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SHORT COMMUNICATION

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C-reactive protein and *n*–3 fatty acids in patients with a previous myocardial infarction

A placebo-controlled randomized study

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Abstract *Background* Dietary intake of the marine long-chain *n*−3 polyunsaturated fatty acids (PUFA) may reduce mortality after a myocardial infarction (MI). This may partly be attributed to their anti-inflammatory properties. *Aim of the study* To investigate the effect of *n*−3 PUFA on C-reactive protein (CRP) in patients with a previous MI. *Methods* In a double-blind design, forty-one patients (mean age 63 ± 7 years) were randomized to receive daily supplements with 5.2 g of *n*−3 PUFA or olive oil (control). Serum CRP was measured with a highly sensitive assay (hs-CRP) before and after 12 weeks supplements. Compliance was monitored by measuring the incorporation of *n*−3 PUFA into platelets. *Results* The content of *n*−3 PUFA in platelets increased significantly in the *n*−3 PUFA supplemented group, whereas no changes were seen in controls. There was a minor increase in hs-CRP in the *n*−3 PUFA group (2.46 vs. 2.70 mg/l) and a small decrease in hs-CRP in the control group (2.52 vs. 1.67 mg/l). The changes, however, were not statistically significant ($P = 0.30$ and 0.43, respectively). *Conclusion* Supplementation with 5.2 g of *n*−3 PUFA for 12 weeks had no hs-CRP lowering effect in patients with a previous MI.

Key words coronary heart disease – C-reactive protein – fish oil – inflammation – *n*−3 fatty acids

Introduction

Chronic low-grade inflammation is involved in the initiation and progression of atherosclerosis [13], and C-reactive protein, measured with a highly sensitive assay (hs-CRP), independently predicts cardiovascular events [11]. The marine *n*−3 polyunsaturated fatty acids (PUFA), EPA (C20:5*n*−3) and DHA (C22:6*n*−3), reduce mortality after a myocardial infarction (MI) [1, 3]. This has been attributed in part to an antiarrhythmic effect of *n*−3 PUFA. However, *n*−3 PUFA have other effects acting in concert to protect against coronary heart disease (CHD), including anti-inflammatory effects [9]. We investigated the effect of *n*−3 PUFA on hs-CRP levels in patients with a previous MI.

Methods

This study is a substudy of a randomized, placebo-controlled study investigating the effect of *n*−3 PUFA on heart rate variability [2]. Patients were included 6 months after discharge after an MI from the Department of Cardiology, Aalborg Hospital, Denmark, and they were all clinically stable at the time of inclusion. The exclusion criteria have previously been published [2]. Patients with CRP values >10 mg/l

Table 1 The content of selected fatty acids in platelets in the two groups before and after supplementation

	n−3 PUFA			Control		
	Before	After	Difference	Before	After	Difference
C18:2*n*−6	6.82 ± 1.59	6.43 ± 1.48*	−0.39 ± 0.98	6.94 ± 0.66	6.70 ± 1.02	−0.26 ± 0.85
C20:4*n*−6	22.45 ± 2.79	19.07 ± 2.17**	−3.37 ± 2.60†	21.77 ± 1.57	21.54 ± 2.10	−0.23 ± 1.87
C20:5*n*−3	1.49 ± 0.67	4.10 ± 1.03**	2.61 ± 1.05†	1.58 ± 0.50	1.46 ± 0.84	−0.13 ± 0.81
C22:5*n*−3	1.43 ± 0.33	2.12 ± 0.44**	0.69 ± 0.42†	1.37 ± 0.35	1.23 ± 0.35	−0.14 ± 0.32
C22:6*n*−3	2.78 ± 0.52	3.54 ± 0.45**	0.76 ± 0.59†	2.83 ± 0.71	2.78 ± 0.69	−0.11 ± 0.56

Data are expressed as percentage of total fatty acids (mean ± SD). Difference = After−Before
Significantly different from before supplementation: * $P < 0.05$, ** $P < 0.01$
Significantly different from the control group: † $P < 0.001$

($n = 11$) were excluded from this substudy, since this was regarded as elevations due to infectious or inflammatory conditions other than atherosclerosis. The study was approved by the regional ethics committee, and informed written consent was obtained from all the patients.

Patients were randomized to receive daily supplements of either 5.2 g of *n*−3 PUFA (as 4.3 g EPA and DHA) in 8 capsules of Pikasol® (EPAX 5500; Pronova Biocare A/S, Norway) or 8 capsules of olive oil (control) for 12 weeks. The incorporation of EPA and DHA into platelet membranes was measured. The lipids were extracted and transesterified as previously described [14]. The fatty acid composition was determined by gas chromatography using a Chrompack CP-9002 gas chromatograph (Chrompack International, Middelburg, The Netherlands). The capillary column used was a Chrompack CP-Wax 58 CB (25m × 0.25 mm ID) with temperature programming from 80 to 200°C. This approach permits quantification of fatty acid methyl esters with 12–24 carbon atoms. hs-CRP was measured using a ADVIA 1650 analyzer (Bayer Corp. USA) and an immunoturbidimetric assay from Randox Laboratories Ltd. UK. Differences within and between groups were tested using Wilcoxon signed rank sum test and Mann–Whitney test, respectively. Differences in nominal data were evaluated by the Chi-squared test. A *P*-value < 0.05 (2-tailed) was considered statistically significant.

Results

Seven women and 34 men with a mean age of 63 ± 7 years [range 48–75 years] were included. The two intervention groups were comparable with regard to age, gender, body mass index, smoking status, treatment with aspirin, serum lipids, and hs-CRP. After the supplements, there was a marked increase in the content of EPA and DHA in platelet membranes in the *n*−3 PUFA group indicating good compliance (Table 1). No changes in fatty acids were seen in the control group. In the fish oil group there was a small increase in median hs-CRP after *n*−3 PUFA (2.46 vs. 2.70 mg/l), while a small decrease was observed in the control group (2.52 vs. 1.67 mg/l). The changes were, however, not statistically significant ($P = 0.30$ and 0.43, respectively), and there was no significant difference between the changes in median hs-CRP in the two groups ($P = 0.22$). The individual results (Fig. 1) also left no indication that hs-CRP levels were lowered by this dose of *n*−3 PUFA.

Discussion

Recently, it has been suggested that hs-CRP should be included in coronary risk assessment to identify individuals at high risk [11]. Other inflammatory markers are associated with atherosclerosis but have not proven as useful for clinical application [11]. There is no direct evidence that a lowering of hs-CRP will reduce cardiovascular risk. However, drugs used in prevention and treatment of CHD disease, such as statins and aspirin, may act partly through anti-inflammatory mechanisms [12]. Similarly, the anti-inflammatory properties of *n*−3 PUFA may contribute to their cardioprotective effects [9].

In this study, the time from the qualifying MI to inclusion was at least 6 months, and hs-CRP should have fallen to baseline levels, reflecting the extent and severity of atherosclerosis in the vasculature and the burden of risk factors. Median hs-CRP was considerably higher than levels reported in healthy individuals [7]. Twelve weeks supplementation with a relatively high dose of *n*−3 PUFA did not reduce hs-CRP levels. All patients in the *n*−3 PUFA group and 86% of the patients in the control group were treated with aspirin. This may have affected the results.

We have previously reported an independent inverse correlation between the content of DHA in granulocytes and hs-CRP in a cross-sectional study with 269 patients referred for coronary angiography

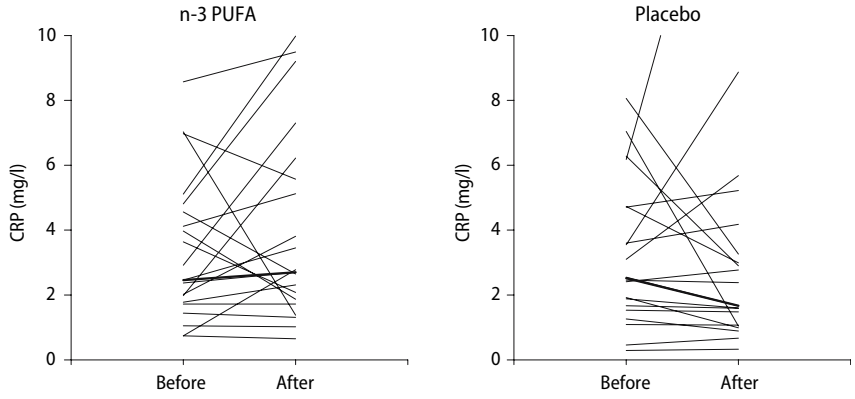

Fig. 1 Serum levels of C-reactive protein (CRP) before and after 12 weeks supplementation with 5.2 g/day *n*−3 polyunsaturated fatty acids (**A**) or olive oil (**B**) in each individual. The bold lines show changes in median hs-CRP

due to clinically suspected CHD [8]. Other authors have also found an inverse correlation in large cross-sectional studies [4, 6]. In a dose-response study involving healthy young subjects, we found no effect on hs-CRP after 12 weeks supplementation with 6.6 g *n*−3 PUFA or 2.0 g *n*−3 PUFA compared to placebo [7]. Similarly, no effect has been found in other intervention studies involving patients with a recent MI [5] and type 2 diabetics [10].

However, since atherosclerosis is largely an inflammatory disease, the effect of *n*−3 PUFA and other compounds with anti-inflammatory activity should be investigated further as possible treatment strategies in patients with or at high risk of developing CHD.

References

1. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM (1989) Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2:757–761
2. Christensen JH, Gustenhoff P, Korup E, Aaroe J, Toft E, Moller J, Rasmussen K, Dyerberg J, Schmidt EB (1996) Effect of fish oil on heart rate variability in survivors of myocardial infarction: a double blind randomised controlled trial. BMJ 312:677–678
3. Dietary supplementation with *n*−3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (1999) Lancet 354:447–455
4. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM (2006) Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 91:439–446
5. Grundt H, Nilsen DW, Mansoor MA, Hetland O, Nordoy A (2003) 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. Pathophysiol Haemost Thromb 33:88–95
6. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB (2004) Consumption of (*n*−3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. J Nutr 134:1806–1811
7. Madsen T, Christensen JH, Blom M, Schmidt EB (2003) The effect of dietary *n*−3 fatty acids on serum concentrations of C-reactive protein: a dose-response study. Br J Nutr 89:517–522
8. Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, Schmidt EB (2001) C-reactive protein, dietary *n*−3 fatty acids, and the extent of coronary artery disease. Am J Cardiol 88:1139–1142
9. Mori TA, Beilin LJ (2004) Omega-3 fatty acids and inflammation. Curr Atheroscler Rep 6:461–467
10. Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, Beilin LJ (2003) Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. Free Radic Biol Med 35:772–781
11. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F (2003) Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107:499–511
12. Prasad K (2006) C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev 24:33–50
13. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340:115–126
14. Schmidt EB, Pedersen JO, Varming K, Ernst E, Jersild C, Grunnet N, Dyerberg J (1991) n−3 fatty acids and leukocyte chemotaxis. Effects in hyperlipidemia and dose-response studies in healthy men. Arterioscler Thromb 11:429–435