Can Fish Oil Reduce the Incidence of Necrotizing Enterocolitis by Altering the Inflammatory Response?

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KEYWORDS

• Necrotizing enterocolitis • Prematurity • Very low birth weight • Fish oil
• Omega-3 long-chain polyunsaturated fatty acids

KEY POINTS

- Despite modern advances in neonatology, necrotizing enterocolitis continues to affect preterm infants, particularly very low-birth-weight infants.
- Necrotizing enterocolitis is characterized by a robust inflammatory response.
- Fish oil, containing docosahexaenoic acid and eicosapentaenoic acid, exerts anti-inflammatory effects via multiple mechanisms of action.
- Previous animal and human studies have demonstrated the potential for fish oil to modulate inflammation; thus, fish oil supplementation may be able to reduce necrotizing enterocolitis incidence.

INTRODUCTION

The final trimester of pregnancy is a time of significant nutritional and metabolic changes for the fetus. With regards to long-chain polyunsaturated fatty acids (LCPUFA), the greatest accretion occurs in the third trimester, a time of rapid growth as well as of development of the brain. As such, infants who are born prematurely do not benefit from this in utero support. Furthermore, after birth, the smallest and sickest preterm infants are often dependent on a parenteral supply of nutrition, and many intravenous lipid preparations in the neonatal intensive care unit (NICU) are devoid of LCPUFA. Although LCPUFA-supplemented enteral feedings are slowly advanced,

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the preterm infant quickly becomes deficient in these important fatty acids. The authors and others have shown that LCPUFA levels drop within 2 weeks of birth,[1] and therefore, preterm infants are quite deficient in LCPUFA compared with normal in utero accretion.

Previous studies demonstrate a benefit for newborns from LCPUFA with respect to brain and eye development,[2–6] in both term and preterm infants, such that LCPUFA supplementation is now standard of care in term and preterm formula. Furthermore, docosahexaenoic acid (DHA) is the main lipid in the central nervous system. There are varying levels of LCPUFA in breast milk, depending on maternal diet.[7]

In addition to the benefits shown on eye and brain development, LCPUFA have been shown to modulate the inflammatory cascade. In vitro studies demonstrate anti-inflammatory effects via several mechanisms, and both animal and human data support an anti-inflammatory role, particularly for the omega-3 LCPUFAs, such as DHA. Recent studies have focused on the interaction between LCPUFA levels and bronchopulmonary dysplasia (BPD),[8] but it is plausible that supplementation with LCPUFA may also modify risk for other neonatal morbidities, such as sepsis and necrotizing enterocolitis (NEC). In this review, the authors examine the evidence to support a role for LCPUFA in modulating the risk for NEC.

Necrotizing Enterocolitis

NEC is a devastating inflammatory bowel necrosis affecting predominantly preterm infants. This disease affects approximately 10% of infants born weighing less than 1500 g, although the incidence varies widely in published studies as well as by center and region. Several risk factors for NEC are well described, including prematurity, formula feeding, intestinal ischemia, and bacterial colonization. Current dogma supports a theory of an imbalance between proinflammatory and anti-inflammatory forces in the preterm infant, with a shift toward a proinflammatory state in the preterm infant as compared with full-term infants.

As such, preventative strategies to shift the balance toward a more anti-inflammatory profile may show promise in reducing NEC risk. Because NEC often presents very acutely and is even fulminant in certain cases, the focus is on prevention rather than treatment. Once diagnosed, the treatment is largely supportive, rather than specific. Human and animal studies have examined the preventative role of probiotics,[9–11] lactoferrin,[12] erythropoietin,[13] and growth factors.[14,15] Because of their anti-inflammatory mechanism of action, LCPUFA, and in particular, the omega-3 LCPUFA, are an intriguing potential preventative measure.

Long-Chain Polyunsaturated Fatty Acids and Inflammation

With regards to LCPUFA, the omega-3 fatty acids eicosapentaenoic acid (EPA) and DHA give rise to eicosanoids, or cell signaling molecules, which are generally anti-inflammatory in nature, whereas the eicosanoids derived from the omega-6 fatty acids, including arachidonic acid (ARA), are more proinflammatory. DHA and EPA are derived from α-linolenic acid, an essential fatty acid, whereas ARA is derived from linoleic acid, another essential fatty acid. The omega-6 fatty acids are precursors for proinflammatory mediators such as the n-4 series of leukotrienes, whereas the omega-3 fatty acids are precursors for prostaglandins and thromboxanes of the n-3 series and leukotrienes of the n-5 series, which reduce platelet aggregation and vascular tone. The omega-3 fatty acids also generate resolvins, which are endogenous mediators thought to regulate inflammation[16] (**Fig. 1**).

DHA and EPA exert many anti-inflammatory effects, as demonstrated in cell culture models. In human kidney cells, both of these fatty acids decreased gram-negative

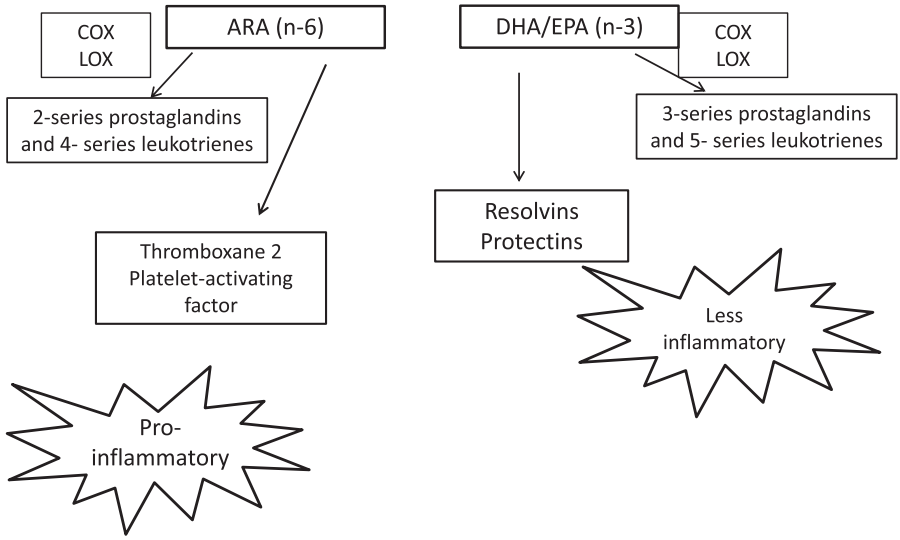

Fig. 1. LCPUFA. Omega-3 and omega-6 LCPUFA compete for cyclo-oxygenase (COX) and lipo-oxygenase (LOX). Depending on the availability of omega-3 LCPUFA, the balance will be shifted toward production of more proinflammatory or less inflammatory eicosanoids.

lipopolysaccharide (LPS)-induced nuclear factor-kappaB (NF-κB) activation,[17] a downstream event inherent to the NEC inflammatory cascade. In a separate study, treatment of human fetal intestinal cells with DHA, EPA, and ARA significantly attenuated proinflammatory cytokine production.[18] Specifically, DHA significantly reduced interleukin-1β (IL-1β)–induced IL-8 and IL-6 protein production compared with controls. Furthermore, DHA treatment of RAW macrophages led to an increase in resolvins, and these resolvins were able to inhibit LPS-induced proinflammatory cytokine expression.[19]

Animal Studies of Necrotizing Enterocolitis

Furthermore, animal models support the role of fish oil in reducing inflammation and modulating the risk for NEC. Using a neonatal rat model of NEC, the authors found a statistically significant reduction in NEC incidence using 3 separate polyunsaturated fatty acids (PUFA)-supplemented formulas, compared with controls ($P<.01$).[20] In a separate study, preterm rats were fed DHA-enriched or EPA-enriched diets compared with a control diet and then subjected to an NEC protocol. Compared with a baseline incidence of NEC of 56% in the control group, the DHA-enriched group demonstrated only a 26.7% NEC incidence, and the EPA-enriched group demonstrated a 20% incidence ($P<.05$).[21] Mouse models have similarly shown a reduction in NEC incidence when chow was supplemented with 10% fish oil for 4 weeks.[22] This study also demonstrated a reduction in intestinal platelet activating factor (PAF) levels in fish oil supplemented mice. PAF is a phospholipid mediator that causes bowel necrosis in NEC models.[23]

LCPUFA likely reduce inflammation via multiple mechanisms. In the authors' study, they found that PUFA supplementation downregulated toll-like receptor (TLR) expression.[20] The TLRs are a family of pattern-recognition receptors that have been well described in the pathogenesis of NEC. Previous work has shown that they are upregulated in animal models of NEC[24] and downregulated in mother-fed pups. The

authors also found that PUFA supplementation of formula in their neonatal rat model decreased plasma endotoxin levels, intestinal phospholipase A_2 II expression at 24 hours, and platelet-activating receptor expression at 48 hours.[25]

Human Studies of Necrotizing Enterocolitis

In humans, several studies have evaluated the effect of LCPUFA in term and preterm infants. However, these studies have used different formulations of LCPUFA, in different target populations, and with varying dosages, so it is difficult to make firm conclusions about the utility of LCPUFA as a modality for reducing inflammation. Smithers and colleagues[26] did a meta-analysis in 2008 and found that LCPUFA-supplemented infants did not have a lower risk of NEC; however, the included studies were not limited to the more preterm infants, and those infants are the most at risk for developing NEC. A more recent meta-analysis systematically evaluated the relationship between omega-3 LCPUFA and common neonatal adverse outcomes.[27] Based on a subgroup analysis looking at preterm infants less than 32 weeks, the investigators found a trend toward a reduction in NEC risk in those infants treated with LCPUFA (pooled relative risk 0.50, 95% confidence interval 0.23–1.10). However, of the 18 randomized trials and 6 observational studies included in the meta-analysis, only 5 specifically looked at infants less than 32 weeks, and NEC was not the primary outcome of interest in the included studies.

More specifically, O'Connor and colleagues[28] randomized 470 preterm infants weighing 750 to 1800 g to one of 3 feeding groups. Two groups were supplemented with ARA and DHA and compared with control. These investigators did not report a difference in NEC in any of the treatment groups, but the study was powered to detect a neurodevelopmental outcome rather than NEC. Furthermore, the DHA dose used in this study was 0.27% and 0.24% of fatty acids in the 2 treatment groups, which is a lower dose than used in other studies. A separate study randomized infants less than 35 weeks' gestation to control formula, formula supplemented with algal-DHA and ARA, or formula supplemented with fish oil DHA and algal DHA.[29] There was no difference in comparison of NEC incidence in any of the groups (control 3% vs algal-DHA 5% vs fish oil DHA 5%). Infants less than 37 weeks' gestation were enrolled into a randomized controlled trial in the United Kingdom, and randomized to either a control formula or a formula supplemented with egg lipid LCPUFA.[30] The primary outcome for this study was neurodevelopmental outcome, but the investigators compared rates for common neonatal morbidities. They found no difference in NEC incidence, with 11% of infants in the control formula group and 19% of infants in the experimental formula group reported to have suspected or confirmed NEC. The same group enrolled infants into a separate study using borage oil supplementation, which contains γ-linolenic acid, a precursor of ARA.[31] Eligible infants had a birth weight of less than 2 kg and were less than 35 weeks' gestation. They were randomized to LCPUFA-supplemented formula and compared with control, and the primary outcome was neurodevelopmental outcome at 18 months as measured by the Bayley Scales of Infant Development II. No difference was noted in the secondary outcome of NEC (2% controls vs 4% in experimental formula group). Of note, the infants in this study were eligible even if they received partial mother's own milk, so this may have confounded results.

More recently, several studies in Australia have evaluated various dosing strategies for LCPUFA supplementation. Collins and colleagues[32] randomized a small group of preterm infants born at less than 30 weeks' gestation to several doses of DHA supplementation and compared them with maternal supplementation and placebo. There was no difference noted in NEC incidence. The same group published a separate

study in which they randomized preterm infants born before 29 weeks' gestation to 60 mg/kg/d of DHA and compared with placebo, to evaluate the effect on BPD.[33] NEC incidence was the same in both groups. The Docosahexaenoic Acid for the Improvement of Neurodevelopmental Outcome in Preterm Infants (DINO) trial enrolled 657 infants born at less than 33 weeks' gestation and randomized them to supplementation with high-dose DHA (1% total fatty acids) or standard-dose DHA (0.3% total fatty acids).[6] The primary outcome of this study was also to evaluate the effect on neurodevelopmental outcome, but NEC was evaluated as a secondary outcome. Again, there was not a statistical difference, but there was not a comparison group that did not receive DHA.

In a separate study by Carlson and colleagues,[34] infants less than 32 weeks' gestation and 725 to 1375 g at birth whose mothers chose not to breast feed were randomly assigned to one of 2 formulas, either a control formula or one that was supplemented with egg phospholipid, containing 0.13% DHA and 0.41% ARA. Compared with infants fed the control formula, infants who were fed the egg phospholipid–containing formula had a significantly lower incidence of NEC (2.9% experimental formula vs 17.6% control formula, $P<.05$). More recently, a randomized controlled trial in Norway evaluated 141 infants with birth weight less than 1500 g and supplementation with DHA and ARA as compared with control, with the primary outcome of cognitive development, as assessed by the Ages and Stages Questionnaire as well as an assessment of memory.[4] These infants were fed human milk and provided LCPUFA supplementation as study oil. With regards to NEC, no significant difference was noted between groups, and the incidence was low in both groups (1.5% in intervention group vs 3% in control group). Finally, Innis and colleagues[35] evaluated a group of premature infants in a double-blind, randomized, multicenter trial. These investigators randomized infants born weighing less than 1560 g who were to be fed formula to one of 3 formulas. One formula contained DHA alone; one had a combination of DHA and ARA, and the control formula had neither DHA nor ARA. The primary outcome was predetermined to be a comparison of growth and visual acuity, but the investigators also collected information on morbidities such as NEC. As in the aforementioned study, the incidence of NEC was quite low, affecting only one baby out of 62 in the control group, 2 of 66 in the DHA supplemented group, and 0 of 66 in the DHA and ARA supplemented group. No statistical difference was noted.

DISCUSSION

Considering the anti-inflammatory effects of the omega-3 LCPUFA, it is plausible that they may have the potential to modulate inflammation in preterm infants. Modulating inflammation may translate to prevention of inflammatory diseases known to affect preterm infants, such as BPD, sepsis, and NEC.

To date, only one single-center human trial has shown a reduction in NEC incidence after LCPUFA supplementation.[34] However, meta-analysis suggests that LCPUFA may reduce NEC. Additional data are needed, with emphasis on very low-birth-weight infants (VLBW; birth weight <1500 g), the most vulnerable population at risk for NEC.

To date, numerous studies have evaluated LCPUFA supplementation in preterm infants, but the primary outcome has largely been neurodevelopmental. Several studies have evaluated NEC as a secondary outcome, and meta-analyses suggest that LCPUFA may reduce the incidence of NEC. In order to more fully elucidate the potential for LCPUFA to modulate inflammation and affect NEC incidence in at-risk preterm infants, a much larger trial is needed, focusing on only the VLBW infants.

There is biologic plausibility that omega-3 LCPUFA can reduce inflammation and potentially modify NEC risk. Animal studies using established NEC models have shown a reduction in NEC, and the omega-3 LCPUFA exert many anti-inflammatory effects. This effect on inflammation likely occurs via several mechanisms, including production of anti-inflammatory eicosanoids, incorporation of omega-3 LCPUFA into membrane phospholipids, production of anti-inflammatory cytokines, and production of resolvins and protectins (Fig. 2).

Both omega-6 and omega-3 LCPUFA produce eicosanoids. However, those produced by omega-3 LCPUFA are much less inflammatory than those produced by omega-6 LCPUFA. Both EPA and ARA use same enzymatic pathways (namely cyclo-oxygenase and lipo-oxygenase) in order to generate prostaglandins, leukotrienes, and thromboxanes.[36] The eicosanoids generated by omega-3 LCPUFA such as EPA, however, are much less inflammatory. Furthermore, supplementation with omega-3 LCPUFA leads to incorporation of these compounds into membrane phospholipids, often replacing ARA, thus limiting its availability for use in production of proinflammatory eicosanoids.

LCPUFA can also modulate inflammation by inhibiting production of proinflammatory cytokines. DHA was shown to reduce IL-1α–induced IL-6 and IL-8 secretion in endothelial cells.[37] Separately, an omega-3 emulsion reduced LPS-induced tumor necrosis factor-α production in RAW macrophages.[38] In this same study, the investigators report a reduction in inhibitor of kappa B (IκB) phosphorylation, and also a reduction in NF-κB activation, both in cells pretreated with the omega-3 emulsion. NF-κB translocates to the nucleus in inflammatory signaling to turn on expression of proinflammatory cytokines, and IκB inhibits this process. In order for NF-κB translocation to occur, IκB must first be phosphorylated. Thus, a reduction in IκB phosphorylation and a reduction in NF-κB activation will effectively blunt proinflammatory cytokine signaling. Furthermore, recent work suggests that peroxisome proliferator–activated receptor alpha (PPARα), a nuclear receptor transcription

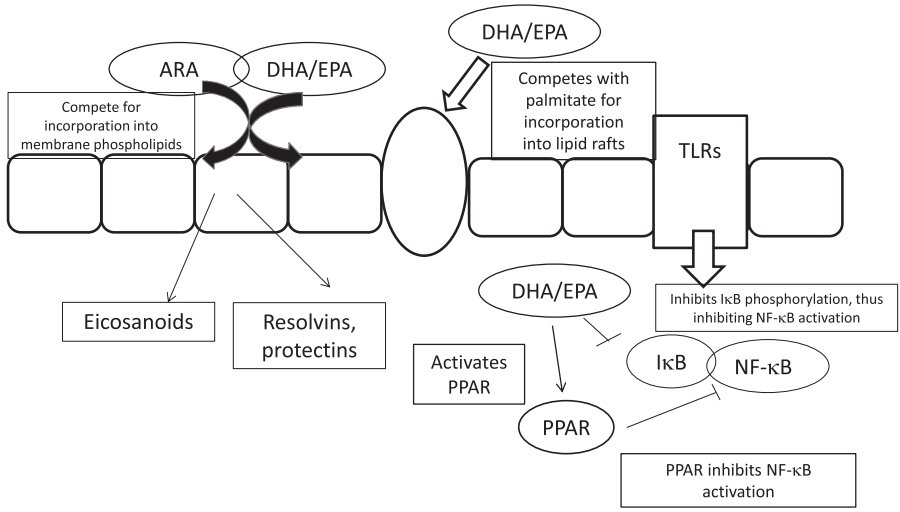

Fig. 2. Potential mechanism of action for fish oil to attenuate inflammation and prevent NEC. Suggested mechanisms include competition for incorporation into membrane phospholipids, competition with palmitate for incorporation into lipid rafts, and inhibition of proinflammatory cytokine production via effects on NF-κB signaling.

factor, is activated by omega-3 LCPUFAs.[39,40] PPARα inhibits NF-κB activation, thus providing another mechanism whereby omega-3 LCPUFAs can reduce inflammation.

LCPUFA can also alter palmitoylation, a posttranslational modification required for effective signaling by many G protein–coupled receptors, including PAF receptor. Palmitoylation targets specific proteins to specialized areas of the plasma membrane called lipid rafts, enabling effective signal transduction. The authors have previously reported that LCPUFA disrupts palmitoylation at least in part, inhibiting PAF receptor signaling.[41]

Last, recent evidence supports the role of resolvins and protectins, mediators derived from omega-3 LCPUFA. These mediators are present especially during the resolution phase of inflammation. They have been shown to reduce inflammatory cytokine expression and reduce neutrophil migration.[19,42]

Despite an abundance of literature supporting the anti-inflammatory role of omega-3 LCPUFA, data for the role of these compounds as prevention for NEC are limited. However, evidence does support their role in prevention of other inflammatory diseases. A recent study by Bisgaard and colleagues[43] supplemented pregnant mothers with fish oil and compared them with placebo-supplemented women. After 3 years, the offspring of the women treated with fish oil had a significantly lower rate of persistent wheezing or asthma (16.9% in fish oil group vs 23.7% in placebo group, $P = .035$). However, this finding has not been supported in similar trials. The DOMInO trial randomized pregnant women to fish oil capsules or vegetable oil capsules without omega-3 LCPUFA.[44] In the follow-up to this trial, the investigators reported on 706 children with a family history of allergic disease. In this particular study, there was no difference in immunoglobulin E–associated allergic disease between the treatment and placebo group (31.5% in both groups, $P = .73$).[45] However, the Bisgaard study used a much higher dose of fish oil (2.4 g), and evidence suggests higher doses are likely necessary in order to exert an anti-inflammatory effect.[46] In further analysis of the DINO trial, the investigators reported on respiratory and allergic outcomes in their study subjects.[47] In those infants treated with high-dose DHA, there was a reduction in BPD both in boys (18.7% high-dose group vs 28% standard-dose group, $P = .03$) and in all infants born at less than 1250 g (34.5% high-dose group vs 47% standard-dose group, $P = .04$). Furthermore, the high-dose group had less hay fever reported at either 12 or 18 months (3.5% high-dose group vs 8.6% standard-dose group, $P = .03$).

Furthermore, low omega-3 LCPUFA levels have been shown to be correlated with common inflammatory morbidities in preterm infants, such as BPD and sepsis.[8,48] In these studies, the omega-6 to omega-3 ratio also correlates with a higher incidence of inflammatory diseases, lending support to the theory that the balance of proinflammatory versus anti-inflammatory mediators contributes to pathogenesis of disease in at-risk infants.

In conclusion, evidence suggests that omega-3 LCPUFA supplementation in preterm infants may lower risk for common inflammatory morbidities in the NICU. However, the human studies to date have used differing products and dosages, making meaningful meta-analyses difficult. Large, multicenter, randomized trials are greatly needed to assess the potential benefit of these compounds. Furthermore, varying doses should be studied, including high doses, because research suggests that the anti-inflammatory benefit may be more robust when giving high-dose LCPUFA. With the survival of smaller and more preterm infants, NEC continues to cause significant morbidity and mortality, and preventative measures are greatly needed.

Best practices

What is the current practice for neonatal necrotizing enterocolitis?

- Preventative measures such as provision of human milk; cautious advancement of feeds
- Recognize signs and symptoms and make prompt diagnosis
- Decompress intestine; withhold enteral feedings; obtain blood culture and start antibiotics; supportive care; surgery as indicated

What changes in current practice are likely to improve outcome?

- Ongoing research into preventative measures to reduce NEC incidence
- Encouragement to mothers to provide mother's own milk as feasible
- Clinical trials into use of LCPUFA, probiotics, growth factors for prevention of NEC

Major recommendations

- Encourage all mothers to provide breast milk for their preterm infants when feasible
- When mother is unable to provide expressed breast milk, encourage use of donor human milk
- Recognize signs and symptoms of NEC promptly and initiate treatment and supportive care
- Continue investigative efforts into preventative measures for reducing NEC incidence

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