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The effects of dietary *n*-3 polyunsaturated fatty acids on neutrophils

Richard I. Sperling*

Department of Medicine, Harvard Medical School, Division of Rheumatology and Immunology, Brigham & Women's Hospital, Boston, MA 02115, USA

The present review will focus on the effects of dietary *n*-3 polyunsaturated fatty acids (PUFA) on the cellular function and leukotriene (LT) synthesis of human neutrophils. Dietary marine fish oils rich in *n*-3 PUFA have been shown to have an anti-inflammatory effect in patients with active rheumatoid arthritis (RA; Kremer *et al.* 1987, 1990; Sperling *et al.* 1987; Cleland *et al.* 1988; van der Tempel *et al.* 1990). This anti-inflammatory effect appears to be primarily due to the inhibitory effects of eicosapentaenoic acid (20 : 5*n*-3; EPA) on the formation of the lipid mediators of inflammation, which are derived predominantly from cellular phospholipids, and on the cellular function of inflammatory cells. The major lipid mediators of inflammation are the arachidonic acid (20 : 4*n*-6; AA) metabolites, the LT thromboxane A_2 and the prostaglandins. Various inflammatory stimuli can activate cells to produce these mediators of inflammation. On cell activation (Fig. 1) phospholipase A_2 (*EC* 3.1.1.4) is activated, which then catalyses the hydrolysis of PUFA from the 2-position of phospholipids yielding 2-lyso-phospholipids and the non-esterified PUFA. The

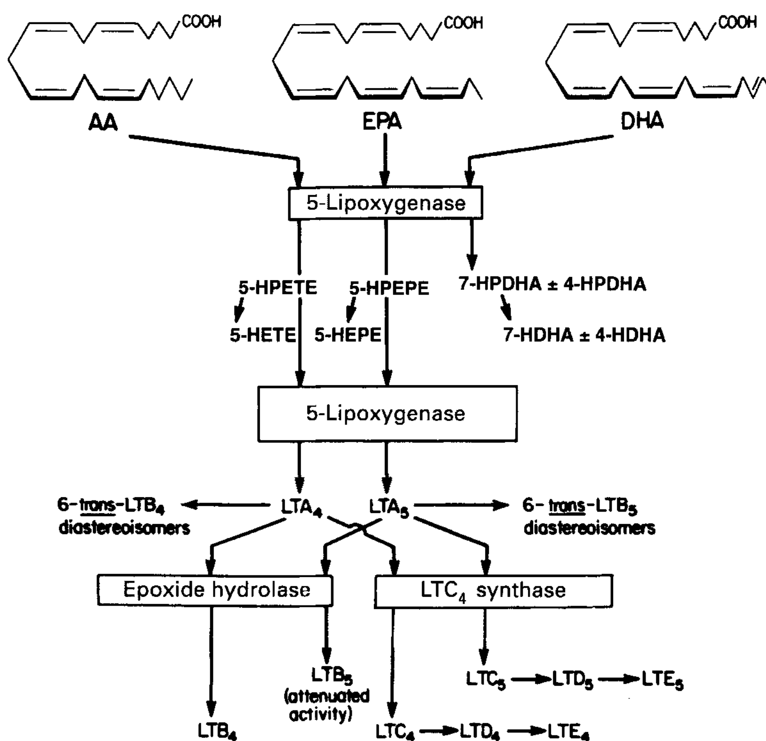

Fig. 1. Oxidative metabolism of polyunsaturated fatty acids arachidonic acid (20 : 4*n*-6; AA), eicosapentaenoic acid (20 : 5*n*-3; EPA) and docosahexaenoic acid (22 : 6*n*-3; DHA) by the 5-lipoxygenase (*EC* 1.13.11.34) pathway. 5-HPETE, 5-*S*-hydroperoxyeicosatetraenoic acid; 5-HETE, 5-*S*-hydroxyeicosatetraenoic acid; 5-HPEPE, 5-*S*-hydroperoxyeicosapentaenoic acid; 5-HEPE, 5-*S*-hydroxyeicosapentaenoic acid; 7-HPDHA, 7-*S*-hydroperoxydocosahexaenoic acid; 4-HPDHA, 4-*S*-hydroperoxydocosahexaenoic acid; 7-HDHA, 7-*S*-hydroxydocosahexaenoic acid; 4-HDHA, 4-*S*-hydroxydocosahexaenoic acid; LT, leukotriene. (Adapted from Lee *et al.* 1985.)

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FMLP, N-formyl-methionyl-leucyl-phenylalanine; 5-HEPE, 5-S-hydroxy-*trans*-8,11,14-*cis*-eicosapentaenoic acid; 5-HETE, 5-S-hydroxy-*trans*-8,11,14-*cis*-eicosatetraenoic acid; 5-HPEPE, 5-S-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosapentaenoic acid; 5-HPETE, 5-S-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid; HPDHA, hydroperoxy-docosahexaenoic acid; IP_3, inositol-1,4,5-triphosphate; LT, leukotriene; PAF, platelet-activating factor; PLC, phospholipase C; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis.

Corresponding author: Dr R. I. Sperling, fax +1 508 822 7975

***Present address:** Health Associates of Taunton, Cutter Place, 152 Dean Street, Taunton, MA 02780, USA.

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non-esterified PUFA may serve as substrates for the cyclooxygenase (*EC* 1.14.99.1) and 5-lipoxygenase (*EC* 1.13.11.34) pathways. AA, the major substrate for both the 5-lipoxygenase and cyclooxygenase pathways in human subjects on a typical Western diet, is derived from *n*-6 PUFA of land-based animals and plants. EPA and docosahexaenoic acid (22 : 6*n*-3; DHA) are the major fatty acids in marine organisms, found primarily in the form of esterified triacylglycerols. The well-established efficacy of the non-steroidal anti-inflammatory drugs in RA underscores the importance of the proinflammatory cyclooxygenase-pathway products in the pathophysiology of this disease. Studies of the synovial fluid from patients with active RA have demonstrated the presence of biologically-significant concentrations of LTB_4 (5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; Klickstein *et al.* 1980; Borenstein & Vanderhoek, 1987) and 5-*S*-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HETE; Klickstein *et al.* 1980). LTB_4 is not only one of the most potent chemotaxins for neutrophils and monocytes (Goetzl & Pickett, 1980; Palmer *et al.* 1980), but also induces the release of lysosomal enzymes, the generation of highly-reactive reduced oxygen species, and also the aggregation of human neutrophil and monocytes (Yasaka *et al.* 1982). 5-HETE is a weak, partial agonist at the LTB_4 receptor. These inflammatory mediators are believed to play a significant role in mediating the symptoms and joint destruction seen in RA (Robinson, 1988). The effects of the *n*-3 PUFA on leucocytes from healthy volunteers and patients with several defined inflammatory disorders has been evaluated. The clinical effects of dietary *n*-3 PUFA on disease activity in RA has been studied in a number of small clinical trials (Kremer *et al.* 1985, 1987, 1990; Sperling *et al.* 1987; Cleland *et al.* 1988; Tulleken *et al.* 1990; van der Tempel *et al.* 1990; Kjeldsen-Kragh *et al.* 1992; Nielsen *et al.* 1992; LG Darlington, personal communication).

Pathway of the synthesis of leukotrienes

Dietary PUFA, including the *n*-3 PUFA, are incorporated into membrane phospholipids, predominantly on the 2-position. On activation of phospholipase A_2, non-esterified fatty acids, primarily long-chain PUFA, and 2-lysophospholipids are released. Although AA and EPA are readily de-esterified from phospholipids, the hydrolysis of DHA-containing phospholipids by phospholipase A_2 is disproportionately less.

AA is initially metabolized by 5-lipoxygenase to its 5-*S*-hydroperoxy derivative, 5-*S*-hydroperoxy-6-*trans*-8,11, 14-*cis*-eicosatetraenoic acid (5-HPETE). Similarly, EPA and DHA are metabolized to their corresponding hydroperoxy derivatives, 5-*S*-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosapentaenoic acid (5-HPEPE) and the corresponding 4- and 7-*S*-hydroperoxy-docosahexaenoic acids (HPDHA) respectively. DHA, however, is oxidatively metabolized by 5-lipoxygenase to a much lesser extent than either AA or EPA. These unstable hydroperoxy-fatty acid intermediates may, at least in part, be reduced to the corresponding alcohols known as 5-HETE, 5-HEPE, and 4- and 7-HDHA. This reduction is the final step in the metabolism of DHA by the 5-lipoxygenase pathway. 5-HPETE, derived from AA, and 5-HPEPE, derived from EPA, may also be metabolized further by the hydroperoxy-fatty acid dehydrase activity of 5-lipoxygenase to the respective 5,6-epoxides known as LTA_4 (5,6-*trans*-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid), and the corresponding LTA_5, derived from EPA (Rouzer *et al.* 1986).

The LTA epoxide LT are unstable in aqueous environments. The non-enzymic hydrolysis of AA-derived LTA_4 mainly generates two minimally-bioactive diastereoisomeric dihydroxy-derivatives (5*S*, 12*R*- and 5*S*, 12*S*- dihydroxy-6,8, 10-*trans*-14-*cis*-eicosatetraenoic acids) known as the 6-*trans*-LTB_4 diastereoisomers. The epoxide hydrolase (*EC* 3.3.2.3), a cytosolic enzyme, is found in some human cells such as neutrophil (Rådmark *et al.* 1984), monocyte, erythrocyte and pulmonary alveolar macrophage and has also been detected in blood plasma (Fitzpatrick *et al.* 1983, 1984). The action of the LTA epoxide hydrolase on LTA_4 generates the bioactive LTB_4 (Fels *et al.* 1982; Godard *et al.* 1983; Williams *et al.* 1984; Borgeat & Samuelsson, 1987). Analogously, the non-enzymic hydrolysis of LTA_5 generates the corresponding 6-*trans*-LTB_5 diastereoisomers, whereas the action of the LTA epoxide hydrolase on LTA_5 generates LTB_5 (Lee *et al.* 1984*a*,*b*; Prescott *et al.* 1985; Strasser *et al.* 1985). The EPA-derived products, 5-HPEPE, 5-HEPE, LTA_5, the 6-*trans*-LTB_5 diastereoisomers and LTB_5, differ structurally from the corresponding AA-derived products only by the presence of an additional *cis*-double bond in the 17-position; however, LTB_5, derived from EPA, possesses less than 10 % of the bioactivity of LTB_4 with respect to chemotaxis and lysosomal enzyme release (Lee *et al.* 1984*a*). In addition, recent evidence has indicated that the EPA-derived LTB_5 can partially inhibit LTB_4-mediated superoxide formation and chemotaxis (Kragballe *et al.* 1987). Thus, LTB_5 may actually act as a partial antagonist at the LTB_4 receptor, in addition to its effects as a weak, partial agonist. LTB_4 and LTB_5 are oxidatively degraded by the neutrophil to their respective 20-hydroxy derivatives by a unique cytochrome P450, referred to as $P450_{LTB}$ (Shak & Goldstein, 1985; Soberman *et al.* 1985). These minimally-bioactive metabolites may be further oxidized by either of two pathways leading to the formation of 20-carboxy-derivatives. However, 20-hydroxy-LTB_5 is metabolized to its 20-carboxy derivative to only a minimal extent. In the monocyte, eosinophil and mast cell, LTA_4 and LTA_5 may be hydrolysed by LTC_4 synthase to LTC_4 and LTC_5 respectively. Sequential cleavage of the glutathione side chain generates the biologically-active LT LTD_4 and LTE_4 from LTC_4, and the bioactive LTD_5 and LTE_5 from LTC_5 (Lee *et al.* 1985).

Effects of *n*-3 polyunsaturated fatty acids on the human 5-lipoxygenase pathway

In vitro *effects of* n-*3 polyunsaturated fatty acids*

In the initial study of the *in vitro* effects of exogenous PUFA on 5-lipoxygenase-pathway product generation (Lee *et al.* 1984*b*), neutrophils were isolated from healthy donors who consumed their usual Western diet, and were activated *ex vivo* with 10 μM-calcium ionophore A23187 for 5 min in the presence of exogenous quantities, 0–40 μg AA, EPA and

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DHA/l. The formation of 5-lipoxygenase-pathway products was quantified by integrated optical density and/or radio-immunoassay after the resolution of products by reverse-phase HPLC. In the absence of exogenous fatty acids, the only products of the 5-lipoxygenase pathway which were detected were the stable products derived from endogenous AA in the neutrophil: 5-HETE, the 6-*trans*-LTB_4 diastereo-isomers and LTB_4. The ω-oxidation products of LTB_4 were not clearly resolved by the HPLC programme and, therefore, were not quantified. The addition of concentrations of exogenous AA to the suspension of the neutrophils augmented the generation of each of these stable AA-derived 5-lipoxygenase products. In the presence of quantities of exogenous EPA, these same 5-lipoxygenase metabolites of AA, derived from cellular lipids, were demonstrated. In addition, small quantities of the analogous metabolites derived from exogenous EPA were detected. In the presence of exogenous EPA, there was a significant and selective reduction in the generation of LTB_4, thus indicating inhibition of the LTA epoxide hydrolase enzyme. Ford- Hutchinson and colleagues (Nathaniel *et al.* 1985) have demonstrated that LTA_5 covalently binds to the LTA epoxide hydrolase, thereby inactivating it. In the presence of exogenous docosahexaenoic acid, the only DHA-derived 5-lipoxygenase products demonstrated were the 4- and 7-hydroxy derivatives, and exogenous DHA did not affect significantly the metabolism of endogenous AA by this pathway.

The effects of dietary n-*3 polyunsaturated fatty acids on the 5-lipoxygenase pathway in healthy individuals*

Seven healthy, male volunteers daily supplemented their usual diet for 6 weeks with 18 g MaxEPA (R. P. Scherer, Troy, NY, USA) fish oil daily, providing 3·2 g EPA and 2·2 g DHA (Lee *et al.* 1985). Leucocytes were isolated and activated *ex vivo* with calcium ionophore in dose- and time-dependent protocols. 5-Lipoxygenase-pathway product generation was quantified by integrated optical density and/or radioimmunoassay after resolution of the products by reverse- phase HPLC. After 3 weeks of dietary fish oil, a 7-fold increase in the EPA content of neutrophil cellular lipids was observed. This change persisted after 6 weeks of dietary fish oil consumption. The changes in neutrophil cellular lipid composition after 6 weeks of the dietary fish oil supplementation were associated with a 60 % decrease in 5-HETE and 6-*trans*-LTB_4 generation, and a greater than 50 % decrease in LTB_4 generation. Only small quantities of the EPA-derived 5-HEPE and LTB_5 were detected after 6 weeks of dietary fish oil consumption. Despite comparable incorporation of EPA into neutrophil cellular lipids at the 3- and 6-week time points and comparable generation of EPA-derived LTB_5 and 5-HEPE, suppression of 5-lipoxygenase-pathway product formation was not observed after 3 weeks of dietary fish oil supplementation. The absence of an inhibitory effect of dietary *n*-3 PUFA on neutrophil 5-lipoxygenase-pathway product generation after the significant incorporation of *n*-3 PUFA into neutrophils which occurs after 3–4 weeks of dietary supplementation was also observed by Prescott *et al.* (1985) and Strasser *et al.* (1985). In their studies, after 3 and 4 weeks of dietary fish oil supplementation respectively, significant increases in neutrophil EPA contents and LTB_5 formation were observed; however, suppression of LTB_4 production was not demonstrated.

Terano *et al.* (1987) studied the effects of 4 weeks of dietary EPA ethyl ester and DHA ethyl ester, individually, on the function of neutrophils from healthy subjects. After 4 weeks of dietary EPA, small quantities of the EPA-derived 5-lipoxygenase-pathway products, 5-HEPE, the 6-*trans*-LTB_5 diastereoisomers and LTB_5, were detected. LTB_4 synthesis by calcium ionophore-activated neutrophils was inhibited by 34 %. No effects were observed on the synthesis of the other AA-derived 5-lipoxygenase-pathway products. In contrast, DHA did not affect neutrophil metabolism of AA by this pathway.

The effects of dietary n-*3 polyunsaturated fatty acids in patients with rheumatoid arthritis on leucocyte function and the 5-lipoxygenase pathway*

In the study by Sperling *et al.* (1987), twelve patients with active RA supplemented their diets for 6 weeks with 20 g MaxEPA fish oil daily. An 18-fold increase in EPA content and a concomitant 33 % decline in AA content was observed after 6 weeks of the fish oil supplementation. Neutrophils were activated *ex vivo* with calcium ionophore in a dose- and time-dependent protocol similar to that in the study of healthy individuals (Lee *et al.* 1985). A 50 % inhibition in LTB_4 generation and only small quantities of LTB_5 were observed after 6 weeks of dietary fish oil supplementation. Although a 50 % inhibition in LTB_4 generation was observed both in healthy volunteers and the patients with RA, the generation of 5-HETE was inhibited by approximately 60 % in the healthy volunteers after 6 weeks (Lee *et al.* 1985), but not in the RA patients (Sperling *et al.* 1987). Similar inhibition by dietary fish oil supplementation on LTB_4 generation by neutrophils of patients with RA were observed by other investigators who employed different study designs (Kremer *et al.* 1987; Cleland *et al.* 1988).

The effects of dietary *n*-3 polyunsaturated fatty acids on neutrophil function

The effects of dietary n-*3 polyunsaturated fatty acids on neutrophil function in healthy subjects*

In the study of healthy volunteers (Lee *et al.* 1985), neutrophil chemotaxis to LTB_4 was assessed *ex vivo* in Boyden microchambers. The 6 weeks of dietary fish oil consumption resulted in a 71% decrease in the maximal neutrophil chemotactic responsiveness to LTB_4, in addition to inhibiting 5-lipoxygenase-pathway product generation. Suppression of the LTB_4-mediated enhancement of neutrophil adherence to endothelial cell monolayers (Hoover *et al.* 1984) was also observed (Lee *et al.* 1985). Neither of these changes were observed at the 3-week evaluation. These findings suggest that dietary fish oil supplementation might be expected to decrease both the margination and diapedesis of neutrophils into an inflammatory site in response to LTB_4. Both the suppression of neutrophil chemotaxis and LTB_4-mediated enhancement of neutrophil–endothelial cell adhesion returned approximately to baseline levels 6 weeks

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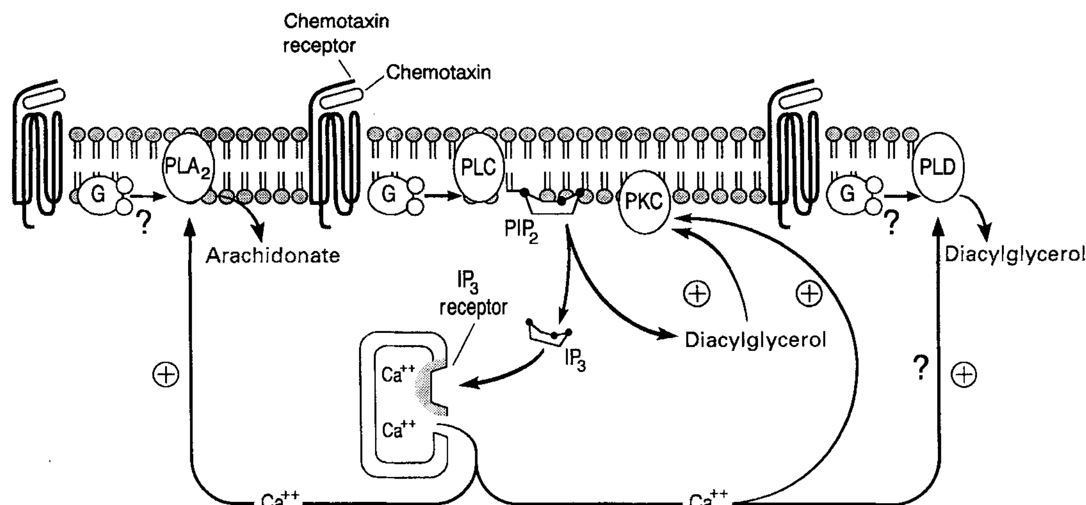

Fig. 2. Mechanisms of chemotaxin-induced activation of neutrophils. G, G-protein; IP_3, inositol-1,4,5-triphosphate; PKC, protein kinase C; PLA_2, phospholipase A_2 (*EC* 3.1.1.4); PLC, phospholipase C (*EC* 3.1.4.3); PLD, phospholipase D (*EC* 3.1.4.4); PIP_2, phosphatidylinositol-4, 5-bisphosphate. ⊕, Activation.

after discontinuing the dietary fish oil, further supporting a causal relationship to the dietary modification. The suppression of neutrophil chemotaxis in the healthy volunteers is difficult to explain, as there is no clear link between chemotaxis and AA metabolism, as measured *ex vivo*, and no changes in neutrophil membrane fluidity were detected by fluorescence polarization in cells treated with the fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene. The mechanism(s) by which dietary *n*-3 PUFA modulate transmembrane stimulation of neutrophils by chemotactic ligands were not addressed in this study.

In the study by Terano *et al.* (1987) of the effects of 4 weeks of dietary EPA ethyl ester and DHA ethyl ester, individually, on the function of neutrophils from healthy subjects, neutrophil chemotaxis to LTB_4 and the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) was assessed in Boyden microchambers. The authors observed a significant suppression of neutrophil chemotaxis to both agonists after 4 weeks of dietary EPA ethyl ester, supporting the findings of the previous study (Lee *et al.* 1985). DHA demonstrated a much less potent effect on neutrophil chemotaxis. The authors attributed the effect of DHA on neutrophil chemotactic responsiveness to a rise in the neutrophil cellular EPA content, observed after 4 weeks of DHA ethyl ester consumption (Terano *et al.* 1987).

Early events in the signal transduction after the binding of the neutrophil chemotactic ligands LTB_4, platelet-activating factor (PAF) and FMLP to their respective receptors are believed to involve the activation of a G-protein which then activates an associated phosphatidylinositol-selective phospholipase C (*EC* 3.1.4.3; PLC; Fig. 2). The activated PLC hydrolyses phosphatidylinositol-4,5-bisphosphate, resulting in the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerols. IP_3 binds to specific receptors on the endoplasmic reticulum, resulting in the release of intracellular stores of Ca and activation of phospholipases A_2 and D (*EC* 3.1.4.4). It is also possible that the receptor–ligand complex may activate phospholipase D and/or phospholipase A_2 directly through a G-protein. The diacylglycerols, in conjunction with the increased cytoplasmic Ca^{2+} concentration, activate protein kinase C. The rise in intracellular Ca^{2+} concentration and the activity of protein kinase C lead to activation of other specific cellular pathways, including the increased polarization of leucocytes, the assembly of elements of the cytoskeleton and cell contraction. It is possible that dietary fish oil supplementation affects any of these steps, resulting in inhibition of chemotaxis.

Sperling *et al.* (1993*a*) investigated the effects of 10 weeks of dietary *n*-3 PUFA supplementation with 20 g Super-EPA (Phenmacaps, Elizabeth, NJ, USA) daily, providing 9 g EPA and 5 g DHA in eight healthy volunteers, three men and five women, aged 25–59 years. Neutrophils and monocytes were isolated for biochemical investigations 4 weeks before starting the dietary supplementation and after 3 and 10 weeks of dietary *n*-3 PUFA supplementation. Increases in the EPA contents of all the major phospholipid classes and subclasses of neutrophils were observed after 3 and 10 weeks of dietary *n*-3 PUFA supplementation. There were no significant differences in the fatty acid contents of neutrophil phospholipids observed between the 3 and 10 week time points. Although the change in the EPA content of the phosphatidylinositol pool, of approximately 2 mol/100 mol, was much smaller than that observed in the other phospholipids, this change was nevertheless highly significant ($P < 0{\cdot}0001$). The AA contents of the major phospholipid classes and subclasses of neutrophils, with the exception of the phosphatidylinositol pool, declined substantially after 3 and 10 weeks of dietary *n*-3 PUFA supplementation. The LTB_4 and PAF receptor affinity and number of neutrophils did not change significantly with dietary *n*-3 PUFA supplementation.

The generation of inositol phosphates by neutrophils activated with 1 μM-LTB_4 or -PAF for 15 s was quantified in [^{3}H]inositol-labelled neutrophils before and after 3 and 10 weeks of dietary *n*-3 PUFA supplementation, by on-line β-scintillation counting after product resolution by anion-exchange HPLC. The three and 10 weeks of dietary *n*-3

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PUFA supplementation profoundly inhibited net formation of inositol monophosphate, diphosphate and IP_3 in neutrophils stimulated with either agonist. Despite the minimal incorporation of EPA into the neutrophil phosphatidylinositol pool, formation of IP_3 by both LTB_4 and PAF-stimulated neutrophils correlated, by subject and time point, very significantly and negatively with the EPA contents of the respective phosphatidylinositol pools, as well as with other phospholipid pools. Dietary *n*-3 PUFA supplementation did not affect neutrophil diacylglycerol formation significantly. Under the conditions we employed, 2 min activation of cytochalasin B-treated neutrophils, most of the diacylglycerol formed would be derived from the phospholipase D pathway. Neutrophil chemotaxis to concentrations of LTB_4 and PAF was assessed *ex vivo* in Boyden microchambers before and after 3 and 10 weeks of dietary *n*-3 PUFA supplementation. Neutrophil chemotaxis to each agonist was significantly inhibited after 3 weeks of dietary *n*-3 PUFA supplementation. A further significant inhibition of neutrophil chemotaxis to each of the ligands was observed after 10 weeks of dietary *n*-3 PUFA supplementation. Neutrophil chemotaxis to each of the ligands correlated significantly with IP_3 formation, as determined for each subject at each time point, and also correlated negatively with the EPA contents of the phosphatidylinositol pools of the neutrophils of the respective subjects (Sperling *et al.* 1993*a*).

The inhibition of chemotaxin-stimulated IP_3 formation, in the absence of an effect on the number of affinity of the respective chemotaxin receptors indicates that dietary *n*-3 PUFA supplementation inhibits the signal transduction pathway between the receptor and phospholipase C. This could occur at the level of the interaction of the receptor with the G-protein, the function of the G-protein itself, the activation of PLC by the G-protein, the intrinsic activity of PLC and/or at the level of PLC substrate availability, or an inhibitory effect on PLC of altered phosphatidylinositol species, the substrate of the enzyme.

The effects of dietary n*-3 polyunsaturated fatty acids on the function of neutrophils in patients with rheumatoid arthritis*

In the study by Sperling *et al.* (1987), in which twelve patients with active RA supplemented their diets for 6 weeks with 20 g of MaxEPA fish oil daily, the effects of dietary fish oil fatty acids on neutrophil chemotaxis to concentrations of LTB_4 and FMLP were evaluated in Boyden microchambers. In the patients with RA, chemotaxis to both LTB_4 and FMLP was suppressed in the pre-diet period as compared with that of healthy volunteers (Lee *et al.* 1985). After 6 weeks of dietary supplementation, neutrophil chemotaxis to LTB_4 and FMLP increased towards normal. One possible explanation of these paradoxical findings is that in the pre-diet period the neutrophils of patients with RA were deactivated, perhaps due to *in vivo* LTB_4 exposure; after the fish oil diet, there was less *in vivo* LTB_4 exposure, and therefore less deactivation. This study, however, did not address the mechanism(s) of the effects of dietary *n*-3 PUFA supplementation on the chemotaxis of neutrophils from patients with RA.

Sperling *et. al.* (1993*b*) studied the mechanism of the differences in the baseline chemotaxin responsiveness of neutrophils from RA patients and previously studied healthy volunteers, and the effects of fish oil PUFA in sixteen patients with RA randomized to receive either 215 mg fish oil PUFA or maize oil PUFA/kg per d in a 10-week double-blind study. The methods used in this study are the same as the methods used in our parallel study in healthy volunteers (Sperling *et al.* 1993*a*). The maximal chemotactic response to LTB_4 by neutrophils from RA patients was decreased by 84 % compared with historical controls ($P < 0{\cdot}0001$). This reflected a decrease in neutrophil LTB_4 receptors and approximately 90 % decrease in the IP_3 signal generated by the LTB_4 receptor in LTB_4-stimulated neutrophils ($P = 0{\cdot}0001$), as assessed by $[^3H]IP_3$ formation in $[^3H]$inositol-labelled polymorphonucleocytes stimulated by LTB_4 and quantified by β-scintillation counting after resolution by HPLC. Similarly, formation of inositol mono- and diphosphates was decreased. In fish oil-PUFA-treated RA patients, an appreciable increase in LTB_4 receptors was seen at week 10, resulting in a small increase in IP_3 formation and an increase in LTB_4-mediated neutrophil chemotaxis. Significant changes in the neutrophils of maize oil-treated RA patients were not observed. The findings suggest that the decreased responsiveness to chemotaxins of neutrophils from RA patients is due to down-regulation of chemotaxin receptor number resulting in decreased signalling via chemotaxin receptors. Dietary fish oil PUFA partially reversed the down-regulation of the chemotaxin receptor of neutrophils of RA patients but had a lesser effect on chemotaxin receptor signalling and function, probably due to a post-receptor inhibition induced by fish oil PUFA, as was previously observed in healthy controls.

The effects of dietary n*-3 polyunsaturated fatty acids on disease activity in patients with rheumatoid arthritis*

Several small clinical studies (Kremer *et al.* 1985, 1987, 1990; Sperling *et al.* 1987; Cleland *et al.* 1988; Tulleken *et al.* 1990; van der Tempel *et al.* 1990; Astorga *et al.* 1991; Kjeldsen-Kragh *et al.* 1992; Nielsen *et al.* 1992; LG Darlington, personal communication) of the effects of low-dose dietary *n*-3 PUFA supplementation in patients with active RA each suggest modest improvement in disease activity. Larger, well-designed, multicentre trials are needed to determine the role of dietary *n*-3 PUFA supplementation in the treatment of RA, but have not been carried out to date due to the high cost of such trials and the lack of funding. Fortin *et al.* (1995) have objectively analysed the data from the studies already completed, using the techniques of meta-analysis and mega-analysis.

Studies were identified by a Medline literature search, checking references of the studies identified by the literature search, and by contacting reseachers in the field regarding any studies which they may be aware of, especially unpublished studies (Fortin *et al.* 1995). As part of the mega-analysis, eleven studies met our inclusion criteria. As part of the meta-analysis, for each publication, the methods and results sections were 'blinded' by the protocol of Chalmers *et al.* (1981). Quality scores for the methods and results were determined using the criteria of Chalmers *et al.* (1981) by two 'blinded', independent observers trained in quality scoring, but who were not otherwise involved in the study. Both the methods and results of each of these studies

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were rated of 'moderate quality' by the independent observers. The data were abstracted from the primary data of the original trials by the principal investigator of the original studies onto standardized data collection forms. Demographic data for the treatment and placebo groups were similar. A Chi-square test of homogeneity was performed for each outcome variable, and then rate differences and standard deviations for 3 months minus baseline were calculated between the treatment and the placebo groups for each outcome, and pooled rate differences with 95 % CI were calculated using the DerSimonian & Laird (1986) method. The meta-analysis revealed significant improvements in the tender/painful joint count and morning stiffness in the fish oil-supplemented group as compared with the control-oil group, after 3 months of treatment. Improvements observed in the other five outcome variables did not reach statistical significance. The results from the mega-analysis, as in the meta-analysis, show significant improvements in the tender or painful joint count and morning stiffness in the fish oil-supplemented group as compared with the control-oil group, after 3 months of treatment. Again, improvements in the other five outcome variables in the treatment group relative to the control group did not reach statistical significance. The results for the mega-analysis, as in the meta-analysis, show significant improvements in the tender or painful joint count and morning stiffness in the fish oil-supplemented group as compared with the control-oil group after 3 months of treatment. Again, improvements in the other five outcome variables in the treatment group relative to the control group did not reach statistical significance (Fortin *et al.* 1995).

Effects of *n*-6 polyunsaturated fatty acids on the human 5-lipoxygenase pathway

γ-Linolenic acid is an *n*-6 PUFA found in significant quantities in some land-based plants. In part, it is elongated to dihomo-γ-linolenic acid, a substrate for cyclooxygenase and 5-lipoxygenase. Ziboh & Fletcher (1992) have shown that dietary supplementation with γ-linolenic acid-rich borage (*Borago officinalis*) oil suppresses LTB_4 generation in calcium ionophore-activated neutrophils. In Dr Zurier's laboratory, similar findings were observed in monocytes (Pullman-Mooar *et al.* 1990), as well as evidence of suppressive effects on lymphocytes (Rossetti *et al.* 1997). This led to a few small clinical studies, spearheaded by Dr Robert Zurier, which suggested that plant oils rich in dihomo-γ-linolenic acid may improve disease activity in patients with active RA (Leventhal *et al.* 1993, 1994; Zurier *et al.* 1996).

Summary and conclusions

The studies of dietary fish oil supplementation in healthy volunteers demonstrate a significant increase in neutrophil EPA content, a concomitant reduction in neutrophil AA content, and suppression of neutrophil LTB_4 synthesis by supplementation with dietary fish oil containing approximately 3–4 g EPA daily for a minimum of 4 weeks. Suppression of neutrophil chemotactic responsiveness to LTB_4 and FMLP was observed after dietary *n*-3 PUFA supplementation at these levels. Dietary EPA is more active than DHA in eliciting these effects in human neutrophils. Dietary *n*-3 PUFA supplementation inhibits neutrophil chemotaxis to these ligands through the inhibition of the signal transduction pathway between the receptor and phospholipase C, as demonstrated by the inhibition of chemotaxin-stimulated IP_3 formation, in the absence of an effect on the number or affinity of the respective chemotaxin receptors.

In patients with RA, dietary supplementation with *n*-3 PUFA resulted in decreased AA content of cellular lipids, with an augmented EPA content and decreased LTB_4 generation by neutrophils. Dietary supplementation with *n*-3 PUFA also resulted in augmentation of depressed neutrophil chemotaxis to LTB_4 and FMLP. Preliminary findings suggest that the decreased responsiveness to chemotaxins of neutrophils from RA patients is due to down-regulation of chemotaxin receptor number, resulting in decreased signalling via chemotaxin receptors. Dietary fish oil PUFA partially reversed the down-regulation of the chemotaxin receptor of neutrophils of RA patients, but had a lesser effect on chemotaxin receptor signalling and function, probably due to a post-receptor inhibition induced by fish oil PUFA, as was previously observed in healthy controls. Several small clinical trials have each suggested that dietary supplementation with *n*-3 PUFA resulted in modest improvements in disease activity. Meta-analysis of these studies confirms statistically significant improvements in tender joint count and morning stiffness after 3 months of dietary fish oil supplementation in patients with RA. Dietary supplementation with γ-linolenic acid-rich oils also inhibits neutrophil LTB_4 formation, has other anti-inflammatory and immunosuppressive effects, and shows promise of therapeutic efficacy in RA.

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