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

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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.


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
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.

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 and control 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 N2 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 (CHCl3) and 1000 µl of methanol that contained butylated hydroxytoluene as antioxidant. After addition of 500 µl of CHCl3 and 500 µl of H2O and brief mixing, the tubes were centrifuged at 1000 × g for 2 min for phase separation. A total of 550 µl of the CHCl3 phase was transferred to a Sep Pak NH2 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 (N2), 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 mx0.25 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 ± 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 χ² 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 (Table 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).

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>
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<tr>
<th>Table 1. Baseline characteristics of the 206 patients in chronic HDa</th>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Female gender</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Months on dialysis</td>
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<tr>
<td>Active smoker</td>
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<tr>
<td>Diabetes</td>
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<td>Hypertension</td>
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<td>systolic BP (mmHg)</td>
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<td>diastolic BP (mmHg)</td>
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<td>CVD</td>
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<td>MI</td>
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<td>CABG/PCI</td>
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<tr>
<td>angina pectoris</td>
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<tr>
<td>stroke/TIA</td>
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<td>peripheral vascular disease</td>
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<td>Laboratory parameters</td>
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<tr>
<td>hemoglobin (mmol/L)</td>
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<tr>
<td>total cholesterol (mmol/L)</td>
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<td>albumin (g/L)</td>
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<tr>
<td>Kt/V</td>
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<tr>
<td>URR (%)</td>
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<td>Concomitant medication (n)</td>
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<td>β blockers</td>
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<tr>
<td>calcium antagonists</td>
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<td>ACE inhibitors</td>
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<td>angiotensin II receptor</td>
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<td>antagonists</td>
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<td>aspirin</td>
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<tr>
<td>statins</td>
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<tr>
<td>aData are mean ± 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.</td>
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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 2. Number of cardiovascular end points in the two groups during follow-up

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

aData are n (%). CI, confidence interval; HR, hazard ratio.
creased incorporation of n-3 PUFA in carotid plaques and mentation with n-3 PUFA before carotid surgery led to in-
ther could support an antithrombotic effect of n-3 PUFA in HD
significant reduction in the number of graft thromboses in HD
treatment, suggesting a potent antithrombotic effect of n-3 PUFA in
achieve antithrombotic effects, in daily clinic, the antithrom-
ındadaumentation with n-3 PUFA before carotid surgery led to in-
carotid plaques and
interestingly decreased inflammation in the plaque. The au-
thors suggested that n-3 PUFA might stabilize the atheroscle-
rotic plaque, a mechanism that also could be a possible expla-
nation 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). Pre-
vious studies with n-3 PUFA in dialysis patients have sug-
gested 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 cardio-
vascular 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, al-
though no effect was shown on total mortality. These studies
suggests that antioxidant 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 com-
pared 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,
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; never-
theless, there are several possible mechanisms that might ex-
plain 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 antithrom-
botic 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 hemosta-
sis 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 treat-
ment, suggesting a potent antithrombotic effect of n-3 PUFA in
this population. In addition, Schmitz et al. (32) showed a sig-
nificant reduction in the number of graft thromboses in HD
patients who received a supplement of n-3 PUFA, which fur-
ther could support an antithrombotic effect of n-3 PUFA in HD
patients. Second, Thies et al. (33) recently showed that supple-
mentation with n-3 PUFA before carotid surgery led to in-
creased incorporation of n-3 PUFA in carotid plaques and

| Table 3. Compliance data with serum phospholipid fatty acid composition at baseline and after 3, 12, and 24 mo of treatmenta |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| n-3 PUFA                                      | Control         |
| Baseline (n = 103)                             | Baseline (n = 103) |
| 3 Mo (n = 86)                                  | 3 Mo (n = 82)   |
| 12 Mo (n = 53)                                 | 12 Mo (n = 53)  |
| 24 Mo (n = 32)                                 | 24 Mo (n = 36)  |
| n-3, 20:5                                     | 1.4 ± 0.6       | 1.6 ± 0.8       | 4.0 ± 1.1       |
| n-3, 22:6                                     | 4.0 ± 1.0       | 5.5 ± 1.2       | 5.6 ± 1.1       |
| n-3, 22:6                                     | 5.5 ± 1.2       | 5.6 ± 1.1       | 5.5 ± 1.3       |
| n-3, 22:6                                     | 5.6 ± 1.1       | 5.5 ± 1.3       | 5.5 ± 1.3       |

aSome patients had more than one adverse event.

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

P < 0.001 versus baseline and control group.
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.

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