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ORIGINAL COMMUNICATION

Increased lipid peroxidation during long-term intervention with high doses of n-3 fatty acids (PUFAs) following an acute myocardial infarction

H Grundt[1,2]*, DWT Nilsen[2], MA Mansoor[1] and A Nordøy[3]

[1]*Department of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway;* [2]*Department of Medicine, Central Hospital in Rogaland, Stavanger, Norway; and* [3]*Department of Medicine, University of Tromsoe, Norway*

Objective: To assess the oxidative burden of a highly concentrated compound of n-3 PUFAs as compared to corn oil by measuring thiobarbituric acid–malondialdehyde complex (TBA–MDA) by HPLC. We also studied the influence on TBA–MDA of statins combined with n-3 PUFAs or corn oil.
Design: A prospective, randomised, double-blind, controlled study.
Setting: One hospital centre in Stavanger, Norway.
Subjects: A total of 300 subjects with an acute myocardial infarction (MI).
Interventions: Gelatine capsules, containing 850–882 mg EPA and DHA as concentrated ethylesters, or 1 g of corn oil, were ingested in a dose of two capsules twice a day for at least 1 y. Alpha-tocopherol (4 mg) was added to all capsules to protect the PUFAs against oxidation.
Results: After 1 y TBA–MDA increased modestly in the n-3 PUFA group ($n=125$), as compared to the corn oil group ($n=130$), $P=0.027$. Multiple linear regression analyses of fatty acids in serum total phospholipids ($n=56$) on TBA–MDA measured after 12 months intervention, showed no dependency. Performing best subsets regression, serum phospholipid concentration of arachidonic acid (20:4 n-6 PUFA) was identified as a predictor of TBA–MDA at 12 months follow-up, $P=0.004$.
We found no impact of statins on TBA–MDA.
Conclusion: TBA–MDA increased modestly after long-term intervention with n-3 PUFAs compared to corn oil post-MI, suggesting biological changes induced by n-3 PUFAs, rather than simply reflecting their concentration differences. The peroxidative potential of n-3 PUFAs was not modified by statin treatment.
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Introduction

Despite several favourable effects of n-3 PUFAs on inflammation and atherothrombosis, concern has been raised, as n-3 PUFAs may have a potential to increase oxidative stress (Harats *et al*, 1991; Meydani *et al*, 1991), resulting in the formation of lipid peroxides. During peroxidation of PUFAs several chain reactions may modify lipids and lipoproteins containing PUFAs (Dargel, 1992; Halliwell & Chirico, 1993). Peroxidation of lipids and lipoproteins, especially low-density lipoprotein (LDL), is thought to play an important role in the development of atherosclerotic plaques (Avogaro *et al*, 1988; Boyd *et al*, 1989; Steinberg *et al*, 1989; Rosenfeld, 1991; Wiklund *et al*, 1991).

In vitro studies of lipid peroxidation suggest an increased susceptibility to oxidation with increasing number of double bonds in fatty acids (Cosgrove *et al*, 1987; Liu *et al*, 1997). However, the impact of n-3 PUFAs supplementation on lipid peroxidation is controversial (Nenseter & Drevon, 1996). Several clinical studies have reported no significant change in the lipid peroxidation following increased consumption of n-3 PUFAs (Nenseter *et al*, 1992; Bittolo-Bon *et al*, 1993;

***Correspondence: H Grundt, Department of Internal Medicine, Central Hospital in Rogaland, POB 8100, 4068 Stavanger, Norway.**
E-mail: heidi@madlalia.no
***Guarantor*: H Grundt.**
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Frankel *et al*, 1994; Eritsland *et al*, 1995; Bonanome *et al*, 1996; Wander *et al*, 1996; Brude *et al*, 1997; Hansen *et al*, 1998). No enhancement of the lipid peroxidation was noted after 9 months supplementation with 4 g n-3 PUFA concentrate in the shunt occlusion trial (Eritsland *et al*, 1995). Moreover, thiobarbituric acid reactive substances (TBARS) as a global marker of peroxidation remained unchanged in healthy males given 4 g purified EPA or DHA for 5 weeks, despite an immediate increase in chylomicron peroxidation *ex vivo* (Hansen *et al*, 1998). On the other hand, Meydani (Meydani *et al*, 1991) found increased TBARS in plasma samples from women given supplements of fish oil for 12 weeks. In agreement with these results, Harats *et al* (1991) observed both an increase in LDL TBARS and an accelerated metabolism of LDL by macrophages. These divergent/inconsistent findings may be because of different experimental settings, as these studies include relatively small sample sizes, different doses and compositions of n-3 PUFAs, short intervention periods, different amounts of antioxidants supplies and different methods used to quantify the oxidative burden and different methods to estimate TBARS.

As the beneficial effects of n-3 PUFAs (Kinsella *et al*, 1990; Schmidt & Dyerberg, 1994) may be opposed by an enhanced lipid peroxidation, the main aim of this study was to prospectively assess the impact of concentrated n-3 PUFAs *vs* corn oil on lipid peroxidation during 12 months of treatment in 300 acute myocardial infarction (MI) patients demonstrating improvement in lipids (Nilsen *et al*, 2001). As a marker of lipid peroxidation, we determined TBA–MDA measured by HPLC.

We decided to include ultrasensitive CRP (μCRP) and plasma total homocysteine (p-tHcy) in the baseline evaluation of our trial subjects, as these parameters have become approved markers of cardiovascular disease (Arnesen *et al*, 1995; Ridker *et al*, 1998).

Finally, we hypothesized that additional statin therapy might favourably reduce lipid peroxidation in subjects on n-3 PUFAs, in view of a recent study demonstrating beneficial effects on serum lipids and lipoproteins by short-term combined therapy (Nordøy *et al*, 1998).

Subjects and methods

Subjects

This prospective study was designed as part of a randomised, parallel, double-blind study evaluating the effects on clinical outcome and serum lipids of a concentrate of n-3 PUFAs, introduced early after an acute MI (Nilsen *et al*, 2001).

A total of 300 subjects (238 men and 62 women), 28–87 y of age, with an acute MI verified by WHO criteria, were recruited at one hospital centre from September 1995 until December 1996. Written informed consent was obtained from each subject. Regular supplementation of other fish-oil products was discontinued prior to intervention. Exclusion criteria have previously been described (Nilsen *et al*, 2001). All participants were included between the 4th and the 6th day following the acute MI. Included subjects were randomly assigned to receive gelatine capsules (Pronova A/S, Oslo, Norway), containing 850–882 mg EPA and DHA as concentrated ethylesters, or 1 g of corn oil, both administered in a dose of two capsules twice a day for at least 1 y (total 3.464 g n-3 PUFAs or 4.000 g corn oil). This dose of n-3 PUFAs has previously been shown to be therapeutically useful in lowering triglyceride levels and blood pressure (Phillipson *et al*, 1985; Eritsland *et al*, 1995; Connor & Connor, 1997). Alpha-tocopherol (4 mg) was added into all capsules to protect the unsaturated fatty acids against oxidation. The study was double-blind. Intervention was initiated immediately after inclusion and collection of baseline blood samples. Details regarding compliance have previously been reported (Nilsen *et al*, 2001).

The phospholipid fatty acid profile was analysed at baseline and after 12 months of interventention in serum from 60 subjects randomly allocated from the study participants. In all, 56 of the allocated subjects had complete data throughout the study period. The study population was compared to 30 healthy individuals from a general out-of-hospital population, matched according to age, gender, smoking habits and diet. Their diet was similar to the average Norwegian diet. These subjects were not taking any supplementation of n-3 PUFAs.

Simvastatin in a dose of 20–40 mg was initiated prior to discharge and continued beyond 12 months follow-up in 95 patients. This subgroup was compared to 79 individuals free of statins during the evaluation period of 12 months.

Clinical examination and laboratory measurements

Clinical follow-up, including a detailed patient history, clinical examination, blood tests, electrocardiogram and capsule counting was performed at inclusion, at 6 weeks, 6 months and at 1 y. All cardiac events (cardiac death, resuscitation, recurrent MI, unstable angina pectoris, revascularisation), noncardiac death, smoking habits, ongoing medication and average fishmeals per week were recorded. The blood samples were preceded by 12 h of fasting.

Serum for analyses of TBA–MDA and ultrasensitive C-reactive protein (μCRP) and plasma for analyses of homocysteine (Hcy) were immediately frozen and stored at −80°C. The analyses were performed by a technician with no knowledge of the randomisation code.

TBA–MDA was measured in duplicate in serum samples heated with thiobarbituric acid at low pH, separated and determined by HPLC-fluorescence detection, based on previously described methods by Wong *et al* (1987).

μCRP was determined in serum samples by an ultrasensitive immunoassay, using a kit from Behring Diagnostics, Marburg, Germany.

Total plasma Hcy was determined using an HPLC assay as previously described (Mansoor *et al*, 1992).

Serum triglycerides and total cholesterol were determined by enzymatic methods with kits provided by Roche Diag-

nostics, Mannheim, Germany (GPO-PAP, CHOP-PAP). HDL-cholesterol was similarly measured after precipitation with dextran sulphate and magnesium chloride (Burstein *et al*, 1970).

Analyses of fatty acids in serum phospholipids were performed at the University of Tromsø, Norway, as previously described (Nordøy *et al*, 1998).

Troponin-T (TnT), which is a sensitive marker of minor myocardial cell injury was quantified by a cardiac-specific second-generation troponin-T ELISA assay, using a high-affinity cardiac-specific TnT isoform antibody (Roche Diagnostics, Mannheim, Germany), (Muller-Bardorff *et al*, 1997), modified by Hetland *et al* (1995).

Creatine kinase-muscle brain (CK-MB) analysis was performed on a Johnson and Johnson Vitros 700 analyser, based upon the immunoinhibition principle (Gerhardt *et al*, 1982).

The study was approved by the Regional Board of Research Ethics and the Norwegian Health Authorities, and conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Statistical methods

Potential differences between intervention groups at baseline regarding quantitative variables were tested by a two-sample Student's *t*-test, or in case of non-normality by the Mann–Whitney rank-sum test. Continuously distributed variables were given as mean and standard deviation (s.d.), while variables with more skewed distributions were given as median and upper and lower quartiles.

Changes in parameter values from baseline to follow-up were calculated for each individual patient and subjected to statistical analysis. As the normality assumption was fulfilled, the *t*-test was used to evaluate whether changes in the fatty acid composition of the serum phospholipids and in TBA–MDA from baseline to 12 months were significantly different from zero, and to compare changes between treatment groups. Also, comparison of TBA–MDA between treatment groups at 12 months was performed in a multiway ANACOVA model with adjustments for age, gender and baseline values of TBA–MDA, triglycerides, HDL-cholesterol, μCRP and p-tHcy.

Multiple linear regression analyses were introduced to identify concentrations of fatty acids in serum phospholipids as potential predictors of TBA–MDA. Pearson's correlation coefficient was calculated to evaluate relations between different variables.

A statistically significant level of $P<0.05$ was applied for all tests. Adjustment for multiple pairwise comparisons has been performed using the Bonferroni correction.

The statistical analyses were performed using the statistical package SAS version 8.2.

Results

Baseline data

As previously reported, 150 subjects in each group, 123 (82%) receiving n-3 PUFAs and 129 (86%) receiving corn oil, fulfilled the criteria for complete compliance after 6 weeks (Nilsen *et al*, 2001). There were no significant differences in baseline demographic or clinical characteristics, and concomitant medication at baseline and follow-up did not differ between the groups, including use of fish oil prior to inclusion. Approximately 30% in the n-3 group and 25% in the corn oil group were taking fish oil supplements prior to inclusion, comparable to a daily dose of about 1 g n-3 PUFAs. The above-mentioned baseline data have previously been described by Nilsen *et al* (2001).

The weekly consumption of fish was two meals or less in the lower quartile and three meals or more in the upper quartile. The median intake consisted of three fish meals per week. The dietary habits of the participants were essentially unchanged throughout the study. The fatty acid composition in serum phospholipids was equal in both intervention groups at baseline (Table 1). However, the content of EPA in serum phospholipids was 25% higher in the group taking

Table 1 Concentrations of fatty acids (μmol/l) (mean (s.d.)) in serum total phospholipids in 56 subjects randomly allocated from the study population

	n-3 group (n=*28*)		*Corn oil group* (n=*28*)	
Fatty acids	*Baseline*	*12 months*	*Baseline*	*12 months*
EPA (20:5 n-3)	62.9 (34.5)	189.6 (63.2)***[c]	67.4 (38.6)	97.4 (91.0)
DHA (22:6 n-3)	180.2 (52.0)	213.4 (45.3)**	207.4 (77.3)	209.9 (71.5)
LA (18:2 n-6)	796.5 (223.2)	748.6 (183.0)	828.1 (216.4)	893.4 (192.1)
AA (20:4 n-6)	264.7 (85.5)	236.0 (53.3)	291.0 (85.6)	317.5 (83.4)*[b]
Total n-3 PUFA	284.3 (84.0)	457.6 (96.3)***[c]	320.8 (117.1)	356.6 (136.5)
Total n-6 PUFA	1189.0 (297.2)	1062.0 (215.7)	1248.8 (294.1)	1332.5 (243.3)[a]
Total saturated FA	2030.7 (446.6)	2023.3 (306.1)	2103.2 (386.7)	2171.3 (324.8)
Total FA	3935.5 (870.2)	3920.9 (583.6)	4136.8 (800.7)	4252.6 (789.2)

LA—linoleic acid; AA—arachidonic acid.
No significant differences between intervention groups at baseline.
Significance of difference from baseline: ***$P<0.001$, **$P=0.011$, *$P=0.035$.
Significance of difference between groups: [c]$P<0.001$, [b]$P=0.004$, [a]$P=0.01$.

Table 2 Contents of fatty acids (μmol/l) (mean (s.d.), and (% of total fatty acids)) in serum phospholipids at baseline and serum total cholesterol, HDL-cholesterol and triglycerides (mmol/l) in 63 subjects randomly allocated from the study population, arranged according to whether fish oil supplementation was given before inclusion or not

	Fish oil supplementation before inclusion	
Fatty acids	*Yes (n=17)*	*No (n=46)*
EPA (20:5 n-3)	81.9 (45.4)* (2.0)	59.0 (28.2) (1.5)
DHA (22:6 n-3)	217.6 (71.2) (5.3)	191.7 (64.3) (4.8)
LA (18:2 n-6)	784.4 (214.5) (19.2)	839.5 (215.6) (20.9)
AA (20:4 n-6)	278.2 (64.5) (6.8)	276.6 (88.6) (6.9)
Total n-3 PUFA	344.0 (116.0) (8.4)	294.8 (95.7)[a] (7.3)
Total n-6 PUFA	1188.0 (293.8) (29.1)	1244.4 (286.4) (31.0)
Total saturated FA	2084.5 (384.6) (51.1)	2079.0 (415.4) (51.8)
Total FA	4079.5 (791.7)	4017.2 (862.2)[b]
Total cholesterol	6.0 (1.2)	5.8 (1.1)[b]
HDL-cholesterol	1.1 (0.3)[c]	1.1 (0.3)[d]
Triglycerides	1.5 (0.7)[c]	1.7 (1.0)[d]

*P=0.047, otherwise no significant differences between groups.
LA—linoleic acid, AA—arachidonic acid.
[a]Three missing values. [b]Seven missing values. [c]One missing value. [d]Nine missing values.

fish oil supplements prior to inclusion as compared to those with no fish oil supplements, $P=0.047$ (Table 2). As previously reported (Nilsen *et al*, 2001), there were no significant differences in other lipid parameters at baseline. The serum concentrations of μCRP (median and 25 and 75 percentiles) were equal in the n-3 PUFAs (20.7 (10.0–49.0) mg/l), ($n=121$) as compared to the corn oil group (18.8 (6.3–49.3) mg/l), ($n=130$). Furthermore, no difference in baseline p-tHcy (median and 25 and 75 percentiles) was seen between the n-3 PUFAs (14.1 (11.4–17.8) μmol/l) ($n=118$) as compared to the corn oil group (13.7 (10.5–16.3) μmol/l) ($n=123$). The healthy reference population ($n=30$) presented with significantly lower levels of μCRP (1.2 (0.6–3.0) mg/l) and p-tHcy (9.3 (7.4–11.4) μmol/l) as compared to baseline values in the MI population, $P\leq0.001$. Moreover, TBA–MDA concentrations at baseline did not differ between the intervention groups (Table 3) and were similar when related to age, gender, thrombolytics and smoking habits (data not shown).

In subgroups with and without statin treatment the use of thrombolytic treatment and size of infarction, as judged by TnT and CKMB levels, were similar, although the statin-treated patients were younger and had higher levels of serum cholesterol and triglycerides at inclusion, $P<0.05$.

Serum concentrations of TBA–MDA in the healthy reference population ($n=30$) were significantly lower than the values in the coronary population, $P=0.001$ (Table 3).

No correlation was found between baseline TBA–MDA and serum lipids, p-tHcy or μCRP (data not shown). However, TBA–MDA at baseline was positively correlated with the fatty acids in serum phospholipids, both unsaturated and saturated (Table 4), but this relation was disrupted during intervention.

Table 3 TBA–MDA complex, (μmol/l) (mean and (s.d.)) at baseline and 12 months after the MI, arranged according to intervention group, as compared to the healthy reference group (n=30)

n-3 Group (n=125)		*Corn oil group (n=130)*		*Reference group (n=30)*
Baseline	12 months	Baseline	12 months	—
1.95 (0.59)	3.63[a] (0.82)	1.92 (0.59)	3.35[a] (0.85)	1.57 (0.61)[b]

Statistically significant intergroup differences; P=0.0274 adjusting for age, gender, baseline values for TBA–MDA, serum lipids, μCRP and p-tHcy (ANACOVA), and P=0.0270 (t-test).
No significant difference between intervention groups at baseline.
[a]$P<0.001$ for intragroup difference from baseline to follow-up (Student's t-test).
[b]Significance of difference between study group at baseline and reference population: $P<0.001$.

Changes in TBA–MDA during intervention

As previously reported (Nilsen *et al*, 2001), a highly significant improvement in serum triglycerides and HDL-cholesterol was found in the n-3 group. TBA–MDA increased after 1 y in both the n-3 PUFAs and corn oil groups, $P<0.001$ (Table 3), but the increase was more pronounced in the n-3 group, $P=0.0270$. Adjusting for possible confounding factors such as age, gender, triglycerides, HDL-cholesterol, p-tHcy, μCRP and TBA–MDA values at baseline, a statistically significant difference between groups after 12 months of intervention was clearly evident, $P=0.0274$.

During 12 months of intervention the incidence of recurrent MI, defined according to the recommendations in the consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction (Alpert & Thygesen, 2000), was almost the same in the n-3 group (18.0%) as compared to the corn oil group (18.7%). Subjects with a recurrent MI over the year had similar TBA–MDA values as compared to those who did not experience a re-event.

Multiple linear regression analyses of fatty acids in serum total phospholipids ($n=56$) (listed in Table 2) on TBA–MDA measured after 12 months supplementation with n-3 PUFAs or corn oil, showed no TBA–MDA dependency. Performing best subsets regression, serum phospholipid concentration of arachidonic acid (20:4 n-6 PUFA) was identified as a predictor of TBA–MDA at 12 months follow-up ($P=0.004$).

Subgroup analysis of patients on statin treatment ($n=95$) as compared to those without a statin ($n=79$), revealed no significant intergroup differences in TBA–MDA, regardless of the background intervention with either n-3 PUFAs or corn oil (Table 5).

Concentrations of fatty acids in serum phospholipids during intervention

In the group receiving n-3 PUFAs, the amount of EPA, DHA and the total amount of n-3 fatty acids in serum phospholipids increased significantly (Table 1), while the total

Table 4 Correlations (Pearson's correlation coefficient; *r*) between TBA–MDA complex and fatty acids in serum total phospholipids (*n*=56) at baseline

	Fatty acid	*18:2* n-6	*20:4* n-6	*Sum* n-6	*20:5* n-3	*22:6* n-3	*Sum* n-3	*Sum saturated*
Baseline	*r*	0.449***	0.296*	0.464***	0.177	0.315*	0.293*	0.333**

*$P<0.05$. **$P<0.01$. ***$P<0.001$.

Table 5 TBA–MDA complex (μmol/l) (mean and (s.d.)) at baseline and 12 months after the MI, in patients on statin treatment, as compared to patients without statin treatment, analysed according to intervention with either n-3 fatty acids (PUFAs) or corn oil

Statin treatment (n=*95*)				*No statin treatment* (n=*79*)			
n-3 PUFAs (n=*47*)		*Corn oil* (n=*48*)		*n-3 PUFAs* (n=*34*)		*Corn oil* (n=*45*)	
Baseline	12 months	Baseline	12 months	Baseline	12 months	Baseline	12 months
2.03 (0.59)	3.55[a] (0.83)	2.00 (0.57)	3.45[a] (0.85)	1.86 (0.53)	3.49[a] (0.78)	1.80 (0.55)	3.18[a] (0.74)

No statistically significant difference between groups at baseline (*t*-test).
[a]$P<0.001$ for intragroup difference from baseline to follow-up (Student's *t*-test).
No statistically significant differences between any of the subgroups at 12 months (ANACOVA).

amount of n-6 fatty acids showed a trend towards an increase in the corn oil group from baseline to 12 months (ns). However, a significant increase in the amount of arachidonic acid (20:4 n-6 acid) was noted (Table 1). Moreover, the changes in the amounts of n-3 and n-6 fatty acids differed significantly between the groups during intervention (Table 1).

Discussion

Using TBA–MDA measured by HPLC as a marker of lipid peroxidation, we found a modest increase in lipid peroxidation in subjects receiving a high-dose concentrate of n-3 PUFAs as compared to those on corn oil. The clinical implications of increased TBA–MDA levels are uncertain, but increased lipid peroxidation might contribute to the lack of clinical prognostic benefit in this study population, as previously suggested (Nilsen *et al*, 2001). Based on findings of increased TBARS and reduced vitamin E, a possible adverse effect of high doses of n-3 PUFAs on cardiac events was hypothesised in a previous study using high-dose ethylester compounds of EPA/DHA (Johansen *et al*, 1999). TBARS may serve as a marker for later restenosis after PTCA (Johansen *et al*, 2001), and several studies indicate that elevated levels of TBARS may be associated with an increased risk of cardiovascular disease (CVD) (Jayakumari *et al*, 1992; Duthie *et al*, 1994; Miwa *et al*, 1995; Cavalca *et al*, 2001). Moreover, the significantly lower serum levels of TBA–MDA in our healthy reference population ($n=30$), as compared to values in the coronary population, may support the clinical relevance of elevated TBARS levels (Diaz-Velez *et al*, 1996; Cavalca *et al*, 2001). However, we could not demonstrate any association between TBA–MDA as a marker of lipid peroxidation and cardiovascular risk factors such as p-tHcy and μCRP, and no relations between TBA–MDA and levels of serum total cholesterol and triglycerides were found.

An intragroup increase in TBA–MDA from baseline to 12 months was observed in both intervention groups. Wander *et al* (1998) have previously shown that linoleic acid is a significant contributor to lipid peroxidation. This n-6 PUFA is present in LDL in an amount far greater than any other fatty acid. In the present study, the change in n-6 PUFAs in serum phospholipids differed significantly between the groups, but the intragroup increase in linoleic acid during 1 y of corn oil treatment did not reach statistical significance. Several studies support the notion that a high intake of n-6 PUFAs may increase lipid peroxidation (Reaven *et al*, 1991, 1993, 1994), and may explain the increase over 1 y also in our control group. Therefore, the choice of control in our study may partly mask the effect of n-3 PUFAs intervention.

The impact of n-3 PUFAs on oxidative stress of the vascular system in humans still remains an unsettled issue. Previous studies included relatively small sample sizes, different doses and compositions of n-3 PUFAs, short intervention periods, different amounts of antioxidants supplies and different methods used to quantify the oxidative burden and different methods to estimate TBARS.

The choice of dose of n-3 PUFAs in our study was based on previous studies investigating the influence of n-3 PUFAs on cardiovascular risk factors and lipid peroxidation (Phillipson *et al*, 1985; Eritsland *et al*, 1995; Connor & Connor, 1997). Later the GISSI-Prevenzione trial demonstrated beneficial effects on CVD of a lower dose (GISSI-Prevenzione Investigators, 1999). Baseline levels of total n-3 PUFAs in our study were reasonably high (Table 3), and a background diet rich in n-3 PUFAs may mask the effect of intervention. However, the baseline TBA–MDA level in our CHD population was of a similar magnitude as noted by others (Nordøy *et al*, 1998; Kharb *et al*, 2000).

It has been argued that the TBARS assay may measure the oxidation or decomposition of specific PUFAs instead of measuring the overall lipid peroxidation, as both the number of double bonds as well as the position of the first double bond from the methyl terminus play a role in the formation of the oxidative products (Yoshino *et al*, 1991; Higdon *et al*, 2000). Lack of MDA–TBA dependency on concentrations of n-3 PUFAs in serum phospholipids during treatment suggests that increased TBA–MDA may reflect actual differences in lipid peroxidation, rather than merely reflecting the differences in concentrations of n-3 PUFAs in serum phospholipids.

The TBARS assay is well accepted for determination of lipid-derived decomposition products (Armstrong & Browne, 1994), but certain methodological problems must be addressed. This biomarker estimates the circulating levels of a range of lipoperoxidation aldehydes secondary to lipid peroxidation (Janero, 1990; Armstrong & Browne, 1994), and the more acidic conditions of the method applied in the present study is likely to favour the release of TBARS precursors from the lipoproteins and protein-bound aldehydes (Lapenna *et al*, 2001). Therefore, in spite of its simplicity and high sensitivity, the TBARS test has been criticised for inadequate specificity to oxidant-driven lipid peroxidation, especially when using human plasma. Greater specificity is achieved by HPLC-separation of the MDA–TBA complex from thiobarbituric acid-reactive substances, as performed in our study, allowing for purified separation of the MDA–TBA complex from extraneous substances (Wong *et al*, 1987; Anoopkumar-Dukie *et al*, 2001). Using an external standard to which the TBA–MDA complex has been compared, provides a more accurate assessment of MDA levels and lipid peroxidation. The TBARS assay is a relatively quick and inexpensive method compared to determination of F2-isoprostanes, which is a tedious and expensive procedure. However, the source of the MDA measured still remains a pitfall, as MDA also is generated *ex vivo* by decomposition of lipid peroxides during the heating stage of the test (Gutteridge, 1986). While the interpretation of the MDA content in studies of lipid peroxidation requires caution, the isoprostanes represent stable products of lipid peroxidation formed *in situ* in cell membranes, which can be measured with great sensitivity and specificity (Meagher and FitzGerald, 2000).

The vast majority of studies investigating the oxidative potential of n-3 PUFAs include short-term supplementation periods (Harats *et al*, 1991; Nenseter *et al*, 1992; Bonanome *et al*, 1996; Wander *et al*, 1996; Hansen *et al*, 1998; Higdon *et al*, 2000). Long-term effects *in vivo* after 9 months of intervention were studied by Eritsland *et al* (1995), and susceptibility to lipid peroxidation induced *in vitro* was evaluated after 180 days of n-3 PUFAs treatment in the study by Palozza (Palozza *et al*, 1996). The present study is the first to examine the peroxidative status after 12 months of intervention with a high-dose of n-3 PUFAs.

Following thrombolytic treatment, reperfusion of ischaemic areas may be associated with reperfusion injury mediated by free-radical-induced oxidation. At the time of blood sampling 4–6 days after the acute MI, TBA–MDA levels were equal in patients who had been given thrombolytic treatment as compared to those not having received thrombolytics. In a study by Kharb *et al* (2000) TBARS were increased within the first hours after thrombolytic treatment, but this increase was no longer evident after 72 h.

Oxygen-free radicals are involved in the pathophysiology of inflammation and atherosclerosis (Dargel, 1992; Halliwell & Chirico, 1993). Treatment with statins has been shown to reduce the risk for cardiovascular events and exhibits beneficial effects on inflammation and plaque stability. In a subgroup of patients receiving simvastatin, no difference in TBA–MDA was observed between individuals on n-3 PUFAs as compared to those on corn oil. Moreover, no difference in TBA–MDA was noted between statin-treated and statin-untreated patients. However, the number of subjects in these subgroups was low, and these data should be interpreted with great care. A previous study has shown no influence on lipid peroxidation of combined treatment of simvastatin and n-3 PUFAs (Nordøy *et al*, 1998).

In conclusion, the main results of this long-term, post-infarct study show a modest increase in lipid peroxidation, measured as TBA–MDA by HPLC, in subjects on a high-dose concentrate of n-3 PUFAs as compared to a control group on corn oil. Increased TBA–MDA may reflect an actual difference in lipid peroxidation caused by n-3 PUFAs, rather than merely reflecting a change in their serum concentrations. The use of a statin did not modify the peroxidation potential of n-3 PUFAs.

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