on in the sponsor's investor presentation on the mineral oil placebo issue.[133]

To the first point above, Amarin provided an analysis as graphics showing a reduction in risk for IPE group compared to both those placebo group subjects that showed an increase in LDL-C from baseline, and to those that showed no change or a decrease in LDL-C from baseline. We redrew this analysis below (see page 4 *here* for the original charts):



[133] https://investor.amarincorp.com/static-files/2e95e517-3545-44a1-abc2-9ed470adc64e



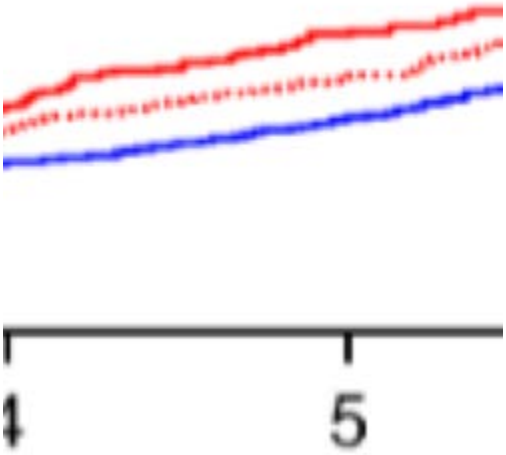
The sponsor argues this is proof that statin absorption was either not affected by dosing with mineral oil, or even if it was, had no material impact on the risk of atherosclerotic cardiovascular disease (ASCVD) events, since even those placebo group subjects with no change or a decrease in LDL-C (hence, we assume their statin meds were working just fine) still performed worse than IPE group.

However, the critical drawback to this subgroup analysis is that it was not pre-specified, and therefore fails to take into account the baseline risk of the placebo subgroup that showed "no change/decrease in LDL-C" relative to the baseline risk of the placebo subgroup that showed an "increase in LDL-C" relative to the IPE group. As such, it remains uninterpretable, given that the baseline characteristics (major prognostic factors) between the subgroups explored are not equivalent. An adjusted analysis was not provided either, which might have induced at least some confidence in the finding.[134]

The sponsor was also not forthcoming on the number of subjects in each placebo subgroup (*number at risk* info is conspicuously absent from both charts). Upon closer inspection, it appears that the "no change/decreased LDL-C" subgroup was much smaller than the "increased LDL-C" subgroup, and will

[134] Agoritsas T, Merglen A, Shah N, et al. Adjusted Analyses in Studies Addressing Therapy and Harm Users' Guides to the Medical Literature. *JAMA*. 2017. [link]

thus have higher inter- and intra-group variability (a negative consequence of small sample size[135, 136, 137]); zooming-in on the original plots that they provide[138] roughly reveals the upticks on the curve:



By our count, it appears to be around 75 key secondary endpoint events for the "no change/decreased LDL-C" placebo subgroup (solid red line above), which leaves about 530 events for the "increased LDL-C" placebo subgroup (in the NEJM paper they enumerate 605 total key secondary endpoint events in placebo group). This is also probably why they avoid any mention of p-values associated with the stated HRs (of course, the FDA will have the full data to examine and will know the exact N per group).

Comparing time-to-event rates between these placebo subgroups and the IPE group and drawing conclusions based on such is therefore flawed, due to a lack of equivalent baseline risk between groups and the potential for exaggeration of observed effects from the smaller sample. The fact that the second chart above shows a marked increase in the incidence of "hard" major coronary events (MCE) in the "no change/decreased LDL-C" subgroup beyond 3-years compared to the "increased LDL-C" subgroup (a counter-intuitive observation) infers that the former is much more likely to be a higher risk group than either of the other two (i.e. higher percentage of secondary prevention patients, higher overall BMI, greater prevalence of male subjects and smokers, higher overall levels of atherogenic and inflammatory markers, etc.). The hierarchy of baseline risk by group would then be (from highest to lowest): "no change/decreased LDL-C" group > IPE group > "increased LDL-C" group. It would be expected, then, for the "no change/decreased LDL-C" group to perform worse than the IPE group, as they are a higher baseline risk group (and apparently much smaller in number). We cannot reasonably conclude anything more than this without grossly overinterpreting these data.[139]

Thus, the analysis fails to prove: (a) that statin malabsorption did not occur in placebo group subjects, or (b) if statin malabsorption did occur, it did not meaningfully impact the placebo group's performance.

---

[135] Katherine S. Button, John P. A. Ioannidis, Claire Mokrysz, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nature*. 2013. [link]
[136] http://www.stat.yale.edu/Courses/1997-98/101/sampinf.htm
[137] http://www.psychology.emory.edu/clinical/bliwise/Tutorials/CLT/CLT/fsummary.htm
[138] https://amarincorp.gcs-web.com/static-files/d2a5fe2f-8dcf-4365-bc69-ce31e1200e21
[139] Yusuf S, Wittes J, Probstfield J, Tyroler HA. Analysis and Interpretation of Treatment Effects in Subgroups of Patients in Randomized Clinical Trials. *JAMA*. 1991. [link]

Post-hoc subgroup analyses such as these—especially when unadjusted—are innately unreliable, and appropriate for hypothesis generating only.[140]

On the second point, regarding the JELIS trial,[141] there are major limitations to the study that make generalizability difficult. It was open-label, and open-label trials are notorious for reporting exaggerated treatment effects;[142,143] and the only significant individual finding from the trial was a reduction in unstable angina, with well over half of the primary endpoint events comprised of these (193 vs 147 events, p=0.014). Therefore, there is an increased likelihood the results were due to performance bias and/or detection bias, resulting from changes in patients' behavior, physicians' treatment, or event ascertainment.[144,145,146] It was also comprised of a 100% Japanese population of patients with poorly controlled LDL-C (182 mg/dL at baseline); nearly 70% were women; all were given very low dose statin regimens, even for an all-Asian population (pravastatin 10 mg/day or simvastatin 5 mg/day), and around 27% of the subjects discontinued statin use during the study (whether or not more control group subjects discontinued statin use earlier than ethyl ester EPA (EPA-E) group subjects was not disclosed).

Importantly, in JELIS, the MCE composite endpoint itself was different from that in REDUCE-IT. It included revascularization and hospitalization for unstable angina, but did not include strokes. In order to make an apt comparison with the REDUCE-IT trial—ignoring for a moment the numerous differences in populations and background therapies—the same composite endpoint should be used. When this is done, with strokes included, the RRR in JELIS decreases sharply from 19% to just 11.5%, insignificant from 914 events ([324 vs 262 non-stroke MCE; HR 0.81] + [162 vs 166 stroke events; HR 1.02] = blended HR 0.885). Comparing the reported 19% RRR in JELIS with the 25% RRR in REDUCE-IT is therefore misleading.

Not only this, but there was no perceived effect on coronary death (HR 0.94, p=0.81) in JELIS, and all-cause mortality trended worse for treatment group (265 vs 286 events, HR 1.09, p=0.33).

It can only be said that in this open-label, 100% Japanese, 70% female-gender study, 1.8 g/d EPA-E (aka "IPE") significantly reduced unstable angina in subjects not optimally treated, with the potential for bias to have overinflated the result. JELIS is not a trial that reliably shows IPE can significantly reduce the risk of ASCVD events, particularly not in a patient population similar to that of REDUCE-IT.

---

[140] Sleight P. Debate: Subgroup analyses in clinical trials: fun to look at - but don't believe them!. *Curr Control Trials Cardiovasc Med*. 2000;1(1):25-27. [link]

[141] Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *The Lancet*. 2007. [link]

[142] Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA*. 1995. [link]

[143] David Moher, Ba' Pham, Alison Jones, et al. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? *The Lancet.* 1998. [link]

[144] J. BEYER-WESTENDORF, H. BÜLLER. External and internal validity of open label or double-blind trials in oral anticoagulation: better, worse or just different? *jth*. 2011. [link]

[145] Jüni P, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. *BMJ*. 2001. [link]

[146] Dariush Mozaffarian. JELIS, fish oil, and cardiac events. *The Lancet*. 2007.

Furthermore, a positive result from one study is often not reproduced in later trials. This is one reason that FDA normally requires at least two adequate and well-controlled studies as proof of efficacy and tolerability.[147]

For example, the GISSI-Prevenzione trial, which tested 1 g/d omega-3 ethyl esters (OM3-E) vs control in over 11,000 Italian post-MI subjects, demonstrated a significant 15% RRR in the primary MCE composite, which included all-cause mortality, MI and stroke. The study showed a 20% reduction in death from any cause and a 30% reduction in CV mortality (each also significant) compared with control. There was also a 44% reduction in sudden death ($p<0.01$). This led to Omacor 1 g/d (aka Lovaza) being indicated for secondary prevention (following MI) in major EU member states. The AHA also began recommending 1 g/d omega-3s for secondary prevention,[148] though there was no formal FDA approval for that indication. However, subsequent placebo-controlled trials did not reproduce the positive findings,[149] and as contrary evidence continued to mount,[150] the EMEA eventually decided to retract their approval.[151]

What happened here? Was the result due to the open-label design? It does not seem possible that that could have affected an endpoint like mortality. Was it due to differences in background therapy between GISSI-P and more modern trials? That could be a plausible explanation, as most of the subjects, especially early on in the study (when much of the benefit in mortality was realized), were not on statin therapy. It is possible that the reduction in sudden death in particular was the result of antiarrhythmic and antifibrillatory properties that have been ascribed to omega-3s.[152] These same properties are also ascribed to statins, particularly at higher doses.[153] There were likely untreated targets present due to lack of statin therapy in GISSI-P subjects. Later trials were conducted in an era where all secondary prevention subjects are given moderate to high-intensity statins following an MI (as well as numerous other treatments and cardiac medications[154]). JELIS enrolled all of its subjects between 1996 and 1999, similar to the GISSI-P trial (1993 – 1997).[155] Would the open-label JELIS trial results involving 1.8 g/d of EPA-E repeat in a placebo-controlled test with optimally treated modern-day subjects of Japanese descent randomized over 20 years after the fact? Perhaps not.

Generalizability of study results is crucial in informing drug regulation.[156] 1 g/d OM3-E offers no additional benefit to secondary or primary prevention patients already on extensive background

---

[147] https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072008.pdf
[148] JOHN H. LEE, JAMES H. O'KEEFE, CARL J. LAVIE, et al. Omega-3 Fatty Acids for Cardioprotection. *Mayo Cin Proc*. 2008. [link]
[149] The ORIGIN Trial Investigators. n–3 Fatty Acids and Cardiovascular Outcomes in Patients with Dysglycemia. *N Eng J Med*. 2012. [link]
[150] Aung T, Halsey J, Kromhout D, et al. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77 917 Individuals. *JAMA Cardiol*. 2018. [link]
[151] https://www.ema.europa.eu/documents/referral/omega-3-fatty-acid-medicines-omega-3-fatty-acid-medicines-no-longer-considered-effective-preventing_en.pdf
[152] R. Marchioli. Treatment with n-3 polyunsaturated fatty acids after myocardial infarction: results of GISSI-prevenzione trial. European Heart Journal. 2001. [link]
[153] Rezaei Y, Gholami-Fesharaki M, Dehghani MR et al. Statin Antiarrhythmic Effect on Atrial Fibrillation in Statin-Naive Patients Undergoing Cardiac Surgery: A Meta-Analysis of Randomized Controlled Trials. *J Cardiovasc Pharmacol Ther*. 2016. [link]
[154] https://www.heart.org/en/health-topics/heart-attack/treatment-of-a-heart-attack
[155] https://www.ncbi.nlm.nih.gov/pubmed/17398308
[156] Kukull WA, Ganguli M. Generalizability: the trees, the forest, and the low-hanging fruit. *Neurology*. 2012. [link]

therapy, even though it proved effective in the GISSI-P trial. The JELIS trial subjects and REDUCE-IT trial subjects are too different and were too differently treated to confer anything from the results of one trial to that of the other.

Another apologetic presented by proponents of icosapent ethyl (IPE), aka "Vascepa," is in comparing studies such as ODYSSEY and FOURIER that demonstrated a regression to the mean in LDL-C with the REDUCE-IT and ANCHOR trials, arguing that the changes in markers seen in the mineral oil (MO) placebo groups of the latter two trials are also regressions to the mean. However, the regressions seen in other trials often involve one or two parameters only, and impact both treatment and control groups. By contrast, in the REDUCE-IT and ANCHOR trials, we find:

- Highly significant elevations in every atherogenic lipid/ lipoprotein and inflammatory marker tested (11 total in ANCHOR) in MO placebo groups of both trials;
- The absence of any of these changes in those randomized to IPE groups in either trial; and
- The confirmation of these effects in two robustly sized studies—even to similar degrees.

As elaborated on previously, a lead-in period helps prevent regressions to the mean from impacting data, which the ANCHOR trial had—and yet, *all eleven* atherogenic/inflammatory markers increased significantly in its placebo group. What has so far been reported from the REDUCE-IT trial is a repeat (and thus, confirmation) of the same phenomenon. The baseline characteristics, including background therapies, of subjects in both trials are extremely similar.

In our view, the only potentially relevant comparison in seeking to prove that the highly significant increases in atherogenic and inflammatory markers in the MO placebo groups in ANCHOR and REDUCE-IT could have been a mere regression to the mean would have to be another trial with an extensive lead-in period that also showed abrupt changes in markers in subjects of approximately equal baseline characteristics. The EVOLVE trial was the closest that we could find.

In EVOLVE, which had a similar lead-in stabilization period as ANCHOR, there was noted a 10% increase in median LDL-C in the olive oil placebo group.[157] However, other atherogenic markers were reduced or did not significantly change in this group. Also, the least squares mean value recorded in the study (which reflects changes across the entire arm) showed an insignificant 3% increase in LDL-C in the placebo group.[158] It seems then that the elevations in LDL-C primarily occurred in those placebo group subjects with baseline values near the median, and little change in the rest.

There could also have been some impact on LDL-C from the modest TG and VLDL-C lowering effect of olive oil (-10% and -11% median change, respectively) by mechanisms similar to those by which DHA can cause an increase in LDL-C along with a decrease in TG and VLDL-C—especially in those with very high triglycerides at baseline.[159] An increase in LDL-C from a triglyceride-lowering therapy is much more likely

---

[157] https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205060s000lbl.pdf

[158] Kastelein, John J.P. et al. Omega-3 free fatty acids for the treatment of severe hypertriglyceridemia: The EpanoVa fOr Lowering Very high triglyceridEs (EVOLVE) trial. *Journal of Clinical Lipidology*. 2013. [link]

[159] Jan Oscarsson, Eva Hurt-Camejo. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. *Lipids in Health and Disease*. 2017. [link]

to occur when the baseline TG levels of subjects are very high.[160] In ANCHOR, the median baseline TG level was ~260 mg/dL, and in EVOLVE it was >700 mg/dL, and so an increase in LDL-C from a TG-lowering therapy would be more likely to occur in EVOLVE than in ANCHOR. The discrepancy in changes in median and mean TG values (-10% vs -4.3%, respectively) from baseline in the olive oil placebo group in EVOLVE also lends credence to the possibility that such a phenomenon affected those with values near the median more than the rest of the group. Thus, the comparison between EVOLVE and the ANCHOR/REDUCE-IT trials breaks down on multiple levels.

The adverse impact on all atherogenic/inflammatory markers tested in ANCHOR, and confirmed in a separate study with patients of equivalent background therapy and characteristics (REDUCE-IT), is much more reminiscent of a treatment effect than a regression to the mean.

Lastly, apologists have pointed out the decrease in LDL-C in some of the statin-treated subjects in the placebo arm of the MARINE trial, causing a net reduction in LDL-C in this subgroup, despite being given 4 g/d MO. However, the number of patients in this subgroup analysis is small (n=18 in the 4 g/d MO arm). As one FDA reviewer noted when examining the same data,[161]

> "Whether mineral oil affects statin absorption has not been formally tested to our knowledge. The applicant submitted data regarding patients who were taking concomitant statin therapy in the MARINE trial and who were randomized to the mineral oil group. Only 18 patients in the mineral oil group were taking a statin. The median percent change in LDL-C was -8% in the statin-treated mineral oil group, with large variability (Q1 -36.0%, Q3 +30.8%); the median change was 0% in LDL-C among the 57 patients not taking statins in the mineral oil group. The applicant contends that if mineral oil reduced statin exposure, then LDL-C should have increased after 12 weeks of treatment, not decreased. While the reduction in LDL-C in this group is somewhat reassuring, the small number of statin-treated patients and the large intra-subject variability do not allow definitive conclusions from this subgroup."

Relatively few patients can significantly impact data from a collective small group (especially when considering the median percent change only). Potential reasons for incidental disparities in values from baseline to end of treatment are numerous, such as cessation or reduction in dose of allowed therapies—or the opposite; adding therapies, including supplements, that may affect other prescribed therapies, increasing or decreasing their potency; changes in diet and exercise habits; and/or any of the aforementioned while also taking their statin medication and mineral oil placebo at far removed times of the day. Three or four subjects out of the 18 analyzed that were on statins and in the 4g/d MO placebo group adopting any of the above could completely shift the subgroup's statistics (the FDA reviewer noted that some of the 18 subjects saw a >30% increase in LDL-C while some saw a >30% decrease in LDL-C). Thus, the subgroup is too small and the variability of data too high to reliably base any conclusions on.

[160] Feingold KR, Grunfeld C. Triglyceride Lowering Drugs. *Endotext*. 2018. [link]
[161] http://epadruginitiative.com/files/FDA_Briefing_Document_for_ADCOM.pdf

However, later, data were reported on changes in LDL-P and non-HDL-C parameters as determined by analysis of blood samples from all subjects in the MARINE trial, and what was observed appears to confirm, rather than disprove, the adverse MO impact hypothesis:[162]

Table 26: Comparison of LDL-C Particle Number (nmol/L) Across Treatment Arms from Baseline to Week 12 Endpoint- MARINE (ITT Population)

	Placebo n=53	Vascepa 2g n=63	Vascepa 4g n=61
Median Baseline LDL-C Particle Number	1310	1374	1418
Median Week 12 Endpoint LDL-C Particle Number	1452	1464	1419
Median Percent Change from Baseline (IQR)	14.4	12.6	-0.1
Treatment Comparisons			
		Estimated Median 95%CI	**p-value**
Vascepa 4g -Placebo		-16.3 (-25.3, -7.0)	0.0006
Vascepa 2g -Placebo		-1.1 (-10.5, +7.4)	0.8202

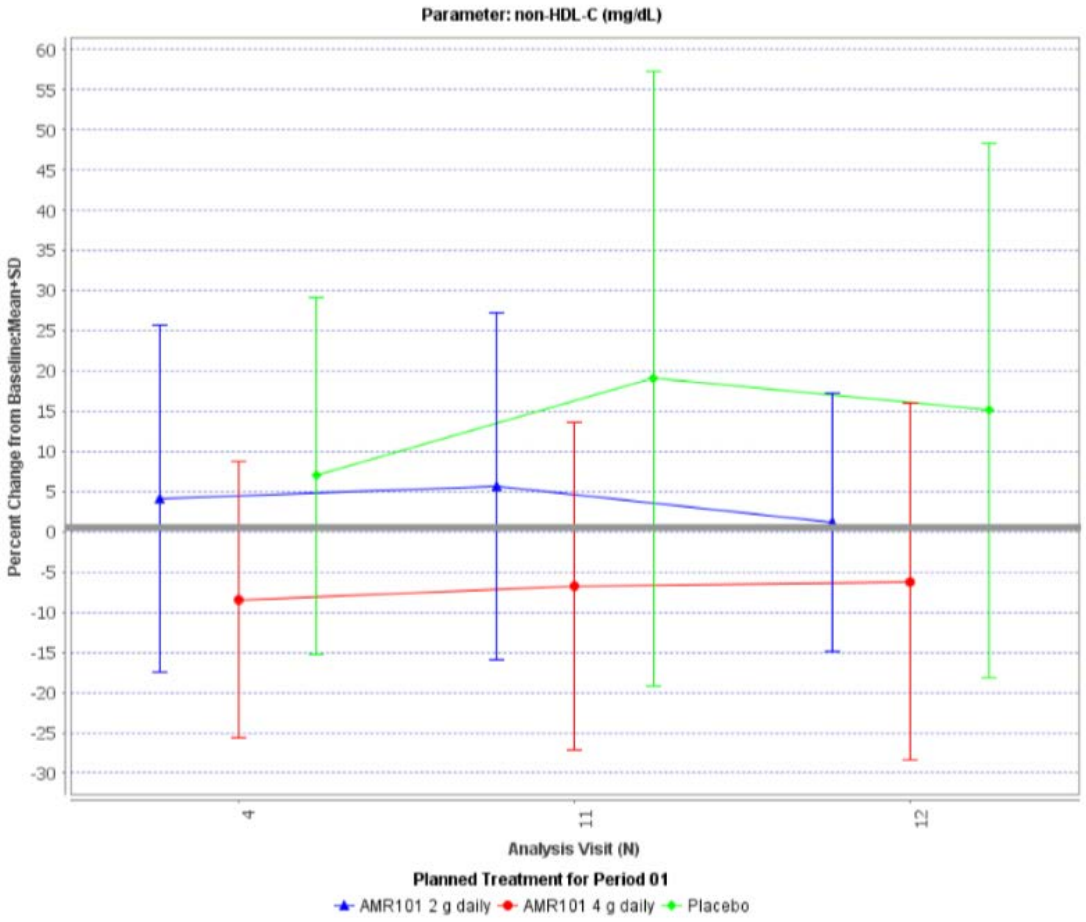
Source: MARINE CSR, Table 18, pg. 86.

[162] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202057Orig1s000MedR.pdf

Table 29: Percent Change in non-HDL-C (mg/dL) From Baseline to Week 12 Endpoint –ITT Population- MARINE

	Placebo n=75	2 grams Vascepa n=73	4 grams Vascepa n=76
Median Baseline non-HDL-C	229 mg/dL	210 mg/dL	225 mg/dL
Median Week 12 Endpoint non-HDL-C	243 mg/dL	214 mg/dL	206 mg/dL
Median Percent Change from Baseline	**7.8**	**0.0**	**-7.7**
Treatment Comparison			
	Estimated Median, 95% CI		**P-value**
Vascepa 4g - Placebo	**-17.7 (-25.0, -11.3)**		**<0.0001**
Vascepa 2g - Placebo	**-8.1 (-15.1, -1.4)**		**0.0182**

Figure 13: Mean Percent Change From Baseline at Various Timepoints in non-HDL-C – ITT Population- MARINE



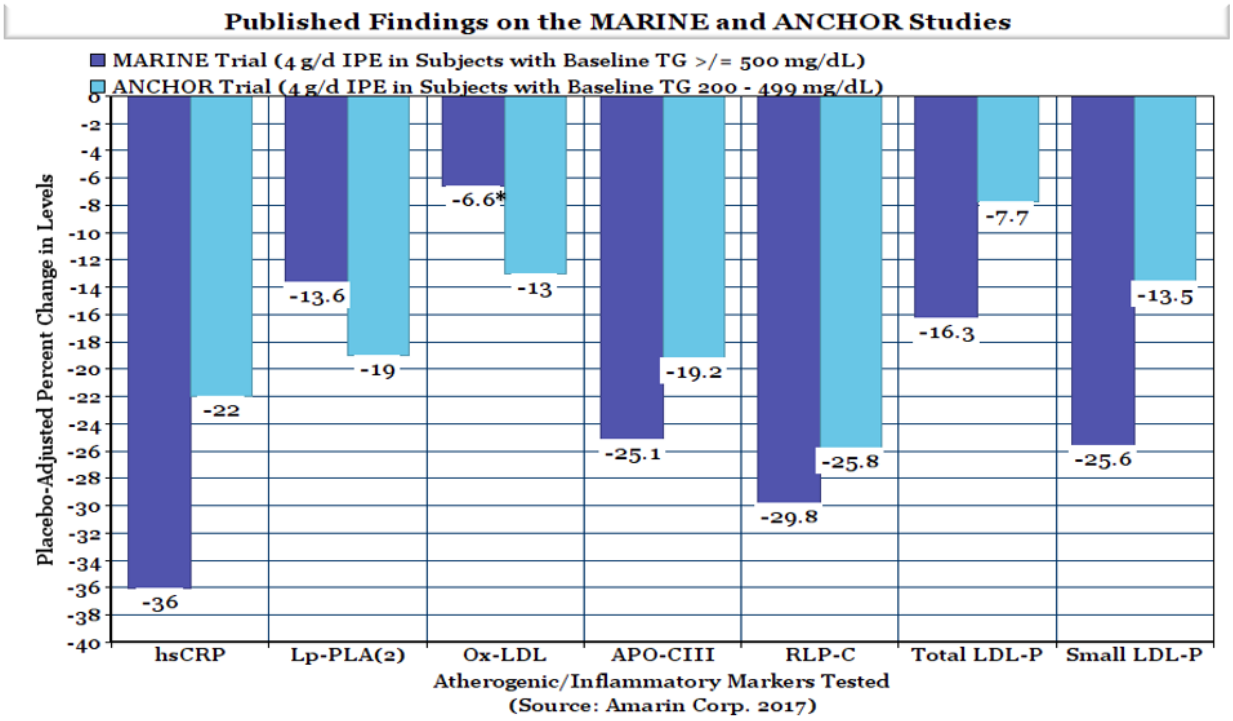
Increases in LDL-P and non-HDL-C are highly predictive of increased prevalence of ASCVD events, even when LDL-C remains unchanged/low.[163,164] The above data show that median LDL-P concentration increased by 14.4% and 12.6% (while mean non-HDL-C increased by 19% and 5%) by week 11 in the 4 g/d MO group and 2 g/d IPE group (who concurrently took 2 g/d MO), respectively. Thus, MARINE trial data seem to confirm rather than disprove the adverse MO impact hypothesis.

As an aside, the sponsor chose to report data from the ANCHOR and MARINE studies in the following manner:[165]

[163] Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J Clin Lipidol*. 2011. [link]

[164] Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. *Circulation*. 2014. [link]

[165] https://investor.amarincorp.com/static-files/23818c55-2c68-423c-8d9f-beaa006b6d6f



**All statistically significant results, except Ox-LDL reduction in MARINE trial*

(The above was redrawn from the original, viewable *here*)

Although they do mention "placebo-adjusted," the clear and obvious message being relayed from the chart (targeting the investment and healthcare communities) is that 4 g/d IPE has a profound impact on atherogenic and inflammatory markers—when in fact, it does not.

For example, hs-CRP levels were lowered insignificantly by ~3 - 4% from baseline in the 4 g/d IPE groups in both studies, yet the slide shows a 36% and 22% reduction in hs-CRP in MARINE and ANCHOR, respectively. This was wholly due to sharp increases in the 4 g/d MO placebo arms, not the result of a CRP-lowering effect of IPE. The graphic also shows that small LDL-P concentration was reduced by 25.6% in MARINE and 13.5% in ANCHOR, but this too was entirely due to increases in the 4 g/d MO placebo arms. In fact, LDL-P actually *increased* insignificantly in the 4 g/d IPE arms from baseline in both studies. Median apoB was also insignificantly lowered by 3.8% and 2.2% from baseline in the 4 g/d IPE arms of MARINE and ANCHOR, respectively. Yet elsewhere, the sponsor has stated that 4 g/d IPE was shown to significantly reduce apoB by 8.5% ($p=0.0019$) and 9.3% ($p<0.001$) in these studies.[166] Once again, this result was predominantly caused by a significant increase in apoB from baseline in the MO-dosed placebo arms, not a significant reduction in IPE arms.

[166] Christie M.Ballantyne, Rene A.Braeckman, Harold 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). *Journal of Clinical Lipidology*. 2014. [link]

The misleading inference that IPE has a pronounced effect on these biomarkers was further exacerbated by a statement made by the principal investigator of the MARINE trial, quoted in an Amarin Corp. press release:[167]

> "Increased apo-B and LDL particle concentration may increase cardiovascular disease risk," said Dr. Bays. "In the previously reported results of the MARINE trial, we observed that AMR101 significantly reduced triglycerides and apo-B, without increasing LDL cholesterol, as compared to placebo, in a most challenging patient population having triglyceride levels ≥500 mg/dL. In this follow-up analysis of the MARINE trial, AMR101 [IPE] reduced both total and small LDL particle concentration, which is not only consistent with its known effects in decreasing apo-B and lack of LDL-cholesterol raising in patients with very high triglyceride levels, but also is suggestive of another potentially favorable lipid effect."

Investigator bias in industry-sponsored clinical trials has far-reaching implications.[168]

We find the reporting of these data by the sponsor and commentary by the principal investigator to be irresponsible. It seems that the sponsor has been relying on the adverse impact of mineral oil on placebo group subjects to make misleading claims as to the efficacy of IPE therapy. The REDUCE-IT trial results may unfortunately prove to be the culmination of this tendency.

1.13 Discussion

There are much data available on the anti-inflammatory and lipid-lowering properties of omega-3 fatty acids, and in particular eicosapentaenoic acid (EPA).[169] Numerous biomarkers, namely TG, VLDL-C, RLP-C, and LpPLA2, have consistently been shown to be lowered by ingesting this fatty acid, especially at the dose of 4 g/d (>95% ethyl ester EPA formulation, taken with food).[170] This most often occurs without any significant effect on LDL-C. Other omega-3 fatty acids, and in particular docosahexaenoic acid (DHA), can significantly increase LDL-C, especially in subjects with persistently high triglyceride levels. Although a lowering-effect on such biomarkers is not an accepted surrogate endpoint for a reduction in ASCVD risk, it is difficult to imagine a scenario in which these effects would not confer a health benefit to any individual, and all the more so for those at higher risk of ASCVD, especially secondary prevention patients with chronically elevated triglyceride levels.

---

[167] https://investor.amarincorp.com/news-releases/news-release-details/amarins-phase-3-marine-study-results-presented-american-heart

[168] Ahn Rosa, Woodbridge Alexandra, Abraham Ann, et al. Financial ties of principal investigators and randomized controlled trial outcomes: cross sectional study. *BMJ*. 2017. [link]

[169] Mullen A, Loscher CE, Roche HM. Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages. *J Nutr Biochem*. 2010. [link]

[170] 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. [link]

There is little question that the safety profile of highly purified (>90%) ethyl ester omega-3s, when looking at data from A&WC studies testing use of the same, is excellent,[171] and that includes "EPA-only" formulations such as icosapent ethyl (>95% EPA-E). Even at doses in excess of 6 g/d, the risk of a bleeding event, or other serious adverse event, has been very low with highly purified omega-3s.[172] Although there is certainly evidence suggesting long-term ingestion of ethyl ester omega-3s, such as Vascepa, can cause organ damage, there have been no studies conducted that have tested this hypothesis directly. From one of the studies on fatty acid ethyl esters (FAEE) cited elsewhere in this paper, the author (Laposata, 1998) writes:

> "V. FATTY ACID ETHYL ESTERS AS FATTY ACID SUPPLEMENTS
>
> There is substantial clinical interest in fatty acid supplements as treatments for a variety of diseases. Oral preparations of FAEE have been made available for fatty acid supplementation and with the introduction of FAEE capsules for oral intake, there were a number of studies to evaluate ethyl ester absorption from the GI tract. In 1991, Nordoy et al. reported that ethyl esters and triglycerides were equally well absorbed from the GI tract in human subjects.[52] In 1992, Yamazaki et al.[53] infused emulsions of ethyl eicosapentaenoate (ethyl EPA) into rat veins and demonstrated that the EPA content in the phospholipids of a variety of organs substantially increased. It has also been shown that treatment of normal volunteers with oral n±3 FAEE, with approximately 3 gm ethyl EPA plus ethyl docosahexaenoic acid (ethyl 22:6), per day results in a marked accumulation of the fatty acids from these ethyl esters in the plasma and in cell lipids within 6 weeks.[54] Harris et al. conducted a randomized, placebo controlled, double blind, crossover trial in which 10 mildly hypertriglyceridemic patients were given capsules containing n±3 FAEE or an olive oil placebo for two 4-week treatment periods with a one week washout phase in between.[55] They found that the n±3 FAEE were effective hypotriglyceridemic agents and that they impact lipoprotein metabolism very quickly. However, they also determined that the incorporation of n±3 FAEE into lipoproteins was associated with an increased susceptibility of the lipoproteins to oxidation. Krokan et al.[56] reported in a 1993 study that there is enrichment of 20:5 n±3 and 22:6 n±3 in total plasma lipids and phospholipids in healthy volunteers after oral ingestion of either a concentrated ethyl ester or a natural triglyceride and that the enteral absorption of 20:5 and 22:6 was very similar for FAEE and triglycerides. Importantly, there were no acute toxic effects associated with oral FAEE intake in any of these studies. There is now evidence of a significant therapeutic effect of n±3 ethyl esters in mice. Paulsen et al. recently demonstrated that administration of ethyl esters of eicosapentaenoic acid (EPA, 20:5 n± 3) and docosahexaenoic acid (DHA, 22:6 n±3) for 18 weeks suppresses the formation and growth of intestinal polyps in a mouse strain genetically predisposed to develop tumors in the gastrointestinal tract.[57] We recently tested the hypothesis that orally ingested supplemental FAEE are rapidly degraded in the gastrointestinal tract and blood to explain the lack of toxicity.[58] Using rats given FAEE as an oil directly into the stomach or within LDL particles directly into the circulation, we demonstrated that FAEE hydrolysis in the gastrointestinal tract and blood is rapid and extensive. The fate of the fatty acid from FAEE hydrolysis was highly dependent on the organ or tissue presented with the FAEE."

[171] https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/

[172] Harris WS. Fish Oils and Bleeding—Where Is the Evidence? *JAMA Intern Med.* 2016. [link]

Of course, it would take an exceptionally long study, following subjects for, we posit, 15-years or more in order to adequately answer the question, and it is doubtful such a study will be conducted in the future. But the connection is certainly there, and is compelling enough in our view to warn the public of the possibility. In any event, for those at high-risk of ASCVD, the potential long-term deleterious effects of FAEE administration (including ethyl ester EPA) are outweighed by the benefit of a 25% relative reduction in the risk of an MCE—*if* we can take the REDUCE-IT results at face-value. But if we cannot, then the benefit-risk assessment becomes obscured, making the possibility of organ damage (as well as the highly significant incidence of atrial fibrillation) from this formulation a weightier matter to consider.

Although arguments presented in this paper have been dismissive of the JELIS trial results,[173] stating that if strokes were included in the primary composite endpoint that the study would have failed with a reported 11.5% RRR for ethyl ester EPA group, it should be noted that JELIS was not designed to include strokes in the MCE composite endpoint. And even though hospitalization for unstable angina, which is a serious event not to be taken lightly,[174] was the only significantly reduced individual component of the MCE composite primary endpoint of the study, the composite endpoint is meant to consider the totality of all components therein, and as such was significantly impacted, with nearly all individual components trending in a favorable direction. Further, some of these were also approaching significance (i.e. "non-fatal MI," HR 0.75; p=0.086 and "coronary death or MI," HR 0.78; p=0.083). However, JELIS, like GISSI-P, was conducted in an era before statins and other modern-day treatments became widespread, and the lack of benefit demonstrated from modern randomized omega-3s trials[175] suggests that omega-3 dosing might offer little additional benefit beyond state-of-the-art pharmacotherapy. The JELIS trial subjects were also all on very low dose statins, and many discontinued the use of statins during the trial. All told, the differences in patient populations (including era-specific background therapies) studied between JELIS and REDUCE-IT are vast; a significant reduction in one or more individual "hard" MCE endpoints would have increased confidence in the finding; and it is impossible to overlook the open-label design, increasing the possibility that bias may have overinflated the positive results.[176]

When considering the rationale behind the REDUCE-IT trial, it appears compelling in a number of ways. For instance, all FDA-approved drugs are effective at a certain dose; there is of course a dose at which all drugs would be rendered ineffective. If a therapeutically effective dose of EPA exists in which an at-risk individual treated with modern-day interventions may significantly reduce their risk of ASCVD by taking it, and if this dose is closer to 4 g/d (the dose at which numerous lipid and inflammatory markers are consistently lowered with high-purity omega-3s), then all of the failed modern-era cardiovascular outcomes studies that tested mixed omega-3 fatty acids[177] may have failed as a result of utilizing a dose of EPA well-below a therapeutically effective threshold (i.e ~450 mg EPA per day vs ~4 g/d). In the same

---

[173] Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *The Lancet*. 2007. [link]

[174] https://www.heart.org/en/health-topics/heart-attack/angina-chest-pain/unstable-angina

[175] Abdelhamid AS, Brown TJ, Brainard JS, et al. Omega 3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2018. [link]

[176] Savović J1, Jones H, Altman D, et al. Influence of reported study design characteristics on intervention effect estimates from randomised controlled trials: combined analysis of meta-epidemiological studies. Health Technol Assess. 2012. [link]

[177] Sradha Kotwal, Min Jun , David Sullivan, et al. Omega 3 Fatty Acids and Cardiovascular Outcomes Systematic Review and Meta-Analysis. *Circulation*. 2012. [link]

vein, many of these studies had a relatively short follow-up period; if it takes a median of approximately 5-years to observe a significant reduction in ASCVD risk from 4 g/d high-purity omega-3 or EPA-only dosing, then the follow-up for these failed outcomes studies may have been insufficient. The successful outcome of the REDUCE-IT trial is consistent with the above logical deductions.

One could also argue that the borderline significant increase in bleeding events and robust reduction in the incidence of stroke in EPA-treated subjects in REDUCE-IT that is so unapparent in other studies testing omega-3s, including JELIS (1.8 g/d of >96% pure ethyl ester EPA), might be related to the high dose of EPA used and the average length of time it was used in the study, reasoning that it is only at such a dose (~4 g/d) over a median of 5-years or more that the antithrombotic effects of ethyl ester EPA may result in a reduction in the incidence of MCE, and stroke in particular, in modern-day treated subjects. Or, as posited in this paper, it could be a red-flag that an inhibition of antithrombotics in the placebo arm took place.

After consideration of multiple points of view, we find that there is too much that is alarming from the REDUCE-IT data to simply overlook, in particular the confirmation of an increase in all atherogenic/inflammatory markers tested in the MO placebo group, just as was reported from ANCHOR. The background therapies, patient characteristics, and era (relevant SOC) in which the ANCHOR and REDUCE-IT studies were conducted are highly similar between the two trials. This could infer an attenuation of statin therapy that equates to a multi-fold reduction in the administered dose, as well as the inhibition of other cardiac medications. Further, the highly significant increase in peripheral edema in IPE group, which is a very common adverse event from antihypertensive therapy, and which is a side-effect that is completely absent from the vast literature on omega-3 studies, is a peculiar and unsettling phenomenon, with one reasonable explanation for the observation being a hindrance of the absorption of antihypertensives in placebo arm, causing a preponderance of these adverse events to appear in the group that de facto received greater doses of these drugs, namely the IPE arm.

The possibility that MO hindered the absorption of much-needed therapies in the placebo group of REDUCE-IT is an unfortunate reality, but one that must be addressed before the superb results of the study can be universally accepted as reliable.

1.14 Conclusions

Considering the information provided herein, we conclude that multiple DDI studies are warranted to exonerate or implicate light paraffin oil, aka mineral oil, in the perceived malabsorption of interventions used in the treatment of ASCVD—specifically, statins, antithrombotics (aspirin, clopidogrel, ticagrelor, dipyridamole, etc.), anti-ischemia agents (nitrates, beta-blockers), and antihypertensives (CCBs, ARBs and ACE inhibitors). Without such studies to supplement the REDUCE-IT trial data, we view the results to be uninterpretable.

Therefore, it is our recommendation that FDA require relevant DDI studies of the sponsor, Amarin Corp., to determine the effect, if any, of dosing with mineral oil (encapsulated) on concomitant therapies before making a decision to expand the Vascepa label. We further recommend a crossover design to increase confidence in the results.

If the above proposed DDI studies prove dosing with mineral oil concurrent with cardiac medications has no effect on their potency, then as far as we can tell the REDUCE-IT results can be relied upon for patient care, and that will be wonderful news. However, should the proposed DDI studies demonstrate an inhibitory effect of mineral oil on drug absorption, then the validity of the REDUCE-IT study results would be dubious, and another CVOT with an inert placebo would be needed to elucidate the effects, if any, of high-purity ethyl ester EPA (aka "icosapent ethyl," aka "Vascepa") on ASCVD risk.

Lastly, in the interest of public safety, we recommend FDA consider placing a clinical hold on the EVAPORATE trial (and any other ongoing studies involving a mineral oil placebo where it is possible that concomitant therapies may be attenuated via concurrent dosing), which is being funded by Amarin Corp., until such time as mineral oil is found to be truly inert in this regard, as subjects could be exposed to immediate and serious harm.

The acceptance of a confounded result by health care professionals and the general public can be pernicious, particularly when the adoption of the resultant practice change would be widespread (millions of patients). Some may forego dosing with ezetimibe due to its adverse effect on omega-3 absorption. Others might lower the dose/frequency of their statin medication—or perhaps stop taking it altogether—reasoning that as long as they are taking 4 g/d EPA (prescription or non), their risk of ASCVD is markedly lowered. Still others may forgo the use of PCSK9 inhibitors, or some other therapy not yet developed, due to it falling short of a 25% RRR in MCE. They may in each case opt for Vascepa instead, and if Vascepa is not as effective as the REDUCE-IT trial results suggest, the outcome would be detrimental to patients and payors, and ultimately, to all US citizens.

C. Environmental Impact

The only environmental impact identified by Medical Research Collaborative that would result from carrying out the recommendations in this petition would be a positive one, in that a potential new burden on the world's anchovy fisheries, which could have unintended negative ripple effects on other species as well, would be averted.

D. Economic Impact

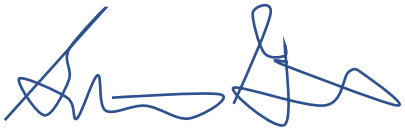
Pursuant to 21 C.F.R. § 10.30(b), an economic impact statement will be submitted only upon the request of the Commissioner.

E. Certification

I certify that, to my best knowledge and belief: (a) this petition includes all information and views upon which the petition relies; (b) this petition includes representative data and/or information known to the petitioner which are unfavorable to the petition; and (c) I have taken reasonable steps to ensure that any representative data and/or information which are unfavorable to the petition were disclosed to me. I further certify that the information upon which I have based the action requested herein first became known to the party on whose behalf this petition is submitted on or about the

following date: November 10th, 2018, the date of the presentation of REDUCE-IT trial results at the AHA Annual Scientific Sessions, simultaneously published in the NEJM. Certain of the information became known to the petitioner later, such as topline blood pressure data revealed by Dr. Bhatt on April 25th, 2019. However, the analysis of data upon which this petition relies took a number of months to conduct. If I received or expect to receive payments, including cash and other forms of consideration, to file this information or its contents, I received or expect to receive those payments from the following persons or organizations: none. I verify under penalty of perjury that the foregoing is true and correct as of the date of the submission of this petition.

Respectfully submitted,



Steven Giardino
President and CEO
Medical Research Collaborative, LLC