REVIEW

The role of marine omega-3 (*n*-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability

Philip C. Calder

Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, MP887 Southampton General Hospital, Southampton, UK

Received: October 22, 2011
Revised: November 16, 2011
Accepted: November 30, 2011

Atherosclerosis has an important inflammatory component and acute cardiovascular events can be initiated by inflammatory processes occurring in advanced plaques. Fatty acids influence inflammation through a variety of mechanisms; many of these are mediated by, or associated with, the fatty acid composition of cell membranes. Human inflammatory cells are typically rich in the *n*-6 fatty acid arachidonic acid, but the contents of arachidonic acid and of the marine *n*-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid have roles in inflammation. EPA also gives rise to eicosanoids and these are usually biologically weak. EPA and DHA give rise to resolvins which are anti-inflammatory and inflammation resolving. EPA and DHA also affect production of peptide mediators of inflammation (adhesion molecules, cytokines, etc.). Thus, the fatty acid composition of human inflammatory cells influences their function; the contents of arachidonic acid, EPA and DHA appear to be especially important. The anti-inflammatory effects of marine *n*-3 polyunsaturated fatty acids (PUFAs) may contribute to their protective actions towards atherosclerosis and plaque rupture.

Keywords:
Cytokine / Eicosanoid / Eicosapentaenoic acid / Docosahexaenoic acid / Inflammation

1 Inflammation contributes to atherosclerosis

Inflammation is now recognised to play an important role in atherosclerosis, which results from interaction between modified lipoproteins, monocyte-derived macrophages, T cells and the normal cellular elements of the vessel wall [1–5] (Figs. 1 and 2). The earliest lesions, fatty streaks, are pure inflammatory lesions consisting only of monocyte-derived macrophages and T lymphocytes. Endothelial injury increases the adhesiveness of the endothelium to leukocytes (and platelets) by upregulating expression of adhesion molecules as well as its permeability. Mice deficient in the adhesion molecules intercellular adhesion molecule 1 (ICAM-1), E-selectin or P-selectin show less atherosclerosis when fed an atherosclerotic diet [1, 3], indicating the importance of endothelial-leukocyte adhesive interactions in the atherosclerotic process. The injured endothelium also produces vasoactive molecules, cytokines and growth factors. Some of these molecules such as monocyte chemoattractant protein 1 (MCP-1), act as chemoattractants attracting monocytes and T cells to the vessel wall (Figs. 1 and 2). The resulting inflammatory response stimulates migration and proliferation of smooth muscle cells (SMCs) that become mixed into the area of inflammation (Fig. 2). The inflammatory response within the vascular wall is mediated by monocyte-derived macrophages and T cells [1, 2, 4, 5]. Continued inflammation results in increased numbers of inflammatory cells that migrate from the bloodstream and proliferate within the lesion. Activation of these cells leads to release of enzymes, cytokines, chemokines, eicosanoids and growth factors continuing the

Correspondence: Professor Philip C. Calder, Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
E-mail: pcc@soton.ac.uk
Fax: +44-2380-795255

Abbreviations: COX, cyclooxygenase; **CRP**, C-reactive protein; **CVD**, cardiovascular disease; **DHA**, docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **ICAM**, intercellular adhesion molecule; **IL**, interleukin; **LOX**, lipoxygenase; **LPS**, lipopolysaccharide; **LT**, leukotriene; **MCP**, monocyte chemoattractant protein; **MMP**, matrix metalloproteinase; **NFκB**, nuclear factor kappa B; **OCEAN**, Omacor Carotid EnArterectomy iNtervention; **PG**, prostaglandin; **PUFA**, polyunsaturated fatty acid; **TNF**, tumour necrosis factor; **VCAM**, vascular cell adhesion molecule

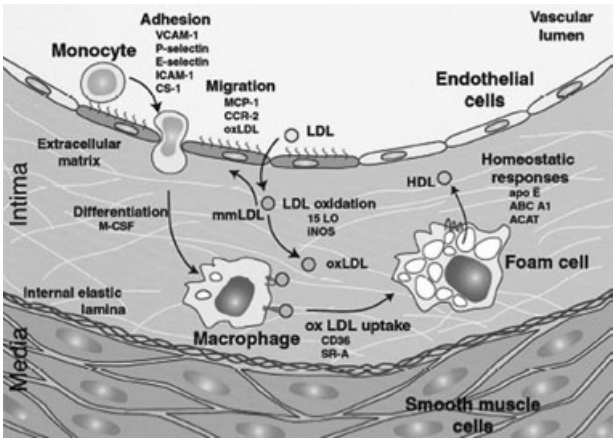

Figure 1. Initiating events in atherosclerosis. Low density lipoproteins (LDL) are subject to oxidative modifications in the subendothelial space, progressing from minimally modified LDL (mmLDL) to extensively oxidised LDL (oxLDL). Monocytes attach to endothelial cells that have been induced to express adhesion molecules by mmLDL and inflammatory cytokines. Adherent monocytes migrate into the subendothelial space and differentiate into macrophages. Uptake of oxLDL via scavenger receptors leads to foam cell formation. Reprinted from [1], Copyright (2001), with permission from Elsevier.

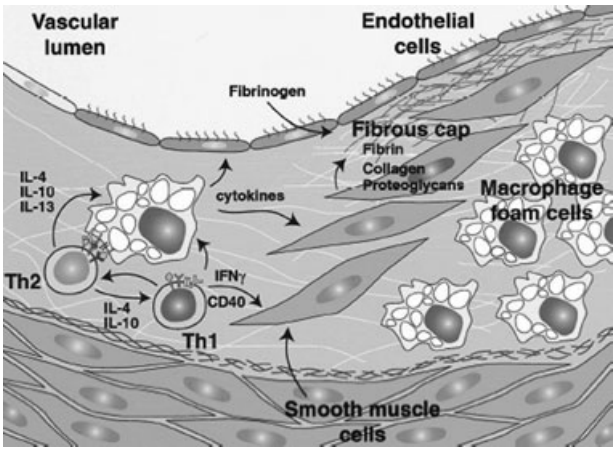

Figure 2. Progression of the atherosclerotic lesion. Interactions between macrophage foam cells and helper T lymphocytes (Th1 and Th2) establish a chronic inflammatory process within the vascular wall. Cytokines secreted by lymphocytes and macrophages exert pro- and anti-atherogenic effects on endothelial and SMCs. SMCs migrate from the media into the intima where they proliferate and secrete intracellular matrix proteins that form the fibrous plaque. Reprinted from [1], Copyright (2001), with permission from Elsevier.

inflammatory process and contributing to the build up of the plaque. Both necrosis and rupture occur within regions of the plaque as it grows and the latter may serve as foci for thrombus formation, suggesting that cycles of rupture and healing thrombosis occur as plaques grow. In accord with the proposed central role of inflammation in development of the atherosclerotic plaque, several inflammatory markers present in the serum or plasma are associated with cardiovascular disease (CVD) risk. Soluble P-selectin was identified as an independent predictor of future CVD risk in the Women's Health Study [6]. sICAM-1 concentration was shown to be an independent predictor of coronary heart disease [7, 8]. Serum interleukin (IL)-6 concentrations may also be predictive [9]. However, of the inflammatory markers, C-reactive protein (CRP) has been most investigated, and has been found to be predictive of future myocardial infarction, stroke, peripheral vascular disease and CVD mortality [3]. CRP has been compared with other inflammatory and lipid risk factors and found to be a strong predictor of risk [3]. CRP has been considered to be simply a marker of inflammation. However, more recent studies have indicated that CRP may play an active role in inflammation by stimulating monocytes to release inflammatory cytokines and by increasing upregulation of adhesion molecule expression and MCP-1 synthesis by endothelial cells [3].

2 Marine *n*-3 fatty acids, inflammatory cell membrane fatty acid composition and lipid mediators

Polyunsaturated fatty acids (PUFAs) are important constituents of the phospholipids of all cell membranes, where they play roles assuring the correct environment for membrane protein function, maintaining membrane fluidity, regulating cell signalling, gene expression and cellular function, and serving as substrates for the synthesis of lipid mediators [10]. The phospholipids of inflammatory cells (e.g. neutrophils, lymphocytes, monocytes) from the blood of humans consuming typical Western diets contain significant amounts (usually 10–20% of fatty acids) of arachidonic acid, with much lower amounts of eicosapentaenoic acid (EPA) (usually 0.5–1%) and docosahexaenoic acid (DHA) (usually 2–4%) [11–22], although there are differences between the different phospholipid classes in terms of the content of these fatty acids [13]. A key role of arachidonic acid in inflammatory cell membranes is as a precursor of eicosanoids. These are long recognised mediators and regulators of inflammation and immunity and include prostaglandins (PG), thromboxanes, leukotrienes (LT) and other oxidised derivatives produced by the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 pathways [23, 24]. In general arachidonic acid-derived eicosanoids act in a pro-inflammatory way, although this is an oversimplification since it is now recognised that PGE_2, for example, has both pro- and anti-inflammatory effects, and that another eicosanoid lipoxin A_4 is anti-inflammatory [25].

The fatty acid composition of inflammatory cells can be modified by increasing intake of marine *n*-3 fatty acids, which leads to a higher content of EPA and DHA [11–18, 20–22].

This occurs in a dose response fashion [17, 22] and over a period of days [26] to weeks [16, 17, 22], with a new steady-state composition reached within a few weeks. Typically the increase in content of *n*-3 PUFAs occurs at the expense of *n*-6 PUFAs, especially arachidonic acid. Such changes in membrane phospholipid fatty acid composition might be expected to influence the function of cells involved in inflammation through alterations in the physical properties of the membrane such as membrane order ("fluidity") and raft structure; effects on cell signalling pathways, either through modifying the expression, activity or avidity of membrane receptors or modifying intracellular signal transduction mechanisms that alter transcription factor activation and gene expression; and alterations in the pattern of the lipid mediators produced [25, 27–29]. The earliest anti-inflammatory action of marine *n*-3 PUFAs described in humans was a reduction in generation of arachidonic acid-derived eicosanoids like PGE_2 and LTB_4 [11–13, 15, 30, 31]. This effect reflects, in part, the decrease in arachidonic acid content seen after a period of increased intake of marine *n*-3 PUFAs. Additionally, EPA acts as an inhibitor of arachidonic acid metabolism via the COX and LOX enzymes.

EPA is also a substrate for the COX and LOX enzymes, and the mediators produced (e.g. PGE_3 and LTB_5) have a different structure from the arachidonic acid-derived mediators, and this influences their potency [32]. Usually the EPA-derived mediators are less biologically active than those produced from arachidonic acid [25, 32] or they may even antagonise the action of those produced from arachidonic acid [33].

Another family of lipid mediators is the resolvins and protectins. EPA and DHA give rise to resolvins (e.g. resolvin E1 and resolvin D1) and DHA to protectins (e.g. protectin D1) through complex pathways involving COX and LOX enzymes [34–36]. These mediators have been demonstrated in cell culture and animal feeding studies to be anti-inflammatory and inflammation resolving [34–36]. The latter effect may be very important because resolution of inflammation is vital in shutting off the ongoing inflammatory process and in limiting tissue damage.

3 Anti-inflammatory effects of marine *n*-3 fatty acids

The forgoing discussion has highlighted the ability of marine *n*-3 PUFAs to decrease production of pro-inflammatory eicosanoids from arachidonic acid and to enhance the production of weak eicosanoids from EPA and potent anti-inflammatory and inflammation resolving resolvins and protectins from EPA and DHA. The altered milieu of lipid mediators that results can have a multitude of downstream effects on inflammatory processes. For example LTB_4, derived from arachidonic acid, is a potent leukocyte chemoattractant and so a marine *n*-3 PUFA-induced reduction in LTB_4 has the potential to decrease the attraction of leukocytes to inflammatory loci. Marine *n*-3 PUFAs may also decrease expression of receptors for chemoattractants on leukocytes. Several trials have reported that marine *n*-3 PUFAs can decrease chemotaxis of human neutrophils and monocytes towards various chemoattractants including LTB_4, bacterial peptides and human serum [11–13, 37–39]. Both the distance of cell migration and the number of cells migrating were decreased.

Adhesion molecule expression on the surface of endothelial cells and leukocytes is promoted by several transcription factors including the prototypical pro-inflammatory transcription factor, nuclear factor kappa B (NFκB) [40, 41]. A number of cell culture studies have reported that both EPA and DHA decrease activation of NFκB in response to inflammatory stimuli like lipopolysaccharide (LPS) and inflammatory cytokines [42–45]. Through this mechanism marine *n*-3 PUFAs could decrease adhesion molecule expression and reduce the leukocyte-endothelial adhesive interaction. Indeed, cell culture [46–49] and animal feeding studies [50] have reported decreased expression of some adhesion molecules on the surface of monocytes [49], macrophages [50] or endothelial cells [46–48] following exposure to marine *n*-3 PUFAs. This has been shown to result in decreased adhesion between leukocytes and endothelial cells [46, 51, 52]. Supplementing the diet of healthy humans with 1.5 g EPA + DHA/day resulted in a lower level of expression of ICAM-1 on the surface of blood monocytes stimulated ex vivo with interferon-γ [53]. Another study showed that 1.1 g EPA + DHA/day decreased circulating levels of soluble VCAM-1 in elderly subjects [54], but it is not clear if this represents decreased surface expression of VCAM-1.

Activated NFκB induces inflammatory cytokine gene expression. In accordance with decreased NFκB activation following exposure to EPA and DHA, these fatty acids are seen to decrease LPS-stimulated production of IL-6 and IL-8 by cultured human endothelial cells [46, 55] and EPA decreased LPS-induced tumour necrosis factor (TNF)-α production by cultured monocytes [42–44]. Feeding marine *n*-3 PUFAs to mice decreased ex vivo production of TNF-α, IL-1β and IL-6 by LPS-stimulated macrophages [56–58]. Several studies in healthy human volunteers involving supplementation of the diet with marine *n*-3 PUFAs have demonstrated decreased production of TNF-α, IL-1β, IL-6 and various growth factors by LPS-stimulated monocytes or mononuclear cells (a mixture of lymphocytes and monocytes) [12, 14, 30, 59, 60].

4 The anti-inflammatory effects of marine *n*-3 PUFAs may be anti-atherogenic

As outlined above, marine *n*-3 PUFAs act in a number of ways to decrease inflammation. They decrease production of eicosanoid mediators from arachidonic acid, many of which have pro-inflammatory roles; increase production of weakly inflammatory or anti-inflammatory eicosanoids from EPA; increase production of anti-inflammatory and inflammation resolving resolvins from EPA and DHA; decrease chemotactic responses of leukocytes; decrease adhesion molecule

expression on leukocytes and on endothelial cells and decrease intercellular adhesive interactions; and decrease production of pro-inflammatory cytokines and other pro-inflammatory proteins induced via the NFκB system. Together, these anti-inflammatory actions may contribute to anti-atherogenic effects of marine *n*-3 PUFAs, since, as shown in Figs. 1 and 2, initiation and progression of that atherosclerotic plaque involve leukocyte chemotaxis, intercellular adhesive interactions and production of inflammatory mediators including cytokines.

5 Marine *n*-3 fatty acids and atherosclerotic plaque stability

Marine *n*-3 PUFAs have been demonstrated to reduce the risks of cardiovascular events and mortality [61–66], including sudden cardiac death [63, 67]. The anti-inflammatory actions of marine *n*-3 PUFAs may play a key role in these effects. Plaque rupture is an acute occurrence that exposes the plaque contents to the highly pro-thrombotic environment of the vessel lumen [4, 5, 68, 69] (Fig. 3), so, initiating thrombosis that may lead to myocardial infarction, stroke or other vascular event. Ruptures are more likely to occur where the fibrous cap is thin and partly degraded. Inflammatory cells (macrophages, T cells, mast cells) are typically abundant at such sites, and these cells produce a range of mediators and enzymes that can thin and weaken the cap making the plaque vulnerable and unstable. Certain matrix metalloproteinases (MMPs) and cysteine proteases appear to play important roles in degradation, thinning and weakening of the fibrous cap. Thus, plaque rupture is essentially, an inflammatory event, and the characteristics of an atherosclerotic plaque that make it vulnerable to rupture are a thin fibrous cap and increased numbers of inflammatory cells such as macrophages [4, 5, 68, 69]. Marine *n*-3 fatty acids might act to stabilise atherosclerotic plaques by decreasing infiltration of inflammatory and immune cells (e.g. monocyte/macrophages and lymphocytes) into the plaques and/or by decreasing the activity of those cells once in the plaque. An intervention study conducted in patients awaiting carotid endarterectomy showed that marine *n*-3 fatty acids are incorporated from dietary fish oil supplements (providing 1.4 g EPA + DHA/day) into advanced atherosclerotic plaques and that this incorporation is associated with structural changes consistent with increased plaque stability [70]. The morphology of carotid plaque sections was characterised according to the American Heart Association classification [69]. Plaques from patients treated with fish oil were more likely to be type IV (fibrous cap atheromas: well-formed necrotic core with an overlaying thick fibrous cap) than those from the placebo group (odds ratio 1.19). Conversely, plaques from patients treated with fish oil were less likely to be type V (thin fibrous cap atheromas – thin fibrous cap infiltrated by macrophages and lymphocytes) than those from the placebo group (odds ratio 0.52). Thus, there were more plaques with a well-formed fibrous cap, rather than a thin-inflamed cap, in the fish oil group. Infiltration by macrophages was investigated using immunohistochemistry. It was found that plaques from patients given fish oil were more likely to be less heavily infiltrated with macrophages than those in the placebo group. A follow-up study, termed OCEAN (Omacor Carotid EnArterectomy iNtervention), using 1.8 g EPA + DHA/day as ethyl esters confirmed the higher EPA content of carotid plaque phospholipids in patients receiving marine *n*-3 fatty acids and the association between a higher EPA content of the plaque and lower plaque inflammation and instability [71]. OCEAN found that mRNA levels for MMP-7, MMP-9 and MMP-12 were lower in plaques from patients who had received marine *n*-3 fatty acids. These two studies suggest that marine *n*-3 fatty acids are incorporated quite quickly into advanced plaques and that plaques with a higher EPA content are more stable.

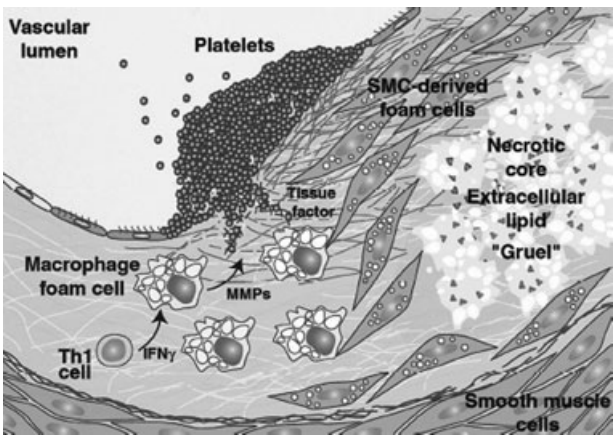

Figure 3. Plaque rupture and thrombosis. Necrosis of macrophages and SMC-derived foam cells leads to formation of a necrotic core and accumulation of extracellular cholesterol. Macrophage secretion of MMPs weakens the fibrous cap. Plaque rupture exposes blood components to tissue factor initiating coagulation, recruitment of platelets and thrombus formation. Reprinted from [1], Copyright (2001), with permission from Elsevier.

The findings of the two human studies have been confirmed in an animal study [72]. Apolipoprotein E deficient or low-density lipoprotein-receptor deficient mice were fed a Western-type diet or the same diet plus EPA for 12 weeks. EPA reduced aortic lipid deposition, consistent with earlier animal studies. EPA resulted in increased plaque collagen and decreased macrophage numbers in the plaque. The authors concluded that EPA had stabilised the atherosclerotic lesions. In separate in vitro studies, EPA attenuated cytokine-induced expression of adhesion molecules by endothelial cells, again confirming earlier studies, and attenuated cytokine-induced expression of MMP-2 and MMP-9 by a macrophage cell line [72]. There was a tendency for dietary EPA to decrease aorta MMP-2 and MMP-9 in apolipoprotein E deficient mice.

Since it is the vulnerability of the plaque to rupture rather than the degree of atherosclerosis, which is the primary determinant of thrombosis-mediated acute cardiovascular events, it is likely that the findings of Thies et al. [70] and OCEAN [71], replicated in an animal model [65], are clinically relevant. This might explain the significant protective effects of marine *n*-3 fatty acids towards both fatal and non-fatal cardiovascular events [61–67], which are so far not fully explained [73, 74].

6 Summary and conclusions

Atherosclerosis has an important inflammatory component and acute cardiovascular events can be precipitated by inflammatory events occurring in advanced plaques. Fatty acids influence inflammation through a variety of mechanisms; many of these are mediated by, or at least associated with, changes in fatty acid composition of cell membranes. Changes in these compositions can modify membrane fluidity, cell signalling leading to altered gene expression and the pattern of lipid mediator production. Human inflammatory cells are typically rich in the *n*-6 fatty acid arachidonic acid, but the contents of arachidonic acid and of the *n*-3 fatty acids EPA and DHA can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid have roles in inflammation. EPA also gives rise to eicosanoids and these are usually biologically weaker than arachidonic acid-derived eicosanoids. EPA and DHA give rise to resolvins which are anti-inflammatory and inflammation resolving. Increased membrane content of EPA and DHA (and decreased arachidonic acid content) results in a changed pattern of production of eicosanoids and probably also of resolvins, although the latter are not well examined in the human context. Changing the fatty acid composition of inflammatory cells also affects production of peptide mediators of inflammation (adhesion molecules, cytokines, etc.). Thus, the fatty acid composition of human inflammatory cells influences their function; the contents of arachidonic acid, EPA and DHA appear to be especially important. The anti-inflammatory effects of marine *n*-3 PUFAs may contribute to their protective actions towards atherosclerosis, plaque rupture and cardiovascular mortality. However, at this stage it is not possible to say which is the most clinically relevant anti-inflammatory effect of marine *n*-3 PUFAs because this is likely to depend upon the stage of the disease process. For example, it would seem that preventing monocyte infiltration into the early lesion would be very important in slowing atherosclerosis and so in preventing the disease. On the other hand, in advanced unstable plaques, reducing the activity of resident inflammatory macrophages and foam cells is likely to be important because it is these cells that weaken the plaque making it more vulnerable to rupture.

The author serves on the Danone Scientific Advisory Board on Immunity and the Scientific Advisory Board of Aker Biomarine; acts as a consultant to the Danone Research Centre for Specialised Nutrition; in the past 5 years has acted as a consultant to Mead Johnson Nutritionals, Vifor Pharma and Amarin Corporation; has received speaking honoraria from Solvay Healthcare, Solvay Pharmaceuticals, Pronova Biocare, Fresenius Kabi, B. Braun, Abbott Nutrition, Baxter Healthcare and Nestle; currently has research funding from Vifor Pharma and Abbott Nutrition; is elected President of the International Society for the Study of Fatty Acids and Lipids, an organisation that is partly supported by corporate membership fees, mainly the food and supplements industries; serves on the Council of the British Nutrition Foundation, on the Board of Directors of the European Neutraceutical Association and on the Board of Directors of ILSI Europe, all organisations that are partly funded by the food and supplements industries.

The author has declared no conflict of interest.

7 References

[1] Glass, C. K., Witztum, J. L., Atherosclerosis: the road ahead. *Cell* 2001, *104*, 503–516.

[2] Ross, R., Mechanisms of disease – atherosclerosis – an inflammatory disease. *N. Eng. J. Med.* 1999, *340*, 115–126.

[3] Blake, G. J., Ridker, P. M., Novel clinical markers of vascular wall inflammation. *Circ. Res.* 2001, *89*, 763–771.

[4] Hansson, G. K., Inflammation, atherosclerosis, and coronary heart disease. *N. Eng. J. Med.* 2005, *352*, 1685–1695.

[5] Hallenbeck, J. M., Hansson, G. K., Becker, K. J. Immunology of ischemic vascular disease: plaque to attack. *Trends Immunol.* 2005, *26*, 550–556.

[6] Ridker, P. M., Buring, J. E., Rifai, N., Soluble P-selectin and the risk of future cardiovascular events. *Circulation* 2001, *103*, 491–495

[7] Ridker, P. M., Hennekens, C. H., Roitman-Johnson, B., Stampfer, M. J. et al., Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 1998, *351*, 88–92.

[8] Hwang, S. J., Ballantyne, C. M., Sharrett, A. R., Smith, L. C. et al., Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 1997, *96*, 4219–4225.

[9] Ridker, P. M., Rifai, N., Stampfer, M. J., Hennekens, C. H., Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000, *101*, 1767–1772.

[10] Calder, P. C., Burdge, G. C., in: Nicolaou, A., Kokotos, G. (Eds.), *Bioactive Lipids* The Oily Press, Bridgewater 2004, pp. 1–36.

[11] Lee, T. H., Hoover, R. L., Williams, J. D., Sperling, R. I. et al., Effects of dietary enrichment with eicosapentaenoic acid and docosahexaenoic acid on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Eng. J. Med.* 1985, *312*, 1217–1224.

[12] Endres, S., Ghorbani, R., Kelley, V. E., Georgilis, K. et al., 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. Eng. J. Med.* 1989, *320*, 265–271.

[13] Sperling, R. I., Benincaso, A. I., Knoell, C. T., Larkin, J. K. et al., Dietary ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J. Clin. Invest.* 1993, *91*, 651–660.

[14] Gibney, M. J., Hunter, B., The effects of short- and long-term supplementation with fish oil on the incorporation of *n*-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. *Eur. J. Clin. Nutr.* 1993, *47*, 255–259.

[15] Caughey, G. E., Mantzioris, E., Gibson, R. A., Cleland, L. G. et al., The effect on human tumor necrosis factor α and interleukin 1β production of diets enriched in *n*-3 fatty acids from vegetable oil or fish oil. *Am. J. Clin. Nutr.* 1996, *63*, 116–122.

[16] Yaqoob, P., Pala, H. S., Cortina-Borja, M., Newsholme, E. A. et al., Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *Eur. J. Clin. Invest.* 2000, *30*, 260–274.

[17] Healy, D. A., Wallace, F. A., Miles, E. A., Calder, P. C. et al., The effect of low to moderate amounts of dietary fish oil on neutrophil lipid composition and function. *Lipids* 2000, *35*, 763–768.

[18] Thies, F., Nebe-von-Caron, G., Powell, J. R., Yaqoob, P. et al., Dietary supplementation with γ-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *J. Nutr.* 2001, *131*, 1918–1927.

[19] Kew, S., Banerjee, T., Minihane, A. M., Finnegan, Y. E. et al., Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. *Am. J. Clin. Nutr.* 2003, *77*, 1278–1286.

[20] Miles, E. A., Banerjee, T., Calder, P. C., The influence of different combinations of gamma-linolenic, stearidonic and eicosapentaenoic acids on the fatty acid composition of blood lipids and mononuclear cells in human volunteers. *Prost. Leuk. Essent. Fatty Acids* 2004, *70*, 529–538.

[21] Kew, S., Mesa, M. D., Tricon, S., Buckley, R. et al., Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *Am. J. Clin. Nutr.* 2004, *79*, 674–681.

[22] Rees, D., Miles, E. A., Banerjee, T., Wells, S. J. et al., Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am. J. Clin. Nutr.* 2006, *83*, 331–342.

[23] Lewis, R. A., Austen, K. F., Soberman, R. J., Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human diseases. *N. Eng. J. Med.* 1990, *323*, 645–655.

[24] Tilley, S. L., Coffman, T. M., Koller, B. H., Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J. Clin. Invest.* 2001, *108*, 15–23.

[25] Calder, P. C., Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie* 2009, *91*, 791–795.

[26] Faber, J., Berkhout, M., Vos, A. P., Sijben, J. W. et al., Supplementation with a fish oil-enriched, high-protein medical food leads to rapid incorporation of EPA into white blood cells and modulates immune responses within one week in healthy men and women. *J. Nutr.* 2011, *141*, 964–970.

[27] Calder, P. C., N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.* 2006, *83*, 1505S–1519S.

[28] Calder, P. C., The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins Leukot. Essent. Fatty Acids* 2008, *79*, 101–108.

[29] Calder, P. C., Fatty acids and inflammation: the cutting edge between food and pharma. *Eur. J. Pharmacol.* 2011, *618*, S50–S58.

[30] Meydani, S. N., Endres, S., Woods, M. M., Goldin, B. R. et al., Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J. Nutr.* 1991, *121*, 547–555.

[31] Von Schacky, C., Kiefl, R., Jendraschak, E., Kaminski, W. E., N-3 fatty acids and cysteinyl-leukotriene formation in humans in vitro, ex vivo and in vivo. *J. Lab. Clin. Med.* 1993, *121*, 302–309.

[32] Wada, M., DeLong, C. J., Hong, Y. H., Rieke, C. J. et al., Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. *J. Biol. Chem.* 2007, *282*, 22254–22266.

[33] Tull, S. P., Yates, C. M., Maskrey, B. H., O'Donnell, V. B. et al., Omega-3 fatty acids and inflammation: novel interactions reveal a new step in neutrophil recruitment. *PLoS Biol.* 2009, *7*, e1000177.

[34] Serhan, C. N., Clish, C. B., Brannon, J., Colgan, S. P. et al., Novel functional sets of lipid-derived mediators with anti-inflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal anti-inflammatory drugs and transcellular processing. *J. Exp. Med.* 2000, *192*, 1197–1204.

[35] Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P. et al., Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. *J. Exp. Med.* 2002, *196*, 1025–1037.

[36] Serhan, C. N., Chiang, N., van Dyke, T. E., Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 2008, *8*, 349–361.

[37] Schmidt, E. B., Pedersen, J. O., Ekelund, S., Grunnet, N. et al., Cod liver oil inhibits neutrophil and monocyte chemotaxis in healthy males. *Atherosclerosis* 1989, *77*, 53–57.

[38] Schmidt, E. B., Varming, K., Pedersen, J. O., Lervang, H. H. et al., Long term supplementation with *n*-3 fatty acids. ii. Effect on neutrophil and monocyte chemotaxis. *Scand. J. Clin. Lab. Invest.* 1992, *52*, 229–236.

[39] Luostarinen, R., Siegbahn, A., Saldeen, T., Effect of dietary fish oil supplemented with different doses of vitamin E on

neutrophil chemotaxis in healthy volunteers. *Nutr. Res.* 1992, *12*, 1419–1430.

[40] Kumar, A., Takada, Y., Boriek, A. M., Aggarwal, B. B., Nuclear factor-kappaB: its role in health and disease. *J. Mol. Med.* 2004, *82*, 434–448.

[41] Sigal, L. H., Basic science for the clinician 39: NF-kappaB-function, activation, control, and consequences. *J. Clin. Rheumatol.* 2006, *12*, 207–211.

[42] Lo, C. J., Chiu, K. C., Fu, M., Lo, R., Helton, S., Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NFκB activity. *J. Surg. Res.* 1999, *82*, 216–222.

[43] Babcock, T. A., Novak, T., Ong, E., Jho, D. H. et al., Modulation of lipopolysaccharide-stimulated macrophage tumor necrosis factor-α production by ω-3 fatty acid is associated with differential cyclooxygenase-2 protein expression and is independent of interleukin-10. *J. Surg. Res.* 2002, *107*, 135-139.

[44] Novak, T. E., Babcock, T. A., Jho, D. H., Helton, W. S. et al., NF-κB inhibition by ω-3 fatty acids modulates LPS-stimulated macrophage TNF-α transcription. *Am. J. Physiol.* 2003, *284*, L84–L89.

[45] Zhao, Y., Joshi-Barve, S., Barve, S., Chen, L. H., Eicosapentaenoic acid prevents LPS-induced TNF-α expression by preventing NF-κB activation. *J. Am. Coll. Nutr.* 2004, *23*, 71–78.

[46] De Caterina, R., Cybulsky, M. I., Clinton, S. K., Gimbrone, M. A. et al., The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler. Thromb.* 1994, *14*, 1829–1836.

[47] Weber, C., Erl, W., Pietsch, A., Danesch, U. et al., Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic cell adhesion to human endothelilal cells stimulated by tumor necrosis factor-α. *Atheroscler. Thromb. Vasc. Biol.* 1995, *15*, 622–628.

[48] Collie-Duguid, E. S., Wahle, K. W., Inhibitory effect of fish oil *n*-3 polyunsaturated fatty acids on the expression of endothelial cell adhesion molecules. *Biochem. Biophys. Res. Commun.* 1996, *220*, 969–974.

[49] Hughes, D. A., Southon, S., Pinder, A. C., (n-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes in vitro. *J. Nutr.* 1996, *126*, 603–610.

[50] Miles, E. A., Wallace, F. A., Calder, P. C., Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis* 2000, *152*, 43–50.

[51] De Caterina, R., Libby, P., Control of endothelial leukocyte adhesion molecules by fatty acids. *Lipids* 1996, *31*, S57–S63.

[52] Yates, C. M., Tull, S. P., Madden, J., Calder, P. C. et al., Docosahexaenoic acid inhibits the adhesion of flowing neutrophils to cytokine stimulated human umbilical vein endothelial cells. *J. Nutr.* 2011, *141*, 1331–1334.

[53] Hughes, D. A., Pinder, A. C., Piper, Z., Johnson, I. T. et al., Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. *Am. J. Clin. Nutr.* 1996, *63*, 267–272.

[54] Miles, E. A., Thies, F., Wallace, F. A., Powell, J. R. et al., Influence of age and dietary fish oil on plasma soluble adhesion molecule concentrations. *Clin. Sci.* 2001, *100*, 91–100.

[55] Khalfoun, B., Thibault, F., Watier, H., Bardos, P. et al., Docosahexaenoic and eicosapentaenoic acids inhibit in vitro human endothelial cell production of interleukin-6. *Adv. Exp. Biol. Med.* 1997, *400*, 589–597.

[56] Billiar, T. R., Bankey, P. E., Svingen, B. A. Curran, R. D. et al., Fatty acid uptake and Kupffer Cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* 1988, *104*, 343–349.

[57] Renier, G., Skamene, E., de Sanctis, J., Radzioch, D., Dietary *n*-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice: modulation of macrophage secretory activities. *Arterioscler. Thomb.* 1993, *13*, 1515–1524.

[58] Yaqoob, P., Calder, P. C., Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cell. Immunol.* 1995, *163*, 120–128.

[59] Baumann, K. H., Hessel, F., Larass, I., Muller, T. et al., Dietary ω-3, ω-6, and ω-9 unsaturated fatty acids and growth factor and cytokine gene expression in unstimulated and stimulated monocytes. *Arterioscler. Thromb. Vasc. Biol.* 1999, *19*, 59–66.

[60] Trebble, T., Arden, N. K., Stroud, M. A., Wootton, S. A. et al., Inhibition of tumour necrosis factor-α and interleukin-6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Brit. J. Nutr.* 2003, *90*, 405–412.

[61] Burr, M. L., Gilbert, J. F., Holliday, R. M., Elwood, P. C. et al., Effects of changes in fat, fish and fibre intake on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989, *ii*, 757–761.

[62] Siscovick, D. S., Raghunathan, T. E., King, I., Weinmann, S. et al., Dietary intake and cell membrane levels of long-chain *n*-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *J. Am. Med. Assoc.* 1995, *274*, 1363–1367.

[63] Anonymous. Dietary supplementation with *n*-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999, *354*, 447–455.

[64] Hu, F. B., Bronner, L., Willett, W. C., Stampfer, M. J. et al., Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *J. Am. Med. Assoc.* 2002, *287*, 1815–1821

[65] Lemaitre, R. N., King, I. B., Mozaffarian, D., Kuller, L. H. et al., n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. *Am. J. Clin. Nutr.* 2003, *77*, 319–325.

[66] Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y. et al., Japan EPA lipid intervention study (JELIS) investigators. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS):

a randomised open-label, blinded endpoint analysis. *Lancet* 2007, *369*, 1090–1098.

[67] Albert, C. M., Campos, H., Stampfer, M. J., Ridker, P. M. et al., Blood levels of long-chain *n*-3 fatty acids and the risk of sudden death. *N. Eng. J. Med.* 2002, *346*, 1113–1118.

[68] Plutzky, J., Atherosclerotic plaque rupture: emerging insights and opportunities. *Am. J. Cardiol.* 1999, *84*, 15J–20J.

[69] Stary, H. C., Chander, A. B., Dinsmore, R. E., The definition of advanced type of atherosclerotic lesions and a histological classification of atherosclerosis. *Circulation* 1995, *92*, 1355–1374.

[70] Thies, F., Garry, J. M. C., Yaqoob, P., Rerkasem, K. et al., Association of *n*-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003, *361*, 477–485.

[71] Cawood, A. L., Ding, R., Napper, F. L., Young, R. H. et al., Eicosapentaenoic acid (EPA) from highly concentrated *n*-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. *Atherosclerosis* 2010, *212*, 252–259.

[72] Matsumoto, M., Sata, M., Fukuda, D., Tanaka, K. et al., Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. *Atherosclerosis* 2008, *197*, 524–533.

[73] Calder, P. C., n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci.* 2004, *107*, 1–11.

[74] Saravanan, P., Davidson, N. C., Schmidt, E. B., Calder, P. C., Cardiovascular effects of marine omega-3 fatty acids. *Lancet* 2010, *376*, 540–550.

