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FASTTRACK CLINICAL RESEARCH
Dyslipidaemias

A possible explanation for the contrasting results of REDUCE-IT vs. STRENGTH: cohort study mimicking trial designs

Takahito Doi[1,2,3,†], Anne Langsted[1,2,3], and Børge G. Nordestgaard [1,2,3,*]

[1]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Borgmester Ib Juuls Vej 73, Herlev 2730, Denmark; [2]The Copenhagen General Population Study, Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Borgmester Ib Juuls Vej 73, Herlev 2730, Denmark; and [3]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, Copenhagen 2200, Denmark

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See page 4818 for the editorial comment for this article 'Investigating contrasting results in REDUCE-IT and STRENGTH: partial answers but questions remain', by K.C. Maki, https://doi.org/10.1093/eurheartj/ehab643.

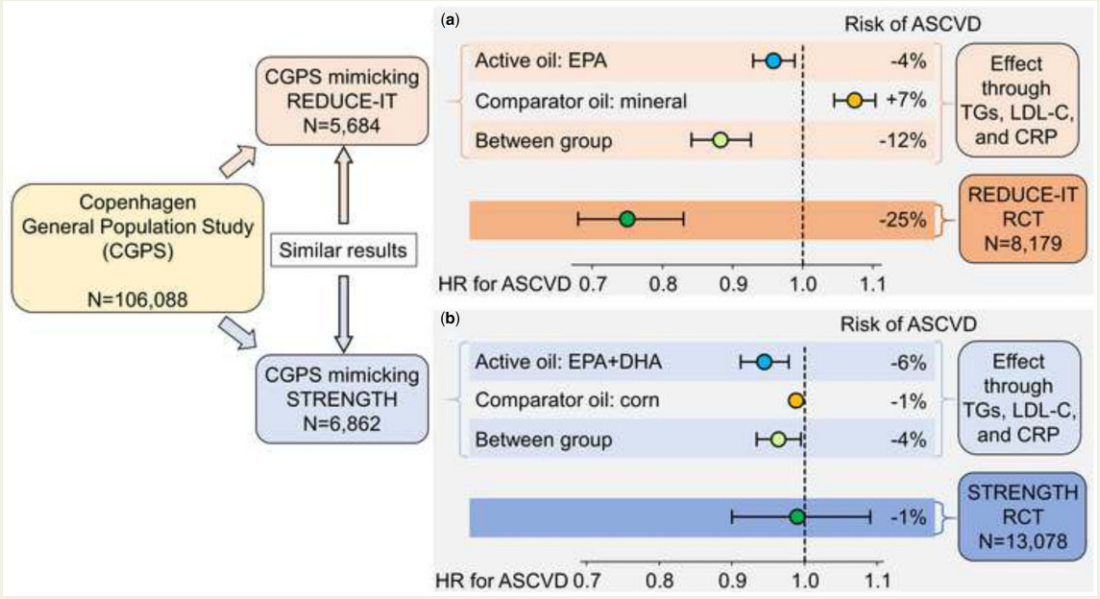
Aims We tested the hypothesis that the contrasting results for the effect of high-dose, purified omega-3 fatty acids on the prevention of atherosclerotic cardiovascular disease (ASCVD) in two randomized trials, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) vs. Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia (STRENGTH), can be explained by differences in the effect of active and comparator oils on lipid traits and C-reactive protein.

Methods and results In the Copenhagen General Population Study (CGPS) with 106 088 individuals, to mimic trial designs we analysed those who met key inclusion criteria in REDUCE-IT ($n = 5684$; ASCVD = 852) and STRENGTH ($n = 6862$; ASCVD = 697). Atherosclerotic cardiovascular disease incidence was followed for the median durations of REDUCE-IT and STRENGTH (4.9 and 3.5 years), respectively. When combining changes in plasma triglycerides, low-density lipoprotein cholesterol, and C-reactive protein observed in the active oil groups of the original studies, estimated hazard ratios for ASCVD in the CGPS were 0.96 [95% confidence interval 0.93–0.99] mimicking REDUCE-IT and 0.94 (0.91–0.98) mimicking STRENGTH. In the comparator oil groups, corresponding hazard ratios were 1.07 (1.04–1.10) and 0.99 (0.98–0.99). Combining these results, the active oil vs. comparator oil hazard ratio was 0.88 (0.84–0.93) in the CGPS mimicking REDUCE-IT compared to 0.75 (0.68–0.83) in the REDUCE-IT. The corresponding hazard ratio was 0.96 (0.93–0.99) in the CGPS mimicking STRENGTH compared to 0.99 (0.90–1.09) in STRENGTH.

Conclusion The contrasting results of REDUCE-IT vs. STRENGTH can partly be explained by a difference in the effect of comparator oils (mineral vs. corn), but not of active oils [eicosapentaenoic acid (EPA) vs. EPA + docosahexaenoic acid], on lipid traits and C-reactive protein. The unexplained additional 13% risk reduction in REDUCE-IT likely is through other effects of EPA or mineral oil.

* Corresponding author. Tel: +45 38683297, Email: boerge.nordestgaad@regionh.dk
†Dr. Doi is also affiliated with Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan.

Graphical Abstract



Explanations for contrasting results of REDUCE-IT vs. STRENGTH using a cohort study mimicking trial designs. We tested the hypothesis that contrasting results in REDUCE-IT vs. STRENGTH can be explained by differences in the effect of active oils (eicosapentaenoic acid vs. eicosapentaenoic acid + docosahexaenoic acid) and comparator oils (mineral vs. corn) on lipid traits and C-reactive protein, or in study populations (high vs. moderate–high risk). To do so, we used the CGPS to mimic the study populations of REDUCE-IT and STRENGTH and followed them for 4.9 and 3.5 years, respectively, for atherosclerotic cardiovascular disease events, corresponding to the median follow-up time of the two trials.[1,2] Combination of percent change in plasma triglycerides, low-density lipoprotein cholesterol, and C-reactive protein in the active oil arm, the comparator oil arm, and between arm difference reported in REDUCE-IT[1] or STRENGTH[2] (Supplementary material online, *Table S1*) were examined in (*A*) the CGPS mimicking REDUCE-IT, or (*B*) the CGPS mimicking STRENGTH. The hazard ratios for atherosclerotic cardiovascular disease are shown in blue dots for active oil arms, yellow dots for comparator oil arms, and light green dots for between arm differences. The dark green dots represent the actual hazard ratios for risk of atherosclerotic cardiovascular disease as reported in REDUCE-IT[1] or STRENGTH.[2] ASCVD, atherosclerotic cardiovascular disease; CGPS, Copenhagen General Population Study; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; RCT, randomized controlled trial; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; STRENGTH, Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia; TGs, triglycerides.

Keywords Fish oil • Remnants • Triglyceride-rich lipoproteins • Very low-density lipoproteins • Inflammation • Cardiovascular disease

Introduction

Recently, two double-blind randomized controlled trials have shown contrasting results for the effect of high-dose, purified omega-3 fatty acids for triglyceride lowering on the prevention of atherosclerotic cardiovascular disease (ASCVD). In REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) including 8179 individuals at high ASCVD risk, administration of 4 g/day icosapent ethyl [an ethyl ester of eicosapentaenoic acid (EPA)] compared to mineral oil for 4.9 years resulted in a 25% lower risk of ASCVD.[1] In contrast, in STRENGTH (Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia) including 13 078 individuals at moderate–high ASCVD risk, administration of 4 g/day EPA (75%) plus docosahexaenoic acid (DHA; 25%) compared to corn oil for 3.5 years had no beneficial effect on the risk of ASCVD.[2,3] Thus, these two trials differed with respect to active oils (EPA vs. EPA + DHA), comparator oils (mineral vs. corn), and study populations (high vs. moderate–high risk).

In both REDUCE-IT and STRENGTH, active and comparator oils independently had different effects on triglyceride-rich lipoproteins (marked by plasma triglycerides), low-density lipoprotein (LDL) cholesterol, and low-grade inflammation (marked by plasma C-reactive protein) (Supplementary material online, *Table S1*),[1,2] all three causally related to the risk of ASCVD.[4–9] Triglyceride-rich lipoproteins together with LDL [which includes lipoprotein(a)] can be summarized as non-high-density lipoprotein (non-HDL) cholesterol or apolipoprotein B, both of which were also reported in REDUCE-IT and STRENGTH. A better understanding of the contrasting results in REDUCE-IT vs. STRENGTH may thus emerge through examining

different effects on lipid traits and C-reactive protein within the two trials.

We tested the hypothesis that contrasting results in REDUCE-IT vs. STRENGTH can be explained by differences in effect of active oils (EPA vs. EPA + DHA) and comparator oils (mineral vs. corn) on lipid traits and C-reactive protein, or in study populations (high vs. moderate–high risk). To do so, we used the Copenhagen General Population Study (CGPS) to mimic the study populations of REDUCE-IT and STRENGTH and followed them for 4.9 and 3.5 years, respectively, for ASCVD events, corresponding to the median follow-up time of the two trials.[1,2] Within these two cohorts, we then examined the difference in ASCVD incidence that could be explained by study-specific observed changes in triglyceride-rich lipoproteins, LDL, and low-grade inflammation within each study arm (active and comparator oil) separately and combined,[1,2] as well as between study populations. Finally, we compared these estimated changes in ASCVD risk with corresponding observed changes in REDUCE-IT and STRENGTH.

Methods

Study population

The CGPS recruited in 2003–15 individuals aged 20–100 invited randomly from the Danish Civil Registration System (43% participation rate). Information on lifestyle and medication including statin therapy was obtained through a questionnaire. Furthermore, participants underwent a physical examination and had non-fasting blood samples drawn for biochemical measurements.[10] We used 106 088 eligible individuals with full baseline information on all lipid traits (triglycerides, LDL cholesterol, non-HDL cholesterol, apolipoprotein B) and C-reactive protein.

For the present study, we included individuals based on key inclusion criteria used in REDUCE-IT and STRENGTH, referred to as the CGPS mimicking REDUCE-IT and the CGPS mimicking STRENGTH, respectively. For the CGPS mimicking REDUCE-IT, we selected individuals on statins at study entry, who were (i) ≥45 years with established ASCVD or (ii) ≥50 years with diabetes and at least one additional risk factor including high age (≥55 years for men or ≥65 years for women), current smoking, hypertension, renal dysfunction (creatinine clearance between 30 and 60 mL/min calculated by the Cockcroft–Gault equation), or ankle-brachial index <0.9.[1] For the CGPS mimicking STRENGTH, we selected individuals on statins at study entry, who (i) had established ASCVD, (ii) had diabetes and were ≥40 years for men or ≥50 years for women with at least one additional risk factor including current smoking and hypertension, or (iii) did not have diabetes but were ≥50 years for men or ≥60 years for women with at least one additional risk factor including family history of premature coronary artery disease, current smoking, or renal dysfunction (estimated glomerular filtration rate <45 mL/min/1.73 m^2 calculated by the Chronic Kidney Disease Epidemiology Collaboration equation).[2] We did not exclude individuals with plasma triglycerides <1.52 mmol/L (135 mg/dL) in the CGPS mimicking REDUCE-IT and <2.03 mmol/L (180 mg/dL) in the CGPS mimicking STRENGTH because we need individuals with both high and low triglycerides in the CGPS to mimic the two trial designs for untreated and treated participants, respectively; however, in the sensitivity analysis, we excluded individuals with such low triglycerides.

In another sensitivity analysis, we further used the inclusion criteria from REDUCE-IT of LDL cholesterol between 1.06 and 2.59 mmol/L (41 and 100 mg/dL) and from STRENGTH of (i) LDL cholesterol <2.59 mmol/L (<100 mg/dL) and (ii) HDL cholesterol <1.09 mmol/L (<42 mg/dL) for men or <1.22 mmol/L (<47 mg/dL) for women. Individuals with triglycerides >5.7 mmol/L (>500 mg/dL) or C-reactive protein >200 mg/L (>20 mg/dL) were excluded as these individuals represent extreme outliers, potentially at risk of other diseases than ASCVD.

Written informed consent was given by each participant. The study was approved by an institutional review board and a Danish Ethical Committee (H-KF-01-144/01). The study was conducted according to the Declaration of Helsinki.

Lipids traits and high-sensitivity C-reactive protein

Plasma total cholesterol, HDL cholesterol, triglycerides, apolipoprotein B, and high-sensitivity C-reactive protein were measured at baseline using standard hospital assay from Konelab, Helsinki, Finland or Roche, Basel, Switzerland. When triglycerides were <4 mmol/L (<354 mg/dL), LDL cholesterol was calculated by the Friedewald equation,[11] and otherwise measured directly. Non-HDL cholesterol was total cholesterol minus HDL cholesterol. Changes in these parameters were identified directly from the original trials (Supplementary material online, *Methods* and Supplementary material online, *Tables S1* and *S2*).

Atherosclerotic cardiovascular disease

We defined ASCVD using the definition of the primary outcome in REDUCE-IT and STRENGTH.[1,2] Atherosclerotic cardiovascular disease included cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, hospitalization for unstable angina, and coronary revascularization. Information on ASCVD was collected until 13 December 2018 by reviewing the national Danish Causes of Death Registry and all hospital admissions and diagnoses entered in the Danish National Patient Registry [World Health Organization International Classification of Diseases, 10th edition (ICD-10) codes I01–I99 for cardiovascular death, I21–I22 for myocardial infarction, I60–I61, I63–I64, and G45 for stroke, and I20.0 for unstable angina], and coronary revascularization registered according to the Nordic Medico-Statistical Committee (NOMESCO) Classification of Surgical Procedures as coronary artery bypass graft (NOMESCO: KFNA–KFNE) and percutaneous coronary intervention (NOMESCO: KFNG00–05). In the Danish health registries, myocardial infarction is more than 99% correct,[12] stroke events were individually validated,[13] and invasive procedures are registered accurately,[14] while the accuracy of registration for cardiovascular death and unstable angina is likely less precise.

Statistical analyses

We used Stata version 15.1 SE (Stata Corp., College Station, TX, USA). Covariates for adjustment were chosen due to known associations with ASCVD, including age, sex, smoking status (current or former/never smoker), and systolic blood pressure. Body mass index, diabetes mellitus, and HDL cholesterol were deliberately omitted as covariates for adjustment because these covariates influence lipid traits and/or C-reactive protein through biological pathways; increasing body mass index and diabetes mellitus lead to increased plasma triglycerides and C-reactive protein, while HDL cholesterol levels are inversely related to plasma triglycerides.[15] However, these three variables were included in the sensitivity analysis. Missing information on covariates (0.1%) for adjustment was imputed; for continuous variables by regression based on age and sex, while categorical variables were assigned a separate category. However, if only individuals with complete data were included, results were similar.

We used Cox proportional hazards regressions with age as the underlying timescale, study entry at baseline examination (left truncation), and

censoring at the occurrence of ASCVD, emigration ($n = 6$ for the CGPS mimicking REDUCE-IT and $n = 10$ for the CGPS mimicking STRENGTH), death, or end of follow-up (4.9 years for the CGPS mimicking REDUCE-IT, 3.5 years for the CGPS mimicking STRENGTH, and 9.1 years for the entire CGPS), whichever came first. On continuous scales, restricted cubic splines with three knots (best fit according to the Akaike information criteria) were applied for all models, with the reference value as the mean of the top quartile in all splines. When analysing the association between percent difference in lipid traits or C-reactive protein levels with the risk of ASCVD, we used a log scale to make a one-unit change correspond to a percent difference, e.g. when analysing the association between 20% lower triglycerides and risk of ASCVD, we used a 0.8 log scale, where a one-unit change corresponds to a 20% lower level. When three changes were combined (e.g. triglycerides, LDL cholesterol, and C-reactive protein), the markers were assumed to act independently on the outcome. The assumption of proportional hazards was tested using Schoenfeld residuals; no major deviations were observed.

Please see Supplementary material online, *Methods* for additional information.

Results

In 106 088 eligible individuals in the CGPS, 5684 fulfilled the key inclusion criteria for REDUCE-IT (the CGPS mimicking REDUCE-IT) and 6862 for STRENGTH (the CGPS mimicking STRENGTH) (*Table 1*).

Active oil: eicosapentaenoic acid vs. eicosapentaenoic acid + docosahexaenoic acid

In the EPA arm of REDUCE-IT, a median -20% change in triglycerides, -1% change in LDL cholesterol, and -14% change in C-reactive protein were observed during the study period (Supplementary material online, *Table S1*).[1] In the CGPS mimicking REDUCE-IT, the hazard ratios [95% confidence interval (CI)] for ASCVD were 0.97 (95% CI 0.95–1.00) for -20% change in triglycerides, 1.00 (1.00–1.00) for -1% change in LDL cholesterol, and 0.97 (0.96–0.98) for -14% change in C-reactive protein (upper estimate of *Figure 1A*). When combining these three changes, the hazard ratio was 0.96 (0.93–0.99) in the CGPS mimicking REDUCE-IT (upper estimate of *Figure 2A*).

In the EPA + DHA arm of STRENGTH, a median -19% change in triglycerides, +1% change in LDL cholesterol, and -20% change in C-reactive protein were observed during the study period (Supplementary material online, *Table S1*).[2] In the CGPS mimicking STRENGTH, the corresponding hazard ratios for ASCVD were 0.97 (95% CI 0.94–1.00), 1.00 (1.00–1.00), and 0.96 (0.95–0.98), respectively (upper estimate of *Figure 1B*). When combining these three changes, the hazard ratio was 0.94 (0.91–0.98) in the CGPS mimicking STRENGTH (upper estimate of *Figure 2B*).

Comparator oil: mineral vs. corn

In the mineral oil arm of REDUCE-IT, a median 0% change in triglycerides, +10% change in LDL cholesterol, and +32% change in C-reactive protein were observed during the study period (Supplementary material online, *Table S1*).[1] In the CGPS mimicking REDUCE-IT, the corresponding hazard ratios for ASCVD were 1.00 (95% CI 1.00–1.00), 1.03 (1.01–1.05), and 1.05 (1.03–1.07) (middle estimate of *Figure 1A*). When combining these three changes, the hazard ratio was 1.07 (1.04–1.10) in the CGPS mimicking REDUCE-IT (middle estimate of *Figure 2A*).

In the corn oil arm of STRENGTH, a median -1% change in triglycerides, -1% change in LDL cholesterol, and -6% change in C-reactive protein were observed during the study period (Supplementary material online, *Table S1*).[2] In the CGPS mimicking STRENGTH, the corresponding hazard ratios for ASCVD were 1.00 (95% CI 1.00–1.00), 1.00 (1.00–1.00), and 0.99 (0.98–0.99), respectively (middle estimate of *Figure 1B*). When combining these three changes, the hazard ratio was 0.99 (0.98–0.99) in the CGPS mimicking STRENGTH (middle estimate of *Figure 2B*).

Between arm difference

The between arm difference in REDUCE-IT resulted in a median -19% change in triglycerides, -10% change in LDL cholesterol, and -40% change in C-reactive protein during the study period (Supplementary material online, *Table S1*).[1] In the CGPS mimicking REDUCE-IT, the corresponding hazard ratios for ASCVD were 0.98 (95% CI 0.95–1.00), 0.97 (0.95–0.99), and 0.91 (0.88–0.95), respectively (lower estimate of *Figure 1A*). When combining these three changes, the hazard ratio was 0.88 (0.84–0.93) in the CGPS mimicking REDUCE-IT (lower estimate of *Figure 2A*), while the hazard ratio for ASCVD in the original REDUCE-IT trial was 0.75 (0.68–0.83).[1]

The between arm difference of STRENGTH resulted in a median -18% change in triglycerides, +3% change in LDL cholesterol, and -11% change in C-reactive protein during the study period (Supplementary material online, *Table S1*).[2] In the CGPS mimicking STRENGTH, the corresponding hazard ratios for ASCVD were 0.97 (95% CI 0.94–1.00), 1.00 (1.00–1.01), and 0.98 (0.97–0.99), respectively (lower estimate of *Figure 1B*). When combining these three changes, the hazard ratio was 0.96 (0.93–0.99) in the CGPS mimicking STRENGTH (lower estimate of *Figure 2B*), while the hazard ratio for ASCVD in the original STRENGTH trial was 0.99 (0.90–1.09).[2]

Combination of other lipid traits and C-reactive protein

In the EPA (active oil) arm of REDUCE-IT, a median -4% change in non-HDL cholesterol and -3% change in apolipoprotein B were observed during the study period (Supplementary material online, *Table S1*).[1] These changes combined with -14% change in C-reactive protein lead to estimated hazard ratios for ASCVD of 0.96 (95% CI 0.95–0.97) and 0.97 (0.95–0.98), respectively (upper estimate of *Figure 2A*). In the EPA + DHA (active oil) arm of STRENGTH, a median -6% change in non-HDL cholesterol and -2% change in apolipoprotein B were observed (Supplementary material online, *Table S1*),[2] leading to corresponding hazard ratios for ASCVD of 0.95 (0.93–0.97) and 0.96 (0.94–0.98), respectively, when combined with -20% change in C-reactive protein (upper estimate of *Figure 2B*).

In the mineral oil (comparator) arm of REDUCE-IT, a median +9% change in non-HDL cholesterol and +8% change in apolipoprotein B were observed during the study period (Supplementary material online, *Table S1*).[1] These changes combined with +32% change in C-reactive protein lead to estimated hazard ratios for ASCVD of 1.08 (95% CI 1.05–1.11) and 1.08 (1.05–1.10), respectively (middle estimate of *Figure 2A*). In the corn oil (comparator) arm of STRENGTH,

Table 1 Baseline characteristics of the study populations

	REDUCE-IT[1,a]	CGPS mimicking REDUCE-IT	STRENGTH[2,b]	CGPS mimicking STRENGTH	Entire CGPS
No.	8179	5684	13 078	6862	106 088
Age (years)	64 (57–69)	70 (63–76)	63[c] (SD 9)	69 (63–75)	58 (48–67)
Women	2357 (28)	2109 (37)	4568 (35)	2731 (40)	58 387 (55)
Body mass index (kg/m2)	31 (28–35)	28 (25–31)	32[c] (SD 6)	27 (25–30)	26 (23–28)
Whites	7379 (90)	5684 (100)	10 723 (82)	6862 (100)	106 088 (100)
ASCVD	5785 (71)	4061 (71)	7316 (56)	3917 (57)	8811 (9)
Diabetes	4787 (59)	2405 (42)	9170 (71)	2327 (34)	4514 (4)
Hypertension	NA[d]	4944 (88)	11 420 (88)	5946 (88)	59 465 (56)
Statins	8179 (100)	5684 (100)	13 078 (100)	6862 (100)	12 759 (12)
Median follow-up (years)	4.9	4.9	3.5	3.5	9.1
Plasma triglycerides					
mmol/L	2.5 (2.0–3.1)	1.6 (1.1–2.3)	2.7 (2.2–3.5)	1.6 (1.1–2.3)	1.4 (1.0–2.1)
mg/dL	217 (177–272)	140 (97–203)	239 (192–307)	142 (98–203)	122 (85–182)
LDL cholesterol					
mmol/L	1.9 (1.6–2.3)	2.1 (1.7–2.6)	1.9 (1.4–2.6)	2.2 (1.7–2.7)	3.2 (2.6–3.8)
mg/dL	74 (62–88)	81 (66–101)	75 (56–99)	85 (67–104)	124 (100–147)
Non-HDL cholesterol					
mmol/L	3.1[e]	2.9 (2.4–3.5)	3.2 (2.7–3.9)	3.0 (2.4–3.6)	3.9 (3.2–4.7)
mg/dL	118[e]	111 (91–133)	125 (104–152)	114 (94–139)	150 (123–180)
Plasma apolipoprotein B (g/L)	0.82[e]	0.88 (0.72–1.07)	0.56 (0.44–0.72)	0.90 (0.74–1.10)	1.05 (0.86–1.28)
Plasma C-reactive protein (mg/L)	2.2 (1.1–4.5)	1.5 (1.0–2.7)	2.1 (1.1–4.2)	1.5 (1.0–2.7)	1.4 (0.9–2.3)
ASCVD during follow-up					
No.	1606[f]	852	1580[f]	697	9418
Events/1000 person-years	40[f,g]	34	35[f,g]	29	10

Values are shown as median (interquartile range) or *n* (%), unless otherwise stated.
ASCVD, atherosclerotic cardiovascular diseases; CGPS, Copenhagen General Population Study; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not available; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; SD, standard deviation; STRENGTH, Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia.
[a]Values for REDUCE-IT represent those for the icosapent ethyl arm except for ASCVD; however, values were similar for the comparator mineral oil arm.
[b]Values for STRENGTH represent those for the omega-3 carboxylic acid arm except for ASCVD; however, values were similar for the comparator corn oil arm.
[c]Mean values.
[d]Prevalence of hypertension was not reported in REDUCE-IT.
[e]Interquartile range was not reported in REDUCE-IT.
[f]Numbers include events from both active and comparator oil arms in REDUCE-IT or STRENGTH.
[g]Events per 1000 person-years in REDUCE-IT or STRENGTH were estimated by number of ASCVD events in both arms during follow-up divided by median follow-up in years multiplied with numbers of individuals.

a median -1% change in non-HDL cholesterol and -1% change in apolipoprotein B were observed (Supplementary material online, *Table S1*),[2] leading to corresponding hazard ratios for ASCVD of 0.99 (0.98–0.99) and 0.99 (0.98–0.99), respectively, when combined with -6% change in C-reactive protein (middle estimate of *Figure 2B*).

The between arm difference resulted in hazard ratios for ASCVD in the CGPS mimicking REDUCE-IT of 0.88 (95% CI 0.84–0.92) for -12% difference of non-HDL cholesterol combined with -40% difference of C-reactive protein and 0.89 (0.85–0.92) for -10% difference of apolipoprotein B combined with -40% difference of C-reactive protein (lower estimate of *Figure 2A*).[1] Correspondingly, the hazard ratios in the CGPS mimicking STRENGTH were 0.97 (0.96–0.99) for -5% difference of non-HDL cholesterol combined with -11% difference of C-reactive protein, and 0.98 (0.97–0.99) for -1% difference of apolipoprotein B combined with -11% difference of C-reactive protein (lower estimate of *Figure 2B*).[2]

Study population

When applying the percent changes in triglycerides, LDL cholesterol, and C-reactive protein in the REDUCE-IT EPA (active oil) arm, the hazard ratio for ASCVD was similar in the CGPS mimicking REDUCE-IT, the CGPS mimicking STRENGTH, and the entire CGPS (upper estimates of *Figure 3A*). This was also true in the mineral oil (comparator) arm and between arms difference (middle and lower estimates of *Figure 3A*). Such results were likewise similar when applying the percent changes in triglycerides, LDL cholesterol, and C-reactive protein in the STRENGTH EPA + DHA (active oil) arm, corn oil (comparator) arm, or between arms difference (*Figure 3B*).

Sensitivity analysis

After additionally applying low triglycerides inclusion criteria in REDUCE-IT to the CGPS mimicking REDUCE-IT and that in STRENGTH to the CGPS mimicking STRENGTH, results were

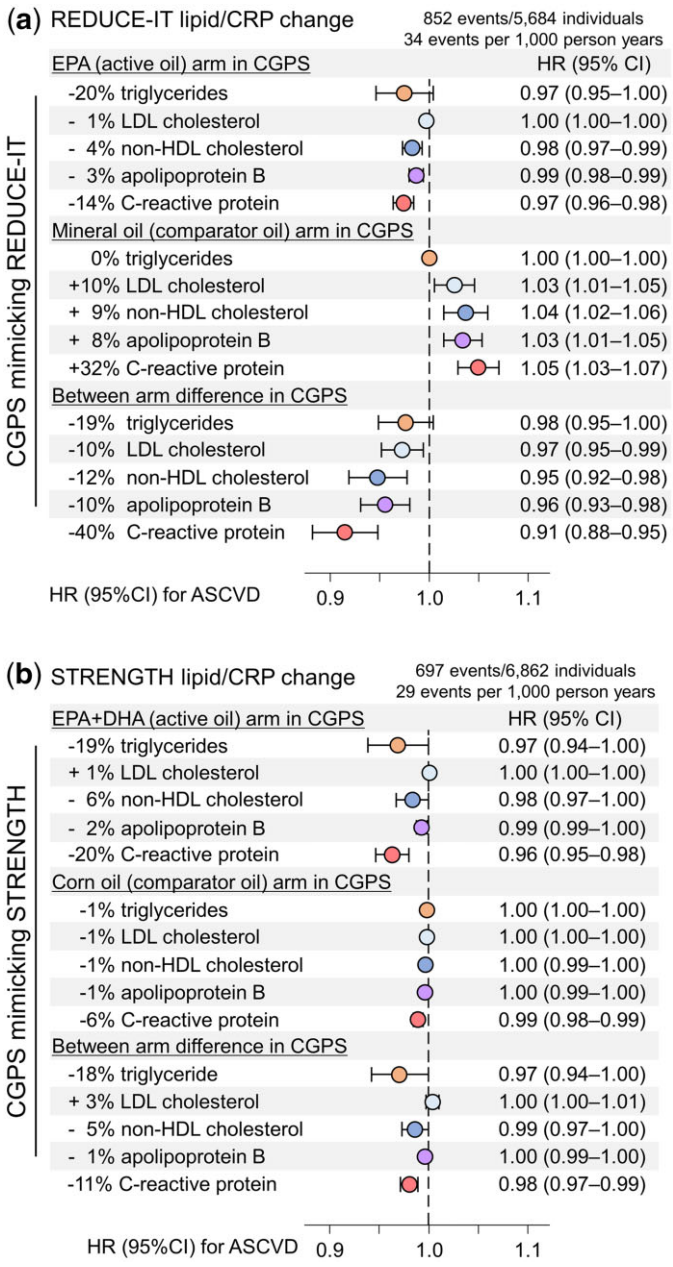


Figure 1 Risk of atherosclerotic cardiovascular disease according to individual percent changes in lipid traits and C-reactive protein in each arm and between arm difference in the CGPS mimicking REDUCE-IT or the CGPS mimicking STRENGTH. We tested the hypothesis that contrasting results in REDUCE-IT vs. STRENGTH can be explained by differences in the effect of active oils (eicosapentaenoic acid vs. eicosapentaenoic acid-+docosahexaenoic acid) and comparator oils (mineral vs. corn) on lipid traits and C-reactive protein. To do so, we used the CGPS to mimic the study populations of REDUCE-IT and STRENGTH and followed them for 4.9 and 3.5 years, respectively, for atherosclerotic cardiovascular disease events, corresponding to the median follow-up time of the two trials.[1,2] Cox regression models were multifactorially adjusted for age (as time scale), sex, smoking status (current, former/never), and systolic blood pressure, but not for other lipid traits or C-reactive protein. Percent change in lipid traits and C-reactive protein levels in active oil arms, comparator oil arms, and between arm differences were as reported in REDUCE-IT[1] or STRENGTH[2] (Supplementary material online, *Table S1*) and examined in (*A*) the CGPS mimicking REDUCE-IT or (*B*) the CGPS mimicking STRENGTH. Hazard ratios are shown in orange dots for percent change in plasma triglycerides, light blue dots for percent change in low-density lipoprotein cholesterol, blue dots for percent change in non-high-density lipoprotein cholesterol, purple dots for percent change in apolipoprotein B, and red dots for percent change in C-reactive protein. ASCVD, atherosclerotic cardiovascular disease; CGPS, Copenhagen General Population Study; CI, confidence intervals; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; STRENGTH, Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia.

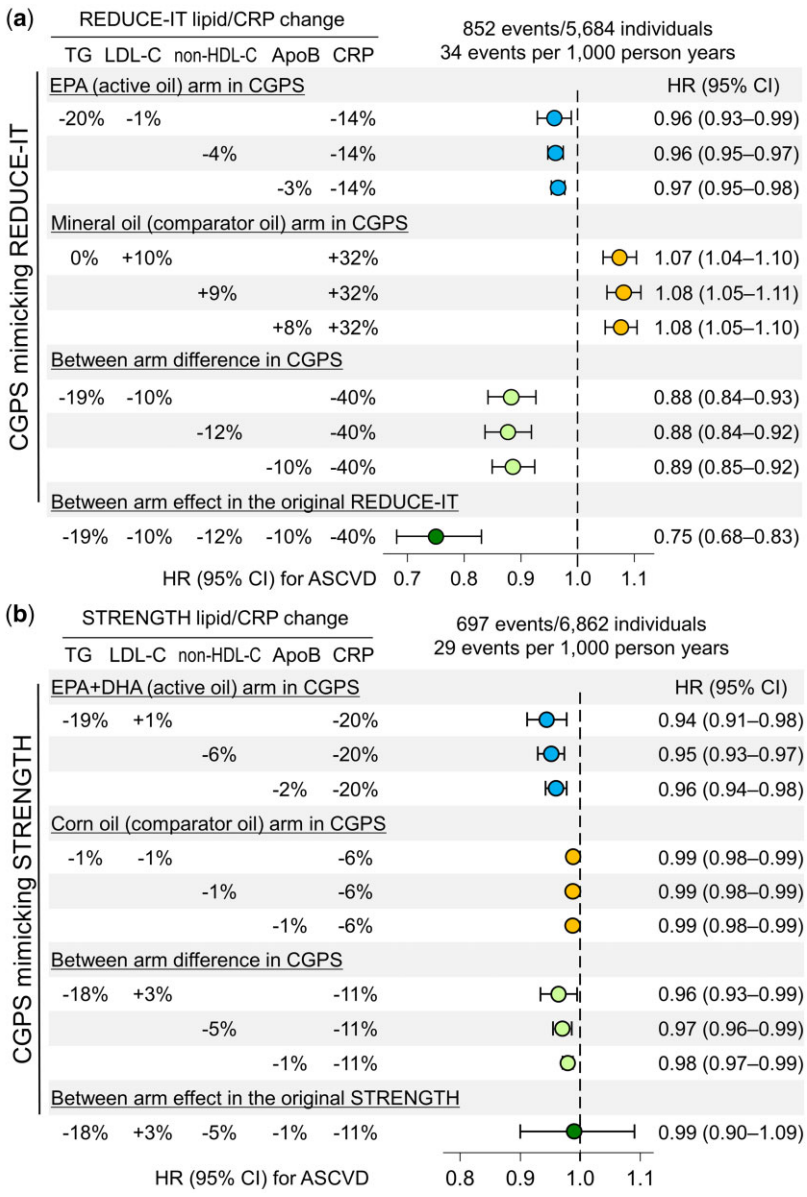


Figure 2 Risk of atherosclerotic cardiovascular disease according to combination of percent changes in lipid traits and C-reactive protein in the CGPS mimicking REDUCE-IT or the CGPS mimicking STRENGTH. We tested the hypothesis that contrasting results in REDUCE-IT vs. STRENGTH can be explained by differences in the effect of active oils (eicosapentaenoic acid vs. eicosapentaenoic acid + docosahexaenoic acid) and comparator oils (mineral vs. corn) on lipid traits and C-reactive protein. To do so, we used the CGPS to mimic the study populations of REDUCE-IT and STRENGTH and followed them for 4.9 and 3.5 years, respectively, for atherosclerotic cardiovascular disease events, corresponding to the median follow-up time of the two trials.[1,2] Cox regression models were multifactorially adjusted for age (as time scale), sex, smoking status (current, former/never), and systolic blood pressure. The blue, yellow, and light green dots represent the hazard ratios for atherosclerotic cardiovascular disease when we examining combinations of percent change in lipid traits and C-reactive protein levels in active oil arms, comparator oil arms, and between arm differences as reported in REDUCE-IT[1] or STRENGTH[2] (Supplementary material online, *Table S1*) and examined in (*A*) the CGPS mimicking REDUCE-IT or (*B*) the CGPS mimicking STRENGTH. The upper estimates in each arm represent the results of changes in triglycerides, low-density lipoprotein cholesterol, and C-reactive protein; the middle estimates in each arm represent the results of changes in non-high-density lipoprotein cholesterol and C-reactive protein; and the lower estimates in each arm represent the results of changes in apolipoprotein B and C-reactive protein. The dark green dots represent the actual hazard ratios for risk of atherosclerotic cardiovascular disease as reported in REDUCE-IT[1] or STRENGTH.[2] ApoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; CGPS, Copenhagen General Population Study; CI, confidence intervals; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; STRENGTH, Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia; TG, triglycerides.

similar to the former results, but with wider 95% CIs due to smaller study population and therefore lower statistical power (compare Supplementary material online, *Figures S1* and *S2* with *Figures 1 and 2*); baseline characteristics of these cohorts are shown in Supplementary material online, *Table S3*. In addition, after additionally applying LDL cholesterol inclusion criteria in REDUCE-IT to the CGPS mimicking REDUCE-IT and LDL and HDL cholesterol inclusion criteria in STRENGTH to the CGPS mimicking STRENGTH (Supplementary material online, *Table S4*), the results were also similar (compare Supplementary material online, *Figures S3 and S4* with *Figures 1 and 2*). When analyses were additionally adjusted for body mass index, diabetes, and HDL cholesterol levels, the results were similar (compare Supplementary material online, *Figures S5 and S6* with *Figures 1 and 2*). Finally, when we used changes in lipid traits and C-reactive protein on absolute scales (Supplementary material online, *Table S2*) rather than percent difference (Supplementary material online, *Table S1*), results were similar (compare Supplementary material online, *Figures S7–S9* with *Figures 1–3*).

Impact of lipids traits and C-reactive protein on atherosclerotic cardiovascular disease

As expected, lower levels of plasma triglycerides, LDL cholesterol, non-HDL cholesterol, apolipoprotein B, and C-reactive protein were all associated with lower risk of ASCVD (Supplementary material online, *Figure S10*). Due to less statistical power, the 95% CI for hazard ratios were naturally wider in the CGPS mimicking REDUCE-IT and the CGPS mimicking STRENGTH than in the entire CGPS.

Discussion

In 5684 and 6862 individuals fulfilling key inclusion criteria of REDUCE-IT (the CGPS mimicking REDUCE-IT) and STRENGTH (the CGPS mimicking STRENGTH), the surprising difference in results between REDUCE-IT and STRENGTH can partly be explained by different effect of comparator oils (mineral vs. corn) on lipid traits and C-reactive protein while effect of different active oils (EPA vs. EPA + DHA) on lipid traits and C-reactive protein and different study populations (high vs. moderate–high risk) could not explain the different results (Graphical Abstract).

Our approach takes the effects of active and comparator oils on lipid traits and C-reactive protein exactly as observed in the two trials[1,2] and insert them in regression models for the risk of ASCVD using the CGPS. In other words, we estimate what changes in risk of ASCVD can be explained by the observed effects on lipid traits and C-reactive protein by active oils and comparator oils. Importantly, our approach does not exclude other possible effects of active and comparator oils on ASCVD unrelated to lipid traits and C-reactive protein. Indeed, in REDUCE-IT, through changes in lipid traits and C-reactive protein, we could only account for 12% of the observed 25% lower risk of ASCVD. Therefore, the unexplained additional 13% risk reduction in REDUCE-IT likely is through other beneficial effects of EPA, or deleterious effects of mineral oil. EPA may have effects on other ASCVD risk factors like blood pressure, platelet activation, oxidative stress, inflammation, endothelial function, plaque phenotype, and lipid levels and metabolism not accounted for, which may delay the onset of atherosclerosis and the clinical sequelae associated with acute plaque rupture.[16–18] In support of this idea, in REDUCE-IT when adjusting for differences between treatment arms in triglycerides, LDL cholesterol, and C-reactive protein, the point estimate of the treatment effect did not change substantially.[19] Also the results from the EVAPORATE (Effect of Vascepa on Improving Coronary Atherosclerosis in People With High Triglycerides Taking Statin Therapy) study, which is a coronary arterial plaque assessment trial comparing high-dose purified EPA plus statins vs. mineral oil plus statins, showed slightly regressed coronary artery plaques in the EPA plus statin group.[20] These observations together support the possibility that effects of EPA beyond reducing lipid traits and C-reactive protein may explain other parts of the risk difference between the EPA and mineral oil arms in the REDUCE-IT trial. That said, we cannot exclude that the unexplained additional 13% risk reduction in REDUCE-IT could represent a chance finding.

Mechanistically, the higher estimated risk of ASCVD in the REDUCE-IT mineral oil arm compared to the STRENGTH corn oil arm is likely explained by the different changes in causal risk factors for ASCVD, that is, triglyceride-rich lipoproteins (marked by plasma triglycerides), LDL cholesterol, and low-grade inflammation (marked by plasma C-reactive protein).[4–9] Indeed, in the REDUCE-IT mineral oil arm, LDL cholesterol, non-HDL cholesterol, apolipoprotein B, and C-reactive protein were all elevated at the end of the study period. In contrast, such changes were minimal in the STRENGTH corn oil arm, even with a trend towards potential protection against ASCVD due to lower C-reactive protein. Surprisingly, changes in the three causal risk factors lead to similar estimated reduced risk of ASCVD in the active oil groups of EPA in REDUCE-IT and of EPA + DHA in STRENGTH. Taken together, increased lipid traits and C-reactive protein may account for increased risk of ASCVD in the mineral oil arm of REDUCE-IT, explaining part of the contrasting results of REDUCE-IT vs. STRENGTH.

One possible explanation of the elevation in lipid traits and C-reactive protein in the mineral oil arm of REDUCE-IT is an inhibited absorption of statins.[21] Mineral oil, which is composed of higher alkanes distilled from petroleum, is hardly absorbable in the human gastrointestinal tract and is considered safe in small amounts by the US Food and Drug Administration (FDA) and by the European Food Safety Authority (EFSA).[22,23] However, as mineral oil interfered with the absorption of lipophilic vitamin A,[24] statins might likewise be less absorbed and therefore in the REDUCE-IT mineral oil arm lead to higher levels of lipid traits and consequently higher C-reactive protein. Also, as mineral oil is used as a mild laxative,[21] mineral oil may lead to larger loss of ingested statins via stools and in consequence lower blood statin levels. Finally, it is not possible to exclude that some mineral oil was absorbed in the intestine leading to deleterious effects including elevated C-reactive protein within the body.[25] In support, in the ANCHOR [Effect of AMR101 (Ethyl Icosapentate) on Triglyceride Levels in Patients on Statins With High Triglycerides Levels (≥200 and <500 mg/dL)] and MARINE [Efficacy and Safety of AMR101 (Ethyl Icosapentate) in Patients With Fasting Triglyceride Levels ≥500 and ≤2000 mg/dL] studies in statin-treated patients, elevation of atherogenic lipoproteins (LDL cholesterol and/or non-HDL cholesterol) and C-reactive protein in the group receiving mineral oil was likewise observed.[26,27] Further, in the EVAPORATE study, coronary plaque progression was found in the mineral oil group.[20] These

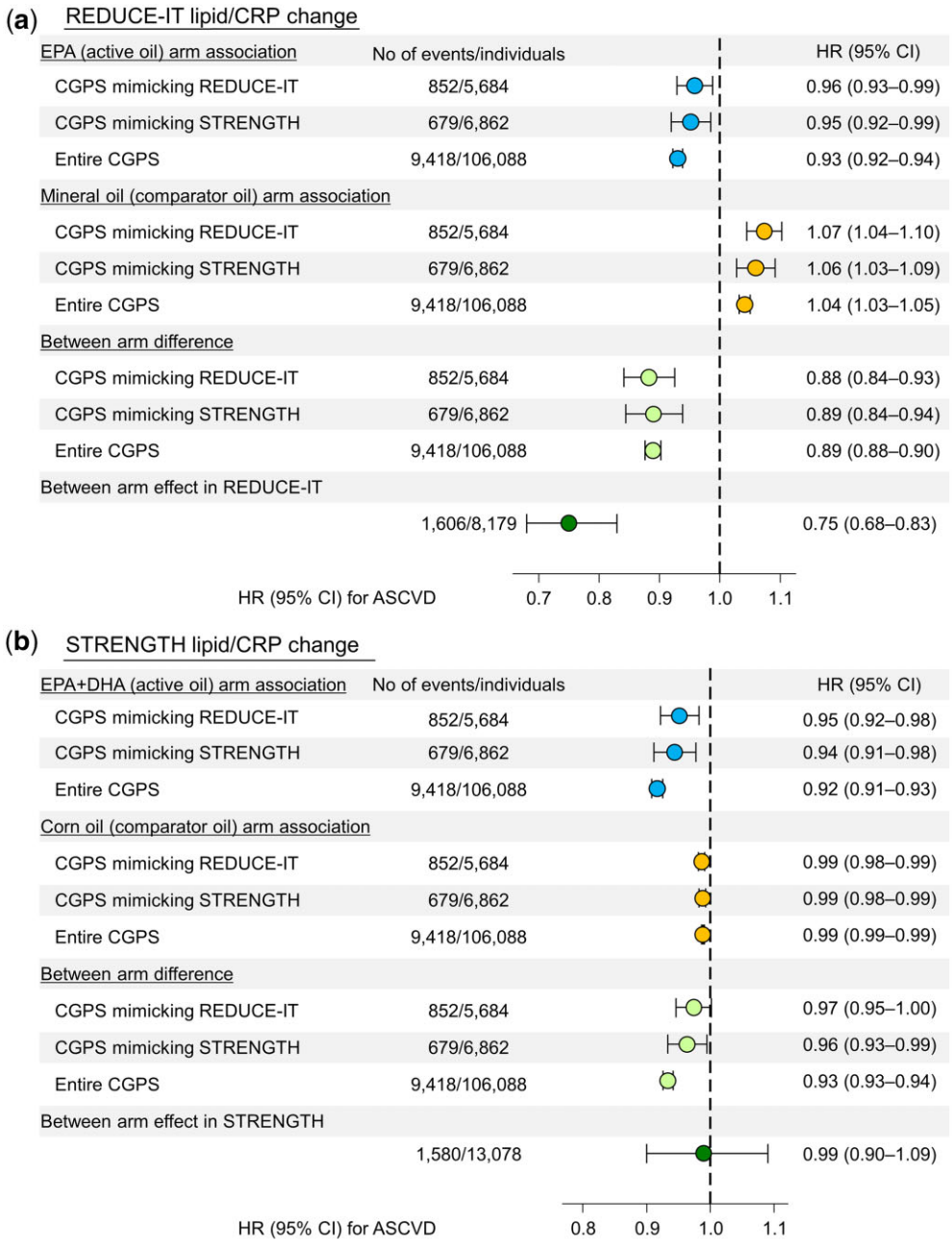


Figure 3 Comparison of study populations. We tested the hypothesis that contrasting results in REDUCE-IT vs. STRENGTH can be explained by differences in study populations (high vs. moderate–high risk). To do so, we used the CGPS to mimic the study populations of REDUCE-IT and STRENGTH and followed them for 4.9 and 3.5 years, respectively, for atherosclerotic cardiovascular disease events, corresponding to the median follow-up time of the two trials.[1,2] Cox regression models were multifactorially adjusted for age (as time scale), sex, smoking status (current, former/never), and systolic blood pressure. Combination of percent change in plasma triglycerides, low-density lipoprotein cholesterol, and C-reactive protein as reported in (*A*) REDUCE-IT[1] or (*B*) STRENGTH[2] (Supplementary material online, *Table S1*) were examined in the CGPS mimicking REDUCE-IT, the CGPS mimicking STRENGTH, or the entire CGPS. The hazard ratios for atherosclerotic cardiovascular disease are shown in blue dots for active oil arms, yellow dots for comparator oil arms, and light green dots for between arm differences. The upper estimates in each arm represent the results adapted in the CGPS mimicking REDUCE-IT study population; the middle estimates in each arm represent the results adapted in the CGPS mimicking STRENGTH study population; and the lower estimates in each arm represent the results adapted in the entire CGPS. The dark green dots represent the actual hazard ratios for the risk of atherosclerotic cardiovascular disease as reported in REDUCE-IT[1] or STRENGTH.[2] ASCVD, atherosclerotic cardiovascular disease; CGPS, Copenhagen General Population Study; CI, confidence intervals; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HR, hazard ratio; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; STRENGTH, Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridaemia.

effects on lipid traits and C-reactive protein by mineral oil may thus have led to progression of coronary artery plaques and thereby increased risk of ASCVD in the REDUCE-IT mineral oil arm, accounting for part of the 'beneficial effect' between the EPA and mineral oil arms in REDUCE-IT.

Both LDL cholesterol and apolipoprotein B decreased in the REDUCE-IT between arm difference,[1] while LDL cholesterol increased slightly and apolipoprotein B decreased minimally in the STRENGTH between arm difference.[2] Moreover, in the STRENGTH EPA + DHA arm, LDL cholesterol levels increased, while apolipoprotein B decreased.[2] In individuals with diabetes, LDL cholesterol is often not elevated as opposed to apolipoprotein B explained by elevated triglyceride-rich lipoproteins. When individuals with diabetes are commenced on EPA and DHA, which decrease hepatic *de novo* lipogenesis, LDL particles become larger, more cholesterol enriched, and triglyceride depleted resulting in increased LDL cholesterol and decreased apolipoprotein B levels.[7,28] Therefore, because apolipoprotein B tracks the risk of ASCVD better than LDL cholesterol in individuals on statin,[29] it seems plausible that the risk of ASCVD was estimated to be lowered even in CGPS mimicking STRENGTH in which LDL cholesterol levels increased slightly.

Based on data from REDUCE-IT, it has previously been estimated that the increase in LDL cholesterol of 0.22 mmol/L (9 mg/dL) in the mineral oil arm could explain a 3% increased risk of ASCVD,[30,31] which is exactly what we observed in the CGPS mimicking REDUCE-IT (Supplementary material online, *Figure S7A*, middle estimate). However, when we examined the combined influence of 0.22 mmol/L (10%) higher LDL cholesterol and 0.5 mg/L (32%) higher C-reactive protein, we estimated that the risk of ASCVD would be higher by 7% (Supplementary material online, *Figure S8A*, middle estimate).

Strengths of our study include (i) high number of individuals fulfilling key inclusion criteria of REDUCE-IT and STRENGTH, (ii) large availability of relevant data, (iii) inclusion of individuals from a general population setting, (iv) endpoints obtained from nationwide Danish health registries, and (v) no individuals lost to follow-up. Potential limitations include that (i) exact statin doses during follow-up were unknown, (ii) use of non-fasting lipid profiles, (iii) we only included white individuals of Danish descent, (iv) lipid changes reported in REDUCE-IT and STRENGTH trials were evaluated over time, while in the CGPS only baseline lipid values were analysed, (v) baseline characteristics were somewhat different between the target populations in REDUCE-IT and STRENGTH and the CGPS mimicking populations regarding parameters such as age, sex, and individuals with diabetes, and (vi) the use and adherence to medications that could affect the outcomes were not taken into consideration in the CGPS; however, such differences will affect the results from the two mimicking studies equally. Ideally, we should also have adjusted for other potential effects of EPA like on endothelial function and platelet activation; however, such information was not available in the CGPS. Finally, total ASCVD events in CGPS mimicking REDUCE-IT and STRENGTH were slightly lower than those in REDUCE-IT and STRENGTH; however, in the entire CGPS with 11- to 14-fold more ASCVD events, results were similar.

A recent meta-analysis including randomized trials of any omega-3 fatty acids with minimal 500 patients and at least 1-year follow-up (including REDUCE-IT and STRENGTH) found an odds ratio for non-fatal myocardial infarction of 0.91 (95% CI 0.83–0.99).[32] Furthermore, in meta-regression, a dose-dependent risk reduction was found for EPA, while DHA was associated with non-fatal myocardial infarction at low, but not at high doses. Interestingly, in bivariate meta-regression including both EPA and DHA, EPA but not DHA conferred a reduced risk for non-fatal myocardial infarction ($P = 0.05$ vs. $P = 0.48$). Such findings[32] together with results from REDUCE-IT[1] support European guideline recommendations to consider use of omega-3 fatty acids (icosapent ethyl 2 × 2 g/day) in combination with a statin in high-risk (or above) patients with triglyceride levels between 1.5 and 5.6 mmol/L (135–499 mg/dL) despite statin treatment.[33]

In conclusion, contrasting results of REDUCE-IT vs. STRENGTH may partly be explained by different effects of comparator oils (mineral vs. corn) on lipid traits and C-reactive protein. This topic is of high clinical relevance, given the uncertainty of the diverging results from REDUCE-IT vs. STRENGTH and the question whether EPA should be recommended as an adjunct to optimal guideline-based therapy for the prevention of ASCVD in patients with hypertriglyceridaemia. Randomized controlled trials comparing EPA vs. EPA + DHA, or vs. corn oil for the assessment of ASCVD prevention are warranted.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: B.G.N. reports consultancies or talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Amarin, Kowa, Denka, Novartis, Novo Nordisk, Esperion, and Silence Therapeutics. T.D. reports talks sponsored by MSD. The other author declared no conflict of interest.

Data availability

The data underlying this article cannot be shared publicly because the Danish data protection agency does not allow open access. However, on reasonable request, additional analyses can be done after contacting the corresponding author.

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