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SHORT COMMUNICATION

Intake of n-3 fatty acids from fish does not lower serum concentrations of C-reactive protein in healthy subjects

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Objective: High-sensitivity C-reactive protein (CRP), a marker of systemic inflammation, is a powerful predictor of cardiovascular risk. We hypothesised that n-3 fatty acids reduce underlying inflammatory processes and consequently CRP concentrations in healthy middle-aged subjects.
Design: Placebo-controlled, double-blind study.
Subjects: A total of 43 men and 41 postmenopausal women aged 50–70 y. Before and after intervention, we measured serum CRP concentrations with an enzyme immunoassay.
Interventions: Capsules with either 3.5 g/day fish oil (1.5 g/day n-3 fatty acids) or placebo for 12 weeks.
Results: The median CRP change in the fish oil group did not significantly differ from that in the placebo group (0.01 *vs* −0.17 mg/l, $P = 0.057$).
Conclusion: The currently available data —including ours— do not support that beneficial effects on CRP are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids as present in fish.
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Introduction

Blood markers of low-grade inflammation are emerging as risk indicators for coronary heart disease (Danesh *et al*, 2000). High-sensitivity C-reactive protein (CRP) is a marker of systemic inflammation produced by the liver and a powerful predictor of cardiovascular risk in healthy populations (Ridker *et al*, 2002; Yeh & Willerson, 2003). Dietary effects on markers of inflammatory processes have hardly been studied and are not yet established (de-Maat *et al*, 2000; Sierksma *et al*, 2002). N-3 fatty acids are suggested to have anti-inflammatory effects; they appear to reduce the production of inflammatory cytokines associated with several chronic diseases, such as rheumatoid arthritis (Kremer, 2000) and inflammatory bowel disease (Belluzzi, 2002). We hypothesised that n-3 fatty acids from fish reduce underlying inflammatory processes and in this way CRP concentrations.

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Subjects and methods

The primary purpose of this placebo-controlled, double-blind study was to study effects of n-3 fatty acids on markers of arrhythmia; details have been published elsewhere (Geelen *et al*, 2002). A total of 43 men and 41 postmenopausal women aged 50–70 y were randomised within strata of habitual fish consumption, diastolic blood pressure, and sex (Geelen *et al*, 2003) to receive either a daily dose of 3.5 g fish oil or placebo (Loders Croklaan, Wormerveer, the Netherlands) for 12 weeks as indistinguishable capsules. The fish oil provided approximately 700 mg eicosapentae-

noic acid (C20:5n-3, EPA), 560 mg docosahexaenoic acid (C22:6n-3, DHA), and 260 mg of other n-3 fatty acids per day. The placebo was high-oleic-acid sunflower oil (C18:1n-9). Subjects were instructed to maintain their usual diet and lifestyle. In diaries, they reported illness, irregular medication use, and other deviations from their usual lifestyle. Intakes of energy and nutrients were estimated by 24-h dietary recalls. Subjects abstained from fish, seafood, or (additional) fish oil supplements from 4 weeks before and during the study. We measured CRP concentrations and n-3 fatty acids in cholesteryl esters in nonfasting blood samples at baseline and end (Zock *et al*, 1997). High-sensitivity CRP concentrations were measured with an enzyme immunoassay (de Maat *et al*, 1996). CRP standard serum was used for calibration. The intra-assay variation was 5.8% for low (~0.5 mg/l), 2.7% for intermediate (~2 mg/l), and 4.8% for high (~10 mg/l) CRP values. Because of the skewed distribution of CRP values, we present values as medians and interquartile ranges. Differences in response between the fish oil and the placebo group were analysed using the Mann–Whitney *U*-test.

Results

Three subjects had CRP concentrations greater than 10 mg/l at one of the two blood collections and reported flu-like symptoms in their diary preceding that blood collection. We excluded them from the data reported here (Yeh and Willerson, 2003). Inclusion of the outliers did not materially affect the results. Compliance was supported by a change in the proportion of EPA (C20:5n-3) in serum cholesteryl esters of 282% (from 0.76±0.29 to 2.91±0.67 g/100 g fatty acids) in the fish oil group and of −6% in the placebo group. Background dietary intake was similar in the two treatment groups. During the study, the fish oil group consumed 0.5% of the energy as n-3 fatty acids, 6% as linoleic acid (C18:2n-6), and 6% as alcohol. In the placebo group, the corresponding figures were 0.4%, 5% and 5%, respectively. The habitual fish intake was 8.4±7.9 g n-3 fatty acids per month in the fish oil group and 8.7±7.3 g n-3 fatty acids per month in the placebo group.

The median CRP change in the fish oil group (0.01 mg/l) was not significantly different ($P = 0.057$) from that in the placebo group (−0.17 mg/l) (Figure 1, Table 1).

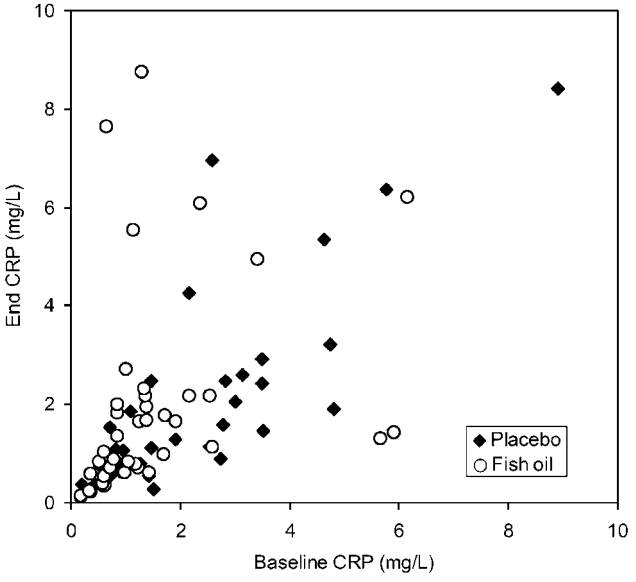

Figure 1 Serum CRP concentrations at baseline and after 12 weeks of dietary supplementation with placebo or fish oil.

Table 1 Serum CRP concentration at the start and after 12 weeks of treatment*

	CRP concentration (mg/l); Median and interquartile range	
	Baseline	*End*
Fish oil	1.14 (0.62–1.71)	1.31 (0.63–2.17)
Placebo	1.47 (0.72–3.02)	1.14 (0.70–2.48)

*The fish oil group received 3.5 g fish oil; the placebo group received 3.5 g high oleic sunflower oil.

Discussion

Supplementation with 1. 5 g of n-3 fatty acids per day did not lower CRP concentrations in healthy middle-aged subjects. The results would point towards an increase rather than a decrease of CRP with n-3 fatty acids relative to placebo. Other studies on fish oil and inflammation provide conflicting results. In healthy volunteers, increasing fish oil intake suppressed the *in vitro* synthesis of interleukin-2, interleukin-1β, interleukin-1α, and tumor necrosis factor-α (Endres *et al*, 1989, 1993). However, placebo-controlled studies found that fish oil does not affect *ex vivo* cytokine production (Blok *et al*, 1997) or the functional activity of neutrophils, monocytes, or lymphocytes in healthy humans (Kew *et al*, 2003). Few studies investigated effects of n-3 fatty acids on CRP. Pischon *et al* (2003) performed a cross-sectional analysis in 859 men and women. They reported a modest inverse relation between intake of EPA + DHA and CRP levels. In obese individuals, 4 g/day fish oil for 6 weeks did not decrease CRP (Chan *et al*, 2002). A recent study in healthy young subjects (mean age 38 y) with lower baseline CRP concentrations (median 0.78 mg/l) than in our study reported no effect of n-3 fatty acids on CRP (Madsen *et al*, 2003). Our study shows that n-3 fatty acids also do not affect CRP in a middle-aged population that might be more prone to low-grade systemic inflammations (median CRP concentration 1.24 mg/l). We cannot exclude that n-3 fatty acids affect other inflammatory markers or CRP at higher concentrations during

systemic inflammation. However, the currently available data —including ours— do not support that beneficial effects on CRP are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids as present in fish.

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References

Belluzzi A (2002): N-3 fatty acids for the treatment of inflammatory bowel diseases. *Proc. Nutr. Soc.* **61**, 391–395.

Blok WL, Deslypere JP, Demacker PN, van der Ven-Jongekrijg J, Hectors MP, van der Meer JW & Katan MB (1997): Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *Eur. J. Clin. Invest.* **27**, 1003–1008.

Chan DC, Watts GF, Barrett PH, Beilin LJ & Mori TA (2002): Effect of atorvastatin and fish oil on plasma high-sensitivity C-reactive protein concentrations in individuals with visceral obesity. *Clin. Chem.* **48**, 877–883.

Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR & Pepys MB (2000): Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ.* **321**, 199–204.

de Maat MP, de Bart AC, Hennis BC, Meijer P, Havelaar AC, Mulder PG & Kluft C (1996): Interindividual and intraindividual variability in plasma fibrinogen, TPA antigen, PAI activity, and CRP in healthy, young volunteers and patients with angina pectoris. *Arterioscler. Thromb. Vasc. Biol.* **16**, 1156–1162.

de-Maat MP, Pijl H, Kluft C & Princen HM (2000): Consumption of black and green tea had no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. *Eur. J. Clin. Nutr.* **54**, 757–763.

Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS & Weber PC (1989): The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* **320**, 265–271.

Endres S, Meydani SN, Ghorbani R, Schindler R & Dinarello CA (1993): Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J. Leukoc. Biol.* **54**, 599–603.

Geelen A, Brouwer IA, Zock PL, Kors JA, Swenne CA, Katan MB & Schouten EG (2002): N-3) fatty acids do not affect electrocardiographic characteristics of healthy men and women. *J. Nutr.* **132**, 3051–3054.

Geelen A, Zock PL, Swenne CA, Brouwer IA, Schouten EG & Katan MB (2003): Effect of n-3 fatty acids on heart rate variability and baroreflex sensitivity in middle-aged subjects. *Am. Heart J.* **146**, e4.

Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R, Williams CM & Calder PC (2003): Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. *Am. J. Clin. Nutr.* **77**, 1287–1295.

Kremer JM (2000): N-3 fatty acid supplements in rheumatoid arthritis. *Am. J. Clin. Nutr.* **71**, 349S–351S.

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.

Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC & Rimm EB (2003): Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation* **108**, 155–160.

Ridker PM, Rifai N, Rose L, Buring JE & Cook NR (2002): Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.* **347**, 1557–1565.

Sierksma A, van-der-Gaag MS, Kluft C & Hendriks HF (2002): Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. *Eur. J. Clin. Nutr.* **56**, 1130–1136.

Yeh ET & Willerson JT (2003): Coming of age of C-reactive protein: using inflammation markers in cardiology. *Circulation* **107**, 370–371.

Zock PL, Mensink RP, Harryvan J, de-Vries JH & Katan MB (1997): Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans. *Am. J. Epidemiol.* **145**, 1114–1122.