Omega-3 fatty acids in inflammation: Actions & impact on rheumatoid arthritis, inflammatory bowel disease and asthma

Philip Calder
Professor of Nutritional Immunology
University of Southampton
What is inflammation?

- Inflammation is a NORMAL response to infection, injury and trauma
- Typified by redness, swelling, heat and pain
- Normally it is protective (and so beneficial)
- Can be acute (i.e. short lived) or chronic (i.e. long term)
- Involves various cells including granulocytes (e.g. neutrophils), macrophages and lymphocytes
- Involves mediators
Diseases or conditions that involve inflammation

- Rheumatoid arthritis
- Crohn’s disease
- Ulcerative colitis
- Cystic fibrosis
- Psoriasis
- Lupus
- Type-1 diabetes
- Childhood asthma
- Adult asthma
- Allergic diseases
- Atherosclerosis
- Acute cardiovascular events
- Post-surgery
- Trauma & sepsis
- Obesity …….
ω-6 PUFA content of human mononuclear cells

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (18:2ω-6)</td>
<td>10</td>
</tr>
<tr>
<td>DGLA (20:3ω-6)</td>
<td>1.5</td>
</tr>
<tr>
<td>Arachidonic acid (20:4ω-6)</td>
<td>20</td>
</tr>
</tbody>
</table>
A major role of arachidonic acid is as a precursor for eicosanoids

Arachidonic acid in cell membrane phospholipid

\[ \text{Phospholipase A}_2 \]

Free arachidonic acid

\[ \begin{align*}
\text{COX} & \rightarrow \text{PGG}_2 \\
\text{15-LOX} & \rightarrow \text{15-HPETE} \\
\text{12-LOX} & \rightarrow \text{12-HPETE} \\
\text{5-LOX} & \rightarrow \text{5-HPETE} \\
\end{align*} \]

\[ \begin{align*}
\text{PGG}_2 & \rightarrow \text{PGH}_2 \\
\text{15-HPETE} & \rightarrow \text{15-HETE} \\
\text{12-HPETE} & \rightarrow \text{12-HETE} \\
\text{5-HPETE} & \rightarrow \text{5-HETE} \\
\end{align*} \]

\[ \begin{align*}
\text{PGH}_2 & \rightarrow \text{PGD}_2 \\
\text{PGD}_2 & \rightarrow \text{PGE}_2 \\
\text{PGE}_2 & \rightarrow \text{PGI}_2 \\
\text{PGI}_2 & \rightarrow \text{TXA}_2 \\
\text{LXA}_4 & \rightarrow \text{LTC}_4 \\
\text{LTC}_4 & \rightarrow \text{LTD}_4 \\
\text{LTD}_4 & \rightarrow \text{LTE}_4 \\
\text{LTA}_4 & \rightarrow \text{LTB}_4 \\
\end{align*} \]
Some pro-inflammatory effects of PGE$_2$

Induces fever
Increases vascular permeability
Increases vasodilation
Causes pain
Enhances pain caused by other agents

Induces its own production
Induces production of IL-6 (a pro-inflammatory cytokine)
## Some pro-inflammatory effects of 4-series leukotrienes

<table>
<thead>
<tr>
<th>Produced by</th>
<th>LTB₄</th>
<th>LTC₄, D₄, E₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils,</td>
<td></td>
<td>Mast cells, basophils, eosinophils</td>
</tr>
<tr>
<td>macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actions</td>
<td>Leukocyte chemotaxis</td>
<td>Bronchoconstriction</td>
</tr>
<tr>
<td></td>
<td>Vascular permeability</td>
<td>Vascular permeability</td>
</tr>
<tr>
<td></td>
<td>Epidermal proliferation</td>
<td>Mucus secretion</td>
</tr>
<tr>
<td></td>
<td>Leukocyte degranulation</td>
<td>Hypersensitivity</td>
</tr>
<tr>
<td></td>
<td>Leukocyte adhesion</td>
<td>Skin vasodilation</td>
</tr>
<tr>
<td></td>
<td>Inflammatory mediator production (Superoxide; Inflammatory cytokines)</td>
<td>Arteriole constriction</td>
</tr>
<tr>
<td></td>
<td>Pain</td>
<td></td>
</tr>
</tbody>
</table>
LINOLEIC ACID

ARACHIDONIC ACID

2-Series PG

4-Series LT

Mediators of inflammation
Allergic inflammation
- role of arachidonic acid-derived mediators

Linoleic acid → Arachidonic acid → 4-series LT → 5-HETE → IgE

Linoleic acid
Arachidonic acid
PGD$_2$

PGE$_2$ → B-cell
A new anti-inflammatory role for PGE$_2$?


A new anti-inflammatory role for PGE$_2$?

ARA

5-LOX

4-series LT

Inflammatory actions

COX

PGE$_2$

Inflammation actions

15-LOX

Lipoxin A$_4$

Inflammation “stop” signal
LINOLEIC ACID

ARACHIDONIC ACID

2-Series PG

4-Series LT

Mediators and regulators of inflammation
Metabolism of \( \omega-6 \) and \( \omega-3 \) PUFA

Linoleic acid (18:2\( \omega-6 \)) \( \rightarrow \) \( \alpha \)-Linolenic acid (18:3\( \omega-3 \))

- 6-desaturase
  - GLA (18:3\( \omega-6 \)) \( \rightarrow \) 18:4\( \omega-3 \)
    - Elongase
      - DGLA (20:3\( \omega-6 \)) \( \rightarrow \) 20:4\( \omega-3 \)
        - 5-desaturase
          - Arachidonic acid (20:4\( \omega-6 \)) \( \rightarrow \) EPA (20:5\( \omega-3 \))
            - DPA (22:5\( \omega-3 \)) \( \rightarrow \) DHA (22:6\( \omega-3 \))
### ω-6 and ω-3 PUFA contents of human mononuclear cells

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (18:2ω-6)</td>
<td>10</td>
</tr>
<tr>
<td>DGLA (20:3ω-6)</td>
<td>1.5</td>
</tr>
<tr>
<td>Arachidonic acid (20:4ω-6)</td>
<td>20</td>
</tr>
<tr>
<td>α-Linolenic acid (18:3ω-3)</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>EPA</td>
<td>1.0</td>
</tr>
<tr>
<td>DHA</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Feeding fish oil decreases the amount of arachidonic acid in human mononuclear cells.

Healthy subjects given fish oil providing 2.1 g EPA + 1.1 g DHA/day for 12 weeks.

Time course of incorporation of EPA and DHA into human mononuclear cell phospholipids

Healthy volunteers given fish oil (2.1 g EPA and 1.1 g DHA/day) for 12 weeks
Dose response of incorporation of EPA and DHA into human neutrophil phospholipids

Healthy volunteers given fish oil (0 to 9 g/day) for 12 weeks
Healy et al. (2000) Lipids 35, 763-768
PGE$_2$ production by human mononuclear cells before and after fish oil supplementation

Miles & Calder, unpublished
Effect of fish oil on 5-LOX metabolite production by human inflammatory cells

Healthy volunteers given fish oil (9.4 g EPA + 5 g DHA/day) for 10 weeks
Classic view of the anti-inflammatory action of long chain ω-3 PUFA

- Arachidonic acid in membrane phospholipids
- Free arachidonic acid
- COX-2
- 2-series PG and TX
- Inflammatory effects
- 5-LOX
- 4-series LT
- Inflammatory effects
- Phospholipase A$_2$
- EPA
- DHA
EPA is also a precursor of eicosanoids

ARACHIDONIC ACID
- 2-Series PG
- 4-Series LT
- 3-Series PG

EPA
- 5-Series LT

Inflammation
Effect of dietary fish oil on eicosanoid production by human inflammatory cells

Healthy volunteers given fish oil (9.4 g EPA + 5 g DHA/day) for 10 weeks
Mediators formed from EPA often have different biological potencies than those formed from arachidonic acid.
Neutrophil chemotaxis: $\text{LTB}_4$ vs. $\text{LTB}_5$

Chemotactic activity (neutrophils per 5 hpf)

$\text{LT} (\log \text{M})$

Lee et al. (1988) Clin. Sci. 74, 467-475
PGE$_2$ vs. PGE$_3$ and induction of COX-2

NIH3T3 fibroblasts at one hour
Bagga et al. (2003) PNAS 100, 1751-1756
**PGE₂ vs PGE₃ and IL-6 production**

RAW 264.7 macrophages with 50 ng/ml PGE

Bagga et al. (2003) PNAS 100, 1751-1756
ARACHIDONIC ACID

2-Series PG  4-Series LT  3-Series PG

Inflammation

EPA

3-Series PG  5-Series LT

Less inflammation
Modified view of the anti-inflammatory action of long chain ω-3 PUFA

- Arachidonic acid in membrane phospholipids
  - Phospholipase A₂
  - Free arachidonic acid
    - 2-series PG and TX
      - Pro- and anti-inflammatory effects
    - 4-series LT
      - Pro-inflammatory effects
    - 3-series PG and TX
      - Less inflammatory effects than 2-series
    - 5-series LT
      - Less inflammatory effects than 4-series

- EPA
  - 5-LOX
  - COX-2

- DHA
  - 5-LOX
  - COX-2

EPA and DHA have less inflammatory effects than 2-series and 4-series.
Resolvins & related compounds

EPA

COX-2 (& presence of aspirin)

E-series resolvins

Anti-inflammatory; inflammation resolving

DHA

D-series resolvins, neuroprotectins etc.

Anti-inflammatory; inflammation resolving
Less arachidonic acid

Lower concentration of 2-series PG and 4-series LT

Cytokines and other mediators → Cellular responses
Leukocyte chemotaxis

- n = 7 (male; 22 to 53 years)
- 5.4 g EPA + DHA/ day for 6 weeks
- Neutrophil chemotaxis to LTB$_4$ decreased

Lee et al. (1985) N. Engl. J. Med. 312, 1217
Pro-inflammatory cytokines

Inflammatory stimulus

- \( \text{TNF-} \alpha \)
- \( \text{IL-1} \beta \)
- \( \text{IL-6} \)

Cause:
- Local inflammation
- Fever
- Activation of T and B cells
- Acute phase protein synthesis
- Hypotension
- Coagulation
- Body wasting
- Bone loss
Inverse relationship between \( \omega-3 \) fatty acid status and human cytokine production

EPA or DHA?
Study of the effect of DHA alone on inflammatory mediator production

Kelley et al. (1999) Lipids 34, 317-324

Healthy men mean age 33 years
N = 7
Consumed 6 g DHA/day (DHASCO) for 12 weeks
Fairly low fat background diet (30% en from fat; DHA < 0.1 g/day)
<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE$_2$ (ng/million cells)</td>
<td>13.1 +/- 2.0</td>
<td>5.0 +/- 1.0*</td>
</tr>
<tr>
<td>LTB$_4$ (pg/million cells)</td>
<td>140 +/- 30</td>
<td>34 +/- 10*</td>
</tr>
</tbody>
</table>
Inflammatory cytokines (pg/ml) (LPS-stimulated PBMCs)

Kelley et al. (1999) Lipids 34, 317-324
Study of EPA vs. DHA


Hypertensive type 2 diabetics; both male & female; non-smokers; aged 40 to 75 years

4 g/d EPA vs. 4 g/d DHA vs placebo (olive oil)

6 weeks

Plasma TNF-α

Some other plasma markers of inflammation (CRP, IL-6) did not change

Plasma TNF-α (% change from baseline)
What about $\alpha$-linolenic acid?
Inflammatory cytokines

- Males aged 22 to 44 years
- Sunflower oil-based diet vs. Flaxseed oil-based diet (13.7 g αLNA/day) for 4 weeks
  Then + 2.9 g EPA + DHA/day 4 weeks
- IL-1β and TNF-α production in response to LPS decreased but decrease greater with long chain n-3 fatty acids

Caughey et al. (1996)
Many studies report no effect of $\alpha$-linolenic acid:

<table>
<thead>
<tr>
<th>Intake (g/d)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>TNF, IL-1 &amp; IL-6 production</td>
<td>Thies et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Monocyte respiratory burst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neutrophil respiratory burst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sCAM concentration</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Neutrophil chemotaxis</td>
<td>Healy et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Neutrophil respiratory burst</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>TNF, IL-1, IL-6 production</td>
<td>Wallace et al. (2003)</td>
</tr>
<tr>
<td>4.5, 9.0</td>
<td>TNF, IL-1, IL-6 production</td>
<td>Kew et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Neutrophil respiratory burst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monocyte respiratory burst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICAM-1 expression on monocytes</td>
<td></td>
</tr>
</tbody>
</table>
These studies suggest that an intake of $\alpha$-linolenic acid of at least 10 g/d is required to see anti-inflammatory effects and even then these effects will be weaker than those exerted by long chain $\omega$-3 PUFA.
Potential clinical benefits of the anti-inflammatory effects of long chain ω-3 PUFA

- Rheumatoid arthritis
- Crohn’s disease
- Ulcerative colitis
- Cystic fibrosis
- Psoriasis
- Lupus
- Type-1 diabetes
- Childhood asthma
- Adult asthma
- Allergic diseases
- Atherosclerosis
- Acute cardiovascular events
- Post-surgery
- Trauma & sepsis
- ……
Fish oil and RA

- Fish oil exerts anti-inflammatory actions in RA patients (e.g. decreased LTB$_4$ production)
- There have been 17 placebo-controlled, double-blind, clinical trials of fish oil in RA
- First was reported in 1985; most recent in 2005
- Used 1 to 7.1 g EPA plus DHA per day (most used about 3.3 g per day)
- Duration of 4 to 52 weeks
- Two trials used more than one dose of fish oil
- Several trials report outcomes at more than one time point
- Report a variety of clinical outcomes
Fish oil and RA

- 16/17 studies report improvement in at least two clinical outcomes
- 6/17 studies report improvement in at least four clinical outcomes
- Studies report decreased number of swollen and tender joints, decreased joint pain, increased grip strength, decreased duration of morning stiffness
- All studies which monitored NSAID use reported a significant reduction
- Two other studies required cessation of NSAID use - patients could endure this
“One can conclude that the findings of benefit from dietary fish oil in RA treatment are robust”

“Thus, dietary fish oil supplements in RA have treatment efficacy”

“Why are fish oil supplements not used more widely in RA?”

“…. dietary fish oil supplements should now be regarded as part of the standard therapy for RA”
“Dietary fish oil supplementation for three months significantly reduced tender joint count (mean difference -2.9; P = 0.001) and morning stiffness (mean difference -25.9 minutes; P = 0.01)”
“no effect on patient report of pain, swollen joint count, disease activity of patient’s global assessment”

“of seven studies that assessed the effect on anti-inflammatory drug or corticosteroid requirement, six demonstrated reduced requirement for these drugs”

……. “n-3 fatty acids may reduce requirements for corticosteroids”

Did not assess tender joint count but reiterated “n-3 fatty acids reduce tender joint counts”
Fish oil and IBD (UC & CD)

- Fish oil exerts anti-inflammatory actions in IBD patients (e.g. decreased LTB$_4$ production by gut mucosa and by leukocytes)
- Uncontrolled or open trials report benefit from fish oil
- There have been 12 placebo-controlled, double-blind, clinical trials of fish oil in IBD (8 in UC; 3 in CD; 1 in UC+CD)
- First was reported in 1989; most recent in 2005
- Used 2.7 to 5.8 g EPA plus DHA per day (most used about 4.5 to 5 g per day)
- Duration of 12 to 104 weeks
- Report a variety of clinical outcomes
Fish oil and IBD

- 7/12 studies report improvement in at least two clinical outcomes
- Studies report improved gut histology, decreased use of corticosteroids, decreased disease activity
- 5/12 studies report no improvement in any clinical outcome
Patients with Crohn’s Disease (in remission) given fish oil

- Patients remaining in remission (%)
- Time (months)

- Fish oil (2.7 g LC n-3 PUFA/day)
- Placebo
AHRQ Report 2004

Reviewed 13 trials published 1989 to 2002

Looked at clinical score, sigmoidoscope score, gut mucosal histology score, induced remission and relapse

Concluded that sufficient data to meta-analyse only relapse and only in UC patients - 5 studies considered and 3 used - “n-3 fatty acids have no effect on relative risk of relapse in ulcerative colitis” .... “there was a statistically non-significant reduction in requirement for corticosteroids for n-3 fatty acids relative to placebo in two studies”
Fish oil and asthma

- Fish oil exerts anti-inflammatory actions in asthma patients (e.g. decreased LTB₄ production and decreased leukocyte chemotaxis)
- Epidemiological evidence of a protective effect on long chain ω-3 PUFA on adult and childhood asthma
- Uncontrolled or open trials report benefit from fish oil in adult asthma
- There have been 9 placebo-controlled, double-blind, clinical trials of fish oil in asthma (7 in adults, 2 in children)
- First was reported in 1988; most recent in 2000
- Studies in adults used 1.0 to 6.0 g EPA plus DHA per day
- Duration of 4 to 52 weeks
- Report a variety of clinical outcomes related to lung function, disease severity etc.
Thien et al. (2002) Cochrane Library

“no consistent effect on FEV$_1$, PEF, asthma symptoms, asthma medication use or bronchial hyper-reactivity” but “one study in children showed improved peak flow and reduced asthma medication use”
Covered 26 trials (both placebo, controlled, randomized and others)

“no definitive conclusion can yet be drawn regarding the efficacy of n-3 fatty acid supplementation as a treatment for asthma in children and adults”
29 children (mean age 10 y) with bronchial asthma
- Fish oil capsules provided for 10 months
- Olive oil placebo
- Asthma score evaluated 4 times daily and totalled for each day
- Each month subjects were challenged with increasing doses of acetylcholine
- FEV₁ measured
Control Fish oil

-30
-20
-10
0
10
20
30

Asthma score

-1000
0
1000
2000
3000

IC20 for acetylcholine (µg/ml)

At entry

After 10 months

Change
Design of study of Broughton et al. (1997)

- 26 non-smoking atopic asthmatics
- Detailed dietary assessment to determine accurately each individual's ω-6 PUFA intake
- Fish oil capsules provided on an individual basis to achieve ω-6:ω-3 PUFA ratios of 10 (low FO) and 2 (high FO); each treatment period lasted 4 weeks
- At baseline and after each treatment period subjects challenged with increasing doses of methacholine
- FVC, FEV₁, PEF and FEF₂₅₋₇₅ measured
- Urinary 4- and 5-series LT measured
The graph illustrates the effect of cumulative dose of methacholine on FEV1 (%). Two lines are shown:

- **Baseline**
- **Low n-3**

The x-axis represents the cumulative dose of methacholine ranging from 0.01 to 100, while the y-axis shows FEV1 (%) ranging from 100 to 0. The graph shows a decrease in FEV1 (%) as the cumulative dose of methacholine increases.
Cumulative dose of methacholine

Baseline

Subset of high n-3

High n-3

FEV1 (%)

Cumulative dose of methacholine

0.01 0.1 1 10 100
### Summary of the results of Broughton et al. (1997)

#### Cumulative dose of methacholine to cause a 20% decline (units)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Low dose fish oil</th>
<th>High dose fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Responders</td>
</tr>
<tr>
<td>FVC</td>
<td>24.1</td>
<td>11.8</td>
<td>&gt; 67</td>
</tr>
<tr>
<td>PEF</td>
<td>17.1</td>
<td>5.9</td>
<td>&gt; 67</td>
</tr>
<tr>
<td>FEV</td>
<td>16.9</td>
<td>1.9</td>
<td>&gt; 67</td>
</tr>
<tr>
<td>FEF</td>
<td>9.0</td>
<td>0.7</td>
<td>&gt; 67</td>
</tr>
</tbody>
</table>
Summary of clinical benefits of long chain ω-3 PUFA in human inflammatory diseases

Evidence in favour of benefit:
Rheumatoid arthritis – therapeutic

Weaker evidence:
Crohn’s disease – prolongs remission
Psoriasis - therapeutic

Some evidence:
Childhood asthma – therapeutic
Cystic fibrosis

Contradictory or no evidence:
Ulcerative colitis
Lupus
Type-1 diabetes
Adult asthma
Eicosanoids derived from arachidonic acid are involved as mediators and regulators of inflammation.

- EPA and DHA from oily fish/fish oil can partially replace arachidonic acid in membrane phospholipids.
- $\omega$-3 fatty acids (especially EPA) lead to decreased production of eicosanoids from arachidonic acid.
- EPA and DHA give rise to anti-inflammatory resolvins (cell culture & animal work).
- $\omega$-3 fatty acids lead to decreased production of inflammatory cytokines.
- Through these effects, $\omega$-3 fatty acids act to decrease inflammation.
- $\omega$-3 fatty acids may protect against and provide therapy for diseases with an overt or covert inflammatory component.
- Evidence for therapeutic benefit from $\omega$-3 fatty acids is reasonably strong in RA but is weaker elsewhere – doses used are quite high (approx. 3.5 g/day).
- $\alpha$-Linolenic acid is not anti-inflammatory at intakes < 10 g/d.