Anti-Inflammatory Effects of Fish Oils

A. Macrophage Production of Eicosanoids
B. w-3 PUFA Supplementation of Macrophages
C. w-3 PUFA in IR & diabetes

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Eicosanoids Contribute to the Inflammatory Response

**Microbial Insult/Injury**

**Initiation/Propagation of Inflammation**

**Resolution of Inflammation**

- Extravasation/Recruitment
- Block Recruitment

- Vasodilation

- Prostaglandins
- Leukotrienes

- Lipoxins
- Resolvins
- Protectins

**COX**

**LOX**

**Non-phlogistic Phagocytosis**

- w-6 Fatty Acids 20:4 and related

- w-3 Fatty Acids 20:5 22:6
Eicosanoid Signaling Pathways in Macrophages

- ATP
- LPS (KLA)
- P2X
- cPLA$_2$
- COX
- COX-2
- NF-$\kappa$B
- Sustained Ca$^{2+}$ Influx
- Eicosanoid metabolites
Basic Macrophage Experimental Scheme

- Macrophages
- KLA (TLR4)
- ATP (P2X7)
- Isolate Media & Solid Phase Extraction (Eicosanoids)
- Eicosanoid enzyme mRNA & protein

LC-MS/MS

Quehenberger et al. J.Lipid Res. 51, 3299-3305 (2010)
Eicosanoid Analytes

184 Total

- AA: 114
- LA: 26
- LLA: 22
- DGLA: 18
- EPA: 13
- DHA: 17
- Internal Standards: 26

Total: 184
Cellular Eicosanoid Metabolism

Eicosanoid Genes to Metabolites

The Beneficial Lipids in Fish Oil

- Fish oil is composed primarily of two \( \omega-3 \) PUFAs:
  - \( \text{Eicosapentaenoic Acid (EPA)} \) (C20:5, \( \omega-3 \))
  - \( \text{Docosahexaenoic Acid (DHA)} \) (C22:6, \( \omega-3 \))

- Humans synthesize from essential fatty acid \( \alpha \)-linolenic acid (C18:3, \( \omega-3 \))
- EPA is found at barely detectable levels in humans
- DHA is more abundant than EPA; found primarily in the brain and retina
Arachidonic Acid Serves as a Precursor for Many Eicosanoids

Are eicosanoids less pro-inflammatory when derived from EPA/DHA?
Effects of PUFA Supplementation on Fatty Acid Membrane Distribution

Membrane PUFA Composition

PUFA Release (2 Hr LPS)
Effects of PUFA Supplementation on Fatty Acid Release

- LPS
- ATP

Graphs showing the release of fatty acids (AA, EPA, DHA) over time (Hr and Min) for different treatments (Control, AA, EPA, DHA).
Effects of PUFA Supplementation on TLR-4 Stimulated Eicosanoid Signaling

Fold Increase

Fold Decrease

Not detected
Effects of PUFA Supplementation on TLR-4 Stimulated COX-2 Signaling

Norris PC et al., PNAS 2012
Effects of PUFA Supplementation on ATP Stimulated Eicosanoid Signaling

Fold Increase

Fold Decrease

Not detected
Effects of PUFA Supplementation on ATP Stimulated Eicosanoid Signaling

Norris PC et al., PNAS 2012
Effect of Fatty Acids on Adipose Inflammation and IR

Adopted from Glass CK and Olefsky JM
Cell Metabolism 2012

Oh DY et al., Cell 2010
Eicosanoids in primary adipocyte conditioned medium

**Free AA**

- **WT**
- **GPR120KO**

**PGE2**

- **WT**
- **GPR120KO**

**15d-PGJ2**

**LTB4**

**PGF2a**

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**Eicosanoids in primary adipocyte conditioned medium**

- **dpm/100mg**

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w-3 Fatty Acids Reduce Inflammatory Stress in Adipose Tissue

- 8-iso PGF2α III
- Free AA
- PGD2
- 5-HETE
- LTB4

pmol/mg Adipose Tissue
Aspirin-Triggered Lipoxinin Formation

Downregulate:
- Neutrophil transmigration
- Cytokine release

Promote resolution:
- Removal of apoptotic cells by macs
- Antifibrotic
- Signals through ALX (FPR2)

Ye et al. BBRC 1992
Omega-3 Fatty Acids Inhibit COX-derived 15-HETE

**TLR4 Stimulation**

- **Eicosanoid (pmol/10^6 cells)**
- **Time (Hr)**: 0, 4, 8, 12, 16, 20, 24

**P2X7 Stimulation**

- **Eicosanoid (pmol/10^6 cells)**
- **Time (Min)**: 0, 15, 30, 45, 60

- **Control**
- **AA**
- **EPA**
- **DHA**
### Global Summary of Fish Oil Effects

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**Supplement**
- Decrease
- No Change
- Increase

Norris PC et al., PNAS 2012
Conclusions on Omega-3 Study

- The global effects of EPA and DHA on normal lipid metabolism can be quantitatively studied.
- EPA and DHA affect the overall eicosadome decreasing production of some, but not all, AA-derived eicosanoids.
- There is a concomitant increase in specific EPA- and DHA-derived metabolites.
- cPLA2 releases EPA/DHA from membrane phospholipids.
- Deciphering the role of fish oil-derived ω-3 EPA and DHA in inflammatory eicosanoid signaling provides insight as to their role as therapeutic agents in human disease.
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