Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors

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Abstract

Objective The aim of the present paper was to review the literature in order to summarize the effects of marine n-3 fatty acids on circulating inflammatory markers among healthy subjects, subjects with high risk of developing cardiovascular disease (CVD) and in patients with CVD in human intervention studies.

Methods A systematic literature search in PubMed was performed. Intervention studies describing the effects of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects, subjects with high risk of CVD and patients with CVD were included. The following exclusion criteria were used: (1) interventions assessing inflammatory markers with ex vivo methods (2) interventions with children (3) articles describing animal or cell culture studies. Twenty-two articles were included. Additionally, 13 papers from their literature lists were included based on the same inclusion and exclusion criteria as the literature search.

Results and conclusion Intervention studies with marine n-3 fatty acids administered from either fish or fish oil demonstrate different results on inflammatory markers. No firm conclusion can be drawn about the effect of marine n-3 fatty acids on circulating inflammatory markers in healthy individuals, individuals with high risk of developing CVD or individuals with CVD related diseases.

Keywords Omega-3 fatty acids · Atherosclerosis · Inflammation · Circulating inflammatory markers · PBMCs

Introduction

Fish consumption reduces the risk of developing cardiovascular disease (CVD) and CVD mortality [1, 2]. Reduced total mortality and major coronary events, including fatal and non-fatal MI, are observed in intervention trials after intake of fish and fish oil containing the marine n-3 fatty acids eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) [3–6]. The association between high intakes of marine n-3 fatty acids and decreased morbidity and mortality from CVD can be explained by the decrease in plasma triglycerides [7, 8], moderate reduction in blood pressure [9] and reduced blood plate aggregation [10, 11]. Protection against cardiac arrhythmias has also been shown but the effect is still under discussion [12, 13].

Atherosclerosis is the underlying cause of most CVD. In atherosclerosis, the combined action of risk factors causes the gradual thickening of the arterial wall due to lipid
accumulation to form an atherosclerotic plaque which can abruptly rupture, causing thrombosis. Inflammation has emerged as a major player in the development and progression of atherosclerosis [14]. There is an association between various biomarkers of inflammation and prospective CVD risk in apparently healthy individuals as well as in patients with CVD or heart failure. Systemic biomarkers of early and late atherosclerosis are of great clinical interest due to their potential for identifying high risk patients. Even though there are some good candidates, only C-reactive protein (CRP) has emerged as a leading biomarker of inflammation for clinical application [15, 16]; other markers with appropriate robustness, sensitivity and specificity have not yet emerged. Based on the fact that endothelial dysfunction is the earliest manifestation of atherosclerosis, along with the involvement of oxidative stress and inflammation at all stages of coronary atherosclerosis development, biomarkers such as soluble intracellular adhesion molecule (sICAM-1) and soluble vascular adhesion molecule (sVCAM-1), soluble E-selectin (sE-sel) (for endothelial activation), interleukin (IL)-6 (transcriptional driver of CRP) and monocyte chemoattractant protein (MCP)-1 (produced by activated vasculature) are of particular interest (Table 1). So far CRP, IL-6, sVCAM-1 and sICAM-1 provide prognostic information beyond that obtained by clinical variables after acute coronary syndromes; these mediators seem to be powerful predictors of subsequent cardiovascular events. [17, 18]. Recently, intake of marine n-3 fatty acids has been associated with reduced plasma levels of inflammatory markers [19–21].

The aim of the present paper was to review the literature in order to summarize the effects of marine n-3 fatty acids on circulating inflammatory markers among healthy subjects, subjects with high risk of developing CVD and in patients with CVD in human intervention studies.

### Methods of this review

The systematic literature search was conducted in PubMed in 2009 using the following terms: “eicosapentaenoic acid OR docosapentaenoic acid OR docosahexaenoic acid OR omega-3 OR fish oil OR cod liver oil AND inflammation”. The following limitations were included in the search: “added to PubMed in the last 10 years, published in the last 10 years, Humans, Clinical Trial, English”. In total, 91 articles were identified. Based on these papers, we included all studies that measured the effect of marine n-3 fatty acids on circulating inflammatory markers in plasma or serum among apparently healthy individuals (Table 2), individuals with high risk of developing CVD (Table 3) or individuals with CVD (Table 4). Interventions with marine n-3 fatty acids given as supplements or in the diet as fish were included. The following exclusion criteria were used: (1) interventions assessing inflammatory markers with ex

### Table 1 Relevant inflammatory markers and their biological function

| Inflammatory markers                        | Abbreviation | Function |
|---------------------------------------------|--------------|----------|
| **Acute-phase protein**                     |              |          |
| C-reactive protein                          | CRP          | CRP is associated with the formation of cytokines, chemokines and the acute-phase response |
| **Cytokines**                               |              |          |
| Interleukin-6                               | IL-6         | Induces acute-phase response (by inducing CRP), anti-body secretion and differentiation |
| Interleukin-1α, Interleukin-1β              | IL-1α, IL-1β | Proliferation and maturation of lymphocytes, involved in inflammation and acute-phase response |
| Interleukin-18                              | IL-18        | Involved in the formation of Th1 cells |
| Tumor necrosis factor-α is a cytokine       | TNF-α        |Induces adhesion molecules and cytokine expression, involved in cell death |
| **Adhesion protein**                        |              |          |
| Soluble intercellular adhesion molecule-1   | sICAM-1      | Binds monocytes and lymphocytes to the endothelium |
| Soluble vascular cell adhesion molecule-1   | sVCAM-1      | Binds monocytes and lymphocytes to the endothelium |
| sE-selectin                                 | sE-sel       | Recruits leukocytes to the inflammatory site |
| sP-selectin                                 | sP-sel       | Recruits leukocytes to the inflammatory site. Indicates monocytes and platelet interactions |
| **Chemokines**                              |              |          |
| Monocyte chemoattractant protein-1          | MCP-1        |Facilitates migration of leukocytes to the intima |
| Granulocyte-macrophage colony-stimulating factor | GM-CSF     | Growth and differentiation of monocytes |
| Interleukin-8                               | IL-8         |Facilitates migration of leukocytes to the intima |
| Study                  | Individuals (n) | Number of groups | Dose n-3 (g/day) | Duration | CRP | Cytokines | Other inflammatory markers |
|-----------------------|-----------------|------------------|------------------|----------|-----|-----------|--------------------------|
| **Interventions with n-3 supplements** |                 |                  |                  |          |     |           |                          |
| Geelen et al. [22]    | 43 men and 41 women, healthy (50–70 years) | 2 groups: sunflower or fish oil | Fish oil: 0.7 g EPA, 0.56 g DHA, 0.26 g other n-3 PUFA | 12 weeks |    |           |                          |
| Vega-Lopez et al. [23] | 80 healthy men and women (20–55 years) | 4 groups: placebo, n-3 PUFA, Vit E or n-3 PUFA/Vit E | n-3 PUFA: 0.6 g EPA, 0.9 g DHA | 12 weeks |    |           |                          |
| Madsen et al. [24]    | 60 healthy men and women (21–57 years) | 3 groups: olive oil, low dose fish oil or high dose fish oil | Low dose fish oil: 0.9 g EPA, 0.8 g DHA, High dose fish oil: 3 g EPA, 2.9 g DHA | 12 weeks |    |           |                          |
| Yusof et al. [25]     | 20 healthy men (35–60 years) | 2 groups: coconut oil or fish oil | Fish oil: 1.8 g EPA, 0.3 g DHA | 8 weeks |    |           | ↔ IL-6 ↓ sICAM-1 ↔ sVCAM-1, sE-sel, sP-sel |
| Fujioka et al. [26]   | 59 men and 82 women, healthy (mean age 38 years) | 2 groups: olive oil or fish oil | Fish oil: 0.6 g EPA, 0.26 g DHA | 12 weeks |    |           | ↔ TNF-R1, TNF-R2 |
| Ciubotaru et al. [27] | 30 healthy women, postmenopausal and hormone treated (mean age 60 years) | 3 groups: sunflower oil, low dose fish oil or high dose fish oil | Low dose fish oil: 1.3 g n-3 PUFA, High dose fish oil: 2.56 g n-3 PUFA | 5 weeks |    | ↓ * ↓ IL-6 * |                          |
| Pot et al. [29]       | 77 healthy elderly men and women (50–70 years) | 2 groups: sunflower oil or fish oil | Fish oil: 0.7 g EPA, 0.56 g DHA, 0.26 g other n-3 PUFA | 12 weeks | ↔ (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, TNF-α, IFN-γ) | ↔ MIF, MCP-1, MIP-1α, RANTES, CCL11, IL-8 | ↔ sVCAM-1 and sICAM-1 |
| Thies et al. [31]     | 46 healthy men and women (55–75 years) | 6 groups: palm and sunflower oils, ALA, GLA, AA, DHA or fish oil | Fish oil: 0.72 g EPA, 0.28 g DHA or DHA 0.7 g | 12 weeks |    | ↓ s-VCAM-1 (Fish oil and ALA) ↔ sE-sel (Fish oil and ALA) ↔ sICAM-1 |                          |
| Study | Individuals (n) | Number of groups | Dose n-3 (g/day) | Duration | CRP | Cytokines | Other inflammatory markers |
|-------|----------------|------------------|-----------------|----------|-----|-----------|--------------------------|
| Miles et al. [32] | 16 men <40 years and 12 elderly men and women >55 years | 2 groups: palm oil and soy bean oils or fish oil | Fish oil: 1.2 g EPA/DHA | 12 weeks |  |  | ← sICAM-1 ↓ sVCAM-1 (older) ↑ sE-sel (young) |
| Michaeli et al. [33] | 15 healthy men (mean age 26 years) | 2 groups: fish oil or not (not-blinded) | Fish oil: 1.1 g EPA 0.7 g DHA | 3–4 weeks followed by LPS stimulation |  | ← TNF-α, IL-6 | |
| Cazolla et al. [34] | 93 healthy young men (18–42 years) and 62 healthy elderly men (53–70 years) | 4 groups: corn oil or 3 different doses EPA oil | EPA oil (EPAX 4510TG): 1.35 g, 2.7 g or 4.05 g EPA | 12 weeks |  |  | ↑ sE-sel (4.05 g EPA, young men) ↓ sICAM-1 (4.05 g EPA, both groups) (tendency) |
| Interventions with n-3 diet | | | | | | | |
| Tsitouras et al. [28] | 12 healthy elderly men and women (60–75 years) | 2 groups: control diet or n-3 diet (Cross-over design) | n-3 diet: 720 g/week fatty fish and sardine oil (4–5 g/day EPA and DHA) | 8 weeks | ↓ IL-6 (tendency) | | |
| Paulo MC [30] | 275 healthy men and women (20–40 years) | 4 groups: sunflower oil, fish oil, lean fish or fatty fish | Fish oil: 0.63 g EPA and 0.43 g DHA Lean fish: 0.05 g EPA and 0.21 g DHA Fatty fish: 0.77 g EPA and 1.37 g DHA | 8 weeks | ↓ sICAM-1 (lean fish) ↑ sVCAM-1 (Fish oil and lean fish) | | |

All the intervention studies with n-3 supplements were placebo-controlled; double-blinded with parallel-design if not otherwise stated.

* Larger change in CRP and IL-6 with fish oil with low content of EPA and DHA compared to fish oil with high content.

ALA α-linolenic acid (C18:3, n-3), GLA γ-linolenic acid (C18:3, n-6), AA arachidonic acid (C20:4, n-6)
Table 3  A review of marine n-3 fatty acid intervention studies and circulating inflammatory markers in individuals with high CVD risk

| Study                  | Individuals (n)                      | Number of groups | Dose n-3 (g/day)                  | Duration | CRP | Cytokines                                      | Other inflammatory markers                          |
|------------------------|--------------------------------------|------------------|-----------------------------------|----------|-----|-----------------------------------------------|------------------------------------------------------|
| Interventions with n-3 supplements |
| Kelley et al. [36]     | 34 hyperlipidemic men (39–66 years) | 2 groups: olive oil or DHA-oil | DHA-oil: 3 g DHA                  | 3 months | ↓   | ↓ IL-6                                        | ↓ GM-CSF, ↑ circulating neutrophils, ↑ MMP-2, ↑ IL-8 |
|                        |                                      |                  |                                   |          | ↓   | IL-1β, IL-2, IL-10, TNF-α                     |                                                      |
| Murphy et al. [37]     | 74 overweight individuals, BMI > 25 and TG > 1.6, (20–65 years) | 2 groups: n-3 enriched diet or no enriched diet | n-3 enriched food: 1 g EPA and DHA | 6 months | ↔ | IL-8                                         |                                                      |
| Plat et al. [38]       | 11 healthy overweight, BMI: 30-35, (mean age 59 years) | 2 groups: sunflower oil or fish oil (cross-over design) | Fish oil: 0.6 g EPA, 0.5 g DHA | 6 weeks   | ↔ | sICAM-1, sE-sel, MCP-1                        |                                                      |
| Browning et al. [39]   | 30 healthy overweight women, BMI > 25 | 2 groups: placebo (LA and OA) or fish oil (cross-over design) | Fish oil: 1.3 g EPA, 2.9 g DHA | 12 weeks  | ↔ | IL-6                                         |                                                      |
| Kabir et al. [40]      | 26 women with type 2 diabetes, 40–60 years | 2 groups: placebo (paraffin oil) or fish oil | Fish oil: 1.08 g EPA, 0.72 g DHA | 2 months  | ↔ | IL-6, TNF-α                                  |                                                      |
| Krebs et al. [47]      | 93 overweight women, BMI > 27, Fasting Insulin > 7, (21–69 years) | 3 groups: placebo (LA and OA), fish oil + weight reduction or placebo + weight reduction | Fish oil: 1.3 g EPA, 2.9 g DHA | 24 weeks  | ↔ | IL-6, TNF-α                                  |                                                      |
| Jellema et al. [42]    | 11 overweight men, BMI: 30-35         | 2 groups: sunflower oil or fish oil (cross-over design) | Fish oil: 1.35 g EPA and DHA | 6 weeks   | ↔ | IL-6, TNF-α (fasting or postprandial)         | sTNF-R55, sTNF-R75 (fasting or postprandial)          |
| Mori et al. [43]       | 59 hypertensive type 2 diabetic patients (40–75 years) | 3 groups: olive oil, EPA or DHA | n-3 PUFA: 4 g EPA (ethyl ester) or 4 g DHA (ethyl ester) | 6 weeks   | ↔ | IL-6, TNF-α                                  |                                                      |
| Chan et al. [44]       | 48 overweight men, BMI > 29, mean age 53 years | 4 groups: corn oil, atorvastatin + corn oil, fish oil, atorvastatin + fish oil | n-3 PUFA: Omacore 4 g/day (1.8 g EPA and 1.56 g DHA ethyl ester) | 6 weeks   | ↔ | IL-6, TNF-α                                  |                                                      |
| Accinni et al. [45]    | 57 dyslipidemic individuals (23–65 years) | 3 groups: placebo, n-3 PUFA/ vitE or n-3 PUFA/vitE and γ-oryzanol/niacin | n-3 PUFA: 0.66 g EPA, 0.44 g DHA | 4 months  | ↓ | TNF-α (n-3 PUFA)                             |                                                      |
| Study                  | Individuals (n)                              | Number of groups | Dose n-3 (g/day)                                                                 | Duration | CRP    | Cytokines                  | Other inflammatory markers                                      |
|-----------------------|----------------------------------------------|------------------|---------------------------------------------------------------------------------|----------|--------|----------------------------|----------------------------------------------------------------|
| Sampson et al. [48]   | 29 individuals with type 2 diabetes and 21 healthy controls | 4 groups: controls and patients were given n-3 PUFA or not. (not placebo controlled) | n-3 PUFA: 1.2 g EPA 0.8 g DHA                                                   | 3 weeks  |        | sVCAM-1, sICAM-1, sE-sel,  |                                                                 |
| Seljefflot et al. [49]| 41 male smokers with hyperlipidemia (45–57 years) | 4 groups: n-3 PUFA and antioxidants, n-3 PUFA, antioxidants, placebo | n-3 PUFA: 4.8 g EPA and DHA (ethyl ester)                                         | 6 weeks  |        | sE-sel, sVCAM-1 (n-3 PUFA) |                                                                 |
| Troseid et al. [35]   | 487 elderly men (64–76 years)                | 4 groups: corn oil, n-3 PUFA, corn oil + dietary intervention or n-3 PUFA + dietary intervention | n-3 PUFA: 0.84 g EPA 0.48 g DHA Diet. intervention: Increase intake of unsaturated fat, fish, fruit and vegetables | 3 years  | ↓ IL-18 (dietary intervention, n-3 PUFA) | ← MCP-1 |                                                                 |
| Hjerkin et al. [46]   | 487 Elderly men with high CVD risk (64–76 years) | 4 groups: corn oil, n-3 PUFA, corn oil + dietary intervention or n-3 PUFA + dietary intervention | n-3 PUFA: 0.84 g EPA 0.48 g DHA Diet. intervention: Increase intake of unsaturated fat, fish, fruit and vegetables | 3 years  | ↓ sICAM-1 (n-3 PUFA, dietary intervention, dietary intervention + n-3 PUFA) |                                                                 |
| Berstad [47]          | 171 Elderly men with high CVD risk (65–75 years) | 4 groups: corn oil, n-3 PUFA, corn oil + dietary intervention or n-3 PUFA + dietary intervention | n-3 PUFA: 0.84 g EPA 0.48 g DHA Diet. intervention: Increase intake of unsaturated fat, fish, fruit and vegetables | 18 months |        | sICAM-1, sVCAM-1 # | ↓ sE-sel (dietary intervention) |

All the intervention studies with n-3 supplements were placebo-controlled; double-blinded with parallel-design if not otherwise stated

# Positive correlation between change in serum n-3 PUFA and sVCAM-1

TG triglycerides, OA oleic acid (C18:1, n-9), LA linoleic acid (C18:2, n-6)
**Table 4** A review of marine n-3 fatty acid intervention studies and circulating inflammatory markers in individuals with CVD and CVD related diseases

| Interventions with n-3 supplements | Individuals (n) | Number of groups | Dose n-3 (g/day) | Duration | CRP | Cytokines | Other inflammatory markers |
|-----------------------------------|----------------|------------------|------------------|----------|-----|-----------|---------------------------|
| Schiano et al. [50]               | 32 men and women with PAD, mean age 66 years | 2 groups: no change in treatment or n-3 PUFA (not blinded) | n-3 PUFA: 0.85 g EPA and DHA (ethyl ester) | 12 weeks | ⇔ | ⇔ | Myeloperoxidase |
| Madsen et al. [51]               | 41 men and women with previous MI, mean age 63 years | 2 groups: olive oil or n-3 PUFA | n-3 PUFA: 4.3 g EPA and DHA | 12 weeks | ⇔ | | |
| Grundt et al. [52]               | 252 men and women with previous MI, (28–87 years) | 2 groups: corn oil or n-3 PUFA | n-3 PUFA: 3.4 g EPA and DHA (ethyl ester) | 12 months | ⇔ | ⇔ | sICAM-1, sE-sel |
| Lee et al. [53]                  | 77 men and women with previous MI, 40 healthy controls, mean age 57 years | 2 groups: no change in treatment or n-3 PUFA (not blinded) | n-3 PUFA: Omacore 1 g/day (ethyl ester) | 12 weeks | ⇔ IL-6 | ⇔ sP-sel |
| Thies et al. [55]                | 162 men and women awaiting carotid endarterectomy, mean age 69 years | 3 groups: palm and soy bean oils, sunflower oil or fish oil | Fish oil: 1.4 g n-3 PUFA | 7–189 days (median 42 days) | ⇔ sICAM-1, sVCAM-1 in plaques | ↓ macrophage infiltration (anti-CD68) in plaques (fish oil) | sVCAM-1, sE-sel (corn oil followed by 4 weeks with n-3 PUFA) |
| Johansen et al. [56]             | 54 men and women with CHD, mean age 58 years | 2 groups: corn oil or n-3 PUFA 5.1 g for 6 months, followed by 4 weeks where both groups received n-3 PUFA | n-3 PUFA: 5.1 g EPA and DHA | 4 weeks | | | |

**Interventions with n-3 diet**

| Seierstad et al. [54]           | 60 men and women with CHD (46–75 years) | 3 groups: all groups got 700 g salmon/week with low, moderate or high content of n-3 PUFA | n-3 content in salmon: Low n-3: 0.5 g EPA + DHA Moderate n-3: 1.5 g EPA + DHA High n-3: 2.9 g EPA + DHA | 6 weeks | ↓ IL-6, (high n-3) | ↓ sVCAM-1 (high n-3) |

All the intervention studies with n-3 supplements were placebo-controlled; double-blinded with parallel-design if not otherwise stated.

*MI* myocardial infarction, *PAD* peripheral arterial disease, *CHD* coronary heart disease
vivo methods (2), interventions with children (3) and articles describing animal or cell culture studies. After reading abstracts of the 91 papers, 22 articles were included. The literature lists of the selected papers were checked and 13 additional relevant papers were included based on the same inclusion and exclusion criteria as the literature search. The search was re-run in August 2010. However, no further papers were included from this search.

Results

Details of the 35 studies selected in this review are given in Tables 2, 3 and 4. Twenty-nine of these are based on marine n-3 fatty acids given as supplements, whereas six studies are dietary interventions with fish. Both categories of interventions are given in the tables.

The systematic review of the literature reveals that seven out of thirteen trials reported an effect on circulating inflammatory markers [22–34] in healthy individuals (Table 2). Six of the studies did not observe any changes in inflammatory markers [22–24, 26, 29, 33] (Table 2). C-reactive protein (CRP) was analyzed in seven of the trials, and in five of these no effect in CRP levels was observed [22–26], while a reduction in plasma concentration of CRP after intake of marine n-3 fatty acids was reported in two of the trials [27, 28]. Five of the thirteen trials analyzed the plasma concentration of IL-6. Two of these reported a reduction in IL-6 levels [27, 28], whereas three did not demonstrate any effect on IL-6 levels [25, 29, 33]. Table 2 also reveals that marine n-3 fatty acids reduced or had no effects on the concentration of sICAM-1 in five of the trials [25, 29, 31, 32, 34]. The concentration of sVCAM-1 was reduced, increased or unchanged after intake of marine n-3 fatty acids among healthy individuals in these trials, whereas the level of sE-selectin increased or remained unchanged [25, 31, 32, 34]. The effects of marine n-3 fatty acids on circulating inflammatory markers among individuals with a high risk of developing CVD are shown in Table 3 [35–49]. Four of fifteen trials reported a reduction in the concentration of inflammatory markers after intake of marine n-3 fatty acids [35, 36, 45, 46], while nine studies did not observe any effects on inflammation [37–44, 48]. An increase in inflammatory markers [49] was reported in one study. One of nine studies where CRP was analyzed demonstrated a reduction in the concentration of CRP after intake of marine n-3 fatty acids [36]. Three trials demonstrated a reduction in plasma cytokines or soluble adhesion molecules [35, 45, 46]. Table 4 reviews the effects of marine n-3 fatty acids on circulating inflammatory markers among individuals with CVD or CVD related diseases [50–56]. Five of seven studies showed no change in the concentration of inflammatory markers after intake of marine n-3 fatty acids [50–53, 55], whereas one study reported a reduction in the levels of IL-6 and sVCAM-1 [54] and one trial showed an increase in the level of sE-selectin and sVCAM-1 [56].

Discussion and conclusion

Effect of marine n-3 fatty acids on circulating inflammatory markers

Intervention trials with marine n-3 fatty acids administered either from fish or fish oil supplements demonstrate different results on circulating inflammatory markers in healthy individuals, individuals with high risk of developing CVD or individuals with CVD related diseases. Based on these findings, a firm conclusion about the effect of marine n-3 fatty acids on circulating inflammatory markers cannot be drawn. The majority of the trials do not show any effect, however a decreased level of circulating CRP and IL-6 are observed in some studies, whereas the circulating level of adhesion molecules inconsistently are elevated, decreased or not changed. The reasons for the different results may be several. First of all, a local inflammatory improvement in the arterial wall will not necessary be reflected in the circulation and may explain the lack of effect observed after intake of marine n-3 fatty acids. Several of the reported trials have a relative small number of study subjects and probably low statistical power. Variation in the results can also be explained by differences in the administration of the marine n-3 fatty acids (supplement vs. diet) as well as the doses used. Furthermore, there is uncertainty regarding the background diet and to what extend the intervention changed the balance between n-3 and n-6 fatty acids or altered the intake of saturated fat in the diet.

Comparing the results on the effects of marine n-3 fatty acids given as supplements on serum CRP in healthy individuals, the baseline level of CRP seems crucial. Ciubotaru et al. [27] reported a significant reduction in the level of CRP in postmenopausal women who had mean baseline levels of CRP above 6 mg/L. This is in contrast to the results from Geelen et al. [22], Vega-Lopez et al. [23] and Madsen et al. [24] who did not observe any changes in the CRP serum levels. The baseline level of serum CRP in the subjects in these studies was approximately 1 mg/L. Vega-Lopez et al. [23] failed to see any effect on the serum CRP level using a daily dose of 0.6 g EPA and 0.9 DHA for 12 weeks, which is in line with the results from Madsen et al. [24] who used two different daily doses (3.0 g EPA + 2.9 g DHA or 0.9 g EPA + 0.8 g DHA). In contrast, Ciubotaru et al. [27] observed in postmenopausal women that the effect on CRP is dependent on the dose of
marine n-3 fatty acids. A larger decrease in CRP was observed in the women who received the highest dose of marine n-3 fatty acids (2.56 g/d) compared to those receiving a daily dose of 1.3 g marine n-3 fatty acids.

It is interesting to note that observed decrease in circulating CRP level was associated with decrease in circulating IL-6 level after intake of marine n-3 fatty acids, both via supplement or diet. In this review there are relatively few studies where marine n-3 fatty acids are administered as fish. When comparing these interventions with the interventions with fish oil, there are indications for a more prominent effect on circulating inflammatory markers with fish containing diet. Reductions or no change in one of the following markers (CRP, IL-6, sICAM-1, sVCAM-1, IL-18, sE-sel) are observed in all the dietary studies included in this review. A possible explanation could be that subjects participating in dietary intervention trials may change their overall dietary pattern resulting in more awareness of healthy lifestyle, which again may influence the circulating inflammatory markers. Fish may also contain other bioactive compounds that may influence outcome.

New strategies for studying effects of marine n-3 fatty acids on inflammation

To further understand if marine n-3 fatty acids have an effect on inflammation in relation to atherosclerosis and the underlying molecular mechanisms responsible for their inflammatory response, new strategies should be considered. When inflammatory markers are measured in the circulation, the local effect in monocytes will not necessary be detected since the monocytes only contribute to about 5% of the total cells in plasma. The peripheral blood mononuclear cells (PBMCs) include monocytes and lymphocytes, which are cells central in inflammation and hence in the atherosclerosis process. The PBMCs are exposed to many of the same environmental factors as the arterial wall. Alterations in gene expression levels in these cells can thus be demonstrated early in the process before signs of inflammation can be seen in vivo, and thus constitute an important source of biomarkers for progression of atherosclerotic disease. PBMCs are easily available, which makes them suitable for studying gene expression of mediators involved in the early development of atherosclerosis [57]. A previous study recently demonstrated that 166 genes among 182 candidate cardiovascular genes were expressed in PBMCs [58].

The ability of marine n-3 fatty acids to alter gene expression has been clearly demonstrated in vitro and may account for its potential beneficial health effects. Fatty acids regulate expression of genes involved in lipid metabolism and inflammation by acting as ligands for the peroxisomal proliferator-activated receptors (PPARs) [59]. Fatty acids are also known to reduce the activation of the transcription factor nuclear factor kappa B (NF-κB) probably by interference with the PPARs [60, 61] or the Toll-like receptors [62, 63]. The G protein-coupled receptor 120 (GPR120) has recently been characterized as an n-3 fatty acid receptor/sensor involved in the anti-inflammatory effects of n-3 fatty acids [64]. PPAR and PPAR target genes are expressed in PBMCs [65]. Dietary supplements containing fish oil have also been shown to affect expression of PI3K, Akt, NF-κB and inflammatory cytokines in mononuclear cells [66].

To obtain a more comprehensive overview of the processes that are modulated by EPA and DHA, new “omics” technologies can be used to detect large scale changes in gene expression profiles (transcriptomics). Whole genome transcriptomic analysis in PBMCs will be valuable in further understanding the inflammatory role of n-3 fatty acids in humans. Recently, a human intervention study among elderly who daily consumed fish oil (containing 1.8 g EPA and DHA) for 26 weeks demonstrated changes in the gene expression profiles in PBMCs. Gene transcripts involved in inflammation and other processes of atherosclerosis were down-regulated among those who had consumed fish oil [67]. The combined use of other “omics” technologies, such as proteomics and metabolomics (lipidomics), in intervention studies will further extend the understanding of the effects of marine n-3 fatty acids on inflammation. Using proteomics technology, a recent study demonstrated that daily intake of 3.5 g fish oil down-regulated a number of proteins involved in the acute phase response and lipid/lipoprotein metabolism [68]. Furthermore, Lankinen et al. [69] previously used a lipidomics approach in an intervention study where 33 individuals randomized to eat fatty fish (150 g four times per week), lean fish (150 g four times per week) or no fish, in 8 weeks. They observed a reduction in the level of lipid metabolites related to inflammation and insulin-signaling among those eating fatty fish.

No firm conclusion about the effect of marine n-3 fatty acids on circulating inflammatory markers can be drawn in this review. A systematic use of transcriptome, proteome, and metabolome technologies for the construction of network based models of biological processes has emerged as an exciting research approach in molecular biology and functional genomics. In future marine n-3 supplementation and dietary intervention trials, systems biology combined with traditional circulating markers may help us to gain more insight into the underlying molecular mechanisms of marine n-3 fatty acids responsible for the inflammatory response in the progression of atherosclerotic disease.
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