In their current guidelines cardiac societies recommend the consumption of the two n-3 fatty acids EPA and DHA to prevent cardiovascular complications. Cardiovascular events are reduced by EPA and DHA, because they are antiarrhythmic, mitigate the course of atherosclerosis and stabilise plaque. As atherosclerosis is considered an inflammatory disorder a number of studies have investigated the anti-inflammatory mechanisms of EPA and DHA in a cardiovascular context in human dietary intervention studies. Pro-inflammatory cytokines, or cytokines reflecting inflammatory processes, e.g. IL-1β, IL-2, IL-6, TNFα, platelet-derived growth factor (PDGF)-A and -B and monocyte chemoattractant protein-1 (MCP-1), are reduced by ingestion of EPA and DHA by human subjects. Interestingly, C-reactive protein remains largely unaltered. However, in in vitro and animal models, but less so in human subjects, soluble cytokines reflecting interactions between blood cells and the vessel wall, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, are reduced. Moreover, in contrast to common expectations, oxidative stress seems to be reduced after ingestion of EPA and DHA, at least as indicated by measurement of urinary F2 isoprostane excretion. Notably, for PDGF-A and -B and for MCP-1 the reduction has been demonstrated to occur at the gene expression level, which indicates that a deliberate change in diet can alter gene expression quantitatively. The precise underlying mechanism, however, remains to be clarified, but might involve PPAR, NF-κB and/or the eicosanoid system. The same holds true for the mechanisms by which levels of other cytokines are altered by EPA and DHA.

Cytokines: Growth factors: Coronary artery disease

The World’s most important cardiac societies have issued guidelines that recommend the intake of the two marine n-3 fatty acids EPA and DHA at 1 g/d for CVD prevention, treatment after a myocardial infarction and prevention of sudden death and secondary disease (De Backer et al. 2003; Priori et al. 2003; Van der Werf et al. 2003; Smith et al. 2006). National cardiac societies have followed suit (Wirth & Gohlke, 2005). These recommendations are based not only on intervention trials with these n-3 fatty acids (Burr et al. 1989, 2003; The GISSI Prevenzione Group, 1999; Marchioli et al. 2002; Yokoyama et al. 2003), but also on a wealth of literature describing mechanisms of actions, animal models, studies with surrogate and intermediate factors and other aspects (von Schacky, 1987, 2003). Taken together, the scientific basis of the current guidelines is so strong that they have been established despite a null result in a recent Cochrane analysis (Hooper et al. 2006).

EPA and DHA have been demonstrated not only to have an antiarrhythmic effect (Leaf et al. 2005; Raitt et al. 2005), but also to mitigate the course of coronary atherosclerosis (von Schacky et al. 1999) and to stabilize unstable plaque, e.g. in carotid arteries (Thies et al. 2003). These two findings are manifestations of an anti-inflammatory effect of EPA and DHA, currently used in the treatment of inflammatory disorders such as rheumatoid arthritis (Kremer, 2000). For some time atherosclerosis has been considered to be a disease with an inflammatory component (for example, see Ross, 1999). The present review considers investigations aimed at unravelling the mechanisms by which EPA and DHA exert their anti-inflammatory effects through alterations of cytokine metabolism. Since differences exist in cytokine metabolism in vitro v. in vivo and in experimental animals v. in human subjects the present review will largely focus on work done in human subjects.

Abbreviations: ICAM, intercellular adhesion molecule; MCP-1, monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; s, soluble; VCAM, vascular cell adhesion molecule.

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Cytokines related to inflammation and atherosclerosis

**IL-1β**

IL-1β is an important cytokine that has a plethora of actions, including a pronounced pro-inflammatory effect and increasing the expression of adhesion molecules (Dinarello, 2006). Levels of IL-1β decrease by 61–90% when healthy volunteers and patients with rheumatoid arthritis ingest doses of between 2.7 and 5.8 g EPA and DHA/d (for review, see James et al. 2000). Furthermore, IL-1β levels associated with stimulation by strenuous exercise (a marathon run) are not influenced by previous ingestion of 3.6 g EPA and DHA/d (Toft et al. 2000), which suggests that IL-1β levels clearly respond to a strong stimulus. These findings might partially explain why infectious complications are not seen more frequently in large-scale intervention trials using EPA and DHA (von Schacky & Harris, 2006).

**IL-6**

IL-6 is a cytokine that together with a soluble IL-6 receptor plays an important role in perpetuating an inflammatory state (Scheller et al. 2006). IL-6 is generally reduced, as assessed ex vivo, after supplementation of the human diet with n-3 fatty acids (for review, see Calder, 2005). In the supernatant fraction of unstimulated mononuclear cells the reduction is more pronounced after 0.3 g EPA and DHA/d than after 1.0 or 2.0 g EPA and DHA/d (Trebble et al. 2003). However, in the supernatant fraction of stimulated mononuclear cells the reduction is clearly dose-related (Trebble et al. 2003).

**IL-10**

An epidemiological study (Ferrucci et al. 2006) has shown that low levels of this anti-inflammatory compound are associated with low levels of DHA. After treatment for 1 year with 3.4 g EPA and DHA/d, the IL-10 levels of forty-five recipients of heart transplants were found to be decreased (Holm et al. 2001). In healthy volunteers after supplementation with 7 g EPA and DHA/d for 4 weeks IL-10 mRNA steady-state levels were found to be unaltered in either unstimulated mononuclear cells or monocytes that had been adherence-activated ex vivo (Baumann et al. 1999). There have been no other reports of studies in human subjects. Thus, there is at present no clear picture of the effects of EPA and DHA on levels of IL-10.

**TNFα**

TNFα is a pro-inflammatory cytokine that has a large number of effects, among them increased body temperature, reduced appetite and stimulation of other immunomodulatory cytokines (Grimble, 1996). TNF is thought to be a propagator of atherosclerosis (Ross, 1999). The effects of fish oil on TNFα production by peripheral blood mononuclear cells have been investigated in eleven studies of healthy volunteers, of which six studies have demonstrated a suppressive effect (for review, see Grimble et al. 2002). These apparently discrepant findings can be explained by the effects of inherent TNFα production and by polymorphisms in the TNFα and lymphotoxin α genes (Grimble et al. 2002). These polymorphisms might also explain an unexpected increase in TNF in recipients of heart transplants after supplementation with 3.4 g EPA and DHA/d for 1 year (Holm et al. 2001). In a dose–response study of healthy volunteers levels of TNFα in the supernatant fraction of unstimulated and stimulated mononuclear cells was shown to decrease (Trebble et al. 2003). Interestingly, the decrease was found to be less pronounced after consuming 2.0 g EPA and DHA/d than after consuming 1.0 g EPA and DHA/d.

DHA, but not EPA, reduces the expression of pro-inflammatory IL-1, IL-6 and TNFα in vitro (De Caterina et al. 2006).

**Platelet-derived growth factor**

Platelet-derived growth factor (PDGF) stimulates smooth muscle cell proliferation and plays a role in the migration of these cells into neointima following injury and in atherosclerosis (Raines, 2004). PDGF is thought to play a major role in the proliferation of atherosclerotic lesions (Ross, 1999). In volunteers ingesting 7 g EPA and DHA/d levels of mRNA coding for PDGF-A and -B were found to be reduced by 58% after 1 week and by 70% after 6 weeks, with these levels remaining constant in controls on an unaltered Western diet (Kaminski et al. 1993). This finding was the first demonstration that a deliberate change in diet can alter gene expression quantitatively. In a subsequent study, with the same dose of EPA and DHA but comparing it with 7 g n-6 fatty acids/d and 7 g n-9 fatty acids/d (Baumann et al. 1999), the reduction in the levels of PDGF-A and -B mRNA was found to be quantitatively less pronounced (for PDGF-A −25 (sd 10) %, for PDGF-B −31 (sd 13) %) in non-stimulated mononuclear cells. However, after cell adherence for 4 h or 20 h the reduction was found to persist to a quantitatively similar extent (Baumann et al. 1999). Lower doses of EPA and DHA (0.3, 0.6 or 0.9 g n-3 fatty acids/d) have no effect on serum mitogenic activity or serum PDGF levels (Wallace et al. 2000).

**Monocyte chemotactic protein-1**

Monocyte chemotactic protein-1 (MCP-1), acting through its receptor chemokine (C–C motif) receptor 2, appears to play an early and important role in the recruitment of monocytes to atherosclerotic lesions and in the formation of intimal hyperplasia after intimal injury (Charo & Taubman, 2004). Supplementation with dietary n-3 fatty acids (EPA and DHA), but not n-6 or n-9 fatty acids, at 7 g /d for 4 weeks reduces MCP-1 mRNA levels in unstimulated mononuclear cells ex vivo by 40% (Baumann et al. 1999). After stimulation of the cells by adherence for 4 h and 20 h MCP-1 mRNA levels are reduced by 35 (sd 10) % and 30 (sd 8) % respectively (Baumann et al. 1999). It is quite likely that the reductions in gene expression translate into reduced levels of MCP-1 before and after stimulation in vivo, although the direct proof will be quite difficult to obtain. Evidence from in vitro studies
C-reactive protein

C-reactive protein is a marker of systemic inflammation that is currently considered to be a risk factor for CVD (Tsimikas et al. 2006). Given the anti-inflammatory and anti-atherosclerotic effects of EPA and DHA, reduced levels of C-reactive protein would be expected to occur after supplementation. While an epidemiological study (Pischon et al. 2003) has shown an inverse relationship between the intake of EPA and DHA and levels of C-reactive protein, human intervention studies (for review, see Mori & Beilin, 2004) have not found a reduction in C-reactive protein levels after ingestion of EPA and DHA. Thus at present no clear picture of the relationship between EPA and DHA and C-reactive protein has emerged.

Cytokines related to endothelial activation

Vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule-1 (ICAM-1) and E-selectin have a role in the pathogenesis of atherosclerosis (Hope & Meredith, 2003). Plasma levels can be measured because soluble (s) VCAM and sICAM are shed from the cell surface. Plasma levels of sE-selectin can also be measured (Roldan et al. 2003). Levels of VCAM and ICAM correlate to some extent with the presence of clinical atherosclerosis (Hope & Meredith, 2003). However, VCAM-1 and ICAM-1 have a role in the early phase of the development of the atherosclerotic lesion, i.e. in monocyte recruitment by endothelial cells, before the appearance of macrophages (foam cells) in the intima (De Caterina & Massaro, 2005). E-selectin is expressed on the endothelial surface slightly earlier in the sequence of events (Roldan et al. 2003). sVCAM-1, sICAM-1 and sE-selectin are considered to be markers of endothelial activation (Hope & Meredith, 2003; Roldan et al. 2003). Whether they are risk factors for atherosclerosis or its clinical events is a matter of debate.

Stimulated endothelial cells express less sVCAM, sICAM or sE-selectin in the presence of n-3 fatty acids, with DHA being more potent than EPA (De Caterina et al. 2004). DHA reduces mRNA levels by inhibition of the activation of the NF-κB system of transcription factors (De Caterina et al. 2004). Corresponding findings from studies in experimental animals have been published (for review, see Calder, 2004).

Studies in human subjects have yielded less clear results. In a randomized study of forty-one male smokers with hyperlipidaemia (Seljeot et al. 1998) 4-8 g EPA and DHA/d was shown to increase levels of sVCAM-1 and sE-selectin. In middle-aged healthy volunteers (Thies et al. 2001) supplementation with 1 g EPA and DHA/d for 12 weeks was found to reduce levels of sVCAM-1 (~28%) and sE-selectin (~17%), while levels of sICAM-1 were unaltered. In another 12-week randomized double-blind study of healthy subjects by the same group (Miles et al. 2001) levels of all three compounds were found to be unaltered after fish oil supplementation. In a 1-year double-blind study of 300 patients after a myocardial infarction (Grundt et al. 2003) treatment with EPA and DHA was not found to reduce levels of sICAM or sE-selectin. In individuals with type 2 diabetes, however, levels of sE-selectin are reduced after treatment with EPA or DHA (Nomura et al. 2003; Woodman et al. 2003). Administration of 2-4 g EPA and DHA/d to 171 elderly men at risk for coronary artery disease has been shown to increase sVCAM (Berstad et al. 2003). A randomized study (Eschen et al. 2004) comparing EPA and DHA at doses of 2-0 g/d and 6-6 g/d in healthy subjects has shown a decrease in sE-selectin only when fed at a dose of 6-6 g/d. In a large (563 elderly subjects) 3-year randomized study that compared (using a factorial design) dietary advice, 2-4 g EPA and DHA/d and no treatment (Hjerkinn et al. 2005) EPA and DHA was found to reduce levels of sICAM.

Thus, while a clear picture has emerged in vitro and in animal studies, demonstrating reductions in plasma levels of sVCAM, sICAM and sE-selectin, investigations in human subjects have yielded mixed results. This disparity may be related to differences in populations studied and doses used.

Oxidative stress

The measurement of urinary F2 isoprostane excretion by GC–MS is currently thought to best reflect oxidative stress in vivo (Mori & Beilin, 2004). In contrast to theoretical concerns and earlier observations with less-refined methodology, oxidative stress has been consistently shown to be reduced after ingestion of EPA and DHA both in combination and individually (Mori & Beilin, 2004). Reduced oxidative stress is thought to contribute to the anti-atherosclerotic actions of n-3 fatty acids, possibly through immunomodulation and decreased leucocyte activation (Mori & Beilin, 2004).

Conclusion

Taken together EPA and DHA reduce levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNFα in human subjects. Moreover, mRNA levels of pro-atherosclerotic growth factors, such as PDGF-A and -B, and MCP-1 are reduced in mononuclear cells after supplementing the human diet with EPA and DHA. The cytokines and growth factors mentioned play a role in the propagation of the atherosclerotic lesion. In vitro the levels of sICAM, sVCAM and sE-selectin are reduced by the presence of EPA or DHA. These cytokines reflect endothelial activation. Data from human studies are less clear cut, probably because endothelial activation is transient. It is currently thought that the effects of EPA and DHA on cytokines and growth factors are the mechanisms responsible for the anti-atherosclerotic action of these n-3 fatty acids.
References


