

N-3 Fatty Acids as Secondary Prevention against Cardiovascular Events in Patients Who Undergo Chronic Hemodialysis: A Randomized, Placebo-Controlled Intervention Trial

My Svensson,* Erik Berg Schmidt,[†] Kaj Anker Jørgensen,[‡] and Jeppe Hagstrup Christensen;* on behalf of the OPACH Study Group

*Department of Nephrology and [†]Department of Cardiology, Center for Cardiovascular Research, Aalborg Hospital, and

[‡]Department of Renal Medicine C, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark

Patients who are treated with chronic hemodialysis (HD) experience premature cardiovascular disease and an increased mortality. N-3 polyunsaturated fatty acids (PUFA) may be effective in the secondary prevention of cardiovascular disease, but the effects of n-3 PUFA has not previously been examined in HD patients. It was hypothesized that secondary prevention with n-3 PUFA would reduce the number of cardiovascular events and death in patients who are treated with chronic HD. A randomized, double-blind, placebo-controlled intervention trial compared the effect of n-3 PUFA and a control treatment as secondary prevention of cardiovascular events in HD patients. The primary outcome was a composite of total cardiovascular events and death. A total of 206 patients were randomly assigned to treatment with n-3 PUFA or control treatment and followed for 2 yr or until reaching a predefined end point. During the trial, 121 (59%) of 206 patients reached a primary end point. N-3 PUFA had no significant effect on the primary composite end point of cardiovascular events and death (62 *versus* 59; NS). In the n-3 PUFA group, a significant reduction was seen in the number of myocardial infarctions (four *versus* 13; $P = 0.036$). This trial was limited by a relatively small number of patients and a large number of withdrawals. However, it is concluded that treatment with n-3 PUFA did not reduce the total number of cardiovascular events and death in this high-risk population. N-3 PUFA significantly reduced the number of myocardial infarctions as a secondary outcome, a finding that might be of clinical interest.

Clin J Am Soc Nephrol 1: 780–786, 2006. doi: 10.2215/CJN.00630206

Patients who are treated with chronic hemodialysis (HD) have a high incidence of cardiovascular disease (CVD) and an increased premature mortality (1,2). Traditional risk factors of CVD are frequent in patients with kidney disease (3); in addition, the uremic milieu results in inflammation (4), specific alterations in lipid metabolism (5), and accumulation of uremic toxins (6), which may contribute to the high risk for CVD. During recent years, there has been focus on the need for intervention trials to prevent CVD and reduce mortality in this high-risk population (7).

Evidence exists from both epidemiologic (8) and interventional studies (9) that n-3 polyunsaturated fatty acids (PUFA) might be effective as secondary prevention of CVD and possibly prevent sudden cardiac death (10). However, a recent Cochrane analysis concluded that there is no clear evidence that n-3 PUFA reduce cardiovascular mortality and that there is a need for additional intervention studies in this area of research

(11). The possible mechanisms of n-3 PUFA include a lipid-lowering effect, with a reduction in plasma triglycerides (12) and a mild antihypertensive effect (13). Several other possible protective mechanisms of n-3 PUFA also have been reported, such as anti-inflammatory (14), antiatherosclerotic (15), anti-thrombotic (16), and antiarrhythmic (17). The effect of n-3 PUFA on CVD has not previously been studied in HD patients, and the need for intervention trials with n-3 PUFA in this population has been emphasized recently (7,18). Therefore, the aim of this study was to examine the effect of n-3 PUFA as secondary prevention of cardiovascular events and death in patients who are treated with chronic HD.

Materials and Methods

Study Objectives

We tested the hypothesis that treatment with n-3 PUFA would reduce the incidence of cardiovascular events and death when given as secondary prevention of CVD in patients who undergo chronic HD.

Study Design

We conducted a randomized, double-blind intervention trial in which patients were recruited from 11 dialysis centers in Denmark. The total population of HD patients was evaluated by reviewing the patients' medical records, and all patients who were eligible for inclusion

Received February 21, 2006. Accepted April 18, 2006.

Published online ahead of print. Publication date available at www.cjasn.org.

Address correspondence to: Dr. My Svensson, Department of Nephrology, Aalborg Hospital, Aarhus University Hospital, Hobrovej 18-20, 9100 Aalborg, Denmark. Phone: +45-99326610; Fax: +45-99326108; E-mail: my_svensson@hotmail.com

were asked to participate (Figure 1). Patients who had established CVD and had been treated with stable HD for at least 6 mo were eligible for inclusion. CVD was defined as previously documented myocardial infarction (MI), angina pectoris, angiographically documented coronary atherosclerosis, stroke, transient ischemic attack (TIA), or peripheral vascular disease. Exclusion criteria were patients who were participating in other clinical trials, patients with active malignant disease, and patients with known poor compliance. The study was approved by the regional ethics committee and conducted according to the Hong Kong amendment to the Declaration of Helsinki. After signed informed consent, the patients were randomly assigned to treatment with n-3 PUFA or control treatment, two capsules daily. Patients were followed for 2 yr with clinical evaluation and blood samples four times during the study period (0, 3, 12, and 24 mo). Serum phospholipid fatty acid composition of n-3 PUFA was determined to evaluate compliance. Patients who received supplement with n-3 PUFA before the study had a washout period of 4 wk before inclusion.

Randomization and Blinding

Randomization was performed using an allocation sequence that was generated by computer at an independent firm, GM pack (Hadsund, Denmark), who also packed and delivered the capsules. The allocation sequence was kept at GM pack, and the investigators did not have access to the allocation sequence until the database was closed in September 2005. Sealed envelopes for each participant were kept in case of emergency situations, but the blinding was not broken during the study. The main investigator enrolled all patients and randomly assigned them by giving them the next consecutive number. All participants, investigators, care providers, and data monitors were blinded, according to treatment, throughout the study. In addition, all analysis of serum phospholipids was performed after the end of the study period, which further ensured blinding.

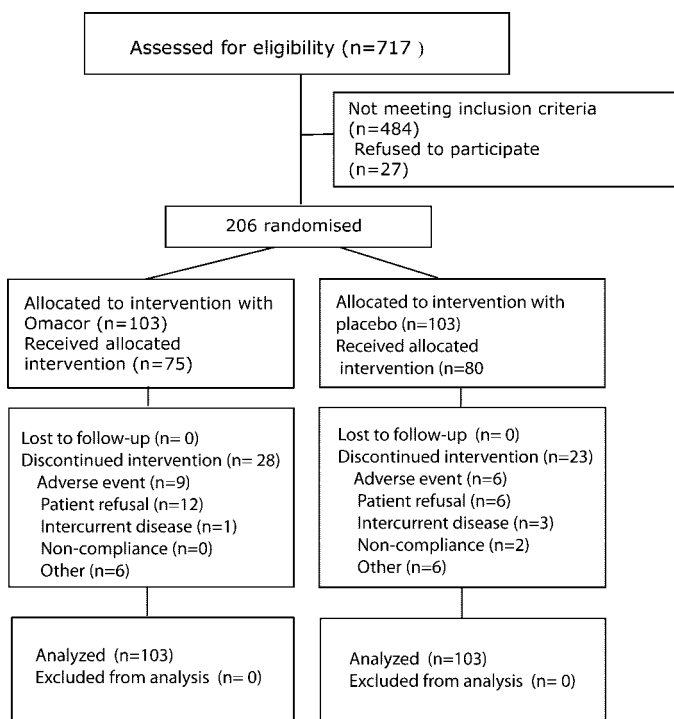


Figure 1. Flowchart illustrating trial profile.

Primary and Secondary Outcomes

Predefined end points were registered continuously throughout the study. The primary outcome was a composite of acute MI, angina pectoris that required coronary investigation or intervention, stroke, TIA, peripheral vascular disease that required surgical intervention, or death. Furthermore, we evaluated the following secondary outcomes: Acute MI; angina pectoris that required coronary investigation or intervention; stroke; TIA; peripheral vascular disease; and major coronary events, as a composite of MI and angina pectoris leading to coronary intervention. A clinical end point committee, whose members were blinded to treatment, evaluated all end points. The definitions of end points were acute MI, documented with two of three findings: (1) Typical chest pain; (2) electrocardiographic changes; and (3) elevated cardiac markers, unstable angina pectoris/crescendo angina that necessitated cardiologic investigation/intervention, TIA (clinical diagnosis defined as sudden onset of focal neurologic symptoms with regression within 24 h), stroke (defined as sudden onset of focal neurologic symptoms with verified infarction by computed tomography imaging and/or sustained symptoms for >24 h), new symptoms of peripheral vascular disease in an extremity not previously affected (verified by peripheral BP measurement or exacerbation of previously affected extremity necessitating surgical investigation or intervention), and death.

Treatment

The treatment with n-3 PUFA was administered as two capsules of Omacor (omega-3-acid ethyl esters 90) with a concentration of 20:5 n-3, eicosapentaenoic acid (EPA) of 45% and a concentration of 22:6 n-3, and docosahexaenoic acid (DHA) of 37.5%, in total 1.7 g/d n-3 PUFA. The control treatment contained 77% 18:1 n-9 (olive oil). n-3 PUFA and control capsules were identically colored gelatin capsules. n-3 PUFA treatment and control capsules were provided by Pronova Biocare (Sandefjord, Norway).

Blood Sampling and Laboratory Methods

Blood was drawn immediately before the patients' usual dialysis session. All safety parameters and standard lipid analyses were performed according to standard routines at the hospital laboratory in Aalborg. Serum was stored at -80°C in tubes that were filled with N_2 to avoid oxidation until analysis in our lipid research laboratory. In brief, total lipids were extracted from serum according to Bligh and Dyer (19) Serum (400 μl) was mixed with 500 μl of chloroform (CHCl_3) and 1000 μl of methanol that contained butylated hydroxytoluene as antioxidant. After addition of 500 μl of CHCl_3 and 500 μl of H_2O and brief mixing, the tubes were centrifuged at $1000 \times g$ for 2 min for phase separation. A total of 550 μl of the CHCl_3 phase was transferred to a Sep Pak NH_2 column (Waters Corporation, Milford, MA), which had been conditioned with hexane. Phospholipids were separated from other lipid classes as described by Kaluzny *et al.* (20). The extracted phospholipids were dried under nitrogen (N_2), redissolved in 250 μl of heptane, and transesterified according to Christoffersen and Glass (21) using 0.5 M sodium methoxide and acetic acid. The fatty acid composition was analyzed by gas chromatography using a Chrompack CP-9002 gas chromatograph (Varian, Middleburg, Netherlands) that was equipped with a Liquid Sampler CP 9050, a flame ionization detector, and a Highpolar CP-sil 88 60 m \times 0.25 mm ID capillary column. Split injection mode, temperature programming 90 to 210°C , and constant pressure were used. Helium was used as carrier gas. This approach permits quantification of fatty acid methyl esters with 14 to 24 carbon atoms and separation of several trans fatty acids. Interassay variation of serum phospholipid fatty acids was 3.5% for EPA and 2.8% for DHA.

Statistical Analyses

The study was designed to have a power of 80% to detect a relative risk of <0.6 and an expected event rate of 40% in 2 yr. This risk reduction was based on the GISSI study (22) and an observational study by Kutner *et al.* (23). The power calculations did not take into account withdrawals. Statistical analyses were performed as intention to treat and included all patients who were randomly assigned to treatment. Continuous data were reported as mean \pm SD. All *P* values were two tailed, and all confidence levels were computed to a 95% level. *P* < 0.05 was considered statistically significant. Comparison of groups was performed using a nonpaired *t* test for continuous variables and a χ^2 test to compare frequencies. The cumulative rate of cardiovascular events in the two groups was analyzed using Kaplan-Meier survival curves and Cox regression analysis. The statistical software used was STATA and SPSS (version 11.0; SPSS, Inc., Chicago, IL).

Results

Patients were enrolled between November 2002 and May 2003. A total of 206 patients were included and randomly assigned to treatment with n-3 PUFA or control treatment. Patients are illustrated in a flowchart (Figure 1). The two groups were well matched according to baseline characteristics and medication (Table 1). The patients were followed for 2 yr or until reaching a primary end point with a median follow-up of 558 d (range 219 to 730).

A total of 121 (59%) of 206 patients reached a primary end point during follow-up (Table 2). No significant difference was seen in the total number of cardiovascular end points and death between the two treatment groups, with 62 end points in the n-3 PUFA group compared with 59 end points in the control group (Figure 2). A total of 17 MI were observed during follow-up, with four MI in the n-3 PUFA group compared with 13 MI in the control group, which was a significant difference (*P* = 0.036; Figure 3). Although NS, there was a tendency toward more strokes in the n-3 PUFA group. Results from the per-protocol analysis, including all patients who adhered to the protocol, were consistent with those from the intention-to-treat analysis (data not shown). Throughout the trial, a significant increase in serum phospholipid EPA and DHA in the n-3 PUFA group was observed compared with the control group (Table 3).

Fifty-one (25%) patients were withdrawn from the study, mainly because of patient refusal for various reasons or adverse events. There was no significant difference between withdrawals in the n-3 PUFA group (*n* = 28) and in the control group (*n* = 23). Occurrence of a primary end point among all withdrawals (61%) did not differ significantly from nonwithdrawals (58%). Withdrawals were similar to nonwithdrawals for all the baseline characteristics of Table 1 (data not shown).

There was a tendency toward more patients with adverse events in the n-3 PUFA group (31 [30%] of 103) compared with the control group (22 [21%] of 103; NS). In the n-3 PUFA group, 12 (12%) of 103 patients had a serious adverse event compared with seven (7%) of 103 patients in the control group (NS). The majority of adverse events reported were gastrointestinal complaints (Table 4).

Table 1. Baseline characteristics of the 206 patients in chronic HD^a

	n-3 PUFA (<i>n</i> = 103)	Control (<i>n</i> = 103)
Age (yr)	66 \pm 11	68 \pm 12
Female gender	34 (33)	39 (38)
BMI (kg/m ²)	24.7 \pm 4.4	24.0 \pm 4.1
Months on dialysis	44 \pm 42	44 \pm 39
Active smoker	29 (28)	28 (27)
Diabetes	23 (22)	26 (26)
Hypertension	79 (77)	81 (79)
systolic BP (mmHg)	148 \pm 28	154 \pm 28
diastolic BP (mmHg)	77 \pm 17	77 \pm 13
CVD		
MI	40 (39)	26 (25)
CABG/PCI	26 (25)	29 (28)
angina pectoris	38 (37)	37 (36)
stroke/TIA	41 (40)	44 (43)
peripheral vascular disease	35 (34)	36 (35)
Laboratory parameters		
hemoglobin (mmol/L)	11.7 \pm 1.3	11.8 \pm 1.3
total cholesterol (mmol/L)	4.9 \pm 1.3	4.9 \pm 1.3
albumin(g/L)	36.2 \pm 3.1	36.0 \pm 5.2
Kt/V	1.42	1.40
URR (%)	74	74
Concomitant medication (n)		
β blockers	52 (50)	59 (57)
calcium antagonists	35 (34)	33 (32)
ACE inhibitors	28 (27)	25 (24)
angiotensin II receptor antagonists	14 (14)	6 (6)
aspirin	69 (67)	78 (76)
statins	21 (20)	20 (19)

^aData are mean \pm SD or *n* (%). ACE, angiotensin-converting enzyme; BMI, body mass index; CABG, coronary artery bypass grafting; CVD, cardiovascular disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; PUFA, polyunsaturated fatty acids; TIA, transient ischemic attack; URR, urea reduction rate.

Discussion

In this study of HD patients with established CVD, secondary prevention with n-3 PUFA had no significant effect on the primary end point of all-cause mortality and total number of cardiovascular events. However, treatment with n-3 PUFA for 2 yr significantly reduced the number of MI in these high-risk patients.

Some evidence suggests that n-3 PUFA might be effective as secondary prevention of cardiovascular events in the general population (8–10). He *et al.* (8) showed in a meta-analysis of cohort studies that there was a reduction in cardiovascular death rates with increasing fish intake. For every 20-g/d increase in fish intake, mortality from CVD was reduced by 7%. However, a recent Cochrane review (11) showed no beneficial effect of n-3 PUFA on CVD. The Cochrane review has been a

Table 2. Number of cardiovascular end points in the two groups during follow-up^a

	n-3 PUFA	Control	HR (95% CI)	P
Primary end points				
Cardiovascular event or death	62 (60.2)	59 (57.3)	1.04 (0.72 to 1.48)	0.85
Secondary end points				
MI	4 (3.9)	13 (12.6)	0.30 (0.10 to 0.92)	0.036
coronary intervention	3 (2.9)	4 (3.9)	0.73 (0.16 to 3.25)	0.68
major coronary events	7 (6.8)	17 (16.5)	0.40 (0.17 to 0.97)	0.043
stroke	7 (6.8)	3 (2.9)	2.23 (0.58 to 8.64)	0.24
TIA	5 (4.9)	2 (1.9)	2.54 (0.49 to 13.1)	0.26
peripheral vascular disease	9 (8.7)	7 (6.8)	1.26 (0.47 to 3.39)	0.65
death, total	34 (33.0)	30 (29.1)	1.12 (0.69 to 1.83)	0.65

^aData are *n* (%). CI, confidence interval; HR, hazard ratio.

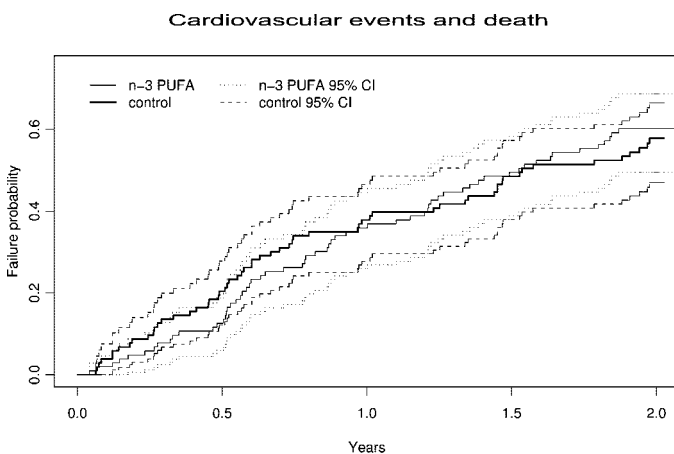


Figure 2. Kaplan-Meier survival curve showing the total number of cardiovascular events and death in the two treatment groups.

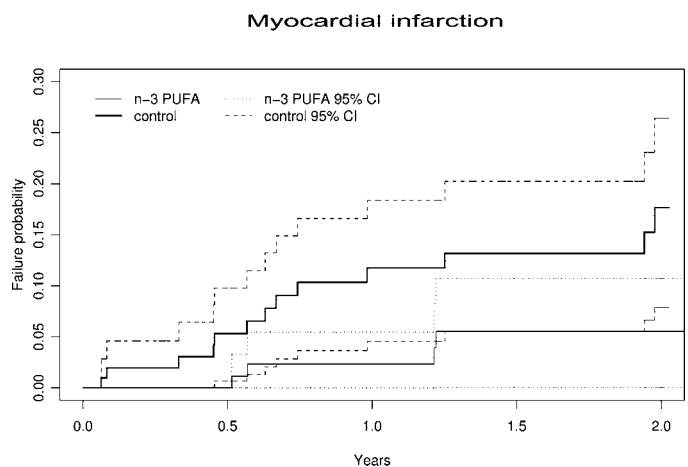


Figure 3. Kaplan-Meier curve showing the incidence of myocardial infarctions in the two treatment groups.

topic of discussion as the negative result mainly was a consequence of the Diet and Reinfarction Trial II (DART II) study, which showed an unexplained increased mortality in patients who had angina pectoris and were randomly assigned to oily fish (24). These results stand in contrast to the first DART study (25), in which patients were given dietary advice after an MI. After 2 yr, a reduction of 29% in total mortality was observed in the group of patients who were advised to eat fatty fish twice a week, compared with a control diet. Similar results were also shown in the large GISSI-Prevenzione trial (22) of MI survivors, with a significant reduction in the primary composite end point of MI, stroke, and cardiovascular death in the group of patients who were treated with one capsule of Omacor (0.85g of n-3 PUFA). So far, few data exist regarding intervention with n-3 PUFA in HD patients. We previously showed beneficial effects of n-3 PUFA on the lipid profile in predialysis patients (26), and Kutner *et al.* (23) showed in an observational study that dialysis patients with a high intake of fish lived longer, compared with patients with a low fish intake. There are several possible beneficial effects of n-3 PUFA in the dialysis population, an area that recently has been reviewed by Friedman and Moe (18). To

our knowledge, no earlier studies addressed the effect of n-3 PUFA on cardiovascular end points and death in HD patients.

In this study, no significant effect was seen on the primary end point of total cardiovascular events and death. A possible explanation for this finding is that this study was designed to show a relatively large risk reduction from n-3 PUFA, based on the GISSI study (22) and observational data from Kutner *et al.* (23). It therefore is possible that we overlooked a smaller risk reduction in the primary end point, which our study was not powered to detect. Moreover, only approximately half of the deaths in the HD population are due to CVD. Therefore, choosing total death as an end point might have weakened the results, because an effect on mortality from noncardiac causes might not be plausible in this population. It also is possible that intervention in fact should begin at an earlier stage to have a preventive effect of CVD in these high-risk patients. Furthermore, the dose of n-3 PUFA might have been too small, although previous data recommend the use of 1 g/d n-3 PUFA as secondary prevention of CVD (27). We chose a dose of 1.7 g/d, because some smaller studies suggested that HD patients might be depleted of fatty acids (28,29) and larger doses might not be

Table 3. Compliance data with serum phospholipid fatty acid composition at baseline and after 3, 12, and 24 mo of treatment^a

	n-3 PUFA				Control			
	Baseline (n = 103)	3 Mo (n = 86)	12 Mo (n = 53)	24 Mo (n = 32)	Baseline (n = 103)	3 Mo (n = 82)	12 Mo (n = 53)	24 Mo (n = 36)
n-3, 20:5	1.4 ± 0.6	3.8 ± 1.2 ^b	3.8 ± 1.3 ^b	3.8 ± 1.4 ^b	1.6 ± 0.8	1.5 ± 0.7	1.3 ± 0.5	1.6 ± 0.7
n-3, 22:6	4.0 ± 1.0	5.5 ± 1.2 ^b	5.6 ± 1.1 ^b	5.5 ± 1.3 ^b	4.0 ± 1.1	4.0 ± 1.1	3.9 ± 0.8	3.8 ± 1.3

^aFatty acids are shown as % of total amount of fatty acids ± SD.

^bP < 0.001 versus baseline and control group.

Table 4. Adverse events in the two treatment groups^a

	n-3 PUFA	Control
Abdominal pain	9	2
Diarrhea	7	7
Nausea and vomiting	10	10
Other gastrointestinal	3	2
Gastrointestinal bleeding	8	5
Cerebral bleeding	2	1
Bleeding, other	5	1
Other various	12	9
Total	56	37

^aSome patients had more than one adverse event.

clinically applicable because the incidence of gastrointestinal complaints increases with larger doses of n-3 PUFA.

Although no effect of n-3 PUFA was seen on the primary end point of total cardiovascular end points and death, we find the significant reduction of MI in the n-3 PUFA group of interest. The secondary nature of this end point and the small total number of MI make interpretations slightly speculative; nevertheless, there are several possible mechanisms that might explain such an effect. First, an antithrombotic effect of n-3 PUFA could be of importance, and previous animal studies showed that n-3 PUFA inhibit thrombus formation (30). Although in human trials large doses of n-3 PUFA have been used to achieve antithrombotic effects, in daily clinic, the antithrombotic effect of n-3 PUFA has not been documented convincingly (16). In addition, in both the DART study (25) and the GISSI trial (22), no effect was seen on the number of MI as a single end point. Conversely, multiple changes in parameters of hemostasis and thrombosis were shown in patients who were treated with chronic HD (31). In our study, the relative risk reduction of an MI was 70% and the absolute risk reduction 8.7% when treatment with n-3 PUFA was compared with control treatment, suggesting a potent antithrombotic effect of n-3 PUFA in this population. In addition, Schmitz *et al.* (32) showed a significant reduction in the number of graft thromboses in HD patients who received a supplement of n-3 PUFA, which further could support an antithrombotic effect of n-3 PUFA in HD patients. Second, Thies *et al.* (33) recently showed that supplementation with n-3 PUFA before carotid surgery led to increased incorporation of n-3 PUFA in carotid plaques and

interestingly decreased inflammation in the plaque. The authors suggested that n-3 PUFA might stabilize the atherosclerotic plaque, a mechanism that also could be a possible explanation of our findings. Finally, we cannot rule out a possible antioxidative effect in this population. It was shown previously that levels of oxidative stress are increased in patients with uremia, a phenomenon that might be part of the increased risk burden in patients who are treated with chronic HD (34). Previous studies with n-3 PUFA in dialysis patients have suggested that n-3 PUFA might increase the antioxidant capacity of LDL particles (35), and Ando *et al.* (36) showed that n-3 PUFA reduced levels of oxidized LDL after 3 mo of treatment with 1.8 g of n-3 PUFA, compared with placebo. In the general population, interventional studies with antioxidant therapies have not shown beneficial effects (37). However, some data from patients who were treated with chronic HD suggested that antioxidant therapy might be effective. Although in the general population, vitamin E has shown no effect on cardiovascular mortality or morbidity (37), the SPACE investigators showed a reduction in the number of MI and cardiovascular end points after treatment with vitamin E in a population of HD patients (38). These results are similar to our findings in several ways. First, the study population was similar in size; second, the effect of the intervention was shown on the number of MI; and, finally, no effect was shown on total mortality. In HD patients, Tepel *et al.* (39) showed a significant reduction in cardiovascular events after treatment with acetylcysteine, although no effect was shown on total mortality. These studies suggest that antioxidative treatment might play an important role in HD patients, and an antioxidative effect of n-3 PUFA therefore might be a possible explanation of the findings in this study.

Strengths and Limitations

One of the strengths of our study was the ability to document compliance, by determination of plasma fatty acid composition. Therefore, we were able to show a significant increase in EPA and DHA in the group that was treated with n-3 PUFA compared with control treatment during the entire study period. However, the strength of measuring compliance is somewhat limited because of the large number of withdrawals for which we do not have blood samples. The study participants are representative of the heterogeneous population of HD patients,

and treatment with n-3 PUFA is added to concomitant medication, which gives the results a good external validity.

There also are several limitations of this study. The power calculations were based on a large risk reduction, and we therefore might have overlooked a smaller difference between groups. The composite primary end point including total death might have blurred the results and thereby failed to show a difference between groups. Although interesting, the significant difference in MI between groups was a secondary outcome and therefore should be interpreted with caution.

Conclusion

In our study of n-3 PUFA as secondary prevention of CVD in patients who were treated with chronic HD, no significant effect was seen on the primary composite end point of death and total number of cardiovascular events. It is interesting that we report that treatment with n-3 PUFA significantly reduced the number of MI as a secondary outcome. The significant reduction in MI might be explained by antithrombotic, anti-inflammatory, or antioxidative effect from n-3 PUFA in this population. These findings need to be confirmed in larger intervention studies with n-3 PUFA that are designed to evaluate MI as a primary outcome.

Acknowledgments

This study was supported by The Danish Heart Foundation, The Danish Kidney Foundation, the Research Foundation of the County of Northern Jutland, and Pronova Biocare.

These data first were presented as an abstract at the annual meeting of the American Society of Nephrology in Philadelphia, PA, November 8 to 13, 2005.

The OPACH Study Group: H. Danielsen and E. Randers (Viborg Hospital, Viborg, Denmark); I. Tietze, T. Gohr, and J.H. Kristiansen (Fredericia Hospital, Fredericia, Denmark); F.T. Nielsen (Odense University Hospital, Odense, Denmark); and S. Saugmann, L.H. Taasti, and R.S. Pedersen (Esbjerg Hospital, Esbjerg, Denmark). Contributors: M. Svensson, J.H. Christensen, E.B. Schmidt, and K.A. Jørgensen took part in writing the protocol, organizing the study, and preparing the manuscript; M. Svensson was responsible for the operative organization, all data collection, and data management.

We thank C. Dethlefsen and A. Gorst-Rasmussen for statistical assistance and I. Aardestrup for invaluable laboratory assistance.

References

1. Vanholder R, Massy Z, Argiles A, Spasovski G, Verbeke F, Lanieri N; European Uremic Toxin Working Group: Chronic kidney disease as cause of cardiovascular morbidity and mortality. *Nephrol Dial Transplant* 20: 1048–1056, 2005
2. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, Mc Cullough PA, Kasiske BL, Kelepouris MJ, Klag MJ, Parfrey P, Pfeffer M, Raji L, Spinosa DJ, Wilson PW: American heart association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology and epidemiology and prevention. Kidney disease as a risk factor for development of cardiovascular disease. AHA scientific statement. *Circulation* 108: 2154–2169, 2003
3. Menon V, Gul A, Sarnak M: Cardiovascular risk factors in chronic kidney disease. *Kidney Int* 68: 1413–1418, 2005
4. Zimermann J, Herrlinger S, Pruy A, Metzger T, Wanner C: Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 55: 648–658, 1999
5. Wanner C, Krane V: Uremia-specific alterations in lipid metabolism. *Blood Purif* 20: 451–453, 2002
6. Vanholder R, De Smet R, Glorieux G, Argiles A, Baumeister V, Brunet P, Clark W, Cohen G, De Deyn PP, Deppish R, Descamps-Latscha B, Henle T, Jones A, Lemke HD, Mazzy ZA, Passlick-Dejean J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W; European Uremic Toxic Work Group (EUTox): Review on uremic toxins: Classification, concentration and interindividual variability. *Kidney Int* 63: 1934–1943, 2003
7. K/DOQI clinical practice guidelines on cardiovascular disease in dialysis patients. *Am J Kidney Dis* 45: S91–S95, 2005
8. He K, Song Y, Daviglius M, Liu K, Van Horn L, Dyer AL, Greenland P: Accumulated evidence on fish consumption and coronary heart disease mortality: A meta-analysis of cohort studies. *Circulation* 109: 2705–2711, 2004
9. Studer M, Briel M, Leimenstoll B, Glass TR, Bucher HC: Effect on different antilipidemic agents and diets on mortality. A systematic review. *Arch Intern Med* 165: 725–730, 2005
10. Albert C, Campos H, Stampfer M, Ridker PM, Manson JE, Willett WC, Ma J: Blood levels of long chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 346: 1113–1118, 2002
11. Hooper L, Thompson RL, Harrison RA, Summerbell CD, Moore H, Worthington HV, Durrington PN, Ness AR, Capps NE, Davey Smith G, Riemersma RA, Ebrahim SB: Omega 3 fatty acids for prevention and treatment of cardiovascular disease [Cochrane review]. *Cochrane Database Syst Rev* 4: CD003/7718, 2004
12. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. *Atherosclerosis* Mar 9, 2006 [epub ahead of print]
13. Geleijnse J, Giltay E, Grobbee D, Donders AR, Kok FJ: Blood pressure response to fish oil supplementation: Metaregression analysis of randomised trials. *J Hypertens* 20: 1493–1499, 2002
14. Calder PC: N-3 fatty acids and inflammation: From molecular biology to the clinic. *Lipids* 38: 343–352, 2003
15. von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H: The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomised, double blind, placebo-controlled trial. *Ann Intern Med* 130: 554–562, 1999
16. Kristensen SD, Bach Iversen AM, Schmidt EB: N-3 polyunsaturated fatty acids and coronary thrombosis. *Lipids* 36: S79–S82, 2003
17. Christensen JH: n-3 fatty acids and the risk of sudden cardiac death: Emphasis on heart variability. *Dan Med Bull* 50: 347–367, 2003
18. Friedman A, Moe S: Review of the effects of omega-3 supplementation in dialysis patients. *Clin J Am Soc Nephrol* 1: 182–192, 2006
19. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911–917, 1959
20. Kaluzny MA, Duncan LA, Merritt MV, Epps DE: Rapid

- separation of lipid classes in high yield and purity using bonding columns. *J Lipid Res* 26: 135–140, 1985
21. Christoffersen SW, Glass RL: Preparation of milk fat methyl esters by alcoholysis in an essentially non-alcoholic solution. *J Dairy Sci* 52: 1289–1290, 1969
 22. GISSI Prevenzione Investigators: Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: Results of the GISSI Prevenzione trial. *Lancet* 354: 447–455, 1999
 23. Kutner NG, Clow PW, Zhang R, Aviles X: Association of fish intake and survival in a cohort of incident dialysis patients. *Am J Kidney Dis* 39: 1018–1024, 2002
 24. Burr M, Ashfield-Watt P, Dunstan F, Feihly AM, Breay P, Ashton T, Zotos PC, Haboubi NA, Elwood PC: Lack of benefit of dietary advice to men with angina: Results of a controlled trial. *Eur J Clin Nutr* 57: 193–200, 2003
 25. 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 infarction; Diet and Reinfarction Trial (DART). *Lancet* 30: 757–761, 1989
 26. Svensson M, Christensen JH, Solling J, Schmidt EB: The effect of n-3 fatty acids on plasma lipids and lipoproteins and blood pressure in patients with CRF. *Am J Kidney Dis* 44: 77–83, 2004
 27. Kris-Etherton P, Harris W, Appel L; American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation* 106: 2747–2757, 2002
 28. Koorts AM, Viljoen M, Kruger MC: Red blood cell fatty acid profile of chronic renal failure patients receiving maintenance haemodialysis treatment. *Prostaglandins Leukot Essent Fatty Acids* 67: 13–18, 2002
 29. Peck LW, Monsen ER, Ahmad S: Effect of three sources of long-chain fatty acids on the plasma fatty acid profile, plasma prostaglandin E2 concentrations, and pruritus symptoms in hemodialysis patients. *Am J Clin Nutr* 64: 210–214, 1996
 30. Jerling JC, Curiel-Martos A, Kroner C, Kloots W: Fish oil inhibits photochemically induced thrombosis in the guinea pig in a dose dependent manner. *Thromb Res* 111: 11–17, 2003
 31. Casserly LF, Dember LM: Thrombosis in end-stage renal disease. *Semin Dial* 16: 245–256, 2003
 32. Schmitz P, McCloud L, Reikes S, Leonard C, Gellens M: Prophylaxis of hemodialysis graft thrombosis with fish oil: Double blind, randomized, prospective trial. *J Am Soc Nephrol* 13: 184–190, 2002
 33. Thies F, Garry JM, Yaqoob P, Rekasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF: Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: A randomised controlled trial. *Lancet* 361: 477–485, 2003
 34. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C: Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. *Nephrol Dial Transplant* 18: 1272–1280, 2003
 35. Bonanome A, Biasia F, De Luca M, Munaretto G, Biffanti S, Pradella M, Pagnan A: n-3 fatty acids do not enhance LDL susceptibility to oxidation in hypertriglycerolemic hemodialyzed subjects. *Am J Clin Nutr* 63: 261–266, 1996
 36. Ando M, Sanaka T, Nihei H: Eicosapentaenoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. *J Am Soc Nephrol* 10: 2177–2184, 1999
 37. Kris-Etherton P, Lichtenstein A, Howard B, Steinberg D, Witzum JL; Nutrition Committee of the American Heart Association Council on Nutrition, Physical Activity and Metabolism: Antioxidant vitamin supplements and cardiovascular disease. *Circulation* 110: 637–641, 2004
 38. Boaz M, Smetana S, Weinstein T, Matas Z, Gafer U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, Green MS: Secondary prevention with antioxidants of cardiovascular disease in end stage renal disease (SPACE): Randomised placebo-controlled trial. *Lancet* 356: 1213–1218, 2000
 39. Tepel M, Van der Giet M, Statz M, Jankowski J, Zidek W: The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal disease. *Circulation* 107: 992–995, 2003