European Heart Journal (2012) **33**, 1582–1588
doi:10.1093/eurheartj/ehr499

Effects of *n*-3 fatty acids on major cardiovascular events in statin users and non-users with a history of myocardial infarction

Simone R.B.M. Eussen[1,2], Johanna M. Geleijnse[3], Erik J. Giltay[4], Cathy J.M. Rompelberg[2], Olaf H. Klungel[1], and Daan Kromhout[3]*

[1]Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands; [2]National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands; [3]Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; and [4]Department of Psychiatry, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands

Received 30 September 2011; revised 30 November 2011; accepted 19 December 2011; online publish-ahead-of-print 1 February 2012

See page 1555 for the editorial comment on this article (doi:10.1093/eurheartj/ehs063)

Aims Recent secondary prevention trials have failed to demonstrate a beneficial effect of *n*-3 fatty acids on cardiovascular outcomes, which may be due to the growing use of statins since the mid-1990s. The aim of the present study was to assess whether statins modify the effects of *n*-3 fatty acids on major adverse cardiovascular events in patients with a history of myocardial infarction (MI).

Methods and results Patients who participated in the Alpha Omega Trial were divided into consistent statin users ($n = 3740$) and consistent statin non-users ($n = 413$). In these two groups of patients, the effects of an additional daily amount of 400 mg eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), 2 g α-linolenic acid (ALA), or both on major cardiovascular events were evaluated. Multivariable Cox's proportional hazard models were used to calculate adjusted hazard rate ratios (HR_{adj}). Among the statin users 495 (13%) and among the statin non-users 62 (15%) developed a major cardiovascular event. In statin users, an additional amount of *n*-3 fatty acids did not reduce cardiovascular events [HR_{adj} 1.02; 95% confidence interval (CI): 0.80, 1.31; $P = 0.88$]. In statin non-users, however, only 9% of those who received EPA–DHA plus ALA experienced an event compared with 18% in the placebo group (HR_{adj} 0.46; 95% CI: 0.21, 1.01; $P = 0.051$).

Conclusion In patients with a history of MI who are not treated with statins, low-dose supplementation with *n*-3 fatty acids may reduce major cardiovascular events. This study suggests that statin treatment modifies the effects of *n*-3 fatty acids on the incidence of major cardiovascular events.
ClinicalTrials.gov number: NCT00127452.

Keywords *n*-3 fatty acids • Eicosapentaenoic acid • Docosahexaenoic acid • α-linolenic acid • Cardiovascular diseases • Statins • Lipids

Introduction

The landmark Scandinavian Simvastatin Survival Study (4S)[1] and subsequent randomized controlled trials[2] showed beneficial effects of statins on mortality and morbidity in patients with and without previous myocardial infarction (MI) or other coronary heart disease (CHD). Ever since, statins have been the first choice of drug treatment for preventing and treating cardiovascular disease (CVD). The benefits of statins were first attributed solely to their ability to inhibit hepatic cholesterol synthesis, thereby improving serum lipid levels. Depending on the type, dose, and baseline levels, statins reduce LDL cholesterol by 18–55% and triglycerides by 7–30% and increase HDL cholesterol up to 15%.[3] Yet, over the years, multiple lipid-independent pleiotropic effects of statins have been described. Statins have, for example, beneficial effects on endothelial function, inflammation, and coagulation, independent of lipid lowering.[4]

* Corresponding author. Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands. Tel: +31 317 483054, Fax: +31 317 483342, Email: daan.kromhout@wur.nl

A healthy lifestyle is promoted for CVD prevention. Lifestyle changes include smoking cessation, increased physical activity level, and adopting a healthier diet. Dietary guidelines emphasize the importance of *n*-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[5] A meta-analysis of both prospective cohort studies and trials showed that 250 mg/day of EPA–DHA reduced fatal CHD by 36% compared with no EPA–DHA.[6] Fish consumption, the major source of EPA–DHA in the diet, was inversely related to incident stroke in a meta-analysis of cohort studies.[7] Less evidence exists for a protective effect of α-linolenic acid (ALA), the plant-derived *n*-3 fatty acid, against CVD.[8,9]

Although adding *n*-3 fatty acids to statin therapy leads to significant reductions in triglyceride levels,[10] it has also been suggested that the use of guideline-concordant statin therapy dilutes the effects of *n*-3 fatty acids such that no additional protective effect is observed.[11] This hypothesis is supported by the reduction in cardiovascular events through either fatty fish or EPA–DHA in trials in which less than one-third of the participants were on statin therapy (DART[12] and GISSI-Prevenzione[13]). *n*-3 fatty acids did not reduce major cardiovascular events in three recently conducted trials with a large number of statin users. The OMEGA trial showed that guideline-adjusted drug treatment—including statin use in >90% of the post-MI patients—resulted in a low risk of cardiovascular events which could not be further reduced by 840 mg EPA–DHA daily.[14] Also in the SU.FOL.OM3 trial, no significant difference was detected in major vascular events between coronary artery disease patients allocated to 600 mg EPA–DHA daily and those allocated to placebo.[15] Finally, we showed in the Alpha Omega Trial no reduction in cardiovascular events in 4837 post-MI patients who were randomized to an additional amount of EPA–DHA (400 mg/day) and/or ALA (2 g/day), compared with placebo.[16]

The aim of the present study was to assess whether the effects of EPA–DHA and/or ALA on major cardiovascular events in the Alpha Omega Trial differed between statin users and statin non-users.

Methods

Study population

The Alpha Omega Trial is a multicentre, double-blind, placebo-controlled trial of low doses of *n*-3 fatty acids (400 mg/day EPA–DHA and/or 2 g/day ALA) on the risk of fatal and non-fatal major cardiovascular events. The trial was approved by a central medical Ethics Committee (Haga Hospital, Leyenburg, The Hague, The Netherlands) and by the Ethics Committee at each participating hospital. All subjects signed informed consent before entering the study. Details of the Alpha Omega Trial have been described elsewhere.[16,17] Briefly, 4837 free-living Dutch post-MI patients aged 60–80 years were randomized to receive one of four margarines: an EPA–DHA-enriched, an ALA-enriched, an EPA–DHA plus ALA-enriched margarine or a placebo margarine. Patients were enrolled from April 2002 through December 2006 and were followed for an average of 41 months.

At baseline, anthropometric measures were obtained and blood pressure, heart rate, lipid and glucose levels were determined. Information on demographic variables, lifestyle habits, current health status and medical history were collected by self-administered questionnaires. Baseline measurements were repeated after 20 months of the intervention in a random sample of 810 participants, and after 41 months in the 2531 participants who completed the trial before 1 January 2009. For the remaining participants, due to budgetary constraints, physical examination and blood sampling were not repeated and data were collected by questionnaires at the end of follow-up.[17]

Assessment of statin use

Questionnaires on medication use were filled out by all participants at baseline and after 41 months. Subjects were asked to record changes in medication use in a structured diary, and medication was checked during structured telephone interviews after 12 and 24 months. All drugs were coded according to the Anatomical Therapeutic Chemical (ATC) classification[18] by two of the authors (S.R.B.M.E. and O.H.K.). Subjects who reported the use of statins (ATC codes C10AA and C10B) at all measurements (at baseline and at 12-, 24-, and after on average 41-month follow-up) were classified as statin users. Those who were not using statins at any time point were classified as non-users. Subjects who initiated or stopped statin therapy during the trial and inconsistent statin users who used statins at some, but not all, time points were excluded.

Endpoint

The vital status of the participants was monitored via a computerized link with municipal registries. For patients who experienced a fatal event during follow-up, general practitioners, hospitals, and family members were approached to ascertain the primary and contributing causes of death. The occurrence of non-fatal major cardiovascular events (MI, cardiac arrest, and stroke) and cardiac interventions (percutaneous coronary intervention and coronary artery bypass grafting) was monitored by annual telephone interviews conducted by research staff and verified against hospital records. The primary endpoint of this study was the rate of major cardiovascular events, which comprised fatal CVDs, non-fatal MI, non-fatal cardiac arrest, non-fatal stroke, and cardiac interventions (percutaneous coronary intervention and coronary artery bypass grafting).[16]

Statistical analysis

Demographic and health characteristics of the participants who received the different margarines, stratified for statin users and non-users, were compared by using Student's *t*-test or the Mann–Whitney *U*-test for continuous variables and the χ^2-test for nominal variables.

Uni- and multivariable general linear regression models were used to assess differences in changes in lipid levels over time among statin users and non-users randomized to *n*-3 fatty acid supplementation or placebo. Uni- and multivariable Cox's proportional hazard models were used to estimate hazard rate ratios (HR) for major cardiovascular events with the placebo group as reference. Fixed effects in the models were the *n*-3 fatty acids and the use of statins. To test whether the effect of EPA–DHA and/or ALA differed between patients with and without statins, the product term of *n*-3 fatty acids and statins was added to the models.

In the multivariable models, we adjusted for age, gender, and diabetes mellitus.[16] In addition, we checked whether inclusion one by one of other potential confounding variables altered the relationship of EPA–DHA and/or ALA with major cardiovascular events by ≥10%. We selected the following potential confounders: baseline levels of body mass index, current smoking (yes/no), physical activity level (≥5 or <5 days/week engaged in physical activity with a Metabolic Equivalent TASK score>3), self-reported history of stroke, dietary EPA–DHA intake, alcohol consumption (≥1 or <1 glass/week), ratio of total to HDL cholesterol, serum triglyceride levels, systolic blood pressure, current use of blood pressure-lowering

medication (ATC codes C02, C03, C07, C08, and C09), antithrombotic agents (B01), and hormone replacement therapy (G03).

Results

Demographic and health characteristics of the patients

Of the 4837 patients who were enrolled in the Alpha Omega Trial, 3740 (77%) patients were consistent statin users and 413 (9%) patients were consistent statin non-users. The remainder of the patients ($n = 684$, 14%) were starters, stoppers, or inconsistent users of statins and were for this reason excluded from the study. The mean age of all participants was 68.9 $\pm$ 5.6 years and 78% were males. The median time since last MI before study entry was 3.7 years (inter-quartile range: 1.7–6.3). For statin users as well as for statin non-users, the four study groups receiving placebo, EPA–DHA only, ALA only, or EPA–DHA plus ALA were similar for most characteristics (*Table 1*). Among statin users, significant differences between study groups were observed for the use of blood pressure-lowering drugs, triglyceride levels, and consumption of fish. Among statin non-users, significant differences between study groups were observed for the percentage of patients with diabetes mellitus, self-reported stroke, the use of antithrombotic drugs, physical activity, and plasma glucose and serum triglyceride levels.

Effects of *n*-3 fatty acids on lipid levels

Table 2 presents the average changes in lipid levels between baseline and 41-month follow-up among statin users and statin non-users, respectively. No significant effects were observed in the groups receiving EPA–DHA only and ALA only. Yet, the combination of EPA–DHA and ALA reduced triglycerides significantly by 0.17 mmol/L in statin users.

Effects of *n*-3 fatty acids on major cardiovascular events

No patient was lost to follow-up and hence all patients' data were included in the Cox proportional hazard analysis. During 12 048 persons-years of follow-up, 495 (13%) statin users had a major cardiovascular event. For statin non-users, 1234 persons-years of follow-up were accumulated and 62 (15%) major cardiovascular events occurred. Among statin users, there was no significant difference in the rate of major cardiovascular events between the four groups (*Table 3*). Supplementation with EPA–DHA only or with ALA only did not reduce major cardiovascular events in statin non-users. However, 9% of the statin non-users who received EPA–DHA plus ALA had a major cardiovascular event during the 41-month follow-up period compared with 18% of the patients in the placebo group. Statin non-users receiving EPA–DHA plus ALA had a 54% lower incidence of major cardiovascular events compared with the placebo group, which was borderline statistically significant (HR_{adj} 0.46; 95% confidence interval: 0.21, 1.01; $P = 0.051$). The effect of the combination of EPA–DHA plus ALA on major cardiovascular events was borderline statistically significantly different between statin users and non-users ($P = 0.057$).

Discussion

The present study suggests that statin treatment modifies the effects of *n*-3 fatty acids on the incidence of major cardiovascular events. In statin users, additional *n*-3 fatty acids had no effect on major cardiovascular events, despite a significant reduction in triglycerides. In statin non-users, reductions in major cardiovascular events due to EPA–DHA alone or ALA alone were 18 and 10%, respectively, and not statistically significant. However, combined effects of EPA–DHA plus ALA accumulated to 54% ($P = 0.051$). This is consistent with the hypothesis that the effects of EPA–DHA and ALA alone are additive and independent, although this has been disputed in a recent review.[19]

The Alpha Omega Trial is the first double-blind placebo-controlled trial in which the effect of adding 400 mg EPA–DHA and/or 2 g ALA/day on major cardiovascular events was investigated. Other large randomized controlled trials have concentrated on the effect of consuming EPA–DHA alone.[20] Apart from the Alpha Omega Trial, also the recently published OMEGA trial[14] and the SU.FOL.OM3 trial,[15] both carried out in cardiac patients, failed to show a reduction in major cardiovascular events after a moderate additional intake of, respectively, 840 and 600 mg EPA–DHA per day. In all three trials, at least 85% of the patients were treated with statins.[20] However, in the 11 years earlier published GISSI-Prevenzione (GISSI-P) trial,[13] treatment with 850–882 mg daily of EPA–DHA decreased major cardiovascular events defined as fatal CVD plus non-fatal MI and non-fatal stroke by 20% in patients after a recent MI. In this trial, the percentage of statin users increased from 5% at baseline to 29% after 6 months and to 46% at the end of the trial. Baseline total to HDL cholesterol ratio was 5.1 and clinically important changes in total and HDL cholesterol were not observed during the course of the trial. In the Alpha Omega Trial, supplementation with 400 mg daily of EPA–DHA did not reduce major cardiovascular events. Eighty-five per cent of the participants in this trial were on statin treatment and baseline ratio of total to HDL cholesterol ratio was 3.9, i.e. 1.2 unit lower than in GISSI-P (*Table 4*). Yet, statin non-users who had an average total to HDL cholesterol ratio of 4.6 experienced 18% fewer major cardiovascular events, a finding which was not statistically significant but in the same order of magnitude as in the GISSI-P trial.

The JELIS trial[21] was carried out in patients with a serum total cholesterol level of 6.5 mmol/L or more and contained a primary and secondary prevention group. The latter one consisted of 3664 cardiac patients who were followed for an average of 55 months. The mean serum total cholesterol at baseline was 6.97 mmol/L and the mean HDL cholesterol level was 1.43 mmol/L. During follow-up, total cholesterol decreased by 19% and HDL increased by 3%. The average total/HDL cholesterol ratio decreased from 4.9 to 3.8. At this low total/HDL cholesterol ratio, an additional amount of 1.8 g EPA per day reduced the number of major cardiovascular events by 19% ($P < 0.05$). The statin users in the Alpha Omega Trial had a very similar average total/HDL cholesterol ratio of 3.7, but in these patients, an additional amount of 400 mg EPA–DHA, 2 g ALA, or both did not reduce the number of major cardiovascular events (*Table 3*). These results suggest that in cardiac patients with

Table 1 Baseline characteristics of users and non-users of statins randomized to placebo or *n*-3 fatty acid supplementation in the Alpha Omega Trial

	Statin users (*n* = 3740)				Statin non-users (*n* = 413)			
	Placebo (*n* = 943)	EPA–DHA (*n* = 920)	ALA (*n* = 930)	EPA–DHA + ALA (*n* = 947)	Placebo (*n* = 113)	EPA–DHA (*n* = 102)	ALA (*n* = 102)	EPA–DHA + ALA (*n* = 96)
Age, years	68.7 ± 5.6	68.7 ± 5.5	68.5 ± 5.3	68.8 ± 5.5	70.4 ± 5.8	71.5 ± 5.2	71.8 ± 6.3	71.2 ± 5.9
Male gender, *n* (%)	745 (79)	718 (78)	734 (79)	751 (79)	85 (75)	72 (71)	78 (76)	66 (69)
Median time since MI, years	3.4 (1.5–6.2)	3.6 (1.7–6.3)	3.6 (1.7–6.3)	3.6 (1.6–6.2)	4.8 (2.8–6.3)	4.7 (2.8–7.3)	5.1 (1.8–7.2)	4.6 (2.2–7.2)
Self-reported history of stroke, *n* (%)	58 (6)	61 (7)	67 (7)	60 (6)	11 (10)[a,b]	10 (10)[a,b]	6 (6)[b]	14 (15)[a]
Use of cardiovascular medication, *n* (%)								
Antithrombotic agents	930 (99)	909 (99)	919 (99)	925 (98)	105 (93)[a,b]	92 (90)[a,b]	98 (96)[b]	82 (85)[a]
Antihypertensive agents	856 (90)[a,b]	853 (93)[a]	835 (90)[b]	856 (90)[a,b]	97 (86)	84 (88)	97 (86)	85 (83)
Blood pressure, mmHg								
Systolic	141.6 ± 20.9	142.3 ± 21.3	141.2 ± 20.9	140.8 ± 22.2	141.5 ± 23.6	141.7 ± 23.7	141.7 ± 23.7	143.5 ± 26.6
Diastolic	80.0 ± 10.5	80.5 ± 11.3	80.3 ± 11.0	80.2 ± 11.1	80.2 ± 13.2	77.4 ± 12.2	77.4 ± 12.2	80.2 ± 11.8
Plasma glucose, mmol/L	6.25 ± 2.10	6.21 ± 2.10	6.17 ± 1.95	6.18 ± 2.27	6.07 ± 1.61[a]	5.64 ± 1.42[b]	6.09 ± 1.73[a]	6.46 ± 2.25[a]
Serum lipids, mmol/L								
Total cholesterol	4.59 ± 0.88	4.61 ± 0.86	4.56 ± 0.84	4.55 ± 0.84	5.61 ± 1.15	5.58 ± 1.14	5.51 ± 1.03	5.50 ± 1.00
LDL cholesterol	2.44 ± 0.73	2.48 ± 0.73	2.43 ± 0.71	2.44 ± 0.70	3.49 ± 1.03	3.42 ± 0.84	3.34 ± 0.94	3.28 ± 0.82
HDL cholesterol	1.29 ± 0.34	1.29 ± 0.35	1.29 ± 0.34	1.29 ± 0.32	1.25 ± 0.41	1.30 ± 0.37	1.26 ± 0.34	1.33 ± 0.41
TC/HDL cholesterol ratio	3.76 ± 1.05	3.76 ± 1.03	3.73 ± 1.03	3.68 ± 0.95	4.75 ± 1.23	4.55 ± 1.25	4.58 ± 1.15	4.47 ± 1.40
Median triglycerides, mmol/L (range)	1.68 (1.22–2.38)[b]	1.62 (1.24–2.29)[a,b]	1.61 (1.19–2.28)[a,b]	1.59 (1.18–2.20)[a]	1.75 (1.37–2.42)[b]	1.59 (1.17–2.18)[a]	1.79 (1.31–2.46)[a,b]	1.52 (1.22–2.25)[a,b]
BMI, kg/m^2	27.9 ± 3.8	27.7 ± 3.7	27.8 ± 3.7	27.8 ± 3.9	27.2 ± 4.7	27.3 ± 3.8	27.9 ± 4.5	27.6 ± 4.8
Diabetes mellitus, *n* (%)	190 (20)	204 (22)	201 (22)	180 (19)	15 (13)[b]	18 (18)[a,b]	20 (20)[a,b]	24 (25)[a]
Physical activity[c] <5 days/week, *n* (%)	197 (21)	188 (20)	194 (21)	217 (23)	22 (19)[a,b]	23 (23)[a]	11 (11)[b]	25 (26)[a]
Current smoker, *n* (%)	169 (18)	153 (17)	165 (18)	142 (15)	24 (21)	21 (21)	19 (19)	13 (14)
Alcohol use ≥1 glass/week, *n* (%)	673 (71)	646 (70)	661 (71)	691 (73)	74 (65)	56 (55)	68 (67)	60 (63)
Median fish consumption, g/day (range)	14.3 (5.9–18.4)[b]	15.0 (7.5–19.8)[a,b]	15.3 (7.8–22.4)[a]	15.1 (6.4–19.4)[a,b]	15.0 (5.3–19.1)	11.7 (5.2–18.3)	13.5 (6.0–17.1)	13.9 (5.9–18.3)
Intake of fish ≥20 g/day, *n* (%)	288 (31)	286 (31)	296 (32)	292 (31)	39 (35)	41 (40)	28 (27)	33 (34)
Median intake of EPA–DHA,[d] mg/day (range)	120 (50–210)[b]	130 (60–205)[a,b]	130 (60–220)[a]	130 (50–210)[a,b]	120 (60–200)	90 (40–190)	105 (45–180)	115 (40–220)

Plus–minus values are means ± SD; range is the inter-quartile range. ALA, α-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MI, myocardial infarction; TC/HDL cholesterol ratio, total to HDL cholesterol ratio.

[a,b]Values within a row with different superscripts were significantly different ($P < 0.05$).

[c]Physical activity with a Metabolic Equivalent Task score >3.

[d]Intake of EPA–DHA outside the study treatment.

Table 2 Unadjusted and adjusted changes in total cholesterol, low-density lipoprotein cholesterol, total to high-density lipoprotein cholesterol ratio, and triglycerides between baseline and 41-month follow-up among statin users and statin non-users randomized to *n*-3 fatty acid supplementation in the Alpha Omega Trial with the placebo group as reference

	Total cholesterol, mmol/L		LDL cholesterol, mmol/L		TC/HDL cholesterol ratio		Triglycerides, mmol/L	
	β (95% CI)	Adj β^a (95% CI)	β (95% CI)	Adj β^a (95% CI)	β (95% CI)	Adj β^a (95% CI)	β (95% CI)	Adj β^a (95% CI)
Statin users ($n = 1893$)								
EPA–DHA	0.032 (−0.049, 0.11)	0.028 (−0.052, 0.11)	−0.0081 (−0.077, 0.061)	−0.0085 (−0.077, 0.060)	−0.067 (−0.14, 0.0088)	−0.062 (−0.14, 0.013)	−0.057 (−0.17, 0.052)	−0.056 (−0.16, 0.053)
ALA	−0.034 (−0.11, 0.047)	−0.036 (−0.12, 0.043)	0.0026 (−0.066, 0.071)	0.0033 (−0.065, 0.071)	0.058 (−0.017, 0.13)	0.063 (−0.012, 0.14)	−0.044 (−0.15, 0.064)	−0.048 (−0.16, 0.059)
EPA–DHA + ALA	−0.069 (−0.15, 0.012)	−0.071 (−0.15, 0.0092)	−0.025 (−0.093, 0.044)	−0.025 (−0.093, 0.043)	−0.037 (−0.11, 0.038)	−0.036 (−0.11, 0.039)	−0.17[b] (−0.28, −0.058)	−0.17[b] (−0.27, −0.058)
Statin non-users ($n = 178$)								
EPA–DHA	0.0082 (−0.24, 0.26)	−0.0012 (−0.25, 0.25)	0.076 (−0.14, 0.29)	0.071 (−0.14, 0.29)	0.090 (−0.15, 0.33)	0.089 (−0.15, 0.32)	−0.082 (−0.42, 0.26)	−0.067 (−0.41, 0.27)
ALA	−0.12 (−0.38, 0.14)	−0.094 (−0.35, 0.16)	0.11 (−0.11, 0.33)	0.12 (−0.099, 0.34)	0.069 (−0.17, 0.31)	0.057 (−0.19, 0.30)	−0.23 (−0.58, 0.12)	−0.21 (−0.56, 0.14)
EPA–DHA + ALA	−0.26 (−0.53, 0.0059)	−0.24 (−0.51, 0.020)	0.017 (−0.21, 0.25)	0.025 (−0.20, 0.25)	0.14 (−0.11, 0.39)	0.12 (−0.13, 0.37)	−0.14 (−0.49, 0.22)	−0.13 (−0.49, 0.23)

[a]Adjusted for age, gender and diabetes mellitus types I and II.
[b]$P < 0.05$ when compared with the baseline value.
ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; TC/HDL cholesterol ratio, total to HDL cholesterol ratio.

hypercholesterolaemia who are effectively treated with statins and obtain a low total/HDL cholesterol ratio, an additional high dose of 1.8 g EPA is needed to significantly reduce the number of major cardiovascular events. Further studies are needed to clarify which dose of EPA or EPA–DHA will effectively lower cardiovascular risk in statin users.

It should be noted that in the present trial, *n*-3 fatty acids were incorporated at the expense of oleic acid into margarines, which replaced the participant's normal spread. Also the placebo group has benefited from an improvement in the quality of bread spread,[16] and consequently, it might have been more difficult to find statistically significant effects of *n*-3 fatty acids on major cardiovascular events in the present study compared with the studies that used fish oil supplements as a source of *n*-3 fatty acids; i.e. the GISSI-P and JELIS trials.

Statins and *n*-3 fatty acids have different effects on blood lipids. Statins reduce CVD risk through improving the total to HDL cholesterol ratio.[2,3,22] *n*-3 fatty acids in large doses (>1 g/day) lower effectively serum triglycerides but their effect on CVD risk is less convincing than that of statins.[23] Besides these effects on lipids, both statins and *n*-3 fatty acids have anti-inflammatory effects, improve endothelial function, and inhibit platelet aggregation.[4,19] Statins and *n*-3 fatty acids share mechanisms such as plaque stabilization that influence atherosclerosis and its complications positively.[24] The results of the present analysis suggest that in patients who do not use statins, the *n*-3 fatty acids EPA–DHA and ALA in amounts comparable to the Recommended Dietary Allowances[25] effectively reduce major cardiovascular events.

The current guidelines recommend statin treatment to all subjects with established CVD unless their LDL cholesterol level is below 2.5 mmol/L.[26,27] In the present study, 86% of the statin non-users had a LDL cholesterol level exceeding 2.5 mmol/L, indicating a considerable level of undertreatment. One could argue that when guidelines are followed more closely, an additional use of *n*-3 fatty acids is redundant. However, for the subset of patients in our trial who do not tolerate statins, an additional amount of 400 mg EPA–DHA plus 2 g ALA daily could be an attractive alternative to reduce their risk of future cardiovascular events.

Some limitations of our study should be acknowledged. First, the use of statins was assessed by questionnaires and telephone interviews. These methods of medication information collection have the disadvantage that they are sensitive to recall bias. Nonetheless, previous validation studies indicated that for drugs used chronically such as statins, the specificity and sensitivity of questionnaires compared with pharmacy records is high.[28] Secondly, due to the high level of statin use in our cohort, there were considerably more statin users than non-users. Nevertheless, despite the relatively small number of statin non-users ($n = 413$), the effect of *n*-3 fatty acids on major cardiovascular events was borderline statistically significant. Finally, some important cardiovascular risk factors, such as diabetes mellitus, serum triglycerides, and physical activity level, were at baseline not equally balanced among the different groups of statin non-users. Therefore, in spite of careful attempts to adjust for confounding, residual confounding cannot be ruled out. Because of these limitations, the results reported here

Table 3 Unadjusted and adjusted hazard rate ratios for major cardiovascular events among statin users and statin non-users randomized to *n*-3 fatty acid supplementation in the Alpha Omega Trial with the placebo group as reference

	Statin users (n = 3740)					Statin non-users (n = 413)				
	No./total (%)	HR (95% CI)	*P*-value	HR^{a}_{adj} (95% CI)	Adj *P*-value	No./total (%)	HR (95% CI)	*P*-value	HR^{a}_{adj} (95% CI)	Adj *P*-value
Placebo (reference)	123/943 (13)	1.00		1.00		20/113 (18)	1.00		1.00	
EPA–DHA	127/920 (14)	1.06 (0.83, 1.36)	0.65	1.05 (0.82, 1.34)	0.72	16/102 (16)	0.84 (0.44, 1.62)	0.60	0.82 (0.42, 1.58)	0.55
ALA	119/930 (13)	0.98 (0.76, 1.27)	0.89	0.98 (0.76, 1.26)	0.87	17/102 (17)	0.94 (0.49, 1.80)	0.85	0.90 (0.47, 1.72)	0.75
EPA–DHA + ALA	126/947 (13)	1.02 (0.79, 1.31)	0.89	1.02 (0.80, 1.31)	0.88	9/96 (9)	0.48 (0.22, 1.06)	0.070	0.46 (0.21, 1.01)	0.051

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a]HR_{adj}, hazard rate ratio adjusted for age, gender, and diabetes mellitus types I and II.

Table 4 Effect of EPA–DHA and/or ALA on major cardiovascular events in post-myocardial patients in the GISSI-Prevenzione Trial[13] and the Alpha Omega Trial[16]

	GISSI-Prevenzione	All patients in Alpha Omega	Statin non-users in Alpha Omega
Patients	11 324	4837	413
Intake of fish ≥1 serving/week or ≥20 g/day[a] (%)	86	31	34
Dose EPA (mg)	289	226	218
Dose DHA (mg)	577	150	145
Dose ALA (mg)	0	1882	1815
Medication use (%)[b]			
Anti-platelet drug	88	84	74
ACE-inhibitors	41	42	36
β-Blockers	41	69	58
Statins	29	85	0
Serum lipids, mmol/L			
Total cholesterol	5.45 ± 1.10	4.73 ± 0.97	5.55 ± 1.08
LDL cholesterol	3.55 ± 0.98	2.59 ± 0.84	3.38 ± 0.92
HDL cholesterol	1.07 ± 0.29	1.29 ± 0.34	1.29 ± 0.38
TC/HDL cholesterol ratio[c]	5.08	3.87 ± 1.13	4.59 ± 1.26
Triglycerides, mmol/L	1.83 ± 0.97	1.92 ± 1.04	1.96 ± 1.03
RR reduction in MCE[d]			
EPA–DHA	0.80 (0.68, 0.95)	1.05 (0.85, 1.29)	0.82 (0.42, 1.58)
ALA		0.94 (0.76, 1.17)	0.90 (0.47, 1.72)
EPA–DHA plus ALA		0.91 (0.74, 1.13)	0.46 (0.21, 1.01)

ACE, angiotensin-converting enzyme; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MCE, major cardiovascular events; RR, relative risk; TC/HDL cholesterol ratio, total to HDL cholesterol ratio.
[a]Fish intake was categorized into ≥1 or <1 serving/week in GISSI-Prevenzione and as ≥20 or <20 g/day in Alpha Omega.
[b]Medication use in GISSI-Prevenzione at 6 months.
[c]Total to HDL cholesterol ratio was not presented in GISSI-Prevenzione but was derived by dividing the total cholesterol level by the HDL cholesterol level.
[d]MCE comprised fatal cardiovascular disease, non-fatal myocardial infarction, and non-fatal stroke in GISSI-Prevenzione. In Alpha Omega, MCE comprised fatal cardiovascular disease, non-fatal myocardial infarction, non-fatal stroke, non-fatal cardiac arrest, and cardiac interventions (percutaneous coronary intervention and coronary artery bypass grafting).

should be regarded as preliminary until these have been confirmed in larger patient populations.

In conclusion, the present study indicates that low-dose supplementation with *n*-3 fatty acids might reduce the risk of major cardiovascular events in statin non-users with a history of MI. These results contribute to the explanation of the inconsistent results on the effects of *n*-3 fatty acids in secondary prevention trials.

Acknowledgements

We thank Janette de Goede, Linda Oude Griep, Lucy Okma, and Eveline Waterham for their contribution to the fieldwork and the data management of the Alpha Omega Trial.

Funding

The Alpha Omega Trial was supported by the Netherlands Heart Foundation, the National Institutes of Health, USA, and Unilever R&D, the Netherlands. The Netherlands Heart Foundation covered the costs of the infrastructure of the trial, baseline examinations, and data collection of primary and secondary fatal outcomes. The National Institutes of Health provided a grant for patient examinations and follow-up. Unilever R&D produced trial margarines and provided an unrestricted grant for the distribution of trial margarines to the patients. The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; in writing the manuscript or in the decision to submit the paper for publication.

Conflict of interest: none declared.

References

1. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;**344**:1383–1389.
2. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;**366**:1267–1278.
3. Expert Panel on Detection, Evaluation and, Treatment of High Blood Cholesterol In Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) expert panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *J Am Med Assoc* 2001;**285**:2486–2497.
4. Ray KK, Cannon CP. The potential relevance of the multiple lipid-independent (pleiotropic) effects of statins in the management of acute coronary syndromes. *J Am Coll Cardiol* 2005;**46**:1425–1433.
5. The U.S. Departments of Agriculture and Health and Human Services. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans. http://www.cnpp.usda.gov/dietaryguidelines.htm (6 July 2011).
6. Mozaffarian D, Rimm E. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *J Am Med Assoc* 2006;**296**:1885–1899.
7. He K, Song Y, Daviglus M, Liu K, Van Horn L, Dyer A, Greenland P. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 2004;**109**:2705–2711.
8. Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. *Heart* 2006;**92**:166–169.
9. Brouwer I, Katan M, Zock P. Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr* 2004;**134**:919–922.
10. Eussen S, Klungel O, Garssen J, Verhagen H, van Kranen H, van Loveren H, Rompelberg C. Support of drug therapy using functional foods and dietary supplements: focus on statin therapy. *Br J Nutr* 2010;**103**:1260–1277.
11. Saravanan P, Davidson N, Schmidt E, Calder P. Cardiovascular effects of marine omega-3 fatty acids. *Lancet* 2010;**376**:540–550.
12. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989;**2**:757–761.
13. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999;**354**:447–455.
14. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H, Gottwik M, Steinbeck G, Del Castillo U, Sack R, Worth H, Katus H, Spitzer W, Sabin G, Senges J. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation* 2010;**122**:2152–2159.
15. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J, Hercberg S. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. *Br Med J* 2010;**341**:c6273.
16. Kromhout D, Giltay E, Geleijnse J. *n*-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010;**363**:2015–2026.
17. Geleijnse J, Giltay E, Schouten E, de Goede J, Oude Griep LM, Teitsma-Jansen A, Katan M, Kromhout D. Effect of low doses of *n*-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients: design and baseline characteristics of the Alpha Omega Trial. *Am Heart J* 2010;**159**:539–546.
18. World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. 2010. http://www.whocc.no/filearchive/publications/2010guidelines.pdf (15 May 2011).
19. De Caterina R. *n*-3 fatty acids in cardiovascular disease. *N Engl J Med* 2011;**364**:2439–2450.
20. Kromhout D, Yasuda S, Geleijnse JM, Shimokawa H. Fish oil and omega-3 fatty acids in cardiovascular disease: do they really work? *Eur Heart J* 2011; doi:10.1093/eurheartj/ehr362.
21. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;**369**:1090–1098.
22. Nicholls S, Tuzcu EM, Sipahi I, Grasso A, Schoenhagen P, Hu T, Wolski K, Crowe T, Desai M, Hazen S, Kapadia S, Nissen S. Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *J Am Med Assoc* 2007;**297**:499–508.
23. Contacos C, Barter PJ, Sullivan DR. Effect of pravastatin and omega-3 fatty acids on plasma lipids and lipoproteins in patients with combined hyperlipidemia. *Arterioscler Thromb* 1993;**13**:1755–1762.
24. Thies F, Garry JMC, Yaqoob P, Rerkasem K, Williams J, Shearman C, Gallagher P, Calder P, Grimble R. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;**361**:477–485.
25. Elmadfa I, Kornsteiner M. Fats and fatty acid requirements for adults. *Ann Nutr Metab* 2009;**55**:56–75.
26. Dutch Institute for Healthcare Improvement and Dutch College of General Practitioners. Dutch Guideline Cardiovascular Risk Management. 2006. http://www.cbo.nl/Downloads/211/guide_cvrm_07.pdf (15 October 2010).
27. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, Ebrahim S, Faergeman O, Graham I, Mancia G, Manger Cats V, Orth-Gomer K, Perk J, Pyorala K, Rodicio JL, Sans S, Sansoy V, Sechtem U, Silber S, Thomsen T, Wood D, Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Heart J* 2003;**24**:1601–1610.
28. Boudreau D, Daling J, Malone K, Gardner J, Blough D, Heckbert S. A validation study of patient interview data and pharmacy records for antihypertensive, statin, and antidepressant medication use among older women. *Am J Epidemiol* 2004;**159**:308–317.