

# Effect of Omega-3 Fatty Acids on Kidney Function after Myocardial Infarction: The Alpha Omega Trial

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## Abstract

**Background and objectives** Kidney function gradually decreases with age, and myocardial infarction accelerates this deterioration. Omega-3 (n-3) fatty acids may slow down the decline of kidney function. The effect of marine and plant-derived n-3 fatty acids on kidney function in patients after myocardial infarction was examined.

**Design, setting, participants, & measurements** In the Alpha Omega Trial, 2344 patients with history of myocardial infarction ages 60–80 years old (81% men) were randomized to one of four trial margarines. The patients received an additional targeted amount of 400 mg/d eicosapentaenoic acid and docosahexaenoic acid, 2 g/d  $\alpha$ -linolenic acid, eicosapentaenoic acid–docosahexaenoic acid plus  $\alpha$ -linolenic acid, or placebo for 40 months. Serum cystatin C and serum creatinine were assessed at baseline and after 40 months. Creatinine–cystatin C-based GFR was estimated with the Chronic Kidney Disease Epidemiology Collaboration equation.

**Results** Patients consumed 19.9 g margarine/d, providing an additional 239 mg/d eicosapentaenoic acid with 159 mg/d docosahexaenoic acid, 1.99 g/d  $\alpha$ -linolenic acid, or both in the active treatment groups. After 40 months, compared with baseline, mean ( $\pm$ SD) creatinine–cystatin C-based GFR was  $-6.9$  ( $\pm 12.6$ ),  $-4.8$  ( $\pm 13.4$ ),  $-6.2$  ( $\pm 12.8$ ), and  $-6.0$  ( $\pm 13.0$ ) ml/min per 1.73 m<sup>2</sup> in the placebo, eicosapentaenoic acid–docosahexaenoic acid,  $\alpha$ -linolenic acid, and eicosapentaenoic acid–docosahexaenoic acid plus  $\alpha$ -linolenic acid groups, respectively. After 40 months, in patients receiving eicosapentaenoic acid–docosahexaenoic acid compared with placebo, the decline in creatinine–cystatin C-based GFR was 2.1 less (95% confidence interval, 0.6 to 3.6;  $P < 0.01$ ) ml/min per 1.73 m<sup>2</sup>; other comparisons were not statistically significant. Odds ratios (95% confidence intervals) of incident CKD ( $< 60$  ml/min per 1.73 m<sup>2</sup>) and rapid decline of kidney function ( $\geq 3$  ml/min per year) for eicosapentaenoic acid–docosahexaenoic acid compared with placebo were 0.83 (0.58 to 1.18) and 0.85 (0.67 to 1.08), respectively.

**Conclusions** Long-term supplementation with 400 mg/d eicosapentaenoic acid–docosahexaenoic acid provides a small beneficial effect on kidney function in patients with a history of myocardial infarction.

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## Introduction

Kidney function gradually decreases with age. Atherosclerosis, diabetes, and hypertension are major causes of accelerated loss of kidney function (1–3). A large cohort study showed a greater decline in eGFR among participants after a first myocardial infarction (MI) compared with those without an event: 2.2 versus 0.5 ml/min per 1.73 m<sup>2</sup> per year (4). Therefore, patients have an increased risk to develop CKD after MI. CKD, defined as an eGFR  $< 60$  ml/min per 1.73 m<sup>2</sup>, is a risk factor of cardiovascular morbidity, ESRD, and all-cause mortality (5,6). Globally, the prevalence rates of CKD are  $> 20\%$  among men and women ages 65–74 years old and  $> 30\%$  among those ages 75–84 years old (1,7). Although angiotensin-converting enzyme inhibitor compared with  $\beta$ -blocker or calcium channel blocker therapy slowed eGFR decline, escalation of drug therapy to a lower BP goal compared with the usual had no effect (8). Identification of novel potential modifiable risk

factors is important for targeted prevention and improved life expectancy.

Results from observational studies suggest that Omega-3 (n-3) fatty acids may prevent or slow down the decline of kidney function (9,10). Gopinath *et al.* (9) showed, in a cross-sectional study, that older adults in the highest compared with the lowest quartile of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake had a 32% lower prevalence of CKD. The prospective Aging in the Chianti Area study showed that, in older adults ( $\geq 65$  years old) with an eGFR  $> 60$  ml/min per 1.73 m<sup>2</sup>, those with a high plasma n-3 fatty acids level had a slower decline of kidney function after 3 years than those with a low level (10). Therefore, we examined in a study ancillary to the Alpha Omega Trial the effect of the marine n-3 fatty acids EPA and DHA and plant-derived  $\alpha$ -linolenic acid (ALA) on the decline of kidney function among older patients who had had an MI (11).

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## Materials and Methods

### Patients

This study was carried out to explore the effect of n-3 fatty acids on the decline of kidney function in the Alpha Omega Trial, a multicenter, randomized, double-blind, placebo-controlled trial conducted between 2002 and 2009 (ClinicalTrials.gov no. NCT00127452). The study has been described in detail elsewhere (11,12). In brief, 4837 free-living Dutch patients with a history of MI ages 60–80 years old at baseline received antihypertensive, antithrombotic, and lipid-modifying drug treatment. Patients were recruited sequentially and randomly assigned to one of four trial margarines for 40 months. For this study, patients were selected in which nonfasting blood was drawn at baseline and after 40 months. Owing to financial constraints, two blood samples were available for only 2344 patients (those randomized before August of 2005, yielding 48% of the cohort) (Figure 1). Of all patients randomized before August of 2005 ( $n=2918$ ), 233 patients died during follow-up, and 341 patients had missing blood samples or refused (Supplemental Table 1). The Alpha Omega Trial was conducted in accordance with the Declaration of Helsinki and approved by a central medical ethics committee in The Netherlands. Written informed consent was obtained from all patients.

### Intervention with n-3 Fatty Acids

Patients were randomly allocated to a daily intake of approximately 20 g trial margarine that provided a targeted additional daily intake of 400 mg EPA-DHA (ratio of 3:2), a targeted additional daily intake of 2 g ALA, a combination of EPA-DHA and ALA, or placebo. Dosages were comparable with the recommended dietary allowances for these n-3 fatty acids (12). Patients were asked to avoid n-3 fatty acid supplements during the trial. Actual treatment was (for logistic reasons) preceded by 4–6 weeks on placebo margarine. Compliance was monitored by margarine tub counts, telephone interviews, and patient diaries. In samples of randomly selected patients at baseline and after 20 and 40 months of follow-up, n-3 fatty acids in serum cholesteryl

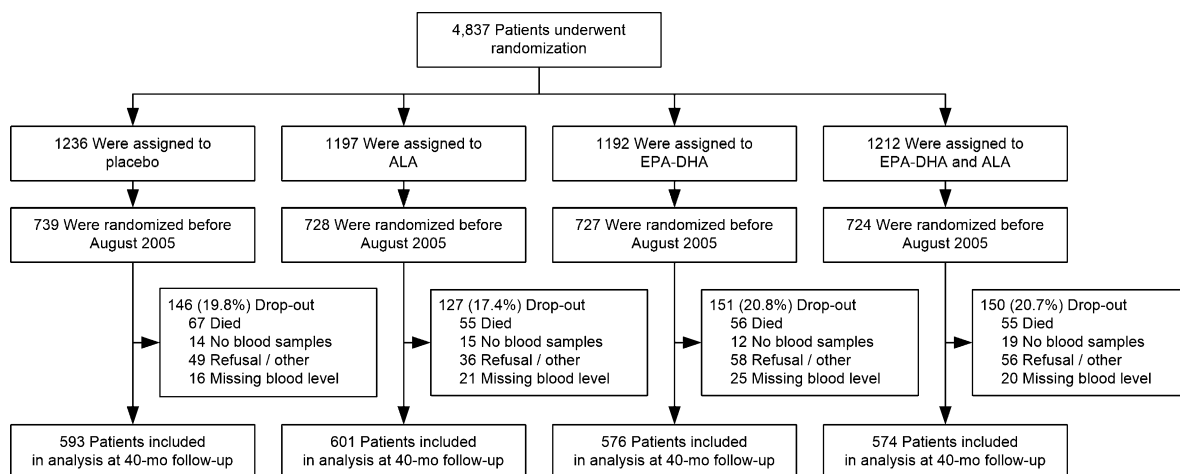
esters were determined as an objective measure of compliance (12).

### Kidney Function Assessment

Standardized blood handling procedures for the Alpha Omega Trial are described in detail elsewhere (13). Briefly, nonfasting blood samples were obtained at the participant's home or the hospital. Tubes were packaged in sealed envelopes and sent by standard postal service to a central laboratory.

At baseline and after 40 months of intervention, serum creatinine (cr) and cystatin C (cysC) were measured from stored blood samples in a central laboratory from September 1, to November 15, 2011 (14,15). Serum cysC was measured by means of a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dimension Vista 1500 Analyzer; Siemens). We used calibrators and assays of the same lot code, which was stable (no downward drift). CysC was calibrated directly using the standard supplied by the manufacturer (traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C) (16). The analytical measurement range of cysC was 0.23–8.00 mg/L. Intra- and interassay variations for low cysC (mean=1.00 mg/L) were 1.3% and 4.2%, respectively, and for high cysC (mean=1.75 mg/L), they were 2.9% and 2.8%, respectively.

Serum cr was measured by the modified kinetic Jaffé method (Dimension Vista 1500 Analyzer; Siemens). We calibrated directly to the standard supplied by the manufacturer from the National Institute of Standards and Technology Standard Reference Material, and postcalibration correction factor was applied (17). Intra- and interassay variations for low cr (mean=0.8 mg/dl) were 1.8% and 2.9%, respectively, and for high cr (mean=3.9 mg/dl), they were 0.8% and 2.2%, respectively. Serum cr values <0.6 mg/dl were unreliable (owing to technical failure or analytical disturbance;  $n=82$ ) and therefore, not reported in accordance with the Standard Operating Procedure of the central laboratory.



**Figure 1.** | Flow chart of the 2344 patients in the Alpha Omega Trial: randomization and follow-up. ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

### Data Collection and Follow-Up Procedures

Patients were interviewed and physically examined by trained research nurses at home or in the hospital at baseline and after 40 months. Information on demographic variables, lifestyle habits, current health status, and medical history was collected by self-administered questionnaires as previously described in detail (12). Ethnicity was categorized as white, black, or other. Medication was coded according to the Anatomical Therapeutic Chemical Classification System. Diabetes mellitus was considered present in case of a self-reported physician diagnosis, use of antidiabetic drugs, and/or elevated blood glucose. High-sensitivity C-reactive protein (hsCRP) levels were measured in stored serum samples as previously described (18).

### Data Analysis

Baseline characteristics in the four groups are presented as mean±SD, median with interquartile range (IQR), or percentage. Differences between groups were tested by ANOVA or chi-squared test depending on the variable. We estimated GFR with the combined cr-cysC-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation from 2012, taking into account age, sex, and race (15).

Analyses were performed according to the intention-to-treat principle. From each individual, the change (or slope) of the eGFR from baseline to 40 months was calculated by subtracting the eGFR at baseline from the eGFR after 40 months. Changes in cr-cysC-based eGFR (eGFR<sub>cr-cysC</sub>) from baseline to 40 months of follow-up in the three n-3 fatty acids groups versus the placebo group were compared along with accompanying 95% confidence intervals (95% CIs) according to the prespecified analysis plan to examine our primary hypothesis. Additional adjustment for potential mediators, such as change of hsCRP, body mass index (BMI), physical activity, or animal protein intake, was performed with analysis of covariance.

We assessed the effect of n-3 fatty acids on change in eGFR after stratification for baseline CKD, diabetes, and use of blockers of the renin-angiotensin-aldosterone system (RAAS) (19). Finally, we calculated the odds ratio (95% CI) of rapid kidney function decline ( $\geq 3$  ml/min per year) and 40-month incident CKD (eGFR $< 60$  ml/min per 1.73 m<sup>2</sup>) in patients without CKD (eGFR $\geq 60$  ml/min per 1.73 m<sup>2</sup>) at baseline with logistic regression for n-3 fatty acids treatment compared with placebo. Interaction was tested by entering a product term in the model. All analyses were done using SPSS 21.0 (SPSS, Inc., Chicago, IL).

## Results

### Descriptive Characteristics

Baseline characteristics of the patients after MI were well balanced over the four study groups (Table 1). Mean age of 2344 patients was 69 years, 81% of patients were men, 19% had diabetes, 23% were obese, and 15% smoked. The median (IQR) time after MI at study entry was 4.0 years (2.0–6.4). Of all patients, 98% used antithrombotic agents, 86% used antiplatelet agents, and 85% used statins. The mean systolic BP was 143 mmHg, and 87% of patients received BP-lowering drugs; 54% of patients used RAAS blockers, of whom 92% of patients persisted on RAAS blockers, which did not differ among the four treatment groups.

The median daily fish intake was 14.7 g (IQR=5.9–18.4 g), which translates to a median intake of 120 mg (IQR=60–200 mg/d) EPA-DHA per day, and did not differ significantly among the four groups. About 21% of all patients had a very low fish intake of  $< 5$  g/d. During the trial, fish oil capsules or supplementation had been used by 123 (5.2%) of 2344 participants, and use was similar among the patients in the four treatment groups. There was no differential mortality or other dropout among the four treatment groups ( $P=0.66$ ) (Supplemental Table 1).

At baseline mean (SD), serum cysC was 0.97 (0.25) mg/L, and serum cr was 1.0 (0.3) mg/dl. The mean (SD) eGFR<sub>cr-cysC</sub> was 78.5 (18.7) ml/min per 1.73 m<sup>2</sup>. The four treatment groups did not differ in eGFR<sub>cr-cysC</sub>. The percentages of patients with eGFR<sub>cr-cysC</sub> $\geq 90$ , 60–90, 30–60, and  $< 30$  ml/min per 1.73 m<sup>2</sup> were 31%, 52%, 16%, and 1%, respectively. Of the patients who died during follow-up, the mean (SD) eGFR<sub>cr-cysC</sub> at baseline was 63.9 (21.6) ml/min per 1.73 m<sup>2</sup> and did not differ among the four study groups ( $P=0.62$ ).

### Intervention with n-3 Fatty Acids

The mean intake of trial margarine was 19.9 (SD=3.8) g/d, and 96% of patients used the margarines  $> 80\%$  of the time. The patients in the EPA-DHA group received, on average, an additional 239 mg EPA and 159 mg DHA per day, and those in the ALA group received an additional 1.99 g ALA per day. Patients participated in the trial for a median of 41.3 (IQR=40.8–41.9) months, including a run-in period on placebo margarine of 1–1.5 months; thus, the treatment itself lasted 40 months. The daily additional amount of n-3 fatty acids during the trial was reflected in the increment of the mass percentages of the serum cholesteryl esters, underscoring the good compliance (Supplemental Figure 1).

### Effect of n-3 Fatty Acids on Kidney Function

After, on average, 40 months of follow-up, the decline of eGFR<sub>cr-cysC</sub> was 6.9 ml/min per 1.73 m<sup>2</sup> in the placebo group (Table 2). Assuming a linear decline in kidney function, the average eGFR<sub>cr-cysC</sub> decline per year in the placebo group was 2.0 (95% CI, 1.7 to 2.3) ml/min per 1.73 m<sup>2</sup> (Figure 2). After 40 months of intervention, the mean difference in eGFR<sub>cr-cysC</sub> was 2.1 (95% CI, 0.6 to 3.6) in the EPA-DHA group compared with the placebo group. Assuming a linear decline in kidney function, the average eGFR<sub>cr-cysC</sub> decline per year in the EPA-DHA group was 1.4 (95% CI, 1.1 to 1.7) ml/min per 1.73 m<sup>2</sup>. Compared with the placebo group, the decline in eGFR<sub>cr-cysC</sub> was reduced by 30% in the EPA-DHA group. We found no effect in the ALA group. The treatment effects between the EPA-DHA and the EPA-DHA plus ALA group did not differ:  $-1.3$  ml/min per 1.73 m<sup>2</sup> (95% CI,  $-2.8$  to 0.3;  $P=0.11$ ). The effect estimates did not differ much after adjustment for change in animal protein intake, physical activity, BMI, or hsCRP (data not shown). There were no changes in important risk factors of kidney function among the four study groups (Supplemental Table 2).

After stratification for baseline CKD (no or yes), the treatment effect of EPA-DHA compared with placebo was 1.3 (95% CI,  $-0.3$  to 2.9) ml/min per 1.73 m<sup>2</sup> in patients without CKD and 4.9 (95% CI, 1.1 to 8.7) ml/min per 1.73 m<sup>2</sup> in patients with CKD ( $P$  for interaction=0.07) (Table 3).

**Table 1. Baseline characteristics of 2344 patients of the Alpha Omega Trial by treatment group**

Variables	Placebo (n=593)	ALA (n=601)	EPA-DHA (n=576)	EPA-DHA plus ALA (n=574)	P Value <sup>a</sup>
<b>Age, yr</b>	68.7±5.3	69.5±5.6	69.0±5.4	69.0±5.5	0.71
≥70 yr old, no. (%)	243 (41)	259 (42)	245 (43)	239 (40)	0.89
Men, no. (%)	491 (82.8)	471 (78.4)	458 (79.5)	470 (81.9)	0.19
Ethnicity (white), no. (%)	587 (99.0)	593 (98.7)	569 (98.8)	570 (99.3)	0.89
Time since myocardial infarction, years	4.6±3.4	4.6±3.2	4.5±3.2	4.3±2.9	0.47
Self-reported history of stroke, no. (%)	40/589 (6.8)	38/596 (6.4)	40/572 (7.0)	35/572 (6.1)	0.99
<b>Diabetes,<sup>b</sup> no. (%)</b>	113 (19)	120 (20)	99 (17)	106 (19)	0.67
Antidiabetic drugs, no. (%)	77 (13.0)	92 (15.3)	69 (12.0)	74 (12.9)	0.38
<b>Body mass index,<sup>c</sup> kg/m<sup>2</sup></b>	27.7±3.7	27.8±3.6	27.6±3.7	27.6±3.6	0.89
≥30 kg/m <sup>2</sup> , no. (%)	133 (22.5)	138 (23.0)	141 (24.5)	119 (20.8)	0.50
Systolic BP, mmHg	145±22	143±21	143±21	143±21	0.26
Diastolic BP, mmHg	82±11	81±11	82±11	81±11	0.67
<b>Use of cardiovascular medication,<sup>d</sup> no. (%)</b>					
Antithrombotic agents	583 (98)	588 (98)	566 (97)	553 (96)	0.09
BP-lowering drugs	514 (86.7)	519 (86.4)	511 (88.7)	495 (86.2)	0.56
ACE inhibitor and/or angiotensin blocker	327 (55.1)	330 (54.9)	301 (52.3)	312 (54.4)	0.75
β-Blockers	391 (65.9)	380 (63.2)	391 (67.9)	365 (63.6)	0.30
Diuretics	135 (22.8)	121 (20.1)	113 (19.6)	117 (20.4)	0.55
Lipid-modifying drugs	503 (84.8)	518 (86.2)	491 (85.2)	501 (87.3)	0.63
Current smoker, no. (%)	91 (15.3)	103 (17.1)	90 (15.6)	77 (13.4)	0.37
Alcohol use ≥1 glass/wk, no. (%)	447/593 (75.4)	445/600 (74.2)	446/576 (77.4)	442/572 (77.3)	0.50
Fish intake (<5 g/d), <sup>e</sup> no. (%)	119/551 (21.6)	97/559 (17.4)	114/532 (21.4)	117/534 (21.9)	0.19
Animal protein intake, g/d	43±15	42±15	43±15	43±15	0.69
Serum cystatin C, mg/L	0.97±0.25	0.97±0.25	0.97±0.23	0.98±0.25	0.68
Serum creatinine, <sup>f</sup> mg/dl	1.01±0.33	1.01±0.31	1.02±0.31	1.04±0.37	0.41
Creatinine–cystatin C-based eGFR, <sup>g</sup> ml/min per 1.73 m <sup>2</sup>	79.2±18.4	79.0±19.2	78.2±18.5	77.6±18.5	0.44
Plasma glucose, <sup>h</sup> mg/dl	110±37	110±36	108±35	109±35	0.30
Total cholesterol, <sup>i</sup> mg/dl	188±36	186±37	188±36	186±35	0.56
LDL, mg/dl	106±32	105±32	107±31	105±29	0.57
HDL, mg/dl	48±12	48±12	49±14	49±12	0.26
Triglycerides, <sup>j</sup> mg/dl	149 (109–211)	143 (110–205)	145 (109–195)	140 (105–198)	0.21
High-sensitivity C-reactive protein, mg/L	1.7 (0.8–3.6)	1.8 (0.9–3.8)	1.5 (0.8–3.3)	1.7 (0.8–3.8)	0.94
Higher education, <sup>k</sup> no. (%)	82 (13.9)	55 (9.2)	72 (12.6)	79 (13.7)	0.31
Physically active (≥3 METs >5 d/wk), <sup>l</sup> no. (%)	128 (21.7)	130 (21.7)	125 (21.8)	138 (24.2)	0.65

Numbers may not total to the column total because of missing values for some variables. Data are reported as mean±SD or median (interquartile range). ACE, angiotensin-converting enzyme; MET, metabolic equivalent task; ALA, α-linolenic acid; EPA-DHA, eicosapentaenoic acid–docosahexaenoic acid.

<sup>a</sup>Chi-squared tests and ANOVA were used to determine statistical significance.

<sup>b</sup>Diabetes was considered to be present if a patient reported having received the diagnosis from a physician, was taking antidiabetic drugs, or had an elevated plasma glucose level (≥126 mg/dl in the case of patients who had fasted >4 hours or ≥200 mg/dl in the case of nonfasting patients).

<sup>c</sup>Body mass index was calculated as weight in kilograms divided by height in meters squared.

<sup>d</sup>Antithrombotic agents: Anatomical Therapeutic Chemical Classification System (ATC) code B01; BP-lowering drugs: ATC codes C02, C03, C07, C08, and C09; lipid-modifying drugs: ATC code C10.

<sup>e</sup>Fish intake <5 g/d equals intake of one fish per month.

<sup>f</sup>To convert the values for creatinine to micromoles per liter, multiply by 88.40.

<sup>g</sup>Combined creatinine–cystatin C eGFR on the basis of the Chronic Kidney Disease Epidemiology Collaboration equation of 2012 (15).

<sup>h</sup>To convert the values for glucose to millimoles per liter, multiply by 0.05551.

<sup>i</sup>To convert the values for cholesterol to millimoles per liter, multiply by 0.02586.

<sup>j</sup>To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

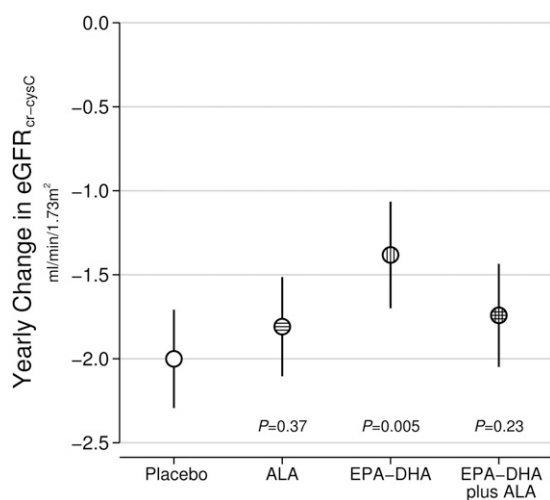
<sup>k</sup>Defined as higher vocational education or university.

<sup>l</sup>Defined as three or more METs during >5 d/wk.

**Table 2. Effect of 40 months of intervention of Omega-3 fatty acids on decline in creatinine–cystatin C-based kidney function in 2344 patients of the Alpha Omega Trial according to treatment group**

Treatment Groups	Creatinine–Cystatin C-Based eGFR (ml/min per 1.73 m <sup>2</sup> ) <sup>a</sup>			
	Pretreatment (Mean±SD)	Post-Treatment (Mean±SD)	Decline (Mean±SD)	Treatment Effect Mean (95% CI) <sup>b</sup>
Placebo (n=593)	79.2±18.4	72.3±20.1	−6.9±12.6	
ALA (n=601)	79.0±19.3	72.7±20.4	−6.2±12.8	0.7 (−0.8 to 2.1)
EPA-DHA (n=576)	78.2±18.5	73.4±19.2	−4.8±13.4	2.1 (0.6 to 3.6)
EPA-DHA plus ALA (n=574)	77.6±18.5	71.6±19.5	−6.0±13.0	0.9 (−0.6 to 2.3)

95% CI, 95% confidence interval.  
<sup>a</sup>On the basis of the Chronic Kidney Disease Epidemiology Collaboration equation of 2012 (15).  
<sup>b</sup>Decline in intervention group minus decline in placebo group with 95% CI.

**Figure 2. | Effect of intervention of n-3 fatty acids on yearly decline in creatinine–cystatin C-based kidney function in 2344 patients of the Alpha Omega Trial according to treatment group.** Yearly mean changes of eGFR<sub>cr-cysC</sub> compared with baseline with 95% confidence intervals. *P* values were obtained by means of the *t* test for independent samples using the placebo group as the reference. eGFR<sub>cr-cysC</sub>, creatinine–cystatin C-based eGFR; n-3, Omega-3.

After stratification at baseline for the presence of diabetes or use of RAAS blockers, we found no evidence for effect modification (data not shown). After 40 months, the incidence of CKD and rapid decline of kidney function were 15% and 34%, respectively. The odds ratios of incident CKD and rapid decline of kidney function for EPA-DHA compared with placebo were 0.83 (95% CI, 0.58 to 1.18) and 0.85 (95% CI, 0.67 to 1.08), respectively (Tables 4 and 5).

## Discussion

In our cohort of stable patients after MI, we found a yearly decline of kidney function of 2.0 ml/min per 1.73 m<sup>2</sup> in patients on placebo, a value similar to that observed in other older cohorts with cardiovascular disease or diabetes (4,20). Patients who increased their daily intake by an estimated 398 mg EPA-DHA (comparable with the recommended dietary allowance) for 40 months had a slower

yearly decline of kidney function of 1.4 ml/min per 1.73 m<sup>2</sup>. After 40 months of supplementation with EPA-DHA, there was a small treatment effect of 2.1 (95% CI, 0.6 to 3.6) ml/min per 1.73 m<sup>2</sup> less kidney function decline compared with placebo. The treatment effect of EPA-DHA plus ALA on kidney function was smaller but not significantly different from the effect established in the EPA-DHA group. We found no beneficial effect of ALA on kidney function. We found evidence for a stronger treatment effect of EPA-DHA on decline of kidney function in patients with CKD compared with patients without CKD (4.9 versus 1.3 ml/min per 1.73 m<sup>2</sup>). In contrast, EPA-DHA did not statistically significantly reduce incident CKD and rapid decline of kidney function, which are both clinically relevant dichotomous outcomes. However, dichotomization of a continuous outcome variable results in loss of statistical power owing to loss of information (21). Taken together, these results do not show an unequivocal beneficial effect of EPA-DHA.

The beneficial effect of EPA-DHA on kidney function decline was attained on top of drug treatment for elevated BP and dyslipidemia, both important risk factors of progression of kidney disease (4,22). The number of patients on RAAS blockers during the trial did not differ much among the groups and, therefore, could not explain the slower decline of kidney function in the EPA-DHA group compared with the placebo group.

We used the combined cr–cysC-based CKD-EPI equation, the most accurate equation to estimate kidney function, because each endogenous marker alone has limitations owing to non-GFR determinants (14,15). Inflammation might be a non-GFR determinant of cysC (23–25). Anti-inflammatory effects of high-dose EPA-DHA may lower serum cysC production (26–30). However, we previously showed that 40 months of supplementation with low-dose n-3 fatty acids did not lower the level of hsCRP in statin users (85% of the patients) (18). In addition, the change of hsCRP did not differ much among the four groups after 40 months. Non-GFR determinants of serum cr, including muscle mass, diet, and physical activity, cannot be accounted for fully by age, sex, and race. However, adjustment for change of BMI, physical activity, or animal protein intake did not materially change our findings.

We found no differential dropout of patients who died among the four study groups. In addition, kidney function

**Table 3. Effect of 40 months of intervention of Omega-3 fatty acids on decline in creatinine–cystatin C-based kidney function in 2344 patients of the Alpha Omega Trial with or without CKD at baseline according to treatment group**

Treatment Groups	Creatinine–Cystatin C-based eGFR (ml/min per 1.73 m <sup>2</sup> ) <sup>a</sup>			
	Pretreatment (Mean±SD)	Post-Treatment (Mean±SD)	Decline (Mean±SD)	Treatment Effect Mean (95% CI) <sup>b</sup>
<b>≥60 ml/min per 1.73 m<sup>2</sup></b>				
Placebo (n=500)	85.0±13.1	77.4±16.5	−7.6±12.5	
ALA (n=503)	85.1±14.1	78.0±17.0	−7.1±12.8	0.6 (−1.0 to 2.1)
EPA-DHA (n=467)	84.9±12.9	78.5±15.8	−6.4±12.6	1.3 (−0.3 to 2.9)
EPA-DHA plus ALA (n=468)	84.2±12.7	77.2±15.2	−7.1±12.7	0.6 (−1.0 to 2.2)
<b>&lt;60 ml/min per 1.73 m<sup>2</sup></b>				
Placebo (n=93)	48.2±10.1	45.4±15.4	−2.8±12.3	
ALA (n=98)	47.7±9.4	45.7±14.9	−2.0±11.7	0.9 (−2.6 to 4.3)
EPA-DHA (n=109)	49.4±8.6	51.5±16.9	2.1±14.8	4.9 (1.1 to 8.7)
EPA-DHA plus ALA (n=106)	48.6±10.2	47.2±17.9	−1.4±13.1	1.4 (−2.2 to 5.0)

<sup>a</sup>On the basis of the Chronic Kidney Disease Epidemiology Collaboration equation of 2012 (15). CKD was defined as eGFR<60 ml/min per 1.73 m<sup>2</sup>.  
<sup>b</sup>Decline in intervention group minus decline in placebo group with 95% CI. The P value for interaction with regard to change of eGFR between CKD at baseline (yes or no) and ALA versus placebo was 0.67; for EPA-DHA versus placebo, P for interaction was 0.07, and for EPA-DHA plus ALA versus placebo, P value for interaction was 0.89.

**Table 4. Odds ratios (95% confidence intervals) of 40 months incident CKD (eGFR<60 ml/min per 1.73 m<sup>2</sup>) among patients without CKD at baseline according to intervention of Omega-3 fatty acids compared with placebo**

Treatment Groups	No CKD at baseline (N)	Incident CKD N (%)	Odds Ratio (95% CI)
Placebo	500	83 (17)	1 (reference)
ALA	503	83 (17)	0.99 (0.71 to 1.39)
EPA-DHA	467	66 (14)	0.83 (0.58 to 1.18)
EPA-DHA plus ALA	468	65 (14)	0.81 (0.57 to 1.15)

**Table 5. Odds ratios (95% confidence intervals) of rapid decline of kidney function (≥3 ml/min per year) during 40 months according to intervention of Omega-3 fatty acids compared with placebo**

Treatment Groups	N	Rapid Decline N (%)	Odds Ratio (95% CI)
Placebo	593	217 (37)	1 (reference)
ALA	601	200 (33)	0.86 (0.68 to 1.10)
EPA-DHA	576	189 (33)	0.85 (0.67 to 1.08)
EPA-DHA plus ALA	574	198 (34)	0.91 (0.72 to 1.16)

did not differ at baseline for deceased patients among the four study groups. Therefore, we conclude that there is no indication of selective mortality among the four study groups.

In contrast to the observational studies showing that EPA-DHA may prevent or slow down the decline of kidney function, clinical trials show conflicting results (31–34). However, a trial in patients with type 2 diabetes (n=97) showed, after 3 months of supplementation with 4 g fish oil/d, 0.05 mg/dl lower serum cr compared with controls (35). A meta-analysis, including 626 patients from 12 trials with predominantly IgA nephropathy and diabetes, showed that 0.7–5.1 g/d fish oil supplementation compared with placebo resulted in an 11% (95% CI, −7% to 29%) slower decline of kidney after a median follow-up of

9 months (36). Compared with the studies included in the meta-analysis, in the Alpha Omega Trial, the daily dose of EPA-DHA was lower, but the effect on kidney function was greater. Differences in underlying causes of renal function decline and longer duration of intervention may explain the stronger effect of EPA-DHA observed in this study compared with the meta-analysis.

Lowering BP, inflammation, and oxidative stress has been suggested as a mechanism that might explain how high doses of EPA-DHA could slow down loss of kidney function (26,27,37,38). We found, however, no effect of low-dose EPA-DHA on BP and hsCRP (Supplemental Table 2) (18). The following mechanisms may explain the potential beneficial effect of low-dose EPA-DHA on loss of kidney function. First, EPA-DHA may alter local renal

hemodynamics by increasing the level of prostaglandins and decreasing thromboxanes, which may have improved GFR. Second, improvement of renal energy metabolism may have occurred through improvement of mitochondrial function and efficiency of ATP generation. Third, improvement of endothelial function may have resulted from the modification of membrane lipid composition (28,39,40). Unfortunately, we could not explore these mechanisms because of lack of data.

This study has limitations. Kidney function was not defined as a primary outcome of the Alpha Omega Trial. Data on decline of kidney function were based on only two measurements available for  $\pm 50\%$  of the patients, which has broadened the confidence intervals of the estimates of our effect parameters (41,42). The targeted dose of ALA may have been too low to show an effect on the indicators of kidney function. We did not measure GFR. We did not have information on proteinuria or the presence of diabetic nephropathy. However, stratification for diabetes did not change the results. Finally, the results of this study are only applicable to individuals with a history of MI and cannot be generalized to other patients or healthy participants.

Our trial also has several strengths. First, randomization led to balanced risk factors, which was evidenced by similar distributions of demographic variables, lifestyle factors, risk factors, and indicators of kidney function. Second, it was the largest trial investigating the effect of n-3 fatty acids on decline of kidney function and had a relative long follow-up. Third, serum cysC and cr were analyzed in a central laboratory.

In summary, we showed a small beneficial effect of an additional amount of 400 mg EPA-DHA per day on kidney function in patients with a history of MI and a low habitual EPA-DHA intake. The absolute treatment effect of 2.1 ml/min per 1.73 m<sup>2</sup> less kidney function decline that we found after 40 months of supplementation with EPA-DHA is clinically not important for an individual patient. Nevertheless, if continuous supplementation with EPA-DHA can persistently retard kidney function decline by 0.6 ml/min per 1.73 m<sup>2</sup> per year, it might prevent or postpone CKD in some high-risk patients, which is clinically relevant. The effect of EPA-DHA on kidney function should be further explored in a trial of longer duration specifically designed for patients with different stages of CKD with and without proteinuria using higher doses of EPA-DHA before a definitive statement can be made.

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