Omega-6 fatty acids and inflammation

Jacqueline K. Innes[a] and Philip C. Calder[a,b*]

[a]Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom.

[b]National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton SO16 6YD, United Kingdom.

*Author for correspondence: 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, United Kingdom

Email: pcc@soton.ac.uk

Phone: +44 23281 205250

Highlights

- The Western diet typically results in a significant concentration of omega-6 polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (ARA), in the cells involved in inflammation.

- High omega-6 PUFA dietary intake has been linked to increased inflammation, mainly because ARA is the precursor of pro-inflammatory lipid mediators.

- Some studies have shown that increased dietary intake of omega-6 PUFAs may not lead to enhanced inflammation and may in fact be linked to reduced inflammation.

- This article investigates the extent to which omega-6 PUFAs are involved in the inflammatory process.

Abstract

Inflammation is a normal process that is part of host defense and tissue healing. However, excessive or unresolved inflammation can lead to uncontrolled tissue damage, pathology and disease. In humans on a Western diet, the omega-6 polyunsaturated fatty acid arachidonic acid (ARA) makes a significant contribution to the fatty acids present in the membrane phospholipids of cells involved in inflammation. ARA is a precursor to a number of potent pro-inflammatory mediators including well described prostaglandins and leukotrienes, which has led to the development of anti-inflammatory pharmaceuticals that target the ARA pathway to successfully control inflammation. Hence, it is commonly believed that increasing dietary intake of the omega-6 fatty acids ARA or its precursor linoleic acid (LA) will increase inflammation. However, studies in healthy human adults have found that increased intake of ARA or LA does not increase the concentrations of many inflammatory markers. Epidemiological studies have even suggested that ARA and LA may be linked to reduced inflammation. Contrastingly, there is also evidence that a high omega-6 fatty acid diet inhibits the anti-inflammatory and inflammation-resolving effect of the omega-3 fatty acids. Thus, the interaction of omega-3 and omega-6 fatty acids and their lipid mediators in the context of inflammation is complex and still not properly understood.

Abbreviations used: ARA, arachidonic acid; ARASCO, arachidonic acid-rich single-cell oil; COX, cyclooxygenase; CRP, C-reactive protein; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; IL, interleukin; LA, linoleic acid; LOX, lipoxygenase; LPS, lipopolysaccharide; LT, leukotriene; LXA_4, lipoxin A_4; NF-kB, nuclear factor kappa B; PBMC, peripheral blood mononuclear cell; PG, prostaglandin; PMN, polymorphonuclear neutrophil; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; sE-selectin, soluble E-selectin; sICAM-1, soluble intercellular adhesion molecule 1; sTNF-R1, soluble tumour necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule 1; TGF-β, transforming growth factor β; TNF, tumour necrosis factor; TX, thromboxane.

1. The inflammatory process: an overview

Inflammation is an essential and normal component of the host's defence mechanism against pathogenic organisms and the response to injury. Inflammation creates an environment that is hostile to pathogens, it initiates pathogen killing, and it induces changes of metabolism in the host. Many cell types are involved in the inflammatory response [1,2]. Furthermore, the response involves the production of, and responses to, a vast number of chemical mediators [1,2]. The earliest steps in the inflammatory response are an increased supply of blood to the site of inflammation and an increase in vascular wall permeability. This permits plasma and large molecules to cross the endothelium, so delivering soluble mediators to the site of inflammation. Leukocytes (white blood cells) migrate from the blood stream into the surrounding tissue. This process is promoted by the release of chemicals that act as leukocyte chemoattractants from the site of inflammation and by the upregulation of adhesion molecules on the endothelium that enable the transient tethering of leukocytes to the endothelium. The newly arrived and activated leukocytes then release chemical mediators at the site of inflammation (Figure 1). These mediators may include lipids (e.g. prostaglandins (PGs), leukotrienes (LTs), endocannabinoids, platelet activating factor), peptides (e.g. cytokines, chemokines), reactive oxygen species (e.g. superoxide anion, hydrogen peroxide), amino acid derivatives (e.g. histamine, nitric oxide) and enzymes (e.g. matrix proteases) depending upon the cell types present, the nature of the inflammatory stimulus, the anatomical site involved, and the stage during the inflammatory response. PGs and LTs are formed from the omega-6 fatty acid arachidonic acid (ARA; 20:4n-6) suggesting that fatty acid nutrition may play a role in promoting or suppressing inflammatory processes.

The influx of cells into the site of inflammatory activity and the presence of the multitude of inflammatory mediators result in the cardinal signs of inflammation: redness, swelling, heat, pain and loss of function. Although designed to be damaging to pathogens, the cellular activities

involved in the inflammatory response and the chemical mediators produced can cause damage to host tissues. However, inflammation is normally self-limiting and resolves, often rapidly. This is because various negative feedback mechanisms are activated as inflammation runs its course. These include the secretion of anti-inflammatory cytokines and pro-resolving lipid mediators which act to inhibit pro-inflammatory signaling; the loss of receptors for inflammatory mediators; and the activation of regulatory cells that dampen the activity of pro-inflammatory cell types. Loss of these regulatory processes can result in excessive, inappropriate or on-going inflammation that can cause irreparable damage to host tissues (Figure 2). As a result of this, inflammation may become pathological and disease can occur. For many diseases, such as rheumatoid arthritis, inflammatory bowel diseases and asthma, that inflammation is central to the pathology is well recognized [1,2]. Individuals with these conditions have heavy infiltration of inflammatory cells at the site of disease activity and elevated concentrations of inflammatory mediators at those sites and in the systemic circulation, and they are treated with anti-inflammatory drugs [1,2]. Interestingly animal models of these diseases can be prevented or treated by administration of pro-resolving mediators [3–6]. Amongst the most potent specialised pro-resolving mediators are the resolvins, protectins and maresins produced from the omega-3 fatty acids eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), again indicating key role for fatty acid nutrition in regulating the evolution of the inflammatory response. In addition to the high grade unresolved inflammatory response associated with the sorts of diseases mentioned above, an elevated but lower grade inflammatory response is involved in many lifestyle-related conditions of ageing [7,8]. Hence, an understanding of the factors that might contribute to the initiation, progression and lack of resolution of inflammatory processes is important to identify the causes and possible preventative and therapeutic strategies for many common conditions.

This article will consider the extent to which omega-6 fatty acids are involved in inflammation.

2. Omega-6 polyunsaturated fatty acids – introduction, dietary sources and intakes

Linoleic acid (LA; 18:2 n-6) is the principal polyunsaturated fatty acid (PUFA) in most western diets. LA is considered to be essential as it cannot be synthesised in higher animals including humans. Rich dietary sources of LA include many vegetable oils, nuts and seeds, and products made from vegetable oils such as margarines. A recent assessment of omega-6 PUFA intake among adults aged 19 to 64 years in the UK was 10.9 $\pm$ 4.7 (mean $\pm$ SD) g/d [9]; most of this (at least 90%) would be LA. This equated to about 5% of energy from omega-6 PUFA, mainly as LA. The estimated contribution of LA to energy intake among adults in the US is about 7% [10].

LA can be metabolised to other omega-6 PUFAs in a pathway involving desaturation to form gamma-linolenic acid (GLA; 18:3n-6), then elongation to form dihomo-gamma-linolenic acid (DGLA; 20:3n-6), and then a further desaturation to form ARA (Figure 3). ARA can be further metabolized to other omega-6 PUFAs (Figure 3). GLA, DGLA and ARA are relatively rare in the diet. GLA is found in certain oils that are available as dietary supplements including borage oil (sometimes called starflower oil), blackcurrant seed oil and evening primrose oil. ARA is found in meat (both red and white including fish), organ meats (e.g. liver, kidney, brain) and eggs. A recent survey reported estimated dietary intake for ARA among adults in 47 developed and 128 developing countries [11]; the study demonstrated that 48% of the 175 countries have an ARA intake of <150 mg/day. Amongst developed countries, mean daily ARA intakes were estimated to be between 100 and 350 mg and ARA contributed < 0.1% of energy intake. Human breast milk also contains ARA: using data from 65 studies in women from different countries around the world, Brenna et al. identified that the mean $\pm$ SD concentration of ARA in breast milk is 0.47 $\pm$ 0.13% of fatty acids with a range of 0.24 to 1.0% [12].

3. Arachidonic acid and inflammation

3.1. Arachidonic acid and eicosanoids

The fatty acid composition of cell membrane phospholipids has a strong influence on cellular responses and cell function. This is achieved through a variety of mechanisms. The fatty acid makeup of membrane phospholipids influences membrane order and lipid raft assembly. Many second messengers are derived from membrane phospholipids - examples include diacylglycerols, endocannabinoids and platelet activating factor - and the fatty acid composition of these messengers affects their biological activity and potency. Finally, some lipid mediators are formed from fatty acids released from membrane phospholipids upon cellular activation. A prime example of these are the eicosanoids produced from ARA, described in more detail below. Through modulation of membrane order, lipid rafts, second messengers and eicosanoids, fatty acids can alter intracellular and extracellular signaling pathways, ultimately affecting gene expression and physiologic and metabolic responses in many different cell and tissue types. As a result of these molecular and cellular actions, fatty acids affect health, disease risk, disease severity and clinical outcome. This is particularly evident within the inflammatory response (Figure 4) [13].

Given its role as a precursor of highly bioactive eicosanoids (see below), the membrane content of ARA is likely to be important. Indeed, ARA is usually the major PUFA in the membranes of cells involved in inflammation in humans. For example, peripheral blood mononuclear cells (PBMCs; a mixture of ~85% lymphocytes and ~15% monocytes) from adults have been reported to have an average ARA content of 16% [14], 18% [15,16] or 20% [17,18] of total fatty acids. Similarly, average neutrophil ARA content has been variously reported as 15% [19,20], 11% [21] and 9% [22] of total fatty acids. However, in common with other fatty acids, the content of ARA in these cell types is variable amongst individuals. For example, Kew et al. reported 10th and 90th percentiles of PBMC ARA content of 13.5 and 23.5% of total fatty acids using samples from 150 healthy British adults [15]. It is also important to note that ARA is found differentially distributed across several different phospholipid species in these cells [23].

LA and DGLA are typically present in lower amounts than ARA (~10% and 2% of total fatty acids in PBMCs, respectively [14–18]) and omega-3 PUFAs are found in much lower amounts than both ARA and LA [14–18].

Phospholipase A2 is activated by many inflammatory stimuli and acts to cleave ARA from the sn-2 position of cell membrane phospholipids, particularly phosphatidylcholine [24]. The ARA that is released acts as a substrate for cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 enzymes to form eicosanoid mediators [25–27]. These include the various PGs, thromboxanes (TXs) and LTs (Figure 5), which, working through specific receptors (Table 1), act as mediators and regulators of inflammatory processes [28,29]. For example, PGE_2 has a number of pro-inflammatory effects including induction of fever, increase in vasodilation and vascular permeability and activation of pain perception as well as enhancement of pain caused by other agents. PGE_2 is also responsible for the induction of its own production as well as induction of the pro-inflammatory cytokine, interleukin (IL)-6. The 4-series LTs also contribute a number of pro-inflammatory effects. LTB_4, which is produced by neutrophils and macrophages, promotes leukocyte chemotaxis, adhesion and degranulation, and enhances vascular permeability, pain perception and the production of inflammatory mediators such as superoxide and inflammatory cytokines. LTC_4, LTD_4 and LTE_4, which are produced by mast cells, eosinophils and basophils, also contribute to inflammatory processes through effecting arteriole- and bronchoconstriction, and enhancing vascular permeability, mucus secretion, hypersensitivity and skin dilation. The concentrations of many ARA-derived eicosanoids are elevated in people with frank inflammatory conditions. For example, eicosanoids produced by both the COX and LOX pathways are found in the synovial fluid of patients with active rheumatoid arthritis [30,31]. Likewise, several ARA-derived eicosanoids including PGE_2, TXB_2, and several hydroxyeicosatetraenoic acids were found to be significantly elevated in the

intestinal mucosa of patients with ulcerative colitis where they were directly correlated with severity of disease [32].

With strong evidence supporting the link between ARA-derived eicosanoids and inflammation, anti-inflammatory drug therapies targeting the various steps in the synthesis of ARA-derived lipid mediators and their action have been developed. These include COX inhibitors like aspirin, non-steroidal anti-inflammatory drugs and some steroids, as well as LOX inhibitors, 5-LOX activating protein inhibitors and LT receptor antagonists. The robust evidence that these drugs work in people with inflammation, either transient or chronic, provides strong evidence that the ARA-derived eicosanoids are central components of the inflammatory process.

Despite this, it is important to appreciate that not all the actions of ARA-derived eicosanoids are pro-inflammatory and that some eicosanoids seem to be important in the resolution of inflammation. For example, whilst PGE_2 is regarded as being a potent pro-inflammatory mediator (see earlier), it also acts as an inhibitor of the production of classic pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α: in cultured human whole blood, lipopolysaccharide-induced TNF-α production was significantly reduced following addition of PGE_2 [33]. Furthermore, through induction of 15-LOX, PGE_2 induces production of lipoxins (LXs) such as LXA_4 [34], which has a temporal role in inflammation resolution following the acute inflammatory response [35] (Figure 6). This has been demonstrated in a murine dorsal pouch model of inflammation, whereby injection of TNF-α led to a rapid increase in LTB_4 and infiltration of leukocytes (primarily neutrophils) together with a more gradual rise in PGE_2 [36]. PGE_2 reached its maximum concentration concurrent with the maximum concentration of infiltrating neutrophils. The more gradual delayed rise in LXA_4 occurred as PGE_2 and polymorphonuclear neutrophil (PMN) numbers decreased, suggesting that LXA_4 is involved in inflammation resolution [36].

3.2 The effect of increased ARA intake on markers of inflammation

Relatively few studies of the impact of increased dietary intake of ARA on inflammation have been performed, particularly in humans. However from the few studies performed to date it seems that increasing ARA intake results in increased content of ARA in cell membranes and increased production of ARA-derived lipid mediators. This is also well illustrated by a rodent study in which ARA levels in splenocytes were altered through feeding the animals diets containing ARA, which resulted in a high splenocyte ARA content, or the omega-3 fatty acids EPA or DHA, which resulted in low splenocyte ARA content [37]. The production of PGE_2 by cultured splenocytes was highly correlated with splenocyte ARA content (Figure 7) [37].

In humans, increased intake of ARA through use of supplements has been shown to increase ARA concentrations in PBMCs: in healthy adults aged 56 to 70 y, supplementation of 0.7 g/d ARA as ARASCO (arachidonic acid-rich single-cell oil) for 12 weeks resulted in an increase of ARA in PBMC phospholipids from ~ 20% of total fatty acids at baseline to ~ 23% at 12 weeks [18]. The increase in ARA appeared to be at the expense of LA which decreased from 9.3 to 6.6% of total fatty acids when ARA was given [18].

In a small cross-over study in healthy American men aged 20 to 38 y, supplementation of 1.5 g/d ARA as ARASCO for 7 weeks resulted in significant increases of lipopolysaccharide (LPS)-stimulated PGE_2 and LTB_4 production by cultured PBMCs. There was however no effect on LPS-stimulated secretion of TNF-α, IL-1β or IL-6 by PBMCs [38]. This lack of effect of ARA on pro-inflammatory cytokine secretion was also reported in another small study investigating supplementation of ~ 0.7 g/d ARA as ARASCO for 12 weeks in older British men and women (56 to 70 years of age): there was no effect of ARA on LPS-stimulated TNF-α, IL-1β or IL-6 production by PBMCs [39]. Furthermore, there was no effect of ARA supplementation on the numbers of inflammatory cells in the bloodstream (i.e. total leukocytes, neutrophils, eosinophils,

basophils, lymphocytes or monocytes) or on the concentrations of soluble adhesion molecules (soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), soluble E-selectin (sE-selectin)) in plasma [39]. In a much larger clinical trial in Japanese subjects aged 45 to 55 y, supplementation of 0.24 g/d or 0.72 g/d ARA from a novel oil for 4 weeks increased plasma phospholipid content of ARA in a dose-dependent manner, but did not affect the plasma concentrations of C-reactive protein (CRP), TNF-α or IL-6 [40]. Likewise, neither dose of ARA significantly altered plasma PGE_2 or LXA_4 concentrations [40].

Taken together, these study results suggest that increased intake of ARA from supplements does not adversely affect circulating inflammatory cell numbers or type, or pro-inflammatory cytokines measured in plasma or *ex vivo* following LPS-stimulation of PBMCs. There may be effects of supplementing ARA on *ex vivo* production of ARA-derived eicosanoids. It is important to note that these conclusions are based on only three human studies, two of which were very small in size, which investigated supplemental doses of ARA of 0.24 to 1.5 g/day. Higher doses and durations beyond 12 weeks have not been explored.

4. Other omega-6 fatty acids and inflammation

4.1. Gamma-linolenic acid (GLA)

GLA is found at only low levels in inflammatory cells and increasing intake of GLA does not increase the amount of GLA in cells [17,18]. GLA does however increase the content of its elongation product DGLA in PBMCs [17,18] and neutrophils [22,41–43]. The lack of incorporation of GLA seen in these studies is likely due to the efficient *in vivo* conversion of GLA to DGLA (Figure 8). DGLA may then be further metabolised via the rate-limiting delta-5 desaturase reaction to ARA (Figure 8). However, most of the studies of increased GLA intake which reported increased DGLA in PBMCs and neutrophils found no effect of GLA on ARA in these cell types [17,18,22,41,42]. In fact, Ziboh et al. reported that neutrophil ARA content was

decreased from 5.8 to 4.0% of total fatty acids after 12 months supplementation with 2 g GLA/d in patients with asthma [43]. DGLA may also act as a substrate for COX, giving rise to PGE_1, and for 15-LOX, giving rise to 15-hydroxy-eicosatrienoic acid, both inflammation-suppressing eicosanoids (see [44] for a review). Thus, via conversion to DGLA, increased GLA intake may have an anti-inflammatory effect due to production of PGE_1 and other eicosanoids from DGLA. Furthermore providing GLA has been demonstrated to decrease *ex vivo* production of LTB_4 by neutrophils [22,42,43].

4.2. Linoleic acid (LA)

Increased intake of LA does not increase PBMC ARA content, despite LA being a substrate for ARA synthesis: in healthy humans, supplementation with 6.5 g/d of LA (as sunflower oil) had no effect on the ARA content of PBMCs [17]. This may be because the pathway of ARA synthesis from LA is already saturated because of the high intake of LA from the diet (likely to be around 10 g/day) so that increasing LA intake even further will have no effect on promoting ARA synthesis. This observation with PBMCs is consistent with findings of a systematic review of 36 RCTs investigating the effect of dietary intake of LA on tissue fatty acid composition in humans consuming a Western diet [45]. The review reported that neither a decrease nor an increase in LA intake affected ARA concentrations in plasma/serum phospholipids or erythrocytes [45]. Decreases in LA intake of up to 90% and increases of up to six-fold were analysed in the systematic review [45].

LA itself can affect inflammation because it is metabolised by LOX to derivatives called hydroxyoctadecadienoic acids (HODEs) (e.g. 9- and 13-HODE) and further to oxo-HODEs (e.g. 9-oxo-HODE and 13-oxo-HODE) and epoxy-HODEs (Figure 8), which play roles in inflammation (see [46,47] for reviews). 9-HODE, 13-HODE and 13-oxoHODE were found in colonic mucosal

biopsies from patients with ulcerative colitis but they were not significantly related to inflammation or disease severity [32].

Regarding the effect of dietary LA intake on inflammatory markers, in a large cross-sectional study in 405 healthy men and 454 healthy women, dietary intake of LA was not significantly associated with the plasma inflammatory markers CRP, IL-6, soluble TNF receptor (sTNF-R) 1 and sTNF-R2 [48]. Interestingly, dietary intake of the anti-inflammatory omega-3 PUFAs EPA and DHA was found to be inversely associated with sTNF-R1 and R2 concentrations, a relationship which was stronger in the presence of high LA intake rather than low LA intake [48].

Similarly, in an epidemiological study of 1,123 Italian subjects, no relationship was found between plasma concentrations of LA and eight markers of inflammation: IL-6, soluble IL-6 receptor, IL-1β, IL-1 receptor antagonist, TNF-α, IL-10, transforming growth factor (TGF)-β and CRP [49]. Interestingly, those subjects in the lowest quartile of plasma LA concentration had the highest pro-inflammatory IL-6 and CRP concentrations and the lowest anti-inflammatory IL-10 and TGF-β concentrations [49]. Furthermore, an inverse relationship was found between plasma ARA and IL-6 and IL-1 receptor antagonist, and a positive relationship found between plasma ARA and TGF-β [49]. These study outcomes support the concept that LA and ARA are involved in both pro- and anti-inflammatory pathways.

Finally, a systematic review of 15 RCTs in healthy non-infant human subjects found no link between dietary LA intake and markers of inflammation such as cytokines (including IL-6, TNF-α, CRP), fibrinogen, plasminogen activator inhibitor type 1, and soluble vascular adhesion molecules [50].

In conclusion, despite long-held belief to the contrary, the available evidence demonstrates that high dietary intake or high plasma concentrations of LA do not appear to result in increased tissue ARA or in increased *in vivo* or *ex vivo* concentrations of inflammatory markers in humans.

5. Interaction between omega-6 and omega-3 PUFAs and impact on inflammation

The omega-3 PUFAs EPA and DHA are generally regarded to be anti-inflammatory [51,52], to promote resolution of inflammation [3–6] and to decrease pain in inflammatory conditions [53–56]. This is thought to be achieved in part via replacement of ARA in membrane phospholipids and through competitive inhibition of ARA metabolism [51,52], shifting metabolism away from potent pro-inflammatory ARA-derived eicosanoids towards EPA and DHA-derived lipid mediators. Consistent with this, in patients with rheumatoid arthritis the anti-inflammatory effects of EPA and DHA were shown to be enhanced when ARA intake was decreased [57]. This suggests that decreasing ARA enhances the effectiveness of EPA and DHA. This makes sense because ARA and the omega-3 PUFAs are directly competing with one another for metabolism (e.g. by phospholipase A2 and COX and LOX enzymes) and their mediators are competing for receptors [58]. The extent of this competition depends upon the concentration of the competing compounds and the relative affinities of the enzymes and receptors for the various substrates and ligands [58]. Furthermore, LA has been shown to limit the synthesis of EPA from alpha-linolenic acid in humans [59], a pathway which is already considered to be inefficient in humans [60]. Thus, a high amount of LA in the background diet might limit endogenous EPA synthesis potentially creating a more inflammatory environment. However, as discussed above, omega-6 PUFAs, including ARA, produce not only pro-inflammatory eicosanoids, but also lipid mediators that play an important role in inflammation resolution. Thus, the interaction between omega-3 and omega-6 PUFAs and their derivatives in the context of inflammation is complex and not fully elucidated.

6. Summary

Whilst inflammation is a normal process that is part of host defense, excessive or unresolved inflammation can lead to uncontrolled tissue damage, pathology and disease. In humans on a

Western diet, the omega-6 PUFA ARA makes a significant contribution to the fatty acids present in the membrane phospholipids of cells involved in inflammation. ARA is a precursor to a number of potent pro-inflammatory mediators including well described PGs and LTs, which has led to the development of anti-inflammatory pharmaceuticals that target the ARA pathway to successfully control inflammation. Hence, it is commonly believed that increasing dietary intake of the omega-6 PUFAs ARA or its precursor LA will increase inflammation. However, studies in healthy human adults have found that increased intake of ARA or LA does not increase the concentrations of many inflammatory markers. Indeed, epidemiological studies have even suggested that ARA and LA may be linked to reduced inflammation. Contrastingly, there is also evidence that a high ARA diet inhibits the anti-inflammatory and inflammation-resolving effect of the omega-3 PUFAs EPA and DHA [57]. Thus, the interaction of omega-3 and omega-6 PUFAs and their lipid mediator derivatives in the context of inflammation is complex and still not properly understood. Additional trials in patients rather than in healthy subjects, and focused on the impact of decreasing omega-6 intake on inflammation may go some way to providing further clarification.

Disclosures

Authors' responsibilities

Both Philip C. Calder and Jacqueline K. Innes drafted the manuscript and approved the final version.

References

[1]   P.C. Calder, R. Albers, J.M. Antoine, S. Blum, R. Bourdet-Sicard, G.A. Ferns, G. Folkerts, P.S. Friedmann, G.S. Frost, F. Guarner, M. Løvik, S. MacFarlane, P.D. Meyer, L. M'Rabet, M. Serafini, W. Van Eden, J. Van Loo, W. Vas Dias, S. Vidry, B.M. Winklhofer-Roob, J. Zhao, Inflammatory disease processes and interactions with nutrition, Br. J. Nutr. 101 Suppl 1 (2009) S1-S45. doi:10.1017/S0007114509990511.

[2]   P.C. Calder, N. Ahluwalia, R. Albers, N. Bosco, R. Bourdet-Sicard, D. Haller, S.T. Holgate, L.S. Jönsson, M.E. Latulippe, A. Marcos, J. Moreines, C. Mrini, M. Müller, G. Pawelec, R.J.J. Van Neerven, B. Watzl, J. Zhao, A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies, Br. J. Nutr. 109 Suppl 1 (2013) S1-S34. doi:10.1017/S0007114512005119.

[3]   G. Bannenberg, C.N. Serhan, Specialized pro-resolving lipid mediators in the inflammatory response: An update, Biochim. Biophys. Acta 1801 (2010) 1260–1273. doi:10.1016/j.bbalip.2010.08.002.

[4]   C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators, Nat. Rev. Immunol. 8 (2008) 349–361. doi:10.1038/nri2294.

[5]   C.N. Serhan, N. Chiang, Resolution phase lipid mediators of inflammation: Agonists of resolution, Curr. Opin. Pharmacol. 13 (2013) 632–640. doi:10.1016/j.coph.2013.05.012.

[6]   J. Dalli, R.A. Colas, C.N. Serhan, Novel n-3 immunoresolvents: Structures and actions, Sci. Rep. 3 (2013) 1940. doi:10.1038/srep01940.

[7]   P.C. Calder, N. Ahluwalia, F. Brouns, T. Buetler, K. Clement, K. Cunningham, K. Esposito, L.S. Jönsson, H. Kolb, M. Lansink, A. Marcos, A. Margioris, N. Matusheski, H. Nordmann, J. O'Brien, G. Pugliese, S. Rizkalla, C. Schalkwijk, J. Tuomilehto, J. Wärnberg, B. Watzl, B.M. Winklhofer-Roob, Dietary factors and low-grade inflammation in

relation to overweight and obesity, Br. J. Nutr. 106 Suppl 3 (2011) S5-78. doi:10.1017/S0007114511005460.

[8]   P.C. Calder, N. Bosco, R. Bourdet-Sicard, L. Capuron, N. Delzenne, J. Doré, C. Franceschi, M.J. Lehtinen, T. Recker, S. Salvioli, F. Visioli, Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition, Ageing Res. Rev. 40 (2017) 95–119. doi:10.1016/j.arr.2017.09.001.

[9]   G.K. Pot, C.J. Prynne, C. Roberts, A. Olson, S.K. Nicholson, C. Whitton, B. Teucher, B. Bates, H. Henderson, S. Pigott, G. Swan, A.M. Stephen, National Diet and Nutrition Survey: Fat and fatty acid intake from the first year of the rolling programme and comparison with previous surveys, Br. J. Nutr. 107 (2012) 405–415. doi:10.1017/S0007114511002911.

[10]   T.L. Blasbalg, J.R. Hibbeln, C.E. Ramsden, S.F. Majchrzak, R.R. Rawlings, Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century, Am. J. Clin. Nutr. 93 (2011) 950–962. doi:10.3945/ajcn.110.006643.950.

[11]   S. Forsyth, S. Gautier, N. Salem Jr., Global estimates of dietary intake of docosahexaenoic acid and arachidonic acid in developing and developed Countries, Ann. Nutr. Metab. 68 (2016) 258–267. doi:10.1159/000446855.

[12]   J.T. Brenna, B. Varamini, R.G. Jensen, D.A. Diersen-Schade, J.A. Boettcher, L.M. Arterburn, Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide, Am. J. Clin. Nutr. 85 (2007) 1457–1464.

[13]   P.C. Calder, Fatty acids and inflammation: The cutting edge between food and pharma, Eur. J. Pharmacol. 668 (2011) S50–S58. doi:10.1016/j.ejphar.2011.05.085.

[14]   C.G. Walker, A.L. West, L.M. Browning, J. Madden, J.M. Gambell, S.A. Jebb, P.C. Calder, The pattern of fatty acids displaced by EPA and DHA following 12 months supplementation varies between blood cell and plasma fractions, Nutrients 7 (2015) 6281–6293. doi:10.3390/nu7085285.

[15]   S. Kew, T. Banerjee, A.M. Minihane, Y.E. Finnegan, C.M. Williams, P.C. Calder, 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. 77 (2003) 1278–1286. doi:10.1093/ajcn/77.5.1278.

[16]   D. Rees, E.A. Miles, T. Banerjee, S.J. Wells, C.E. Roynette, K.W. Wahle, P.C. Calder, Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men, Am. J. Clin. Nutr. 83 (2006) 331–342. doi:10.1093/ajcn/83.2.331.

[17]   P. Yaqoob, H.S. Pala, M. Cortina-Borja, E.A. Newsholme, P. Calder, Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions, Eur. J. Clin. Invest. 30 (2000) 260–274. doi:10.1046/j.1365-2362.2000.00623.x.

[18]   F. Thies, G. Nebe-von-Caron, J.R. Powell, P. Yaqoob, E.A. Newsholme, P.C. Calder, Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older Humans, J. Nutr. 131 (2001) 1918–1927.

[19]   D.A. Healy, F.A. Wallace, E.A. Miles, P.C. Calder, P. Newsholme, Effect of low-to-moderate amounts of dietary fish oil on neutrophil lipid composition and function, Lipids 35 (2000) 763–768. doi:10.1007/s11745-000-0583-1.

[20]   S. Kew, M.D. Mesa, S. Tricon, R. Buckley, A.M. Minihane, P. Yaqoob, Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans, Am. J. Clin. Nutr. 79 (2004) 674–681.

[21]   M. Gibney, B. Hunter, 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. 47 (1993) 255–9.

[22]   V. Ziboh, M. Fletcher, Dose-response effects of dietary γ-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B4, Am. J. Clin. Nutr. 55

(1992) 39–45. doi:10.1093/ajcn/55.1.39.

[23]   R.D. Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF, Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils, J. Clin. Invest. 91 (1993) 651–660. doi:10.1172/JCI116245.

[24]   E.A. Dennis, Liberating chiral lipid mediators, inflammatory enzymes, and LIPID MAPS from biological grease, J. Biol. Chem. 291 (2016) 24431–24448. doi:10.1074/jbc.X116.723791.

[25]   A. Nicolaou, Prostanoids, in: A. Nicolaou, G. Kafatos (Eds.), Bioactive Lipids, The Oily Press, Bridgewater, 2004: pp. 197–222.

[26]   S. Fiore, Leukotrienes and lipoxins, in: A. Nicolaou, G. Kafatos (Eds.), Bioactive Lipids, The Oily Press, Bridgewater, 2004: pp. 223–243.

[27]   P.C. Calder, Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology?, Br. J. Clin. Pharmacol. 75 (2013) 645–662. doi:10.1111/j.1365-2125.2012.04374.x.

[28]   S.L. Tilley, T.M. Coffman, B.H. Koller, Mixed messages: Modulation of inflammation and immune responses by prostaglandins and thromboxanes, J. Clin. Invest. 108 (2001) 15–23. doi:10.1172/JCI200113416.

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

[30]   H. Sano, T. Hla, J.A.M. Maier, L.J. Crofford, J.P. Case, T. Maciag, R.L. Wilder, In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis, J. Clin. Invest. 89 (1992) 97–108. doi:10.1172/JCI115591.

[31]   R. Sperling, Eicosanoids in rheumatoid arthritis, Rheum. Dis. Clin. N. Am. 21 (1995) 741–758.

[32]   M. Masoodi, D.S. Pearl, M. Eiden, J.K. Shute, J.F. Brown, P.C. Calder, T.M. Trebble,
         Altered colonic mucosal polyunsaturated fatty acid (PUFA) derived lipid mediators in
         ulcerative colitis: New insight into relationship with disease activity and pathophysiology,
         PLoS One 8 (2013) 1–13. doi:10.1371/journal.pone.0076532.

[33]   E.A. Miles, E. Allen, P.C. Calder, In vitro effects of eicosanoids derived from different 20-
         carbon fatty acids on production of monocyte-derived cytokines in human whole blood
         cultures, Cytokine 20 (2002) 215–223. doi:10.1006/cyto.2002.2007.

[34]   I. Vachier, P. Chanez, C. Bonnans, P. Godard, J. Bousquet, C. Chavis, Endogenous anti-
         inflammatory mediators from arachidonate in human neutrophils., Biochem. Biophys.
         Res. Commun. 290 (2002) 219–24. doi:10.1006/bbrc.2001.6155.

[35]   A.T. Gewirtz, L.S. Collier-Hyams, A.N. Young, T. Kucharzik, W.J. Guilford, J.F.
         Parkinson, I.R. Williams, A.S. Neish, J.L. Madara, Lipoxin A4 analogs attenuate induction
         of intestinal epithelial proinflammatory gene expression and reduce the severity of
         dextran sodium sulfate-induced colitis, J. Immunol. 168 (2002) 5260–5267.
         doi:10.4049/jimmunol.168.10.5260.

[36]   B.D. Levy, C.B. Clish, B. Schmidt, K. Gronert, C.N. Serhan, Lipid mediator class
         switching during acute inflammation: Signals in resolution, Nat. Immunol. 2 (2001) 612–
         619. doi:10.1038/89759.

[37]   L.D. Peterson, N.M. Jeffery, F. Thies, P. Sanderson, E.A. Newsholme, P.C. Calder,
         Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid
         composition and prostaglandin E2 production but have different effects on lymphocyte
         functions and cell-mediated immunity, Lipids 33 (1998) 171–180. doi:10.1007/s11745-
         998-0193-y.

[38]   D.S. Kelley, P.C. Taylor, G.J. Nelson, B.E. Mackey, Arachidonic acid supplementation
         enhances synthesis of eicosanoids without suppressing immune functions in young
         healthy men, Lipids 33 (1998) 125–30. doi:10.1007/s11745-998-0187-9.

[39]   F. Thies, E.A. Miles, G. Nebe-von-Caron, J.R. Powell, T.L. Hurst, E.A. Newsholme, P.C. Calder, Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults, Lipids 36 (2001) 1183–1193. doi:10.1007/s11745-001-0831-4.

[40]   S. Kakutani, Y. Ishikura, N. Tateishi, C. Horikawa, H. Tokuda, M. Kontani, Supplementation of arachidonic acid-enriched oil increases arachidonic acid contents in plasma phospholipids, but does not increase their metabolites and clinical parameters in Japanese healthy elderly individuals : a randomized controlled study, Lipids Health Dis. 10 (2011) 1–12. doi:10.1186/1476-511X-10-241.

[41]   T. Chilton-Lopez, M.E. Surette, D.D. Swan, A.N. Fonteh, M.M. Johnson, F.H. Chilton, Metabolism of gammalinolenic acid in human neutrophils, J. Immunol. 156 (1996) 2941–2947. http://www.ncbi.nlm.nih.gov/pubmed/8609415.

[42]   M. Johnson, D. Swan, M. Surette, J. Stegner, T. Chilton, A. Fonteh, F. Chilton, Dietary supplementation with gamma-linolenic acid alters fatty acid content and eicosanoid production in healthy humans, J. Nutr. 127 (1997) 1435–1444. doi:10.1093/jn/127.8.1435.

[43]   V.A. Ziboh, S. Naguwa, K. Vang, J. Wineinger, B.M. Morrissey, M. Watnik, M.E. Gershwin, Suppression of leukotriene B4 generation by ex-vivo neutrophils isolated from asthma patients on dietary supplementation with gammalinolenic acid-containing borage oil: Possible implication in asthma, Clin. Dev. Immunol. 11 (2004) 13–21. doi:10.1080/10446670410001670445.

[44]   S. Sergeant, E. Rahbar, F.H. Chilton, Gamma-linolenic acid, dihommo-gamma linolenic, eicosanoids and inflammatory processes, Eur. J. Pharmacol. 785 (2016) 77–86. doi:10.1016/j.ejphar.2016.04.020.

[45]   B.S. Rett, J. Whelan, Increasing dietary linoleic acid does not increase tissue arachidonic

acid content in adults consuming Western-type diets : a systematic review, Nutr. Metab. (Lond). 8 (2011) 1–15. doi:10.1186/1743-7075-8-36.

[46]   V.N. Vangaveti, H. Jansen, R.L. Kennedy, U.H. Malabu, Hydroxyoctadecadienoic acids: Oxidised derivatives of linoleic acid and their role in inflammation associated with metabolic syndrome and cancer, Eur. J. Pharmacol. 785 (2016) 70–76. doi:10.1016/j.ejphar.2015.03.096.

[47]   G.C. Shearer, R.E. Walker, Omega-6 oxylipins – Synthesis in physiological PUFA mixtures and potential benefits, Prostagland. Leukot. Essent. Fatty Acids (2018).

[48]   T. Pischon, S.E. Hankinson, G.S. Hotamisligil, N. Rifai, W.C. Willett, E.B. Rimm, Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women, Circulation 108 (2003) 155–160. doi:10.1161/01.CIR.0000079224.46084.C2.

[49]   L. Ferrucci, A. Cherubini, S. Bandinelli, B. Bartali, A. Corsi, F. Lauretani, A. Martin, C. Andres-Lacueva, U. Senin, J.M. Guralnik, Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers, J. Clin. Endocrinol. Metab. 91 (2006) 439–446. doi:10.1210/jc.2005-1303.

[50]   G.H. Johnson, K. Fritsche, Effect of dietary linoleic acid on markers of inflammation in healthy persons: A systematic review of randomized controlled trials, J. Acad. Nutr. Diet. 112 (2012) 1029–1041. doi:10.1016/j.jand.2012.03.029.

[51]   P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance, Biochim. Biophys. Acta Mol. Cell. Biol. Lipids. 1851 (2015) 469–484. doi:10.1016/j.bbalip.2014.08.010.

[52]   P.C. Calder, Omega-3 fatty acids and inflammatory processes: from molecules to man, Biochem. Soc. Trans. 45 (2017) 1105–1115. doi:10.1042/BST20160474.

[53]   R.J. Goldberg, J. Katz, A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain, Pain 129 (2007)

210–223. doi:10.1016/j.pain.2007.01.020.

[54]   M. Abdulrazaq, J.K. Innes, P.C. Calder, Effect of ω-3 polyunsaturated fatty acids on

arthritic pain: A systematic review, Nutrition 39–40 (2017) 57–66.

doi:10.1016/j.nut.2016.12.003.

[55]   N.K. Senftleber, S.M. Nielsen, J.R. Andersen, H. Bliddal, S. Tarp, L. Lauritzen, D.E.

Furst, M.E. Suarez-Almazor, A. Lyddiatt, R. Christensen, Marine oil supplements for

arthritis pain: A systematic review and meta-analysis of randomized trials, Nutrients 9

(2017) 42. doi:10.3390/nu9010042.

[56]   C. Ramsden, K.R. Faurot, D. Zamora, C.M. Suchindran, B.A. Macintosh, S. Gaylord, A.

Ringel, J.R. Hibbeln, A.E. Feldstein, T.A. Mori, A. Barden, C. Lynch, R. Coble, E. Mas, O.

Palsson, D.A. Barrow, J.D. Mann, Targeted alteration of dietary n-3 and n-6 fatty acids for

the treatment of chronic headaches: a randomized trial, Pain 154 (2013) 2441–2451.

doi:10.1016/j.pain.2013.07.028.

[57]   O. Adam, C. Beringer, T. Kless, C. Lemmen, A. Adam, M. Wiseman, P. Adam, R.

Klimmek, W. Forth, Anti-inflammatory effects of a low arachidonic acid diet and fish oil in

patients with rheumatoid arthritis, Rheumatol. Int. 23 (2003) 27–36. doi:10.1007/s00296-

002-0234-7.

[58]   M. Wada, C.J. DeLong, Y.H. Hong, C.J. Rieke, I. Song, R.S. Sidhu, C. Yuan, M.

Warnock, A.H. Schmaier, C. Yokoyama, E.M. Smyth, S.J. Wilson, G.A. FitzGerald, R.M.

Garavito, X.S. De, J.W. Regan, W.L. Smith, Enzymes and receptors of prostaglandin

pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates

and products, J. Biol. Chem. 282 (2007) 22254–22266. doi:10.1074/jbc.M703169200.

[59]   J.K. Chan, B.E. McDonald, J.M. Gerrard, V.M. Bruce, B.J. Weaver, B.J. Holub, Effect of

dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and

thrombogenesis, Lipids 28 (1993) 811–817. doi:10.1007/BF02536235.

[60]   E.J. Baker, E.A. Miles, G.C. Burdge, P. Yaqoob, P.C. Calder, Metabolism and functional

effects of plant-derived omega-3 fatty acids in humans, Prog. Lipid Res. 64 (2016) 30–56. doi:10.1016/j.plipres.2016.07.002.

Table 1. ARA derived mediators associated with inflammation and their receptors

Class	Mediator	Pathway of synthesis	Receptor(s)
Prostanoid	PGD_2	COX	DP1, DP2
	PGE_2	COX	EP1, EP2, EP3, EP4
	$PGF_{2\alpha}$	COX	FP
	PGI_2	COX	IP
	TXA_2	COX	TP
Leukotriene	5-HETE	5-LOX	BLT2
	5-HPETE	5-LOX	OXE
	LTB_4	5-LOX	BLT1, BLT2
	LTC_4, D_4, E_4	5-LOX	CysLT1, CysLT2
	15-HETE	15-LOX	BLT2
	15-HPETE	15-LOX	BLT2
	12-HETE	12-LOX	BLT2
Lipoxin	LXA_4	15-LOX and 5-LOX or 5-LOX and 12-LOX (transcellular)	FPR2/ALX

Abbreviations: COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; TX, thromboxane.

Note that the listing of mediators is not exhaustive.

Figure captions

Figure 1. Simplified scheme of inflammation. Reproduced from Calder et al. [1] with permission from ILSI Europe.

Figure 2. Scheme depicting the normal course of inflammation which is self-resolving and chronic inflammation where resolution is lost.

Figure 3. Pathway of conversion of linoleic acid to longer chain and more unsaturated omega-6 fatty acids.

Figure 4. General overview of the mechanisms by which fatty acids can influence inflammatory cells. PL, phospholipid; DAG, diacylglycerol.

Figure 5. Overview of the pathways of eicosanoid (and endocannabinoid) synthesis from arachidonic acid. AEA, arachidonoyl ethanolamine (also called anandamide); 2-AG, 2-arachidonoyl glycerol; ARA,arachidonic acid; COX, cyclooxygenase; CYTP450, cytochrome P450 enzymes; DAG, diacylglycerol; DHET, dihydroxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; LOX, lipoxygenase; LT, leukotriene; PE, phosphatidylethanolamine; PG, prostaglandin; TX, thromboxane. Note that not all enzymes are named and that not all metabolites are shown. Modified from Calder [27] with permission from John Wiley & Sons.

Figure 6. Scheme depicting the role of prostaglandin E_2 in regulating metabolism of arachidonic acid by lipoxygenase enzymes. Prostaglandin (PG) E_2 (produced by cyclooxygenase (COX) inhibits 5-lipoxygenase (LOX) activity and promotes 15-LOX activity. This switches LOX metabolism away from pro-inflammatory 4-series leukotrienes (LTs) to the pro-resolving lipoxin A_4.

Figure 7. Relationship between rat splenocyte ARA content and production of prostaglandin E_2 by those splenocytes when activated in culture. Male rats were fed for 6 weeks on a control diet (Control) or diets containing gamma-linolenic acid (GLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), each at 4.4% by weight of total fatty acids. All diets contained 17.8% by weight of fat and the contents of palmitic, stearic, oleic and total saturated, monounsaturated and polyunsaturated fatty acids were kept constant as was the ratio of omega-6 to omega-3 polyunsaturated fatty acids. Thus, in the diets containing GLA or ARA, these fatty acids partly replaced linoleic acid, while in the diets containing EPA or DHA, these fatty acids replaced ALA. Splenocytes were prepared and cultured for 48 hours; cells were stimulated with a mitogen. PGE_2 concentration in the supernatant was measured using a commercial immunoassay kit. The fatty acid composition of splenocytes was determined by gas chromatography. Data are taken from Peterson et al. [37].

Figure 8. General overview of the synthesis of lipid mediators from omega-6 fatty acids. HODE, hydroxyoctadecadienoic acid; LT, leukotriene; PG, prostaglandin.