Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kromhout D, Giltay EJ, Geleijnse JM. n–3 Fatty acids and cardiovascular events after myocardial infarction. N Engl J Med 2010;363:2015-26. DOI: 10.1056/NEJMoa1003603.

Webappendix

Manuscript title: N–3 Fatty Acids and Cardiovascular Events after Myocardial Infarction

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Table 1. Chemically Analyzed Fatty Acid Composition of Trial Margarines.[a]

Variable	Margarine type			
	EPA–DHA and ALA	EPA–DHA	ALA	Placebo
Palmitic acid, %[b]	13.2 ± 0.5	13.1 ± 0.4	13.0 ± 0.6	12.6 ± 0.5
Stearic acid, %	3.7 ± 0.2	3.6 ± 0.3	3.7 ± 0.2	3.6 ± 0.3
Oleic acid, %	21.1 ± 1.6	32.3 ± 1.6	23.4 ± 1.7	38.9 ± 1.5
Linoleic acid, %	37.7 ± 1.5	39.7 ± 1.7	39.8 ± 1.9	38.9 ± 1.9
Alpha-linolenic acid, %	13.5 ± 0.8	0.2 ± 0.1	13.7 ± 0.9	0.2 ± 0.1
Eicosapentaenoic acid, %	1.6 ± 0.2	1.6 ± 0.2	--[c]	--
Docosapentaenoic acid, %	0.2 ± 0.1	0.2 ± 0.1	--	--
Docosahexaenoic acid, %	1.2 ± 0.2	1.2 ± 0.1	--	--
Trans fatty acids, %	0.83 ± 0.34	0.64 ± 0.16	0.65 ± 0.29	0.40 ± 0.15
Other fatty acids, %	7.0 ± 1.1	7.6 ± 1.3	5.8 ± 1.5	5.4 ± 0.9
Total fat, g/100g	80.2 ± 0.6	79.7 ± 1.8	80.1 ± 0.6	80.1 ± 1.0
Vitamin E equivalents, mg/100 g	46.7 ± 8.8	47.7 ± 6.0	47.8 ± 6.8	47.2 ± 5.3

Plus–minus values are means ± standard deviations (SD);
ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid;
[a] Based on random sampling from 16 batches that were produced during the trial (2002-2009);
[b] Percentage of total fat content; values in column may not add up to 100% because of rounding;
[c] Not present in that type of margarine.

Table 2. **Changes in Cardiovascular Risk Factors After 40 Months of Intervention, According to N–3 Fatty-Acid Supplementation.**

Variable	n	EPA–DHA and ALA	EPA–DHA	ALA	Placebo
Body mass index, kg/m^2	2,518	0.10 ± 0.07	0.02 ± 0.07	0.07 ± 0.06	–0.08 ± 0.07
Systolic blood pressure, mmHg	2,526	–0.01 ± 0.84	–0.64 ± 0.80	–0.24 ± 0.80	–2.29 ± 0.85
Diastolic blood pressure, mmHg	2,526	–2.26 ± 0.44	–3.17 ± 0.42	–1.79 ± 0.41	–2.75 ± 0.41
Heart rate, bpm	2,526	0.71 ± 0.44	–0.26 ± 0.45	1.31 ± 0.45	0.56 ± 0.43
Plasma glucose, mmol/L[a]	2,433	0.71 ± 0.08	0.70 ± 0.08	0.69 ± 0.09	0.53 ± 0.08
Serum total cholesterol, mmol/L[b]	2,419	–0.30 ± 0.04	–0.26 ± 0.04	–0.30 ± 0.04	–0.28 ± 0.04
Serum LDL cholesterol, mmol/L[b]	2,249	–0.37 ± 0.03	–0.41 ± 0.03	–0.38 ± 0.03	–0.39 ± 0.03
Serum HDL cholesterol, mmol/L[b]	2,418	0.14 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Serum triglycerides, mmol/L[c]	2,419	–0.14 ± 0.03	–0.08 ± 0.05	–0.11 ± 0.04	–0.05 ± 0.04

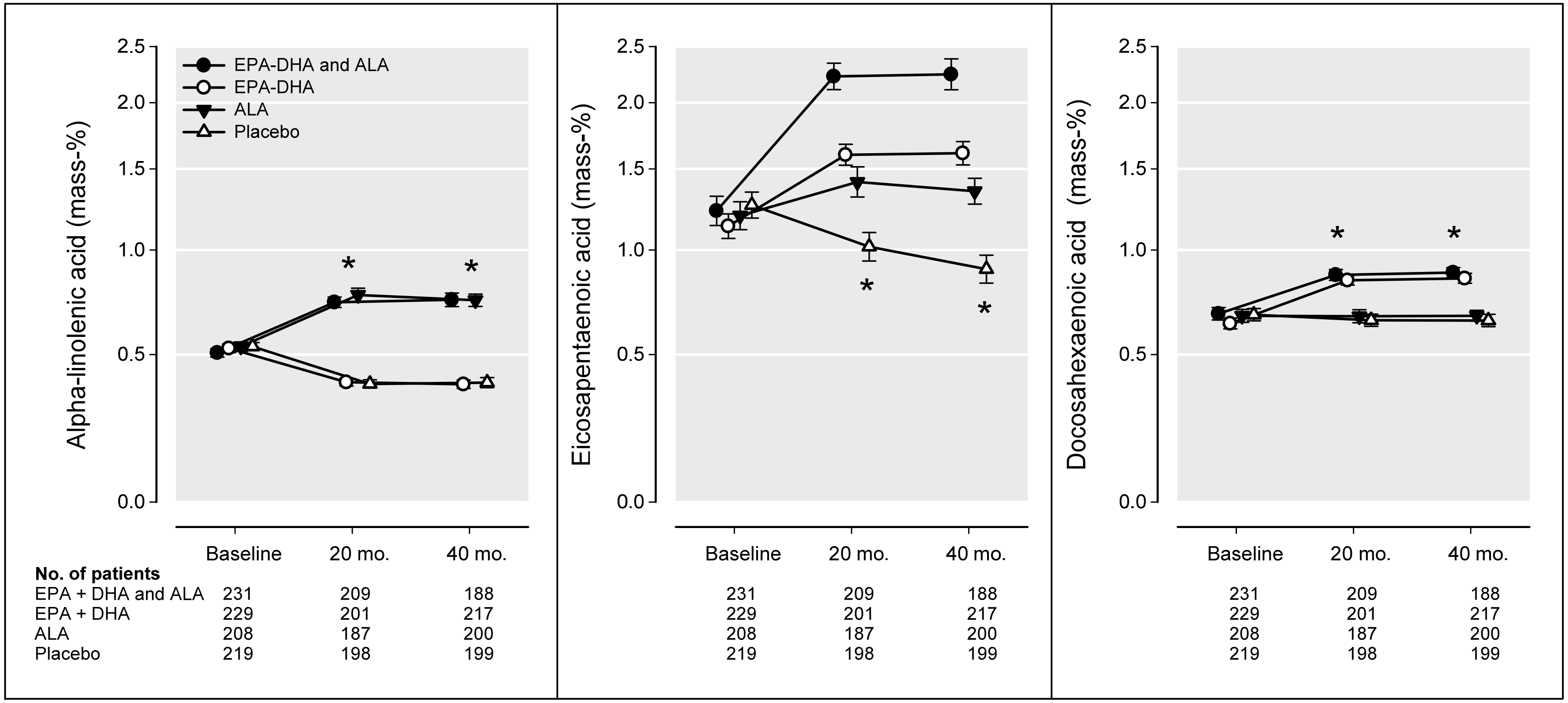
Plus–minus values are means ± standard error (SE);
Changes in active treatment groups were not statistically different from placebo (all P > 0.05);
ALA, alpha-linolenic acid; bpm, beats per minute; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid;
HDL, high-density lipoprotein; LDL, low-density lipoprotein;
[a] Non-fasting; to convert the values for glucose to milligrams per deciliter, divide by 0.05551;
[b] Non-fasting; to convert the values for cholesterol to milligrams per deciliter, divide by 0.02586;
[c] Non-fasting; to convert the values for triglycerides to milligrams per deciliter, divide by 0.01129.

Table 3. Adverse Events During the Alpha Omega Trial, According to N–3 Fatty-Acid Supplementation.

Variable	EPA–DHA and ALA (n=1,212)	EPA–DHA (n=1,192)	ALA (n=1,197)	Placebo (n=1,236)	P Value
Gastrointestinal problems, n (%)	16 (1.3)	18 (1.5)	9 (0.8)	10 (0.8)	0.20
Incident prostate cancer, n (% of men)	15 (1.6)	11 (1.2)	8 (0.9)	8 (0.8)	0.35
Cancer mortality, n (%)	30 (2.5)	33 (2.8)	32 (2.7)	27 (2.2)	0.80
Side effect reported to DSMB[a], n (%)	0 (0.0)	0 (0.0)	1 (0.1)	3 (0.2)	0.12

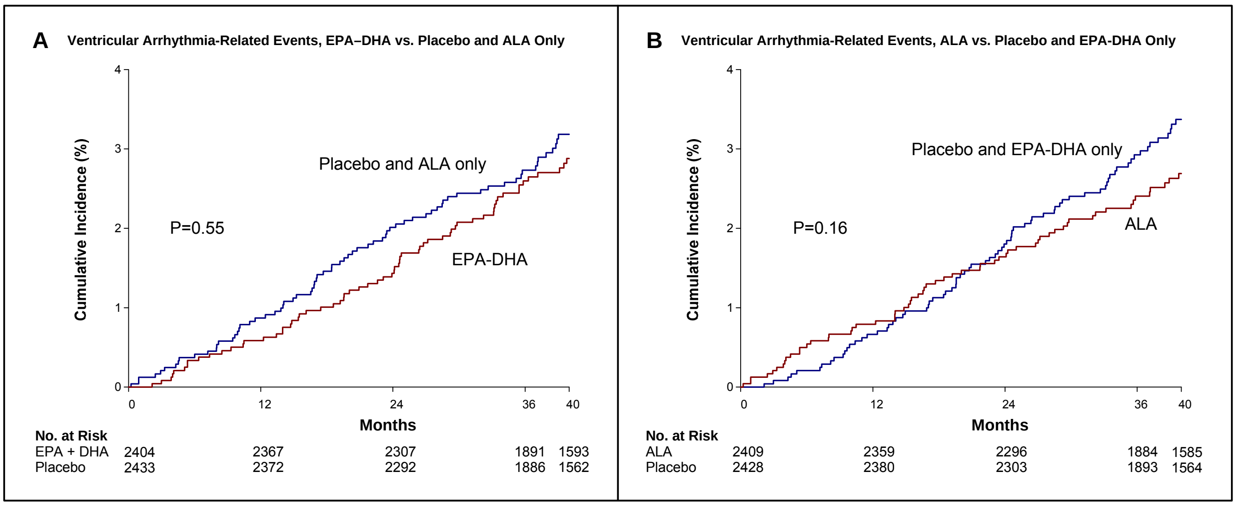
ALA, alpha-linolenic acid; DSMB, Data Safety and Monitoring Board; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid;
P-value obtained by Chi^2 test;
[a] Side effects reported to DSMB do not overlap with other adverse effects.

Figure 1. ALA, EPA and DHA Concentrations in Plasma Cholesteryl Esters at Baseline, 20 and 40 Months in Random Samples of Post-MI Patients, According to N–3 Fatty-Acid Supplementation.



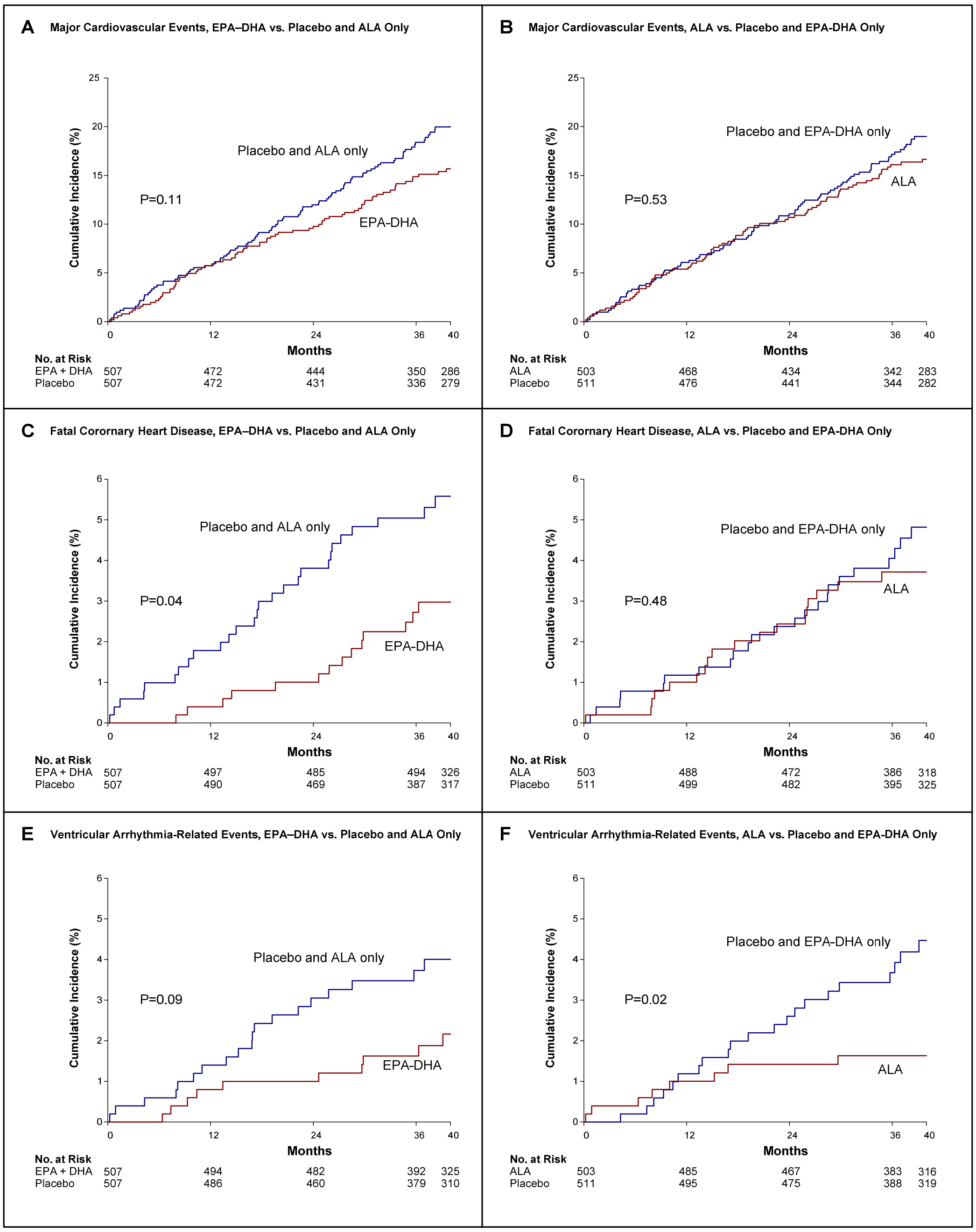
Geometric mean values (expressed as mass percentage) with error bars indicating 95% confidence intervals, on a logarithmic scale;
After 20 months, ALA supplementation in the margarine increased serum ALA by 69.6% and EPA by 43.9% as compared with placebo and EPA–DHA only, and EPA–DHA supplementation increased serum EPA by 53.3% and serum DHA by 28.6%, as compared with placebo and ALA only;
After 40 months, ALA supplementation in the margarine increased serum ALA by 68.8% and EPA by 41.6% as compared with placebo and EPA–DHA only, and EPA–DHA supplementation increased serum EPA by 61.6% and serum DHA by 30.0%, as compared with placebo and ALA only;
* P < 0.001 for group difference at that time point, obtained by ANOVA.

Figure 2. Kaplan-Meier Curves for the Secondary End Point of Ventricular Arrhythmia-Related Events, According to EPA–DHA and ALA Supplementation.



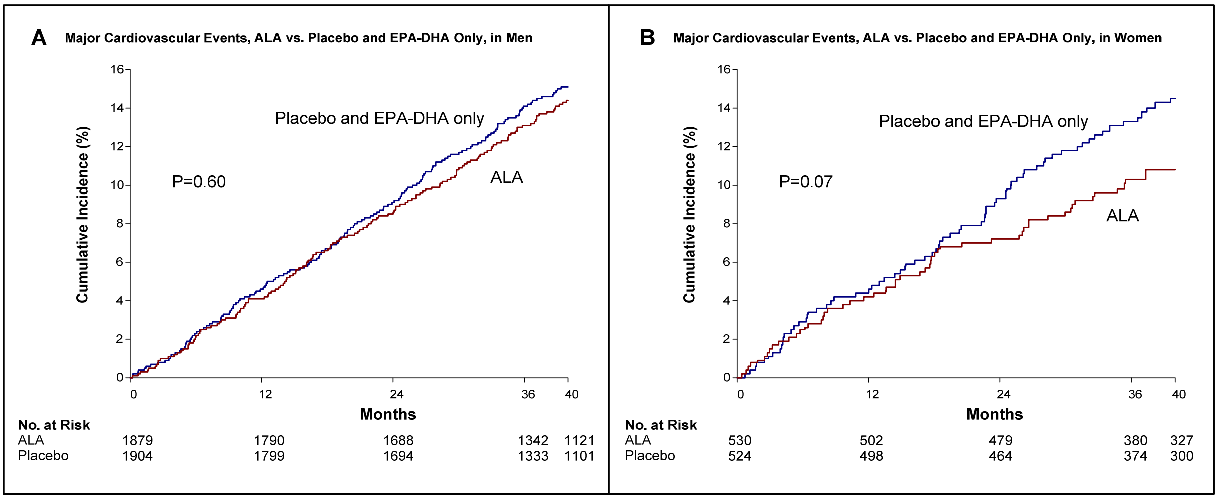
Panel A: findings for EPA–DHA vs. placebo and ALA alone;
Panel B: findings for ALA vs. placebo and EPA–DHA alone;
Ventricular arrhythmia-related events comprise nonfatal and fatal cardiac arrest, sudden death, and implantable cardioverter-defibrillator placement.

Figure 3. Kaplan-Meier Curves for Primary and Secondary End Points in Patients with Diabetes Mellitus, According to EPA–DHA and ALA Supplementation.



Panel A: findings for major cardiovascular events for EPA–DHA *vs.* placebo and ALA alone;
Panel B: findings for major cardiovascular events for ALA *vs.* placebo and EPA–DHA alone;
Panel C: findings for fatal coronary heart disease for EPA–DHA *vs.* placebo and ALA alone;
Panel D: findings for fatal coronary heart disease for ALA *vs.* placebo and EPA–DHA alone;
Panel E: findings for ventricular arrhythmia-related events[a] for EPA–DHA *vs.* placebo and ALA alone;
Panel F: findings for ventricular arrhythmia-related events[a] for ALA *vs.* placebo and EPA–DHA alone;
[a] Ventricular arrhythmia-related events comprise nonfatal and fatal cardiac arrest, sudden death, and implantable cardioverter-defribrillator placement.

Figure 4. **Kaplan-Meier Curves for the Primary End Point of Major Cardiovascular Events, According to ALA Supplementation and Gender.**



Panel A: findings for ALA vs. placebo and EPA–DHA alone for men;
Panel B: findings for ALA vs. placebo and EPA–DHA alone for women.

Power Calculations for the Alpha Omega Trial

Originally, we calculated the sample size needed to detect an effect of EPA–DHA on fatal CHD, with the following assumptions: (1) incidence of CHD mortality of 4% per year in post-MI patients aged 60 to 80 years (on placebo), based on trials carried out in the 1990s; (2) relative risk of CHD mortality of 0.75 (i.e., a 25% risk reduction compared to placebo) for low-dose treatment with EPA–DHA and (3) treatment duration of 3 years. For a two-sided α of 0.05 and a power of 80%, at least 3,408 subjects would be needed to demonstrate an effect of EPA–DHA, and we targeted at a sample size of 4,000 subjects. For ALA, under the assumption of a relative risk of 0.80, the power for demonstrating an effect on CHD mortality with this number of subjects was 67%.

During the course of the study, the overall mortality rate was 23 per 1,000 person-years (Geleijnse et al, Am Heart J 2010). This rate was substantially lower than anticipated, likely due to improved therapy of MI patients in the acute phase and more effective pharmacologic treatment of hyperlipidemia and hypertension in recent years. For this reason, the Steering Committee approved an increase in sample size to a total of 4,800 subjects and an extended duration of intervention, that is, from 36 to 40 months.

Based on the actual figures obtained in the trial, we performed several post-hoc power calculations. For major cardiovascular events, we had a power of 96% to detect a true hazard ratio of 0.75 and a power of 82% to detect a hazard ratio of 0.80. These calculations are based on a total of 4,837 patients, 40 months of follow-up, and a two-sided α of 0.05. For fatal CHD, we had more than 75% power for detecting hazard ratios below 0.60. For a hazard ratio of 0.75 for fatal CHD, however, the power was only 35% due to the limited number of cases that occurred in the Alpha Omega Trial.

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Executive Committee: D. Kromhout (principal investigator), E.G. Schouten (from 2002-–2005, coprincipal investigator), J.M. Geleijnse (trial coordinator), J. de Goede (trial assistant), L.M. Oude Griep (data quality monitor), A.M. Teitsma-Jansen (logistics manager), E. Waterham (data manager) – all at the Division of Human Nutrition, Wageningen University, Wageningen; E.J. Giltay (study physician; Department of Psychiatry, Leiden University Medical Center, Leiden).
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