Reduction in Homocysteine by n–3 Polyunsaturated Fatty Acids after 1 Year in a Randomised Double-Blind Study following an Acute Myocardial Infarction: No Effect on Endothelial Adhesion Properties

Heidi Grundt^{a,b} Dennis W.T. Nilsen^b Mohammad A. Mansoor^a
Øyvind Hetland^a Arne Nordøy^c

Departments of ^aClinical Chemistry and ^bMedicine, Central Hospital in Rogaland, Stavanger, and University of Bergen, Bergen, and ^cDepartment of Medicine, University of Tromsø, Tromsø, Norway

Key Words

n–3 polyunsaturated fatty acids · Homocysteine · Circulating intercellular adhesion molecule-1 · Circulating E-selectin · Ultrasensitive C-reactive protein · Myocardial infarction

Abstract

We hypothesized that n–3 polyunsaturated fatty acids (n–3 PUFAs) as compared to corn oil administered for 1 year following an acute myocardial infarction (MI) may reduce plasma total homocysteine (p-tHcy), ultrasensitive C-reactive protein (μCRP), and the adhesive properties of the endothelium, expressed as soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1). In a prospective, randomised, double-blind study, 300 acute MI patients were allocated to highly concentrated n–3 PUFAs (n = 150) or corn oil (n = 150). After 1 year on treatment there was an intergroup difference in p-tHcy in favour of the n–3 group (n = 118), p = 0.022. However, sE-selectin, sICAM-1 and μCRP were unaffected by the treatment. In conclusion, reduction of p-tHcy by long-term n–3 PUFAs treatment was not associated with demonstrable effects on markers of endothelial adhesion properties.

Introduction

n–3 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have several beneficial effects on the atherothrombotic risk profile, including anti-inflammatory properties [1], and have been found to reduce total and cardiovascular mortality [2]. Homocysteine (Hcy) may play an important role in the atherothrombotic process [3–6]. The beneficial actions of n–3 PUFAs on endothelial function may counteract the endothelial toxicity of substances such as Hcy, but only few and short-term studies have been published on the effects of n–3 PUFAs on Hcy [7–9].

Only Olszewski et al. [7] have demonstrated a beneficial effect on Hcy by fish oil supplements rich in n–3 PUFAs in hyperlipidaemic men. Additionally, plasma Hcy levels have been lowered following fish oil administration in rats [10].

Mechanisms linking a modestly elevated concentration of Hcy to the development of premature cardiovascular disease (CVD) have not been clearly described. Hcy may induce endothelial dysfunction by an unfavourable effect on the antithrombotic features of the endothelium [11]. Several factors are likely to be involved [12–15],

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1424–8832/03/0332–0088\$19.50/0

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Heidi Grundt
Department of Medicine
Central Hospital in Rogaland, POB 8100
NO–4068 Stavanger (Norway)
Tel. +47 51518000, Fax +47 51519906, E-Mail heidi@madlatia.no

including increased neutrophil adhesion to the vascular vessel wall [13]. Moreover, in an animal model using genetic and dietary approaches to produce hyperhomocysteinaemia in mice, Lentz et al. [16] have shown an association between moderate hyperhomocysteinaemia and marked impairment of endothelial function in mice. Furthermore, it has recently been demonstrated that induction of a mild to moderate increase in Hcy concentration by methionine loading in healthy subjects may modify the adhesive properties of the endothelium, expressed by circulating intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [11]. Soluble forms of cellular adhesion molecules are considered to be markers of endothelial activation and may even serve as predictors of early atherosclerosis [17]. Endothelial adhesion molecules mediate adhesion and transmigration of leukocytes, proposed to be essential for the initiation and propagation of atherosclerosis [18, 19]. Increased concentrations of Hcy may induce adhesion between neutrophils and endothelial cells in vitro, resulting in increased neutrophil migration across the endothelium [13]. Moreover, a slight, but significant age-adjusted correlation has been found between soluble ICAM-1 (sICAM-1) and Hcy in healthy individuals enrolled in the Physician's Health Study [17].

Beneficial effects of n-3 PUFAs on cellular adhesion molecules have been demonstrated in cell cultures [20], but results from clinical trials are conflicting. Abe et al. [21] have demonstrated improvement in endothelial function, expressed by reduced concentrations of sE-selectin and sICAM-1, in a hypertriglyceridaemic population treated with high doses of n-3 PUFAs for a period of at least 7 months. These changes were paralleled by a decline in serum triglycerides and total cholesterol. However, after 6 weeks intervention, the levels of sE-selectin were increased, while sICAM-1 remained unchanged in that study. Additionally, soluble forms of E-selectin and VCAM-1 were found to increase in two clinical trials evaluating the impact of concentrated n-3 PUFAs in hyperlipidaemic male smokers [22] and asymptomatic post-PTCA coronary patients [23].

In view of the anti-inflammatory, antiatherogenic and antithrombotic effects of concentrated n-3 PUFAs on the one hand, and the association between hypertriglyceridaemia, atherosclerosis, inflammation and endothelial dysfunction on the other hand, we hypothesized an improvement of markers of inflammation and endothelial dysfunction, such as μ CRP, total plasma Hcy (p-tHcy), sICAM-1 and sE-selectin, by long-term treatment with a high dose of concentrated n-3 PUFAs. We have therefore assessed the impact of highly concentrated n-3 PUFAs on

these parameters in a prospective randomised clinical trial, in which we had previously demonstrated a triglyceride-lowering effect of n-3 PUFAs as compared to corn oil [24].

Subjects and Methods

Subjects

This prospective study was designed as part of a randomised, parallel, double-blind study also evaluating the effects on clinical outcome and serum lipids of a concentrate of n-3 PUFAs, introduced early after an acute MI [24].

Three hundred patients (238 men and 62 women) 28–87 years of age, with an acute MI verified by WHO criteria, were recruited at one hospital center from September 1995 until December 1996. Written informed consent was obtained from each patient. Regular supplementation of other fish-oil products was discontinued prior to intervention. Exclusion criteria included (1) assumed non-compliance to the protocol, due to factors such as alcoholic abuse or serious psychiatric disorder, (2) expected survival less than 2 years due to heart failure NYHA class IV, malignancy or other reasons, (3) ongoing gastrointestinal bleeding and/or verified stomach ulcer, (4) thrombocytopenia (blood platelets $< 100 \times 10^9/l$), (5) liver insufficiency, (6) participation in any other study or (7) residence outside the recruitment area of this study. 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 daily dose of 2 capsules twice a day for at least 1 year. This dose of n-3 PUFAs has previously been shown to be therapeutically useful in lowering triglyceride levels and blood pressure [8]. Alpha-tocopherol (4 mg) was added to each capsule to protect the unsaturated fatty acids against oxidation. Treatment was initiated immediately after inclusion and collection of baseline blood samples. Compliance was monitored at 6 weeks, 6 and 12 months follow-up, counting remaining capsules. Moreover, the patients reported any discrepancy in recommended and ingested dose, using patients' log-book. Serum samples from 60 patients were randomly drawn to have the fatty acid profile in phospholipids analysed at baseline and after 12 months on treatment. Fifty-six out of these patients had complete data on fatty acids.

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 1 year. All cardiac events (cardiac death, resuscitation, recurrent MI, unstable angina pectoris, revascularisation), non-cardiac death, smoking habits, ongoing medication and average fish meals per week were recorded. Blood sampling was preceded by 12 h of fasting.

The blood samples for p-tHcy were collected in heparin-containing vials and placed on ice prior to centrifugation at 4°C, performed within 30 min. p-tHcy was determined using an HPLC assay based on precolumn derivatization with monobromobimane, followed by ion-pair reversed-phase liquid chromatography and fluorescence detection, as previously described [25]. The analytical recovery was close to 100%, and the within-day precision corresponded to a coefficient of variation of 6%.

Table 1. Demographics, biochemical infarct characteristics and frequency of thrombolytic treatment at baseline, as well as the proportion of patients treated with statins at baseline and at follow-up, arranged according to treatment groups

Characteristics	n-3 group	Corn oil group
Sex		
Male	115 (76.7)	123 (82.0)
Female	35 (23.3)	27 (18.0)
Age at infarction		
[year; median (25% and 75% percentiles)]	66.6 (54.4–75.0)	65.0 (55.3–72.0)
Smoking status		
Non-smoker	34 (22.7)	39 (26.0)
Ex-smoker	57 (38.0)	54 (36.0)
Current smoker	59 (39.3)	57 (38.0)
Fish oil before inclusion		
No	104 (69.8)	113 (75.3)
Yes	45 (30.2)	37 (24.7)
Clinical background		
Angina pectoris	49 (32.7)	57 (38.0)
Heart failure	15 (10.0)	11 (7.3)
Previous MI	32 (21.3)	38 (25.3)
Revascularisations	12 (8.0)	15 (10.0)
Hypertension	40 (26.7)	33 (22.0)
Hypertension treatment	29 (19.3)	26 (17.3)
Family history of coronary disease	98 (65.3)	97 (64.7)
Diabetes mellitus	18 (12.0)	13 (8.7)
BMI, kg/m ²		
Mean	25.9	26.0
Range	16.9–41.8	19.4–33.6
Peak creatine kinase MB, U/l		
Mean	164.9	164.5
Range	0–985	0–838
Peak troponin T, µg/l		
Mean	9.5	8.9
Range	0–76	0.4–58
Thrombolysis first 24 h	57 (38.0)	58 (38.7)
Statin use		
At discharge	62 (41.3)	67 (44.7)
At 12 months	90 (71.4)	85 (65.4)

No statistically significant differences between groups at baseline. Figures in parentheses are percentages.

Serum for analyses of µCRP and adhesion molecules, soluble forms of E-selectin and ICAM-1 was immediately frozen and stored at –80 °C. The analyses were performed by an investigator with no knowledge of the randomisation code. Soluble forms of E-selectin and ICAM-1 were analysed in duplo by an ELISA technique (R&D Systems, Abingdon, UK). µCRP was determined in serum samples by an ultrasensitive immunoassay, using a kit (N Latex CRP mono) from Behring Diagnostics, applying the Behring nephelometer II system. Serum triglycerides and total cholesterol were determined by enzymatic methods with kits provided by Boehringer, Mannheim (GPO-PAP, CHOP-PAP). HDL-cholesterol was similarly measured after precipitation with dextran sulphate and magnesium chloride [26].

Analyses of fatty acids in serum phospholipids were performed at the University of Tromsø, Norway, as previously described by Nordøy et al. [27].

The concentrations of vitamin B₁₂ in serum and of folate in serum and blood were determined by automated chemiluminescence assays (Access Immunoassay System Vitamin B₁₂ and Folate, Beckman, USA), in which vitamin B₁₂ or folate competed for binding to compounds covalently coupled to paramagnetic particles. Body mass index (BMI) was calculated as kg/m².

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 in quantitative variables between groups at baseline were tested by a two-sample Student's t-test, or in case of non-normality, by the Mann-Whitney Rank-Sum test. Normally distributed variables were given as mean and standard deviation

Table 2. Concentrations of fatty acids (mean and SD, in parentheses) in serum total phospholipids in 56 patients randomly allocated from the study population

Fatty acids, $\mu\text{mol/l}$	n-3 group (n = 28)		Corn oil group (n = 28)	
	baseline	12 months	baseline	12 months
EPA (20:5 n-3)	62.9 (34.5)	189.6 (63.2)***	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)*, ²
Total n-3 PUFA	284.3 (84.0)	457.6 (96.3)***, ¹	320.8 (117.1)	356.6 (136.5)
Total n-6 PUFA	1,189.0 (297.2)	1,062.0 (215.7)	1,248.8 (294.1)	1,332.5 (243.3) ³
Total saturated FA	2,030.7 (446.6)	2,023.3 (306.1)	2,103.2 (386.7)	2,171.3 (324.8)
Total FA	3,935.5 (870.2)	3,920.9 (583.6)	4,136.8 (800.7)	4,252.6 (789.2)

LA = Linoleic acid; AA = arachidonic acid.

Significance of difference from baseline: *** $p < 0.001$, ** $p = 0.011$, * $p = 0.035$.

Significance of difference in changes from baseline between groups: ¹ $p < 0.001$, ² $p = 0.004$, ³ $p = 0.01$.

(SD), 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. If the normality assumption was fulfilled according to the Shapiro Wilk's test for normality, the t-test was used to evaluate whether changes in parameter values from baseline to follow-up were significantly different from zero, and to compare these changes between the treatment groups. If the assumption of normality was not fulfilled, the Wilcoxon Signed Rank Test was used to evaluate whether changes in parameter values from baseline to follow-up were significantly different from zero, and the Mann-Whitney Rank-Sum test was used to compare changes between treatment groups. Analyses were performed according to intention to treat.

Additionally, adjustments for possible confounding factors such as age, BMI, smoking habits, statin use, serum creatinine, serum and erythrocyte folates and serum vitamin B₁₂ values at baseline have been performed using a multiway ANACOVA model [28].

Linear regression analyses were introduced to identify concentrations of fatty acids in serum phospholipids as potential predictors of p-tHcy and the adhesion molecules. Spearman's rank order correlation coefficients were calculated to evaluate relationships between different variables.

The statistical analyses were performed using the statistical package SAS version 8.2. A statistically significant level of $p < 0.05$ was applied for all tests.

Results

Baseline Data

Of the 150 patients in each group, 123 (82%) receiving n-3 PUFAs and 129 (86%) receiving corn oil, fulfilled the criteria for complete compliance after 6 weeks, as assessed by capsule counting. Among survivors at 12 months, 86%

in the n-3 PUFAs group and 84% in the corn oil group had a mean drug compliance of $>90\%$ after 12 months.

There were no significant differences in baseline demographic or clinical characteristics between subjects of the two treatment groups (table 1), and as previously reported [24], concomitant medication at baseline and follow-up did not differ between the groups, including the use of β -blockers, angiotensin-converting enzyme (ACE) inhibitors, diuretics, acetyl salicylic acid, warfarin and statins, as well as use of fish oil before inclusion.

The fatty acid profiles in serum phospholipids did not differ between the treatment groups at baseline (table 2). As previously reported [24], there were no significant differences in the other lipid parameters at baseline (table 3). Moreover, p-tHcy concentrations at baseline did not differ between the groups (table 3). Plasma total Hcy values of $15 \mu\text{mol/l}$ or higher were found in 94 individuals (39.0%); 52 subjects in the n-3 and 42 subjects in the corn oil group. None had a blood concentration of folate in erythrocytes lower than 200 nmol/l , but 6 subjects were excluded from the analyses due to deficiency of vitamin B₁₂ ($<120 \text{ pmol/l}$). Concentrations of sE-selectin, sICAM-1 and μCRP at baseline were similar in the n-3 and corn oil group (table 3), respectively, and did not differ between smokers and non-smokers at baseline.

Correlation analyses at baseline showed a positive association between sICAM-1 and sE-selectin ($r = 0.466$, $p < 0.0001$). The baseline level of sE-selectin correlated positively with serum triglycerides ($r = 0.167$, $p = 0.009$), while sICAM-1 was negatively associated to the ratio HDL-cholesterol/cholesterol ($r = -0.134$, $p = 0.037$).

Table 3. Plasma-tHcy, serum concentrations of μ CRP, sE-selectin and sICAM-1, vitamin B₁₂ and folate, erythrocyte folate (median and 25% and 75% percentiles, in parentheses) and serum lipids (mean and SD in parentheses) 4–6 days after an acute MI (baseline) and after 12 months of treatment with a high dose of n-3 PUFAs or corn oil

Parameter	n-3 group		Corn oil group	
	baseline	12 months	baseline	12 months
p-tHcy ³ , μ mol/l	14.1 (11.4–17.8) (n = 118)	12.6 (10.8–16.9)	13.7 (10.5–16.3) (n = 123)	13.9 (11.3–16.4)
By age <65 years ²	13.4 (11.0–16.4) (n = 65)	11.8** (9.9–15.0)	13.4 (10.2–15.9) (n = 65)	13.8 (10.9–15.3)
≥ 65 years	15.1 (12.0–19.3) (n = 53)	14.6 (12.0–20.9)	14.0 (11.2–18.0) (n = 58)	14.3 (11.6–18.1)
μ CRP, mg/l	20.7 (10.0–49.0) (n = 124)	1.5** (0.9–3.2)	18.8 (6.3–49.3) (n = 129)	1.5** (0.9–3.3)
By age <65 years	17.6 (7.0–48.6) (n = 66)	1.3** (0.8–2.3)	17.4 (7.3–44.4) (n = 67)	1.4** (0.9–3.0)
≥ 65 years	21.3 (11.0–46.7) (n = 58)	2.0** (1.1–3.9)	22.7 (5.9–54.4) (n = 62)	1.6** (0.9–3.6)
sE-selectin, μ g/l	31.6 (23.6–48.8) (n = 123)	38.7 (28.9–50.5)	33.8 (25.4–46.3) (n = 127)	39.4 (27.6–54.4)
By age <65 years	32.4 (23.8–49.2) (n = 65)	37.8 (30.1–52.0)	33.8 (24.6–50.3) (n = 67)	41.3 (24.5–54.4)
≥ 65 years	30.5 (20.4–47.2) (n = 58)	39.5 (27.7–49.5)	34.1 (25.6–43.7) (n = 60)	37.3 (28.4–52.6)
sICAM-1, μ g/l	215.7 (182.8–257.0) (n = 124)	250.9 (196.7–305.4)	221.6 (187.4–265.2) (n = 127)	238.2 (196.8–298.9)
By age <65 years	212.8 (183.1–279.4) (n = 66)	241.6 (182.4–305.2)	223.2 (187.4–265.2) (n = 67)	244.4 (206.5–300.5)
≥ 65 years	216.8 (182.0–241.4) (n = 58)	253.4 (222.1–307.9)	214.2 (188.3–264.9) (n = 60)	228.1 (192.4–292.5)
Triglycerides ¹ , mmol/l	1.64 (0.83) (n = 120)	1.31 (0.60)	1.55 (0.82) (n = 121)	1.82 (1.05)
HDL cholesterol ¹ , mmol/l	1.08 (0.30) (n = 119)	1.28 (0.33)	1.16 (0.35) (n = 120)	1.24 (0.33)
Total cholesterol, mmol/l	5.94 (1.18) (n = 123)	5.27 (1.09)	5.99 (1.20) (n = 123)	5.59 (1.18)
Vitamin B ₁₂ , pmol/l	283.0 (230.0–370.0) (n = 118)	291.0 (236.0–378.9)	272.0 (210.5–343.5) (n = 123)	272.0 (231.4–346.5)
Erythrocyte folate, nmol/l	523.0 (440.0–625.0) (n = 118)	511.5 (424.0–613.0)	545.0 (444.0–681.0) (n = 123)	551.0 (450.0–682.0)
Serum folate, nmol/l	10.0 (8.0–12.0) (n = 118)	10.0 (8.0–11.0)	10.0 (8.0–14.0) (n = 120)	10.0 (8.0–13.0)

n = Number of patients with complete data at inclusion and follow-up. No statistically significant differences between groups at baseline. Significance of difference from baseline to 12-month value between treatment groups, ¹ p < 0.0001, ² p = 0.005, ³ p = 0.022. Significance of difference from baseline to 12 months: ** p < 0.001, * p < 0.005.

Otherwise we noted no relations between adhesion molecules and p-tHcy, μ CRP or serum lipids. Neither serum triglycerides nor serum cholesterol showed any correlation with p-tHcy. Nevertheless, p-tHcy was negatively correlated with serum HDL-cholesterol ($r = -0.173$, $p = 0.007$) and with the ratio HDL-cholesterol/cholesterol ($r = -0.194$, $p = 0.003$). BMI correlated positively with sE-selectin ($r = 0.191$, $p = 0.004$) and μ CRP ($r = 0.150$, $p = 0.024$).

Serum Concentrations of Phospholipid Fatty Acids 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 2), while the total amount of n-6 fatty acids showed an increasing tendency in the corn oil group (n.s.). Moreover, the changes in the amounts of n-3 and n-6 fatty acids differed significantly between the groups during intervention (table 2).

Changes in p-tHcy and Markers of Endothelial Cell Function during Intervention

As previously reported [24], a favourable effect on the concentrations of serum triglycerides and HDL-cholesterol was found in the n-3 group (table 3). Overall, p-tHcy decreased after 1 year on n-3 PUFAs, $p = 0.001$, while p-tHcy remained unchanged in the corn oil group (table 3), and the intergroup difference in changes from baseline reached statistical significance, $p = 0.022$. The reduction of p-tHcy within the n-3 PUFA group was restricted to patients younger than 65 years of age (median age of the population; $n = 65$; $p < 0.001$), while p-tHcy remained unchanged in the same age group on corn oil ($n = 65$). In the younger population there was also a significant intergroup difference in p-tHcy change between the intervention groups, $p = 0.005$, whereas no intergroup difference in change could be detected in patients ≥ 65 years of age (table 3). Adjusting for possible confounding factors such as age, BMI, smoking, statin use, serum creatinine, serum and erythrocyte folates, and serum vitamin B₁₂ values at baseline, a statistically significant difference between groups after 12 months of intervention was still clearly evident, $p = 0.034$.

However, there were no significant correlations between changes in p-tHcy and serum concentrations of n-3 PUFAs, and also regression analyses failed to identify a dependency of p-tHcy on any of the PUFAs.

After 12 months of treatment, the concentrations of sE-selectin, sICAM-1 and μ CRP did not differ significant-

ly between the n-3 and corn oil group, respectively, and we noted no intergroup difference in change from baseline to follow-up, irrespective of age group (table 3). Linear regression analyses showed no dependency of sE-selectin or sICAM-1 on fatty acids in serum total phospholipids (listed in table 2) measured after 12 months of intervention.

No statistically significant correlations were found between changes in markers of endothelial dysfunction and changes in p-tHcy or serum lipids.

The concentrations of vitamin B₁₂ in serum and of folate in serum and blood remained unchanged during 12 months of intervention (table 3). Serum creatinine did not differ between the groups at baseline and remained stable during intervention in both treatment groups (data not shown).

Discussion

This prospective, randomised, double-blind, controlled, clinical study is the first to show a significant decrease in p-tHcy after 1 year of treatment with a high dose of n-3 PUFAs as compared to corn oil, introduced early after an acute MI, $p = 0.022$.

In a previous study of only 12 weeks duration, the same dose (4 g daily) of n-3 PUFAs given to a high-risk population with combined hyperlipidaemia ($n = 56$), failed to reduce the p-tHcy concentration [8]. However, the limited number of individuals may be regarded as a weakness in that study. Moreover, the p-tHcy concentration was not altered by a daily supplementation with 5 g n-3 PUFAs for 6 weeks in male smokers with hyperlipidaemia [9]. Olszewski et al. [7] reported that 3 weeks of fish oil supplements rich in n-3 PUFAs reduced serum Hcy by at least 36% in 15 type IIa or IIb hyperlipidaemic men. Thus, different doses, unequal intervention periods and non-comparable populations render interpretation of studies on the effect of n-3 PUFAs on Hcy difficult. The pharmacological, high doses of n-3 PUFAs used in the present and the above-mentioned studies are especially noted for their triglyceride-reducing ability [24, 29]. In the context of an association between hypertriglyceridaemia and endothelial dysfunction, and of a positive correlation of p-tHcy to triglycerides [6] and to cholesterol [30], high doses of n-3 PUFAs might be expected to exert a beneficial effect also on p-tHcy. However, the possible p-tHcy-reducing mechanisms by n-3 PUFAs, are not obvious. Tentatively, n-3 PUFAs may modulate gene expression [31], interacting with the formation of p-tHcy. Changes in

concentrations of vitamin B₁₂ in serum and of folate in serum and blood during intervention did not differ between the groups, and cannot explain the intergroup difference in p-tHcy change observed between the intervention groups. Moreover, serum creatinine, of importance for the excretion of Hcy, did not differ between the groups at baseline or during intervention. The failure to demonstrate a p-tHcy decreasing effect by n-3 PUFAs in subjects belonging to the elderly part of the population (above the median age of 65 years) might be due to significantly higher levels of serum creatinine in that population, $p < 0.001$. A lower renal function in the elderly may mask the response to n-3 PUFAs due to affected excretion of p-tHcy. However, serum creatinine has been adjusted for in the multivariate model.

Also, the exact mechanisms underlying the association between Hcy and CVD are still unknown. Results from in vitro studies suggest that Hcy unfavourably influences endothelial cell function, promoting the development of CVD [11–15]. Hypothetically, the anti-inflammatory properties of n-3 PUFAs might counteract the unfavourable effects of p-tHcy on endothelial function. The p-tHcy-lowering potential of n-3 PUFAs in the present study was not accompanied by a reduction in soluble adhesion molecules or μ CRP after 1 year of treatment, lending no support to this hypothesis. Furthermore, no statistically significant correlations were found between p-tHcy and markers of endothelial dysfunction at baseline. These results are consistent with data from Brude et al. [9]. However, these authors demonstrated a negative correlation between p-tHcy and n-3 PUFAs in serum phospholipids. In contrast, a slight, but significant, age-adjusted correlation between sICAM-1 and p-tHcy has been noted in healthy individuals enrolled in the Physician's Health Study [17]. However, as endothelial damage already is established in MI patients, it is possible that 1 year of treatment is not sufficient to influence the endothelial markers. Moreover, our findings do not exclude the possibility that n-3 PUFAs may essentially affect other reflections of the endothelium, such as nitric oxide (NO)-dependent endothelial function. Additionally, the low number of individuals with available PUFA data in this study may be regarded as a statistical limitation when assessing the relationship between sCAMs or p-tHcy and serum PUFAs.

Choice of control is also critical. Corn oil has commonly been used as treatment control. Whether corn oil may exert beneficial effects should be considered. Various experimental and clinical studies implicate a direct link between endothelial adhesion and the intake and compo-

sition of dietary fat [32, 33]. Corn oil as treatment control may therefore partly mask the effect of n-3 PUFAs intervention. Moreover, baseline levels of total n-3 PUFAs in our study were reasonably high (table 2), and a background diet rich in n-3 PUFAs may also reduce the effect of intervention.

Finally, the acute manifestations of inflammation the first days and weeks after an acute coronary ischemic event affect markers relating to inflammation and endothelial dysfunction. The vast majority of studies suggesting that endothelial dysfunction may be triggered by high levels of Hcy [11–15], and studies demonstrating beneficial effects of n-3 PUFAs on cellular adhesion molecules [34–36], are based on cell cultures and in vitro models. In contrast to reports demonstrating beneficial effects of n-3 PUFAs on cell culture studies, serum concentrations of the soluble adhesion molecules E-selectin and VCAM-1 were increased by a daily dose of 5.1 g n-3 PUFAs given to asymptomatic post-PTCA coronary patients [23]. Nevertheless, Abe et al. [21] found that sE-selectin and sICAM-1 were reduced by concentrated n-3 PUFAs in a daily dose of 4 g for ≥ 7 months.

Hypertriglyceridaemia has been proposed to be independently associated with increased concentrations of cellular adhesion molecules [21]. We observed a positive correlation between sE-Selectin and serum triglycerides in the present study, in agreement with the findings reported in the ARIC study [37].

In the present study, serum samples for sICAM-1 and sE-selectin were stored at -80°C for up to 3 years prior to use. Serum may be stored at these conditions for up to 12 years without affecting the measurements [17], and the use of the same kits will secure reproducibility.

In conclusion, the favourable influence of n-3 fatty acids as compared to corn oil on plasma total Hcy after 1 year of treatment was not associated with a beneficial effect on endothelial adhesion properties.

Acknowledgements

We are grateful for the financial support by Pharmacia A/S and Pronova A/S, Oslo, Norway. Thanks are due to Bodil Hauff at the Department of Clinical Chemistry, Central Hospital in Rogaland, Norway, for analyses of μ CRP. We also acknowledge the support of Ole Kristensen, MD, and the skilful assistance of his staff at the Department of Clinical Chemistry, Central Hospital in Rogaland. We thank Hans Fagertun, 3S-Scandinavian Statistical Services AS, Norway, for assistance in statistical analyses. Finally, we thank the project nurse, Solfrid Moen, Hjertelaget Research Foundation, Norway, and the steering committee of the 'OFAMI' study: Dennis W.T. Nilsen, Knud Landmark, Leik Woie, and Michael Abdelnoor.

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